The FCGR2C allele that modulated the risk of HIV-1 infection in the Thai RV144 vaccine trial is implicated in HIV-1 disease progression

Ria Lassaunière1,2,3 • Maria Paximadis1,2 • Osman Ebrahim2,4 • Richard E. Chaisson5 • Neil A. Martinson6,7 • Caroline T. Tiemessen1,2

Received: 27 July 2018 / Revised: 7 November 2018 / Accepted: 16 November 2018 / Published online: 19 December 2018 © The Author(s) 2018. This article is published with open access

Abstract

In the HIV-1 Thai RV144 vaccine trial—the only trial to demonstrate any vaccine efficacy to date—a three-variant haplotype within the Fc gamma receptor 2C gene (FCGR2C) modulated the risk of HIV-1 acquisition. A similar vaccine regimen is currently being evaluated in South Africa in the HVTN702 trial, where the predominant population is polymorphic for only a single variant in the haplotype, c.134-96C>T. To investigate the significance of c.134-96C>T in HIV-specific immunity in South Africans, this study assessed its role in HIV-1 disease progression. In a cohort of HIV-1-infected South African controllers (n = 71) and progressors (n = 73), the c.134-96C>T minor allele significantly associated with increased odds of HIV-1 disease progression (odds ratio 3.80, 95% confidence interval 1.90–7.62; \( P = 2 \times 10^{-4}, P_{\text{Bonf}} = 2.4 \times 10^{-3} \)). It is unlikely that the underlying mechanism involves wild-type FcγRIIC function, since only a single study participant was predicted to express wild-type FcγRIIC as determined by the FCGR2C c.798+1A>G splice-site variant. Conversely, in silico analysis revealed a potential role for c.134-96C>T in modulating mRNA transcription. In conclusion, these data provide additional evidence towards a role for FCGR2C c.134-96C>T in the context of HIV-1 and underscore the need to investigate its significance in the HVTN702 efficacy trial in South Africa.

Introduction

Immune effector functions recruited through the Fc portion of immunoglobulin G (IgG) are increasingly recognised as an important component of HIV-1 protective immunity [1].

In murine and non-human primate models, Fc-mediated mechanisms have been demonstrated to augment the in vivo activity of broadly neutralizing antibodies [2, 3], whereas robust antibody-dependent cellular cytotoxicity (ADCC) responses in humans have been associated with reduced risk of HIV-1 infection following immunization [4], reduced perinatal HIV-1 transmission risk [5] and slower disease progression [6, 7].

IgG Fc-mediated effector functions are predominantly recruited through the engagement of Fc gamma receptors (FcγRs)—a family of polymorphic glycoproteins encoded by five genes, FCGR2A/B/C and FCGR3A/B. In the phase 3 Thai RV144 vaccine trial, which evaluated a prime-boost vaccination regimen of ALVAC-HIV (vCP1521) and HIV-1 gp120 AIDSVAX B/E, a novel FCGR2C haplotype significantly modified vaccine efficacy [8, 9]. This haplotype—hereafter referred to as the Thai FCGR2C haplotype—comprised three single-nucleotide variants that were in complete linkage disequilibrium, including c.353C>T (p. T118I, rs138747765) in FCGR2C exon 3, c.134-96C>T (rs114945036) upstream of exon 3 and c.391+111G>A (rs78603008) downstream of exon 3. Thai vaccinees who possessed at least one minor allele of the c.134-96C>T tag
variant, had an estimated vaccine efficacy of 91% against CRF01_AE 169K HIV-1 and 64% against any HIV-1 strain, compared to 15 and 11% in vaccinees homozygous for the wild-type allele, respectively [8].

Building on the successes of the RV144 trial, a similar prime-boost vaccine regimen—ALVAC-HIV (vCP2438) + bivalent subtype C gp120/MF59—is currently being evaluated in the HVTN702 phase 2b efficacy trial in South Africa. The Thai and South African populations are, however, distinctly different at the FCGR2C gene locus. The three variants in the Thai FCGR2C haplotype are not in complete linkage disequilibrium in the predominant black South African population. Only the c.134-96C>T variant has an appreciable minor allele frequency of 24.9%, whereas the other two variants—c.391+111G>A and p. T118I—rarely occur [10]. It is unclear if the population differences will differentially affect HIV-1 vaccine protection in Thais and South Africans, since the causal variant associated with the Thai FCGR2C haplotype is unknown and the significance of the c.134-96C>T variant in Africans has not been studied. Here we investigated the c.134-96C>T variant and other functional FCGR2C variants in the context of HIV-1 immunity in black South Africans by determining their association with HIV-1 disease progression.

