HIV/AIDS MAJOR ARTICLE

Duffy-Null–Associated Low Neutrophil Counts Influence HIV-1 Susceptibility in High-Risk South African Black Women

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Background. The Duffy-null trait and ethnic netropenia are both highly prevalent in Africa. The influence of pre-seroconversion levels of peripheral blood cell counts (PBCs) on the risk of acquiring human immunodeficiency virus (HIV)–1 infection among Africans is unknown.

Methods. The triangular relationship among pre-seroconversion PBC counts, host genotypes, and risk of HIV acquisition was determined in a prospective cohort of black South African high-risk female sex workers. Twenty-seven women had seroconversion during follow-up, and 115 remained HIV negative for 2 years, despite engaging in high-risk activity.

Results. Pre-seroconversion neutrophil counts in women who subsequently had seroconversion were significantly lower, whereas platelet counts were higher, compared with those who remained HIV negative. Comprising 27% of the cohort, subjects with pre-seroconversion neutrophil counts of $<$2500 cells/mm^3 had a $\sim$3-fold greater risk of acquiring HIV infection. In a genome-wide association analyses, an African-specific polymorphism (rs2814778) in the promoter of Duffy Antigen Receptor for Chemokines (*DARC* $-46T > C$) was significantly associated with neutrophil counts ($P = 7.9 \times 10^{-11}$). *DARC* $-46C/C$ results in loss of DARC expression on erthyrocytes (Duffy-null) and resistance to *Plasmodium vivax* malaria, and in our cohort, only subjects with this genotype had pre-seroconversion neutrophil counts of $<$2500 cells/mm^3. The risk of acquiring HIV infection was $\sim$3-fold greater in those with the trait of Duffy-null–associated low neutrophil counts, compared with all other study participants.

Conclusions. Pre-seroconversion neutrophil and platelet counts influence risk of HIV infection. The trait of Duffy-null–associated low neutrophil counts influences HIV susceptibility. Because of the high prevalence of this trait among persons of African ancestry, it may contribute to the dynamics of the HIV epidemic in Africa.

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The malarial parasite *Plasmodium vivax* requires Duffy Antigen Chemokine Receptor (DARC) to gain entry into red blood cells (RBCs) [1, 2]. In the homozygous state, the *DARC*-null allele (due to the $-46T > C$ polymorphism in the *DARC* promoter) imparts selective loss of DARC expression on RBC (DARC- or Duffy-null or -negative phenotype). Consequently, the *DARC*$-$*46C/C* genotype results in resistance to *P. vivax* infection [1, 2]. Notably, the *DARC*-null allele is highly

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prevalent among Africans, and there is a compelling case for the *DARC* locus to have been affected by selection [3]. However, the impact of this selection on present-day infectious diseases remains largely unknown.

Interestingly, there is a striking geographical overlap between the prevalence of the *DARC*-null allele and human immunodeficiency virus (HIV) infection in Africa [4, 5]. In addition, DARC is a binding protein for multiple chemokines, and RBC-DARC regulates intravascular levels of pro-inflammatory chemokines that influence HIV cell entry [6] and inflammation [1, 2, 7, 8]. Substantiating its possible role in inflammatory processes relevant to HIV pathogenesis [9], RBC-DARC modifies chemokine and coagulation responses following intravenous administration of endotoxin to humans [10, 11]. Furthermore, HIV-1 adsorbs to erythrocyte DARC [12, 13] and thence can be transferred to $CD4^+$ target T cells [12], suggesting that RBCs might act as carriers of infectious HIV-1 particles to susceptible cells.

Our original evaluation of the associations of *DARC−46C/C* with susceptibility to HIV/AIDS [12] conducted in a well-established natural history cohort of HIV from the United States revealed that, among HIV-infected African American persons, the *DARC*-null phenotype associated with a survival advantage. By contrast, *DARC−46C/C* was associated with an increased risk of acquiring HIV infection [12]. A mechanistic model was envisaged whereby DARC impacted on HIV/AIDS susceptibility by mediating *trans*-infection of HIV-1, affecting chemokine-HIV interactions, and influencing chemokine-driven inflammation [12].

However, the association of *DARC* genotype with susceptibility to HIV/AIDS was not observed by others [14–17] who regarded this association as a false-positive result because of the methods used for adjustment of population admixture between African American HIV-infected patients versus HIV-uninfected control subjects [15–17]. However, population stratification was unlikely to explain our original results because the case patients and control subjects were epidemiologically similar (eg, they were US Air Force personnel), the genetic markers used to adjust for admixture predicted self-reported ethnicity with >98% accuracy [12], and additional analyses with a validated panel of 96 ancestry informative markers [18] revealed no evidence for population stratification [19]. Also, genetic associations for even one of the most intensively scrutinized HIV disease-retarding polymorphisms in the HIV field, namely the *CCR5-Δ32* allele, exhibits extensive heterogeneity across cohorts [20, 21]. Therefore, in our riposte [19], we posited that differences between our and others' study end points and cohort characteristics [19] may have accounted for the disparate associations and obscured complex *DARC* genotype-phenotype relationships pertinent to HIV/AIDS.

