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Fc Gamma Receptor Polymorphisms Modulated the Vaccine Effect on HIV-1 Risk in the HVTN 505 HIV Vaccine Trial

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ABSTRACT HIV Vaccine Trials Network (HVTN) 505 was a phase 2b efficacy trial of a DNA/recombinant adenovirus 5 (rAd5) HIV vaccine regimen. Although the trial was stopped early for lack of overall efficacy, later correlates of risk and sieve analyses generated the hypothesis that the DNA/rAd5 vaccine regimen protected some vaccinees from HIV infection yet enhanced HIV infection risk for others. Here, we assessed whether and how host Fc gamma receptor (FcγR) genetic variations influenced the DNA/rAd5 vaccine regimen's effect on HIV infection risk. We found that vaccine receipt significantly increased HIV acquisition compared with placebo receipt among participants carrying the FCGR2C-TATA haplotype (comprising minor alleles of four *FCGR2C* single-nucleotide polymorphism [SNP] sites) (hazard ratio [HR] = 9.79, $P = 0.035$) but not among participants without the haplotype (HR = 0.86, $P = 0.67$); the interaction of vaccine and haplotype effect was significant ($P = 0.034$). Similarly, vaccine receipt increased HIV acquisition compared with placebo receipt among participants carrying the FCGR3B-AGA haplotype (comprising minor alleles of the 3 *FCGR3B* SNPs) (HR = 2.78, $P = 0.058$) but not among participants without the haplotype (HR = 0.73, $P = 0.44$); again, the interaction of vaccine and haplotype was significant ($P = 0.047$). The FCGR3B-AGA haplotype also influenced whether a combined Env-specific $CD8^+$ T-cell polyfunctionality score and IgG response correlated significantly with HIV risk; an *FCGR2A* SNP and two *FCGR2B* SNPs influenced whether anti-gp140 antibody-dependent cellular phagocytosis correlated significantly with HIV risk. These results provide further evidence that Fc gamma receptor genetic variations may modulate HIV vaccine effects and immune function after HIV vaccination.

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IMPORTANCE By analyzing data from the HVTN 505 efficacy trial of a DNA/recombinant adenovirus 5 (rAd5) vaccine regimen, we found that host genetics, specifically Fc gamma receptor genetic variations, influenced whether receiving the DNA/rAd5 regimen was beneficial, neutral, or detrimental to an individual with respect to HIV-1 acquisition risk. Moreover, Fc gamma receptor genetic variations influenced immune responses to the DNA/rAd5 vaccine regimen. Thus, Fc gamma receptor genetic variations should be considered in the analysis of future HIV vaccine trials and the development of HIV vaccines.

KEYWORDS Fc gamma receptor, HIV/AIDS vaccine trial, HVTN 505, genetic polymorphisms

Fc gamma receptors (FcγRs) are expressed on the leukocyte surface and interact with the Fc domain of immunoglobulin G (IgG) antibodies. Interaction of FcγRs with IgG immune complexes initiates intracellular signaling pathways that lead to a variety of downstream events, including cellular activation and cytokine/chemokine production (1, 2). These events can have either immunostimulatory or immunosuppressive effects, making FcγRs major players that modulate a range of processes, including antibody production, antigen presentation, and activation of B cells and innate immune effector cells (3). Interestingly, FcγR genetic variation can have significant functional implications, such as modulating affinity of FcγR binding to antibodies and affecting the level of FcγR expression and effector functions in specific types of immune cells (4–7). Moreover, our sequencing and analysis of exons and the areas surrounding FcγR genes identified significant associations of single-nucleotide polymorphisms (SNPs) in *FCGR2C* with vaccine efficacy (VE), defined as one minus the vaccine/placebo hazard ratio of HIV-1 acquisition (HR), in the RV144 trial (8).

Considering that FcγR genetic variations have broad functional implications (4–7, 9), we hypothesized that FcγR polymorphisms also influence HIV-1 acquisition risk in vaccine recipients in other vaccine efficacy trials. The HIV Vaccine Trials Network (HVTN) 505 phase 2b trial evaluated the efficacy of a multiclade DNA prime, recombinant adenovirus serotype 5 vector boost (DNA/rAd5) vaccine regimen in circumcised, Ad5-seronegative men and transgender women who have sex with men in the United States (10). While this trial was unblinded early due to lack of overall VE, recent studies identified several correlates of HIV-1 acquisition risk in HVTN 505, including Env-specific $CD8^+$ T-cell response magnitude and polyfunctionality score (PFS) (11), Env-specific humoral IgG responses (12) and antibody Fc effector functions (antibody-dependent cellular phagocytosis [ADCP] and FcγRIIa binding) (76). Moreover, the vaccine/placebo hazard ratio of HIV-1 acquisition significantly varied by the type of HIV-1 virus, defined by amino acid sequence distance of the HIV-1 CD4 binding site to the vaccine insert sequence, a "sieve effect" (13). Cumulatively, these findings suggest that the DNA/rAd5 vaccine regimen has had differential effects on HIV-1 acquisition depending on immunologic and virologic markers.

Together with the evidence from two previous HIV-1 vaccine efficacy trials that an rAd5 vaccine increased the risk of HIV-1 acquisition compared to placebo in a subset of individuals (14), these results raise the possibility that the DNA/rAd5 vaccine regimen has enhanced the risk of HIV-1 acquisition in some individuals while conferring a certain degree of protection in others (i.e., individuals who generated relatively strong immune responses upon vaccination and who were exposed to HIV-1 viruses sufficiently similarly to the vaccine strains), averaging out to the observed null efficacy. Our hypothesis is that genetic variations in FcγRs could explain, in part, potential variation in the vaccine's effect on HIV-1 acquisition.

To test this hypothesis, we genotyped HVTN 505 HIV-1-infected cases and uninfected controls (including both vaccine and placebo recipients) for FcγR genes, as previously described for RV144 cases and controls (8). We assessed whether and how FcγR SNPs were modified according to (i) the vaccine/placebo HR of HIV-1 acquisition risk, (ii) the previously identified associations of immune response biomarkers or Fc

effector functions with HIV-1 acquisition risk in vaccine recipients (11, 12), (iii) vaccine-induced immune responses, including Fc effector functions in vaccine recipients, and (iv) the previously identified Env-gp120 sieve effects (13). We also investigated the potential functional mechanisms of the identified FcγR SNPs by investigating their associations with FcγR expression in human B cells.

RESULTS

FCGR2C and FCGR3B genetic variants modified the effect of the DNA/rAD5 vaccine on HIV-1 acquisition risk. We genotyped HVTN 505 cases and controls for 162 loci over five FcγR genes (*FCGR2A*, *FCGR2B*, *FCGR2C*, *FCGR3A*, and *FCGR3B*). Of these loci, 33 SNPs passed selection criteria and tests (details are in Materials and Methods) and were included in further analysis. We began by assessing whether any of these 33 FcγR SNPs significantly modified the effect of the DNA/rAd5 vaccine regimen on HIV-1 acquisition risk. We found that 7 FcγR SNPs significantly modified the vaccine/placebo hazard ratio (HR) of HIV-1 acquisition after adjusting for the 33 statistical tests ($P \leq 0.05$ and false discovery rate [FDR]-adjusted q value of ≤ 0.2) (Fig. 1; see also Table S1 in the supplemental material). These SNPs are located in two FcγR genes: four in *FCGR2C* (FCGR2C-exon06-441-C/T [rs138747765], FCGR2C_intron06_590-G/A, [rs78603008], FCGR2C-intron15-403-C/T [rs373013207], and FCGR2C-intron15-433-G/A [rs201984478]) and three in *FCGR3B* (FCGR3B-5′utr222-G/A [rs34085961], FCGR3B-5′utr44-T/A [rs34322334], and FCGR3B-5′utr99-C/G [rs61803026]). For each of these SNPs, the vaccine/placebo HR was significantly greater than one (HR = 2.6 to 9.8) in participants carrying a minor allele(s) in these *FCGR2C* and *FCGR3B* loci (Table 1), suggesting that the DNA/rAd5 vaccine have enhanced the risk of HIV-1 acquisition in these subgroups of individuals, whereas HR was less than one (HR = 0.65 to 0.88) in participants not carrying a minor allele.