Results

Study design and subjects

A case–control candidate gene association study was undertaken to assess the significance of FCGR2C variants in HIV-1 disease progression. Here we employed selective genotyping, whereby HIV-1-infected individuals at the extreme ends of the HIV-1 disease progression phenotype, that is HIV-1 controllers and chronic progressors, were selected and their FCGR2C genotypes were compared. This approach, also called 'extreme phenotype sampling', has increased statistical power over random sampling of the same sample size and is effective for detecting genetic effects in complex disease traits [11, 12].

HIV-1 progressors (n = 73) were selected from the Soweto Lung Cohort (n = 756) recruited at Chris Hani Baragwanath hospital using the following criteria: (i) CD4 T-cell decline from >500 cells/μl to <350 cells/μl; (ii) initiated antiretroviral therapy and (iii) >10,000 HIV-1 RNA copies/ml at the time of antiretroviral therapy initiation. The HIV-1 controller phenotypes were defined as follows: elite controllers (n = 23) had at least one HIV RNA determination of <50 copies/ml and CD4 T-cell counts >500 cells/μl; viraemic controllers (n = 37) had viral load set points between 50 and 2000 copies/ml and CD4 T-cell counts >500 cells/μl and high viral load long-term non-progressors (n = 11) had multiple viral load measurements >10,000 RNA copies/ml and CD4 T-cell counts >500 cells/μl without apparent CD4 T-cell decline for a period of ≥7 years.

While the three HIV-1 controller groups differ in their ability to control viraemia, all maintained comparatively high CD4 T-cell counts that were significantly higher compared to the chronic progressor group (P<0.0001 for all comparisons, Table 1). Overall, the study participants were predominantly female (84.7%) with a median age of 38 years (interquartile range [IQR] 33–42.5 years). Gender and age did not differ significantly between the HIV-1 controllers (total and individual controller groups) and HIV-1 progressors (P>0.05 for all comparisons).

FCGR2C copy number does not associate with HIV-1 disease progression

The low-affinity FCGR locus on chromosome 1q23 is subject to copy number variation, with genes duplicated or deleted within distinct genomic copy number variable regions (CNRs). In this study cohort, the gain or loss of an FCGR2C gene copy was observed only within the previously designated CNR1 or CNR2 (Fig. 1), where CNR1 encompasses a complete copy of FCGR2C and CNR2 an incomplete copy (lacks the last exon). The two

| Table 1 Clinical and demographic characteristics of HIV-1 disease phenotype groups |
|-----------------------------------|----------------|----------------|-------------------|------------------|
|                                    | Elite controllers | Viraemic controllers | High viral load long-term non-progressors | Chronic progressors |
| N                                  | 23              | 37              | 11                | 73               |
| Age (years) at enrolment            | 40.7 (9.7)      | 35.4 (8.7)      | 40.3 (6.9)        | 38.4 (7.3)       |
| Gender % Females                    | 78.3            | 91.9            | 81.8              | 83.6             |
| CD4 T-cell count (cells/μl)*        | 784 (371)       | 735 (233)       | 682 (109)         | 173 (63)         |
| Viral load (copies/ml)*             | < 20            | 598 (237–1270)  | 22410 (11,370–81,325) | 39,322 (19,822–105,195) |
| Time since diagnosis (years)        | 10 (4–12)       | 3 (2–11)        | 8 (8–11)          | 6 (1–7)          |

Mean with standard deviation is reported for 'Age' and 'CD4 T cell count'; median with interquartile range is reported for 'Viral load' and 'Time since diagnosis'. For HIV-1 controllers the CD4 T cell count and viral load at study enrolment was used, whereas for HIV-1 chronic progressors the CD4 T cell count and viral load prior to initiation of antiretroviral therapy was used.
CNRs further differ with regard to the syntenic genes that are duplicated/deleted as well as the associated phenotypic and/or functional consequences. The variability of CNR1 and CNR2 was therefore assessed independently.

