FC GAMMA RECEPTORS: GENETIC VARIATION AND ROLE IN HIV-1 INFECTION

Maria Magdalena Lassaunière

A thesis submitted to the Faculty of Medicine, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy

Johannesburg, 2015

DECLARATION

I _____ hereby declare that:

a) The research reported in this thesis, except where otherwise indicated is my original work.

b) This thesis has not been submitted for any degree or examination to any other University

c) This thesis does not contain other people's data, pictures, graphs or information, unless specifically stated as being of sourced from other people.

d) This thesis does not contain another person's writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

   I. Their words have been re-written but the general information attributed to them has been referenced;

   II. Where their exact words have been used, their writing has been placed inside quotation marks and referenced.

e) Where I have reproduced a publication of which I am an author, co-author or editor, I have indicated in detail which part of the publication was written by myself alone and have fully referenced such publications.

f) This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being listed in the thesis and in the References section.

Signed_____ Date_____

ABSTRACT

Low affinity Fcγ receptors (FcγR) mediate key immune effector mechanisms through the engagement of the Fc portion of immunoglobulin G (IgG). These receptors are involved in multiple biological processes, including clearance of antigen/antibody immune complexes, enhancement of antigen presentation, antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis, regulation of antibody production, and activation of inflammatory cells. FcγR phenotypic variability modulates these processes through altering receptor IgG subclass binding affinity (FcγRIIa-H131R and FcγRIIIa-F158V), subcellular localization (FcγRIIb-I232T), post-translational modification (FcγRIIIb-HNA1a/b/c), expression of an otherwise pseudogene (FcγRIIc), and receptor surface density (gene copy number variability and promoter haplotypes). Accumulating data suggest that FcγR-mediated effector functions play a significant role in HIV-1 protective immunity, which is substantiated by the association of FcγR phenotypic variants with HIV-1 disease outcome. This study set out to characterize FcγR functional variability in the South African population, and to investigate the potential role thereof in HIV-1 transmission and disease progression in South African Black individuals.

Since the only known determinant of FcγRIIIa surface density – *FCGR3A* gene copy number – is rare, this study investigated novel genetic determinants of FcγRIIIa expression by flow cytometry and nucleotide sequencing. FcγRIIIa expression on peripheral blood mononuclear cells was characterized for 32 South African Caucasian individuals and 22 South African Black individuals (Chapter 3). Significant differences in the proportion of FcγRIIIa-positive monocytes and FcγRIIIa expression levels on natural killer (NK) cells were observed between the population groups. A novel four-variant *FCGR3A* intragenic haplotype that associated with increased surface expression of FcγRIIIa on NK cells was detectable in Caucasian individuals, but not Black individuals and may account for the observed population differences.

Further exploration of genetic diversity at the low affinity *FCGR* gene locus was extended to include all currently known functional variants of FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIIb using a commercial multiplex ligation-dependent probe amplification assay (Chapter 4). Thirty-two South African Caucasian individuals and 131 South African Black

individuals were genotyped for all known functional *FCGR* nucleotide variants and gene copy number variability. The data from South African individuals were compared to that of published works and the 1000 Genome Project for European Caucasian individuals and Black individuals from Nigeria and Kenya. The findings corroborated known ethnic diversity at the low affinity *FCGR* gene locus and also describe newly identified differences not only between Caucasian and Black individuals, but also among populations from different geographic regions in Africa. South African Black individuals do not possess the *FCGR2B* 2B.4 promoter haplotype associated with increased FcγRIIb expression and inhibitory function or the recently identified *FCGR2C* haplotype associated with vaccine efficacy of the HIV-specific prime-boost regimen that showed modest efficacy in Thai individuals (RV144 trial). Moreover, South African Black individuals were not predicted to express functional FcγRIIc compared to ~32% of Caucasian individuals. However, the presence of this additional activating FcγR on NK cells from select Caucasian individuals did not potentiate *in vitro* NK cell-mediated ADCC capacity, suggesting a lesser role for FcγRIIc in this response. Caucasian individuals exhibited moderate to strong linkage disequilibrium between FcγR variants that confer enhanced effector functions for the activating FcγRIIa, FcγRIIc, and FcγRIIIa, but also enhanced inhibitory function of FcγRIIb. Conversely, Black individuals did not display significant linkage disequilibrium and possessed FcγRIIb profiles representative of reduced inhibitory function. This study highlighted (i) the extensive ethnic diversity at the low affinity *FCGR* gene locus, (ii) potential differences between population groups in maintaining the activation/inhibition balance conferred by FcγR variants, and (iii) that variation among African populations precludes the use of any one African population as proxy for FcγR diversity in Africans.

The significance of FcγR variability, and by inference FcγR-mediated effector functions, in modulating HIV-1 infection risk was assessed in the context of mother-to-child transmission, studying both the transmitter (mother) and recipient (infant). The study cohort comprised 217 HIV-1 infected, antiretroviral treatment naïve Black mothers and their infants. Here it was demonstrated that, following adjustment for confounding factors, the maternal FcγRIIa-131R and FcγRIIIa-158F variants that exhibits reduced antibody binding affinity and effector capacity associated with a 3 to 4 times higher odds of HIV-1

transmission to the infant, whereas the alternative alleles associated with >50% reduction in the odds of HIV-1 transmission. Moreover, we demonstrate through an allele scoring system that the combined effect of multilocus FcγR variants modulates HIV-1 transmission risk such that mothers bearing an overrepresentation of low responder FcγR alleles had a ~4 times higher odds of transmitting HIV-1 to their infants compared to mothers with an overrepresentation of high responder FcγR alleles. Protection from HIV-1 acquisition in the infant associated with homozygosity for the high responder FcγRIIIb-HNA1a allele that exhibits enhanced phagocytosis and respiratory burst capacity. The conclusion of this study is that FcγR variants modulate the infectiousness of an HIV-1 infected mother and the susceptibility of an exposed foetus/infant, and thus provide indirect evidence for a role of FcγR-mediated effector functions in modulating the risk of perinatal HIV-1 transmission.

The association between FcγR variants and cross-sectional markers of HIV-1 disease progression were determined in treatment naïve, Black South African women that formed part of the aforementioned perinatal HIV-1 transmission cohort (Chapter 6). In contrast to that observed for protection from HIV-1 acquisition in the infant, homozygosity for the low responder FcγRIIIb-HNA1b allotype associated with lower viral loads and higher $CD4^+$ T cell counts compared to all other allotype combinations. Furthermore, HIV-1 infected women with an overrepresentation of either high or low responder FcγR alleles had higher viral loads compared to women bearing a 'neutral' FcγR variability profile. This study thus demonstrates a role for FcγRIIIb allotypes in modulating HIV-1 disease course and that perturbation of the balance between high and low responder FcγR variants associates with higher viral loads.

Overall, our data highlights the extensive ethnic diversity at the *FCGR* locus and provide further support for a role of FcγR-mediated effector functions in HIV-1 transmission and disease progression.

PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS FROM THIS THESIS

Lassaunière R, Shalekoff S, Tiemessen CT. *A novel FCGR3A intragenic haplotype is associated with increased FccRIIIa/CD16a cell surface density and population differences.* Human Immunology. 2013; 74:627-634

Lassaunière R, Tiemessen CT. *Variability at the low affinity* FCGR *locus: Characterization in Black South Africans and evidence for ethnic variation in and out of Africa.* (in preparation)

Lassauniere R, Gray GE, Kuhn L, Tiemessen CT. *FcγR functional variability impacts on mother-to-child transmission of HIV-1.* (in preparation)

PRESENTATIONS AT MEETINGS

Lassaunière R, Tiemessen CT. *FcγR variability in the South African population – will this impact on HVTN097 vaccine efficacy?* [Poster Presentation] HIV Research for Prevention (HIV R4P) Conference. 28-31 October 2014, Cape Town, South Africa

Lassauniere R, Gray GE, Kuhn L, Tiemessen CT. *FcγR functional variability impacts on mother-to-child transmission of HIV-1.* [Oral Poster Discussion] HIV Research for Prevention (HIV R4P) Conference. 28-31 October 2014, Cape Town, South Africa

Lassaunière R, Spencer D, Tiemessen CT. *Fc receptor functional allelic variant associated with control of HIV-1 infection.* [Oral Presentation] Virology Africa 2011. 28 November to 2 December 2011, Cape Town Waterfront, South Africa

Lassaunière R, Shalekoff S, Tiemessen CT. *A novel FCGR3A intragenic haplotype is associated with increased FcγRIIIa/CD16a cell surface density and population differences.* [Poster Presentation] EMBO Global Exchange Lecture Course on Innate Immunity – evolution and advances in clinical medicine. 2-5 September 2012, Stay City, Johannesburg, South Africa

Lassaunière R, Shalekoff S, Tiemessen CT. *A novel FCGR3A intragenic haplotype is associated with increased FcγRIIIa/CD16a cell surface density and population differences.* [Poster Presentation] 8th Conference of the Federation of African Immunological Societies. 3-5 December 2012, Durban, South Africa

To Josh

ACKNOWLEDGEMENTS

With much gratitude, I would like to thank the following for their valuable contribution to this work.

To my supervisor, Prof. Caroline Tiemessen, for the opportunity to study under her guidance, for her unwavering support, advice and above all the confidence she had in me

To all my co-authors

To all the volunteers, without whom this work would not have been possible

To my fellow colleagues,

Dr. Sharon Shalekoff, who introduced me to flow cytometry and was always willing to help with assay designs and data analysis

Dr. Diana Schramm, without whom the lab would be in chaos

Dr. Maria Paximadis, for helping with complex molecular analysis

Dr. Leonard Damelin, who has patiently guided me through new methods

Dr. Bela Picton, for sharing her knowledge on genotypic analysis

Avani, Heather, and Nikki – it has been great fun going on this PhD journey with you, thank you for all the laughs and chats in the office

To Dr. Justin Pollara and Prof. Guido Ferrari for generously sharing the ADCC assay protocol

To MacroGenics for the anti-FcγRIIb/c antibody

To the funders of the project and my studies: Medical Research Counsel and the Poliomyelitis Research Foundation for project funding; National Research Foundation and Poliomyelitis Research Foundation for scholarships and bursaries

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

ABBREVIATIONS

3TC	lamivudine
ABC	antibodies bound per cell
ADCC	antibody-dependent cellular cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
ADCVI	antibody-dependent cellular virus inhibition
AIDS	acquired immunodeficiency virus syndrome
AMR	1000 genomes project admixed American super population
AOR	adjusted odds ratio
ARV	antiretroviral therapy
ASN	1000 genomes project East Asian super population
AZT	zidovudine
BCR	B cell antigen receptor
BLNK	B cell linker protein
C1, C2, C3	intracytoplasmic region exon 1-3
CA	nucleocapsid
CD	cluster of differentiation
CH	constant heavy
CI	confidence intervals
CNR	copy number regions
CNV	copy number variability
CR3	complement receptor 3
Ct	crossing threshold
DNA	deoxyribonucleic acid
dNK	decidual natural killer
EC1, EC2	extracellur region exons 1 and 2
EDTA	ethylenediaminetetraacetic acid
EUR	1000 genomes project Caucasian super population
Fab	antigen binding fragment
Fc	crystallisable fragment
FcγR	Fc gamma receptor
FCGR	Fc gamma receptor
FcR	Fc receptor
FcRn	neonatal Fc receptor
G-CSF	granulocyte colony stimulating factor
GWAS	genome-wide association studies
H_2O_2	hydrogen peroxide
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
HIV-2	human immunodeficiency virus type 2
HNA	human neutrophil antigens
IFNγ	interferon gamma
Ig	immunoglobulin
IgG	immunoglobulin G
IH	intragenic haplotype

IN	integrase
ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibitory motif
IVIG	intravenous immunoglobulin G
LD	linkage disequilibrium
LWK	1000 genomes project Luhya Kenyans
MA	matrix
Mabs	monoclonal antibodies
Mac-1	macrophage antigen 1
MACS	magnet-activated cell separation
MLPA	multiplex ligation-dependent probe amplification
mRNA	messenger ribonucleic acid
MTCT	mother-to-child transmission
NA	neutrophil antigens
Nef	negative regulatory factor
NETs	neutrophil extracellular traps
NK	natural killer cells
NT	non-transmitting
O_2^-	superoxide anion
OR	odds ratio
ORF	open reading frame
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
PLCγ	phosphoinositide phospholipase C gamma
PMTCT	prevention of mother-to-child transmission
Pprox	proximal promoter
PR	protease
R	Spearman's correlation coefficient
Rev	regulator of virion
RNA	ribonucleic acid
RT	reverse transcriptase
S1, S2	signal peptide exons 1 and 2
SA	South African
sdNVP	single dose nevirapine
sFcγRIIIb	soluble Fc gamma receptor IIIb
SHIP	SH2 domain-containing inositol 5' phosphatase
SIV	simian immunodeficiency virus
SIV_{cpz}	SIV from chimpanzees
SIV_{sm}	SIV from sooty mangabeys
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
SLP-75	lymphocyte cytosolic protein 2
SSP	sequence-specific primer
Tat	trans-activator of transcription

TCR	T-cell receptor
TE	Tris/ethylenediaminetetraacetic acid
TM	transmembrane exon
TM/C	transmembrane region
TR	transmitting mothers
VE	vaccine efficacy
Vif	viral infectivity factor
VL	viral load
Vpr	viral protein r
Vpu	viral protein u
YRI	1000 genomes project Yoruba Nigerians

Amino acid abbreviations

Amino acid	Three-letter code	One-letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

CHAPTER 1

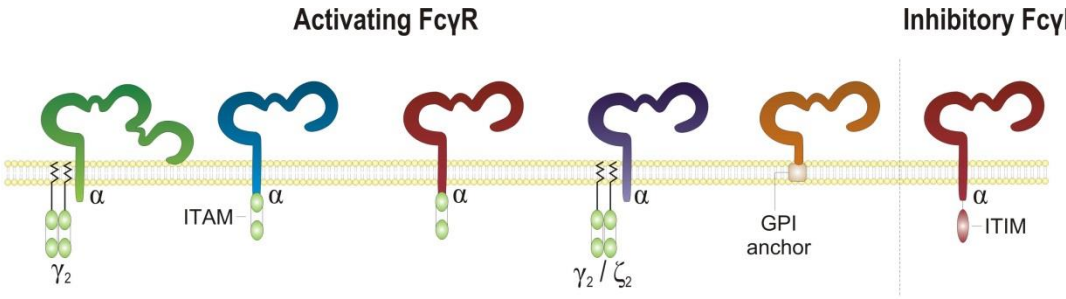
INTRODUCTION

1.1. FC GAMMA RECEPTORS

1.1.1. Overview

Receptors for the crystallisable fragment (Fc) of immunoglobulin (Ig) G, Fcγ receptors (FcγRs), link the humoral and cellular arms of the immune response. They are widely expressed on haematopoietic cells where upon engagement with IgG they initiate a variety of pro- and anti-inflammatory responses. Three classes of FcγRs exist, each with different isoforms: FcγRIa/b/c, FcγRIIa/b/c, and FcγRIIIa/b (Figure 1.1). All FcγRs are glycoproteins belonging to the Ig superfamily and consists of a ligand-binding α-chain with two (FcγRII and FcγRIII) or three (FcγRI) extracellular Ig-like domains, a transmembrane domain, and intracytoplasmic domain. The activating or inhibitory signalling motifs are located either within the α-chain (FcγRII) or associated signalling subunits (FcγRI and FcγRIIIa) (Lanier et al 1991).

Overall, FcγRI exhibits a high affinity for monomeric IgG (K_A: ~1×10^7), while FcγRII and FcγRIII bind IgG through low affinity (K_A: ~1×10^6), high avidity interactions. Cross-linking of FcγRs on the cell surface through multivalent interactions with immune complexes initiates a plethora of responses that include antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), respiratory burst, release of inflammatory mediators, and regulation of antibody production. The capacity of FcγRs to mediate these effector functions is affected by genotypic variants that alter receptor surface density, antibody binding affinity, cellular distribution, or subcellular localization. The impact of these variants on immune homeostasis is validated by their association with autoimmune diseases (Takai 2002), infectious diseases (Adu et al 2012, Zhao et al 2014), and response to immunotherapy (Cartron et al 2002). This review will focus on the low affinity FcγRs (FcγRII and FcγRIII) and the variants that modulate their function.



Name	FcγRI	FcγRIIa	FcγRIIc	FcγRIIIa	FcγRIIIb	FcγRIIb
CD	CD64	CD32a	CD32c	CD16a	CD16b	CD32b
Gene	*FCGR1A*	*FCGR2A*	*FCGR2C*	*FCGR3A*	*FCGR3B*	*FCGR2B*
Affinity	High	Low to medium	Low to medium	Low to medium	Low to medium	Low to medium
Alleles	¤	H_{131} R_{131}	Q_{57} $stop_{57}$	F_{158} V_{158}	HNA1a/b/c	I_{232} T_{232}
IgG1	6×10^7	5×10^6 3×10^6	1×10^5 -	1×10^6 2×10^6	2×10^5	1×10^5 ND
IgG2	-	4×10^5 1×10^5	2×10^4 -	3×10^4 7×10^4	-	2×10^4 ND
IgG3	6×10^7	9×10^5 9×10^5	2×10^5 -	8×10^6 **1×10^7**	1×10^6	2×10^5 ND
IgG4	3×10^7	2×10^5 2×10^5	2×10^5 -	2×10^5 2×10^5	-	2×10^5 ND
Expression pattern:						
B cell	-	-	-	-	-	+
NK cell	-	-	+#	+	-	-*
Mono/Macro	+	+	+#	+	-	+/-
Neutrophil	(+)	+	+#	-	+	+/-
Dendritic cell	+	+	-	-	-	+
Basophil	-	+	-	-	+/-	+
Mast cell	(+)	+	-	-	-	-
Eosinophil	-	+	-	-	-	-
Platelet	-	+	-	-	-	-

Figure 1.1. Summary of FcγR characteristics. ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; GPI, glycosyl-phosphatidylinositol; *Binding affinities for the different IgG subclasses*: given as M-1; - , no binding; ND, not determined. *Expression patterns:* +, indicates expression; -, no expression; (+), inducible expression; +/-, very low expression or expressed by rare subsets; #, in individuals bearing the *FCGR2C* expression variants (van der Heijden et al 2012); *, expressed in individuals bearing a *FCGR2C-FCGR3B* gene deletion. Redrawn and modified from (Gillis et al 2014).

1.1.2. IgG

IgG occurs as four subclasses, named in order of abundance in serum IgG1, IgG2, IgG3 and IgG4. The subclasses have different effector functions and present with a general activating capacity order of IgG3 > IgG1 >> IgG2 > IgG4, and are differentially induced upon

antigenic challenge (Ferrante et al 1990, Horton & Vidarsson 2013, Siber et al 1980). Protein antigens primarily induce IgG1, accompanied by low levels of mostly IgG3 and IgG4. Antibody responses to bacterial capsular polysaccharides are dominated by, and sometimes restricted to, IgG2. IgG3 is a potent inducer of effector functions. It has a short half-life and, together with IgG1, predominates in the early antibody responses to antigen. As in the case of viral infections, IgG3 is found only in association with early infection. In contrast, IgG4 antibodies are generally not associated with a primary response to antigen but are usually formed following repeat exposure to antigen.

1.1.3. IgG-Fc/FcγR interaction

X-ray crystallographic studies of the IgG-FcγR complex show that the membrane-proximal Ig-like domain of FcγR contact the horseshoe Fc fragment at both tips of the constant heavy (CH)-2 domain, at the residues most proximal to the hinge region (Figure 1.2A and B) (Maxwell et al 1999, Ramsland et al 2011, Sondermann et al 2000). Engagement of an FcγR with IgG introduces an asymmetry within the Fc and rearranges the hinge that ensures a 1:1 stoichiometry of the resulting complex.

FcγRs do not bind the four IgG subclasses equally. Most bind IgG1 and IgG3 with a higher affinity than IgG2 and IgG4 (Bruhns et al 2009). While the IgG subclasses are highly homologous they exhibit notable differences in hinge length and flexibility (Figure 1.2C). Overall, the flexibility of the Fab arms with respect to the Fc portion ranks as IgG3 > IgG1 > IgG4 > IgG2, which reflects their relative binding to FcγRs. Due to the close proximity of the hinge region to the FcγR binding site, its flexibility may, in part explain the varying affinity for the different IgG subclasses.

The *N*-linked glycan found at Asn^{297} in both of the CH2 domains is essential for binding to all FcγRs by maintaining the quaternary structure and thermostability of the Fc. The glycan consists of a core sugar structure with variable additions of sugar residues such as N-acetylglucosamine, fucose, sialic acid, and galactose. The individual components of the sugar moiety have been demonstrated to differentially affect FcγR-mediated antibody activity. Galactosylation and fucosylation modulate FcγRIIIa binding and ADCC (Nimmerjahn et al 2007, Shields et al 2002, Shinkawa et al 2003), while increased

sialylation of the glycan can switch IgG from a pro-inflammatory to an anti-inflammatory species by inducing expression of the inhibitory FcγRIIb (Kaneko et al 2006). IgG alters its Fc glycosylation pattern during the course of an antibody response (Vestrheim et al 2014). In the steady state, however, it appears that IgG assumes an anti-inflammatory role as is validated by the use of pooled serum from healthy donors – IVIG (intravenous IgG) – to treat inflammatory diseases (Nimmerjahn & Ravetch 2008a).

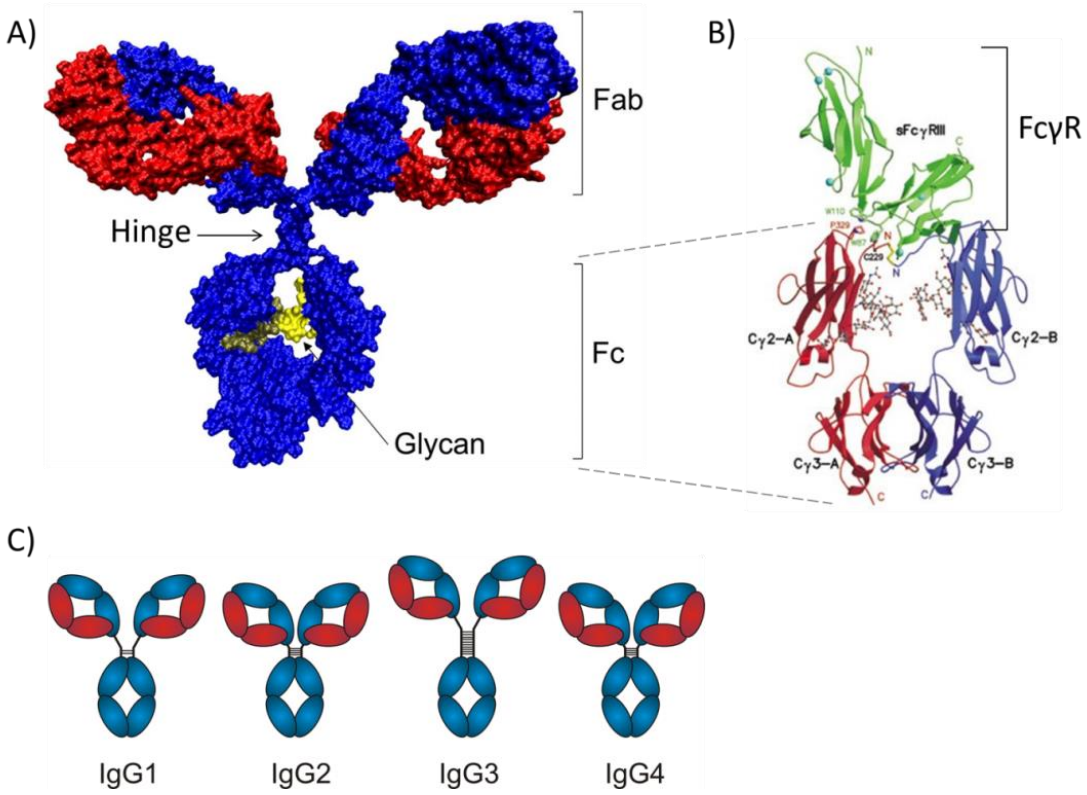


Figure 1.2. IgG structure and FcγR binding. A) IgG structure with heavy chains (blue), light chains (red), and the position of the Asn297 *N*-linked glycan position. B) The FcγR binds the Fc portion of IgG at both tips of the constant heavy (CH)-2 domain, at the residues most proximal to the hinge region. C) The different IgG subclasses differ with respect to hinge length and flexibility. The IgG1 hinge region comprises 15 amino acids and is very flexible. IgG2 has the shortest hinge comprised of 12 amino acids. Its flexibility is restricted by the presence of a poly-proline helix, stabilized by up to four extra inter-heavy chain disulfide bridges. The hinge region of IgG4 also contains 12 amino acids with an intermediate flexibility between that of IgG1 and IgG2. IgG3 has the longest hinge region, containing up to 62 amino acids and limited flexibility. Reproduced from (Collin et al 2008, Sondermann et al 2000, Vidarsson et al 2014).

1.1.4. FcγR-mediated cell activation and inhibition

Initiation of FcγR-mediated signal transduction requires cross-linking and aggregation of FcγRs through multivalent interactions with IgG immune complexes (Daeron 1997). Upon engagement with immune complexes, FcγRs translocate to (if not constitutively reside in) lipid rafts – detergent resistant, cholesterol- and sphingolipid-enriched signalling microdomains – where they co-localize with other signalling molecules (Aman et al 2001, Barabe et al 2002, Fernandes et al 2006, Galandrini et al 2002, Kondadasula et al 2008). This FcγR-lipid raft association modulate immune complex binding and is required for signal transduction events (Bournazos et al 2009a).

FcγRs are divided according to their ability to induce or inhibit cell activation through transmitting their signals via immunoreceptor tyrosine-based activation (ITAM) or inhibitory motifs (ITIM), respectively. FcγRIIa and FcγRIIc are single chain receptors that each bears an ITAM in their cytoplasmic domain, while the ligand binding α-chain of FcγRIIIa associates with the ITAM-bearing FcRγ-chain or TCRζ-chain for signal transduction (Lanier et al 1991). Figure 1.3 summarizes FcγR-mediated cell activation. Cross-linking of activating FcγRs triggers a signalling cascade initiated by tyrosine phosphorylation of the ITAMs themselves by the Src family kinases, in turn creating SH2 sites for docking and activation of Syk kinases. This leads to the recruitment and phosphorylation of a variety of intracellular substrates including phospholipid kinases and phospholipases (PLCγ), adaptor molecules (SLP-76 and BLNK), and proteins associated with the cytoskeleton (Falasca et al 1998, Ferguson et al 1995).

FcγRIIb represents the sole inhibitory FcγR, bearing an ITIM in its cytoplasmic domain. Inhibitory signalling events resulting from crosslinking of FcγRIIb include phosphorylation of its ITIM by Lyn (Muta et al 1994), followed by the recruitment and activation of inositol 5' phosphatase (SHIP) (Ono et al 1996). The latter hydrolyses key intermediates in the ITAM signalling pathway and thereby dampening downstream effector functions and cell proliferation (Van den Herik-Oudijk et al 1995). Engagement of both activating and inhibitory FcγRs co-expressed on the same cell will trigger activating and inhibitory signalling pathways and, thus, set a threshold for cell activation and regulate responses such as ADCC, ADCP, degranulation, and antigen presentation (Ravetch & Bolland 2001).

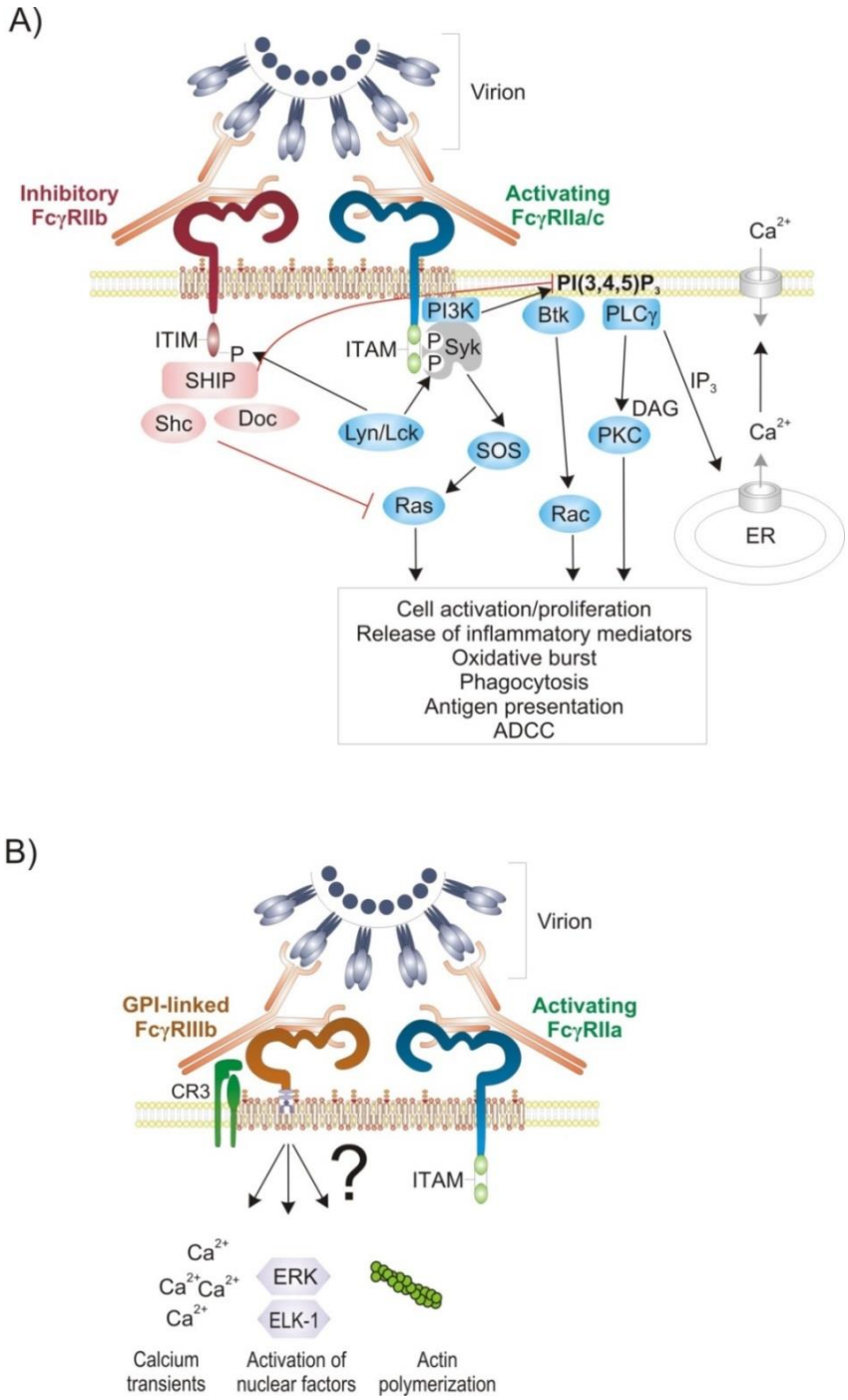


Figure 1.3. FcγR-mediated signal transduction. A) Ligation of activating and inhibitory FcγRs co-expressed by monocytes, granulocytes, and macrophages will simultaneously trigger both activating and inhibitory signalling pathways. Red lines indicate the inhibitory signalling pathway interfere with the activating pathway. Redrawn and modified from (Nimmerjahn & Ravetch 2007). B) Signalling of FcγRIIIb likely occurs through FcγRIIa and complement receptor 3 (CR3). However, independent cross-linking of FcγRIIIb triggers cell activation in the form of calcium transients, actin polymerization, and activation of nuclear factors (illustration in Figure 1.3B by Ria Lassaunière).

