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CCL3L1 copy number is a strong genetic determinant of HIV seropositivity in Caucasian intravenous drug users

Kristi HUIK[1,*], **Maarja SADAM**[1], **Tõnis KARKI**[1], **Radko AVI**[1], **Tõnu KRISPIN**[1], **Piret PAAP**[2], **Kristi RÜÜTEL**[3], **Anneli UUSKÜLA**[4], **Ave TALU**[3], **Katri ABEL-OLLO**[3], and **Irja LUTSAR**[1,*]

[1] Department of Microbiology, Faculty of Medicine, University of Tartu, Tartu, Estonia

[2] Hospital of Tartu Prison, Tartu, Estonia

[3] National Institute for Health Development, Tallinn, Estonia

[4] University of Tartu Department of Public Health, University of Tartu, Tartu, Estonia

Abstract

BACKGROUND—A high copy number of *CCL3L1*, the most potent HIV-suppressive chemokine, associates with reduced HIV susceptibility. Whether *CCL3L1* influences acquisition of multiple blood-borne infections (HCV, HIV-1, HBV) that occurs commonly among intravenous drug users (IDUs) is unknown.

METHODS—We determined *CCL3L1* copy number by real-time PCR among 374 Caucasian IDUs from Estonia of whom 285 were HCV-positive, 208 HIV+, 177 HCV+/HIV+, and 57 HCV−/HIV−.

RESULTS—In univariate and multivariate analyses, HCV and HBV seropositivity, and duration of IDU each strongly predicted HIV seropositivity. A high *CCL3L1* copy number (>2) associated with a 80% reduced risk of acquiring HIV, after adjusting for age, gender, HCV/HBV status, *CCR5-Δ32* polymorphism and IDU duration (OR=0.20; 95% CI=0.09–0.45). By contrast, *CCL3L1* gene dose did not influence HCV seropositivity. Among HCV+ IDUs, there was a 3.5-fold over- and 65% under-representation of a high *CCL3L1* copy number among HCV+/HIV− and HCV+/HIV+ subjects, respectively.

CONCLUSION—Among IDUs exposed heavily to HCV/HIV, *CCL3L1* copy number is a major determinant of HIV seropositivity, but not HCV seropositivity. The contrasting distribution of a protective high *CCL3L1* copy number among HCV+/HIV− vs HCV+/HIV+ IDUs may reflect that HIV preferentially selects for subjects with a low *CCL3L1* gene dose.

Keywords

chemokine copy number; HIV; HCV; IDU

*Corresponding author: Kristi Huik, Department of Microbiology, Faculty of Medicine, University of Tartu, Ravila 19, 50411 Tartu, Estonia. Tel: + 372 53 43 24 53. Fax: + 372 7 374 172. kristi.huik@ut.ee and Irja Lutsar, Department of Microbiology, Faculty of Medicine, University of Tartu, Ravila 19, 50411 Tartu, Estonia. Tel: + 372 7 374 171. Fax: + 372 7 374 172. irja.lutsar@ut.ee. Affiliation has changed since completion of the study: Maarja Sadam, Estonian Forensic Science Institute, Tallinn, Estonia

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INTRODUCTION

Complex interactions between three parameters -- environment, microbial agent, and host genotype --are thought to be a key determinant of the outcome to infectious challenge [1]. Conceptually, environmental factors may be parameters that are intrinsic to the host such as co-infections. Abundant data supports the relevance of investigating the factors that influence HIV pathogenesis in the context of microbial co-pathogens [2–8], especially HCV and HBV, which are frequent co-infections in HIV-infected subjects [9]. However, few studies have been undertaken to define the impact of these complex interactions on HIV-1 susceptibility.

With respect to host genotypes that may influence HIV susceptibility, significant attention has been focused on variations in CCR5, the primary co-receptor for the cell entry of HIV [10–12]. CC ligand 3 like-1 (CCL3L1), CCL3, CCL4, CCL4L1 and CCL5 are the main ligands of CCR5 and they inhibit entry of HIV into CD4+ T-cells in vitro [10,11,13–16]. In addition, CCR5 ligands may affect HIV pathogenesis by influencing several facets of the immune response, including cell-mediated immunity [17–22]. Notably, among the CCR5 chemokine ligands, CCL3L1 is the most potent and has maximal HIV-suppressive properties in vitro [14–16]. The region on chromosome 17q that encodes several CCR5 ligands is a hot-spot for segmental duplications such that individuals vary with respect to the number of *CCL3L1*-containing segmental duplications [16,23–27]. Hence individuals differ with regards to the copy number of genes that are found in this segmental duplication, including chemokine genes such as *CCL3L1* and *CCL4L1* [16,23–27]. Previous studies found that persons of European origin possess between one to six *CCL3L1* gene-containing segmental duplications (henceforth designated as *CCL3L1* copies) with an average of two copies [24–26,28]. By contrast, those of African descent possess higher *CCL3L1* copy numbers than those of European descent [24,26,29–31].