Similar to healthy HIV-uninfected South Africans and other population groups, copy number variability of CNR1 was observed more frequently than CNR2 in the 144 HIV-1 infected study participants (29.2 vs. 4.2%). The copy number distribution of CNR1 was comparable between HIV-1 controllers (total and phenotype groups) and HIV-1 progressors (P > 0.05 for all comparisons) (Fig. 1), whereas an association between CNR2 and HIV-1 disease progression was not assessed due to the low frequency of CNR2 variability (<5%).

**FCGR2C c.134-96C>T (rs114945036) associates with increased odds of HIV-1 disease progression**

Study participants were genotyped for the following functionally and/or clinically significant FCGR2C nucleotide variants: (i) c.–386G>C (rs149754834) that modulates gene...
expression levels; (ii) c.134-96C>T (rs114945036) that associated with risk of HIV-1 acquisition in the RV144 HIV-1 vaccine trial; (iii) c.169T>C (p.X57Q) (rs759550223) that alters the open-reading frame and predicts FcγRIIc expression together with (iv) c.798+1A>G (rs76277413) that modifies pre-mRNA splicing. The latter had a minor allele frequency of <0.1% and was thus not included in further analysis. Variable FCGR2C copy number individuals were included in subsequent analysis as follows: (i) individuals with more than two FCGR2C copies were considered homozygous when all gene copies carried the same allele and heterozygous when both alleles were present; (ii) individuals bearing a single FCGR2C copy were considered homozygous. Associations of FCGR2C variants were unadjusted for FCGR2C copy number since the latter did not independently associate with HIV-1 disease progression.

The genotype distribution of c.134-96C>T was significantly different between HIV-1 controllers and progressors \( (P = 4.2 \times 10^{-4}, \text{Bonf} = 0.005) \) (Fig. 1). In particular, the c.134-96T allele was overrepresented in HIV-1 progressors compared to HIV-1 controllers (37.7% vs. 23.5%) and significantly associated with increased odds of HIV-1 disease progression in a dominant model (odds ratio [OR] 3.80, 95% confidence interval [CI] 1.90–7.62; \( P = 2.0 \times 10^{-4}, \text{Bonf} = 2.4 \times 10^{-3} \)). This association was the strongest for the elite controller group (OR 7.02, 95% CI 2.43–20.25; \( P = 1.8 \times 10^{-4}, \text{Bonf} = 2.2 \times 10^{-3} \)) compared to the viremic controller group (OR 2.61, 95% CI 1.15–5.93; \( P = 0.023, \text{Bonf} > 0.05 \)) or high viral load LTNP group (OR 4.33, 95% CI 1.14–16.37; \( P = 0.037, \text{Bonf} > 0.05 \)) (Fig. 1).

A weaker association was also observed for the c.169T>C (p.X57Q) variant (\( P = 0.019, \text{Bonf} > 0.05 \); Fig. 1). The c.169C allele, which maintains the open-reading frame and is required for expression of functional FcγRIIc, was overrepresented in HIV-1 progressors compared to HIV-1 controllers (19.8% vs. 10.1%) and associated with increased odds of HIV-1 disease progression in a dominant model \( (\text{OR} = 2.46, \text{95% CI} 1.18–5.14; P = 0.017, \text{Bonf} > 0.05) \). The association was not significant when HIV-1 controller phenotypes were assessed independently \( (P > 0.05 \text{ for all comparisons}) \). Notably, whilst 44/144 (30.6%) study participants carried a c.169C allele, only one expressed membrane-bound FcγRIIc as predicted by the c.798+1A>G splice-site variant. Thus, the observed association between the c.169C allele and HIV-1 disease progression is likely unrelated to expression of membrane-bound FcγRIIc.

**Strong linkage disequilibrium between FCGR2C c.134-96C>T and c.169T>C**

To determine if the associations observed for c.134-96C>T and c.169T>C were linked due to co-inheritance of minor alleles, we next assessed the linkage disequilibrium between the different FCGR2C variants (Fig. 2). Indeed, strong linkage disequilibrium existed between c.134-96C>T and c.169 T>C, both when unadjusted for copy number variability \( (D’ = 1 \text{ and } r^2 = 0.382, P < 0.001) \) and when individuals with only two FCGR2C copies were considered \( (D’ = 1 \text{ and } r^2 = 0.227, P < 0.001) \). In particular, the less frequent c.169C (p.57Q) allele always occurred in the presence of the more frequent c.134-96T allele. Following adjustment for c.169T>C in a multivariate logistic regression model, the association between c.134-96C>T and HIV-1 disease progression remained significant \( (\text{OR} = 3.62, \text{95% CI} 1.54–8.54; P = 0.003, \text{Bonf} = 0.036) \).

