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Authors
An, P
Goedert, JJ
Donfield, S
et al.

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Regulatory Variation in HIV-1 Dependency Factor \textit{ZNRD1} Associates With Host Resistance to HIV-1 Acquisition

Ping An,\textsuperscript{1} James J. Goedert,\textsuperscript{2} Sharyne Donfield,\textsuperscript{3} Susan Buchbinder,\textsuperscript{4} Gregory D. Kirk,\textsuperscript{5} Roger Detels,\textsuperscript{6} and Cheryl A. Winkler\textsuperscript{1}

\textsuperscript{1}Basic Research Laboratory, Leidos Biomedical Research, Inc, Frederick National Laboratory for Cancer Research, and \textsuperscript{2}Infections and Immunepidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; \textsuperscript{3}Rho, Inc, Chapel Hill, North Carolina; \textsuperscript{4}San Francisco Department of Public Health, California; \textsuperscript{5}Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland; and \textsuperscript{6}Department of Epidemiology, School of Public Health, University of California, Los Angeles

\textbf{Background.} ZNRD1 was identified as a host protein required for the completion of the human immunodeficiency virus (HIV) lifecycle in a genome-wide screen using small interfering RNA gene silencing. Subsequently, a genome-wide association study (GWAS) of host determinants for HIV-1 disease identified an association of single nucleotide polymorphisms (SNPs) in the \textit{ZNRD1} region with CD4$^+$ T-cell depletion.

\textbf{Methods.} We investigated the effects of SNPs in the \textit{ZNRD1} region on human immunodeficiency virus type 1 (HIV-1) infection and progression to clinical outcomes in 5 US-based HIV-1 longitudinal cohorts consisting of men who have sex with men, males with hemophilia, and injection drug users (IDUs) ($n = 1865$). SNP function was evaluated by electrophoretic mobility shift assay and promoter luciferase assay.

\textbf{Results.} A haplotype in the \textit{ZNRD1} gene showed significant association with a 35% decreased risk of HIV-1 acquisition (OR = 0.65, 95\% CI, 0.47–0.89), independent of \textit{HLA-C rs9264942}, in European Americans. The SNP \textit{rs3132130} tagging this haplotype, located in the \textit{ZNRD1} 5' upstream region, caused a loss of nuclear factor binding and decrease in \textit{ZNRD1} promoter activity. \textit{ZNRD1} variants also affected HIV-1 disease progression in European- and African-American cohorts.

\textbf{Conclusions.} This study provides novel evidence that \textit{ZNRD1} polymorphism may confer host resistance to HIV-1 acquisition.

\textbf{Keywords.} HIV-1; infection; host susceptibility; AIDS; SNP; single nucleotide polymorphism; \textit{ZNRD1}; genetic association.

Individual variation in susceptibility to human immunodeficiency virus type 1 (HIV-1) acquisition, control of viral replication, and rate of disease progression is well documented [1–4]. The underlying biological mechanisms to individual susceptibility are multifactorial, with contributions from host genetic factors in addition to viral factors and environmental exposures [1–4]. Candidate gene association studies and genome-wide association studies (GWAS) have identified variation in host genes encoding HIV-1 coreceptor CCR5, and HLA- B (\textit{HLA-B*57}), HCP5 (\textit{rs2395029}), and HLA-C (\textit{rs9264942}) that influence viral load and progression to AIDS [1, 2, 5–7]. However, only homozygosity for the CCR5 32 base-pair deletion (CCR5-Δ32), found on European ancestry chromosomes, has been securely identified as a resistant genetic factor to HIV acquisition [8, 9]. Other genetic factors associated with altered risk of HIV acquisition have not been consistently validated in independent studies [2, 10–13].