FcγRIIIb is unique among the family of FcγR in that it is linked to the outer plasma membrane layer by a glycosylphosphatidylinositol anchor. It lacks intrinsic signalling domains and does not associate with FcRγ-chain. The mechanism by which it transduces a signal is not clear. However, despite lacking an intrinsic cytoplasmic signalling domain FcγRIIIb is capable of inducing several cell responses, including calcium transients (Rosales & Brown 1992), actin filament assembly (Salmon et al 1991), and activation of nuclear factors (Garcia-Garcia et al 2009). It has been demonstrated that FcγRIIIb constitutively resides in lipid rafts where it is thought to co-localize and signal through association with adaptor molecules and transmembrane receptors (Figure 1.3B), such as FcγRIIa and the complement receptor 3 (CR3, mac-1, $\alpha_M\beta_2$, CD11b/CD18) (Chuang et al 2000, Fernandes et al 2006, Zhou et al 1993).

1.1.5. FcγR-mediated effector functions

Effector responses mediated by FcγRs include ADCC, ADCP, respiratory burst, release of pro-inflammatory mediators, and regulation of B cell activation and antibody production (Figure 1.4). The biological responses triggered by FcγRs appear to be dependent on the cell type more than the FcγR isoform. Cell populations often co-express different FcγRs with overlapping ligand affinities and will likely be simultaneously engaged and activated under patho-physiological conditions (immune complexes).

In brief, ADCC is induced when IgG forms a bridge between a foreign antigen on a cell surface and an FcγR-bearing cytotoxic effector cell. Ligation and cross-linking of activating FcγRs subsequently initiate cell activation and a lytic attack on the antibody-coated target cell. ADCP involves the engulfing of pathogens and cell debris, internalization into phagosomes (acidified cytoplasmic vesicles) and their subsequent fusion with lysosomes, where lysosomal enzymes destroy the ingested matter. This process contributes to antigen presentation. During ADCP, professional phagocytes increase their oxygen uptake and generate superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) through a process called the respiratory burst. These oxygen metabolites give rise to other reactive oxygen species with potent antimicrobial activity (Babior 1984). In addition to the production of reactive oxygen species, FcγR-mediated cell activation induces the production of pro-inflammatory mediators that modulates the function of other immune cells (Hogarth 2002). Effector

processes mediated by activating FcγRs are regulated by the inhibitory FcγRIIb. On B lymphocytes, FcγRIIb regulates antibody production through inhibiting B cell receptor-mediated signalling or inducing apoptosis (Amigorena et al 1992, Muta et al 1994, Tzeng et al 2005).

1.1.6. FcγR biology and variability

The genes that encode the three FcγRII molecules (*FCGR2A*, *FCGR2B*, and *FCGR2C*) and two FcγRIII molecules (*FCGR3A*, *FCGR3B*) are clustered on chromosome 1 band 1q23.3. The diversity at this locus is thought to be the result of segmental duplication and recombination events, and thus the genes are highly homologous (Qiu et al 1990). They encode, however, structurally and biochemically distinct molecules with different cellular distributions and affinities for IgG subclasses. Variants with functional significance have been described for all low affinity FcγRs.

1.1.6.1. FcγRII

The 40 kDa FcγRII molecules are the most widely expressed FcγR (Cassel et al 1993). The three isoforms are encoded by *FCGR2A* (FcγRIIa), *FCGR2B* (FcγRIIb), and *FCGR2C* (FcγRIIc). Each gene spans 15-19 kilobases (kb) and consists of eight exons that encode the signal peptide (S1 and S2), extracellular region (EC1 and EC2), transmembrane domain (TM), and intracytoplasmic region (C1, C2, and C3). *FCGR2A* and *FCGR2B* likely originated from duplication and divergence of a common ancestral gene (Qiu et al 1990). The molecules they encode are highly homologous in their extracellular and transmembrane domains (>90% amino acid identity), but differ significantly in the cytoplasmic region (8% amino acid identity). *FCGR2C* is the product of an unequal cross-over event between *FCGR2A* and *FCGR2B*, with the putative recombination site ~300 nucleotides downstream of the C1 exon (Warmerdam et al 1993). As a result the signal peptide, extracellular region, and transmembrane domain of FcγRIIc are identical to FcγRIIb, while its intracellular domain shares a 98% amino acid sequence identity with that of FcγRIIa. Despite a high degree of similarity the three FcγRII molecules have distinct cellular distributions, functions and allelic variability.

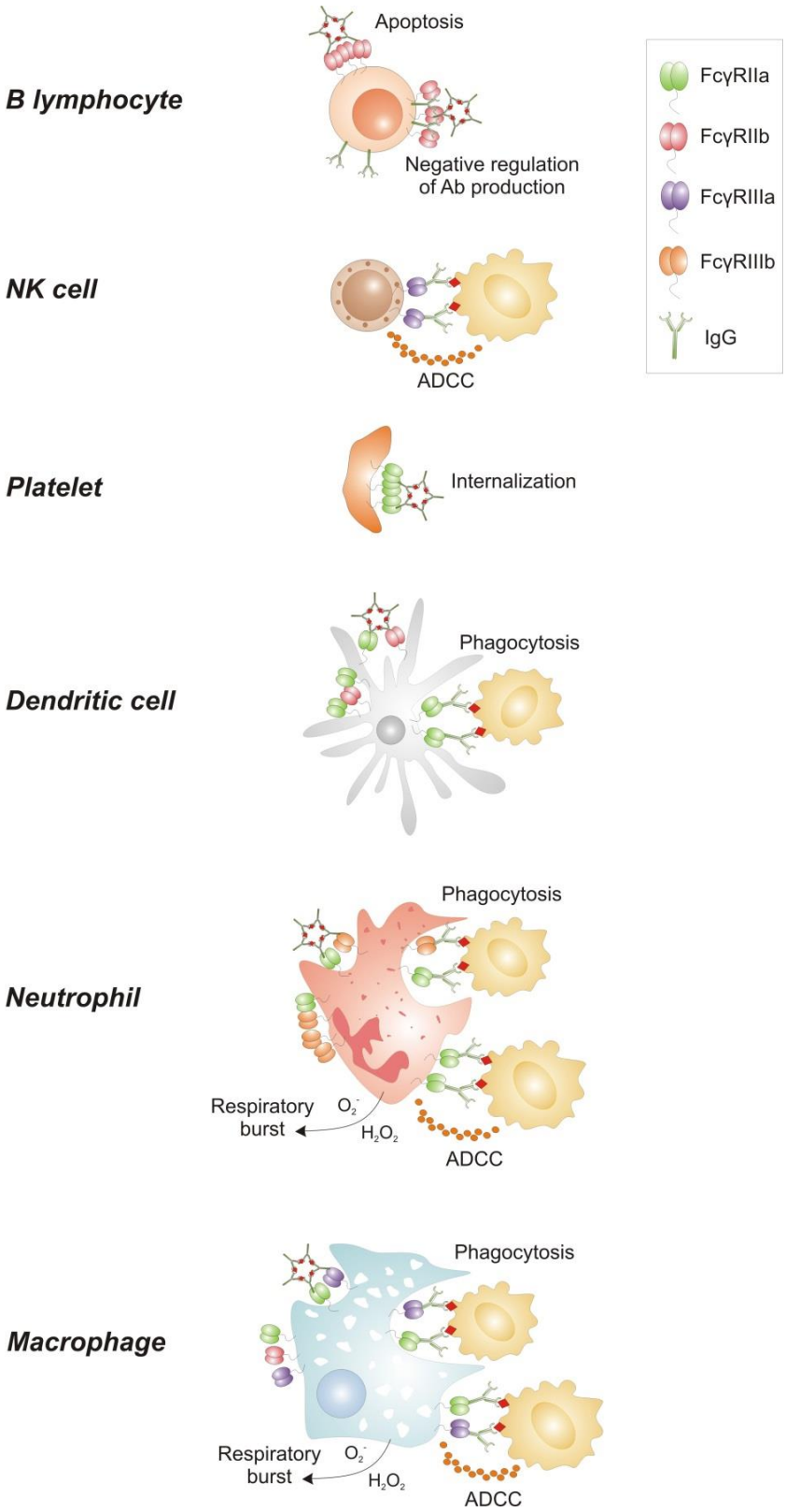


Figure 1.4. FcγR isoform expression and responses mediated by FcγR-bearing leukocytes (illustration by Ria Lassaunière).

1.1.6.1.1. FcγRIIa

Characteristics. *FCGR2A* yield two transcripts, FcγRIIa1 and FcγRIIa2, of which the former predominates in myeloid cells. FcγRIIa2 lacks the transmembrane coding region (TM exon) and is thought to produce a soluble FcγRIIa molecule (Cassel et al 1993, Rappaport et al 1993). The mature membrane-bound FcγRIIa protein of 282 amino acids comprises an extracellular region of two Ig-like domains, each bearing an *N*-linked glycosylation site, followed by a 28 amino acid hydrophobic transmembrane domain and a 76 amino acid intracellular region bearing an intrinsic ITAM (Brooks et al 1989, Van den Herik-Oudijk et al 1995). The FcγRIIa molecule forms a dimer on the cell surface, a configuration that juxtaposes the ITAM motifs and contributes to FcγRIIa aggregation and initiation of signal transduction (Maxwell et al 1999, Powell et al 2006). This activating FcγR is expressed on monocytes, macrophages, dendritic cells, neutrophils, basophils, mast cells, eosinophils, megakaryocytes and platelets (Gillis et al 2014). The wide cellular distribution reflects the diversity of immunological functions attributed to FcγRIIa, including phagocytosis, ADCC, production of reactive oxygen species, cytokine release, platelet activation, and dendritic cell maturation (Anderson et al 1990, Antczak et al 2011, Boruchov et al 2005, Löfgren et al 1999, van de Winkel et al 1989). Compared to other FcγRs, FcγRIIa exhibits the highest affinity IgG2 (K_A: >1 × 10^5 M^{-1} *versus* ~2 × 10^4 M^{-1}) (Bruhns et al 2009), a major component in antibody responses against bacterial capsular polysaccharide, and is thus a significant contributor to FcγR-mediated effector functions in responses against bacterial pathogens. FcγRIIa has the highest affinity for IgG1 (K_A: ~4 × 10^6 M^{-1}) followed by IgG2 (K_A: 1-5 × 10^6 M^{-1}), IgG3 (K_A: 9 × 10^5 M^{-1}), and IgG4 (K_A: 2 × 10^5 M^{-1}) (Bruhns et al 2009).

Variability. An arginine to histidine substitution at amino acid 131 in the membrane-proximal Ig-like domain alters the affinity of FcγRIIa for IgG2 (Clark et al 1989, Warmerdam et al 1990). This polymorphic position is directly involved in Fc binding where the different residues potentially affect ligand contact (Maxwell et al 1999). Moreover, the allelic variants alter FcγRIIa dimerization and consequently the availability of the ligand binding surfaces, which may affect the number of IgG ligands bound by the dimer (Maxwell et al 1999, Ramsland et al 2011). As a result leukocytes that express FcγRIIa-131H bind

IgG2 efficiently, whereas those that express FcγRIIa-131R do not. At high IgG concentrations (30 μg/ml), however, it has been demonstrated that FcγRIIa-131R binds IgG2 immune complexes, but at a ~4-fold lower affinity than FcγRIIa-131H (K_A: 1×10^5 M^{-1} *versus* 4×10^5 M^{-1}) (Bruhns et al 2009). Functionally, phagocytes from FcγRIIa-131RR donors exhibit reduced binding and internalization of IgG2-containing immune complexes compared to phagocytes from FcγRIIa-131HH donors (Bredius et al 1994b, Salmon et al 1992). Furthermore, monocyte derived dendritic cells from FcγRIIa-131RR donors display decreased levels of maturation upon stimulation with IgG compared to FcγRIIa-131HH/HR donors (Boruchov et al 2005).

Clinical significance of variability. The FcγRIIa-H131R variant has been associated with inflammatory diseases, autoimmune diseases and susceptibility to infections. In particular, the high responder FcγRIIa-131H allele is a potential risk factor for Kawasaki disease – systemic vasculitis of unknown aetiology (Onouchi et al 2012, Shrestha et al 2012). The low responder FcγRIIa-131R allele is associated with increased risk for lupus nephritis and systemic lupus erythematosus (SLE) – the prototype systemic autoimmune disease that is characterized by circulating immune complexes and inflammatory pathologies in multiple organs (Karassa et al 2002).

The FcγRIIa-131R allele has also been linked to susceptibility and disease course of bacterial infections where the antibody response is dominated by IgG2, such as *Streptococcus pneumoniae* (Sanders et al 1995, Yee et al 2000) and *Neisseria meningitides* infection (Bredius et al 1994a, Domingo et al 2002, Platonov et al 1998). Conversely, the FcγRIIa-131H allele have been identified as a potential protective factor against blood-stages of malaria infection (Zhao et al 2014).

1.1.6.1.2. FcγRIIb

Characteristics. Alternative splicing of *FCGR2B* transcripts produce three isoforms. FcγRIIb1 and FcγRIIb2 are identical with the exception of a 19 amino acid in-frame insert (encoded by exon C1) in the cytoplasmic domain of FcγRIIb1. FcγRIIb3 differs from FcγRIIb2 by lacking the last seven amino acids of the signal peptide (encoded by exon S2) (Brooks et al 1989). Expression of isoform FcγRIIb1 is restricted to B lymphocytes, while

both FcγRIIb1 and FcγRIIb2 isoforms are expressed on monocytes, macrophages, activated neutrophils, and under specific conditions on natural killer cells (discussed in Section 1.6.3.). The extracellular domain of FcγRIIb shares a 96% amino acid homology with FcγRIIa, predicts three *N*-linked glycosylation sites, is followed by a transmembrane domain and an intracellular region unrelated to FcγRIIa. In contrast to FcγRIIa, FcγRIIb bears the conserved 13-amino-acid ITIM in its cytoplasmic domain (Van den Herik-Oudijk et al 1995). Co-aggregation of FcγRIIb with ITAM-bearing receptors sets a threshold for pro-inflammatory processes initiated by activating FcγRs (Muta et al 1994, Nimmerjahn & Ravetch 2007). In addition, FcγRIIb regulates B lymphocyte activation and antibody production by two mechanisms: 1) Co-ligation of FcγRIIb and the B cell antigen receptor (BCR) by immune complexes inhibit BCR signalling (Amigorena et al 1992, Muta et al 1994); and 2) In the absence of BCR ligation, cross-linking of FcγRIIb can induce apoptosis of mature B cells (Tzeng et al 2005). Compared to other FcγRs, FcγRIIb has the lowest affinity for IgG, binding IgG1, IgG3, and IgG4 with near equivalent affinities at K_A: 1-2 × 10^5 M^{-1} and IgG2 with a lower affinity of K_A: 2 × 10^4 M^{-1} (Bruhns et al 2009).

Variability. The ability of FcγRIIb to translocate to lipid rafts is affected by an amino acid substitution (isoleucine to threonine) at residue 232 within the transmembrane region (Floto et al 2005, Kono et al 2005). Exclusion of the FcγRIIb-232T variant from lipid rafts impairs the receptor's inhibitory potential (Floto et al 2005). This is likely due to decreased quantitative participation of FcγRIIb in lipid raft-based signalling complexes. Compared to FcγRIIb-232II and FcγRIIb-232IT donors, B lymphocytes from FcγRIIb-232TT donors show reduced inhibition of B cell receptor-triggered proliferation and enhanced macrophage phagocytic capacity (Floto et al 2005, Kono et al 2005).

An additional variant modulating FcγRIIb function is located in the *FCGR2B* promoter region. The less common *FCGR2B* promoter haplotype (-386C/-120A, designated 2B.4) exhibit increased binding of transcription factors and higher receptor expression levels compare to the more frequent haplotype (-386G/-120T, designated 2B.1) (Su et al 2004a, Su et al 2004b). Increased expression of FcγRIIb confers a greater inhibitory function, likely due to enhanced efficiency of receptor cross-linking (Su et al 2004a).

Clinical significance of variability. The variants modulating the inhibitory effect of

FcγRIIb have been linked to infectious disease and autoimmune conditions. In particular, the FcγRIIb-232T allele is associated with aggressive periodontitis and susceptibility to SLE (Chu et al 2004, Lee et al 2009, Siriboonrit et al 2003, Yasuda et al 2003). In addition, the 2B.4 promoter haplotype has been found to be overrepresented in SLE patients compared to controls (Su et al 2004b).

1.1.6.1.3. FcγRIIc

Characteristics. The *FCGR2C* gene is composed of 5' exons >99% identical to that of *FCGR2B* (5' untranslated, signal, extracellular, and transmembrane), and 3' exons >95% identical to *FCGR2A* (cytoplasmic and 3' untranslated) (Brooks et al 1989). Four different alternatively spliced transcripts have been identified (Metes et al 1998). Of these, transcript FcγRIIc1 expresses a functional molecule, FcγRIIc2 has a 14-nucleotide insertion between the intracytoplasmic exons C2 and C3, FcγRIIc3 has a spliced out C2 exon, and FcγRIIc4 possesses an 85-nucleotide insertion between the second extracellular exon (EC2) and transmembrane exon (TM). *FCGR2C* is considered a pseudogene (a gene that lacks essential DNA sequences required for function) as the functional receptor is only expressed in approximately 30% of individuals where it is then detectable on B lymphocytes, NK cells, neutrophils, and monocytes (Metes et al 1998, van der Heijden et al 2012). When expressed, FcγRIIc adds to the repertoire of activating receptors that mediates innate immune effector functions such as ADCC (van der Heijden et al 2012). On B cells it counterbalances the negative feedback of FcγRIIb and enhances humoral responses to immunization as demonstrated in a human anthrax vaccine trial (Li et al 2013).With its extracellular domain identical to that of FcγRIIb, it binds the IgG subclasses with equal affinity to FcγRIIb (Bruhns et al 2009, Metes et al 1999). In contrast to FcγRIIb, however, it initiates cell activation through an intrinsic ITAM (Metes et al 1999). On NK cells it is capable of mediating ADCC, while on B lymphocytes it counterbalances the negative feedback of FcγRIIb (Li et al 2013, Metes et al 1998, van der Heijden et al 2012).

Variability. Functional variability of FcγRIIc largely pertains to sequence variants that predict the expression of a functional FcγRIIc molecule. A nonsynonymous variant located in the EC1 extracellular exon (c.169C>T; p.Q57*), changes the common allele (c.169T), which encodes a translation termination codon at residue 57, to c.169C, which encodes an

open reading frame for glutamine (Metes et al 1998). However, not all individuals bearing the p.Q57 allele express functional FcγRIIc. Two intronic sequence variants each located within a splice site further define FcγRIIc expression through alternative splicing of the FcγRIIc transcript. These variants are c.798+1G>A at the donor and c.799-1G>C at the acceptor splice site located in the intron linking exons C2 and C3 (Ernst et al 2002, van der Heijden et al 2012). For individuals bearing the c.798+1A/c.799-1G combination, the intracytoplasmic exon C2 is spliced from the transcript and a premature stop codon inserted in exon C3. Similarly, the c.798+1A/c.199-1G combination causes splicing of the C2 exon from the transcript, but inserts an additional 62 nucleotides. As a result, only the c.169C/c.798+1G/c.799-1G genotype yields a functional FcγRIIc molecule, while the c.169C/c.798+1A/c.799-1G and c.169C/c.798+1A/c.799-1C genotypes do not.

One constituent of the *FCGR2B* 2B.4 promoter haplotype, c.-386G>C, is detectable in the promoter region of *FCGR2C* (Breunis et al 2008). It is thought to also modify FcγRIIc expression levels, but this has not been formally demonstrated. A variant that does affect FcγRIIc expression levels is *FCGR2C* gene copy number variation (discussed in Section 1.6.3.)

Clinical significance of variability. The FcγRIIc-mediated enhancement of B lymphocyte activation likely predisposes to humoral autoimmunity as suggested by the association of functional FcγRIIc with SLE (Li et al 2013). Furthermore, expression of this activating FcγR has been associated with pathologies characterized by excessive or inappropriate leukocyte activation. In particular, expression of the functional receptor has been associated with idiopathic thrombocytopenic purpura and disease severity in rheumatoid arthritis (Breunis et al 2008, Stewart-Akers et al 2004).

1.1.6.2. FcγRIII

The FcγRIII molecules are extensively glycosylated with an apparent molecular weight of 50-80 kDa (Ravetch & Perussia 1989). The two FcγRIII isoforms are encoded by distinct genes, *FCGR3A* (FcγRIIIa) and *FCGR3B* (FcγRIIIb), each comprising five exons: two encode the signal peptide (S1 and S2), two encode the extracellular region (EC1 and EC2), and one encodes the transmembrane region (TM/C). Nucleotide differences in the promoter and

intronic enhancer regions of *FCGR3A* and *FCGR3B* confer tissue-specific transcriptional activities (Gessner et al 1995b). *FCGR3A* and *FCGR3B* share a ~97% sequence identity in both the coding and flanking regions, and encode molecules that are almost identical (Gessner et al 1995a, Ravetch & Perussia 1989). The amino acid differences between the isoforms yield different *N*-linked glycosylation patterns and membrane expression (Ravetch & Perussia 1989). In particular, a C to T substitution at nucleotide 733 introduces a translation termination codon at amino acid 233 in FcγRIIIb, yielding a 25 amino acid shorter molecule (Ravetch & Perussia 1989). As a result, FcγRIIIb lacks a transmembrane domain and is linked to the surface via a glycosylphosphatidylinositol anchor, whereas FcγRIIIa is a transmembrane protein possessing an intracytoplasmic domain.

1.1.6.2.1. FcγRIIIa

Characteristics. Alternative splicing and usage of alternate transcriptional start sites in two discrete 5' terminal exons yield four different FcγRIIIa transcripts (FcγRIIIa1-4). Of these, FcγRIIIa1 translates a functional membrane protein (Gessner et al 1995b). Efficient surface expression of the ligand-binding FcγRIIIa α-chain requires the presence of the FcRγ-chain and/or the CD3ζ-chain for protection from degradation in the endoplasmic reticulum (Hulett & Hogarth 1994, Kurosaki & Ravetch 1989, Ra et al 1989). FcγRIIIa is constitutively expressed on NK cells, macrophages, γδ T-cell receptor (TCR) T lymphocytes, and a subset of monocytes. It is also present on pre-B lymphocytes and small subsets of $CD4^+$ and $CD8^+$ T lymphocytes (Chauhan & Moore 2012, Clemenceau et al 2008, de Andres et al 1999, Gessner et al 1995b). FcγRIIIa mediates ADCC, phagocytosis, and cytokine release (Anderson et al 1990, Wu et al 1997). Compared to other low affinity FcγRs, FcγRIIIa has the highest affinity for IgG3 (K_A: ~9×10^6 *versus* ≤1×10^6 M^{-1}). It binds IgG1 with an affinity of K_A: 1-2 × 10^6 M^{-1}, IgG4 with K_A: 2×10^5 M^{-1} and IgG2 with K_A: 5×10^6 M^{-1} (Bruhns et al 2009).

Variability. The affinity of FcγRIIIa for IgG and the efficiency by which it initiates effector mechanisms is affected by a polymorphism that predicts a phenylalanine (F) to valine (V) at amino acid residue position 158 (Breunis et al 2009, Koene et al 1997, Wu et al 1997). Compared to the FcγRIIIa-158F allele, the FcγRIIIa-158V allele displays a higher affinity for IgG1 and IgG3 as well as a capacity to bind IgG4 (Wu et al 1997). Consequently,

NK cells bearing the FcγRIIIa-258V allele exhibit enhanced IgG-induced NK cell activation and ADCC capacity (Vance et al 1993, Wu et al 1997). In addition, *FCGR3A* is subject to gene copy number variability (discussed in Section 1.6.3.).

Clinical significance of variability. The low-binding FcγRIIIa-158F allele has been identified as a risk factor for SLE, although the association appears to be ethnically confined (Li et al 2010), while the FcγRIIIa-158V allele has been associated with rheumatoid arthritis susceptibility and severity, and idiopathic inflammatory myopathies (Bronner et al 2009, Lee et al 2008, Morgan et al 2000). FcγRIIIa-mediated ADCC has been demonstrated to contribute substantially to the therapeutic action of rituximab – a chimeric anti-CD20 IgG1 monoclonal antibody used for treatment of non-Hodgkin's lymphoma (Clynes et al 2000). Individuals homozygous for the FcγRIIIa-158V allele had an enhanced clinical and molecular response to rituximab compared to individuals bearing an FcγRIIIa-158F allele (Cartron et al 2002).

1.1.6.2.2. FcγRIIIb

Characteristics. FcγRIIIb is unique among the family of FcγR in that it lacks a transmembrane region and is linked to the outer plasma membrane layer by a glycosylphosphatidylinositol anchor. FcγRIIIb is encoded by a single transcript (Gessner et al 1995a). Its expression is restricted to neutrophils and basophils (low levels) (Meknache et al 2009). At present, the biological function of FcγRIIIb on basophils is unknown. On neutrophils, however, it is involved in phagocytosis, degranulation, respiratory burst, and the formation of neutrophil extracellular traps (NETs) (Behnen et al 2014). FcγRIIa and FcγRIIIb are both constitutively expressed by neutrophils with overlapping ligand specificity (Bruhns et al 2009). These receptors appear to have a complementary and synergistic relationship for mediating phagocytosis, respiratory burst, and ADCC (Hunt et al 2003, Kushner & Cheung 1992, Marois et al 2011, Nagarajan et al 2000, Salmon et al 1991, Salmon et al 1995). Evidence suggests a preponderant role for FcγRIIIb in mediating a respiratory burst response compared to phagocytosis (Hundt & Schmidt 1992, Marois et al 2011). FcγRIIIb binds IgG3 with a greater affinity than IgG1 (K_A: 1×10^6 M^{-1} versus 2×10^5 M^{-1}) and has no detectable affinity for IgG2 and IgG4 (Bruhns et al 2009).

Variability. FcγRIIIb bears the human neutrophil antigens (HNA)-1a, -1b, and -1c (formerly neutrophil antigen [NA] 1, NA2, and SH, respectively) (Bux et al 1997, Ory et al 1989b). Auto- and allo-immunization against these antigens result in autoimmune neutropaenia, neonatal immune neutropaenia and transfusion acute lung injury (Youinou et al 2002). Several lines of evidence suggest that these allotypes are not only significant antigenically, but also functionally (Bredius et al 1994b, Salmon et al 1990, van der Heijden et al 2014).

HNA1a and HNA1b differ at five nucleotides and four amino acid residues – p.36R^{a}>S^{b}, p.65N^{a}>S^{b}, p.82D^{a}>N^{b}, and p.106V^{a}>I^{b} (Ory et al 1989a, Ravetch & Perussia 1989). HNA1b and HNA1c differ at one additional position, p.78A^{b}>D^{c} (Bux et al 1997). The differences between HNA1a and HNA1b potentially alter the *N*-linked glycosylation pattern and primary protein structure, while the amino acid change in HNA1c is believed to affect the receptor's tertiary structure (Bux et al 1997, Ory et al 1989a, Ravetch & Perussia 1989). The different allotypes do not display detectable differences in antibody binding affinity or IgG subclass specificity, likely due to all the variants occurring distal to the ligand contact regions (Bruhns et al 2009, Sondermann et al 2000). Nevertheless, independent of FcγRIIa phenotype, neutrophils from HNA1b homozygous individuals display an approximately 20% lower phagocytic capacity compared to those of HNA1a homozygous individuals (Bredius et al 1994b, Salmon et al 1990). Furthermore, neutrophils from HNA1a homozygous individuals exhibit a greater respiratory burst response compared to neutrophils from HNA1b homozygous individuals (Urbaczek et al 2014). A recent study demonstrated that IgG-induced production of reactive oxygen species by resting neutrophils is determined by the FcγRIIa/FcγRIIIb haplotype, such that production levels are in the rank order of 131HH-HNA1b|1b > 131RR-HNA1a|1a > 131HH-HNA1a|1a > 131RR-HNA1b|1b (van der Heijden et al 2014). These differences, however, are not apparent for pre-activated neutrophils. Interestingly, pre-activated neutrophils display allotype-specific differences in FcγRIIIb surface shedding and induced expression of the inhibitory FcγRIIb (van der Heijden et al 2014). Sloughing of FcγRIIIb molecules from the cell surface is reportedly greater for HNA1a homozygous and heterozygous individuals compared to HNA1b homozygous individuals. Furthermore, activated neutrophils from

HNA1a individuals express the highest levels of the inhibitory FcγRIIb, followed by heterozygous individuals, and HNA1b homozygous individuals with near undetectable levels. At present, the functional significance of HNA1c is unknown.

Clinical significance of variability. In adult periodontitis, a disease caused by periodontopathic bacteria and concomitant host inflammatory responses, homozygosity for the low responder FcγRIIIb-HNA1b allotype is a risk factor for disease recurrence and severity (Dimou et al 2010, Kobayashi et al 1997). In addition, homozygosity for the FcγRIIIb-HNA1b allotype has been associated with severe Guillain–Barrè syndrome (van Sorge et al 2005).