**FCGR2C c.134-96C>T is in strong linkage disequilibrium with other FCGR2C variants in native Africans**

The other constituents in the Thai FCGR2C haplotype, p. T118I (rs138747765) and c.391+111G>A (rs78603008), have minor allele frequencies of <1% in native African populations \[10\]. Where detected in Africans, the minor alleles occur in complete linkage disequilibrium. Therefore,
Fig. 3 Global patterns of linkage disequilibrium between FCGR2C variants identified to be in strong-to-complete linkage disequilibrium with c.134-96C>T in this study (c.113-1058 T>C and c.113-684C>T) and in Thai vaccinees (p.T118I and c.391+111G>A). Population data were obtained from the 1000 Genomes Project. Values indicate $r^2 \times 100$ and $D' \times 100$; n/a: not applicable, minor allele frequency too low.

| EAST ASIAN | $r^2$ | $D'$ |
|------------|-------|------|
| Kinh in Ho Chi Minh City, Vietnam | 100 100 100 100 | 100 100 100 100 |
| Chinese Dai in Xishuangbanna, China | 100 100 100 100 | 100 100 100 100 |
| Southern Han Chinese, China | 100 100 100 100 | 100 100 100 100 |
| Han Chinese in Beijing, China | 97 97 100 100 | 100 100 100 100 |
| Japanese in Tokyo, Japan | 97 97 100 100 | 100 100 100 100 |

| AFRICAN | $r^2$ | $D'$ |
|---------|-------|------|
| Esan in Nigeria | 100 100 n/a n/a | 100 100 n/a n/a |
| Yoruba in Ibadan, Nigeria | 96 96 n/a n/a | 100 100 n/a n/a |
| Luhya in Webuye, Kenya | 95 100 n/a n/a | 100 100 n/a n/a |
| Mende in Sierra Leone | 92 96 n/a n/a | 100 100 n/a n/a |
| Gambian in Western Division, The | 90 90 n/a n/a | 100 100 n/a n/a |
| African Caribbean in Barbados | 89 89 20 20 | 100 100 99 99 |
| African Ancestry in Southwest US | 72 72 20 20 | 99 99 99 99 |

| EUROPEAN | $r^2$ | $D'$ |
|----------|-------|------|
| Utah residents with European ancestry | 87 87 97 97 | 97 97 100 100 |
| British in England and Scotland | 87 87 100 100 | 99 99 100 100 |
| Toscani in Italy | 86 86 100 100 | 97 97 100 100 |
| Finnish in Finland | 80 82 98 100 | 97 100 100 100 |
| Iberian populations in Spain | 67 67 93 93 | 100 100 98 98 |

| AMERICAN | $r^2$ | $D'$ |
|----------|-------|------|
| Puerto Rican in Puerto Rico | 88 88 95 95 | 100 100 100 100 |
| Mexican Ancestry in Los Angeles, CA | 87 87 97 100 | 96 96 100 100 |
| Peruvian in Lima, Peru | 86 86 100 100 | 97 97 100 100 |
| Colombian in Medellin, Colombia | 75 75 98 97 | 99 99 100 100 |

| SOUTH ASIAN | $r^2$ | $D'$ |
|-------------|-------|------|
| Sri Lankan Tamil in the UK | 88 88 94 97 | 100 100 97 100 |
| Indian Telugu in the UK | 87 87 100 100 | 99 99 100 100 |
| Punjabi in Lahore, Pakistan | 84 84 98 98 | 99 99 100 100 |
| Bengali in Bangladesh | 80 83 100 100 | 99 99 100 100 |
| Gujarati Indian in Houston, TX | 69 71 94 94 | 99 99 97 97 |

p.T118I was used as a tag variant for c.391+111G>A in the present study. A comparably low prevalence of the p.T118I minor allele was observed in the HIV-1-infected cohort (0.7%), which suggests that the observed association of the c.134-96C>T variant with HIV-1 disease progression in South Africans was independent of the other Thai FCGR2C haplotype variants.