In HVTN 505 participants, the four identified *FCGR2C* SNPs were in high linkage disequilibrium (LD; $D' = 0.91$ to 1.0, $r^2 = 0.68$ to 1.0), likely representing one signal, and the three *FCGR3B* SNPs were also in high LD ($D' = 1.0$, $r^2 = 0.84$ to 1.0), likely representing a second signal. Further analysis showed that the four *FCGR2C* SNPs formed one haplotype block, while the three *FCGR3B* SNPs formed another haplotype block (Fig. 2). We found that the haplotypes FCGR2C-TATA (comprising all minor alleles in the four *FCGR2C* SNPs with haplotype frequency of 20.7%; Fig. 2) and FCGR3B-AGA (comprising all minor alleles of the 3 *FCGR3B* SNPs with haplotype frequency of 35%; Fig. 2) also significantly modified the DNA/rAd5 vaccine effect on HIV-1 acquisition (FCGR2C-TATA, HR = 9.79 and $P = 0.035$ for presence, HR = 0.86 and $P = 0.67$ for absence, with interaction $P = 0.034$; FCGR3B-AGA, HR = 2.78 and $P = 0.057$ for presence and HR = 0.73 and $P = 0.44$ for absence, with interaction $P = 0.047$) (Table 1). These results indicate that vaccine recipients carrying the FCGR2C-TATA haplotype or the FCGR3B-AGA haplotype had an increased risk of HIV-1 acquisition compared with that of placebo recipients carrying the same haplotype.

The FCGR3B-AGA haplotype modified the association of Env-specific IgG and CD8$^+$ T-cell PFS with HIV-1 acquisition risk. To examine the potential functional impact of these FcγR SNPs, we assessed whether any of the 33 FcγR SNPs modified the immune correlates of the Env-specific CD8$^+$ T-cell PFS and Env-specific IgG with HIV-1 acquisition risk identified previously (11, 12). We found that none of the 33 SNPs significantly modified the correlate of Env-specific CD8$^+$ T-cell PFS with HIV-1 acquisition risk (Table S2) or the correlate of Env-specific IgG with risk (Table S3) after adjusting for the 33 statistical tests for each immune response variable. Fong et al. also found that vaccine recipients with a high CD8$^+$ T-cell PFS generally had low risk of HIV acquisition, whereas vaccine recipients with a low CD8$^+$ T-cell PFS and a low Env-specific IgG response had the highest risk (12). Therefore, we assessed whether any of the 33 FcγR SNPs modified the correlate with HIV-1 risk of a combination Env-specific IgG and CD8$^+$ T-cell PFS (low in both, denoted as 0, versus medium/high in at least one variable, denoted as 1). We found that after adjusting for the 33 statistical tests, five *FCGR3B* SNPs (FCGR3B-5′utr425-T/G [rs76732376], FCGR3B-5′utr473-G/A [rs74127076], and the three

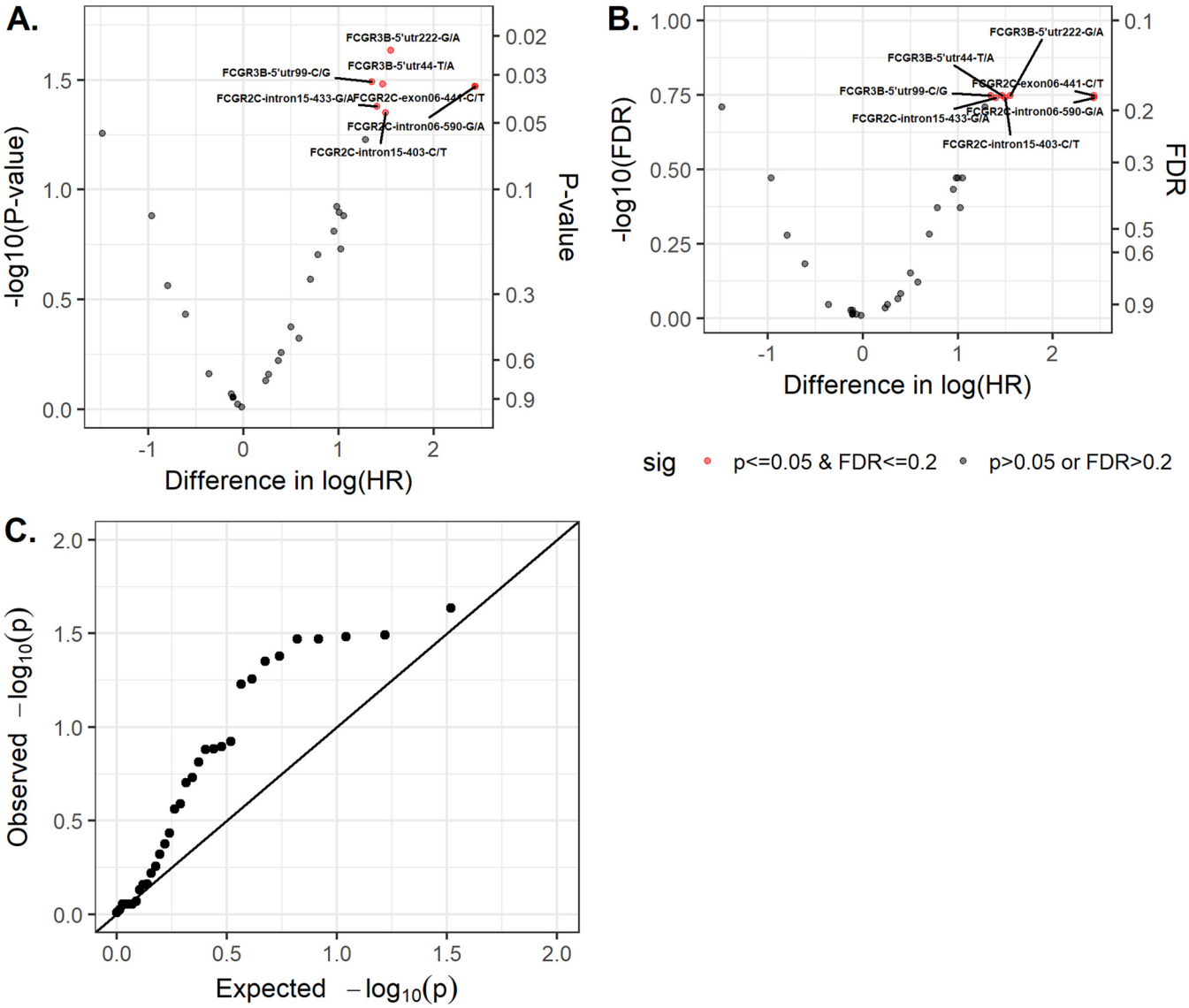


FIG 1 Assessment of whether and how each of the 33 FcγR SNPs modified the hazard ratio (vaccine/placebo) of HIV acquisition in HVTN 505. (A) Volcano plot of *P* value for the interaction between genotype and treatment arm (*y* axis, $-\log_{10}$ scale) versus difference in estimated log(HR) (vaccine/placebo) between genotype groups (*x* axis). (B) Volcano plot of false discovery rate (FDR) (*y* axis, $-\log_{10}$ scale) versus difference in estimated log(HR) between genotype groups (*x* axis). Red dots represent FcγR SNPs that significantly modify the HR (vaccine/placebo). (C) Quantile-quantile plot of the observed and expected *P* values ($-\log_{10}$ scale for both).