Of special relevance was the demonstration that the DARC-null state is a strong genetic basis for ethnic leucopenia and/or neutropenia [22, 23], a hematologic condition observed commonly in persons of African descent [24–26]. We therefore evaluated whether there is a triangular relationship among the *DARC*-null state, leucopenia, and HIV disease course [27]. These analyses demonstrated that during HIV infection, *DARC−46C/C* genotype associated with a survival advantage but mainly in a specific cellular context defined by a low WBC/neutrophil count [27].

For these reasons, we hypothesized that there was a similar triangular relationship among *DARC*-null, low neutrophil levels, and HIV susceptibility. However, the high occurrence of leukopenia and neutropenia during HIV infection [27, 28] precluded a cross-sectional analysis of leukocyte levels in HIV-infected versus HIV-uninfected subjects as a means to investigate whether pre-infection peripheral blood cell (PBC) counts influenced the future risk of acquiring HIV infection. For these reasons, we investigated a prospective cohort of high-risk women (HRW), the majority of whom (80%) were sex workers (SWs) from South Africa [29]. During the intensive prospective follow-up period of 2 years, 19% of the evaluable HRW/SWs had seroconversion, whereas the other subjects remained uninfected despite high-risk activity. These epidemiological characteristics made this cohort ideal for evaluating the proposed triangular relationship among *DARC*-null, low neutrophil levels, and HIV susceptibility. Our results affirmed this relationship.

METHODS

Study Cohort

We evaluated a cohort of 245 black HRW/SWs (Figure 1A) from South Africa who were part of the CAPRISA 002 acute infection study [29]. Of these subjects, 27 HRW/SWs had seroconversion during prospective follow-up, and 115 participants remained HIV negative for 2 years and comprised the study group investigated here.

Genetic Analyses

Genetic data were obtained from a genome-wide association study (GWAS) using the Human Duo-1M Illumina beadchip according to the Infinium HD protocol. Of the 1,144,696 markers typed, 874,956 markers passed quality control (see Supplementary Methods) and were used for final analyses.

Statistical Analyses

We used baseline (initial) values of the PBC (ie, RBC, platelet, and white blood cell [WBC] counts) or the main components of WBC as predictors of subsequent HIV acquisition risk, where "baseline" refers to values obtained when all subjects were HIV seronegative at cohort entry. These associations were investigated using multivariate unconditional logistic regression models. The normal range for neutrophils in healthy persons is 2500–6000 cells/mm^3 [30], and we used the lower end of this

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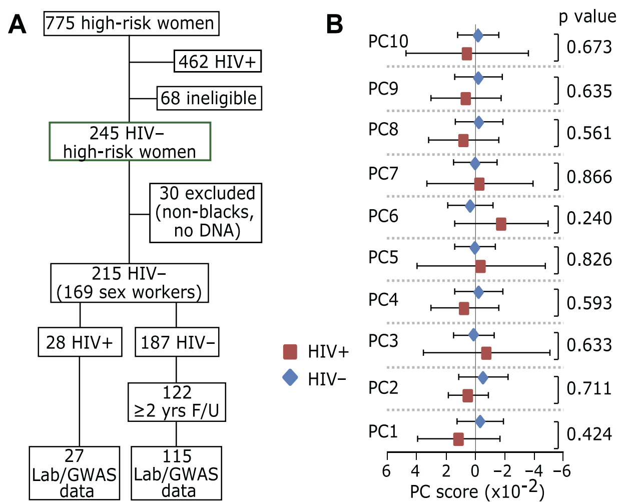