Full FCGR2C sequences of the South African study cohort were not available to enable identification of additional variants in linkage disequilibrium with c.134-96C>T. Thus, FCGR2C genotypic data from African populations in the 1000 Genomes Project were assessed. In native Africans and other population groups, two variants within FCGR2C intron 1—c.113-1058T>C (rs2169052/rs115953596) and c.113-684C>T (rs111828362)—were in strong-to-complete linkage disequilibrium with c.134-96C>T ($D' \geq 0.960$ and $r^2 \geq 0.900$; and $D' \geq 0.960$ and $r^2 \geq 0.670$, respectively) (Fig. 3). Notably, complete linkage disequilibrium ($D' = 1$ and $r^2 = 1$) was observed between c.134-96C>T, c.113-1058T>C, c.113-684C>T, p.T118I and c.391+111G>A in two mainland Southeast Asia populations (Kinh in Vietnam and Chinese Dai in China) that share common ancestry with Thai populations [13]. These additional linkage patterns may be of significance for elucidating the mechanisms underlying the association of c.134-96C>T with vaccine efficacy in the Thai RV144 vaccine trial and HIV-1 disease progression; however, it requires confirmation with FCGR2C gene-specific approaches that account for gene copy number variability.
In silico analysis of c.134-96C>T and linked variants

To explore the potential mechanism(s) underlying the associations of c.134-96C>T with HIV-1 acquisition and disease progression, we studied in silico the potential impact of c.134-96C>T and linked variants on transcriptional regulation and splicing (summarized in Fig. 4).

Chromatin profiling locates the three variants in the Thai FCGR2C haplotype—c.134-96C>T, p.T118I, c.391+111G>A—in a weak enhancer site, where the variants flanking exon 3 were predicted to disrupt binding of transcription factors CTCF and NRSF (presence/absence of coloured domes above the sequences). The p.T118I variant was predicted to disrupt an exonic splice enhancer (ESE) and create an exonic cryptic donor site, whereas

| Variant | Location | Minor allele | Major allele |
|---------|----------|--------------|--------------|
| c.113-1058T>C | intron 1 | CTCF | GATA |
| c.113-684C>T | intron 1 | Myc | Gata |
| c.134-96C>T | intron 2 | CTCF | GATA |
| p.T118I | exon 3 | NRSF | CTCF |
| c.391+111G>A | intron 3 | NRSF | CTCF |

**LD**

| Thai vaccines | Potential complete LD* | Established complete LD |
|---------------|------------------------|------------------------|
| South Africans | Potential strong/complete LD* | Rarely polymorphic (<1%) |

The intron 1 variants c.113-1058T>C and c.113-684C>T were predicted to create an intronic ESE and intronic cryptic donor site, respectively. Bold underlined letters—nucleotide variant; capital letters shaded in green—exon sequences; small letters—intron sequences; nucleotide sequences shaded grey—ESEs; LD—linkage disequilibrium. *Inferred from the 1000 Genomes Project data for geographically close populations.

**Fig. 4** In silico-predicted implications of FCGR2C c.134-96C>T and linked variants on transcriptional regulatory motifs and splicing. Chromatin marks place the Thai FCGR2C haplotype—in a weak enhancer site, where the variants flanking exon 3 were predicted to disrupt binding of transcription factors CTCF and NRSF (presence/absence of coloured domes above the sequences). The p.T118I variant was predicted to disrupt an exonic splice enhancer (ESE) and create an exonic cryptic donor site, whereas

**Discussion**

Accumulating evidence suggests that FCGR2C genetic variability is of clinical significance. The minor allele of FCGR2C c.169T>C (p.X57Q), which predicts expression of functional FcγRIIC, has been associated with autoimmune diseases [14, 15], HIV-associated tuberculosis [16] and antibody responses following vaccination [15]. Moreover, in the Thai phase 3 RV144 HIV-1 vaccine trial, an intragenic FCGR2C haplotype with c.134-96C>T (rs114945036) as tag variant, associated with reduced risk of HIV-1 infection following vaccination [8]. Here we describe an additional association between the FCGR2C c.134-96C>T tag variant and disease progression in HIV-1-infected South Africans. A weaker association was also observed for the c.169T>C (p.X57Q) variant. However, this may be the result of strong linkage disequilibrium with c.134-96C>T (D‘ = 1). The c.169T>C (p.X57Q) variant’s functional significance is also unclear since only a single study participant was predicted to express FcγRIIC based on the donor splice-site variant in intron 6 (c.789–1A>G) [10, 17]. An independent association for c.169T>C with HIV-1 disease progression cannot be
excluded. However, in this study, the c.169T>C minor allele was not detected in the absence of the c.134-96C>T minor allele, which precluded such an analysis. Conversely, an independent association for the more prevalent c.134-96C>T variant was confirmed in a multivariate logistic regression model.