FCGR3B SNPs reported above) passed the significance threshold ($P \leq 0.05$ and $q \leq 0.2$) (Table S4).

The minor alleles at these two newly identified *FCGR3B* SNPs were in linkage with the FCGR3B-AGA haplotype. As shown in Table 2, the FCGR3B-AGA haplotype also significantly modified the association of the combined Env-specific IgG and $CD8^+$ T-cell PFS variable with HIV-1 risk (interaction *P* value of 0.044). Medium/high responders to Env-specific IgG and/or $CD8^+$ T cells had a larger reduction in HIV-1 risk than nonresponders or low-level responders to both: 98% (odds ratio [OR] of 0.02) in recipients without the FCGR3B-AGA haplotype and 76% (OR of 0.24) in recipients with the FCGR3B-AGA haplotype (Table 2). We observed similar trends for the FCGR2C-TTATA haplotype, but the result for the test for effect modification was not statistically significant (data not shown).

To better understand how the FCGR3B-AGA haplotype modified the association of the combined binary variable with HIV-1 acquisition risk, we examined the Env-specific

TABLE 1 Modification of the vaccine/placebo hazard ratio of HIV-1 acquisition by FcγR SNPs and haplotypes in HVTN 505

Gene	SNP	Genotype or haplotype	No. of cases (vaccine:placebo)	HR[a]	95% CI	*P* value[b]	Interaction *P* value[c]	Interaction *q* value[c]
FCGR3B	FCGR3B-5′utr44-T/A (rs34322334)	TT	10:16	0.66	0.29, 1.5	0.317	**0.033**	**0.178**
		TA/AA[d]	14:5	2.84	0.98, 8.18	0.054		
	FCGR3B-5′utr99-C/G (rs61803026)	CC	9:14	0.68	0.29, 1.6	0.375	**0.032**	**0.178**
		CG/GG[d]	18:7	2.6	1.06, 6.37	0.036		
	FCGR3B-5′utr222-G/A (rs34085961)	GG	10:16	0.65	0.29, 1.49	0.308	**0.023**	**0.178**
		GA/AA[d]	15:5	3.07	1.07, 8.75	0.036		
FCGR2C	FCGR2C-exon06-441-C/T (rs138747765)	CC	17:20	0.86	0.44, 1.7	0.667	**0.034**	**0.178**
		CT/TT[d]	9:1	9.79	1.17, 81.78	0.035		
	FCGR2C-intron06-590-G/A (rs78603008)	GG	17:20	0.86	0.44, 1.7	0.667	**0.034**	**0.182**
		GA/AA[d]	9:1	9.79	1.17, 81.78	0.035		
	FCGR2C-intron15-403-C/T (rs373013207)	CC	15:18	0.88	0.43, 1.78	0.719	**0.045**	**0.182**
		CT/TT[d]	12:3	3.92	1.08, 14.16	0.037		
	FCGR2C-intron15-433-G/A (rs201984478)	GG	13:17	0.82	0.39, 1.73	0.601	**0.042**	**0.182**
		GA/AA[d]	14:4	3.33	1.08, 10.29	0.036		
		CT/TT[d]	11:4	2.14	0.6, 7.55	0.239		
FCGR3B		FCGR3B-AGA						
		−	11:16	0.73	0.33, 1.62	0.439	**0.047**	
		+	14:5	2.78	0.97, 7.94	0.057		
FCGR2C		FCGR2C-TATA						
		−	17:20	0.86	0.44, 1.7	0.667	**0.034**	
		+	9:1	9.79	1.17, 81.78	0.035		

[a]Vaccine/placebo hazard ratio of HIV-1 acquisition in HVTN 505 for individuals with the given genotype at the given SNP.
[b]*P* value of testing for HR = 1 for individuals with the given genotype at the given SNP.
[c]Interaction *P* value of testing for a difference in HR between the two indicated genotype groups. Boldface indicates $P \leq 0.05$ and $q \leq 0.2$.
[d]The genotypes containing at least one minor allele.

IgG and $CD8^+$ T-cell PFS responses separately. Interestingly, the inverse correlation of Env-specific IgG response with HIV-1 risk previously observed (12) was only observed in vaccine recipients without the FCGR3B-AGA haplotype (OR of 0.38 and *P* value of 0.034 for recipients without and OR of 1.41 and *P* value of 0.50 for vaccine recipients with the FCGR3B-AGA haplotype; interaction *P* value of 0.035) (Table 2). For vaccine recipients without the FCGR3B-AGA haplotype, one standard deviation (SD) increase in Env-specific IgG score was associated with an estimated 62% (OR of 0.38) decrease in the risk of HIV-1 acquisition, which was larger than the estimated 40% (OR of 0.60) decrease in the risk of HIV-1 acquisition when all vaccine recipients were considered together (12). However, for vaccine recipients with the FCGR3B-AGA haplotype, there was an estimated 1.41 times increase (albeit nonsignificant) in the risk of HIV-1 acquisition (Table 2). The inverse association of Env-specific $CD8^+$ T-cell PFS with HIV-1 acquisition risk shown in reference 11 was observed in both haplotype groups but with a stronger correlate in the group without the FCGR3B-AGA haplotype (HR = 0.19, $P = 0.005$) than in the group with the FCGR3B-AGA haplotype (HR = 0.36, $P = 0.018$), although the difference was not significant.

To better understand these modification effects, we plotted the distributions of these immune response biomarkers according to case/control status and FCGR3B-AGA haplotype status (present or absent) (Fig. 3). In vaccine recipients without the FCGR3B-AGA haplotype, the magnitude of the Env IgG response (Fig. 3A), the magnitude of the $CD8^+$ T-cell PFS (Fig. 3B), and the percentage of Env IgG and/or $CD8^+$ T-cell PFS medium/high-level responders (Fig. 3C) were all significantly lower in cases than controls. Similarly, we observed lower responses in cases than in controls for $CD8^+$ T-cell PFS magnitude (Fig. 3B) and the percentage of Env IgG and/or $CD8^+$ T-cell PFS responders (Fig. 3C) for vaccine recipients with the FCGR3B-AGA haplotype but not in the magnitude of the Env IgG response (Fig. 3A). Further, among controls, $CD8^+$ T-cell PFS magnitude was lower in vaccine recipients with the FCGR3B-AGA haplotype than in those without the FCGR3B-AGA haplotype ($P = 0.04$) (Fig. 3B). These results suggest that DNA/rAd5 vaccination induced differential Env-specific $CD8^+$ T-cell responses but not IgG responses between the