Figure 1. Study subjects and principal component analyses for population stratification. *A,* Study subjects. CAPRISA 002 Acute Infection Study screened 775 high risk women from Durban, KwaZulu-Natal, South Africa, who self-identified as sex workers or who reported >3 sexual partners in the prior 3 months [29]. Four hundred sixty-two of these were human immunodeficiency virus (HIV)–positive subjects ,and 68 met exclusion criteria as described previously (e.g. pregnant, declined follow-up for 2 years) [29]. Two hundred forty-five subjects *(green box)* were enrolled in the Acute Infection study [29]. Thirty were excluded for the present study because they were either not black or DNA was unavailable. Of the 215 HIV-negative women who were observed prospectively, 169 (78.6%) were self-reported sex workers, and at the end of the 2-year follow-up period, 28 women had experienced seroconvesion and 122 remained HIV seronegative (exposed uninfected). 65 women who had less than 2 years of follow-up were excluded from the current analyses. Baseline peripheral blood cell and GWAS data were available on 27 HIV-seroconverting women and 115 HIV nonseroconverting women. The sexual risk behavior and other characteristics of these study subjects were similar to those as described previously [29]. *B,* Evaluation of population stratification in the study groups. Red squares and blue diamonds indicate the mean principal component (PC) scores for the top 10 PCs for HIV-infected and -uninfected subjects, respectively. Error bars represent the 95% confidence interval. Numbers at the right side are significance values obtained using the Student *t* test.

threshold (2500 cells/mm^3) as a cutoff in some of the statistical analyses.

Methods for adjustment of population admixture using 3160 ancestry informative markers (Supplementary Tables 1 and 2) are described in the Supplementary Methods**.** In brief, from these ancestry informative markers we derived and compared the values of the top 10 principal components (PCs) obtained by EIGENSTRAT analysis [31] between HIV-infected and HIV-uninfected subjects. We then adjusted the multivariate models for the scores for the top 10 PCs and excluded subjects who were classified as outliers (n = 4) (see Supplementary Methods for details).

Using PLINK software [32], we determined the association of each polymorphism with the quantitative hematological traits

Table 1. Association of Major Peripheral Blood Cell (PBC) Components With Future Risk of Acquiring Human Immunodeficiency Virus (HIV) Infection

	All subjects			Excluding outliers		
PBC	OR	95% CI	*P*	OR	95% CI	*P*
RBC count	1.08	.29–3.95	.911	1.19	.30–4.76	.801
Platelet count	2.31	1.31–4.08	.006	2.61	1.38–4.93	.003
WBC count	0.72	.55–.93	.014	0.68	.51–.91	.009

NOTE. Unconditional multivariate logistic regression modeling using baseline PBC values from 142 individuals; outliers are as described in the methods section. An odds ratio (OR) that is >1 or <1 indicates that a higher value of the hematological parameter is associated with a higher or lower risk of acquiring HIV infection, respectively. ORs are per 10^6 RBCs, per 10^5 platelets, or per 10^3 WBCs (all in cells/mm^3). CI, confidence interval; RBC, red blood cell; WBC, white blood cell.

(neutrophil and platelet counts; see Results) by using the asymptotic Wald test. We also conducted the GWAS analyses using multivariate linear regression models by adjusting for the top 10 PCs.

RESULTS

PBC Counts and Risk of HIV Infection

Evaluation of the association of the baseline (preinfection) values of the 3 major PBCs—namely WBC, RBC, and platelet counts—revealed that a higher baseline WBC count was associated with a reduced risk of acquiring HIV infection (Table 1). Specifically, each 1000-cell/mm^3 increment in the baseline WBC count was associated with a 28% reduction in the risk of subsequently acquiring HIV (odds ratio [OR], 0.72; 95% confidence interval [CI], 0.55–0.93). By contrast, an initial high platelet count was associated with an increased future risk of acquiring HIV infection, with each additional 100,000-cell/mm^3 increment in the platelet count associating with a ~2 fold increased risk of HIV infection (Table 1). A significant association between RBC count and HIV risk was not detected (Table 1).

To determine which specific leukocyte subset contributed to the association observed for the WBC count, we replaced the baseline WBC count in the multivariate logistic regression model with the baseline counts of its major components (eg, neutrophil and lymphocyte counts). This model revealed that each 1000-cell/mm^3 increase in the baseline neutrophil count was associated with a 36% lower risk of subsequently acquiring HIV infection (OR, 0.64; 95% CI, 0.45–0.94; Table 2, model 1). An association between the baseline values of other WBC components was not detected (Table 2, model 1). In a backward elimination stepwise regression model, the only 2 PBCs that remained statistically significant were platelet and neutrophil counts (Table 2, model 2). In the multivariate logistic regression models, when we replaced the baseline lymphocyte count with baseline counts for CD4$^+$ or CD8$^+$ T cells, only neutrophil and

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Table 2. Association of Peripheral Blood Cell (PBC) Counts and White Blood Cell Components With Future Risk of Acquiring Human Immunodeficiency Virus (HIV) Infection