1.1.3.6. Copy number variability

Gene copy number variability (CNV) is a recognized contributor to inter-individual differences with CNV regions accounting for ~5% of the human genome (McCarroll et al 2008). *FCGR2C*, *FCGR3A*, and *FCGR3B*, but not *FCGR2A* and *FCGR2B* have been detected at variable copy number (Breunis et al 2008). Distinct overlapping segments within the *FCGR3A-FCGR2C-FCGR3B* genomic region are duplicated and deleted (Figure 1.5). The most common of these encompasses *FCGR2C* and *FCGR3B*, previously designated CNV Variant I and copy number region 1 (CNR1) (Breunis et al 2009, Niederer et al 2010). Deletion of this section juxtaposes the 5'-regulatory sequences of *FCGR2C* with the coding sequence of *FCGR2B*, creating a chimeric gene, *FCGR2B'* (Mueller et al 2013). The result is that FcγRIIb, which is otherwise absent from cytotoxic NK cells, is expressed on this cell subset where it inhibits cell activation and ADCC, possibly due to co-engagement of FcγRIIIa and FcγRIIb (Mueller et al 2013, van der Heijden et al 2012). In addition to modifying the cellular distribution of FcγRIIb, CNV at the *FCGR* locus contributes to inter-individual FcγR phenotypic and functional differences through a gene-dosage effect. FcγRIIIa gene copy number has been found to correlate with FcγRIIIa expression levels on NK cells as well as antibody-dependent cytotoxic capacity (Breunis et al 2009). Similarly, CNV of *FCGR3B* directly correlates with protein expression, neutrophil uptake of and adherence to immune complexes (Breunis et al 2009, Huizinga et al 1990b, Willcocks et al 2008).

Clinical relevance of variability. *FCGR3A* variability is associated with anti-glomerular basement membrane antibody disease (Zhou et al 2010), while low *FCGR3B* copy number is a risk factor for lupus nephritis and SLE (Aitman et al 2006, Niederer et al 2010), rheumatoid arthritis (Graf et al 2012), and systemic sclerosis (McKinney et al 2012).

1.2. HUMAN IMMUNODEFICIENCY VIRUS

Human immunodeficiency virus (HIV) is a member of the genus *Lentivirus*, in the family *Retroviridae*, subfamily *Orthoretrovirinae*. Phylogenetic analysis indicates that multiple transmission events from simian species have introduced two genetically diverse types of HIV into the human population: HIV-1, which is closely related to simian immunodeficiency virus (SIV) from chimpanzees (SIV_{cpz}), and HIV-2, which is closely related to SIV from sooty mangabeys (SIV_{sm}). HIV-2 is characterised by reduced transmissibility and virulence (Gilbert et al 2003). Epidemiologically, HIV-2 infections are largely confined to West Africa, while HIV-1 extends worldwide. Further discussion in this review will focus on HIV-1.

1.2.1. Epidemiology

Using the earliest HIV-1 archival sample obtained from a 1959 Kinshasa resident, a recent study has traced the origins of the human immunodeficiency virus type 1 (HIV-1) pandemic to 1920's Kinshasa, Democratic Republic of Congo (Faria et al 2014, Zhu et al 1998). It is believed that rapid population growth, the sex trade, and railways allowed HIV-1 to spread in the community and to other regions of the world. Since the start of the pandemic, more than 78 million people have been infected with HIV-1 and 39 million people have died (UNAIDS 2014a). While prevention and treatment programmes have seen to a decline in new infections, HIV/AIDS remains a significant global burden. At the end of 2013, an estimated 35 million people were living with HIV-1 with 2.1 million new infections and 1.5 million AIDS-related deaths (UNAIDS 2014b). Sub-Saharan Africa remains the region hardest hit by the epidemic, with an estimated 24.7 million HIV-1 infected individuals. Of all affected countries, South Africa bears the greatest burden with 6.3 million people living with HIV-1, 18% of the global total (UNAIDS 2014b).

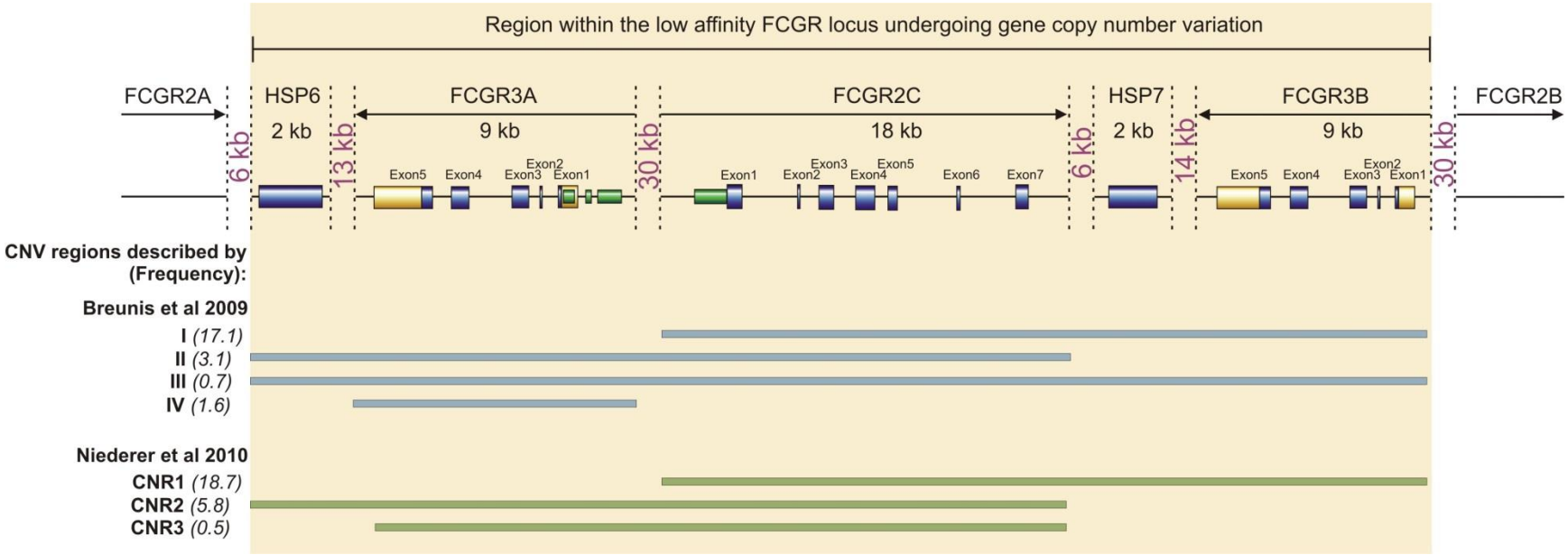


Figure 1.5. *FCGR* gene copy number variability. The gene cluster that encompasses *HSP6*, *FCGR3A*, *FCGR2C*, *HSP7* and *FCGR3B* is subject to copy number variation which results from duplication or deletion of large genomic segments (45,000 – 68,000 base pairs). These copy number regions (CNR) were previously designated Variant I-IV and CNR1-3 (Breunis et al 2009, Niederer et al 2010). The FCGR genes are arranged according to their position on chromosome 1. The black arrows indicate the orientation of the gene. Distances between genes are shown. Illustration by Ria Lassaunière.

1.2.2. The virus

HIV-1 is a 100 to 120 μm enveloped virus containing a cone-shaped, cylindrical capsid (Gonda et al 1985). Each virion bears two copies of a ~9.7 kilobase positive-sense, single-stranded RNA which encodes 15 proteins: structural proteins that include matrix (MA), nucleocapsid (CA), and envelope proteins gp120 and gp41; viral enzymes that include reverse transcriptase (RT), integrase (IN), and protease (PR); accessory proteins that include viral protein u (Vpu), viral infectivity factor (Vif), viral protein r (Vpr), negative regulatory factor (Nef), regulator of virion (Rev), trans-activator of transcription (Tat), and P6 . The structural proteins and enzymes are initially synthesized as polyproteins: Gag, comprising the viral capsid proteins; Pol, comprising the viral enzymes; and Env, comprising the envelope associated proteins. The polyprotein precursors are processed by viral or cellular proteases into mature, particle-associated proteins. The accessory proteins are the primary translation products of spliced mRNA (Freed & Martin 2013).

1.2.3. HIV-1 life cycle

The primary target of HIV-1 is activated $CD4^+$ T lymphocytes. Other cells bearing CD4 and chemokine receptors are also susceptible to infection, including resting $CD4^+$ T lymphocytes, monocytes and macrophages, and dendritic cells. Efficient infection of a target cell begins with the adsorption of cell-free virions to the cell surface and the sequential interaction of the gp120 envelope glycoprotein with CD4 and chemokine co-receptors (Figure 1.5) (Maddon et al 1986). This is followed by fusion of the viral and cellular membranes and the partial uncoating of incoming virions (Didigu & Doms 2012). The viral core undergoes rearrangement to become the reverse transcription complex which comprises the viral capsid and nucleocapsid, viral genome, reverse transcriptase, integrase, protease, and the viral accessory proteins Vif, Nef, and Vpr. Reverse transcription of the viral genome occurs in the cytoplasm, and the double-stranded DNA product within a pre-integration complex is transported to the nucleus where integration into host chromosomal DNA is mediated by the virus encoded integrase (Bowerman et al 1989). Integrated viral DNA, also known as the provirus, serves as a template for transcription and translation of viral proteins. Envelope and Gag plus Gag-Pol polyproteins are transported

to the plasma membrane, where progeny virus buds from cells and are released as immature particles. Maturation of virions is completed following proteolysis by virion-encoded protease (Freed & Martin 2013).

1.2.4. Transmission

The majority of HIV-1 infections occur through sexual exposure when HIV-1 containing fluids (semen, vaginal secretions, and anal secretions) cross mucosal surfaces in the genital tract and rectum. Virus likely crosses mucosal epithelium by transcytosis, through direct contact with dendrites of intraepithelial dendritic cells, or cell-to-cell contact of infected T lymphocytes in seminal, vaginal, or anal fluids (Anderson et al 2010, Cunningham et al 2013, Gupta et al 2013). HIV-1 transmission also occurs through blood transfusions, needle-sharing injection drug-use, percutaneous needle stick, and vertically from an HIV-1 infected mother to her foetus/infant (Smith et al 2005).

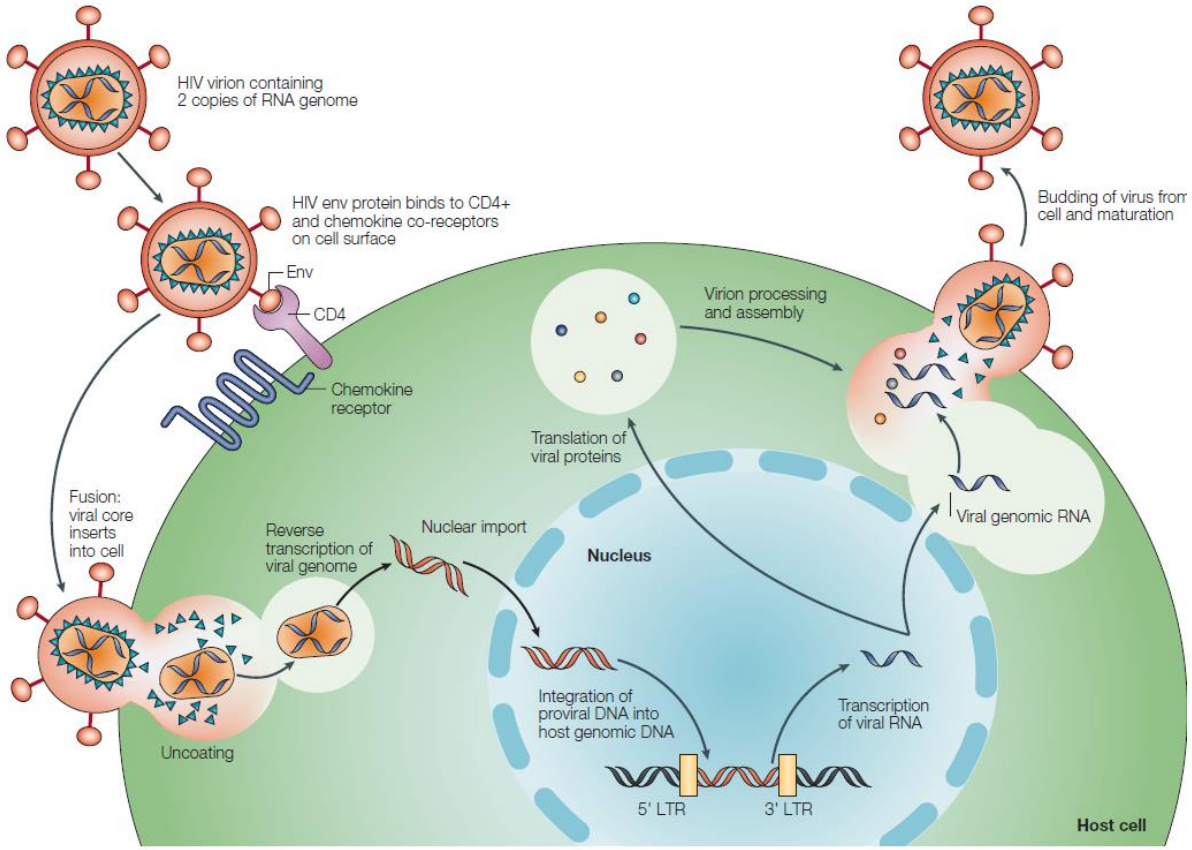


Figure 1.6. HIV-1 life cycle. Reproduced from (Rambaut et al 2004).

1.2.5. HIV-1 infection and disease progression

The clinical course of an HIV-1 infection generally includes three phases: 1) primary infection, 2) clinical latency, and 3) AIDS-defining illness. In ~80% of individuals, productive clinical HIV-1 infection is caused by a single transmitted/founder virus (Abrahams et al 2009, Keele et al 2008, Salazar-Gonzalez et al 2009). It is still unclear whether HIV-1 is transmitted as cell-free or cell-associated virus, but simian immunodeficiency virus (SIV) can be transmitted in either form (Sodora et al 1998). Following transmission, infection is likely established in local tissue(s) at the site of exposure as demonstrated for SIV in rhesus macaques (Whitney et al 2014). Virus in the systemic circulation remains undetectable for approximately 10 days. At the end of this eclipse phase, cell-free and/or cell-associated virus reaches the draining lymph node where infection of activated $CD4^+$ T lymphocytes occurs. HIV-1 replication increases rapidly and the virus is efficiently disseminated throughout the body to other lymph nodes. Plasma viraemia increases exponentially to reach a peak after ~4 weeks, which is followed by a decrease in viral load over 12-20 weeks to reach a more stable level, known as the viral set point (Fiebig et al 2003). During the exponential phase of viral replication, CD4+ T lymphocytes are progressively depleted, but rebound to near normal levels when the viral set point is reached and the virus is under immunological control. Through a balance between virus turnover and the immune response, this set point is maintained for a number of years and is known as the clinical latency phase. During this period, circulating $CD4^+$ T lymphocytes slowly decline (Embretson et al 1993, Pantaleo et al 1993). Eventually control of the virus is lost leading to increasing viraemia, a rapid loss of $CD4^+$ T lymphocytes, and AIDS defining symptoms.

In the absence of treatment, HIV-1 infected individuals show variable rates of disease progression and virus control (Mellors et al 1996). The majority (70-80%) of HIV-1 infected individuals experience a period of clinical latency for 6-8 years and are referred to as typical progressors (Pantaleo & Fauci 1996). Ten to 15% of HIV-1 infected individuals display unusually rapid immunological and clinical progression, with two to three years from primary infection to AIDS. In contrast, a small percentage (>5%) maintain good control of infection and remain asymptomatic for several years (slow progressors or viral

controllers). The determinants are likely multifactorial and include a complex interplay between the virulence of the infecting virus and the immune capability of the infected individual as determined, in part, by host genetic factors (O'Brien & Hendrickson 2013). Generally, the immunological control following peak viraemia during acute HIV-1 infection associates with the rate of disease progression. A higher viral set point associates with rapid disease progression, whereas a low viral set point is observed in cases of slow progression to AIDS (Mellors et al 1996). Viral elite controllers suppress plasma HIV-1 RNA levels to undetectable limits (<50 RNA copies/ml) in the absence of antiretroviral treatment.

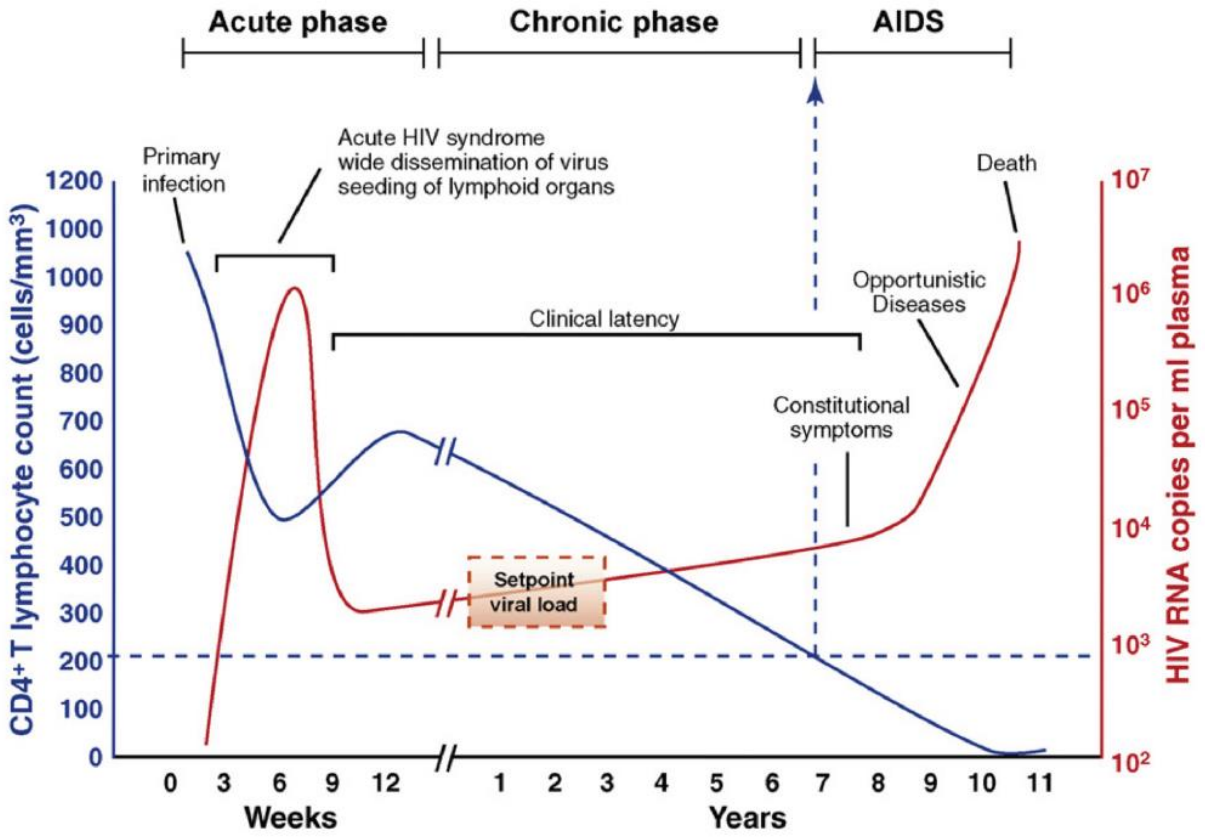


Figure 1.7. Typical disease course in an HIV-1 infected individual. Reproduced from (O'Brien & Hendrickson 2013).

1.2.6. Antibody response to HIV

Overview. The first B cell responses occur within one week of detectable viraemia (~18 days after transmission) and are detected as IgM and IgG containing immune complexes (Tomaras et al 2008). Free antibody against *env*-gp41 occurs a few days later, with anti-gp120 antibodies delayed an additional few weeks (Tomaras et al 2008). Initial anti-HIV-1 antibodies are of the IgM class and are followed by a class switch to IgG and IgA (Tomaras et al 2008). Mathematical modelling indicate that these early HIV-1-specific responses are ineffective against HIV-1 and do not contribute to the initial decline in plasma viral load (Tomaras et al 2008). Autologous neutralising antibody responses develop slowly and do not occur until months after HIV-1 transmission (Gray et al 2007, Richman et al 2003, Wei et al 2003). While these antibody responses can control the virus quasispecies present at the time of their appearance, the narrow neutralization capacity allows for rapid viral escape (Richman et al 2003, Wei et al 2003). Broadly neutralizing anti-HIV-1 antibodies eventually develop in ~20% of patients, many years after transmission has occurred (Gray et al 2009, Stamatatos et al 2009).

IgG subclasses. The IgG subclasses are differentially induced during an HIV-1 infection. IgG1 is the dominant subclass in anti-HIV-1 responses and remain stable during acute and chronic HIV-1 infection, but decline in progressing patients (Broliden et al 1989, Klasse & Blomberg 1987, Mergener et al 1987, Sundqvist et al 1986, Yates et al 2011). Anti-gp120 IgG2 occurs during various stages of HIV-1 infection and is detectable in the majority of patients, albeit at lower levels compared to IgG1 (Forthal et al 2007b, Klasse & Blomberg 1987, McDougal et al 1987). Anti-HIV-1 IgG3 antibodies peak during acute infection and decline thereafter. Anti-HIV-1 IgG4 occurs through all stages of HIV-1 infection, but at lower levels and is not detectable in all individuals (Ljunggren et al 1988, McDougal et al 1987).

HIV-1-specific IgG1 efficiently mediate ADCC of infected cells, whereas IgG3 isolated from chronically infected individuals does not (Ljunggren et al 1988). This is likely attributable to differences in subclass protein specificity and half-life. The response to Env proteins is nearly restricted to IgG1 (Ngo-Giang-Huong et al 2001), whereas anti-HIV-1 IgG3 is primarily directed against Gag (peak: 4.67 μg/ml) and gp41-specific IgG3 (1.49

μg/ml), with low peak titres (<0.1 μg/ml) for IgG3 against p31 (integrase), p66 (reverse transcriptase), and gp120 (Broliden et al 1989, Yates et al 2011). Moreover, the half-life of gag-specific IgG3 is much longer compared to Env-specific IgG3 (Yates et al 2011).

Interestingly, enhanced levels of HIV-1-specific IgG2 have been associated with long-term viral control (Ngo-Giang-Huong et al 2001). These IgG2 responses did not exhibit neutralizing activity and is thought to rather be the consequence or marker of the beneficial Th1 anti-HIV-1 cellular response (Ngo-Giang-Huong et al 2001). The association of these IgG2 responses with effector functions have not been explored.

Immune complexes. As a result of the high level of HIV-1-specific IgG, a proportion of circulating virus occurs as immune complexes. During the acute phase of infections approximately 22% of circulating plasma virions occur as immune complexes, whereas IgG-HIV-1 immune complexes comprise 17-67% of circulating virus during chronic HIV-1 infection (Liu et al 2011). Insufficiently neutralized IgG-HIV-1 immune complexes remain infectious *in vitro* (Liu et al 2011). In addition, cell-bound IgG-HIV-1 has been demonstrated to be highly infectious for T lymphocytes (Jakubik et al 2000). Thus, despite being coated by IgG, HIV-1 likely retains its infectivity.

1.2.7. FcγR in HIV-1 infection

Beyond neutralization, HIV-1-specific IgG has the capacity to recruit potent innate immune effector functions through engagement of its Fc portion with FcγRs. The significance of FcγR-mediated effector functions have been demonstrated in murine and non-human primate models where these mechanisms augment the *in vivo* ability of broadly neutralizing antibodies to block viral entry, suppress viraemia, and confer therapeutic activity (Bournazos et al 2014, Hessell et al 2007). Accumulating data suggest that FcγR-mediated effector functions play a significant role in HIV-1 protective immunity, for both blocking acquisition of HIV-1 and post-infection control of viraemia (Lewis 2014).

Blocking acquisition. Investigating the capacity of FcγR-mediated effector functions in blocking HIV-1 acquisition *in vivo* is limited to animal models and human vaccine candidates that showed efficacy. In a recent study, two non-neutralizing monoclonal antibodies (mAbs; 246-D and 4B3) with potent ADCC and Fc-mediated inhibitory activities

were tested for their ability to protect macaques from vaginal challenge with SHIVSF162P3 (Moog et al 2014). Topical vaginal application of this antibody combination did not prevent infection, but reduced plasma viral load. These findings suggest that non-neutralizing antibodies affected subsequent viral replication and dissemination through other effector functions. Moreover, they potentially protected components of the immune system in early infection, allowing maturation of responses and effective post-infection control of viraemia. Similarly, FcγR-mediated effector functions correlated with post-infection control of viraemia in vaccinated non-human primates (Barouch et al 2012, Demberg et al 2013, Florese et al 2009, Gomez-Roman et al 2005, Xiao et al 2010).

In humans, two vaccines have shown a correlation between antibody-dependent cellular virus inhibition (ADCVI) and ADCC antibody titres. In the VAX004 trial, vaccine-induced ADCVI activity correlated inversely with HIV-1 infection risk, although no overall protection was observed (Forthal et al 2007a). Similarly, in the RV144 trial where a modest overall efficacy of 31% was observed, HIV-1 infection risk inversely correlated with ADCC responses in vaccinees (Haynes et al 2012, Rerks-Ngarm et al 2009). The target for these responses was similar in the majority of vaccinees and involved the epitope recognized by A32 mAb, since A32 Fab fragment blocked ADCC activity in 96% of vaccinees with ADCC responses (Bonsignori et al 2012). The A32 mAb was isolated from a chronically HIV-1 infected person and is a potent mediator of ADCC (Ferrari et al 2011). Further support for a role of FcγR-mediated effector functions in blocking acquisition comes from the inverse correlation of breast milk ADCC titres and HIV-1 transmission to exposed infants (Mabuka et al 2012).

Post-infection control of viraemia. FcγR-mediated effector functions have also been associated with a favourable outcome of HIV-1 disease in natural infection cohorts and non-human primates. Particularly, ADCC appears to contribute to post-infection control of viraemia (Ahmad et al 1994, Ahmad et al 2001, Broliden et al 1993, Forthal et al 2001, Lambotte et al 2013, Ljunggren et al 1987, Sawyer et al 1990). At present, data on the involvement of other FcγR-mediated mechanisms in HIV-1 acquisition and disease progression are limited. A role for phagocytosis is suggested by the FcγRIIa-dependent inhibition of HIV-1 replication in immature monocyte-derived dendritic cells in the

presence of HIV-1-specific IgG (Holl et al 2006a, Holl et al 2006b). It is unclear when phagocytosis first occurs in an HIV-1 infection. The increased expression of FcγRIIa and FcγRIIIa on monocytes and dendritic cells, and the concomitant enhanced phagocytic capacity, during acute infection suggests a role for phagocytosis early in infection (Dugast et al 2011). However, phagocytosis is impaired during chronic infection and is associated with the downregulation of FcγRIIa and FcγRIIIa on monocytes and dendritic cells (Dugast et al 2011). Further studies are needed to define the contribution of antibody-dependent phagocytosis to HIV-1 protective immunity.

1.2.8. Mother-to-child transmission of HIV-1

Mother-to-child transmission (MTCT), or vertical transmission, is the leading cause of HIV-1 infection in children under 10 years of age. In the absence of any interventions, 25-40% of infants acquire HIV-1 perinatally (Connor et al 1994, Forbes et al 2012). The determinants of MTCT risk are multi-factorial and include viral, host, and obstetric factors. Through effective antiretroviral therapy, elective caesarean section and avoidance of breastfeeding, MTCT rates have been reduced to 1-3% in Northern America and Europe (Forbes et al 2012, Townsend et al 2008). In 2011, MTCT rates were 2.8% in South Africa (Barron et al 2013). However, in resource-limited countries MTCT rates remain high.

Timing. Perinatal transmission of HIV-1 can occur while the child is *in utero*, during labour and delivery (intrapartum), or through breastfeeding. *In utero* transmission is distinguished from intrapartum transmission based on virologic detection in the first two days of life *versus* after the first week. *In utero* transmission is the least common form of MTCT with transmission rates ranging from 5-10% (Lehman & Farquhar 2007). It is thought to occur primarily in the last few weeks before delivery and result from transplacental transfer of the virus or HIV-1 infected amniotic fluid crossing foetal mucous surfaces (Rouzioux et al 1995). Intrapartum transmission rates are approximately 10-20% and account for approximately two-thirds of MTCT (Lehman & Farquhar 2007). Exposure to the virus occurs through contact with maternal blood and cervicovaginal secretions as the neonate passes through the birth canal. While HIV-1 levels in breast milk are significantly lower than those in circulation, breast feeding remains a major risk for MTCT of HIV-1 (Rousseau et al 2004). Postnatal transmission rates of 5-15% are reported for

mothers who breastfeed (Lehman & Farquhar 2007). It should be noted that the timing of initial exposure of the foetus/infant to HIV-1 may not necessarily be when infection is established, as was suggested by a study that detected unintegrated, biologically active virus in the peripheral blood mononuclear cells of approximately 18% of exposed-uninfected infants (Lee et al 2004).

Mechanism. The exact biological mechanisms of perinatal HIV-1 transmission are unclear. Several lines of evidence suggest that cell-free and cell-associated viruses are transmitted to the infant (Milligan & Overbaugh 2014). Both infectious components have been detected in maternal peripheral blood, genital secretions, and breast milk, with virus levels in all these fluids correlating with MTCT (Garcia et al 1999, John et al 2001, Rousseau et al 2004). Transmission of cell-free virus through amniotic fluid during pregnancy is unclear and controversial. As such, it is hypothesized that *in utero* infection occurs across the placenta with a preponderant role for cell-associated virus. *In vitro* studies have demonstrated that placental trophoblasts are not very permissive to infection by cell-free virus, while cell-associated virus is able to cross the placenta and cause productive infection in target cells (Ayouba et al 2008). However, recent findings suggest a role for the neonatal Fc receptor (FcRn) in mediating transcytosis of infectious HIV-IgG complexes across epithelial cells (Gupta et al 2013). FcRn is expressed on syncytiotrophoblasts where it facilitates transplacental transfer of maternal IgG to the foetus (Simister 2003). It remains to be determined if FcRn provides a potential route for placental transfer of HIV-1. Intrapartum transmission is thought to occur across infant mucosal surfaces when exposed to maternal blood and cervicovaginal secretions. HIV-1 RNA (cell-free) and DNA (cell-associated) levels correlate with intrapartum transmission (Chuachoowong et al 2000, John et al 2001, Montano et al 2003, Panther et al 2000, Tuomala et al 2003). However, studies that examined both HIV-1 DNA and RNA levels in cervicovaginal secretions reported a stronger correlation between HIV-1 DNA levels and intrapartum transmission, compared to HIV-1 RNA levels (John et al 2001, Tuomala et al 2003).