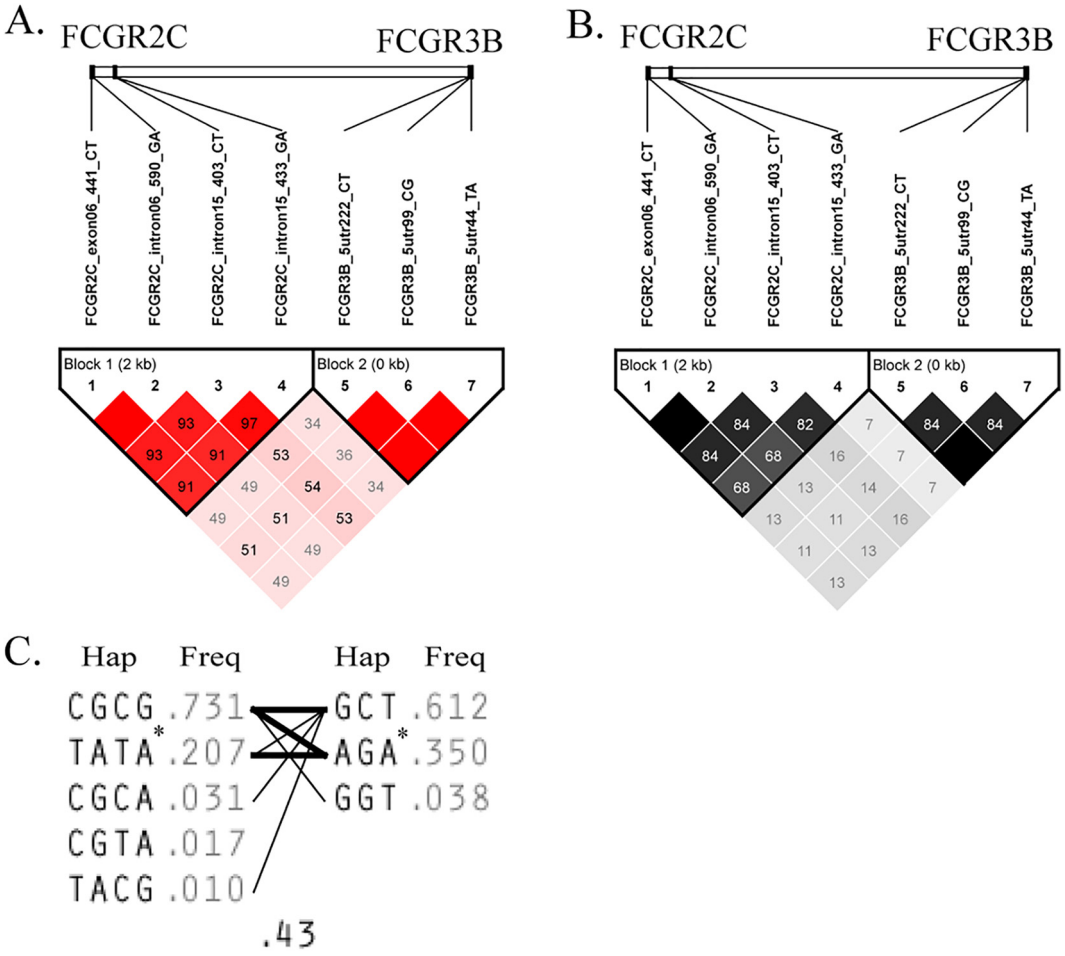


FIG 2 SNP composition of the two haplotype blocks observed in HVTN 505 participants. One of the haplotype blocks was comprised of five *FCGR2C* SNPs, and the other haplotype block was comprised of three FCGR3B SNPs. (A) Linkage disequilibrium measurements (D′) between the 8 SNP genotypes. (B) Correlation (r^2) between the 8 SNP genotypes. (C) Haplotypes and frequencies within *FCGR2C* and *FCGR3B* haplotype blocks. Shown in each haplotype block are the haplotypes with a frequency of ≥1%. The haplotype designated with an asterisk in each haplotype block contains all minor alleles from the composite FcγR SNPs. Lines connecting haplotypes across haplotype blocks indicate the frequency of haplotype linkage, where line width represents frequency magnitude (thin lines for a frequency of ≥1% and thick lines for a frequency of ≥10%). Plots were generated using Haploview.

two FCGR3B-AGA haplotype groups. Thus, for individuals with the FCGR3B-AGA haplotype (and, to a lesser extent, the FCGR2C-TTATA haplotype), vaccination was less likely to induce the potentially protective Env-specific IgG and/or CD8$^+$ T-cell responses than for individuals without the FCGR3B-AGA haplotype.

TABLE 2 Modification of identified immune correlates of risk in HVTN 505 by the FCGR3B-AGA haplotype

Immune variable	FCGR3B-AGA haplotype	Unit	OR[a]	95% CI	*P* value	Interaction *P* value[e]
Env-specific CD8$^+$ T-cell PFS[b]	−	Per 1-SD	0.19	0.06, 0.6	0.005	0.377
	+	Per 1-SD	0.36	0.15, 0.84	0.018	
Env-specific IgG response[c]	−	Per 1-SD	0.38	0.16, 0.93	0.034	**0.035**
	+	Per 1-SD	1.41	0.62, 3.17	0.412	
Env-specific IgG and/or CD8$^+$ T-cell PFS[c,d]	−	1 vs 0[b]	0.02	0, 0.15	0.0002	**0.044**
	+	1 vs 0[b]	0.24	0.06, 0.99	0.049	

[a]Odds ratio of HIV-1 infection for the given immune response and genotype group in the vaccine recipients.
[b]From Janes et al. (11).
[c]From Fong et al. (12).
[d]PFS is a binary variable: 0, low on both Env-specific IgG (in 1st tertile) and Env-specific CD8 PFS (in 1st tertile); 1, all others.
[e]Boldface indicates a *P* value of ≤0.05.

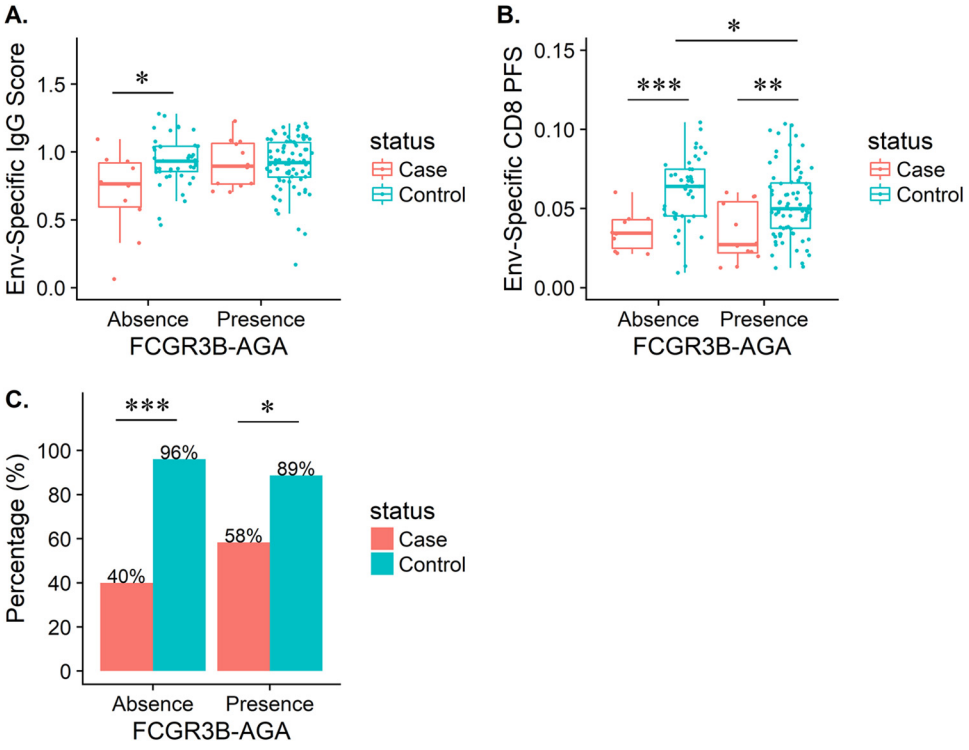
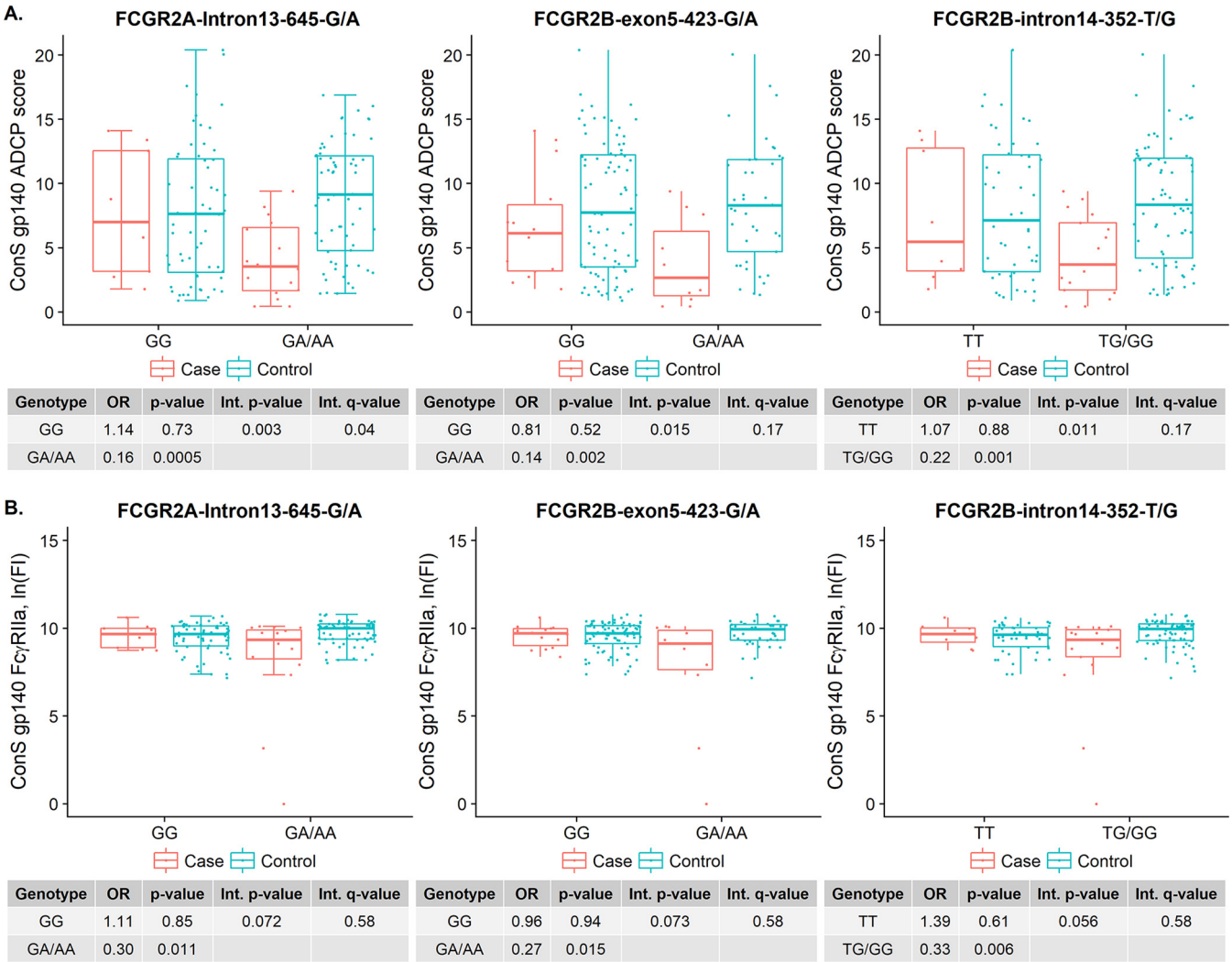