	All subjects			Excluding outliers		
PBC	OR	95% CI	*P*	OR	95% CI	*P*
Model 1: baseline (pre-seroconversion) cell counts at study entry						
RBCs	1.11	.27–4.54	.887	1.19	.27–5.31	.817
Platelets	2.48	1.42–4.33	.004	2.77	1.44–5.35	.002
Neutrophils	0.64	.45–.94	.021	0.62	.42–.93	.022
Lymphocytes	0.61	.28–1.33	.217	0.58	.25–1.32	.194
Monocytes	1.28	.89–1.89	.212	1.18	.78–1.81	.429
Eosinophils	2.01	.25–16.2	.511	1.91	.22–16.9	.560
Basophils	0.89	.74–1.07	.209	0.91	.75–1.06	.345
Model 2: Final model from backward stepwise regression						
Platelets	2.15	1.22–3.80	.008	2.40	1.31–4.40	.004
Neutrophils	0.69	.50–.95	.024	0.65	.46–.92	.016
Model 3: Model 2 adjusted for PC1–PC10 from EIGENSTRAT						
Platelets	2.73	1.44–5.17	.002	2.90	1.48–5.69	.002
Neutrophils	0.64	.46–.90	.011	0.58	.39–.86	.006

NOTE. An odds ratio (OR) that is >1 or <1 indicates that a higher value of the hematological parameter is associated with a higher or lower risk of acquiring HIV infection, respectively). ORs are per 10^6 RBCs, per 10^5 platelets, per 10^3 neutrophils or lymphocytes, per 100 monocytes, and per 10 basophils (all in cells/mm^3). Model 1, full unconditional multivariate logisitc regression model. Model 2, backward elimination stepwise regression model with a probability retention criterion of 0.1. Model 3, final model after adjustment for top 10 principal components from EIGENSTRAT. CI, confidence interval.

platelet counts were retained in the final models (data not shown).

These associations were unlikely to be due to differences in population admixture of the HIV-infected versus HIV-negative HRW. First, none of the mean scores for the top 10 PCs for HIV-positive and HIV-negative groups differed significantly (Figure 1B). Second, the associations for neutrophil and platelet counts observed in the final model of the stepwise regression for PBCs shown in Table 2 (model 2) remained unchanged after inclusion of the top 10 PCs as covariates in this regression model (Table 2, model 3). Third, all the aforementioned associations remained unchanged when we excluded from the multivariate model subjects who were classified as outliers (Table 2).

Normal ranges of blood differential counts vary depending on age, sex, population group, and other factors, and there are inter-laboratory differences in reference intervals. Nonetheless, 2500 cells/mm^3 is widely used as the lower limit of normal neutrophil counts [30], and a neutrophil count <1500 cells/mm^3 has become the commonly accepted definition of neutropenia [25]. With these general guidelines in mind, we determined the threshold of baseline neutrophil counts below which future risk of HIV infection increased (Figure 2A). Approximately 31% of the HRW with a baseline neutrophil count of <1500 or 1500–2500 cells/mm^3 subsequently had

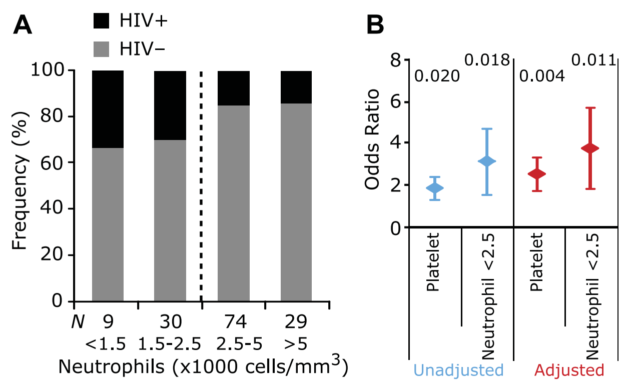

Figure 2. Association of the initial or baseline neutrophil and platelet counts with risk of acquiring human immunodeficiency virus (HIV) infection. *A,* Proportion of HIV-positive and HIV-negative subjects in the indicated categories of baseline neutrophil counts. *B,* Multivariate logistic regression analyses for the association of platelet counts and low neutrophil counts (defined as <2500 neutrophils/mm^3) before *(blue)* and after *(red)* adjustment for potential population stratification of the top 10 principal component (PC) scores. Adjustment was done by including the top 10 PCs as covariates in the logistic regression model. Numbers at the top are the significance values. The reference group for neutrophil counts was the category of ⩾2500 cells/mm^3. Platelet count was included in the model as a continuous variable of increments of 100,000 cells/mm^3.

seroconversion, whereas only ~15% of those with a neutrophils count >2500 cells/mm^3 did so (Figure 2A). By logistic regression analyses, in addition to platelet counts, an initial neutrophil count of <2500 cells/mm^3 was associated with a 3-fold greater future risk of subsequently acquiring HIV infection before or after adjusting for population admixture (Figure 2B). Thus, in our study population, an initial neutrophil count of 2500 cells/mm^3 reflected a threshold for altered HIV risk.