Risk factors. Risk factors for perinatal transmission of HIV-1 are well defined (Kourtis & Bulterys 2010). Maternal virologic and immunologic factors that include high plasma viral load, low $CD4^+$ T cell count, and advanced disease increase the risk of MTCT. While

transmission risk directly correlates with maternal plasma viral load, there is no threshold above which transmission is an absolute certainty or a threshold below which there is no risk of transmission (Garcia et al 1999). Additional risk factors include inflammation of the placenta, maternal genitourinary lesions, breast pathology, vaginal birth, invasive obstetrical procedures, and duration of membrane rupture (Kourtis & Bulterys 2010). Genetic polymorphisms that associate with maternal infectiousness and infant susceptibility have been described for a variety of immunologically relevant molecules [reviewed by (De Souza et al 2012)].

Interventions. Highly active antiretroviral therapy, obstetric interventions, and alternatives to breastfeeding effectively reduce MTCT (Forbes et al 2012, Townsend et al 2008). Initiation of antiretroviral therapy before conception or early in pregnancy is able to reduce maternal plasma viral load to very low or undetectable limits. Short-course zidovudine (AZT) given daily during late gestation acts to reduce maternal viral load by delivery in both plasma and genital secretions. Single dose nevirapine given at the onset of labour cannot reduce maternal viral load by delivery. However, it is rapidly absorbed and crosses the placenta where it is thought to act as pre- and/or post-exposure prophylaxis to the infant. It has a long half-life (45 hours in neonates), and thus provide protection during the first few days of life. Additional interventions that further reduce the risk of MTCT include caesarean section before labour and before rupture of membranes and avoidance of breastfeeding (Read & Newell 2005).

MTCT as a model for protective immunity. The majority of persons exposed to HIV-1 do not become infected. HIV-1 transmission per coital act is less than 1% in heterosexual couples, while 65-85% of infants born to HIV-1 infected mothers escape infection (Boily et al 2009). Understanding the mechanisms that confer this natural resistance to HIV-1 infection will complement rational vaccine design and the development of novel preventative and therapeutic anti-HIV-1 strategies. Investigating correlates of protection for sexual transmission is hindered by the difficulty of quantitating HIV-1 exposure and collecting relevant biological samples and accurate behavioural data at the time of exposure. Conversely, mother-to-child transmission is an attractive model in which to study immune correlates of protection since both members of the transmitting dyad are

known, timing of transmission can be ascertained with reasonable precision, and it affords the opportunity to assess factors contributing to both the infectiousness of the transmitter (mother) and susceptibility of the recipient (infant) (Aldrovandi & Kuhn 2010, Braibant & Barin 2013). Limitations of this model are that transmission occurs between genetically similar individuals, exposure to the HIV-1 occurs at a time of early immune development, and immune circumstances during pregnancy that are associated with tolerance of the foetal allograft (Tiemessen & Kuhn 2006). Nevertheless, it provides a unique opportunity to investigate the role of FcγR-mediated effector functions, since the individual (foetus/infant) at risk is passively immunized with HIV-1-specific antibodies through transplacental transfer of IgG from the HIV-1 infected mother and the model is not confounded by interspecies differences as observed for non-human primate studies (Trist et al 2014).

1.3. OBJECTIVES OF THIS STUDY

The overall aim of this work was to characterize *FCGR*/FcγR variability in the South African population and to indirectly assess the contribution of FcγR-mediated effector functions in HIV-1 protective immunity by evaluating FcγR functional variants in the context of HIV-1 transmission and disease progression.

- *FCGR* gene copy number is a determinant of FcγR surface density and functionality. However, *FCGR3A* gene copy number variability is rare, and thus the first objective of this study was to identify novel determinants of FcγRIIIa expression.
- The second objective was to characterize, in two divergent South African population groups, all known functional *FCGR* variants as well as recently identified *FCGR* variants that associated with vaccine efficacy in a human HIV-1 vaccine trial.
- The third objective was to assess the significance of *FCGR* variability in modulating HIV-1 infection risk in the context of mother-to-child transmission, studying both the transmitter (mother) and recipient (infant).

- The last objective was to determine if functional *FCGR* variants impacted on HIV-1 disease progression in a cohort of treatment naïve, HIV-1 infected women.

CHAPTER 2

MATERIALS AND METHODS

2.1. Study participants

Ethical clearance was obtained from the University of the Witwatersrand Ethics Committee (Appendix A.1) and written informed consent was obtained from all participants. Participant ethnicity was self-reported.

Healthy participants. *FCGR* variability is well characterized in Dutch, British and European Caucasian individuals of which South African Caucasian individuals are descendants, whereas there is a paucity of data for South African Black individuals. Thus, a larger cohort of 137 South African Black individuals and a smaller cohort of 32 South African Caucasian individuals were recruited for the characterization of *FCGR* variability in South Africans. Fresh ethylenediaminetetraacetic acid (EDTA) anticoagulated blood was available for 22 South African Black individuals and 32 South African Caucasian individuals for the characterization FcγRIII surface expression. Based on specific genotypic characteristics, select individuals were included in further phenotyping and functional assays.

Other population groups. To investigate population differences for *FCGR* genetic variants, genotypic data were obtained from published works as well as the 1000 Genomes Project for 379 Caucasian individuals (EUR super population: Tuscani, British, Finnish, and Spanish), 286 East Asians (ASN super population: Han Chinese in Beijing, Southern Han Chinese, and Japanese), 181 admixed Americans (AMR super population: Colombians, Mexicans, and Puerto Ricans), 88 Yoruba Nigerians (YRI), and 97 Luhya Kenyans (LWK). Published data were used to compare *FCGR3A* gene copy number between South African Black individuals and Kenyans (Niederer et al 2010), British Caucasian individuals (Niederer et al 2010), or Dutch Caucasian individuals (Breunis et al 2009).

Perinatal HIV-1 transmission cohort. A nested case-control study was undertaken to investigate low affinity *FCGR* variability in mothers and infants recruited as part of four perinatal cohorts at two hospitals in Johannesburg, South Africa. Genotypic data from HIV-1 infected mothers with HIV-1 infected infants (transmitting cases) were compared with HIV-1 infected mothers with uninfected infants (non-transmitting controls). All study participants were South African Black individuals. The four perinatal cohorts are described in detail in Appendix A.2. Cohort 1 comprised mothers and infants who both received one

of three short courses of zidovudine and lamivudine combination therapy or placebo. Treatment of the mothers started after 34 weeks gestation (Petra Study 2002). Cohort 2 comprised treatment-naïve HIV-1 infected mothers whose infants received either zidovudine or nevirapine as postexposure prophylaxis, and HIV-1 infected mothers and their infants that both received single dose nevirapine as part of a demonstration of antiretroviral therapy initiative (Gray et al 2005, Schramm et al 2006). Cohort 3 included HIV-1 infected mothers who were either treatment-naïve or received single dose nevirapine. All infants received nevirapine as postexposure prophylaxis. Cohort 4 included HIV-1 infected mothers that received either single dose nevirapine or triple drug combination therapy. All infants received nevirapine as postexposure prophylaxis. Over the four cohorts, 83 out of 849 (10%) mothers transmitted HIV-1 to their infants. *FCGR* variability was determined for 73 out of the 83 of the transmitting pairs, the remaining 10 transmitting pairs were excluded due to poor sample quality. For comparison, approximately two non-transmitting (NT) pairs matched by cohort were randomly selected for each transmitting (TR) pair: Cohort 1 – 6 TR and 12 NT; Cohort 2 – 22 TR and 40 NT; Cohort 3 – 25 TR and 51 NT; and Cohort 4 – 20 TR and 41 NT.

2.2. HIV-1 infection status of infants born to HIV-1 infected mothers

Maternal HIV-1 RNA levels were determined using the Roche Amplicor RNA Monitor assay version 1.5 (Roche Diagnostic Systems, Inc., Branchburg, New Jersey, USA) with a lower detection limit of 400 HIV-1 RNA copies/ml. CD4 T-cell counts were determined using the FACSCount System from Becton Dickinson (San Jose, CA, USA).

Infant samples were tested for HIV-1 DNA using the Roche Amplicor Monitor version 1.5 qualitative PCR assay (Roche Diagnostic Systems). Infants that tested HIV-1 positive at 6 weeks of age but negative at birth were considered to be infected intrapartum, while infants that tested HIV-1 positive at birth were considered infected *in utero*. Infants that were HIV-1 positive at 6 weeks but had no birth sample were categorized as 'undetermined'. For transmitting pairs with undetermined mode of transmission, 19/24 (79.2%) mothers received single dose nevirapine and 2/24 (8.3%) received triple drug combination therapy. Since it is known that these interventions reduce intrapartum transmission (Forbes et al 2012, Guay et al 1999, Townsend et al 2008), it can be concluded

that the majority of infants in that group were likely infected *in utero*. Thus, in the present study, non-transmitting pairs were compared to *in utero* and intrapartum pairs but also to a third group where the *in utero* and undetermined mode of transmission groups were combined, hereafter referred to as the *in utero* enriched group.

2.3. DNA extraction

Genomic DNA was extracted from EDTA anticoagulated blood samples using the QIAamp DNA Mini Kit (Qiagen, Dusseldorf, Germany).

2.4. Identification of *FCGR3A* nucleotide variants

The 9.4 kilobase region that encompasses all elements of the *FCGR3A* gene were PCR amplified using the Expand High Fidelity PCR System (Roche, Mannheim, Germany). Sequence-specific primer (SSP)-PCR was used to distinguish between the highly homologous *FCGR3A* and *FCGR3B*. All PCR reactions were performed using the same reaction conditions. In brief, the PCR reaction consisted of ~20 ng genomic DNA as template, 2.6 U Expand High Fidelity enzyme mix, 5 μl 10× PCR buffer (1.5 mM $MgCl_2$), 200 μM of each deoxynucleotide, 0.4 μM of each oligonucleotide primer (Appendix A.3), and molecular grade water to a final volume of 50 μl. PCR cycling conditions were 94°C for 2 min, followed by 10 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1.5 minutes, followed by a further 25 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1.5 minutes + 5 seconds/cycle increment, and finally 72°C for 7 min. PCR amplicons were sequenced bidirectionally (primer sequences are listed in Appendix 2.4). The *FCGR3A* sequences were aligned with the Genbank *FCGR3A* reference sequence (RefSeqGene NG_0009066).

2.5. *FCGR* gene copy number variability and nucleotide variant detection

Gene copy number and nucleotide variants within the low-affinity *FCGR* genes were determined using the *FCGR*-specific multiplex ligation-dependent probe amplification (MLPA) assay (MRC Holland, Amsterdam, The Netherlands). The principle of the assay is illustrated in Figure 2.1. In two multiplex reactions, this assay detects copy number variation of the *FCGR2A, FCGR2B, FCGR2C, FCGR3A* and *FCGR3B* genes as well as functional allelic variants: FcγRIIa-H131R (rs1801274); FcγRIIb-I232T (rs1050501), FcγRIIIa-F158V

(rs396991), FcγRIIIb-HNA1a/b/c, and promoter variants within FcγRIIb/c (g.-386G>C [rs3219018] and g.-120T>A [rs34701572]). Furthermore, the assay detects two *FCGR2C* gene expression variants i.e. c.169T>C in exon 3 and c.798+1A>G (rs76277413).

The assays were performed according to manufacturer's instructions using the One-Tube protocol (Breunis et al 2008, Schouten et al 2002). Genomic DNA was diluted to a concentration of 20 ng/ µl in TE (10 mM Tris-HCl, pH 8.2 + 0.1 mM EDTA), of which 5 µl was denatured at 98 °C for 5 minutes and cooled to 25 °C; 1.5 µl MLPA buffer and 1.5 µl probemix were added to each sample and incubated for 1 minute at 95 °C followed by an 18 hour incubation at 60 °C to allow probe hybridization. Adjacently hybridized probes were ligated by adding 32 µl Ligase-65 reaction mix (25 µl dH20 + 3 µl Ligase buffer A + 3 µl Ligase buffer B + 1 µl Ligase-65 enzyme to each sample while at 54 °C, followed by 15 minutes at 54 °C and 5 minutes at 98 °C. Following ligation, samples were cooled to 20 °C and the 10 µl polymerase master mix (7.5 µl dH2O + 2 µl SALSA PCR primer mix + 0.5 µl SALSA polymerase) was added. PCR amplification was started directly after addition of the polymerase mix with 35 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds and 72 °C for 1 minute, followed by 20 minutes at 72 °C.

One µl PCR product diluted in 9.2 µl Hi-Di Formamide with 0.2 µl LIZ size standard were separated by capillary electrophoresis on an ABI Genetic Analyzer 3130. Capillary electrophoresis reagents and the instrument were from Applied Biosystems. Peak height of the amplicons was used as a measure of gene copy number and was analyzed with Coffalyzer.NET software (MRC Holland).

2.6. *FCGR2B/C* promoter variant discrimination

Due to the high sequence homology between the promoters of the *FCGR2B* gene and *FCGR2C* gene the MLPA assay does not discriminate between these genes at positions g.-386G>C and g.-120T>A. In the event that the minor allele at either position was detected, a long-range SSP-PCR and sequencing of the promoter region was performed as previously described with modifications (Breunis et al 2008).

The 15 kilobase region was PCR amplified with the Expand Long Template PCR System using Buffer 3 (Roche). Two PCR reactions were performed per sample using a common

nonspecific *FCGR2B/C* sense primer (5'-GCCATCCTGACATACCTCCT-3'), and an *FCGR2B*-specific antisense primer (5'-CCCAACTTTGTCAGCCTCATC-3') or the *FCGR2C*-specific antisense primer (5'-CTCAAATTGGGCAGCCTTCAC-3'). In brief, the PCR reaction consisted of ~20 ng genomic DNA as template, 3.75 U Expand Long Template enzyme mix, 5 µl 10× PCR buffer 3 (2.75 mM $MgCl_2$), 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 minutes, 10 cycles of 94°C for 10 seconds, 60°C for 15 seconds, 68°C for 12 seconds, followed by 25 cycles of 94°C for 15 seconds, 60 °C for 15 seconds, 68°C for 12 seconds, with an elongation of each cycle with 20 seconds at 68°C, and finally 72°C for 7 min.

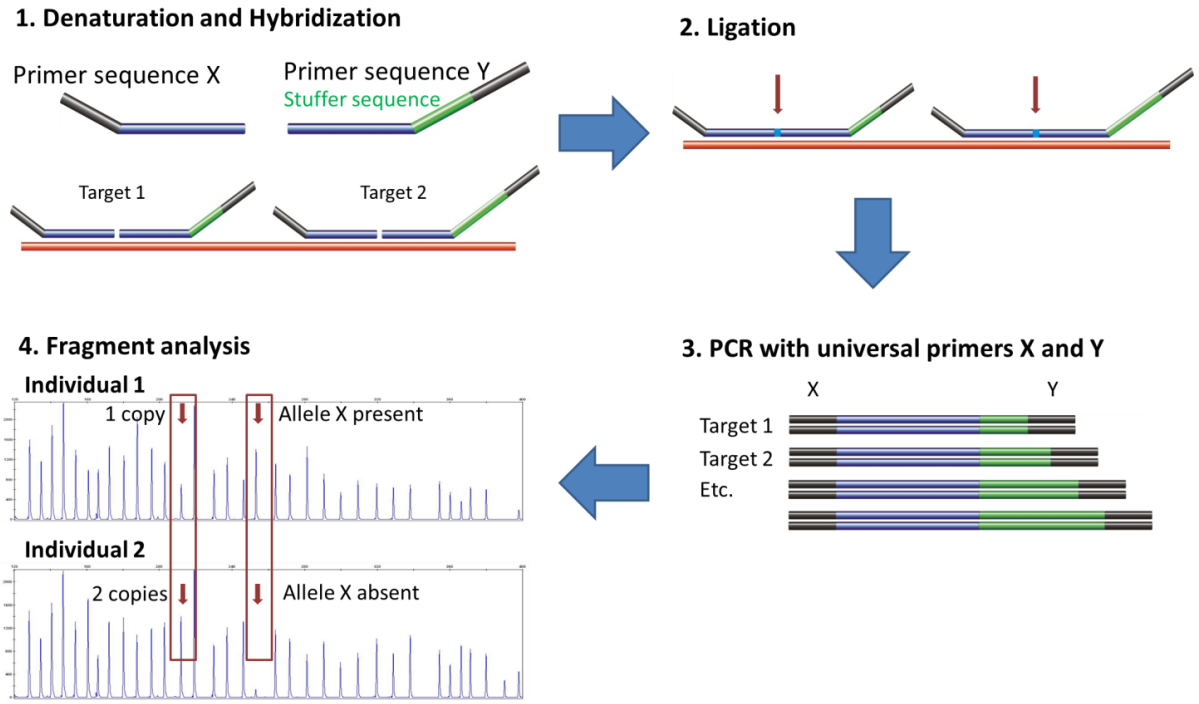


Figure 2.1. Multiplex ligation-dependent probe amplification assay principle. Genomic DNA is denatured and two sequence specific oligonucleotide probes anneal to adjacent DNA target sequences. The two probes are ligated, generating a single DNA molecule that is amplified exponentially during a PCR reaction. The presence of a stuffer sequence in each probe determines the length of the PCR fragment and is specific for each target region on the genome. The variable length fragments are separated and quantified by capillary electrophoresis. Differences between DNA samples are detected by comparing the resulting MLPA peak patterns. Adapted from (Schouten et al 2002).

2.7. *FCGR3A* intragenic haplotype detection

A tag variant (g.-1405G>A, rs56199187) was targeted as representative of the haplotype. Two separate real-time SSP-PCR reactions were performed per donor using the Maxima SYBR Green qPCR Master Mix (Thermo Scientific), with a common antisense primer (5'-CAGGAAGTGGGAGGGTTGTT-3') and two sequence-specific sense primers: 5'-AGAACTGGAATGGCTGCCTA-3' for the minor allele and 5'-AACTGGAATGGCTGCCTG-3' for the major allele. Genotypes were assigned following crossing threshold (Ct) shift analysis. Real-time PCR cycling was performed on an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems) with the following cycling conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 5 sec, and 72°C for 30 sec.

2.8. *FCGR2C* c.134-96C>T (rs114945036)

Genotyping of the *FCGR2C* c.134-96C>T variant (rs114945036) and those reported to be in complete linkage disequilibrium with it, rs138747765 and rs78603008 (Li et al 2014), was done through nucleotide sequencing of the long range SSP-PCR amplicon generated for *FCGR2B/2C* promoter discrimination (described above).

2.9. Synteny of *FCGR2C* variants

The synteny (occurrence on the same chromosome) of nucleotide variants within the *FCGR2C* gene was determined through an SSP-PCR assay targeting a sequence variant (c.134-26T) 62-bp upstream and in linkage disequilibrium with the c.169C variant. In brief, a ~11-kb fragment was amplified with the Expand Long Template PCR System using Buffer 3 (Roche). The sequence-specific sense primer (5'-CTGGGCTTCCTCTTCTTCAT-3') annealed in intron 2 and an antisense primer (5'-CAGCATCCCTTCGTCTTCCT-3') in intron 8. In brief, the PCR reaction consisted of ~20 ng genomic DNA as template, 3.75 U Expand Long Template enzyme mix, 5 µl 10× PCR buffer 3 (2.75 mM $MgCl_2$), 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 10 cycles of 94°C for 10 sec, 60°C for 30 sec, 68°C for 10 min, and 25 cycles of 94°C for 15 sec, 60°C for 30 sec, 68°C for 10 min, with an elongation of each subsequent cycle with 20 sec, and a final

elongation at 72°C for 7 min. Nucleotide variants were identified through nucleotide sequencing of the genomic regions encompassing the variants of interest.

2.10. Nucleotide sequencing

PCR amplicons were purified with the MSB Spin PCRapace (Stratec, Berlin, Germany) and sequenced with BigDye Terminator v3.1 cycle sequencing using the automated 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were analyzed on Sequencher™ version 4.5 (Gene Codes Corporation, Ann Arbor, MI). Multiple sequence alignments were performed using MAFFT (Katoh & Standley 2013), and subsequently analyzed for the presence of single nucleotide variants in BioEdit version 7.0.0 (Ibis Biosciences, Carlsbad, CA).

2.11. Monoclonal antibodies and reagents

The following monoclonal antibodies (MAbs) were used to phenotype cell populations: anti-CD3 clone UCHT1, anti-CD4 clone SK3, anti-CD8 clone SK1, anti-CD19 clone MΦP9, anti-CD14 clone 4G7, anti-CD45 clone 2D1, anti-CD56 clone NCAM16, anti-CD16 clone 3G8 (aforementioned MAbs were all obtained from BD Biosciences, San Jose, CA, USA), and anti-CD32b/c clone 2B6 (a gift from MacroGenics, Rockville, MD, USA). For the cytotoxicity assays, the anti-HIV-1 A32 MAb (IgG1) was used (NIH AIDS Research and Reference Reagent Program).

2.12. Quantitation of FcγRIIIa cell surface density

EDTA anticoagulated blood from participating volunteers was processed within 2 hours on the day of collection. FcγRIIIa expression on peripheral blood mononuclear cells in whole blood was quantitated by flow cytometry using the Becton Dickinson QuantiBRITE™ system. In brief, four tubes containing 50µl of EDTA blood were differentially stained with monoclonal antibodies for the identification of CD4+ and CD8+ T lymphocytes, B lymphocytes, NK cells, and monocytes. Saturating amounts of a quantifiable FcγRIII/CD16-PE monoclonal antibody (3G8 clone) with a ≥95% 1:1 PE-to-monoclonal antibody ratio was added to each tube and incubated for 15 minutes in the dark at room temperature. Red blood cells were lysed with 2 mL FACS lysing solution for 7 minutes in the dark at room temperature. Leukocytes were collected by centrifugation at 200×g for 5 minutes, washed

and resuspended in 150 µL of 1% paraformaldehyde and stored at 4°C until acquisition on a FACSCalibur four-colour flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). Following acquisition, data were analyzed using FlowJo version 7.6.1 software (Tree Star, San Carlos, CA). To calculate FcγRIIIa surface density as Antibodies Bound per Cell (ABC), a Becton Dickinson QuantiBRITE PE sample containing four levels of PE was acquired with each experiment. Using the geometric mean and PE molecules per bead as provided by the manufacturer for each of the four PE levels, a standard curve was calculated on CellQuest software. The slope (m) and intercept (c) was used in the following equation:

$$\text{Antibodies bound per cell} = \text{antilog}_{10}\left(\frac{\text{Log10 FcγRIIIa-PE geometric mean - m}}{\text{c}}\right)$$

A PE-labeled mouse $IgG_{1\kappa}$ isotype control and fluorescence-minus-one (FMO) control was used to optimize the identification of FcγRIII-positive cells. All antibodies were obtained from BD Biosciences (San Jose, CA). Monocyte subpopulations, $CD14^{dim}FcγRIIIa^{bright}$ and $CD14^{bright}FcγRIIIa^{dim}$, were defined as previously described (Ogonda et al 2010).

2.13. Isolation of NK cells

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood using Ficoll-Paque™ PLUS (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) and washed three times with phosphate buffered saline (PBS). NK cells were negatively selected from PBMCs by magnet-activated cell separation (MACS) using the NK Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. A double-step purification was performed and NK cell purity verified by flow cytometry. Viable NK cell numbers were determined by trypan blue exclusion through direct counting. The enriched NK cell fragment was immediately used in the cytotoxicity assays without resting.

2.14. HIV-1 gp120 coated target cells

The $CEM.NK^R$ cell line (NIH AIDS Research and Reference Reagent Program) was used as target cells in HIV-1-specific ADCC assays and maintained in RPMI 1640 (Life Technologies,

Carlsbad, CA) supplemented with 10% foetal bovine serum (Life Technologies). Recombinant gp120 HIV-1 protein representing the subtype B HIV-1 envelope (BaL, NIH AIDS Research and Reference Reagent Program) was added to 1×10^6 CEM.NK^R target cells in a final volume of 1 ml and incubated for 75 minutes at 37°C, mixed gently every 30 min. Cells were washed twice and resuspended in culture medium prior to use in cytotoxicity assays.

The optimum amount to coat target cells was determined as previously described (Pollara et al 2011). In brief, aliquots of 1×10^6 CEM.NK^R target cells were incubated with 2-fold serial dilutions of recombinant HIV_{BaL} gp120 from 10 µg/ml to 1.25 µg/ml for 75 minutes at 37°C with gentle mixing every 30 min. Cells were washed twice with PBS and incubated in the presence of saturating amounts of a FITC-labelled anti-CD4 clone SK3 (epitope in gp120 binding site on CD4) for 15 minutes at room temperature, followed by centrifugation at 1000 rpm for 5 minutes. Cells were washed with PBS, reconstituted in 150 µl 1% paraformaldehyde and acquired on the FACS Aria II flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). The concentration of recombinant HIV_{BaL} gp120 that resulted in a >50% reduction in anti-CD4 FITC fluorescence relative to an unstained control was used to coat target cells in subsequent cytotoxicity assays.

2.15. Cytotoxicity assays

ADCC activity was measured as previously described with minor modifications (Pollara et al 2011). The principle of the assay is illustrated in Figure 2.2. In brief, 1×10^6 HIV-1 gp120 coated CEM.NK^R target cells were stained with a fluorescent target cell marker (TFL4, OncoImmunin, Gaithersburg, MD) and a viability marker (NFL1; OncoImmunin) for 15 min at 37°C. After two washes with culture medium, viable target cells were counted and 1×10^4 cells dispensed to each assay well of a 96-well plate. NK cells were added to target cell suspensions at variable effector to target cell ratios (25 µl final volume) followed by addition of 75 µl PanCyToxiLux™ Substrate solution (detects both granzyme B and upstream caspase activities) and 5 min incubation at room temperature. Twenty-five µl anti-HIV-1 A32 (0.5 µg/ml) was added to the effector/target cell suspension. After incubation for 15 min at room temperature, the plates were centrifuged for 1 min at 300 × g and incubated for 1.5 h at 37°C and 5% CO_2. Cells were washed and resuspended in 200

µl Wash Buffer, and stored at 4°C until acquisition on the FACS Aria II flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). A minimum of 2.5×10^3 events representing viable target cells were acquired and data analyzed using FlowJo version 7.6.1 software (Tree Star, San Carlos, CA). All assays were performed in triplicate. Controls included TFL4 labelled target cells alone to set the proper gate and target and effector cells without antibody to determine the baseline granzyme B activity. The gating strategy is described in detail by Pollara et al (Pollara et al 2011).

2.16. Overall FcγR variability profile: Allele scoring system

To assess the effect of the overall FcγR variability profile, individuals were categorized as possessing an overall inhibitory profile, neutral profile, or activatory profile through scoring each allele a negative or positive value base on its contribution to the extent of cell activation as determined in previously described functional assays. High responder variants (FcγRIIa-131H, FcγRIIb-232T, FcγRIIIa-158V, and FcγRIIIb-HNA1a) were assigned a positive value of one for each allele. Low responder variants (FcγRIIa-131R, FcγRIIb-232I, FcγRIIIa-158F, and FcγRIIIb-HNA1b) were assigned a negative value of one for each allele. For gene copy number variability, two copies were considered 'neutral', as such one copy number was assigned a negative one value, whereas three gene copies were assigned a positive value of one, four copies a positive value of two, etc. This approach considers allele dose for genes that occur at multiple copies. Once the additive score was determined individuals were categorized into profiles of inhibitory (overall negative score), neutral (score of zero), and activatory (overall positive score).