FIG 3 FCGR3B-AGA haplotype modified immune correlates of risk in HVTN 505. (A) Distributions of Env-specific IgG responses, as assessed by binding antibody multiplex assay, plotted according to case/control HIV infection outcome status and FCGR3B-AGA haplotype status. (B) Distributions of Env-specific CD8 polyfunctionality scores (PFS), plotted according to case/control status and FCGR3B-AGA haplotype status. (C) Percentages of HVTN 505 vaccine recipients having medium or high responses for either Env IgG or CD8 PFS in HVTN 505 cases and controls, plotted according to case/control status and FCGR3B-AGA haplotype status.

FCGR2A and FCGR2B SNPs modified the association of gp140-specific antibody-dependent cellular phagocytosis (ADCP) activity with HIV-1 acquisition risk. We recently found that gp140-specific antibody-dependent cellular phagocytosis (ADCP) activity and FcγRIIa binding were each inversely correlated with HIV-1 acquisition risk in HVTN 505, and that an FCGR2A SNP (FCGR2A-intron13-645-G/A [rs2165088]) significantly modified the association of ADCP with HIV-1 acquisition risk (Neidich et al., submitted). Here, we broadened the analysis of the seven FCGR2A SNPs examined in Neidich et al. (submitted) to evaluate whether any of the 33 FcγR SNPs significantly modified the associations of these two antibody Fc effector functions with HIV-1 acquisition risk. The modification of *FCGR2A* SNP (FCGR2A-intron13-645-G/A [rs2165088]) on the association of ADCP with HIV-1 acquisition risk remained significant after adjusting for 33*2 (33 SNPs versus 2 Fc effector functions) statistical tests (interaction *P* value of 0.003, interaction *q* value of 0.04) (Fig. 4A). In addition, two *FCGR2B* SNPs (FCGR2B-exon5-523-G/A [rs6665610] and FCGR2B-intron14-352-T/G [rs6666965]) significantly modified the correlation of ADCP activity with HIV-1 acquisition risk (interaction *P* values of 0.015 and 0.011 and interaction *q* values of 0.017 and 0.017) (Fig. 4A). The inverse correlation of ADCP with HIV-1 acquisition risk reported by Neidich et al. (submitted) was observed only in vaccine recipients with minor alleles at these SNP loci (OR of 0.16, 0.14, and 0.22 per one standard deviation [per 1-SD] increase in ADCP score in GA/AA of FCGR2A-intron13-645-G/A, GA/AA of FCGR2B-exon5-523-G/A, and TG/GG of FCGR2B-intron14-352-T/G, respectively) (Fig. 4A). Similarly, the inverse correlation of FcγRIIa binding with HIV-1 risk identified by Neidich et al. (submitted) was also observed only in vaccine recipients with minor alleles at the same SNP loci (OR of 0.30, 0.27, and 0.33 per 1-SD increase in FcγRIIa binding, respectively), but the interaction testing for the lack of SNP effect modification of the correlation was not



Genotype	OR	p-value	Int. p-value	Int. q-value
GG	1.14	0.73	0.003	0.04
GA/AA	0.16	0.0005		

Genotype	OR	p-value	Int. p-value	Int. q-value
GG	0.81	0.52	0.015	0.17
GA/AA	0.14	0.002		

Genotype	OR	p-value	Int. p-value	Int. q-value
TT	1.07	0.88	0.011	0.17
TG/GG	0.22	0.001		

Genotype	OR	p-value	Int. p-value	Int. q-value
GG	1.11	0.85	0.072	0.58
GA/AA	0.30	0.011		

Genotype	OR	p-value	Int. p-value	Int. q-value
GG	0.96	0.94	0.073	0.58
GA/AA	0.27	0.015		

Genotype	OR	p-value	Int. p-value	Int. q-value
TT	1.39	0.61	0.056	0.58
TG/GG	0.33	0.006		

FIG 4 FcγR SNPs (FCGR2A-intron13-645-G/A [rs2165088], FCGR2B-exon5-523-G/A [rs6665610], and FCGR2B-intron14-352-T/G [rs6666965]) modified the association of Fc effector functions (ConS gp140 ADCP score and FcγRIIa binding) with HIV-1 acquisition risk. (A and B) ConS gp140 ADCP score (A) and ConS gp140 FcγRIIa binding (B). On each panel, distributions of Fc effector functions were plotted according to case/control HIV infection outcome status and genotype groups.

significant (all *P* values were slightly greater than 0.05) (Fig. 4B). These results suggest that for individuals carrying the minor alleles at these identified *FCGR2A* and *FCGR2B* SNP loci, the vaccine-induced antibodies in individuals who later became HIV-1 infected were less capable of engaging effector immune cells, potentially involving less efficient antibody Fc binding for FcγRs like FcγRIIa.

Similar analysis for the additional immune response variables studied in reference 12 showed that none of the 33 FcγR SNPs passed the significance threshold after multiplicity corrections (interaction *q* values of >0.2). Also, these 33 FcγR SNPs did not significantly modify the reported Env-gp120 sieve effects in HVTN 505 (13) after multiplicity correction (interaction *q* values of >0.2) or vaccine-induced immune responses or antibody Fc effector functions (*q* values of >0.2).