A low *CCL3L1* copy number is associated with reduced chemokine levels and a higher proportion of CCR5 expressing CD4+ cells [24,25]. Genetic association studies found that a low *CCL3L1* copy number was associated with an increased risk of acquiring HIV infection in European-, African-, Hispanic-American adults infected primarily through the mucosal route [24]; hemophiliacs from Japan [32]; as well as Argentinean [24], South African [29,30] and Ukrainian [28] children exposed perinatally to HIV.

Previous studies have found that a genetic factor may confer differential effects, depending on the route of infection. For example, Martin et al showed that a DC-SIGN promoter polymorphism was associated with increased risk for parenteral, but not mucosal, acquisition of HIV infection [33]. Although the results of the studies in hemophiliacs are suggestive [32], most of the prior studies which defined the association of a high *CCL3L1* copy number with decreased HIV susceptibility were primarily in subjects infected via the mucosal or perinatal route [24,28–30,32]. To determine whether there was a relationship between *CCL3L1* copy number and risk of acquiring infection following parenteral exposure of HIV, we examined intravenous drug users (IDUs).

We investigated IDU's of European descent from Estonia, a geographic region in which the HIV epidemic is relatively new [34]. The outbreaks in Estonia were first detected among IDUs in 2000 and HIV infection is still concentrated among this population group [34]. Furthermore, infection with HIV among these IDUs is accompanied by a very high frequency of viral co-infections with HCV and HBV [35]. This provided the unique opportunity to examine whether there was an independent association of *CCL3L1* copy number with these two viral infections. In addition, to the copy number of *CCL3L1*, we determined the association of the 32-bp deletion mutation in *CCR5* (*CCR5-Δ32*) with HIV susceptibility because previous studies have demonstrated that homozygyosity for this polymorphism is associated with resistance to HIV

infection [10–12]; the associations of *CCR5-Δ32/Δ32* with HCV susceptibility vary between cohorts [36–39]. The results of our study confirm and extend significantly the notion that *CCL3L1* plays a critical role in HIV susceptibility and also underscore the importance of accounting for complex interactions among HCV, HIV and *CCL3L1* genotype when analyzing genotype-phenotype (HIV susceptibility) relationships.

METHODS

Subjects and sample collection

The study subjects were recruited in 2006 and 2007 from the syringe-exchange programs and three Estonian prisons; 374 ancestrally Caucasian IDUs (301 male; 55 female and gender unknown in 18; median age of 26 years; 95% antiretroviral therapy naïve) were enrolled. The data on the length of IV drug use (IVDU) was available for 249 subjects. All study subjects reported use of IV drugs for a minimum of one year, with most having longer periods of IVDU. From each subject two samples of 8 ml of venous blood were collected and transported immediately to the laboratory. The first blood sample was used for detection of HIV-1, HCV, and HBV serostatus and the second sample was used later for genotyping analyses.

HIV, HCV and HBV serostatus

HIV testing was performed in the Estonian Central HIV Reference Laboratory using a fourth generation enzyme-linked immunoassay (Vironistica HIV Uniform II Ag/Ab, BioMerieux, Marcy Etoile, France); all positive results were confirmed by a immunoblotting assay (INNO LIA HIV I/II Score Westernblot (Microgen Bioproducts Ltd, Surrey, UK). The presence of HCV antibodies was tested with the ETI-AB-HCVK-3 anti-HCV test (DiaSorin, Vercelli, Italy). HBV seropositivity was assessed by ETI-MAK-4 HBsAg (DiaSorin, Saluggia, Italy) and ETI-AB-COREK Plus (anti-HBc core) (DiaSorin, Saluggia, Italy).