Genome-wide Association for Neutrophil and Platelet Counts

Because of the observed associations of neutrophil and platelet counts with risk of HIV, we used a GWAS approach to identify polymorphisms that may associate with variability in these 2 traits. GWAS for neutrophil counts revealed only 1 genetic marker, rs2814778 on chromosome 1, that associated significantly with neutrophil counts, and it represents the T-46C polymorphism in *DARC* ($P = 1.4 \times 10^{-8}$) (Figure 3A, Supplementary Figures 1–3 and Supplementary Table 3). Even after removal of subjects classified as outliers and adjusting for the top 10 PCs, the association of the *DARC* polymorphism with neutrophil counts remained highly significant ($P = 7.9 \times 10^{-11}$) (Figure 3B). Furthermore, the strong association between rs2814778 and neutrophil counts is unlikely to have been inflated due to potential systematic errors (eg, population admixture) as suggested by Q-Q plots analysis (Figure 3C). Moreover, this *DARC* polymorphism explained 26% (R^2 by univariate model) of variability in neutrophil counts. An association for platelet counts that met the genome-wide level for

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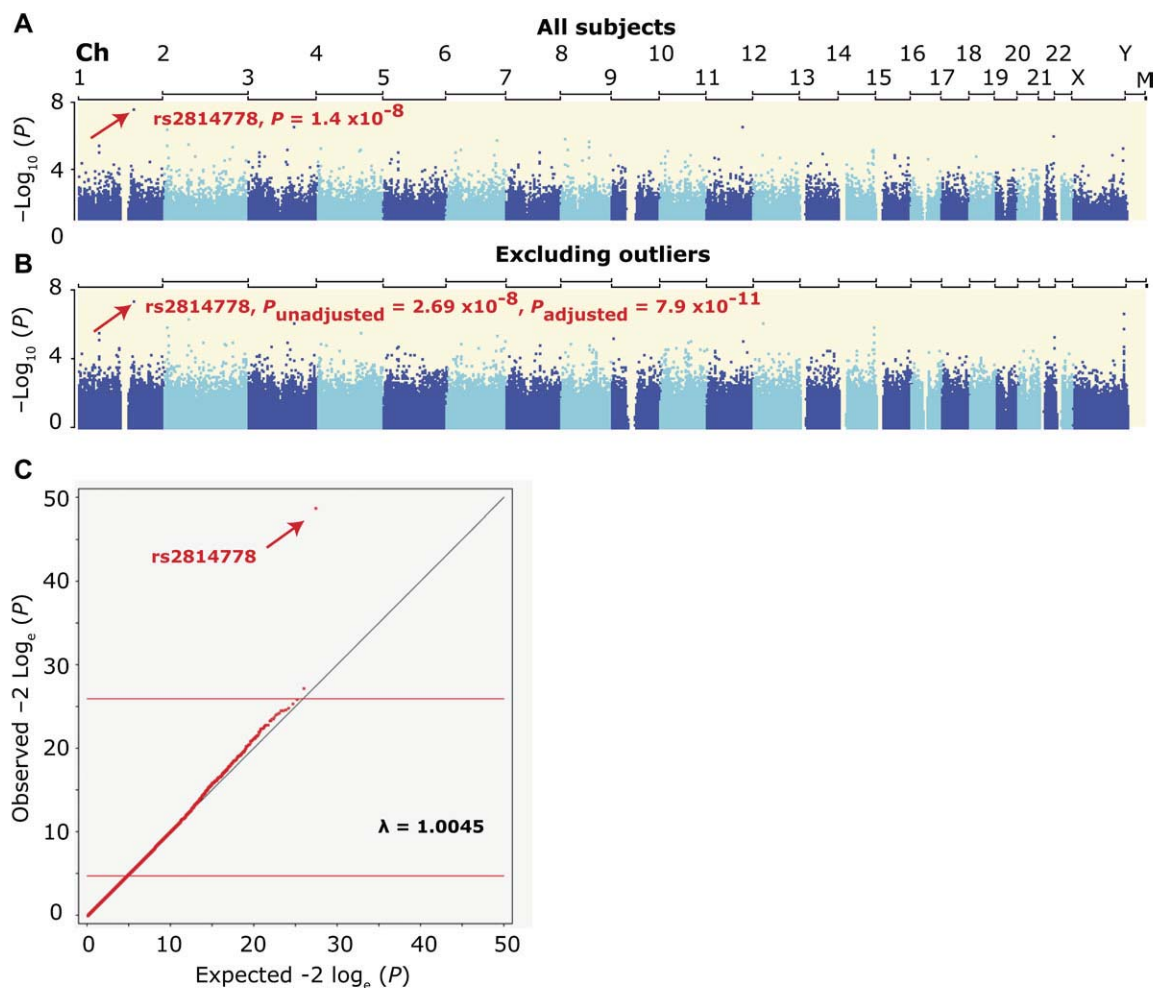