The identified FcγR SNPs could regulate FcγR gene expression. Similar to what we found in reference 15, the identified *FCGR3B* variants were positively correlated with *FCGR2A/C* expression but negatively correlated with *FCRLA* expression (Fig. S1 and Table S5). However, for the three SNPs that modified the correlation of ADCP activity with the HR of HIV-1 acquisition, only one in *FCGR2B* (FCGR2B-exon5-523-G/A [rs6665610]) had a significant expression quantitative trait locus (eQTL), for which the

minor alleles were associated with decreased expression of *FCGR2A* as measured by transcriptome deep sequencing (RNA-seq) analysis (Table S5). These results indicate that both sets of identified FcγR SNPs could regulate FcγR gene expression, but their collective effect is unclear.

Since we did not genotype all genetic variants in the FcγR region, we searched in the FcγR region for other SNPs that were not genotyped in this study but were in high LD ($r^2 > 0.8$) with each of the identified FcγR SNPs using genotyping data publicly available from the 1000 Genomes Project (16). Interestingly, for all SNPs identified in this study that modify the HR of HIV-1 acquisition and/or the association of vaccine-induced immune responses or antibody Fc effector functions with HIV-1 acquisition risk, their linked SNPs were predicted to have FcγR gene regulatory potential (e.g., 5_prime_UTR_variant, upstream_gene_variant, and downstream_gene_variant) but were not predicted to alter FcγR protein sequences (Tables S6 and S7). Again, together these results imply that the identified FcγR SNPs and haplotypes have modulated the selective expression of FcγRs on immune cells.

DISCUSSION

Our previous finding that *FCGR2C* polymorphisms were associated with VE in RV144 raised the question of whether FcγR genetic variations are functionally relevant only for the RV144 vaccine regimen or more broadly in HIV-1 vaccine development. As reported in this study, several FcγR SNPs significantly modified the HR of HIV-1 acquisition in HVTN 505, an efficacy trial that differed from RV144 in a number of respects (canarypox prime/protein boost and general low-risk Thai population in RV144 versus DNA prime/rAd5 boost and high-risk U.S. population of men who have sex with men [MSM] in HVTN 505). Moreover, three of the *FCGR2C* SNPs (FCGR2C-exon06-441-C/T [rs138747765], FCGR2C-intron06-590-G/A [rs78603008], and FCGR2C-intron15-403-C/T [rs373013207]) significantly modified the vaccine's effect on HIV-1 acquisition in both RV144 and HVTN 505. This indicates that FcγR genetic variations have broad impact, given the major differences in the vaccine regimens and populations.

This study also suggests that the functional impact of a given FcγR polymorphism on the risk of HIV-1 acquisition is context specific, dependent on the specific vaccine regimen and possibly other factors, such as demographics, virus quasispecies, and genetic background. Compared to the previous RV144 study, the effect modification of the tag *FCGR2C* SNP (FCGR2C-intron06-126-C/T [rs114945036]) identified in RV144 on the HR in HVTN 505 did not pass the significance threshold, even though it had a trend similar to that of the other *FCGR2C* SNPs (see Table S1 in the supplemental material). This result was expected, since this tag SNP was on intron 6 and in high LD with the other two *FCGR2C* SNPs on intron 6 described above but with reduced correlation ($D' = 1$ and $r^2 = 0.6$) in HVTN 505 compared to the correlation in RV144. Furthermore, we identified additional FcγR SNPs in HVTN 505. *FCGR3B* SNPs significantly modified the vaccine effect on HIV-1 acquisition risk in HVTN 505 but not in RV144. These *FCGR3B* SNPs also modified the association of Env-specific IgG and $CD8^+$ T-cell PFS with HIV-1 acquisition risk in HVTN 505. In addition, *FCGR2A/2B* SNPs significantly modified the immune correlate of ADCP with HIV-1 acquisition risk in HVTN 505. These findings indicate that multiple FcγRs influence the vaccine's effect on HIV-1 acquisition in HVTN 505.

It is intriguing that in HVTN 505 HIV-1 acquisition, risk was increased in vaccine recipients who carried certain *FCGR2C* minor alleles compared with their placebo counterparts. Similarly, in the earlier Vax004 trial, FcγR genetic polymorphisms were associated with HIV risk (17). In that study, conducted in North America among MSM, vaccination with a bivalent recombinant gp120 was associated with a 3.5-fold increased risk in HIV acquisition among low-risk participants homologous for valine (V) at amino acid position 158 of FcγRIIIa, an isoform with greater affinity for monomeric IgG1 and IgG3 and certain IgG isotype immune complexes (4), compared to that of individuals homozygous for phenylalanine (F) or the VF isoform (17). In that report, FcγRIIa

genotypes did not influence HIV risk among vaccine recipients, and FcγRIIa or FcγRIIIa genotypes did not influence HIV risk among placebo recipients.

One mechanism that could explain the increased risk for infection in these two vaccine studies is that HIV vaccination potentially induces nonneutralizing or poorly neutralizing antibodies. Such antibodies could theoretically mediate antibody-dependent enhancement (ADE), a phenomenon where antibodies bind a virion but fail to neutralize it and then the resultant antibody-virus complexes attach to Fc receptor-bearing target cells (e.g., monocytes and macrophages), thereby increasing infection of such cells. Such a phenomenon has been observed *in vitro* for many viruses, perhaps most notably dengue virus (18, 19) and also Zika virus (20, 21), coronavirus (22), Ebola virus (23), and coxsackievirus B (24). In the case of dengue virus, an FcgRIIa polymorphism was shown to be significantly associated with dengue disease (versus subclinical infection); moreover, compelling evidence was recently published to support that ADE enhances disease severity in humans (25). Importantly, ADE has also been described for HIV *in vitro* (26, 27). Although it remains unknown whether ADE could increase HIV infection risk in humans (28), it has been found that patients with HIV who have antibodies capable of ADE have more rapid disease progression (29). For ADE to mediate enhanced infection risk after HIV vaccination, the vaccine regimen would need to induce nonneutralizing or poorly neutralizing antibodies; it has been shown that the HVTN 505 regimen did induce such antibodies (namely, gp41 reactive and nonneutralizing) (30). Further work would need to be done to test the hypothesis that an effect of FcγR polymorphisms of ADE of HIV infection could explain the results presented here.

Another potential mechanism is that FcγR polymorphisms may increase risk of HIV infection through modulation of innate immunity. HIV founder viruses are intrinsically resistant to type I interferons (31, 32). Plasmacytoid dendritic cells (pDC) produce high levels of interferon following HIV infection, which can restrict virus replication (33, 34). Although expression of FcγRs on pDC is low (35), opsonized viral particles can suppress type I and III interferon production by pDC, as well as other myeloid cells, through FcγR-mediated signaling (36). A decrease in the level of interferon production in pDC or other myeloid cells following signaling through FcγR engagement could lower the threshold of interferon resistance required of founder viruses to establish infection.

These hypothesized mechanisms for the increased risk of HIV infection in HVTN 505 among participants with selected FcγRIIc alleles fail to explain the protective effect observed in RV144 vaccine recipients carrying the same minor alleles. It is unclear if these same variants functioned differently in each trial or were linked to other factors that influenced risk. Various factors, such as the diversity and complexity of expression of FcγRs on immune effector cells, the consequences of their binding influenced by IgG isotypes in vaccine recipients, and antibody levels and their binding characteristics, will change during the course of vaccination. Therefore, the balance between risk and protection is not likely to be static over time. The role of FcγRs in modulating natural or vaccine-induced immunity has been evaluated in very few models of viral infections and for even fewer infections in humans. These findings, together with other published studies (17, 37, 38), strongly support the idea that host genetics should be considered in the analysis of future HIV vaccine trials to address these questions.