Genotyping of *CCR5* and *CCL3L1* variations

Genomic DNA was extracted from peripheral blood mononuclear cells (courtesy of Professor Ismo Virtanen, University of Helsinki) using the Qiagen QIAamp DNA minikit (Qiagen, Hilden, Germany). *CCR5-Δ32* mutation was assessed using PCR-RFLP as described previously [40]. *CCL3L1* copy number was determined using quantitative real-time PCR with primers/probes and experimental conditions exactly as described previously [24].

Statistical analyses

Differences in gene copy numbers and other covariates were assessed by the Chi-square tests. Univariate and multivariate logistic regression models were used to determine the association of *CCR5* or *CCL3L1* genotype and other covariates with HIV or HCV status. Interactive multivariate logistic regression analyses were also conducted to assess the interactions between duration of IVDU and *CCL3L1* copy number. STATA 6.0 (Stata Corporation, College Station, Texas, USA) was used for the statistical analyses. The study was approved by the Ethics Committee of Tallinn; all participants signed the informed consent.

RESULTS

HCV/HIV/HBV serostatus

Among the study subjects, 76%, 56% and 15% were infected with HCV, HIV and HBV, respectively (Table 1). Approximately one-third (133/374) were HIV+/HCV+, 1% (4/374) were HIV+/HBV+, 12% (44/374) were HIV+/HCV+/HBV+, and 7% (27/374) were HIV+ but HCV−/HBV− and 27% (100/374) were HCV+ but HIV−/HBV−. Of the remaining 66 subjects, 53 were HIV−/HCV−/HBV− or had other combinations of viral infection.

CCR5-Δ32 genotype and HIV infection

CCR5-Δ*32* homozygotes were not detected among HIV+ subjects whereas four of 162 HIV− subjects had the *CCR5*-Δ*32*/Δ*32* genotype. The prevalence of *CCR5*-Δ*32* heterozygotes among HIV+ and HIV− subjects was similar (19% and 20%, respectively). The distribution of *CCR5*-Δ*32* homozygosity or heterozygosity between HIV−/HCV+ vs HIV−/HCV− subjects was similar (3/105 vs 1/56 for Δ*32*/Δ*32* and 24/108 vs 9/57 for Δ*32* heterozygosity).

Distribution of *CCL3L1* copy numbers

The median *CCL3L1* copy number in the entire study population was two (range from 0 to 6). The distribution of the *CCL3L1* copy numbers between HIV+ and HIV− subjects was significantly different ($\chi^2 = 4.99$; $p < 0.05$) such that HIV− subjects possessed higher gene copy numbers than HIV+ subjects (Figure 1a). By contrast, the distribution of *CCL3L1* copy numbers in HCV+ vs HCV− subjects was similar (Figure 1b).

Association of *CCL3L1* copy number and HIV and HCV serostatus

We observed a clear shift in the frequency distributions of *CCL3L1* copy number between 2 and 3 copies in HIV+ vs HIV− subjects (Figure 1a). Compared to those with ≤2 *CCL3L1* copies, those with three copies had a nearly 50% lower risk of HIV seropositivity (odds ratio (OR) = 0.50; 95% confidence intervals (CI) = 0.26–0.96; P = 0.037). Similarly protective associations were observed for those with 4 (OR = 0.50; 95% CI = 0.19–1.29; P = 0.15) or 5–6 (OR = 0.39; 95% CI = 0.11–1.38; P = 0.14) copies, but these associations did not achieve statistical significance at $p < 0.05$.

Based on these results, and the distribution pattern of *CCL3L1* copy number in HIV+ vs HIV − IDUs (Figure 1a), subjects were categorized into two groups - those with a *CCL3L1* copy number between 0 to 2 and those with >2 (high *CCL3L1* copy number). In univariate analyses, predictably, co-infection with HCV (OR = 3.01; 95% C I= 1.83–4.96) and HBV (OR = 5.10; 95% CI = 2.42–10.75), and longer duration of IVDU (OR = 1.08, 95% CI = 1.01–1.15) were each associated with a significantly increased likelihood of HIV seropositivity (Table 2). Whereas a copy number of *CCL3L1* that was greater than two was associated with a 50% decreased likelihood of HIV seropositivity (OR = 0.49, 95% CI = 0.29–0.81; Table 2). By contrast, gender, age and *CCR5-Δ32* genotype were not associated with HIV serostatus (Table 2). When examining the outcome of HCV serostatus, we found that co-infection with HIV and HBV, and longer duration of IVDU (OR = 1.24, 95% CI = 1.13–1.36) were also each associated with increased odds of HCV seropositivity, but >2 *CCL3L1* copies was not associated with HCV (Table 2) or HBV serostatus (OR = 0.73; 95% CI, 0.34–1.56).