Figure 3. GWAS for the traits of neutrophil counts. Manhattan plots indicate the -$\log_{10}$ *P* value of the association statistic plotted by the chromosomal location of the marker (x-axis). Chromosome numbers are shown at the top. Red arrows indicate the marker with strongest association that was statistically significant at the genome-wide significance threshold level of 5.8×10^{-8}. *A,* Significance value based on the Wald statistic for all subjects. *B,* Analyses after removing 4 subjects who were classified as outliers. The significance values obtained after adjustment for the top 10 principal components derived from the 3073 ancestry informative markers is also shown. *C,* Q-Q plot analysis. We generated a Q–Q plot of observed versus expected *P* values for the association of neutrophil counts. *P*-values are transformed using the inverse χ^2 distribution with 1 degree of freedom. Scatter of dots close to the diagonal line indicate negligible likelihood of population admixture. The single point at the top (highlighted using red arrow) represents the association of rs2814778 with neutrophil count. Estimated λ genomic inflation factor is shown, indicating whether systematic biases, such as population stratification, are present [33]. In this case, $\lambda = 1.0045$, suggested negligible overall effect of stratification or other systematic biases.

statistical significance was not detected (Supplementary Figure 4 and Supplementary Table 4).

DARC −46C/C, Neutrophil Counts and Risk of HIV

We next investigated whether HIV risk might differ according to the initial neutrophil count. Consistent with the GWAS findings, DARC-negative subjects had significantly lower neutrophil counts than DARC-positive individuals (Table 3). Although the leukopenia associated with the *DARC−46C/C* is attributable mainly to low neutrophil counts [22, 23], subjects with this genotype also had lower monocyte counts (Table 3).

The cumulative distributions of neutrophil counts in DARC-negative and DARC-positive subjects were significantly different (Figure 4A). None of the 50 DARC-positive subjects had a baseline neutrophil count <2500 cells/mm^3 (Figure 4A). These low neutrophil counts were found only in DARC-negative subjects, and those DARC-negative subjects with a low neutrophil count comprised nearly 27% of the entire cohort (Figure 4B). Notably, a neutrophil count of 2500 cells/mm^3 is the same neutrophil threshold that is associated with altered risk of HIV (compare Figures 4B and 2A). Predictably, compared with all other subjects, the DARC-negative subjects with a baseline neutrophil count of <2500 cells/mm^3 had a 2.6-fold higher risk of acquiring HIV infection (OR, 2.61; 95% CI, 1.09–6.24; $P = .028$), resulting in an overrepresentation of those with the "DARC-negative-low baseline neutrophil" genotype-phenotype

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Table 3. Association of Peripheral Blood Cell (PBC) Counts With *DARC* Genotype

PBC[a]	DARC+		DARC−		P
	No. of subjects	Mean ± SE	No. of subjects	Mean± SE	
RBC count, × 10^6 cells/mm^3	50	4.32 ±0.05	92	4.37 ±0.038	.453
Platelet count, × 10^5 platelets/mm^3	50	3.22 ±0.11	92	3.02 ±0.88	.179
WBC count, × 10^3 cells/mm^3	50	7.99 ±0.27	92	5.90 ±0.19	<.001
Neutrophil count, × 10^3 cells/mm^3	50	5.01 ±0.21	92	3.03 ±0.17	<.001
Lymphocyte count, × 10^3 cells/mm^3	50	2.25 ±0.09	92	2.23 ±0.07	.807
Monocyte count, × 10^2 cells/mm^3	50	0.46 ±0.02	92	0.36 ±0.01	<.001
Eosinophil count, × 10^2 cells/mm^3	50	0.20 ±0.02	92	0.26 ±0.03	.386
Basophil count, × 10 cells/mm^3	50	0.06 ±0.01	92	0.05 ±0.003	.273

NOTE. RBC, red blood cell; SE, standard error; WBC, white blood cell.

[a] Mean values of initial pre-seroconversion hematological parameters in HRW/CSWs possessing the *DARC −46C/C* genotype (DARC− on RBC) compared with those lacking this genotype (DARC+ on RBC).

relationship among subjects who had seroconversion, contrasting with an underrepresentation of this genotype-phenotype correlate among those who remained HIV negative (Figure 4C). In addition, those with DARC-negative-associated low baseline neutrophil counts seroconverted 2.4 times faster than all other subjects (Figure 4D).