The association between the c.134-96C>T variant and HIV-1 disease progression was primarily determined by the elite controller group; however, weaker associations were also observed for the viraemic controller and high viral load long-term non-progressor groups. Since the controller groups had distinctly different viral loads, it is unclear if the c.134-96C>T variant plays a role in natural control of HIV-1 viraemia. Conversely, the three HIV-1 controller groups also observed for the viraemic controller and high viral load elite controller group; however, weaker associations were linked variant alleles will be required to establish a role for altered splicing.

Consideration of population differences when studying the functional significance of c.134-96C>T is paramount. In Thai vaccinees, this variant was in complete linkage disequilibrium with p.T118I and c.391+111G>A [8], whereas in South Africans, the latter loci are rarely polymorphic [10]. Consequently, there may be different possibilities regarding causality. One is that p.T118I and c.391 +111G>A within the haplotype bear little or no functional significance and that c.134-96C>T is causal or in linkage with a functional variant elsewhere, potentially c.113-684C>T and c.113-1058T>C in FCGR2C intron 1. Alternatively, the association observed in Thai vaccinees involves p.T118I and c.391+111G>A and consequently a separate mechanism. Nevertheless, both studies indicate the relevance for c.134-96C>T in the context of HIV-1 and warrant further investigation.

It should be noted that factors modulating the risk of HIV-1 acquisition following immunization and HIV-1 disease progression once infection is established may not necessarily overlap. For example, in Thai vaccinees, the HLA class I A*02 allele associated with protection from acquisition of HIV-1 CRF01_AE in the RV144 trial [22], but the same allele did not affect progression to disease in Thai HIV-1 CRF01_AE infected military recruits [23].

The findings of the present study are therefore not necessarily indicative of a deleterious role for c.134-96C>T in the HVTN702 vaccine trial. However, the data suggest that South African HVTN702 trial participants who acquire HIV-1 in the placebo arm may be at increased risk of disease progression if they carry the FCGR2C c.134-96C>T minor allele and are not treated. This increased risk may also occur for breakthrough infections in the vaccine arm should the vaccine have no impact on HIV-1 disease progression. Long-term follow-up of Thai RV144 trial participants with breakthrough infections will potentially elucidate the role of the complete Thai FCGR2C haplotype in HIV-1 disease progression. Characterizing the mechanisms underlying the associations of c.134-96C>T in Thais and South Africans is imperative, particularly considering the genetic differences between the population groups. Moreover, establishing whether c.134-96C>T modifies the risk of HIV-1 acquisition in other models of persistent HIV-1 exposure, such as infants born to HIV-1-infected mothers and serodiscordant couples, may provide additional insight into its significance in the HVTN702 trial.
Materials and methods

Study participants

All study participants were HIV-1-infected black individuals recruited from hospitals and clinics in the city of Johannesburg, South Africa. HIV-1 controllers (n = 71) were recruited from hospitals in Soweto and Johannesburg. HIV-1 progressors (n = 73) were selected from the Soweto Lung Cohort (n = 756) recruited at Chris Hani Baragwaneth hospital. Ethical clearance was obtained from the University of the Witwatersrand Human Research Ethics Committee and all participants provided written informed consent.

Genotyping

Genomic DNA from each participant was isolated from ethylenediaminetetraacetic acid (EDTA) whole blood obtained by venepuncture. FCGR2C copy number, expression variants c.169T>C (p.X57Q) and c.798+1A>G (rs76277413) and the promoter variant c.-386G>C (rs3219018) were genotyped using the FCGR-specific multiplex ligation-dependent probe amplification assay (MRC Holland, Amsterdam, The Netherlands) according to the manufacturer’s instructions. Amplicons were separated by capillary electrophoresis on an ABI Genetic Analyzer 3130 (Life Technologies, Applied Biosystems, Foster City, CA, USA) and fragments were analyzed with the Coffalyzer.NET software (MRC Holland) using peak height as a measure of gene/allele copy number.