To assess the independent influence of the covariates analyzed in Table 2 on HIV and HCV seropositivity we considered the following. Because infection with HCV, HBV and HIV are all highly dependent on length of IVDU, and because these co-infections frequently occur concurrently (Table 1), we considered the possibility that the simultaneous inclusion of all these highly correlated variables in a single multivariate logistic regression model may confound the analyses. Hence, we conducted stepwise multivariate regression analyses in which we included these covariates sequentially (Tables 3 and 4).

For the outcome of HIV seropositivity, we found that in models that did not include the duration of IV drug use, HCV and HBV infection status associated with 2- and 4-fold higher risk of HIV seropositivity, respectively (Table 3, models 1 and 4). Conversely, in the model that did not include HCV or HBV infection status, length of IVDU was associated with an increased risk of HIV seropositivity (Table 3, model 5). By contrast, and consistent with our prediction, inclusion of IVDU in the same model with HCV and HBV led to negative associations for each of these highly correlated parameters for the outcome of HIV seropositivity (Table 3, models

2 and 3). However, >2 *CCL3L1* copies associated with a lower risk of HIV seropositivity, independent of length of IVDU and HCV/HBV co-infection status (Table 3, models 3–5).

Table 4 shows that HIV seropositivity is a strong predictor of HCV seropositivity but only in those models which do not include the length of IVDU (models 1 and 4); a similar trend is observed for co-infection with HBV infection. However, these positive associations of HIV and HBV infection status with HCV seropositivity are not observed after inclusion of length of IVDU into the model (compare models 1 and 4 vs models 2 and 3). In a model that accounts for the effects of gender, HIV and HBV serostatus, the *CCL3L1* copy number was not associated with HCV seropositivity (Table 4, model 4). However, when length of IVDU was included in the model with or without HIV/HBV serostatus, a high *CCL3L1* copy number was associated with an increased risk of HCV seropositivity (Table 4, models 3 and 5).

Selection of high copy number in HCV+/HIV− IDUs

Table 4 shows that the associations of *CCL3L1* copy number with HCV seropositivity differs before and after accounting for the length of IVDU. The latter observations raised a conundrum with respect to whether *CCL3L1* copy number is associated with HCV serostatus. We surmised that a high *CCL3L1* copy number was not associated with an increased risk of HCV infection because when length of IVDU was excluded from the statistical models, a high *CCL3L1* copy number did not associate with HCV serostatus (Table 4, model 4). Instead, the data in Table 3 and 4 may reflect three concurrent events that occur as the duration of exposure to both HIV and HCV increases. First, predictably, increasing durations of IVDU increases risk of HCV (Table 4, models 2, 3 and 5) and HIV (Table 3, model 5) infection. Second, however, those who resist acquiring HIV, are more likely to be those who also happen to have a high *CCL3L1* copy number, and this might explain the increased odds ratios for a high *CCL3L1* copy number when both it and duration of IVDU are placed concurrently in the same model (Table 4, models 3 and 5). Third, those subjects who acquire HCV and are also susceptible to HIV are less likely to possess a high *CCL3L1* copy number (Table 3, models 2–5). In this scenario, there should be an overrepresentation of subjects with a high *CCL3L1* copy number among HCV+ IDUs who resist acquiring HIV infection, and conversely an underrepresentation of high *CCL3L1* copy numbers in those who are both HCV+ and HIV+.

To test this premise, we conducted two analyses. First, to determine whether there is an overrepresentation and underrepresentation of high *CCL3L1* copy number among HCV+/HIV − vs HCV+/HIV+ subjects, respectively, we stratified HCV+ subjects according to their HIV status, and conducted the logistic regression analyses. The results showed (Table 5) that, consistent with our hypothesis, there was a 3.5-fold overrepresentation of a high *CCL3L1* copy number among HCV+/HIV− subjects (OR = 3.57; 95% CI = 1.47–8.67), whereas there was a 65% lower representation of a high *CCL3L1* copy number among HCV+/HIV+ subjects (OR = 0.35; 95% CI = 0.14–0.85).