Ideally, the comparator group for DARC-negative subjects with neutrophil counts <2500 cells/mm^3 would be DARC-positive subjects with neutrophil counts <2500 cells/mm^3. However, no such individuals were present in our study population. To capitalize on the entire range of baseline neutrophil counts, we used an alternative statistical approach in which the *DARC*-negative-associated low neutrophil count is considered as an inherited trait, and a higher probability of possessing this trait is conceptualized as being associated with a higher risk of acquiring HIV infection (see Supplementary Methods). We estimated the probability of possessing the *DARC−46C/C* genotype for a given neutrophil count and then used receiver operating characteristic curve to determine the cutoff value above which the probability of possessing this trait increased the likelihood of acquiring HIV infection (Supplementary Figure 5). These analyses revealed that a high probability for this trait associated with a nearly 3-fold increased risk and rate of acquiring HIV infection (Supplementary Table 5).

DISCUSSION

Investigation of a well-characterized prospective cohort of high-risk black South African women revealed several major findings. Preinfection high platelet and low WBC counts, attributable mainly to low neutrophil counts, were associated with increased future HIV acquisition risk. The association of platelet counts with HIV risk may relate to platelets serving as an interface between coagulation and inflammation or immunity [34, 35]. Neutrophils, by serving as first defenders against infections, may play a critical role in instructing the adaptive immune responses relevant to HIV pathogenesis (reviewed in [36, 37]). Furthermore, low neutrophil counts in persons of African ancestry are associated with higher levels of interleukin (IL)–8 and granulocyte colony-stimulating factor [38]: both biomarkers correlate with inflammation [38, 39] and IL-8 increases HIV-1 transmission ex vivo [40]. Thus, low neutrophil counts, by altering the inflammatory milieu, may influence HIV risk. As a corollary to our findings, high neutrophil counts associate with resistance to acquiring HIV in high-risk men in the MACS cohort [41].

Interestingly, GWAS analyses demonstrated that possession of the *DARC*-null allele is highly predictive of low neutrophil counts. This finding is consistent with an extensive admixture mapping study that demonstrated that the *DARC*−46T/C polymorphism is significantly more predictive of neutrophil count than of ancestry and that the DARC-null allele was causal for low neutrophil counts in persons of African descent [23]. In our study participants, only DARC-null subjects had pre-infection neutrophil counts <2500 cells/mm^3. The association of *DARC−46C/C* with ethnic leukopenia/neutropenia may relate to DARC's chemokine-binding and/or transport function, impacting the levels of chemokines that influence neutrophil trafficking [1, 2, 7].

In addition, we found the increased HIV risk associated with the DARC-null state is mainly in those with a low neutrophil count; such subjects constituted 27% of our study population. The trait of DARC-null-associated low neutrophil counts associated with a ~3-fold higher risk and ~3-fold accelerated rate of acquiring HIV. Given the high prevalence of this unfavorable genotypic hematological determinant among persons of African ancestry, this trait may contribute to the HIV epidemic in Africa.

Although the biological mechanism for the trait of DARC-null-associated low neutrophil counts remains unknown, it is noteworthy that leukopenia/neutropenia is the usual response to

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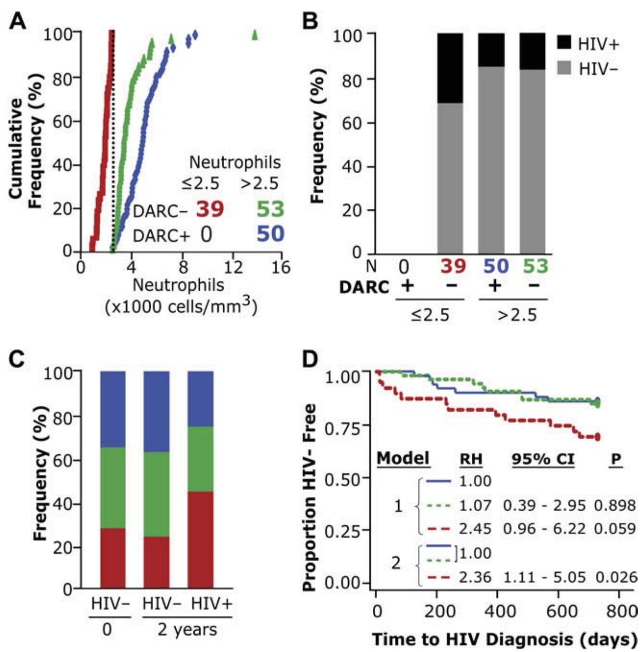