\textit{ZNRD1}, a zinc ribbon domain-containing 1 protein, is a DNA-dependent RNA polymerase that catalyzes
the transcription of DNA into RNA. ZNRD1 was first identified as one of 250 HIV-1 dependency factors required for HIV-1 replication in a genome-wide small interfering RNA (siRNA) knock-down experiment [14]. A separate siRNA knock-down experiment targeted only at ZNRD1 demonstrated a >50% reduction of R5 or X4-tropic HIV-1 replication in HeLa derived cells or lymphoid cells, likely through inhibition of viral transcription [15]. Subsequently, a GWAS of HIV-1 identified several SNPs near ZNRD1 on chromosome 6 that are associated with CD4+ T-cell counts [6]; however, it is still unresolved whether the ZNRD1 association is independent or tracks other variants in the human leukocyte antigen (HLA) region through linkage disequilibrium; it is also not known if ZNRD1 influences host susceptibility/resistance to HIV-1 acquisition.

We examined the effects of variation in the ZNRD1 gene on HIV-1 acquisition and disease progression in 1865 participants enrolled in 5 natural history, treatment-naive HIV-1 cohorts. We demonstrate by both regression and a more conservative stratified analysis robust statistical evidence for an association of ZNRD1 variation with HIV-1 acquisition. We further demonstrate that a haplotype-defining SNP shows differential transcriptional factor binding and altered ZNRD1 gene promoter activity.

MATERIALS AND METHODS

Study Participants
Details about the cohorts have been described elsewhere [16]. Study participants were enrolled in 5 US-based, treatment-naive natural history HIV/AIDS cohorts during 1978–1989: AIDS Link to the Intravenous Experience (ALIVE), an intravenous injection drug user cohort in Baltimore [17], consisting of mainly Africa Americans; Multicenter AIDS Cohort Study (MACS), a longitudinal prospective cohort of men who have sex with men (MSM) [18]; The San Francisco City Clinic Study (SFCC), a cohort of MSM [19]; Hemophilia Growth and Development Study (HGDS), a multicenter prospective study that enrolled children and adolescents with hemophilia who received contaminated blood products [20]; and The Multicenter Hemophilia Cohort Study (MHCS), a prospective study of hemophiliacs [21]. The latter 4 cohorts mainly comprise European Americans. Study protocols were approved by the Institutional Review Boards of participating institutions and informed consent was obtained from all study participants.

The study group comprises HIV-1 seroconverters (infected after study enrollment), seroprevalents (infected at study enrollment), at-risk seronegatives, and highly exposed uninfected. HIV-1 uninfected individuals were classified into 2 categories based on an individual’s documented exposure levels to HIV-1. Highly exposed uninfected subjects were those with documented repeated exposure through sharing of injection equipment [17], anal receptive sex with multiple partners [22–24], or numerous transfusions with factor VIII replacement products prior to 1984, when viral inactivation procedures were initiated [25]. At-risk seronegatives subjects are those enrolled in the cohorts who remained HIV-seronegative despite ongoing or prior risk activities. The number of subjects studied in each category was as follows: seroconverters = 609 European Americans, 269 Africa Americans; at-risk seronegatives = 296 European Americans, 276 Africa Americans; highly exposed uninfected = 140 European Americans, 81 Africa Americans; and seroprevalents = 123 European Americans, 292 Africa Americans.

SNP Genotyping
Twelve SNPs in ZNRD1 region (chromosome 6 position 30 078 567 to 30 148 987, spanning 70 kb), and HLA-C rs9264942, known to be strongly associated with HIV viral load set point [6, 7] were genotyped using ABI TaqMan assays (Table 1, Figure 1). SNPs were selected if they were haplotype-tagging or altered protein coding, transcription, splicing, or microRNA binding (as predicted by SNPinfo web server [http://snpinfo.niehs.nih.gov]), or have published associations [6, 15, 26, 27, 29, 31].

Genetic Association Analysis
The genetic effects of SNPs and haplotypes on HIV-1 infection susceptibility were assessed by comparing frequencies between the HIV-1 infected group composed of seroconverters and seroprevalent persons and the HIV-1 uninfected group composed of highly exposed uninfected and seronegatives. To avoid frailty bias in the seroprevalent group, which had an overrepresentation of long-term nonprogressors, potentially leading to the enrichment of progression resistant factors, we restricted the HIV-1-positive group to HIV-1 seroconverters, which consist of unselected patients with natural disease progression spectrum in a sub-group comparison. As the effect of resistance factors may be dependent on the exposure level, we compared seroconverters with highly exposed uninfected and seronegative groups of high or low HIV-1 exposure levels separately. Odds ratios (OR) and P values for were obtained by using a conditional logistic regression test.