Second, to affirm further that *CCL3L1* copy number does not influence risk of HCV infection, despite increased durations of IVDU, we conducted the analyses shown in Table 6. The median duration of IVDU was 8 years among the study subjects. Relative to those with IVDU of ≤8 years, those with a history of >8 years had a 3-fold greater risk of HCV seropositivity (Table 6, model 1). We next determined the association of *CCL3L1* with HCV serostatus, but for the reasons discussed in the preceding paragraphs, in this model we also accounted for the possible interaction between duration of IVDU and *CCL3L1* copy number (Table 6, model 2). These data indicated that length of IVDU was a strong predictor of HCV seropositivity, but *CCL3L1* copy number was not (Table 6, model 2).

DISCUSSION

In this study, by examining an IDU population of Caucasian ancestry, we demonstrate that a high *CCL3L1* copy number is associated with strong resistance to acquiring HIV infection. Notably, this association of *CCL3L1* copy number with HIV serostatus is independent of the length of IVDU and co-infection with HCV and HBV. The specificity of this association is highlighted by the observation that by contrast to its impact on HIV susceptibility, *CCL3L1* copy number does not influence risk of acquiring HCV infection.

The study was conducted in Estonia, a geographic region in which HIV is thought to be recent, with HIV incidence peaking in 2001 [34,35,41]. Thus, infection with HCV and HBV antedated HIV infection among Estonian IDU's [35,42]. This is reflected by both the higher prevalence of HCV (76%) than HIV (56%) infection in our study subjects and that the duration of IVDU was a stronger predictor of HCV than HIV seropositivity, with each additional year of IVDU increasing the risk of HCV and HIV by 27–35% and 8%, respectively. Predictably then, concordant with studies conducted in other countries [9,41,43], we found that among Estonian IDUs, HCV status was a strong predictor of HIV seropositivity, and conversely, HIV seropositivity associated strongly with HCV serostatus. Hence, given the very heavy exposure to multiple viral infections, and the relatively recent nature of the HIV epidemic in Estonia, this IDU study group was ideal to examine the host genetic factors that confer resistance to infection of HIV and/or HCV infection.

Another strength of this study is the relative homogeneity of both the viral and host population [34]. The HIV epidemic in Estonia commenced in 2000 and was characterized by concentrated one-source infections introduced into the IDU population during the early years of the epidemic [34]. Recent epidemiological studies have confirmed the low sequence heterogeneity of this mainly monophyletic population of recombinant circulating form HIV-1 viruses CRF06_cpx [44–46]. This provides an advantage as it allows the study of the influence of host genetic factors on HIV susceptibility in the context of minimal viral genetic heterogeneity. Another strength of this study was its homogeneous study population consisting mainly of young male IDUs of Caucasian descent. In contrast to some previous studies where distribution of *CCL3L1* copy numbers in HIV+ adults was compared with the distribution in a HIV-negative reference population, in this study all subjects had documented exposure risk factors (e.g. IVDU) [24].

Our results not only affirm the previously reported strong association of a high *CCL3L1* copy number with a reduced likelihood of HIV seropositivity, but also extend them significantly in three notable ways. First, our study provides the first evidence of this association among IDUs with heavy parenteral exposure to both HCV and HIV, a risk-group that is highly understudied with respect to the host genetic factors that influence susceptibility to viral infections. Most of the prior studies that have examined the association of *CCL3L1* with HIV susceptibility have focused on either men with risk of mucosal transmission [24] or children exposed perinatally to HIV [24,28–30]. Our results are consistent with those of Nakajima et al who found that a high *CCL3L1* copy number was associated with a lower risk of acquiring HIV infection among Japanese hemophiliacs [32].

Second, the strength and magnitude of the association was high, and depending on the statistical model, a high *CCL3L1* copy number afforded a nearly 60–80% lower odds of HIV seropositivity. One possible mechanism for this strong association is the extensive *in vitro* data showing that among the chemokines that bind to CCR5, CCL3L1 displays the most prominent HIV suppressive activity *in vitro* [14–16]. However, other mechanisms are possible. Chemokines influence immune responses [18–22,47], and Dolan et al showed that subjects who lacked or had a low *CCL3L1* copy number had reduced cell-mediated immune responses,

as estimated by delayed type hypersensitivity skin test reactions to the neoantigen KLH [17]. Thus, it also possible that a low *CCL3L1* copy may associate with increased HIV susceptibility because of both low chemokine production and impaired cell mediated immune responses.