The FCGR2C c.134-96C>T (rs114945036) and p.T118I (rs138747765) variants were genotyped through nucleotide sequencing. In brief, a 6374 base-pair fragment was amplified with the Expand Long Template PCR System (Roche, Mannheim, Germany) using the FCGR2B/C sense primer 5′-ATGTATGGGGTGCTGTGTGTC-3′ and FCGR2C-specific antisense primer 5′-CTCAATTGGG-CAGCCCTCAC-3′ [14]. The PCR reaction consisted of ~20 ng of genomic DNA as template, 3.75 U Expand Long Template enzyme mix, 5 µl of 10× PCR buffer 3 (2.75 mM MgCl2), 500 µM of each deoxynucleotide, 0.3 µM of each oligonucleotide primer and molecular-grade water to a final volume of 50 µl. The PCR conditions were 94 °C for 2 min, followed by ten cycles of 94 °C for 10 s, 60 °C for 15 s and 68 °C for 7 min, and 25 cycles of 94 °C for 15 s, 60 °C for 15 s and 68 °C for 7 min with an elongation of each subsequent cycle with 20 s, and a final elongation at 72 °C for 7 min. The internal antisense primer 5′-CCTCCACTGAC-CAGAAAGC-3′ was used in standard BigDye Terminator v3.1 Cycle Sequencing reactions. Sequences were analyzed in Sequencher version 4.5 (Gene Codes Corporation, Ann Arbor, MI, USA) and area under the curve of the electropherogram was used to determine allele count for individuals bearing more than two FCGR2C copies.

Genetic variance description

Genetic variance description is according to the recommendations by the Human Genome Variation Society [24]. Gene, transcript and protein sequence accession numbers used to designate polymorphic variants are as follows: FCGR2C—NG_011982.1, NM_201563 and NP_963857.3. Variant coordinates are according to Ensembl human genome assembly GRCh38.p10.

Statistical and computational analysis

Linkage disequilibrium was analyzed in Haploview and Arlequin v3.5 [25, 26]. Genotypic data for individuals with multiple gene copies were considered as homozygous if all copies carried the same allele or heterozygous when both alleles were detected. Data from the 1000 Genomes Project (phase 3) were used to assess linkage disequilibrium in other population groups. HaploReg v4.1 was used to identify potential effects of genetic variants on regulatory motifs and Human Splicing Finder v3.0 to predict alteration or creation of motifs involved in splicing [27].

The D’Agostino–Pearson omnibus normality test was used to determine the distribution of continuous variables. The t test was used to compare normally distributed continuous variables, the Mann–Whitney U test to compare non-normally distributed continuous variables, the Fisher’s exact test for categorical data and multivariate logistic regression to adjust for confounders. All statistical tests were two-sided. Analysis of genetic association between FCGR2C variants and HIV-1 control was restricted to variants with minor allele frequencies greater than 5% to reduce the number of tests and increase statistical power. Given the moderate sample size and the low frequency of minor allele homozygotes, the minor allele’s effect was tested under a dominant model of inheritance. We applied Bonferroni corrections to significant associations (indicated by P_{Bon}fi), which considered 12 independent tests—three unrelated clinical subgroups and four loci (CNR1, c.–386G>C, c.134-96C>T and c.169T>C [p.X57Q]). All analyses were performed in STATA version 10.1 (StataCorp LP, Texas, USA).

Acknowledgements We would like to thank Johanna Ledwaba for her expert assistance with the genetic analyzer.