Kaplan–Meier survival statistics and the Cox proportional hazards model were used to assess the effects of SNPs and haplotypes on the rate of progression to AIDS among seroconverters. Two endpoints reflecting AIDS progression were considered for seroconverters: (1) HIV-1 infection plus a decline of CD4+ T-cell counts to <200 cells/mm3 (CD4 <200); (2) the 1987 Centers for Disease Control and Prevention (CDC) definition of AIDS (AIDS-87): HIV-1 infection plus AIDS-defining illness [32].

Electrophoretic Mobility Shift Assays (EMSA)
The EMSA was performed in HeLa and Jurkat cell nuclear extracts using oligonucleotides carrying ZNRD1 rs3132130 G/C with an Infrared EMSA Kit (LI-COR).
Gene Promoter Reporter Assay

DNA fragments of 1965 bp in the ZNRD1 5′ upstream region (spanning −1689 to +276 based on transcription start site (TSS)) carrying rs3132130 G or C at position −247 were cloned into a pLightSwitch promoter reporter vector (Switch-Gear Genomics) harboring Renilla luminescent reporter gene (RenSP). HeLa and 293 cells were transfected in triplicates. Promoter activity of Renilla luciferase activity was measured 24 hours later using the LightSwitch Dual Assay System and was normalized by the Cypridina luciferases activity (Cluc) from a cotransfected pTK-CLuc Vector (SwitchGear Genomics).

A more detailed description of the Materials and Methods is available in the Supplementary Data.

RESULTS

Linkage Disequilibrium of ZNRD1 Region SNPs

SNPs in the ZNRD1/RNF39 region showed weak to strong correlation with each other ($r^2 = 0.02–1.0$; Figure 1); however, all had low correlation ($r^2 = 0.01–0.05$) with the HLA-C rs9264942, previously shown to be strongly associated with control of HIV viral load (VL) [6], 1.2 MB downstream of the ZNRD1/RNF39 region. These 9 SNPs formed 5 common haplotypes with frequencies >3.0% in European Americans. Haplotypes of ZNRD1/RNF39 region were inferred by the tag SNPs 2 (rs9261174), 4 (rs3757327), 5 (rs3132130), 6 (rs10745), and 9 (rs8321). We performed association analyses on haplotypes, with and without adjustment for VL-affecting HLA-C rs9264942 allele.

ZNRD1 Haplotype and HIV-1 Acquisition

To determine the effect of ZNRD1 haplotypes on the risk to HIV-1 acquisition, we compared the haplotype frequencies between HIV-1 uninfected persons belonging to HIV-1 risk groups representing a range of exposures (n = 296) and HIV-1 infected persons (n = 732). A subgroup of 140 individuals comprised a group of highly exposed uninfected subjects with documented repeated parenteral or mucosal exposures to HIV-1. The HIV-1 infected group included 609 seroconverter cases with midpoint imputed dates of infection and 123 seroprevalent cases with unknown infection dates.

Among the 5 haplotypes, Hap2 showed evidence of association with resistance to HIV-1 acquisition (Figure 2). We first compared the HIV-infected group to the HIV-uninfected group and observed a statistically significant increase of Hap2 frequency in the uninfected group (OR = 0.65, 95% confidence interval [CI], .47–.88, $P = .005$). To avoid distortions in allele frequencies due to potential frailty bias in the seroprevalent group, we performed a more conservative analysis comparing only seroconverters to the highly exposed uninfected group and found a similar effect size (OR = 0.64, $P = .035$). The Hap2 frequency was consistently higher in the HIV-1 uninfected group in all 3 subgroup comparisons: seroconverters vs highly exposed uninfected, seroconverters vs