Third, we provide experimental evidence for the previous suggestion [24] that among population groups that are under continuous and high exposure to HIV infection, such as among IDUs, there will be a shift in the distribution of *CCL3L1* copy number over time, such that subjects who resist HIV infection will be enriched for a high *CCL3L1* copy number. Given the heavy exposure to both HCV and HIV among IDUs, HCV+/HIV− subjects reflect subjects traditionally categorized as highly exposed, HIV-uninfected. When we examined HCV+ subjects according to their HIV infection status, we found that there was a 3-fold overrepresentation of subjects with a high *CCL3L1* copy number among exposed HIV-uninfected HCV+ subjects and conversely, an underrepresentation of IDUs with a high *CCL3L1* copy number among HCV+/HIV+ subjects. That is, the increased frequency of a higher *CCL3L1* gene dose among HCV+/HIV− subjects resulted from resistance to infection with HIV among subjects who also happen to be at high risk for acquiring HCV infection. Thus, the overrepresentation of *CCL3L1* among HCV+/HIV− subjects does not reflect increased susceptibility to HCV. This scenario is similar to studies of Zhang et al, who suggested that the increased frequency of the protective *CCR5-Δ32/Δ32* genotype among HCV +/HIV− hemophiliacs resulted from resistance to infection with HIV, and not increased susceptibility to HCV [39]. In this respect it is noteworthy that consistent with prior reports [12,39], subjects with the *CCR5-Δ32/Δ32* genotype were not found among HIV+ subjects and that among the four HIV-negative *CCR5-Δ32/Δ32*-bearing subjects, three were HCV+/HIV−.

In conclusion, these data suggest that among individuals at high risk for infection with both HCV and HIV, those with a low *CCL3L1* copy number are preferentially infected with HIV, and conversely, those with a high *CCL3L1* copy number resist infection with HIV. However, a high *CCL3L1* copy number does not afford protection against acquiring infection with HCV. In turn, this differential impact of *CCL3L1* copy number on risk of HIV and HCV seropositivity shifts the distribution of *CCL3L1* copy number wherein HCV+/HIV− subjects have a higher *CCL3L1* copy number whereas HCV+/HIV+ subjects are underrepresented for subjects with a high *CCL3L1* copy number. Together, these findings suggest a possible selection by HIV for subjects with a low *CCL3L1* copy number, underscore the pivotal importance of *CCL3L1*, and by extension, its cognate receptor CCR5 in HIV susceptibility. Additionally, our results highlight the importance of investigating the genotype-phenotype relationships for HIV serostatus in the context of microbial co-pathogens.

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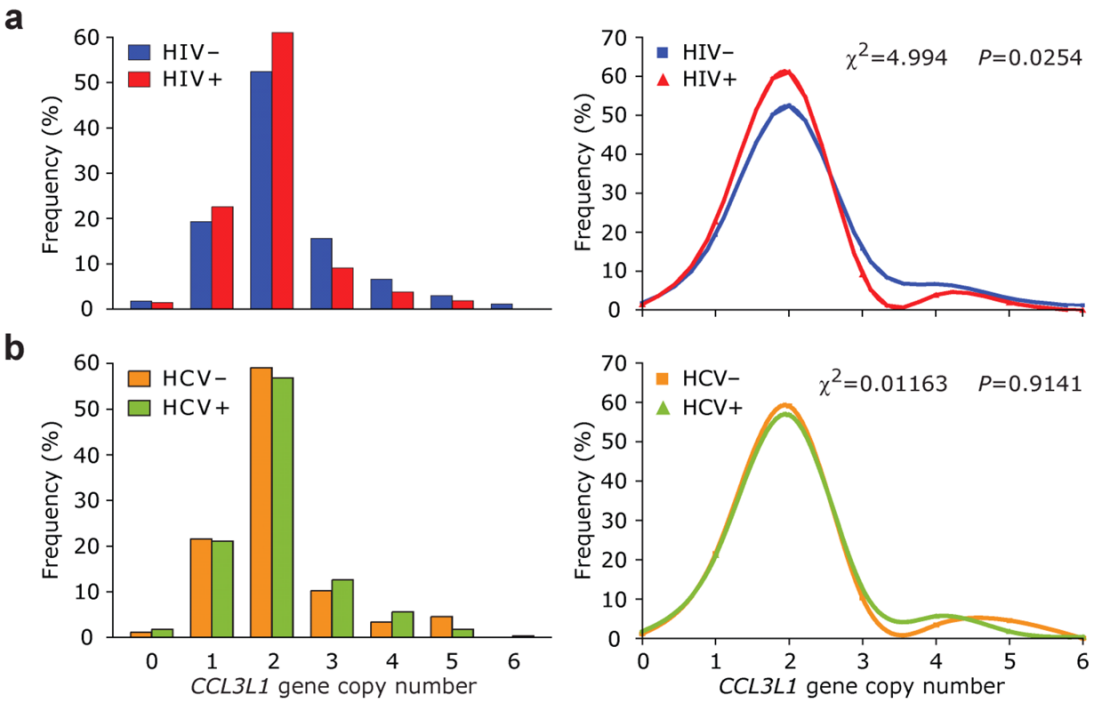