Another potential contributor to host genetics in this regard is the variability of the immunoglobulin constant heavy G chain (IGHG) sequences (39). While it is recognized that FcγR variation can affect binding affinity to the Fc region of IgG with functional consequence (4, 6), the level of variation in the constant regions of the individual IgG genes (IGHG3, IGHG1, IGHG2, and IGHA2, comprising the IGH locus on chromosome 14) suggests IGHG variability also affects FcγR binding (4, 40). The population-specific IGHG variation that has been observed (39) in turn is suggestive that IGHG variation in conjunction with FcγR variation plays a role in vaccine efficacy or disease susceptibility that itself is population specific. Thus, while FcγR variation may be common between populations, IGHG variation may differ and, consequently, yield distinct functional outcomes.

Because none of the FcγR SNPs that we found to modify the HR of HIV-1 acquisition or correlates of HIV-1 acquisition in HVTN 505 introduced any changes in the corresponding FcγR protein sequence, the observed effects of the FcγR polymorphisms are likely related to modulation of FcγR expression levels, perhaps through transcriptional regulation, as suggested by the associations of the *FCGR3B* SNPs and increased *FCGR2A/C* expression. However, since we did not identify any SNP associated with differential expression of its corresponding FcγR gene, it is also plausible that these SNPs are linked to some unknown functional polymorphisms in the FcγR region. Interestingly, a nonexpressed allelic form of *FCGR2C* (*FCGR2C*.nc-ORF) was previously shown to be associated with a decreased risk of alloimmunization in patients with sickle cell disease, perhaps related to lower expression of FcγRIIc on B cells in individuals with *FCGR2C*.nc-ORF (41). Analyses such as cell type-specific FcγR expression on autologous immune cells will be needed to facilitate the identification of causal FcγR variants (i.e., variants that impact HIV-1 acquisition risk) and their functional mechanisms after HIV-1 vaccination.

The genomic and functional complexity of the FcyRs present a major challenge for uncovering the underlying mechanisms for the effects of the FcγR variants described. While different FcγRs have distinct functions and mechanisms of regulation, they share highly similar sequences. Variations are present in both FcγR sequences and copy numbers (42, 43). While FcγR genetic variations are clearly linked to host defense against infectious diseases and other important immune functions (7, 44, 45), current approaches measure only a small portion of the existing FcγR variations. This study and our previous RV144 study (8) were by far the most comprehensive in this regard, measuring ~10 kb of the region, including functional exons and flanking sequences from the five FcγR genes. However, the complete FcγR region extends over 200 kb, leaving considerable room for analysis of additional variations, including those that are bona fide causal variations. While there are currently over 20,000 SNPs documented in the FcγR region (Ensembl Variation database, https://www.ensembl.org/info/genome/variation/index.html; accessed 20 September 2017), the lack of phasing of these data subverts their direct utility for causal variant identification. To fully define the extent of human FcγR genetic variation, it is necessary to obtain complete haplotype-resolved FcγR genomic sequences across vaccine trials using approaches such as those outlined in references 46 and 47. Even with the complete FcγR genomic sequences, significant effort is still needed to locate causal variants of a particular phenotype, such as control of FcγR expression levels. A potential solution is to use parallel transcriptome analysis to systematically identify variants affecting FcγR gene expression, as illustrated in references 15 and 48, and to assess the collective effects of multiple FcγR variants across individual immune cell types.

Also, the identified FcγR polymorphisms might have influenced the development of HIV-1-specific IgG antibodies after vaccination. For example, the difference in Env-specific IgG responses between cases and controls in vaccine recipients without the FCGR3B-AGA haplotype did not exist in vaccine recipients with the FCGR3B-AGA haplotype. This finding suggests that FcγR polymorphisms influence the development of the binding antibody response that was associated with decreased risk of infection. Also, the differences in gp140-specific ADCP activity and FcγRIIa binding between cases and controls were only present in one genotype group of each of the identified *FCGR2A* and *FCGR2B* SNPs. This indicates that FcγR polymorphisms also contribute to the variability of the Fc domain in vaccine-induced IgG antibodies. For example, Fc glycosylation modulates the interaction between antibody and FcγR. Fc glycosylation of HIV-specific antibodies has been shown to vary naturally in HIV-1-infected individuals (49, 50) and can be regulated by HIV-1 vaccination (51). Further, the FcyR SNPs identified here were associated with changes in gene expression levels of *FCGR2A/C* and *FCRLA* in B cells. While the function of *FCGR2A/C* is relatively well studied, very little is known about *FCRLA* (52). *FCRLA* is located in the same region of chromosome 1 as other FcγRs but does not have a transmembrane region. Instead, *FCRLA* resides in the endoplasmic reticulum and can associate with multiple Ig isotypes, including IgM, IgG, and IgA (53). Considering the abundant expression of *FCRLA* in human germinal center

B cells, *FCRLA* is likely involved in B cell and antibody development (52). Therefore, modulation of *FCRLA* expression could also be a mechanism by which FcγR genetic variation influences the development of HIV-1-specific antibodies during vaccination.

The activities of FcγRs can regulate B cell selection and IgG affinity maturation, such as the demonstrated effects of FcγRIIb expression on B cell signaling (1). Moreover, susceptibility to autoimmune diseases, such as systemic lupus erythematosus, has been linked to differential expression of functional FcγRIIb, driven by genetic variations in the *FCGR2B* promoters (54, 55), FcγR copy number variation (56), or amino acid changes in FcγRIIb (57). A recent study of IgG antibodies elicited by administration of the trivalent influenza virus vaccine in healthy subjects showed that the abundance of sialylated Fc glycans (sFc) predicted the quality of vaccine response (58). Further, sFc within immune complexes has been shown to trigger the upregulation of FcγRIIb on B cells (58). Increased levels of FcγRIIb on B cells can elevate the threshold for B-cell antigen receptor affinity, which is required for B cell survival and production of higher-affinity protective IgG antibodies (58). In addition, one recent study analyzed the temporal changes in Fc effector functionality of HIV-specific IgG antibodies in HIV-1-infected individuals (59). As early as 6 months postinfection, individuals who later developed broadly neutralizing antibodies (bnAbs) already had significantly higher levels of antibody-dependent complement deposition (ADCD) and cellular trogocytosis (ADCT), which correlated with antibody binding to C1q and FcγRIIa, respectively (59). Antibodies from these bnAb-developing individuals also showed more IgG subclass diversity, which was correlated with Fc polyfunctionality (59). Interestingly, germinal center activity as represented by CXCL13 levels and expression of activation-induced cytidine deaminase has been found to be associated with neutralization breadth, Fc polyfunctionality, and IgG subclass diversity (59). Together, these findings suggest that it would be informative to investigate how FcγRs are involved in regulating germinal center activities that link IgG Fab and Fc domains and to evaluate if this regulation could be exploited to aid HIV-1 vaccine development.

Our statistical analysis included a large number of hypothesis tests and was not prespecified in the HVTN 505 protocol or statistical analysis plan (SAP). Although a sequel analysis plan was specified prior to data analysis and methods of multiple testing adjustment were employed, it cannot be ruled out that some significant results are false positives. Therefore, this study should be viewed as hypothesis-generating and exploratory, providing guidance for future studies in which these hypotheses are tested and can advance knowledge of the influence of FcγR genetics on vaccine effects. Another utility of this work is the preparation of laboratory and statistical methodology for application to the ongoing HIV-1 vaccine efficacy trials. For instance, it will be particularly relevant to consider FcγR genetics when assessing correlates of vaccine efficacy in the HVTN 702 trial, which is evaluating a pox-protein vaccine regimen similar to that tested in RV144, and in the HVTN 705/VAC89220HPX2008 trial of an Ad26/gp140 mosaic vaccine regimen. Each of these regimens has been shown to induce T-cell and nonneutralizing antibody responses.