<table>
<thead>
<tr>
<th>Table 1. SNPs in the ZNRD1/RNF39 and HLA-C Region Interrogated in the Study</th>
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<tbody>
<tr>
<td>SNP no.</td>
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<tr>
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<tr>
<td>SNP1</td>
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<td>SNP2</td>
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<tr>
<td>SNP11</td>
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<tr>
<td>SNP12</td>
</tr>
<tr>
<td>SNP13</td>
</tr>
</tbody>
</table>

Abbreviations: nsSNP, nonsynonymous SNP; SNP, single nucleotide polymorphism; TFBS, transcription factor binding site.

$^a$ Predicated by SNPinfo web server (http://snpinfo.niehs.nih.gov).
seronegatives, and all HIV-1 infected vs all uninfected, confirming that the association was not due to bias in 1 particular group. To address the issue of false discovery due to multiple comparisons, we performed a permutation test; the 5 haplotypes were included in a Fisher test with permutation resampling implemented in PROC MULTTEST of SAS software [33]. The permutation adjusted $P$ value was .014 comparing the HIV-1 uninfected group and the HIV-1 infected group.

As HLA-C rs9264942 was not associated with infection (HIV-1 infected vs uninfected, OR = 1.18, 95% CI, .81–1.72, $P = .40$), the Hap2 association with HIV-1 infection status is not a result tracking by linkage disequilibrium with rs9264942. The effect size of the Hap2 remained similar even after stratifying on rs9264942 carriage. In addition, no epistatic interaction was observed among ZNRD1 haplotypes and rs9264942 in a Fisher test with permutation resampling. No other allele in the nearby HLA locus has been reported to affect HIV-1 acquisition [5], suggesting that the ZNRD1 associations are likely independent. Based on the haplotype configuration, the Hap2 association signal might stem from SNP5 (rs3132130 in the promoter region) or SNP7 (rs9261269 in intron 4) that are unique to Hap2 (Figure 1). The Hap 2 frequency is 2-fold higher in European Americans (12%) than in African Americans (6%); in African Americans, Hap2 was also associated with lower risk of infection but was not significant (OR = 0.82, 95% CI, .44–1.54).

**ZNRD1 Haplotype and HIV-1 Disease Progression**

To assess the impact of ZNRD1 haplotypes on disease progression, the time from seroconversion to 2 AIDS progression endpoints: CD4+$^+$ T-cell dropping to <200/mm$^3$ (CD4 < 200) and AIDS diagnosis with the CDC 1987 case definition (AIDS-87), were evaluated using Kaplan–Meier survival statistics and the Cox proportional hazards model. SNP rs9261174 was previously reported to affect progression to CD4 < 350 [6] or initiation of highly active antiretroviral therapy (HAART) with CD4 < 500 [7] ($P = 3.9E - 07$ or $1.8E - 08$). In the current study, rs9261174 showed a nonsignificant protective trend on AIDS-87 (Cox model, RH = 0.81, 95% CI, .63–1.05, $P = .11$; survival analysis, $P$ (log–rank test) = .20, Figure 3A), a more stringent and definitive endpoint reflecting AIDS progression; the direction of the effect is similar to those reported [6, 7].

![Figure 1. SNPs analyzed in the ZNRD1, RNF39, and HLA class I locus (6p21) region. A. Genomic structure and SNP locations. The colored blocks indicate exons, empty blocks, untranslated regions (UTR), horizontal arrows, the direction of transcription and vertical arrows, the positions of SNPs. B. Linkage disequilibrium matrix in European Americans (EA). Red block indicates $D^′ = 1.0$, and the number in the blocks indicates the value of $r^2$. The linkage disequilibrium block depicted by black triangle was based on the 95% Confidence interval criteria [30]. The intensity of the red color in each box is proportional to the strength of the linkage disequilibrium estimates ($D^′$) for the SNP pair. C. Haplotype configuration and frequency in EA and in African American (AA). The haplotype-tagging SNPs are underlined. Abbreviation: SNP, single nucleotide polymorphism.](https://academic.oup.com/jid/article-abstract/210/10/1539/2192860)
However, the effect size diminished after adjusting for HLA-C rs9264942 (P = .36).