Figure 1. Distribution of *CCL3L1* copy number in study subjects according to HIV or HCV serostatus

Histograms and the cubic-spline smoothed frequency curves for the distribution of *CCL3L1* copy number among HIV+ (a) and HCV+ (b) subjects.

Table 1

HIV, HCV and HBV serostatus in study subjects

	HCV+	HCV−	HBV+	HBV−	TOTAL
HIV+	177	31	48	160	208
HIV−[a, b]	108	57	9	153	166
TOTAL	285	88	56	313	374

[a] One person had no HCV data collected

[b] Four persons had no HBV data collected

[*] 44 subjects had HIV/HCV/HBV triple-infection

Table 2

Associations by univariate logistic regression analyses of demographic factors, IV drug use duration, viral coinfection status, and *CCL3L1* or *CCR5* genotype with HIV or HCV serostatus

Covariate	Outcome: HIV serostatus OR; 95% CI; *P*	Outcome: HCV OR; 95% CI; *P*
Gender		
Male*	1.0	1.0
Female	1.08; 0.60–1.94; 0.797	0.52; 0.27–0.99; 0.0455
Age		
<26 years*	1.0	1.0
≥26 years	1.10, 0.70–1.72; 0.691	1.18; 0.69–2.02; 0.5378
HCV status		
HCV−*	1.0	
HCV+	3.01; 1.83–4.96; 1.45×10^{-5}	
HIV status		
HIV−*		1.0
HIV+		3.01; 1.83–4.96; 1.45×10^{-5}
HBV status		
HBV−*	1.0	1.0
HBV+	5.10; 2.42–10.75; 1.85×10^{-5}	3.57; 1.38–9.25; 0.0088
IV drug use duration		
Years[b]	1.08; 1.01–1.15; 0.025	1.24; 1.13–1.36; 1.10×10^{-5}
***CCL3L1* copy number**		
0–2*	1.0	1.0
3–6	0.49; 0.29–0.81; 0.006	1.15; 0.62–2.12; 0.6558
***CCR5* genotype**		
wt/wt*	1.0	1.0
wt/Δ32 or	0.81; 0.49–1.33; 0.398	1.47; 0.78–2.78; 0.2363

*NOTE., reference group; OR, odds ratio; CI, confidence interval; P, significance value.

[a]Years was categorized as a continuous variable in full-years, and data reflects an increase in OR with each additional year of IV drug use.

Table 3

Associations by multivariate logistic regression analyses of gender, IV drug use duration, viral coinfection status, and *CCL3L1* copy number with HIV serostatus.

Covariate	Model 1 OR; 95% CI P	Model 2 OR; 95% CI P	Model 3 OR; 95% CI P	Model 4 OR; 95% CI P	Model 5 OR; 95% CI P
Gender					
Male*	1.0	1.0	1.0	1.0	1.0
Female	1.29; 0.687–2.430	1.3; 0.519–3.255	1.297; 0.497–3.389	1.343; 0.706–2.554	1.031; 0.449–2.369
	0.425	0.5751	0.5949	0.3690	0.9424
HCV status					
HCV−*	1.0	1.0	1.0	1.0	
HCV+	2.08; 1.211–3.572	**	**	2.138; 1.234–3.705	
	0.008	0.9459	0.9441	0.0067	
HBV status					
HBV−*	1.0	1.0	1.0	1.0	
HBV+	4.13; 1.943–8.777	0.922; 0.221–3.851	0.657; 0.156–2.772	4.006; 1.875–8.56	
	0.0002	0.9115	0.5674	0.0003	
IV drug use duration					
Years[a]		1.016; 0.944–1.093	1.014; 0.938–1.095		1.08; 1.009–1.157
		0.6665	0.7315		0.0276
***CCL3L1* copy number**					
0–2*			1.0	1.0	1.0
3–6			0.204; 0.093–0.445	0.421; 0.244–0.727	0.267; 0.124–0.574
			<0.0001	0.0019	0.0007