As discussed in references 9, 60, and 61 and elsewhere, the role of antibody and Fc receptor interactions in HIV prevention and therapy is becoming better appreciated. Our results further demonstrate that detailed analyses of FcγR genetics and the impact of FcγR genetic variation on HIV-1 acquisition risk and/or vaccine efficacy against HIV-1 acquisition will be important for understanding correlates of protection in ongoing and future phase 2b/3 trials of preventative HIV-1 vaccines.

MATERIALS AND METHODS

FcγR SNP genotyping. A total of 193 peripheral blood mononuclear cell (PBMC) samples (from 145 controls [defined as HIV negative at month 24] and 48 cases (defined as diagnosis of HIV-1 infection sometime between week 28 and month 24) in HVTN 505 were genotyped for five FcγR genes (*FCGR2A*, *FCGR2B*, *FCGR2C*, *FCGR3A*, and *FCGR3B*) using the same platform as that used in reference 8. Of the 145 control samples, 20 were from placebo recipients and 125 were from vaccine recipients. Among the 48 case samples, 21 were from placebo recipients and 27 were from vaccine recipients. A total of 162 loci across the 5 FcγRs were sequenced. After excluding indels, double mutations, and SNPs that failed to pass the minor allele frequency (MAF) threshold (≥5%) and the Hardy-Weinberg equilibrium (HWE) test

($P \geq 0.00001$) based on the control samples, 33 SNPs remained in the analyses. Haploview (62) was used to generate FcγR haplotypes and linkage disequilibrium (LD) plots.

Immune response correlates of risk in HVTN 505. In the primary T-cell correlates analysis, Janes et al. measured HIV-specific $CD4^+$ and $CD8^+$ T-cell responses by intracellular cytokine staining of PBMCs isolated from vaccine recipients at 2 weeks after the last vaccination (month 6.5) from the case-control cohort, comprised of 25 primary endpoint vaccine cases and 125 randomly sampled frequency-matched vaccine controls (HIV-negative at month 24), where controls were frequency matched to cases with respect to treatment group, body mass index, and race/ethnicity (11). Janes et al. also determined the COMPASS Env-specific $CD4^+$ and $CD8^+$ T-cell PFS using the method described in reference 63.

In the primary humoral correlates analysis, Fong et al. measured IgG and IgA binding antibody multiplex assay (BAMA) responses (64) to HIV-1 envelope antigens using serum samples collected 1 month after the last vaccination (month 7) from the same case-control cohort (12). Low-, medium-, and high-immune-response tertiles were defined in reference 12. Fong et al. also derived and analyzed the primary tier variables (12).

In the primary Fc effector function analysis, Neidich and colleagues (submitted) measured antibody-mediated FcγRIIa tetramer binding and gp140-specific ADCP activity and found that ADCP by monocytes and Env IgG binding to FcγRIIa significantly correlated with decreased HIV-1 risk (OR of 0.47 and $P = 0.001$ as well as OR of 0.48 and $P < 0.001$, respectively).

Statistical association analysis. The case-only method (65) was employed to assess whether and how each FcγR SNP modified the vaccine/placebo hazard ratio of HIV-1 acquisition risk (HR) between week 28 and month 24. The case-only method was also used to assess whether and how each FcγR SNP modified the hazard ratio of Env sequence-specific HIV-1 acquisition risk studied in the sieve analysis (13).

To evaluate whether and how each FcγR SNP modified the previously identified associations of $CD8^+$ T-cell PFS, IgG, and two Fc effector functions (ConS gp140 ADCP and FcγR2a binding) with HIV-1 acquisition risk in vaccine recipients (11, 12), we applied the same model and covariate adjustment as that used in the primary humoral immune correlates of risk analysis (12). As described in reference 12, this model used logistic regression methods (66) that accounted for the case-control biomarker sampling design (implemented in the R package osDesign, available at the Comprehensive Archive R Network). Pseudo-likelihood-based inference methods were chosen and sandwich variance estimates were used to estimate confidence intervals (CI) and evaluate statistical significance. Prior to regression analysis, all immune response variables were means centered and standard deviations scaled (based on distributions among vaccine recipients), such that a unit change in the variable represents a change of one standard deviation. All regression models included the following baseline covariates to adjust for potential confounding: participant age, race (white versus black versus Hispanic/other), body mass index, and the behavioral risk score defined and applied in the primary publication of HVTN 505 (10). For the primary tier humoral correlate analysis, the $CD4^+$ and $CD8^+$ T-cell PFS were adjusted in the models in addition to the baseline covariates.

Given the small sample size of the study, for each SNP, participants were divided into two genotype groups: homozygous genotype of the major allele and the two genotypes containing at least one minor allele (e.g., for FCGR2C-intron6-126-C/T, the two genotype groups were CC and CT/TT).

Bar charts and boxplots were generated to display the distributions of the immune response rates and magnitudes by case/control status and by genotype group. Response rates for controls were estimated with inverse probability weighting to account for the case-control cohort sampling. Logistic regression and the Wilcoxon rank-sum test with inverse probability weighting (67) were used to compare the immune response rates and magnitudes between genotype groups among control vaccine recipients. For immune response variables (e.g., IgA) with some positive responses but many negative responses (response rate of $\leq$20%), the choplump test (68) was used instead of the Wilcoxon rank-sum test.

For each of the four types of null hypotheses, false discovery rates (q values) were calculated to adjust for the number of statistical tests performed (69), which are detailed in Results. False discovery rates (q values) were calculated using a resampling method that retains the correlations between the SNPs and immune response variables (70, 71). Any P value less than 0.05 with a q value less than 0.2 was considered to be significant and hypothesis generating.

Association of FcγR polymorphisms with FcγR gene expression in human B cells. To examine the associations between FcγR SNPs and FcγR gene expression in human B cells, we queried a large-scale B-cell RNA sequencing database of 462 samples from individuals curated by the 1000 Genomes Project (48), as done in reference 15. RNA-seq read mapping, genotypes, expression quantification, and expression quantitative trait locus (eQTL) mapping results reported in reference 48 were downloaded from EBI ArrayExpress (accession number E-GEUV-1). As detailed in reference 48, mRNA expression was quantified at different levels. For exon quantification, overlapping exons of a gene were first merged into meta-exons. Transcripts and splice junctions were quantified by the Flux Capacitor approach (72). Gene expression was quantified as the sum of all transcript reads per kilobase per million (RPKMs) for each gene. Before eQTL analysis, expression quantifications were normalized by PEER correction (73) and transformed into standard normal distributions. eQTLs were mapped using a linear model in Matrix eQTL (74), and permutations were used for FDR estimation.

Other SNPs in LD with the identified FcγR SNPs. To investigate the potential functions of identified FcγR SNPs, we also searched for other SNPs in the FcγR region that were not genotyped in this study but were in LD with the FcγR SNPs found to modify the HR of HIV-1 acquisition and/or the association of vaccine-induced immune responses or antibody Fc effector functions with HIV-1 acquisition risk. To match the B-cell RNA-seq data used in the eQTL analysis (48), we used the genotyping data for the same 373 individuals of the European population from the 1000 Genomes project (16). We calculated the

pairwise LD between SNPs in the FcγR region using PLINK 1.9 (75). For each of the FcγR SNPs identified above, we defined their linked SNPs as the SNPs that were in high LD ($r^2 > 0.8$) with them. The predicted consequences of all linked SNPs were obtained from the Ensembl Variation database (https://www.ensembl.org/info/genome/variation/index.html; accessed 21 June 2018).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JVI.02041-18.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.
SUPPLEMENTAL FILE 2, XLSX file, 0.01 MB.
SUPPLEMENTAL FILE 3, XLSX file, 0.02 MB.
SUPPLEMENTAL FILE 4, XLSX file, 0.01 MB.

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