Among 5 haplotypes tested in a dominant Cox model, Hap3 was associated with accelerated progression to the CD4 < 200 endpoint in EA (RH = 1.50, P = .005, Table 2). With conditioning on HLA-C rs9264942, plus inclusion of 6 other genetic factors securely associated with progression to AIDS (Table 2, footnote), the Hap3 effect on CD4 < 200 remained statistically robust. In contrast, Hap3 had no effect using the clinical AIDS-87 endpoint. Conversely, rs9264942 had a strong effect on AIDS-87 and death but had only marginal effect on CD4 < 200 [34]. These suggest independent effects from the 2 genes. SNP9 (rs3132130) tagging Hap3 showed a clear separation of survival curves along the clinical course, with T/G heterozygotes having accelerated CD4⁺ T-cell depletion compared to the G allele carrying sequence, in HeLa cells (Figure 4A) and HEK293 cells (data not shown), respectively (RH = 1.67, 95% CI, 1.04–2.67, P = .027) and with additional adjustment for rs9264942 (RH = 1.36, 95% CI, 1.01–1.83, P = .04). In African Americans, a different haplotype, Hap4, was associated with accelerated CD4⁺ T-cell depletion (RH = 1.90, P = .01; Table 2, Figure 3C).

SNP rs3132130 in the 5' Upstream Region Alters ZNRD1 Promoter Activity

To test whether rs3132130 influences ZNRD1 promoter activity, we performed luciferase gene promoter assays. Reporter constructs containing the 1.9-kb ZNRD1 promoter sequence with the 2 SNP rs3132130 G or C allele (Figure 4D and 4E) showed that the normalized luciferase activity of the rs3132130 C allele carrying sequence was decreased by 61.8% and 61.3%, compared with that of the G allele carrying sequence, in HeLa cells (Figure 4F) and HEK293 cells (data not shown), respectively (P = .005 and .006). This indicates that rs3132130 is within the ZNRD1 promoter region and the C minor allele of the rs3132130 has a transcription rate 2-fold lower than that of the G major allele.

DISCUSSION

We investigated genetic variants in ZNRD1 in well-characterized, treatment-naive HIV-1 natural history cohorts for association with infection status and rate of progression to clinical outcomes. The findings from this case-cohort study provide...
evidence that the ZNRD1 gene may influence HIV acquisition and rate of CD4+ T-cell decline. The differential impact of ZNRD1 haplotypes on CD4+ T-cell depletion and susceptibility to HIV-1 acquisition provides additional support that ZNRD1 may be important in modulating HIV-1 pathogenesis [6, 14].

This is the first report to our knowledge that ZNRD1 variation affects host resistance to HIV-1 acquisition. ZNRD1 Hap2 containing rs3132130 in the 5′ upstream region was enriched in the exposed but uninfected group compared to those who were HIV-1 infected. Our results suggest that the ZNRD1 associations are independent of the nearby HLA alleles; HLA-C rs9264942 had no impact on infection and none of the other alleles in the HLA B/C locus had an apparent impact on HIV-1 acquisition [5]. Based on an odds ratio of 0.65 and a population frequency of 12% for the Hap2 allele among European Americans, the corresponding population attributable fraction in providing protection to European Americans is 6.0%, which is substantial from a public health point of view. For comparison, the population attributable fraction contributed by CCR5-Δ32 homozygosity (frequency = 1% in European Americans), conferring near complete resistance to HIV-1 acquisition, is 1.0% [8, 9].