Funding This work is based on the research supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa, and the Strategic Health Innovation Partnerships (SHIP) Unit of the South African Medical Research Council (a grantee of the Bill & Melinda
Gates Foundation). The Longitudinal Study of HIV-Associated Lung Infections in Soweto (progressor group) was funded by the National Institutes of Health, USA (R01HL090312 and P30AI094189: R. E. Chaissen). Ria Lassaunière is the recipient of bursaries from the South African National Research Foundation, the Poliomyelitis Research Foundation and a University of the Witwatersrand postgraduate merit award.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Lewis GK. Role of Fc-mediated antibody function in protective immunity against HIV-1. Immunology. 2014;142:46–57.
2. Bournazos S, Klein F, Pietzsch J, Seaman MS, Nussenzweig MC, Ravetch JV. Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for in vivo activity. Cell. 2014;158:1243–53.
3. Hessel AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, Bakker JM, et al. Fc receptor but not complement binding is important in antibody protection against HIV. Nature. 2007;449:101–4.
4. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med. 2012;366:1275–86.
5. Babuka J, Nduati R, Odem-Davis K, Peterson D, Overbaugh J. HIV-specific antibodies capable of ADCD are common in breastmilk and are associated with reduced risk of transmission in women with high viral loads. PLoS Pathog. 2012;8:e1002739.
6. Baum LL, Cassutt KJ, Knigge K, Khattri R, Margolich J, Rinaldo C, et al. HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. J Immunol. 1996;157:2168–73.
7. Lambotte O, Ferrari G, Moog C, Yates NL, Liao HX, Parks RJ, et al. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. AIDS. 2009;23:897–906.
8. Li SS, Gilbert PB, Tomaras GD, Kijak G, Ferrari G, Thomas R, et al. FCGR2C polymorphisms associated with HIV-1 vaccine protection in RV144 trial. J Clin Invest. 2014;124:3879–90.
9. Rerks-Ngarm S, Pititsuttitham P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med. 2009;361:2209–20.
10. Lassaunière R, Tiemessen CT. Variability at the FCGR locus: characterization in Black South Africans and evidence for ethnic variation in and out of Africa. Genes Immun. 2016;17:93–104.
11. Peloso GM, Rader DJ, Gabriel S, Kathiresan S, Daly MJ, Neale BM. Phenotypic extremes in rare variant study designs. Eur J Hum Genet. 2016;24:924–30.
12. Van Gestel S, Houwing-Duistermaat JJ, Adolfsson R, van Duijn CM, Van Broeckhoven C. Power of selective genotyping in genetic association analyses of quantitative traits. Behav Genet. 2000;30:141–6.
13. Wangkumhang P, Shaw PJ, Chaicoompu K, Ngamphiw C, Assawamakini A, Nuinoon M, et al. Insight into the peopling of Mainland Southeast Asia from Thai population genetic structure. PLoS ONE. 2013;8:e79522.
14. Breunis WB, van Mirre E, Bruin M, Geissler J, de Boer M, Peters M, et al. Copy number variation of the activating FCGR2C gene predisposes to idiopathic thrombocytopenic purpura. Blood. 2008;111:1029–38.
15. Li X, Wu J, Puacek T, Redden DT, Brown EE, Alarcon GS, et al. Allelic-dependent expression of an activating Fc receptor on B cells enhances humoral immune responses. Sci Transl Med. 2013;5:216ra175.
16. Machado LR, Bowdrey N, Ngaimisi E, Habtewold A, Minzi O, Makonnen E, et al. Copy number variation of Fc gamma receptor genes in HIV-infected and HIV-tuberculosis co-infected individuals in sub-Saharan Africa. PLoS ONE. 2013;8:e78165.
17. van der Heijden J, Breunis WB, Geissler J, de Boer M, van den Berg TK, Kuipers TW. Phenotypic variation in IgG receptors by nonclassical FCGR2C alleles. J Immunol. 2012;188:1318–24.
18. Peng X, Li SS, Gilbert PB, Geraghty DE, Katze MG. FCGR2C polymorphisms associated with HIV-1 vaccine protection are linked to altered gene expression of Fcγ receptors in human B cells. PLoS ONE. 2016;11:e0152425.
19. Ong C-T, Corces VG. CTCF: An architectural protein bridging genome topology and function. Nat Rev Genet. 2014;15:234–46.
20. Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. Cell. 2005;121:645–57.
21. Zatkova A, Messiaen L, Vandenbroucke I, Wieser R, Fometrico C, Krainer AR, et al. Disruption of exonic splicing enhancer elements is the principal cause of exon skipping associated with seven nonsense or missense alleles of NF1. Hum Mutat. 2004;24:491–501.
22. Gartland AJ, Li S, McNevin J, Tomaras GD, Gottardo R, Janes H, et al. Analysis of HLA A*02 association with vaccine efficacy in the RV144 HIV-1 vaccine trial. J Virol. 2014;88:8242–55.
23. Gandhi RT, Bosch RJ, Rangsin R, Chuenchitra T, Sirisopana N, Kim JH, et al. HLA class I alleles associated with mortality in Thai military recruits with HIV-1 CRF01_AE infection. AIDS Res Hum Retrovir. 2016;32:44–9.
24. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat. 2000;15:7–12.
25. Barrett JC, Fry B, Maller J, Daly MJ. Haplowlow: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21:263–5.
26. Excoffier L, Laval G, Schneider S. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol Bioinform Online. 2005;1:47–50.
27. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. Nucleic Acids Res. 2016;44(D1):D877–81.