Figure 4. Association of *DARC* −*46C/C*–associated low neutrophil counts with risk and rate of acquiring human immunodeficiency virus (HIV) infection. *A,* Cumulative frequency distribution of neutrophil counts based on whether subjects were DARC negative or DARC positive and had a baseline neutrophil count of ≤2500 or >2500 cells/mm^3. The number of subjects in each of the 4 possible *DARC* genotype-neutorphil groups is shown and color-coded to match the frequency plots. *B,* Proportion of HIV-positive and HIV-negative subjects according to *DARC* genotype and baseline neutrophil counts of ≤2500 or >2500 cells/mm^3. *C,* Prevalence of DARC-negative-low neutrophil *(red),* DARC-negative-high neutrophil (*green*)*,* and DARC-positive subjects *(blue)* phenotypes at enrollment (time 0 day), and 2 years after enrollment. *D,* Kaplan-Meier plots for time to HIV diagnosis from enrollment into the cohort for the same 3 color coded groups shown in panel A. RH, relative hazard, CI, confidence interval; *P,* significance values derived by Cox proportional hazard models. In model 1, the reference (RH = 1) for the Cox models are DARC-positive subjects. In model 2, comparison of persons with DARC-negative-low baseline neutrophil versus all other subjects (RH = 1).

malarial infection, whereas higher WBC and/or neutrophil counts associate with parasite density and severe disease [42, 43]. In addition, early neutrophil depletion prevents experimental cerebral malaria [44], suggesting that high neutrophil counts may contribute negatively to aspects of malaria pathogenesis. Thus, from an evolutionary perspective, 2 phenotypic traits associated with the DARC-null state, namely resistance against parasite entry and low neutrophil counts, may have been selected for because together they may have afforded protection against an ancestral malarial infection [3]. However, reflecting an evolutionary trade-off, the ancestral beneficial impact of DARC-null in the present-day era of HIV/AIDS emergence is offset by its detrimental association with an increased HIV acquisition risk. Notably, double-edged genotype-phenotype relationships have also been described for the HIV resistance *CCR5-Δ32* allele, which affords protection against HIV but susceptibility with other infectious agents, such as West Nile virus [45].

It is important to note that because of the close association of Duffy-null with low neutrophil counts in our study population, it is difficult to ascribe an independent effect of low neutrophil counts or the DARC-null state on HIV risk. Hence, these 2 host factors may act independently or as a conjoint genetic-cellular determinant of HIV susceptibility. Nonetheless, together with prior results [12, 27], these findings underscore the extant intricate triangular relationship among DARC-null state, low neutrophil counts, and susceptibility to HIV/AIDS. Consequently, depending on the cohort analyzed, failure to account for this intricate relationship as well as inter-cohort differences in prevalence of the trait of DARC-null-associated low neutrophil counts may obscure the ability to discern true associations of *DARC-46C/C* with susceptibility to HIV/AIDS.

Our study has limitations. The sample size was small, and we could not account for the effect of intercurrent sexually transmitted infections on HIV acquisition risk. In addition, it is difficult to document the degree of exposure to HIV prior to or after cohort entry—that is, we cannot distinguish whether those who remained HIV negative, despite high-risk activity, did so because of genetically-mediated resistance to HIV or limited exposure to HIV. Notwithstanding these limitations, as noted above and reported previously [29], the cohort we evaluated has many strengths that tend to offset these shortcomings. The study participants were recruited after screening a large number (n = 775) of HRW with a much higher prevalence of HIV infection (∼60%) than the prevalence rates of HIV among other South African women [29]. Thus, this cohort, despite its small size, favors the identification of host factors with strong effects on HIV susceptibility. In addition, the prevalence of the DARC-null allele is not fixed among South Africans, allowing comparisons of DARC-positive and DARC-negative subjects.

Given the high prevalence of the Duffy-null state in Africans, it is conceivable that this genetic state could modify the population dynamics of the HIV epidemic in Africa by 2 means. First, by serving as a causal mechanism for low neutrophil counts, the DARC-null trait associates with an increased HIV acquisition risk. Second, by imparting a survival advantage to leukopenic HIV-infected subjects [12, 27], it prolongs the infectious state. Together, these 2 effects would lead to increased HIV prevalence within the population, facilitating continued HIV transmission from a larger pool of infected individuals for a longer period of time. It is well known that viruses have evolved means for subverting or exploiting the immune system to coexist with their hosts [46, 47]. Thus, the targeting by HIV of the null state for the chemokine-binding protein DARC may impart a selective advantage for the virus, akin to the effects of targeting the chemokine system by other viral pathogens [46, 47].

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Supplementary Material

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). 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.

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Potential conflicts of interest. V.R. received a K-RITH travel award. RAW has just been appointed to the Board of Directors of K-RITH. The authors do not consider this to be a conflict of interest, because the function of RAW had just started while this manuscript was in review. All other authors: no conflicts.

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