2.17. Computational and statistical analysis

Comparison of continuous data between two groups was performed by the nonparametric Mann-Whitney U test for non-normally distributed data or the t-test for data transformed to fit a normal distribution (for example $\log_{10}$ HIV-1 RNA copies/ml). Correlation analysis of continuous data between two groups was assessed using the nonparametric Spearman rank correlation coefficient. Comparison of categorical data was performed with the Fisher's exact test or the χ^2-test. For individuals with two gene copies, Hardy Weinberg equilibrium was calculated with the exact test as described by Haldane

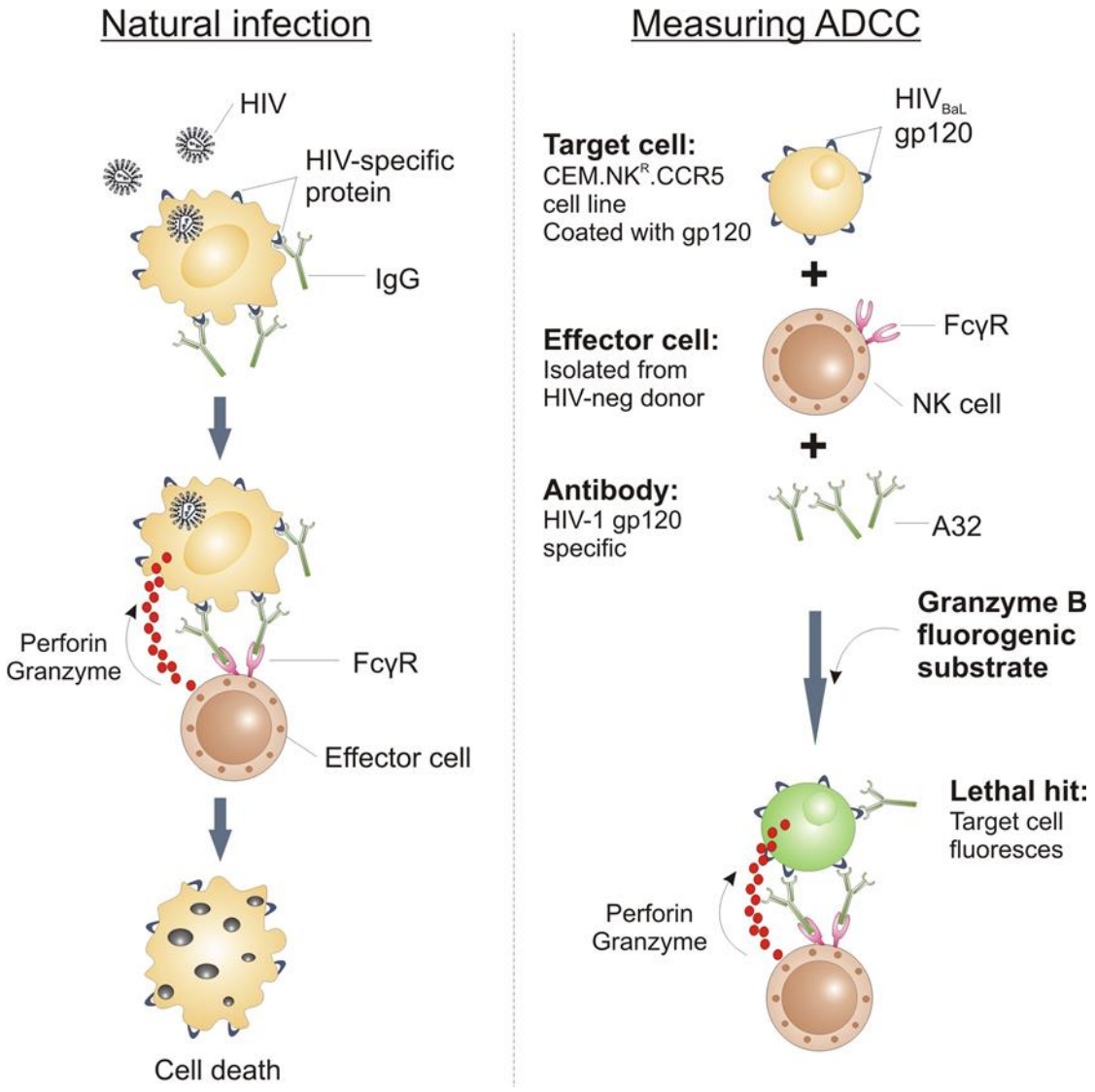


Figure 2.2. Antibody-dependent cellular cytotoxicity (ADCC) assay principle. During the course of a natural HIV-1 infection, HIV-specific IgG will bind to HIV-1 antigens expressed on the surface of an infected cell. Engagement of the antigen-bound IgG-Fc with an FcγR-bearing effector cell will result in targeted cell death through the release of perforin and granzyme by the effector cell. In the *in vitro* ADCC assay, a human T-lymphoblastoid cell line is coated with HIV-1 recombinant gp120 and combined with gp120-specific IgG1, and natural killer cells as effectors. The cell suspension is incubated in the presence of a cell-permeable granzyme B fluorogenic substrate, which generates a fluorescent signal when it gets hydrolysed by granzyme B when a target cell receives a lethal hit by an effector cell. ADCC activity is measured by determining the percentage of granzyme B positive target cells by flow cytometry. Illustration by Ria Lassaunière.

(Haldane 1954) for biallelic variants using the Tools for Population Genetic Analysis (version 1.3) software. Logistic regression was used to assess the association between the outcome (transmission) and the main exposure (genotype). The analysis was adjusted for potential confounders as found to be significantly different between groups in the univariate analysis. Logistic regression analyses and the t-test were performed in STATA version 10.1 (StataCorp LP, College Station, USA). Correlation analysis and the Mann-Whitney U test were performed with SPSS statistical software (IBM Corporation, Armonk, NY). P-values less than 0.05 were considered statistically significant (2-tailed tests).

For comparison of genotype frequencies, homozygosity for the common allele was considered the reference genotype. Where a clear common allele could not be identified as in the case of FcγRIIa-H131R and FcγRIIIb allotypes, the chimpanzee ortholog allele was considered the reference allele. Due to the large number of possible FcγRIIIb-HNA1a/b/c allotype combinations resulting from *FCGR3B* gene copy number variation, participants were categorized according to the presence or absence of each of the three allotypes irrespective of gene copy number. The most prevalent combination was selected as reference combination. Linkage disequilibrium (LD) of diallelic loci was analyzed in Haploview (Barrett et al 2005) where 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.

CHAPTER 3

A NOVEL *FCGR3A* INTRAGENIC HAPLOTYPE IS ASSOCIATED WITH INCREASED FcγRIIIa CELL SURFACE DENSITY AND POPULATION DIFFERENCES

3.1. INTRODUCTION

Fc-gamma receptor IIIa (FcγRIIIa/CD16a) plays an important role in immunity against infection and tumors. It mediates key effector mechanisms through the interaction with the Fc portion of IgG, which include antibody-dependent cellular cytotoxicity (ADCC), phagocytosis, endocytosis and cytokine release. FcγRIIIa is constitutively expressed on natural killer (NK) cells, macrophages, γδ T-cell receptor (TCR) T lymphocytes, and a subset of monocytes. FcγRIIIa is also present on pre-B lymphocytes and small subsets of $CD4^+$ and $CD8^+$ T lymphocytes (Chauhan & Moore 2012, Clemenceau et al 2008, de Andres et al 1999, Gessner et al 1995b). However, the proportion of circulating T and B lymphocytes expressing FcγRIIIa in the absence of inflammation, as well as the relative expression levels of FcγRIIIa on these and other cell types remain largely undefined.

FcγRIIIa displays low affinity for monomeric IgG and preferentially bind aggregated IgG through multimeric low-affinity, high-avidity interactions which are important in recognizing and binding immune complexes. The affinity of FcγRIIIa for IgG and the efficiency by which it initiates effector mechanisms is affected by FcγRIIIa variability (Breunis et al 2009, Koene et al 1997, Wu et al 1997). The most extensively studied FcγRIIIa variant is a polymorphism that predicts a phenylalanine (F) to valine (V) at amino acid residue position 158. Compared to the FcγRIIIa-158F allotype, the FcγRIIIa-158V allotype displays a higher affinity for IgG1 and IgG3 as well as a capacity to bind IgG4 (Wu et al 1997). Furthermore, the FcγRIIIa-158V allotype is associated with significantly higher IgG-induced NK cell activity compared to the FcγRIIIa-158F allotype (Vance et al 1993, Wu et al 1997). The clinical relevance of this polymorphism is validated by studies in lymphoma patients, where possession of the high-affinity allele is associated with better clinical responses to the B lymphocyte depleting CD20-specific antibody (Rituximab) (Cartron et al 2002). A second polymorphism which is located within the membrane-distal Ig-like domain, represents a triallelic variant predicting a leucine (L) to arginine (R) or histidine (H) at residue position 48 (de Haas et al 1996). Linkage disequilibrium is observed between the FcγRIIIa-L48RH and FcγRIIIa-F158V polymorphism and has thus made it difficult to investigate a possible functional role for FcγRIIIa-L48RH (Koene et al 1997).

Variable FcγRIIIa surface densities have also been associated with differences in NK cell activation through FcγRIIIa stimulation (Breunis et al 2009, Liu et al 2009). This is likely attributed to the activation of FcγRIIIa-bearing cells requiring aggregation of multiple FcγRIIIa molecules on their cell surfaces, which primarily results from the cross-linking of multiple FcγRIIIa molecules by immune complexes (Daeron 1997). Higher FcγRIIIa surface density therefore allows FcγRIIIa-bearing cells to respond more efficiently to target cells while lower levels of FcγRIIIa may impair this response. At present little is known about the clinical relevance of FcγRIIIa surface density. This is likely attributable to the lack of an easily measurable marker for variable FcγRIIIa surface density. One such genetic marker is *FCGR3A* gene copy number. However, variation at this locus is generally low within different population groups (2.2% to 9.6%) and is difficult to analyze (Niederer et al 2010). To date, there has been no investigation into the possible role of genetic variances within the untranslated regions of the *FCGR3A* gene (promoters, flanking regions and introns) and FcγRIIIa surface density.

While frequencies of the IgG subclass defining FcγRIIIa-F158V alleles are well described worldwide, differences in FcγRIIIa cell surface density as well as the proportion of circulating leukocytes expressing FcγRIIIa in different ethnic groups is currently undefined. With the increasing use of therapeutic antitumor antibodies in oncology (Carter 2006), and the possible role of FcγRIIIa-mediated effector mechanisms in protection from HIV-1 infection and disease progression (Forthal et al 2007a, Lambotte et al 2009), it is becoming increasingly important to gain insight into these possible differences. This study therefore describes and compares the proportion of circulating FcγRIIIa-positive cell subsets, and relative cell-specific FcγRIIIa surface density among healthy individuals from two different ethnic groups (South African Caucasian and Black individuals). Our data highlight important differences between these population groups. Furthermore, we describe genetic variances within the *FCGR3A* gene, and report the identification of a novel intragenic haplotype that is associated with the highest densities of FcγRIIIa expression on NK cells and monocytes.

3.2. MATERIALS AND METHODS

3.2.1. Study participants

Refer to Chapter 2, Materials and Methods section 2.1. In brief, 53 healthy, HIV-1 uninfected adult volunteers, of whom 22 were Black individuals and 31 Caucasian individuals, were recruited for characterization of FcγRIIIa surface density on peripheral blood leukocytes. A smaller number of individuals from each population group was selected for investigating FCGR3A nucleotide variants associated with FcγRIIIa surface density.

3.2.2. Quantitation of FcγRIIIa cell surface density

FcγRIIIa expression on peripheral blood mononuclear cells was quantitated using flow cytometry as described in Chapter 2, Materials and Methods sections 2.11. and 2.12. In brief, whole blood was stained with fluorescently labelled antibodies that allowed distinction between $CD4^+$ and $CD8^+$ T lymphocytes; B lymphocytes; NK cells; and monocytes. A quantifiable FcγRIII/CD16 monoclonal antibody was used to quantitate FcγRIIIa surface expression levels.

3.2.3. Copy number variation

FCGR3A gene copy number was determined using the *FCGR*-specific multiplex ligation-dependent probe amplification (MLPA) assay as described in Chapter 2, Materials and Methods sections 2.3. and 2.5.

3.2.4. Identification of *FCGR3A* nucleotide variants

Polymorphisms within the *FCGR3A* gene were investigated through nucleotide sequencing as described in Chapter 2, Materials and Methods sections 2.4 and 2.10.

3.2.5. Statistical analysis

Statistical analysis was performed as described in Chapter 2, Materials and Methods section 2.17. In brief, continuous data were compared using the Mann-Whitney U test, correlations between groups was performed with the Spearman rank correlation coefficient, and categorical data were analysed using the Fisher's exact test. The significance level was set at $P < 0.05$ (2-tailed tests).

3.3. RESULTS

3.3.1. Cohort

To control for the potential confounding effect of age and gender on FcγRIIIa surface density, individuals between population groups were age and gender matched. Demographic characteristics of participants within the two South African ethnic groups are described in Table 3.1.

Table 3.1. Demographic characteristics of participants within the two South African population groups

	South African Caucasian individuals n = 31	South African Black individuals n = 22	P - value
Age			
Mean	35 years	39 years	
Median	32 years	38 years	P = 0.237#
Range	22 - 61 years	24 - 66 years	
Sex (% males)	40.9%	32.3%	P = 0.559*

#Mann-Whitney test, *Fisher's exact test

3.3.2. Relative FcγRIIIa cell surface density on leukocytes

T and B Lymphocytes. FcγRIIIa cell surface expression was detected on a small proportion of circulating B lymphocytes (median: 10.1%), $CD4^+$ T lymphocytes (median: 6.4%), $CD8^+$ T lymphocytes (median: 10.6%), and a larger proportion of $CD3^+CD4^-CD8^-$ T lymphocytes which constitutes a combination of γδ and αβ TCR T lymphocytes (median: 26.0%) (Figure 3.1A). Comparable low FcγRIIIa surface densities were observed for B lymphocytes (median: 1319 FcγRIIIa antibodies bound per cell (ABC)), $CD4^+$ T lymphocytes (median: 1299 ABC), $CD8^+$ T lymphocytes (median: 1177 ABC), and $CD3^+CD4^-CD8^-$ T lymphocytes (median: 1569 ABC) (Figure 3.1B). Black individuals had a smaller median proportion of $FcγRIIIa^+CD8^+$ T lymphocytes compared to Caucasian individuals (9.6% vs. 11.7%; P = 0.030). Interestingly, proportions of $FcγRIIIa^+CD8^+$ T lymphocyte subset appears to increase with age in Black individuals but not Caucasian individuals (R = 0.636, P = 0.001; and R = 0.007, P = 0.970, respectively).

Innate immune cells. A median of 17.6% of circulating monocytes expressed FcγRIIIa. Black individuals had a significantly larger median FcγRIIIa^{+} monocyte population compared to Caucasian individuals (24.2% vs. 16.3%, P < 0.001) (Figure 3.1A). However, Black individuals have significantly less FcγRIIIa molecules on the surface of CD14^{dim}FcγRIIIa^{bright} monocytes (31358 vs. 41268 ABC, P = 0.001) and a trend towards less on CD14^{bright}FcγRIIIa^{dim} monocytes compared to Caucasian individuals (11139 vs. 13291 ABC, P = 0.061) (Figure 3.1B). The majority of CD56^{+} NK cells expressed FcγRIIIa on their surfaces (median: 96.5%). Compared to Caucasian individuals, Black individuals had a marginally lower median proportion of FcγRIIIa^{+}CD56^{+} NK cells (95.2% vs. 96.9%, P = 0.017) and a significantly lower median FcγRIIIa surface density (23835 vs. 36495 ABC, P < 0.001) (Figure 3.1).

3.3.3. Correlation between NK cell and monocyte FcγRIIIa cell surface density

For both population groups a positive correlation was observed for FcγRIIIa cell surface density on monocytes and CD56^{+} NK cells (R = 0.607, P < 0.001; and R = 0.638, P = 0.002, respectively) (Figure 3.2A). This correlation was stronger for CD14^{dim}FcγRIIIa^{bright} monocytes and CD56^{dim}FcγRIIIa^{bright} NK cells (Black individuals: R = 0.733, P < 0.001; Caucasian individuals: R = 0.808, P < 0.001) (Figure 3.2B) compared to CD14^{bright}FcγRIIIa^{dim} monocytes and CD56^{dim}FcγRIIIa^{bright} NK cells (Black individuals: R = 0.445, P = 0.043; Caucasian individuals: R = 0.548, P = 0.001) (Figure 3.2C).

3.3.4. *FCGR3A* gene copy number

In this study, a very broad range of FcγRIIIa cell surface density was observed for NK cells (10,032-90,142 ABC). Since a gene-dosage effect is observed for the *FCGR3A* gene and FcγRIIIa levels expressed (Breunis et al 2009), the *FCGR3A* gene copy number was determined for all individuals. The contribution of gene copy number to the observed variation in FcγRIIIa surface densities appeared to be negligible, since only 2/53 (3.8%) individuals had three *FCGR3A* gene copies, while all other individuals had two copies per diploid genome. Both individuals with three copies were Caucasian females and had intermediate FcγRIIIa surface densities (23,206 and 36,395 ABC).

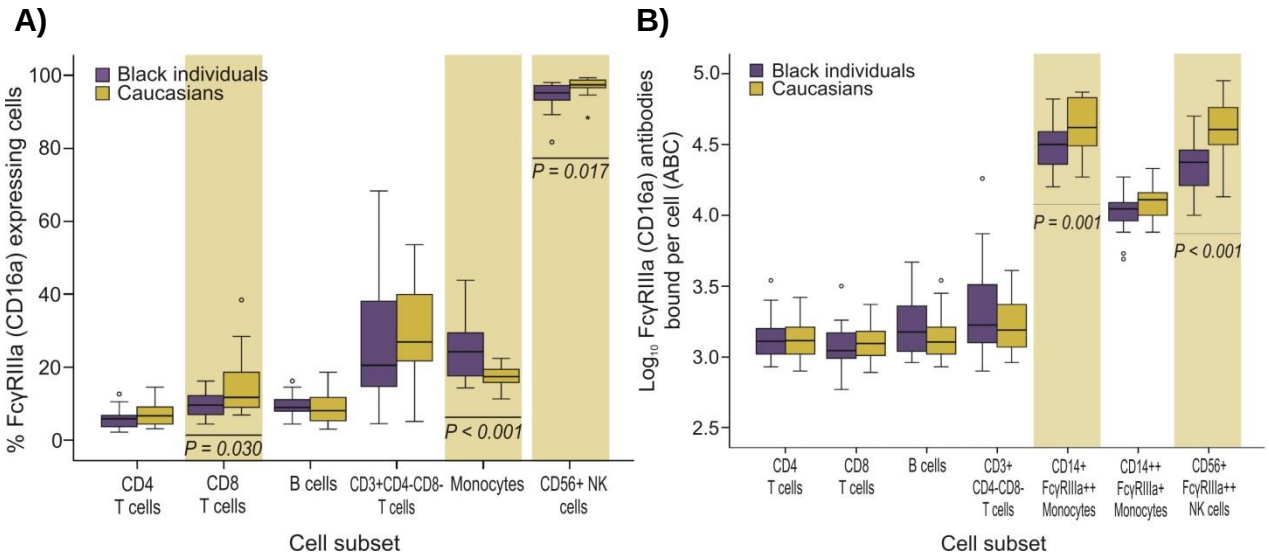


Figure 3.1. Proportions of FcγRIIIa-positive peripheral mononuclear cells and their corresponding FcγRIIIa surface densities observed in two South African population groups. (A) Comparison of the proportion of FcγRIIIa/CD16a expressing mononuclear cell subsets observed in 22 Black individuals and 31 Caucasian individuals. (B) Comparison of FcγRIIIa/CD16a cell surface expression levels (Log_{10} antibodies bound per cell) between Black individuals and Caucasian individuals. The cell subsets analysed are indicated on the x-axes. Box-whisker plots depicting the median (horizontal black line), 25th and 75th percentile (margins of the box) and the 10th and 90th centiles (whiskers). Outliers are indicated with (○). P-values have been indicated.

3.3.4. An FCGR3A *intragenic haplotype is overrepresented in individuals with increased FcγRIIIa surface densities*

To investigate polymorphisms associated with FcγRIIIa surface density, the full-length ~9.4 kb *FCGR3A* gene sequence was determined for individuals with the highest and lowest FcγRIIIa surface densities observed on CD56^{dim}FcγRIIIa^{bright} NK cells. These included 4 individuals with high (61,153-90,142 ABC), 7 individuals with intermediate (21,331-50,455 ABC), and 6 individuals with low (10,032-15,304 ABC) FcγRIIIa surface densities. Figure 3.3 summarizes the genetic variances identified in these 17 healthy control individuals (eight Caucasians and nine Black individuals).

An *FCGR3A* intragenic haplotype (IH) comprising three SNPs and an indel was found to be overrepresented in individuals with high FcγRIIIa surface densities (4/4 individuals),

compared to individuals with intermediate (2/7 individuals) and low surface densities (0/6 individuals). The *FCGR3A*-IH allelic variants include a 5'UTR SNP -1405A (rs56199187), an intronic indel +690-691InsC (rs33959719), two alleles of the triallelic nonsynonymous SNP +1194A or +1194G (rs10127939, FcγRIIIa-48RH), and a second intronic SNP +1842T (rs77825069). Of these, the 5'UTR SNP -1405A and intronic indel +690-691InsC are located within putative regulatory regions, a silencer and enhancer region, respectively (Figure 3.3).

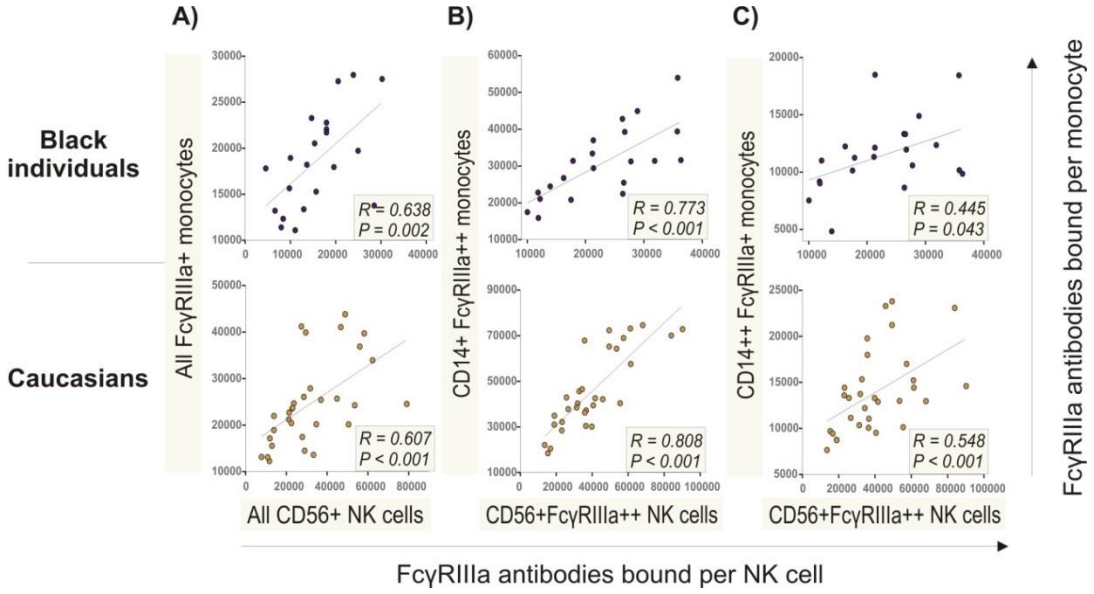


Figure 3.2. Spearman's correlation between FcγRIIIa/CD16a cell surface expression levels on monocytes and NK cells for 22 Black individuals and 31 Caucasian individuals. (A) A positive correlation for FcγRIIIa/CD16a cell surface densities on FcγRIIIa-positive monocytes and $CD56^{+}$ NK cells. (B) A stronger positive correlation for FcγRIIIa/CD16a cell surface densities on the $CD14^{dim}FcγRIIIa^{bright}$ monocyte subset and $CD56^{dim}FcγRIIIa^{bright}$ NK cell subset. (C) A slightly weaker correlation for FcγRIIIa/CD16a cell surface densities on the $CD14^{bright}FcγRIIIa^{dim}$ monocyte subset and $CD56^{dim}FcγRIIIa^{bright}$ NK cell subset. Spearman's correlation coefficient (R) and P-values have been indicated.

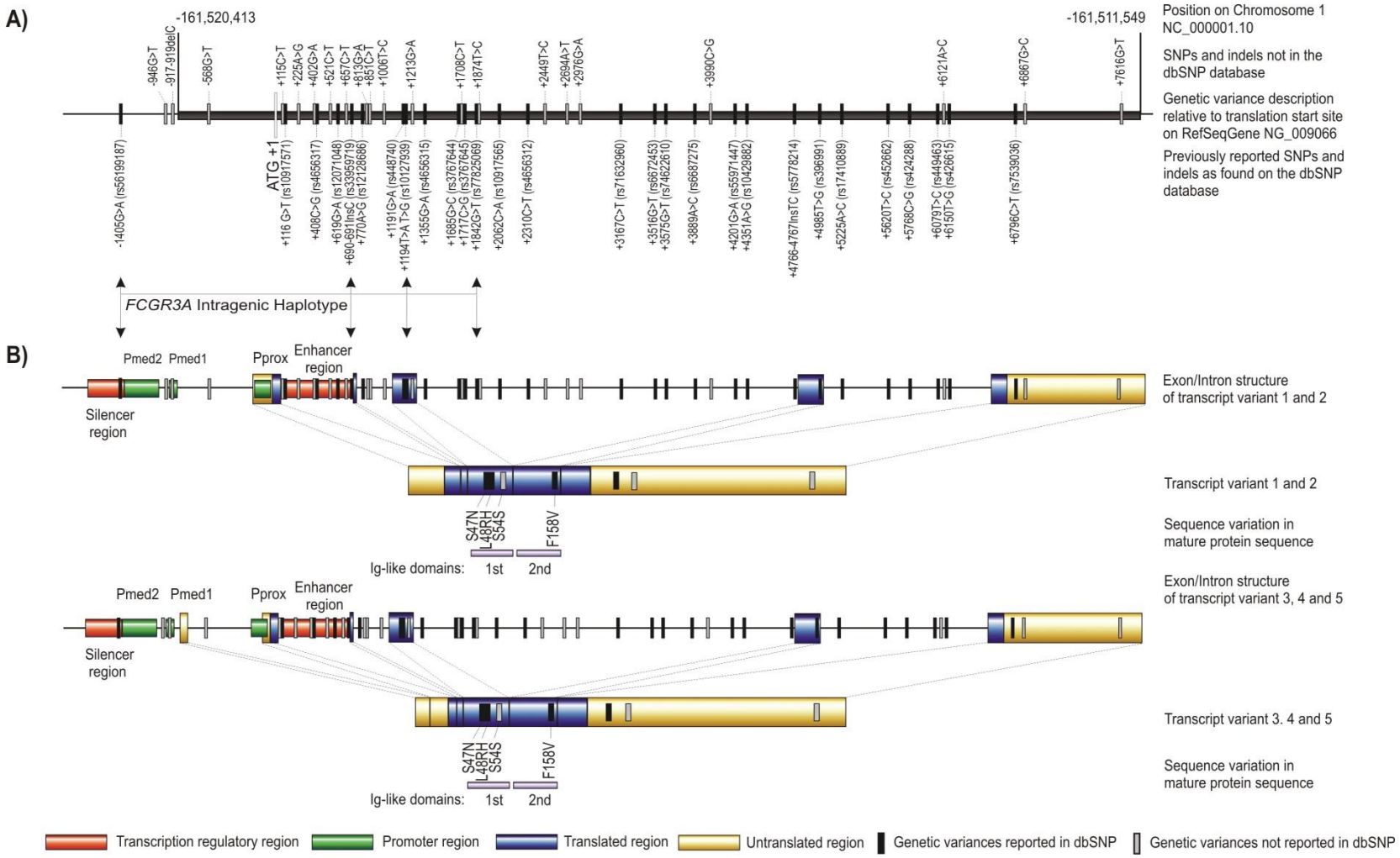


Figure 3.3. A schematic representation of genetic variances identified within the *FCGR3A* gene of 17 healthy adult volunteers. (A) The *FCGR3A* gene with (solid line) newly identified (above solid line) and previously documented (below solid line) single nucleotide polymorphisms and insertions/deletions (indels) in dbSNP. The novel 4-variant (3-SNP/1-indel) *FCGR3A* intragenic haplotype is indicated with black arrows (below solid line). (B) Genetic variances relative to gene elements including transcriptional regulatory regions, promoter regions, exons, introns, translated regions and untranslated regions.

Allelic variants within the *FCGR3A*-IH were subsequently determined for all individuals with two *FCGR3A* gene copies by sequencing the regions encompassing the four variants within the *FCGR3A*-IH. The *FCGR3A*-IH was detected in 13/29 (44.8%) Caucasian individuals at an allele frequency of 0.22. It was not detected in the present cohort of 22 Black individuals, neither in a larger cohort of 115 Black individuals (data not shown). Complete linkage of all four variants that comprise the *FCGR3A*-IH was observed in all individuals positive for the haplotype. All *FCGR3A*-IH positive individuals were heterozygous at all positions and had significantly higher FcγRIIIa surface densities on $CD56^{dim}$FcγRIIIa^{bright} NK cells and $CD14^{dim}$FcγRIIIa^{bright} monocytes compared to *FCGR3A*-IH negative individuals ($P < 0.0001$, Figure 3.4A). When stratified according to decreasing FcγRIIIa surface density on $CD56^{dim}$FcγRIIIa^{bright} NK cells, the haplotype occurred at a significantly higher frequency in individuals in the highest quartile (11/13 [84.6%]) compared to those in the lowest quartile (2/38 [5.3%]) (Odds Ratio = 99, $P < 0.0001$).

To determine the influence of the *FCGR3A*-IH on the observed population differences, FcγRIIIa surface density on $CD56^{dim}$FcγRIIIa^{bright} NK cells were compared between Caucasian individuals and Black individuals in the presence and absence of the *FCGR3A*-IH. In the absence of the *FCGR3A*-IH, the previously observed significant difference between the two population groups was no longer significant ($P = 0.209$ vs. $P < 0.001$) (Figure 3.1B and 3.4B).

3.3.5. FcγRIIIa-F158V (rs396991) and FcγRIIIa cell surface density

The FcγRIIIa-158F allele was detected at a frequency of 0.68 in Black individuals and 0.67 in Caucasian individuals, while the FcγRIIIa-158V allele was detected at a frequency 0.32 in Black individuals and 0.33 in Caucasian individuals. In Caucasian individuals, a genetic linkage was observed between the *FCGR3A*-IH and the functional FcγRIIIa-F158V. All FcγRIIIa-158FF donors were negative for the *FCGR3A*-IH, while all *FCGR3A*-IH positive donors carried at least one FcγRIIIa-158V allele (Table 3.3). Due to the linkage between the *FCGR3A*-IH and FcγRIIIa-158V variant it was not possible to determine the independent role of these variants on FcγRIIIa surface density in Caucasian individuals.

In Black individuals, however, the *FCGR3A*-IH was not detected, thus, allowing for the study of the association between the FcγRIIIa-158V variant and FcγRIIIa surface densities. In this population group, individuals with at least one FcγRIIIa-158V allele had significantly higher FcγRIIIa surface densities compared to FcγRIIIa-158F homozygous carriers (27713 vs. 17040 ABC, P = 0.011) (Figure 3.4C).