We showed that SNP rs3132130, located in the ZNRD1 5′ upstream region affected nuclear factor binding, as revealed by EMSA. Using the luciferase promoter reporter assays, we further demonstrated that the upstream region of ZNRD1 bearing the SNP rs3132130 processes promoter activity, which was significantly lower in the minor allele C-bearing than the common G-bearing fragments. The longer C-bearing fragment had a promoter activity comparable to a shorter standard ZNRD1 promoter. These results suggest that the C allele-bearing promoter had lower promoter activity, possibly due to loss of certain transcription activator factor binding as revealed in EMSA. It is reasonable to predict that the C allele-bearing promoter

Figure 3. Survival curve analysis of the impact of ZNRD1 SNPs on AIDS progression. A, Impact of ZNRD1 rs9261174 on clinical AIDS in EA; B, Impact of ZNRD1 rs83211(hap3) on CD4 < 200 in EA; C, Impact of ZNRD1 hap4 on CD4 < 200 in AA. The x axis represents the time to progression endpoints since HIV-1 seroconversion (years), and the y axis is the percentage of individuals surviving for the outcome endpoint. P values were obtained from a log–rank test. Abbreviations: AA, African American; EA, European American; HIV-1, human immunodeficiency virus type 1; SNP, single nucleotide polymorphism.
could produce a lower level of ZNRD1 gene expression than the G allele-bearing promoter. It is thus biologically plausible that lower expression of ZNRD1, which enhances HIV-1 replication, would be unfavorable for HIV-1 infection. This functional mechanism is in support of the population genetic epidemiological finding that rs3132130C carriers had decreased risk of HIV-1 acquisition. However, it remains to be determined whether the in vitro regulatory effect identified leads to in vivo gene expression changes in the relevant target cells such as CD4+ T cells, mucosal macrophage and epithelial cells.

Investigating unique genetic and immunological features among highly exposed uninfected individuals may uncover host factors underlying the resistance to HIV-1. Highly exposed uninfected are persons who are at high-risk for HIV-1 acquisition but remain seronegative [1–4, 35, 36]. HIV-1 is obligated to use cellular proteins (HIV-1 dependency factors) to complete its replication cycle at every stage, including viral entry, transcription, viral integration, virion maturation, and budding. On the other hand, the host activates innate and acquired immune defense systems (restriction factors, such as APOBEC3, TRIM5, HLA) to combat retroviral infections. Genetic changes in the key pathways in viral-host interaction that disrupt HIV-1 dependency factors or strengthen the defending factors would largely be the same in patients with different transmission routes [37]. A multicenter GWAS focused on HIV-1 acquisition compared HIV-1 infected individuals to population controls identified only signals of association in the HLA-B region; however, this result was likely due to enrichment of progression protective HLA-B alleles among the HIV-1 controller group [12]. When the study was limited to only seroconverters, these associations were not observed [12]. Because these GWAS did not identify common variants reaching genome-wide significance, it was suggested that low frequency variants or variants with low effect sizes might explain the variation in HIV acquisition susceptibility.

Our study was a case-cohort design where every HIV-1 infected individual belonged to 1 or more HIV-1 risk groups and our HIV-1-positive cases comprised only seroconverters to limit bias. The enrichment of HIV-1 resistant variants rs3132130/rs9261269 was found in the exposed, uninfected populations (highly exposed uninfected and at-risk seronegatives). The limitation of our study includes the relatively small sample sizes in highly exposed uninfected groups as examined in this study [8, 9]. With the exception of CCR5 Δ32, no new HIV-1 genetic variants that affect HIV-1 acquisition have been revealed by GWAS [10, 12, 37]. In one GWAS, HIV-1 negative African adults recruited from sexually transmitted infection clinics, considered as at-risk for HIV-1 exposure, were compared with HIV-1 positive controls [10]. Another GWAS compared HIV-1 exposed uninfected heterosexuals with HIV-1 positive homosexuals, assuming resistant genes would largely be the same in patients with different transmission routes [37].