NOTE. Models #1-5 depict multivariate logistic regression analyses with the indicated covariates for the outcome of HIV serostatus. For example, model 1 contains gender and HCV/HBV status whereas model 4 also includes *CCL3L1* copy number. Although gender did not associate with HIV serostatus in univariate analyses (Table 2), it was included in the model to remain consistent with the models shown in Table 4.

* reference group.

** values reflected those of correlated variables (OR >999.99; 95% CI: <0.001–>999.99). OR, odds ratio; CI, confidence interval; P, significance value.

[a] Years was categorized as a continuous variable in full-years, and data reflects an increase in OR with each additional year of IV drug use.

Table 4

Associations by multivariate logistic regression analyses of gender, IV drug use duration, viral co-infection status, and *CCL3L1* copy number with HCV serostatus

Covariate	Model 1 OR; 95% CI P	Model 2 OR; 95% CI P	Model 3 OR; 95% CI P	Model 4 OR; 95% CI P	Model 5 OR; 95% CI P
Gender					
Male*	1.0	1.0	1.0	1.0	1.0
Female	0.493; 0.252–0.966	0.412; 0.122–1.39	0.288; 0.075–1.106	0.488; 0.249–0.958	0.512; 0.18–1.458
	0.0394	0.1528	0.0698	0.0370	0.2100
HIV status					
HIV−*	1.0	1.0	1.0	1.0	
HIV+	2.088; 1.217–3.583	**	**	2.159; 1.246–3.738	
	0.0075	0.9333	0.9282	0.0060	
HBV status					
HBV−*	1.0	1.0	1.0	1.0	
HBV+	2.535; 0.950–6.760	1.549; 0.159–15.113	2.549; 0.255–25.474	2.551; 0.956–6.808	
	0.0631	0.7066	0.4257	0.0615	
IV drug use duration					
Years[a]		1.272; 1.125–1.439	1.331; 1.158–1.531		1.351; 1.191–1.534
		0.0001	<0.0001		<0.0001
***CCL3L1* copy number**					
0–2*			1.0	1.0	1.0
3–6			7.64; 2.052–28.447	1.248; 0.641–2.429	2.995; 0.91–9.862
			0.0024	0.5148	0.0712

NOTE. Models #1-5 depict multivariate logistic regression analyses with the indicated covariates with outcome of HCV serostatus.

* reference group.

** values reflected those of correlated variables (OR >999.99; 95% CI: <0.001–>999.99). OR, odds ratio; CI, confidence interval; P, significance value.

[a] Years was categorized as a continuous variable in full-years, and data reflects an increase in OR with each additional year of IV drug use.

Table 5

Likelihood of high *CCL3L1* copy number among HCV+ subjects according to HIV infection status.

	n	OR	95% CI	*P*
HIV−	**166**			
CCL3L1 0–2 copies	122	1.0		
CCL3L1 3–6 copies	44	3.57	1.47–8.67	0.005
HIV+	**208**			
CCL3L1 0–2 copies	177	1.0		
CCL3L1 3–6 copies	31	0.35	0.14–0.85	0.020

NOTE. OR, odds ratio; CI, confidence interval; P, significance value

Table 6

Association of IV drug use length and *CCL3L1* copy number with HCV infection

	OR	95% CI	*P*
Model 1: IVDU duration with outcome of HCV			
IVDU ≤ 8 years*	1		
IVDU >8 year	3.321	1.654–6.666	0.0007
Model 2: IVDU x CCL3L1 for outcome of HCV			
IVDU*	3.010	1.46–6.20	0.0028
*CCL3L1***	2.174	0.68–6.91	0.1879
IDUx*CCL3L1*	§	§	0.9717

NOTE. Model 1 is a univariate analysis of the association of duration of IV drug use (IVDU) with HCV status. Model 2 is an interactive multivariate logistic regression model with the indicated covariates.

* IVDU was categorized according to median duration of IV drug use.

** *CCL3L1* copy number is categorized as 0–2 vs >2 copies. OR, odds ratio; CI, confidence interval; P, significance value. X, interactio