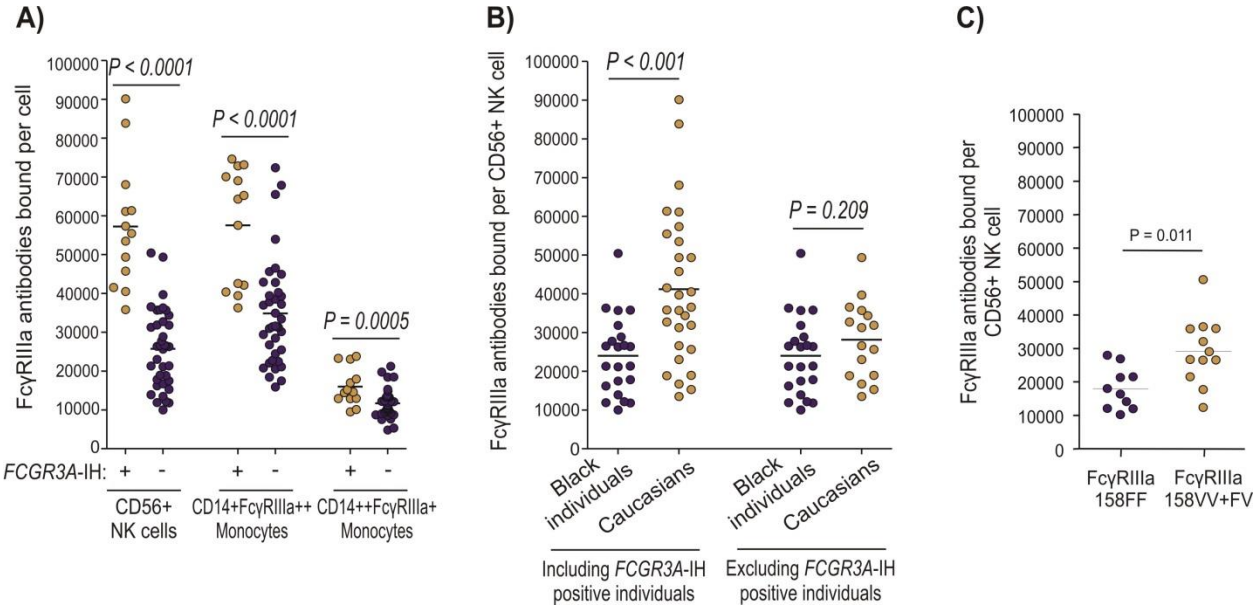


Figure 3.4. FcγRIIIa cell surface densities in relation to genetic variances. (A) FcγRIIIa surface densities (FcγRIIIa antibodies bound per cell) on $CD56^{+}$ NK cells, $CD14^{dim}FcγRIIIa^{bright}$ monocytes and $CD14^{bright}FcγRIIIa^{dim}$ monocytes observed for *FCGR3A* intragenic haplotype positive and negative individuals. (B) The *FCGR3A* intragenic haplotype contributes to the observed significant population differences for FcγRIIIa surface densities. (C) An association between the FcγRIIIa-158V allele and FcγRIIIa surface densities in individuals negative for the *FCGR3A* intragenic haplotype.

Table 3.3. FcγRIIIa haplotype and 158FV allotype combinations for 22 Black and 29 Caucasian individuals

FcγRIIIa Phenotype	*FCGR3A Intragenic Haplotype (IH) Positive or Negative*								
	All Individuals			**Black Individuals**			**Caucasian individuals**		
	n	***IH -***	***IH+***	***n***	***IH -***	***IH+***	***n***	***IH -***	***IH+***
158FF	*25*	25	-	*10*	10	-	*15*	15	-
158FV	*19*	11	8	*10*	10	-	*9*	1	8
158VV	*7*	2	5	*2*	2	-	*5*	-	5

3.4. DISCUSSION

Fc receptors functionally link the humoral and cellular components of the immune system and have a key role in the activation and modulation of the immune response. Variations within Fc-gamma receptors have been implicated in autoimmune diseases, immunotherapy of cancers and viral infections (Bournazos et al 2009b). It is widely accepted that FcγRIIIa binding affinity, as determined by the FcγRIIIa-F158V variant, is a strong predictor of its function and is of clinical importance. However, the role of FcγRIIIa cell surface density in this regard is less clear and requires further elucidation. The purpose of this investigation was therefore to assess variability in proportions of FcγRIIIa-expressing leukocytes and the relative densities of these molecules, in two ethnically divergent South African populations, thereby providing baseline data.

FcγRIIIa-bearing T lymphocytes have long been recognized (Braakman et al 1992, Lanier et al 1985). While FcγRIIIa is constitutively expressed on γδ TCR T lymphocytes, it is also detectable at low levels on subsets of $CD4^+$ and $CD8^+$ αβ TCR T lymphocytes (Bjorkstrom et al 2008, Chauhan & Moore 2012, Clemenceau et al 2008). In our overall study population, 6.4% of $CD4^+$ and 10.6% of $CD8^+$ T lymphocytes expressed FcγRIIIa. Of interest, the proportion of $Fc\gamma RIIIa^+CD8^+$ T lymphocytes reported here was notably higher than previously described (3.8%) (Bjorkstrom et al 2008). The discrepancy is likely due to variations in assays used to detect surface FcγRIIIa molecules as well as possible population differences. While $Fc\gamma RIIIa^+CD4^+$ T lymphocytes are currently uncharacterized

and the function of FcγRIIIa on this cell subset unclear, FcγRIIIa^{+}CD8^{+} T lymphocytes have been characterized as ADCC-mediating terminally differentiated effector memory CD8^{+} T lymphocytes (Clemenceau et al 2008). Together with γδ TCR T lymphocytes, this αβ TCR T lymphocyte subset constitute one-fourth of all peripheral lymphocytes capable of FcγRIIIa-mediated ADCC (Clemenceau et al 2008). In individuals chronically infected with Hepatitis C virus, FcγRIIIa^{+}CD8^{+} T lymphocytes display clonal expansion within peripheral blood and infected tissues and may be an underestimated contributor of ADCC (Bjorkstrom et al 2008). In light of this, it needs to be determined if the observed lower proportion of FcγRIIIa^{+}CD8^{+} T lymphocytes in Black individuals compared to Caucasian individuals is of clinical relevance.

NK cells are the primary mediators of ADCC in peripheral blood and their capacity to perform ADCC depends, in part, on FcγRIIIa surface densities (Breunis et al 2009, Liu et al 2009). While elevated levels of FcγRIIIa surface density potentially allow NK cells to respond more efficiently to target cells, lower levels of FcγRIIIa may impair this response. The significantly lower FcγRIIIa surface densities on NK cells from Black individuals therefore suggest that they may have a lower NK cell-mediated ADCC capacity compared to Caucasian individuals. Since NK cell-mediated ADCC responses are increasingly recognized as an important component of immune control of HIV-1 (Baum et al 1996, Lambotte et al 2009), this immune capability becomes particularly important in sub-Saharan African populations where the HIV-1 burden is significant. Furthermore, it has been postulated that ADCC played a contributory role in the protection against HIV-1 infection in the Thai RV144 gp120 vaccine efficacy trial (Karnasuta et al 2005, Rerks-Ngarm et al 2009). These population differences in FcγRIIIa surface densities may also have implications for future HIV-1 vaccines where mode of protection may involve inducing ADCC-mediating antibodies.

Of interest, FcγRIIIa surface densities on NK cells correlated strongly with that of monocytes in both population groups, indicating similar FcγRIIIa expression regulation in these cell types. While the significantly lower FcγRIIIa surface densities on CD14^{dim}FcγRIIIa^{bright} monocytes from Black individuals may indicate reduced FcγRIIIa-mediated phagocytic capability, the significantly larger proportion of CD14^{dim}FcγRIIIa^{bright}

monocytes in these individuals may compensate for this. On the other hand, it has been demonstrated that FcγRIIIa-positive monocytes are more permissive to productive HIV-1 infection than the majority of monocytes that do not express FcγRIIIa (Ellery et al 2007). While Black individuals may have a larger monocyte population capable of FcγRIIIa-mediated effector functions, it also presents a larger population of monocytes permissive to HIV-1 infection as well as a potential larger HIV-1 reservoir population.

The number of *FCGR3A* gene copies per diploid genome directly influences FcγRIIIa surface density. In the present study, the contribution of *FCGR3A* gene copy number variation to the observed large variation of FcγRIIIa surface densities was negligible due to the low frequency thereof. However, occurring at a higher frequency was a 3-SNP/1-indel *FCGR3A* intragenic haplotype that displayed a strong association with augmented FcγRIIIa surface expression. The observed association is likely attributable to two *FCGR3A* intragenic haplotype polymorphisms, -1405G>A SNP (rs56199187) and +690-691InsC (rs33959719), that are located within transcriptional regulatory regions. The -1405G>A SNP is located within in a ~200 bp putative silencer region (-1579 to -1376 region) (Gessner et al 1996). With the use of luciferase reporter assays, it has been demonstrated that the deletion of this ~200 bp region results in enhanced luciferase activity (>2-fold) (Gessner et al 1996). It is possible that the -1405G>A SNP may disrupt binding of repressors to the silencer element, resulting in increased FcγRIIIa expression. Similarly, the +690-691InsC indel is located within a regulatory region, a ~700 bp intronic enhancer region (+10 to +712 region) that enhances the proximal promoter (Pprox) activity and subsequently transcription (Gessner et al 1995b). The presence of the +690-691InsC indel in this enhancer region may augment binding of activators and subsequently result in upregulation of transcription as has been reported for a polymorphism in a different human enhancer region (Alberobello et al 2011).

Global allele frequencies are available from the 1000 Genomes Project for one *FCGR3A* intragenic haplotype variant located within the putative silencer region (SNP rs56199187) (1000_Genomes_Project_Consortium 2010). While complete linkage of all variants within the *FCGR3A* intragenic haplotype in all individuals is not certain, this SNP may serve as an approximation of *FCGR3A* intragenic haplotype frequencies in different population groups.

According to data from the 1000 Genomes Project the minor allele of SNP rs56199187 that forms part of the *FCGR3A* intragenic haplotype occurs at a frequency of 0.14 (108/758 alleles) in Europeans, 0.07 (23/339 alleles) in Americans, 0.01 (5/492 alleles) in Africans (East Africans, West Africans and African Americans) and 0.002 (1/572 alleles) in Asians. The low detection rate of this SNP in Africans is in agreement with our *FCGR3A* intragenic haplotype findings. However, we were not able to detect the haplotype in an extended total of 137 Black individuals, suggesting that frequencies of this allele are possibly even lower in Southern African individuals.

The two alleles of the triallelic polymorphism, FcγRIIIa-48R and -48H, which form part of the *FCGR3A* intragenic haplotype, are of particular interest since this polymorphism has been investigated previously. Of interest, notably higher cytophilic IgG (naturally associates with FcγRIIIa) has been observed on NK cells from combined FcγRIIIa-48LH and -48LR donors compared to FcγRIIIa-48LL donors (FcγRIIIa-F158V was constant between groups) (Koene et al 1997). Since cytophilic IgG naturally associates with FcγRIIIa, higher cytophilic IgG levels may thus be indicative of high NK cell FcγRIIIa surface density in FcγRIIIa-48LH and -48LR donors. In the present study, FcγRIIIa-48LH and -48LR donors (identified as *FCGR3A* intragenic haplotype positive donors) had higher FcγRIIIa surface densities compared to FcγRIIIa-48L/L donors (identified as *FCGR3A* intragenic haplotype negative donors), thus supporting our findings of an association between the *FCGR3A* intragenic haplotype and FcγRIIIa increased surface densities.

In addition to the association between the *FCGR3A* intragenic haplotype and surface densities, we observed a weaker association for the FcγRIIIa-158V allele. This is in agreement with other studies that used the same anti-FcγRIIIa antibody clone (3G8) to quantitate FcγRIIIa on NK cell surfaces (Hatjiharissi et al 2007, Vance et al 1993). However, the 3G8 epitope includes the FcγRIIIa-F158V polymorphic site and the increased affinity of the 3G8 clone for the FcγRIIIa-158V allele may erroneously display an association with surface density (Congy-Jolivet et al 2008, Tamm & Schmidt 1996). As demonstrated by others, the association between the FcγRIIIa-158V allele and surface densities is no longer significant when FcγRIIIa is quantitated by monoclonal antibodies targeting different regions in the FcγRIIIa molecule (Congy-Jolivet et al 2008, Wu et al 1997). It is, however,

unlikely that the observed association between the *FCGR3A* intragenic haplotype and increased surface density is due to variable binding affinity of 3G8 for its epitope. The 3G8 epitope is situated within the membrane-proximal Ig-like domain of FcγRIIIa and the only nonsynonymous polymorphism included in the *FCGR3A* intragenic haplotype (triallelic FcγRIIIa-L48RH polymorphism) is located within the membrane-distal Ig-like domain.

In conclusion, our findings show baseline inter-individual and population differences in FcγRIIIa surface densities on peripheral blood mononuclear cells and in proportions of FcγRIIIa-positive leukocytes. Future studies investigating FcγRIIIa-mediated effector mechanisms relating to antitumor antibody therapy, autoimmune diseases and protective immunity against pathogens need to consider these differences. Importantly, we have identified an *FCGR3A* intragenic haplotype that displayed a significant association with increased FcγRIIIa cell surface densities and accounted for the observed population differences. As high densities translate to improved cell function (Breunis et al 2009), this haplotype represents an important genetic marker for study in various disease outcomes.

CHAPTER 4

VARIABILITY AT THE LOW AFFINITY *FCGR* LOCUS: CHARACTERIZATION IN BLACK SOUTH AFRICANS AND EVIDENCE FOR ETHNIC VARIATION IN AND OUT OF AFRICA

4.1. INTRODUCTION

Low affinity Fcγ receptors (FcγR) mediate key immune effector mechanisms through the engagement of the Fc portion of IgG. They are involved in multiple biological processes, including clearance of antigen/antibody immune complexes, enhancement of antigen presentation, antibody-dependent cellular cytotoxicity (ADCC), phagocytosis, regulation of antibody production, and activation of inflammatory cells. Two families of low affinity FcγR exist, each with different isoforms – FcγRIIa/b/c and FcγRIIIa/b – that display distinct cellular distributions and effector functions.

Functionally, FcγR are divided into activating or inhibitory receptors depending on the presence of an immunoreceptor tyrosine-based activation (ITAM) or inhibitory motif (ITIM) either in the receptor itself or associated signaling subunits (Daeron 1997). FcγRIIb is the only inhibitory receptor, while all other FcγR are activating receptors. The dual signals from co-engaged activating and inhibitory FcγR set a threshold for cell activation, regulating responses such as phagocytosis, ADCC, and release of inflammatory mediators (Nimmerjahn & Ravetch 2007). FcγR variability may modulate this balance and ultimately effector function capacity.

Functional variability of FcγR has been ascribed to variable surface density that affect cross-linking and aggregation of these receptors on the cell surface as well as nonsense mutations that affect their antibody binding affinity or subcellular localization. Variable density of FcγR on the cell surface affects cell signaling and subsequently effector functions (Breunis et al 2009, Willcocks et al 2008). Genetic determinants of FcγR surface density have been described and include gene copy number variation of *FCGR2C*, *FCGR3A*, and *FCGR3B*, and nucleotide variants in transcriptional regulatory regions of *FCGR2B* (2B.4 haplotype [g.-386C/g.-120A]) and *FCGR3A* (four-variant intragenic haplotype) (Breunis et al 2009, Lassauniere et al 2013, Su et al 2004a). Amino acid changes in the ligand binding domains of FcγRIIa (p.H131R) and FcγRIIIa (p.F158V) change their affinity for IgG subclasses. The FcγRIIa-131H variant displays a higher affinity for IgG2 as well as increased phagocytosis of IgG2 opsonin (Bruhns et al 2009, Sanders et al 1995, Warmerdam et al 1991), while the increased affinity of the FcγRIIIa-158V variant for IgG1, IgG2 and IgG4 has been associated with a greater level of NK cell activation (Bruhns et al

2009, Wu et al 1997). Unlike the aforementioned variants, the functional polymorphism in FcγRIIb (p.I232T) is located in its transmembrane domain where a threonine severely impairs the receptor's inhibitory function through exclusion of FcγRIIb from lipid rafts (Floto et al 2005). The different allotypes of FcγRIIIb – HNA1a, HNA1b, HNA1c – arise from a combination of amino acid changes. While they do not display variable affinity or specificity for IgG subclasses (Bruhns et al 2009), a higher phagocytic capacity has been observed for HNA1a compared to HNA1b (Salmon et al 1990), and increased FcγRIIIb surface density associated with HNA1c (Koene et al 1998).

Unlike other FcγR, FcγRIIc occurs predominantly as a pseudogene with a combination of minor alleles determining its expression on NK cells, monocytes, neutrophils and B cells (Li et al 2013, van der Heijden et al 2012). These include the c.169T>C (p.*57Q) variant in exon 3 and two splice-site mutations in intron 6 i.e. c.798+1A>G and c.799-1G>C. When expressed, FcγRIIc adds to the repertoire of activating receptors that mediates innate immune effector functions such as ADCC (van der Heijden et al 2012), while on B cells it counterbalances the negative feedback of FcγRIIb and enhances humoral responses to immunization as demonstrated in a human anthrax vaccine trial (Li et al 2013). These findings, together with an intronic *FCGR2C* tag variant (c.134-96C>T, alias *FCGR2C* 126C>T) recently associated with vaccine efficacy (VE) in the Thai phase III RV144 HIV-1 vaccine trial (Li et al 2014, Rerks-Ngarm et al 2009), suggest an important role for *FCGR2C* variability in immunization. With the immunogenicity of the RV144 vaccine regimen being evaluated in South Africa (HVTN097 trial) it is important to investigate *FCGR2C* variability in Africans.

Overall, a paucity of data exists for Africans at the *FCGR* locus. While select data are available for certain African populations, studying genetic variants in Africans is complicated by the significant genetic diversity among different African populations. This study therefore comprehensively investigates FcγR variability in South African Black individuals and South African Caucasian individuals and assesses differences between populations of different geographical regions in Africa.

4.2. MATERIALS AND METHODS

4.2.1. Study participants

Refer to Chapter 2, Materials and Methods section 2.1. In brief, *FCGR* variability was studied in 137 South African Black individuals and 32 South African Caucasian individuals. Data from the South African cohort was compared to those of other global populations using data from published works and the 1000 Genomes project.

4.2.2. *FCGR* gene copy number variability and nucleotide variant detection

FCGR gene copy number and nucleotide variants were determined using the *FCGR*-specific multiplex ligation-dependent probe amplification (MLPA) assay as described in Chapter 2, Materials and Methods sections 2.3. and 2.5. Discrimination of *FCGR2B/C* promoter variants were achieved through gene specific PCR amplification and nucleotide sequencing as described in Chapter 2, Materials and Methods section 2.6. and 2.10.

4.2.3. *FCGR3A* intragenic haplotype detection

A tag variant (g.-1405G>A, rs56199187) was targeted as representative of the haplotype and detected by sequence-specific primer PCR (SSP-PCR) as described in Chapter 2, Materials and Methods section 2.7.

4.2.4. *FCGR2C* c.134-96C>T (rs114945036)

Genotyping of the *FCGR2C* c.134-96C>T variant (rs114945036) and those reported to be in complete linkage disequilibrium with it, rs138747765 and rs78603008 (Li et al 2014), was done through nucleotide sequencing as described in Chapter 2, Materials and Methods sections 2.6. and 2.8.

4.2.5. Synteny of *FCGR2C* variants

The synteny (occurrence on the same chromosome) of nucleotide variants within the *FCGR2C* gene was determined through a sequence-specific primer PCR assay as described in Chapter 2, Materials and Methods sections 2.9. and 2.10.

4.2.6. Monoclonal antibodies and reagents

Refer to Chapter 2, Materials and Methods section 2.11.

4.2.7. Isolation of NK cells

NK cells were negatively selected from peripheral blood mononuclear cells as described in Chapter 2, Materials and Methods section 2.13.

4.2.8. HIV-1 gp120 coated target cells

The target cells used in the HIV-1 specific cytotoxicity assays were coated with recombinant HIV-1 gp120 envelope protein as described in Chapter 2, Materials and Methods section 2.14.

4.2.9. Cytotoxicity assays

ADCC activity was measured by flow cytometry using a previously described method (Pollara et al 2011). In brief, HIV-1 gp120 coated cells were incubated with anti-HIV-1 IgG and isolated NK cells in the presence of a fluorogenic granzyme B substrate. In target cells that received a lethal hit, the substrate will be cleaved by granzyme B and a fluorescent signal generated. The percentage target cells killed were determined by flow cytometry.

4.2.10. Computational and Statistical analysis

Computational and statistical analysis was performed as described in Chapter 2, Materials and Methods section 2.17. In brief, the χ^2-test was used to compare demographic characteristics, overall genotype distribution and gene copy number distribution between the study cohorts. Categorical data were analysed with the Fisher's exact test. Hardy Weinberg equilibrium was calculated with the exact test as described by Haldane (Haldane 1954) for biallelic variants.

4.3. RESULTS

4.3.1. Duplication/deletion of genomic regions in the low affinity *FCGR* locus

Due to sample quality six out of 137 South African Black individuals could not be genotyped. Three distinct duplicated/deleted regions of between 45,000 and 68,000 base pairs were identified (Figure 4.1). The most common of these regions, previously designated CNR1 (Niederer et al 2010), encompasses the *FCGR2C-HSP7-FCGR3B* genes and occurred in 8/32 (25%) Caucasian individuals and 34/131 (30%) South African Black

individuals. CNR1 duplications occurred at almost double the frequency of deletions in both Caucasian individuals (62.5% vs. 37.5%) and South African Black individuals (70.6% vs. 29.4%). A second region encompassing the *HSP6-FCGR3A-FCGR2C* genes was detected in 3/32 (9.4%) Caucasian individuals and 5/131 (3.8%) South African Black individuals. Only exons 1 to 6 but not exon 7 of the *FCGR2C* gene were detected and corresponds to the previously described CNR2 (Niederer et al 2010), where the *FCGR3A* gene was duplicated but not the 3'UTR of the *FCGR2C* gene. One South African Black donor carried a deletion of CNR1 and a duplication of CNR2. CNR3 was not detected in our cohorts. However, a novel copy number region, CNR4, was identified in an African individual where only the complete *FCGR2C* gene was duplicated. The frequency of duplications/deletions of CNR1, CNR2 or CNR4 did not differ significantly between the two population groups ($P > 0.05$ for all comparisons).

4.3.2. Low affinity *FCGR* gene copy number variation

CNV is a significant contributor to FcγR variability. It displays a gene-dosage effect and alters the cellular distribution of FcγRIIb (CNRI deletion). In concordance to previous studies (Breunis et al 2008, Breunis et al 2009), CNV was observed for the *FCGR2C*, *FCGR3A* and *FCGR3B* genes but not the *FCGR2A* and *FCGR2B* genes (Table 4.1). Overall, *FCGR3A* CNV was low (8/163 [4.9%]). Conversely, CNV of the *FCGR3B* gene and *FCGR2C* gene was much more frequent, respectively occurring in 8/32 (25%) and 10/32 (31.3%) Caucasian individuals, and 34/131 (26%) and 39/131 (29.8%) South African Black individuals. *FCGR3A* CNV did not differ significantly between South African Black individuals and Kenyans, British Caucasian individuals or Netherlands Caucasian individuals ($P > 0.05$ for all comparisons). While a greater proportion of South African Black individuals had more than two *FCGR3B* gene copies compared to Dutch Caucasian individuals (18.4% vs. 10.9%), this was not statistically significant ($P = 0.085$).

4.3.3. Haplotypes predicting FcγRIIb and FcγRIIIa surface density

Neither the *FCGR2B* 2B.4 promoter haplotype, nor the *FCGR3A* intragenic haplotype (IH) previously associated with increased expression of these molecules were detected in 131 South African Black individuals while occurring in 13/32 (40.6%) and 16/32 (50%) South

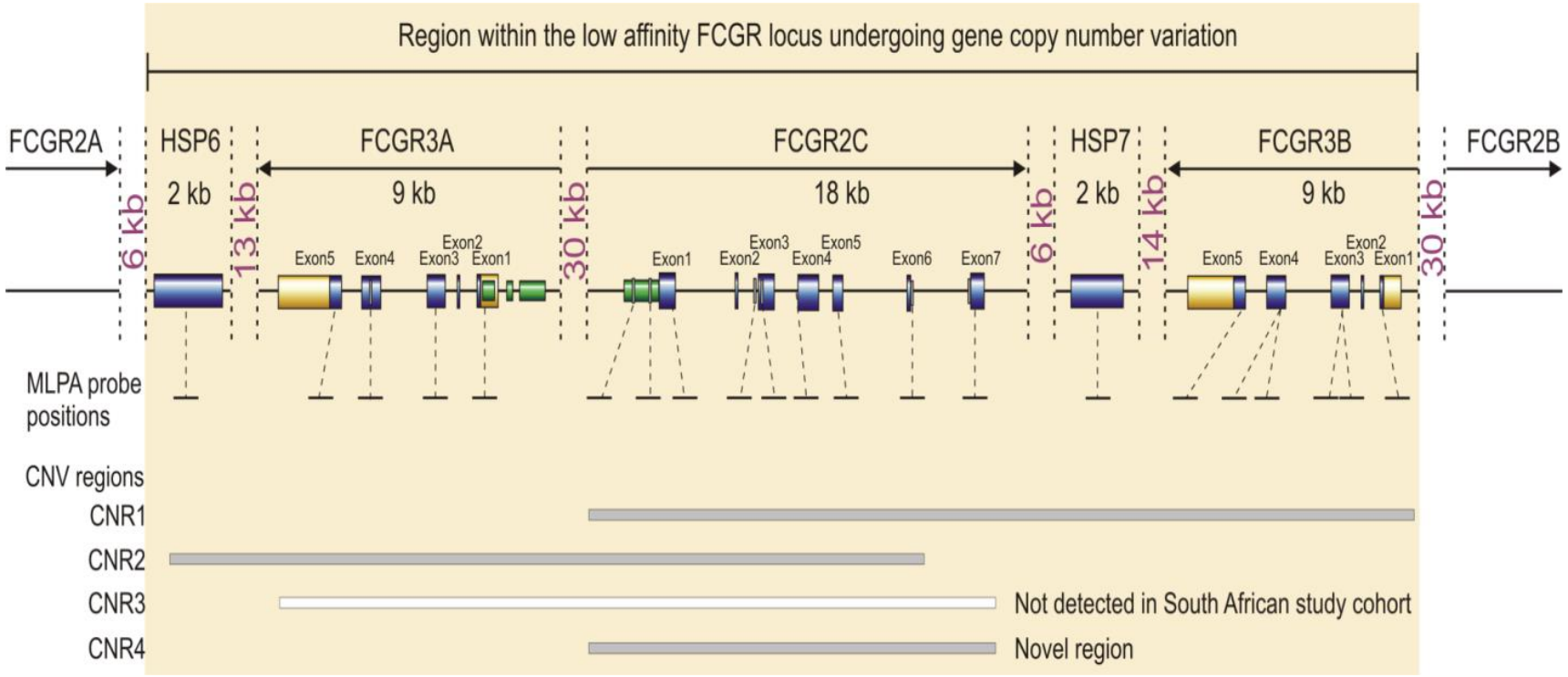


Figure 4.1. A schematic representation of the *FCGR* locus and the regions subject to duplication/deletion. The gene cluster that encompasses *HSP6*, *FCGR3A*, *FCGR2C*, *HSP7* and *FCGR3B* is subject to copy number variation which results from duplication or deletion of large genomic segments (45,000 – 68,000 base pairs). These copy number regions (CNR) were previously designated CNR1-3 (Niederer et al 2010). CNR1, CNR2 and a novel region, designated CNR4, were detected in South African individuals (grey bars) while the previously described CNR3 was not (white bar). CNR1 and CNR2 correspond to CNV regions previously identified by MLPA (Breunis et al 2008). The *FCGR* genes are arranged according to their position on chromosome 1. The black arrows indicate the orientation of the gene. Distances between genes are shown. The vertical dotted lines ending in solid horizontal bars indicate the position of MLPA probes used to determine copy number variation and sequence variations within the respective genes. The grey bars representing CNR1, 2, and 4 indicate regions as detected by consecutive MLPA probes, the true ends of these regions are not known.

African Caucasian individuals, respectively (Table 4.2). Similarly, in the 1000 Genomes populations, the *FCGR3A*-IH tag variant was not detected in 88 Yoruba Nigerians (YRI) and in only 1/97 (1%) Luhya Kenyans (LWK), while occurring in 102/379 (26.9%) Caucasian individuals (EUR super population). 1000 Genomes frequency data was not available for the *FCGR2B* 2B.4 haplotype. However, it has been detected in 66/366 (18%) Caucasian controls in the study that originally described the *FCGR2B* 2B.4 haplotype (Su et al 2004b).

Table 4.1. Copy number variation in the *FCGR2* and *FCGR3* genes in the present study and other studies

	Present study		**Other studies**		
	South African Black individuals n=131	**South African Caucasian individuals n=32**	**Kenyan Black individuals† n=833†**	**United Kingdom Caucasian individuals† n=1484†**	**Netherlands Caucasian individuals#* n=146#, n=129***
FCGR2A					
2 gene copy	**131** *(100%)*	**32** *(100%)*	**-**	**-**	-
FCGR2B					
2 gene copy	**131** *(100%)*	**32** *(100%)*	**-**	**-**	**146#** *(100%)*
FCGR2C					
1 gene copy	**9** *(6.9%)*	**3** *(9.4%)*	**-**	**-**	**10#** *(6.8%)*
2 gene copies	**93[a]** *(71%)*	**21** *(65.6%)*	**-**	**-**	**116#** *(79.5%)*
3 gene copies	**25[b]** *(19.1%)*	**6[c]** *(18.8%)*	**-**	**-**	**20#** *(13.7%)*
4 gene copies	**4** *(3.1%)*	**1** *(3.1%)*	**-**	**-**	**0#** *(0%)*
5 gene copies	**0** *(0%)*	**1** *(3.1%)*	**-**	**-**	**0#** *(0%)*
FCGR3A					
1 gene copy	**0** *(0%)*	**0** *(0%)*	**4†** *(0.4%)*	**20†** *(1.3%)*	**2*** *(1.6%)*
2 gene copies	**126** *(96.2%)*	**29** *(90.6%)*	**814†** *(97.7%)*	**1389†** *(93.7%)*	**122*** *(94.6%)*
3 gene copies	**5** *(3.8%)*	**3** *(9.4%)*	**15†** *(1.8%)*	**75†** *(5.1%)*	**5*** *(3.9%)*
FCGR3B					
1 gene copy	**10** *(7.6%)*	**3** *(9.4%)*	**-**	**-**	**9#** *(6.2%)*
2 gene copies	**97** *(74%)*	**24** *(75%)*	**-**	**-**	**121#** *(82.9%)*
3 gene copies	**20** *(15.3%)*	**3** *(9.4%)*	**-**	**-**	**16#** *(10.9%)*
4 gene copies	**4** *(3.1%)*	**1** *(3.1%)*	**-**	**-**	**0#** *(0%)*
5 gene copies	**0** *(0%)*	**1** *(3.1%)*	**-**	**-**	**0#** *(0%)*

[a]One, [b]Four, and [c]Three individuals had a deletion of CNR1 and a duplication of CNR2, thus one *FCGR2C* copy is incomplete

†(Niederer et al 2010)

#(van der Heijden et al 2012)

*(Breunis et al 2009)

Table 4.2. Genotype and allele frequencies of the *FCGR2B* promoter haplotypes

	South African Black individuals n=131	South African Caucasian individuals n=32
Genotype Frequency		
Homozygous -386G-120T (2B.1)	**131** *(100)*	**19** *(59.4)*
Heterozygous	**0** *(0)*	**12** *(37.5)*
Homozygous -386C-120A (2B.4)	**0** *(0)*	**1** *(3.1)*
Haplotype Frequency		
-386G-120T (2B.1)	**262** *(100)*	**50** *(78.1)*
-386C-120A (2B.4)	**0** *(0)*	**14** *(21.9)*

4.3.4. *FCGR* functional variants in different populations

Genotype and allele frequencies of the low-affinity *FCGR* functional variants observed in the two South African cohorts together with data from the 1000 Genomes Project and other studies are listed in Table 4.3. Due to the small South African Caucasian cohort, population differences were investigated using published data from larger cohorts of Dutch, British and European Caucasian individuals of which South African Caucasian individuals are descendants.