Table 2. Impact of Haplotypes of ZNRD1 and RNF39 on AIDS Progression

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Population</th>
<th>CD4 &lt; 200a RH (95% CI)</th>
<th>P Value</th>
<th>AIDS-87a 95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hap1</td>
<td>European American</td>
<td>.93 (.65–1.33)</td>
<td>.69</td>
<td>.96 (.66–1.39)</td>
<td>.83</td>
</tr>
<tr>
<td></td>
<td>African American</td>
<td>1.22 (.63–2.34)</td>
<td>.56</td>
<td>.91 (.41–2.05)</td>
<td>.92</td>
</tr>
<tr>
<td>Hap2</td>
<td>European American</td>
<td>1.13 (.87–1.46)</td>
<td>.38</td>
<td>.95 (.71–1.28)</td>
<td>.95</td>
</tr>
<tr>
<td></td>
<td>African American</td>
<td>.71 (.40–1.27)</td>
<td>.25</td>
<td>.86 (.40–1.83)</td>
<td>.69</td>
</tr>
<tr>
<td>Hap3</td>
<td>European American</td>
<td>1.50 (1.13–1.98)</td>
<td>.005</td>
<td>1.08 (.79–1.47)</td>
<td>.65</td>
</tr>
<tr>
<td></td>
<td>European American adj.</td>
<td>1.53 (1.15–2.02)</td>
<td>.003</td>
<td>1.11 (0.81–1.52)</td>
<td>.52</td>
</tr>
<tr>
<td></td>
<td>African American</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hap4</td>
<td>European American</td>
<td>.76 (.55–1.06)</td>
<td>.1</td>
<td>.81 (.56–1.17)</td>
<td>.26</td>
</tr>
<tr>
<td></td>
<td>African American</td>
<td>1.94 (1.16–3.25)</td>
<td>.012</td>
<td>1.44 (.69–2.99)</td>
<td>.33</td>
</tr>
<tr>
<td></td>
<td>African American adj.</td>
<td>2.06 (1.21–3.48)</td>
<td>.008</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hap5</td>
<td>European American</td>
<td>1.24 (0.80–1.91)</td>
<td>.33</td>
<td>1.09 (.68–1.74)</td>
<td>.72</td>
</tr>
<tr>
<td></td>
<td>African American</td>
<td>1.19 (0.80–1.77)</td>
<td>.38</td>
<td>.94 (.54–1.61)</td>
<td>.81</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NA, not analyzed due to rarity (1%) in African American.

a Results were for a dominant genetic model from a Cox proportional hazards model analysis; Hap3 was tagged by rs8321.

b Adjusted for the covariates HLA-C rs9264942, HLA-B*57, B*27, class I homozygosity, CCR5Δ32, CCR5P1, and CCR2-64I.

c Adjusted for the covariates HLA-B*57 and class I homozygosity (see Supplementary data).
acquisition has not been tested. In the first HIV-1 GWAS, Fellay et al found a group of correlated SNPs including rs9261174 (SNP2) located near \textit{ZNRD1} among top hits for CD4 T-cell depletion [6, 7]. Van Manen et al observed that rs2074479 was associated with the CD4+ T-cell count but not with viral load or disease progression to AIDS [27]. Limou et al performed GWAS in the French GRIV cohort of extreme phenotypes and found that rs8321 was associated with CD4+ T-cell drop [29]. Catano et al found that rs9261174 affected the rate of CD4+ T-cell loss in the WHMC cohort [26]. By genotyping a subset of the alleles that contribute to HLA-A10, this report suggested the \textit{ZNRD1} association was attributable to linkage disequilibrium with the
HLA-A10 serogroup [26]; this finding was not supported by subsequent studies in other well-characterized HIV cohorts [7, 31]. Ballana et al showed that rs1048412 was associated with long-term nonprogression [15]. Our results, together with others, indicated that ZNRD1 variation mainly affects CD4+ T-cell depletion rather than the development of frank AIDS, but the mechanism underpinning this association requires further study.

This study provides the first evidence that ZNRD1 variation may affect host susceptibility to HIV-1 acquisition. This result is supported by prior functional findings that ZNRD1 is necessary for HIV-1 replication in cell culture [14, 15] and the functional evidence of the associated SNPs. These results, together with the observation that ZNRD1 silencing does not affect cell viability, suggest that the ZNRD1 gene or its protein may be a druggable target for antiretroviral therapy to prevent acquisition or to limit replication by HIV.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Conflict of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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