Significant differences were observed between Caucasian individuals and Africans (Appendix B.1). Similar to previous reports, the FcγRIIb-232T allele was overrepresented in Africans (20.5 – 30.9%) compared to Caucasian individuals (11.5 – 12.3%), with a higher proportion of Africans (38.7 – 56.7%) carrying at least one FcγRIIb-232T allele compared to Caucasian individuals (19.0 – 21.9%). At the FcγRIIIa-F158V locus, Kenyans had an underrepresentation of the FcγRIIIa-158V allele (13.9%) compared to EUR and Dutch Caucasian individuals (26.9 – 30.5%, $P < 0.001$), while South African Black individuals had an overrepresentation of the FcγRIIIa-158V allele (36.7%) compared to EUR Caucasian individuals (26.9%, $P < 0.01$). The FcγRIIIb-HNA1a was the dominant allotype in South African Black individuals (50.6%), while the HNA1b allotype dominated in South African and Dutch Caucasian individuals (60.9% and 62.3%, respectively; $P < 0.01$). Carriage of at least one HNA1c allotype was significantly higher in South African Black individuals compared to South African Caucasian individuals (29.8% vs. 6.3%, $P < 0.05$).

Table 4.3. Genotype and allele frequencies of functional variants for FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb in healthy individuals from South Africa, Kenya, Nigeria and Europe.

	Present study		Black individual genotype/allele frequencies from other studies		Caucasian genotype/allele frequencies from other studies	
	South African Black individuals n=131	South African Caucasian controls n=32	1000 genomes Luhya Kenyans LWK group n=97	1000 genomes Yoruba Nigerians YRI group n=88	1000 genomes Caucasian individuals, EUR supergroup n=379	Netherlands Caucasian individuals (ref 2) n=100
FCGR2A (rs1801274)						
H131R Genotype Frequency						
131RR	**41** *(31.3)*	**5** *(15.6)*	**23** *(23.7)*	**23** *(26.1)*	**98** *(25.9)*	**20** *(20)*
131HR	**64** *(48.9)*	**13** *(40.6)*	**43** *(44.3)*	**47** *(53.4)*	**183** *(48.3)*	**52** *(52)*
131HH	**26** *(19.8)*	**14** *(43.8)*	**31** *(32)*	**18** *(20.5)*	**98** *(25.9)*	**28** *(28)*
Hardy Weinberg EQ	*P = 0.469*	*P = 1.000*	*P = 0.309*	*P = 0.668*	*P = 0.538*	*P = 0.692*
H131R Allele Frequency						
131R	**146** *(55.7)*	**23** *(35.9)*	**89** *(45.9)*	**93** *(52.8)*	**379** *(50)*	**92** *(46)*
131H (ref allele)	**116** *(44.3)*	**41** *(64.1)*	**105** *(54.1)*	**83** *(47.2)*	**379** *(50)*	**108** *(54)*
FCGR2B (rs1050501)						
I232T Genotype Frequency						
232II	**61** *(46.6)*	**26** *(81.3)*	**42** *(43.3)*	**54** *(61.4)*	**296** *(78.1)*	**81** *(81)*
232IT	**59** *(45)*	**5** *(15.6)*	**50** *(51.5)*	**32** *(36.4)*	**73** *(19.3)*	**15** *(15)*
232TT	**11** *(8.4)*	**1** *(3.1)*	**5** *(5.2)*	**2** *(2.3)*	**10** *(2.6)*	**4** *(4)*
Hardy Weinberg EQ	*P = 0.682*	*P = 0.307*	*P = 0.058*	*P = 0.508*	*P 0.052*	***P = 0.022***
I232T Allele Frequency						
232I (ref allele)	**181** *(69.1)*	**57** *(89.1)*	**134** *(69.1)*	**140** *(79.5)*	**665** *(87.7)*	**177** *(88.5)*
232T	**81** *(30.9)*	**7** *(10.9)*	**60** *(30.1)*	**36** *(20.5)*	**93** *(12.3)*	**23** *(11.5)*
FCGR3A (rs396991)						
F158V Genotype Frequency						
158FF	**50** *(38.2)*	**14** *(43.8)*	**74** *(76.3)*	**46** *(52.3)*	**212** *(55.9)*	**48** *(48)*
158FV	**63** *(48.1)*	**8** *(25)*	**19** *(19.6)*	**40** *(45.5)*	**130** *(34.3)*	**41** *(41)*
158VV	**13** *(9.9)*	**7** *(21.9)*	**4** *(4.1)*	**2** *(2.3)*	**37** *(9.7)*	**7** *(7)*
158FFF	**0** *(0)*	**1** *(3.1)*	-	-	-	**1** *(1)*
158FFV	**1** *(0.8)*	**1** *(3.1)*	-	-	-	**0** *(0)*
158FVV	**4** *(3.1)*	**1** *(3.1)*	-	-	-	**1** *(1)*
Hardy Weinberg EQ (2 gene copies only)	*P = 0.335*	***P = 0.044***	*P = 0.081*	*P = 0.083*	***P = 0.013***	*P = 0.805*
F158V Allele Frequency						
158F (ref allele)	**169** *(63.3)*	**42** *(62.7)*	**167** *(86.1)*	**132** *(75)*	**554** *(73.1)*	**141** *(69.5)*
158V	**98** *(36.7)*	**25** *(37.3)*	**27** *(13.9)*	**44** *(25)*	**204** *(26.9)*	**57** *(30.5)*
FCGR3B						
Genotype Frequency						
HNA1a+/1b-/1c-	**31** *(23.7)*	**7** *(21.9)*	-	-	-	**17** *(17)*
HNA1a-/1b+/1c-	**15** *(11.4)*	**14** *(43.8)*	-	-	-	**38** *(38)*
HNA1a-/1b-/1c+	**6** *(4.6)*	**0** *(0)*	-	-	-	-
HNA1a+/1b+/1c-	**46** *(35.1)*	**9** *(28.1)*	-	-	-	**45** *(45)*
HNA1a+/1b-/1c+	**21** *(16)*	**1** *(3.1)*	-	-	-	-
HNA1a-/1b+/1c+	**10** *(7.6)*	**0** *(0)*	-	-	-	-
HNA1a+/1b+/1c+	**2** *(1.5)*	**1** *(3.1)*	-	-	-	-
Allele Frequency						
HNA1a (ref allele)	**137** *(50.6)*	**25** *(36.2)*	-	-	-	**77** *(37.7)*
HNA1b	**91** *(33.6)*	**42** *(60.9)*	-	-	-	**127** *(62.3)*
HNA1c	**43** *(15.9)*	**2** *(2.9)*	-	-	-	**4** *(4)*
Carriage of at least one allele						
≥1 HNA1a	**100** *(76.3)*	**18** *(56.3)*	-	-	-	-
≥1 HNA1b	**73** *(55.7)*	**24** *(75)*	-	-	-	-
≥1 HNA1c	**39** *(29.8)*	**2** *(6.3)*	-	-	-	-

Significant differences were also observed between individuals from different geographic regions in Africa (Figure 4.2, Appendix B.1). Yoruba Nigerians had a lower frequency of the FcγRIIb-232T allele and individuals carrying at least one FcγRIIb-232T allele (20.5% and 38.7%, respectively) compared to Luhya Kenyans (30.1% and 56.7%, respectively; $P < 0.05$) and South African Black individuals (30.9% and 53.4%, respectively; $P < 0.05$). Compared to Luhya Kenyans, South African Black individuals were more likely to carry at least one FcγRIIa-131R allele (80.2% vs. 68%, $P < 0.05$). Luhya Kenyans also had a lower frequency of the FcγRIIIa-158V allele and individuals carrying at least one FcγRIIIa-158V allele (13.9% and 23.7%, respectively) compared to South African Black individuals (36.7% and 61.8%, respectively; $P < 0.0001$) and Yoruba Nigerians (25% and 47.8%, respectively; $P < 0.01$). Compared to Yoruba Nigerians, South African Black individuals had a greater frequency of the FcγRIIIa-158V allele (36.7% vs. 25%, $P < 0.05$) and more individuals carrying at least one FcγRIIIa-158V allele (61.8% vs. 47.8%) though not statistically significant ($P = 0.052$). For comparisons at the FcγRIIIa-F158V locus, *FCGR3A* CNV was not taken into account for the Kenyan and Nigerian data, however the influence thereof was considered negligible due to its low frequency (3.8% in South African Black individuals and 2.2% in Kenyans).

4.3.5. Africans do not express FcγRIIc

A combination of three minor alleles in *FCGR2C* predicts the expression of functional FcγRIIc (Figure 4.3A). To accurately predict expression of FcγRIIc, the synteny (occurrence on the same chromosome) of expression variants was determined for donors carrying the c.169C allele (exon 3 p.57Q allele). In all Caucasian individuals that possessed the c.169C allele (53%), it was syntenic with the c.798+1G/c.799-1G splice-site alleles, representing the previously designated *FCGR2C*-ORF genotype that yields functional FcγRIIc (Figure 4.3B). In all South African Black individuals, however, the c.169C allele was syntenic with the c.798+1**A**/c.799-1G alleles, representing the non-classical *FCGR2C*-ORF1 genotype that does not yield functional FcγRIIc (Figure 4.3B). Thus, while 35/131 (26.7%) South African Black individuals carried the c.169C allele (classical marker for FcγRIIc expression) none of them were predicted to express FcγRIIc based on the c.798+1**A** splice variant. As expected, FcγRIIc surface expression on $CD56^{dim}$ NK cells from donors with the non-classical *FCGR2C*-

ORF1 genotype clustered with those homozygous for the c.169T allele (exon 3 p.57*, Figure 4.3C).

Data from the 1000 Genomes Project indicates that the splice variant required for expression, c.789+G, is subject to significant ethnic variation (Table 4.4). In Caucasian populations 9-32.9% of individuals carried at least one c.798+1G allele, while 6.5-14.2% of admixed Americans (AMR) and only 0-1.2% of Africans (YRI+LWK) and 0-1% of East Asians (ASN) carried this allele. This suggests that expression of FcγRIIc is rare to absent in Africans and East Asians compared to up to 32.9% of Caucasian individuals.

Interestingly, one South African Caucasian donor with a CNR2 duplication (lacks *FCGR2C* exon 7) carried a *FCGR2C* gene copy predicted to express FcyRIIc, but had no detectable levels of FcyRIIc on his NK cells (data not shown). This suggests that the *FCGR2C* gene copy predicted to express FcyRIIc formed part of CNR2, was likely incomplete, and thus not expressed on the cell surface.

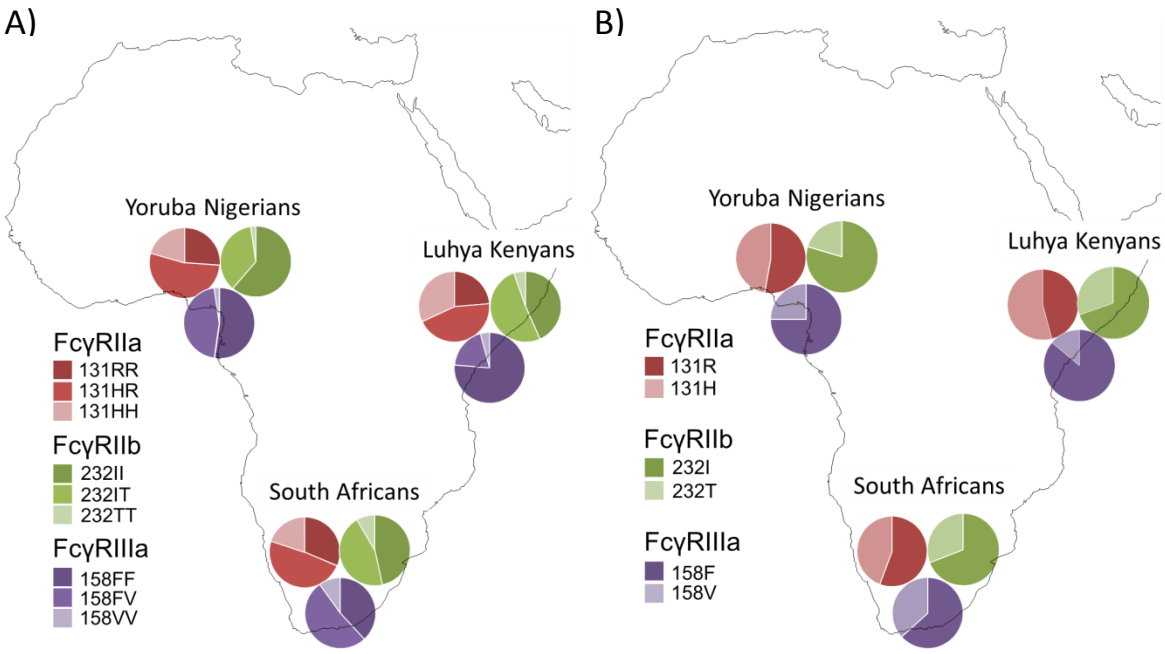


Figure 4.2. A) Genotype and B) Allele distributions of FcγRIIa-R131H, FcγRIIb-I232T, and FcγRIIIa-F158V in African individuals from different geographical regions. Luhya Kenyans in East Africa, Yoruba Nigerians in West Africa and South African Black individuals.

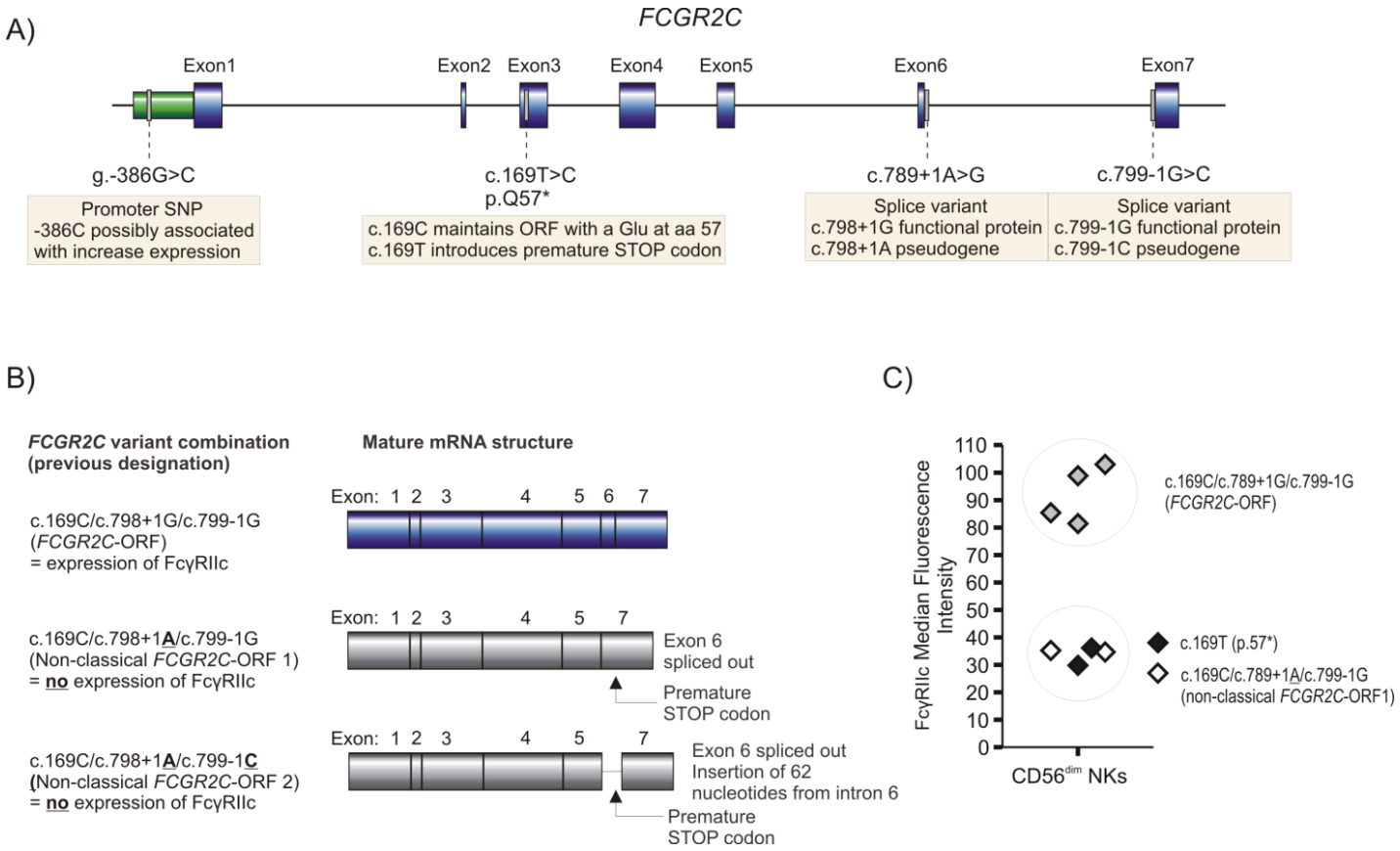


Figure 4.3. Functional genetic variants within *FCGR2C* that determine its expression on natural killer cells, monocytes and neutrophils (van der Heijden et al 2012). A) *FCGR2C* gene structure indicating the location of expression variants. B) Three genotypes have been identified for individuals with the c.196C allele (previously designated ORF allele) depending on the combination of splice variants located in intron 6. The c.169C/c.798+1G/c.799-1G genotype results in expression of a functional FcγRIIc (blue bar). The two grey bars represent the exon assembly in the mature mRNA from the c.169C/c.798+1A/c.799-1G genotype and c.169C/c.798+1A/c.799-1C genotype. Both result in splicing of exon 6 from the mRNA, however, the latter genotype also results in the addition of 62 nucleotides from intron 6. While the latter two genotypes contain the c.169C allele, neither yield functional FcγRIIc due to the modifications to the processed mRNA. C) FcγRIIc surface expression on $CD56^{dim}$ NK cells from donors with different *FCGR2C* expression genotypes.

Table 4.4. Genotype and allele frequencies of the *FCGR2C* c.798+1A>G splice variant in the 1000 Genomes Project super populations

	Caucasian individuals (EUR)[a]	Admixed American (AMR)[b]	African Americans (ASW)[c]	East Asians (ASN)[d]	Africans (YRI+LWK)[e]
FCGR2C (rs76277413)					
Genotype Frequency					
c.798+1AA	69.4 – 89.9%	87.3 - 91.7%	95.1%	99 - 100%	100%
c.798+1AG	9 – 28.6%	5 - 10.9%	4.9%	0 - 1%	0%
c.798+1GG	0 – 4.3%	1.5 - 3.3%	0%	0%	0%
Allele Frequency					
c.798+1A	83.7 – 94.4%	92.7 - 94.7%	97.5%	99.5 - 100%	100%
c.798+1G	5.6 – 16.3%	5.3 - 7.3%	2.5%	0 - 0.5%	0%

[a]Consisting of: CEU – Utah residents with Northern and Western European ancestry; FIN – Finnish in Finland; GBR – British in England and Scotland; IBS – Iberian population in Spain
[b]Consisting of: MXL – Mexican Ancestry from Los Angeles USA; PUR – Puerto Ricans from Puerto Rico; CLM – Colombians from Medellin; Colombia
[c]ASW – Americans of African Ancestry in South West USA. As a separate group in table to illustrate the difference between African Americans and Africans
[d]Consisting of: CHB – Han Chinese in Beijing, China; JPT – Japanese in Tokyo, Japan; CHS – Southern Han Chinese
[e]Two African populations combined: YRI – Yoruba in Ibadan, Nigeria; LWK – Luhya in Webuye, Kenya

4.3.6. The complete *FCGR2C* Thai haplotype is rare to absent in Africans

A three variant haplotype, with *FCGR2C* c.134-96C>T reported as the tag variant, has recently been associated with vaccine efficacy (VE) in the RV144 HIV-1 vaccine trial in Thailand, with an estimated 91% VE in individuals carrying at least one T allele compared 15% VE in those that did not (Li et al 2014). In the present study, at least one *FCGR2C* c.134-96T allele was observed in 56/115 (48.7%) South African Black individuals at an allele frequency of 24.9%. However, the complete *FCGR2C* Thai haplotype was not observed in any of the South African Black individuals as two loci in the haplotype – p.T118I (rs138747765) and c.391+111G>A (rs78603008) – were not polymorphic in this population group. This was also the case for Yoruba Nigerians, while Luhya Kenyans had a haplotype allele frequency of only 0.5%.

Of the 23/28 (82.1%) South African Caucasian individuals that carried at least one *FCGR2C* c.134-96T allele, only 12/28 (42.3%) Caucasian individuals possessed the complete *FCGR2C* Thai haplotype. This difference resulted from the *FCGR2C* c.134-96T tag allele occurring at a higher frequency than the other constituents of the haplotype (48.7% vs. 30.3%) and was therefore not in complete linkage disequilibrium with p.T118I or c.391+111G>A in either South African Caucasian individuals (D' = 1; r^2 = 0.282) or EUR

Caucasian individuals (D' = 0.992, r^2 = 0.832). The p.T118I and c.391+111G>A were, however, in complete linkage disequilibrium (D' = 1, r^2 = 1), with this haplotype occurring at a frequency of 30.3% in South African Caucasian individuals and 25.3% in the 1000 genomes EUR group.

Individuals that carried minor alleles of the *FCGR2C* Thai haplotype would not necessarily express FcγRIIc. The *FCGR2C* c.134-96T allele occurred in 62.5% of Caucasian individuals that did not express FcγRIIc and 86.7% in those that did (p = 0.297). However, the minor alleles of *FCGR2C* p.T118I and *FCGR2C* c.391+111G>A occurred more frequently in Caucasian individuals that do not express FcγRIIc (9/13 [69.2%]) compared to those that do express FcγRIIc (3/15 [20%], p = 0.020). For the latter donors, the variants predicting FcγRIIc expression was syntenic with the major alleles of the aforementioned variants.

4.3.7. Strong linkage disequilibrium between *FCGR* genetic variants in Caucasian individuals but not Africans

FcγRIIc-T118I, *FCGR2C* c.798+1A>G, *FCGR2B* g.-386G>C and *FCGR2B* g.-120T>A were not polymorphic in South African Black individuals leading to notable differences in linkage disequilibrium (LD) between the population groups (Figure 4.4A). These differences were reflected in LD for Caucasian individuals (EUR super population) and Africans (combined Nigerians [YRI] and Kenyans [LWK]) in the 1000 Genomes Project (Figure 4.4B).

In Caucasian individuals, LD was strong across variants in *FCGR3A* and *FCGR2C* as well as the promoter region of *FCGR2B*. In particular, certain alleles were overrepresented in donors that expressed FcγRIIc compared to those that do not (Appendix B.2), including the *FCGR3A* intragenic haplotype (IH) previously associated with increased surface density of FcγRIIIa (94.1% vs. 0%; D' = 0.908, r^2 = 0.758), the FcγRIIIa-158V allele (88.2% vs. 13%; D' = 0.786, r^2 = 0.420), the *FCGR2C* g.-386C allele (94.1% vs. 0%; D' = 0.912, r^2 = 0.766), and the *FCGR2B* g.-386C/g.-120A haplotype (70.6% vs. 6.7%; D' = 0.661, r^2 = 0.335). The combination of alleles suggest a stronger activation phenotype for FcγRIIIa and FcγRIIc, which may be countered by a stronger inhibitory phenotype of FcγRIIb.

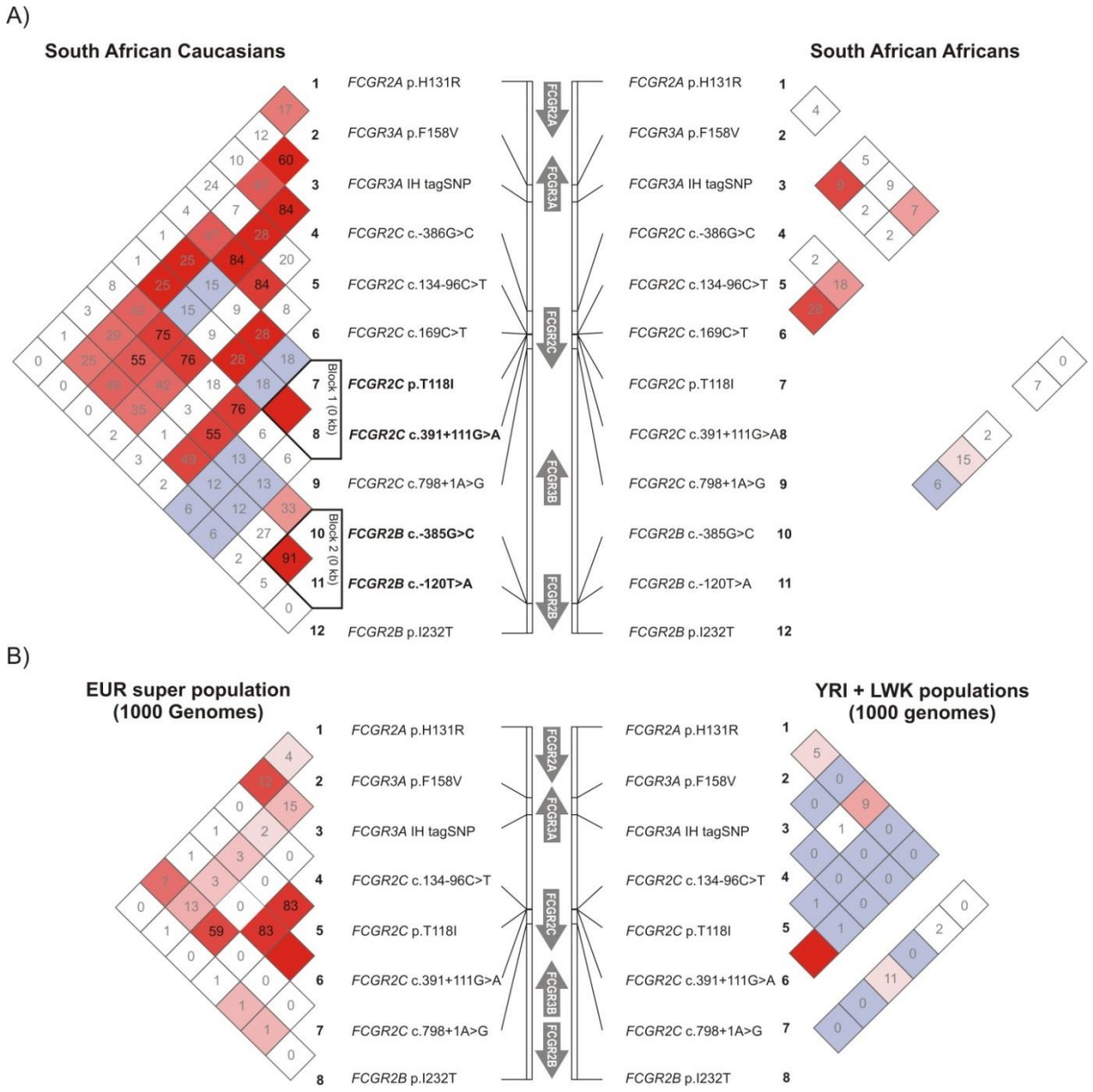


Figure 4.4. Linkage disquilibrium for *FCGR* variants in Caucasian individuals and Black individuals. Linkage disequilibrium plots of variants studied in the low affinity FcγR gene locus in A) South African Caucasian individuals (left) and South African Black individuals (right); and B) 1000 Genomes EUR super population (left) and combined YRI and LWK populations (right). The *FCGR3A*-IH tag variant (rs56199187), *FCGR2C*-p.T118I (rs1387477765), *FCGR2C* c.798+1A>G (rs3219018), *FCGR2B* c.-386G>C (rs3219018), and *FCGR2B* c.-120T>A (rs34701572) were not polymorphic in the South African Black individuals cohort. For variants studied in the South African cohorts, 1000 Genomes data were available for those indicated with an asterisk. Values and colours reflect r^2 (×100) and *D'*/LOD measures of LD, respectively. The black triangle depicts a haplotype block.

As the strong LD between the *FCGR3A*-IH and FcγRIIc expression variants suggest, Caucasian individuals predicted to express functional FcγRIIc had significantly higher FcγRIIIa surface densities compared to those that did not express FcγRIIc (49 386 antibodies bound per cell (ABC) vs 31 313 ABC, P = 0.0005, Figure 5A). A similar LD between the *FCGR3A*-IH tag variant and FcγRIIc c.798+1G expression variant (D' = 0.864, r^2 = 0.598) in the EUR super population suggest this linkage is maintained across all Caucasian individuals.

4.3.8. FcγRIIc and FcγRIIIa do not act synergistically for NK cell mediated ADCC

Since FcγRIIc is an activating receptor capable of mediating ADCC, a possible synergy may exist between FcγRIIc and FcγRIIIa when co-expressed on NK cells. Moreover, the concomitant increased FcγRIIIa surface density on NK cells that express FcγRIIc may further enhance ADCC capacity. To test this hypothesis, the ADCC capacity of $CD56^{dim}$ NK cells from Caucasian donors that express FcγRIIc and had high FcγRIIIa levels (mean MFI: 22354; range: 16805 – 27472) were compared to that of Black donors that do not express FcγRIIc and had low FcγRIIIa levels (mean MFI: 12604; range: 10984 – 14096). To control for the confounding effect of other variants on ADCC capacity, donors across the two groups were matched for the FcγRIIIa-F158V genotype and none possessed a CNR1 deletion previously shown to result in expression of FcγRIIb on NK cells (van der Heijden et al 2012, Wu et al 1997).

The enriched $CD56^{dim}$ NK cell fractions isolated from PBMCs exhibited reduced FcγRIIIa surface density, with a greater loss associated with higher *in vivo* levels (Figure 5B). Due to the disproportionate loss of FcγRIIIa surface molecules and a reduction in the mean density difference between Caucasian individuals and Black individuals from 43% *in vivo* to 26% *ex vivo*, the functional contribution of FcγRIIIa density could not be accurately assessed. FcγRIIc surface density, however, remained constant (Figure 5C). Co-expression of FcγRIIc and FcγRIIIa does not appear to have a synergistic effect on ADCC capacity since the cytotoxic capacity of NK cells that expressed FcγRIIc was comparable to that of NK cells that did not express FcγRIIc (Figure 5D). NK cell purity was >70% (mean: 84.6%), contaminating cells were primarily granulocytes as determined by flow cytometric forward and side scatter data.

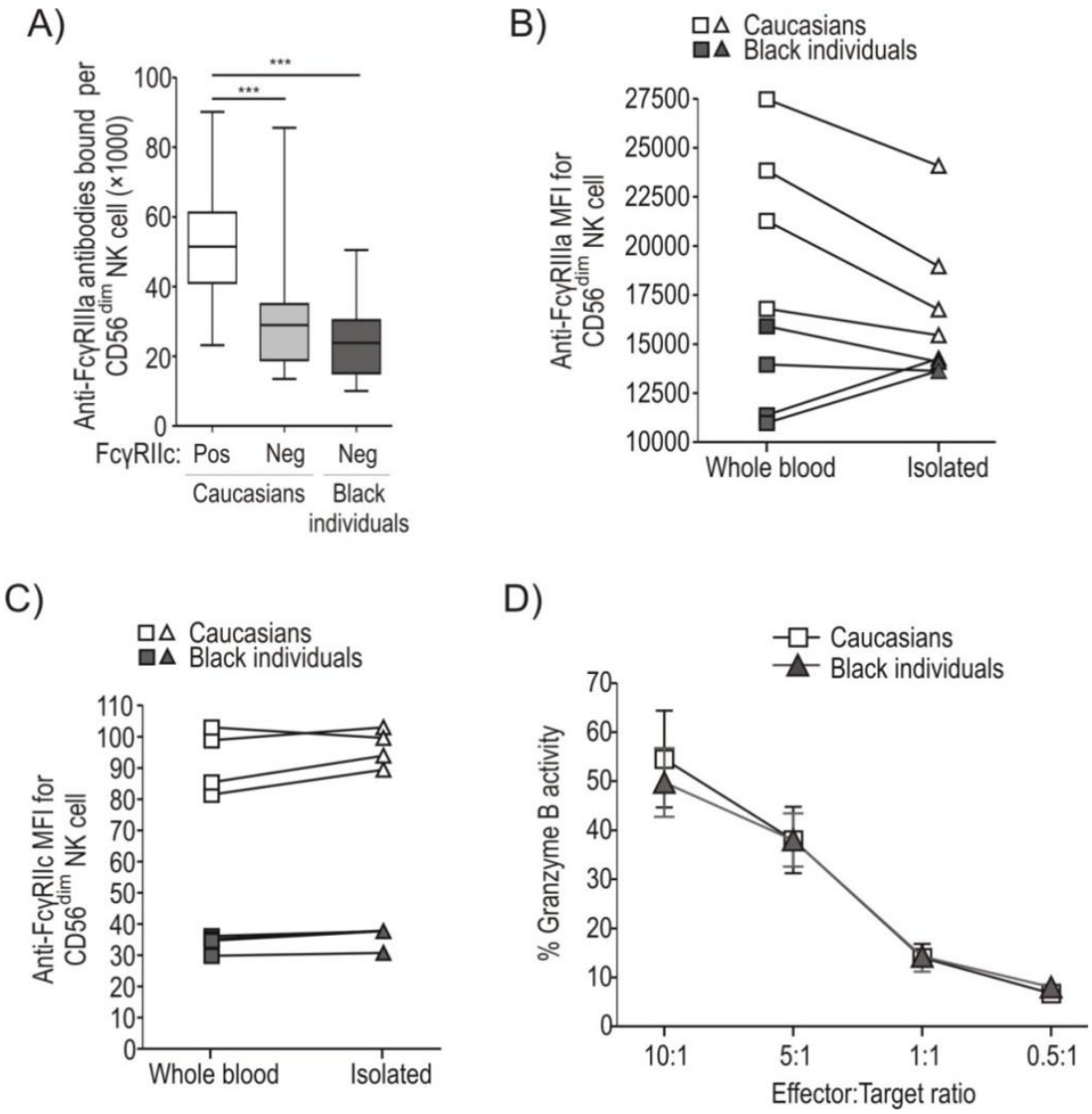


Figure 4.5. Phenotypic and functional analysis of FcγRIIIa and FcγRIIc on NK cells. A) Comparison of FcγRIIIa surface density on CD56^{dim} NK cells in whole blood from donors that express FcγRIIc compared to those that do not. Four Caucasian donors (FcγRIIc +, high FcγRIIIa) and four Black donors (FcγRIIc -, low FcγRIIIa) were selected to investigate the functional significance of the different NK cell phenotypes. To determine whether the *ex vivo* NK cell phenotypes were comparable to the *in vivo* phenotypes, B) FcγRIIIa and C) FcγRIIc surface density were measured in whole blood and isolated CD56^{dim} NK cell fractions. D) The cytotoxic capacity (% granzyme B activity) of NK cells isolated from Caucasian individuals (FcγRIIc +, high FcγRIIIa) and Black donors (FcγRIIc -, low FcγRIIIa) as measured in cytotoxicity assays.

4.3.9. Summary of variability in activating and inhibitory FcγR in South African Black individuals and Caucasian individuals

Figure 4.6 gives a collective representation of the differences observed for FcγR variability in two South African population groups. Overall, Caucasian individuals had higher frequencies of FcγRIIc and FcγRIIIa variants that enhance cell activation compared to Black individuals. Conversely, Black individuals had higher frequencies of FcγRIIb and FcγRIIIb variants that enhance cell activation compared to Caucasian individuals as well as the absence of a variant that enhances inhibitory effect of FcγRIIb.

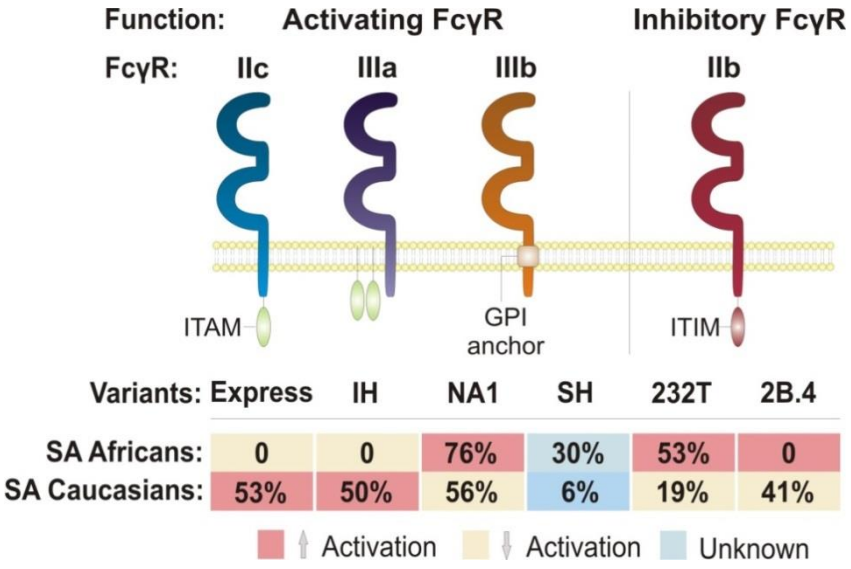


Figure 4.6. Summary of FcγR variants with significantly different distributions between South African Black and Caucasian individuals. Below each receptor are the variants that displayed significant differences between these population groups as well as the frequency of individuals carrying at least one of these variants. The shade of the blocks represents the effect of the variant on cell activation. ITAM - immunoreceptor tyrosine-based activation motif; ITIM - immunoreceptor tyrosine-based inhibitory motif; GPI – glycosylphosphatidylinositol.

4.4. DISCUSSION

This study describes the first extensive characterization of variability at the low affinity *FCGR* locus in South African Black individuals and comparison of FcγR variability between different African populations. The findings of this study highlighted (i) the extensive ethnic diversity at the low affinity FCGR gene locus, (ii) potential differences between population groups in maintaining the activation/inhibition balance conferred by FcγR variants, and (iii) that variation among African populations precludes the use of any one African population as proxy for FcγR diversity in Africans.

Gene copy number variability and high sequence homology between genes at the *FCGR* locus makes it a complex region to study. Collectively, six regions of genomic duplication/deletion have been identified at the *FCGR* locus to date (Breunis et al 2009, Niederer et al 2010), of which not all contain complete *FCGR* gene copies. Copy number region (CNR) 2 lacked *FCGR2C* exon 7, which together with exon 6 encode the main parts of the cytoplasmic domain responsible for signal transduction. An individual predicted to express FcγRIIc may not do so should the copy carrying the expression variants form part of CNR2 as was observed for a Caucasian donor in the present study.

CNV not only displays a gene-dosage effect but can also affect the cellular distribution of FcγRIIb. In the presence of a CNR 1 deletion, the 5'-regulatory sequences of *FCGR2C* is juxtaposed with the coding sequence of *FCGR2B*, creating a chimeric gene, *FCGR2B'* (Mueller et al 2013). The result is that FcγRIIb, which is otherwise absent from cytotoxic NK cells, is expressed on this cell subset where it inhibits cell activation and ADCC, possibly due to co-engagement of FcγRIIIa and FcγRIIb (Mueller et al 2013, van der Heijden et al 2012). In the present study, 7.6% of South African Black individuals and 9.4% of South African Caucasian individuals carried a CNR1 deletion as reflected by carriage of only one *FCGR3B* and *FCGR2C* gene copy. However, the prevalence of *FCGR2B'* may be underestimated. Unless family trios are studied, CNV phase cannot be determined. It is therefore plausible that an individual with two copies of CNR1 may carry both a duplication and deletion. This hypothesis is supported by a higher detection rate of CNR1 duplications compared to CNR1 deletions as well as the identification of individuals carrying both duplications and deletions of different CNRs in this study and others

(Niederer et al 2010). With the potential role of *FCGR2B'* in diseases such as systemic lupus erythematosus (Mueller et al 2013), an assay that accurately detects this variant may be of value in future association studies.

FCGR copy number and genotypic variability is well described in the South East Kenyan population (Niederer et al 2010). However, these data are not representative of all Africans as even geographically close African populations display extensive genetic variation (Tishkoff et al 2009). This diversity is driven by demographic factors such as fluctuation of population size, admixture and migration, as well as region-specific selection pressure from living in diverse environments and exposure to infectious diseases (Tishkoff & Williams 2002). This African population subdivision is reflected at the *FCGR* locus. Africans carrying at least one FcγRIIb-232T allele ranged from 38.7% in Nigerians to 56.7% in Luhya Kenyans, while those carrying at least one FcγRIIIa-158V allele ranged from 23.7% in Luhya Kenyans to 61.8% in South African Black individuals. These differences further emphasize that a single African population cannot be used as a proxy for diversity across Africa.

The ethnic variation between Caucasian individuals, Africans and Asians at specific sites within the *FCGR* locus is well documented (Adu et al 2012, Bux et al 1997, Chu et al 2004, Floto et al 2005, Kaset et al 2013, Li et al 2003, Matsuhashi et al 2012, Nielsen et al 2012, Osborne et al 1994, Siriboonrit et al 2003, Tong et al 2003). The FcγRIIa-131H allele occurs at a higher frequency in Asians (72-77%) compared to Caucasian individuals (50%), while the FcγRIIb-232T allele is overrepresented in both Asians (22%) and African Americans (29%) compared to Caucasian individuals (10-13%), and the FcγRIIIb-SH allotype occurs more frequently in Africans (20%) compared to Caucasian individuals (2.5%) and Asians (0-0.4%). The findings of our study support the aforementioned ethnic variation, but also show that the FcγRIIIb-NA1/NA2 allotype distribution differs significantly between South African Black individuals and Caucasian individuals. Compared to the NA2 allotype, the NA1 allotype increase neutrophil-mediated phagocytosis (Salmon et al 1990). The overrepresentation of the NA1 allotype in SA Africans compared to Caucasian individuals may translate to variable neutrophil phagocytic capacity between these populations.

A significant finding in the present study is the considerable ethnic variation observed for *FCGR2C* variants – Thai haplotype and FcγRIIc expression. The complete *FCGR2C* Thai haplotype was absent from South African Black individuals while detected at an allele frequency of 30.3% in South African Caucasian individuals and previously in 13.5% of Thai vaccines in the RV144 trial (Li et al 2014). The functional significance of the association between the *FCGR2C* Thai haplotype and vaccine efficacy in the RV144 trial has yet to be elucidated. A possible explanation is that the haplotype is a marker for functional variants located elsewhere (Li et al 2014). However, since it is unclear which variant in the haplotype is causal (or marker thereof), it is of significance that two of the three variants that constitute the haplotype were rare to absent in South African Black individuals, Yoruba Nigerians and Luhya Kenyans. With the RV144 regimen scheduled for testing in South Africa (HVTN 702 trial), these population differences should be taken into consideration. For future studies investigating the *FCGR2C* Thai haplotype, a different tag variant should be used in Africans and Caucasian individuals due to the observed difference between detection of the c.134-96C>T variant and possession of the complete haplotype. In addition to the latter haplotype, significant population differences were observed for FcγRIIc expression. While African individuals are polymorphic at the *FCGR2C* exon 3 locus (c.169T>C; p.*57Q) previously used to predict FcγRIIc expression, none possess the minor allele at the donor splice site in intron 7 (c.798+1A>G) that is also required for expression of FcγRIIc. Thus, based on Africans (South Africans, Kenyans, and Nigerians) not being polymorphic at this locus, none express FcγRIIc compared to up to 33% of Caucasian individuals. The minor allele of this splice variant also only occurs in <1% of Asians (Han Chinese, Southern Han Chinese, and Japanese) and therefore FcγRIIc expression would be expected to be rare to absent. The findings of this study strengthens the importance of not only using the FcγRIIc c.169T>C (p.*57Q) variant alone is not sufficient to predict FcγRIIc expression in particular for African and East Asian individuals, but also for Caucasian individuals (van der Heijden et al 2012).

When expressed, FcγRIIc adds to the repertoire of activating receptors on B cells, NK cells, monocytes, and neutrophils (Li et al 2013, van der Heijden et al 2012). Its presence on B cells enhance antibody responses to immunization in transgenic mice as well as in a

human Anthrax vaccine trial, where individuals homozygous for the FcγRIIc expression variants exhibited a 2.5-fold increase in primary antibody response (Li et al 2013). Since FcγRIIc expression is rare to absent in Africans and Asians, this may have implications for vaccine efficacy in different population groups. In contrast, the findings of our study demonstrate that the presence of FcγRIIc on NK cells does not appear to significantly affect ADCC capacity in donors that express FcγRIIc compared to those that do not. While FcγRIIc mediate ADCC, it has been demonstrated that its ability to induce ADCC is notably decreased compared to FcγRIIIa, a finding consistent in two independent studies that used two different anti-FcγRIIc antibody clones (KB61 and AT10) in reverse ADCC assays (Ernst et al 2002, van der Heijden et al 2012). FcγRIIc's effective counterbalancing of FcγRIIb-mediated inhibition of antibody responses, but lack of synergism with FcγRIIIa on NK cells, may be ascribed to antibody affinity. The extracellular region and antibody binding affinity of FcγRIIb and FcγRIIc are identical. Thus, FcγRIIc can effectively compete with FcγRIIb for ligand binding and counterbalance the inhibitory effect of FcγRIIb. However, compared to FcγRIIIa, FcγRIIc has an approximate 10-fold decrease in its affinity for IgG1 (2×10^5 M^{-1} vs. 1.5×10^6 M^{-1}) and IgG3 (1.7×10^5 M^{-1} vs. 5×10^6 M^{-1}) (Bruhns et al 2009). A moderate 2.5-fold change in FcγRIIIa antibody affinity conferred by the FcγRIIIa-F158V variant significantly affects NK cell mediated ADCC capacity (Bruhns et al 2009, Vance et al 1993, Wu et al 1997). Therefore, with a 10-fold difference in antibody affinity, FcγRIIc may be outcompeted by FcγRIIIa for ligand binding and thus have a nominal contribution to NK cell mediated ADCC. However, the contribution of FcγRIIc to ADCC capacity was not investigated at variable antibody levels. The high concentrations of the A32 monoclonal antibody used in our assays may potentially mask differences between NK cells that are FcγRIIc positive and negative and, thus, further studies are needed. Furthermore, the functional significance of FcγRIIc needs to be evaluated for other cell types and effector functions.

Although Africans lack expression of this additional activating FcγR, other mechanisms may contribute to increased cell activation in this population. Overall, it appears that the balance between FcγR-mediated cell activation and inhibition is maintained differently in Africans and Caucasian individuals. For activating FcγRs, Caucasian individuals have

unique phenotypes, increased FcγRIIIa surface density predicted by *FCGR3A*-IH and FcγRIIc expression, conferring an enhanced activatory phenotype for these receptors (Breunis et al 2009, Ernst et al 2002, Lassauniere et al 2013). However, occurring at equal frequency was the FcγRIIb 2B.4 promoter haplotype associated with a more inhibitory profile for FcγRIIb (Su et al 2004a). Interestingly, all of the aforementioned variants are in strong linkage disequilibrium in Caucasian individuals suggesting that these loci may have evolved together to maintain a balance between FcγR-mediated cell activation and inhibition. In contrast, genetic variants associated with increased surface density of FcγRIIIa and expression of FcγRIIc are rare to absent in Africans. However, phenotypes that decrease FcγRIIb-mediated inhibition occurs at a higher frequency in Africans compared to Caucasian individuals i.e. the FcγRIIb-232T allele and absence of the FcγRIIb 2B.4 promoter haplotype. It should be noted that, while there appears to be a balance, the activating and inhibitory FcγRs are not always expressed on the same cells and the differences observed between Africans and Caucasian individuals may lead to an imbalance in activation in certain cell subsets.

Further evidence toward differential evolution of the *FCGR* locus in Africans and Caucasian individuals is that, compared to Caucasian individuals, Africans were not polymorphic at loci *FCGR2C* c.391+111G>A (rs78603008), *FCGR2C*-T118I (rs1387477765), *FCGR2C* c.798+1G>A (rs3219018) or either of the haplotypes previously associated with increased expression of FcγRIIb (haplotype 2B.4) and FcγRIIIa (intragenic haplotype). This is unexpected since Africa is the most genetically diverse region in the world and non-Africans display less genetic variation compared to Africans (Sirugo et al 2008, Tishkoff & Williams 2002). The acquisition of these variants in Caucasian individuals, or loss thereof in Africans, together with the significant ethnic variation seen for other FcγR variants suggest that the *FCGR* locus is under differential selective pressure in different regions of the world. It has been proposed that malaria may be a contributing factor (Clatworthy et al 2007, Willcocks et al 2010).

Taken together, the findings of this study demonstrate novel differences for FcγR variability between Caucasian individuals and Africans, but also for African populations in different geographical regions. This may translate to variable capacities of FcγR-mediated

effector functions between population groups and potentially contribute to the observed ethnic variation of diseases such as systemic lupus erythematosus and severe lupus nephritis that display strong association with FcγR (Bournazos et al 2009b, Korbet et al 2007, Lau et al 2006). Due to the significant contribution of FcγR-mediated effector functions to protective immunity, these population differences may have implications for responsiveness to vaccination and immunotherapy, as well as the susceptibility and prognosis of infectious diseases.

CHAPTER 5

MOTHER-TO-CHILD TRANSMISSION OF HIV-1: *FCGR* VARIABILITY ASSOCIATES WITH INFECTIOUSNESS AND SUSCEPTIBILITY

5.1. INTRODUCTION

In the absence of any interventions, the risk of perinatal HIV-1 transmission is approximately 30 - 40% (Lehman & Farquhar 2007). The infant can acquire HIV-1 while *in utero*, during labour and delivery (intrapartum), and through breastfeeding. Despite significant advances in the prevention of mother-to-child transmission, a large number of infants are still infected every year. Delineating immunological factors that increase the risk of perinatal HIV-1 transmission and acquisition may aid in identifying women at risk of transmitting HIV-1 to their infants.

The role of maternal HIV-1-specific antibodies in preventing mother-to-child transmission (MTCT) is unclear. Studies have primarily focussed on the involvement of neutralizing antibodies and presented conflicting data (Braibant & Barin 2013). Investigations into the contribution of effector functions mediated by the crystallisable fragment (Fc) of IgG have been largely neglected. The Fc portion of IgG has the capacity to recruit potent effector functions of the innate immune system through engagement with Fcγ receptors (FcγR), which are widely expressed throughout the haematopoietic system. Directly or indirectly FcγRs mediate antiviral processes that include antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), respiratory burst, antigen display, antibody production, cell activation, and release of inflammatory mediators.

Accumulating data suggest that FcγR-mediated effector functions are a key component in HIV-1 protective immunity (Lewis 2014). In murine and non-human primate models, FcγR-mediated mechanisms augment the *in vivo* ability of broadly neutralizing antibodies to block viral entry, suppress viraemia, and confer therapeutic activity (Bournazos et al 2014, Hessell et al 2007). FcγR-mediated effector functions alone may not be sufficient to provide complete protection from HIV-1 infection (Burton et al 2011, Moog et al 2014). However, the observed inverse correlation between ADCC responses and HIV-1 infection risk in a human vaccine trial with modest efficacy (RV144), suggests that a certain degree of protection can be achieved by non-neutralizing antibodies and their effector functions (Haynes et al 2012, Rerks-Ngarm et al 2009, Tomaras et al 2013).

In vivo FcγR-mediated effector functions are governed by a balance between activating and inhibitory FcγRs (Nimmerjahn & Ravetch 2007). This balance is perturbed by functionally significant polymorphisms that include amino acid changes that alter the binding affinity for antibody subclasses (FcγRIIa-H131R and FcγRIIIa-F158V), subcellular localization (FcγRIIb-I232T), glycosylation pattern (FcγRIIIb-HNA1a/b/c), or expression of the functional molecule (FcγRIIc-Q57*). In addition, duplication and deletion of the genes that encode FcγRIIc, FcγRIIIa, and FcγRIIIb display a gene-dosage effect that correlates with effector function (Breunis et al 2009, Willcocks et al 2008). The implications of FcγR variability for HIV-1 acquisition has been demonstrated in a human vaccine trial (VAX004) and perinatal HIV-1 acquisition where homozygosity for the FcγR alleles that confer enhanced antibody binding of FcγRIIIa (p.158V) and FcγRIIa (p.131H), respectively, were identified as risk factors for HIV-1 acquisition (Brouwer et al 2004, Forthal et al 2012). To date, studies have only focussed on one or two FcγR variants and their association with HIV-1 acquisition. However, linkage disequilibrium observed between loci may lead to a collective effect of FcγR variants.

Several lines of evidence suggest that HIV-1 can be transmitted to the infant as cell-free and cell-associated virus (Milligan & Overbaugh 2014). Both these infectious components have been detected in maternal peripheral blood, genital secretions, and breast milk, with virus levels in all these fluids correlating with MTCT (Lehman & Farquhar 2007). Given the role of FcγR-mediated effector functions in eliminating cell-free and cell-associated virus, these processes may modify the infectiousness of an HIV-1 infected mother. Moreover, anti-HIV-1 IgG transferred across the placenta may recruit innate immune effector functions in the infant through engaging FcγRs expressed on foetal/infant immune cells, and thus modify infant susceptibility. Through investigating the association of all known FcγR functional variants in a perinatal HIV-1 transmission cohort, this study indirectly assesses the potential role of FcγR-mediated effector functions in mother-to-child transmission of HIV-1.

5.2. MATERIALS AND METHODS

5.2.1. Study populations

Refer to Chapter 2, Materials and Methods section 2.1. In brief, a nested case-control study was undertaken to investigate low affinity FcγR variability in mothers and infants recruited as part of four perinatal cohorts at two hospitals in Johannesburg, South Africa. All study participants were South African Black individuals. Genotypic data from HIV-1 infected mothers with HIV-1 infected infants (transmitting cases) were compared with HIV-1 infected mothers with uninfected infants (non-transmitting controls). FcγR variability was determined for 73 of the transmitting pairs. For comparison, approximately two non-transmitting pairs matched by cohort were randomly selected for each transmitting pair.

5.2.2. Cohort HIV-1 infection status

Maternal HIV-1 RNA levels and infant HIV-1 status were determined as described in Chapter 2, Materials and Methods section 2.2.

5.2.3. **FCGR** *gene copy number variability and nucleotide variant detection*

Gene copy number and nucleotide variants within the low-affinity *FCGR* genes were determined using the *FCGR*-specific multiplex ligation-dependent probe amplification (MLPA) assay as described in Chapter 2, Materials and Methods sections 2.3. and 2.5. Discrimination of *FCGR2B/C* promoter variants were achieved through gene specific PCR amplification and nucleotide sequencing as described in Chapter 2, Materials and Methods section 2.6. and 2.10.

5.2.4. Overall FcγR variability profile: Allele scoring system

To assess the effect of the overall FcγR variability profile, individuals were categorized as possessing an overall inhibitory profile, neutral profile, or activatory profile. Refer to Chapter 2, Materials and Methods section 2.26. In brief, the high responder FcγR variants (FcγRIIa-131H, FcγRIIb-232T, FcγRIIIa-158V, FcγRIIIb-HNA1a, and high copy number) were each assigned a +1 value, whereas the low responder variants (FcγRIIa-131R, FcγRIIb-232I, FcγRIIIa-158F, FcγRIIIb-HNA1b, and low copy number) were each assigned a

-1 value. The sum of the allele scores were determined for each individual, which were subsequently categorized as possessing an overall inhibitor profile (total score ≤ -1), neutral profile (total score = 0), and activatory profile (total score ≥ 1).

5.2.5. Statistical analysis

Statistical analysis was performed as described in Chapter 2, Materials and Methods section 2.17. In brief, multivariable logistic regression was used to determine the association between FcγR functional variants and perinatal HIV-1 transmission. The t-test was used to compare normally distributed continuous variables and the Fisher's exact test for categorical data. All analyses were performed in STATA version 10.1 (StataCorp LP, College Station, USA) and a p-value of less than 0.05 was considered statistically significant (2-tailed tests).

5.3. RESULTS

5.3.1. Cohort

Of the 217 mother-infant pairs selected, six infants and three mothers could not be genotyped due to poor DNA quality or limited sample availability. Thus, 211 mother-infant pairs were analysed with six unmatched mothers and three unmatched infants. The characteristics of non-transmitting and transmitting mother-infant pairs in the nested case-control study are reported in Table 5.1.

Transmitting mothers had higher viral loads and lower $CD4^+$ T cell counts compared to non-transmitting mothers ($P < 0.0001$ and $P = 0.030$, respectively). When evaluated according to the mode of transmission, viral loads but not $CD4^+$ T cell counts differed significantly between non-transmitting mothers and intrapartum transmitting mothers ($P = 0.0003$ and $P = 0.092$, respectively), *in utero* transmitting mothers ($P = 0.0001$ and $P = 0.115$, respectively), and *in utero* enriched transmitting mothers ($P = 0.0002$ and $P = 0.100$, respectively). Antiretroviral use was similar across non-transmitting mothers, total transmitting mothers and *in utero* transmitting mothers. However, fewer mothers in the intrapartum transmission group received single dose nevirapine (sdNVP) compared to mothers in the non-transmitting and *in utero* transmitting groups ($P = 0.041$ and $P = 0.046$,

respectively). Since maternal sdNVP reduces intrapartum transmission (Guay et al 1999), the association between increased intrapartum transmission rates and lower sdNVP use was to be expected. Maternal age, parity, reported breast feeding, mode of delivery, and infant birth weight did not differ significantly between transmitting mothers (total, intrapartum or *in utero*) and non-transmitting mothers.

Table 5.1. Demographic and clinical characteristics of HIV-1 non-transmitting and transmitting pairs

	Non-Transmitting		Total Transmitting		Intrapartum Transmitting		*In Utero* Transmitting		*In Utero* Enriched Transmitting	
	N		N		N		N		N	
Maternal viral load (log_{10} copies/ml)										
Median (IQR)	132	3.97 (3.18-4.56)	66	4.79 (3.74-5.37)***	26	4.79 (3.85-5.30)***	18	4.89 (4.20-5.47)***	40	4.81 (2.60-5.44)***
Maternal $CD4^+$ cell count										
Mean (std)	131	503 (266)	64	421 (221)*	25	402 (181)	15	409 (276)	39	433 (245)
Maternal age (years)										
Mean (std)	142	26.9 (5.3)	72	26.1 (5.2)	28	26.8 (5.1)	20	27.8 (5.7)	44	28.1 (5.1)
Parity										
Mean (std)	142	2.1 (1.0)	71	2.2 (1.2)	27	2.3 (1.3)	20	2.2 (1.2)	44	2.2 (1.2)
Gestation *N* (%)										
Preterm <37 weeks	133	16 (12.0)	65	12 (18.5)	24	7 (29.2)	19	4 (21.1)	41	5 (12.2)
Mode of delivery *N* (%)										
Caesarean section	139	15 (10.8)	71	9 (12.7)	27	1 (3.7)	20	3 (15.0)	42	8 (19.0)
Birth weight (g)										
Mean (std)	141	2958 (453)	72	2897 (451)	28	2946 (413)	20	2784 (320)*	43	2852 (471)
Breast fed *N* (%)										
> 3 days	143	23 (16.1)	72	7 (9.7)	28	4 (14.3)	20	2 (10.0)	44	3 (6.8)
Antiretrovirals										
Nevirapine	144	81 (56.3)	73	42 (57.5)	29	10 (34.5)*	20	13 (65.0)	44	32 (72.7)*
Triple drug therapy	144	4 (2.8)	73	2 (2.7)	29	0	20	0	44	2 (4.5)
Other drugs	144	8 (5.6)	73	5 (6.8)	29	1 (3.4)	20	2 (10)	44	4 (9.1)

N – Data were not available for all participants
For comparisons with Non-Transmitting mothers: *, P < 0.05; ***, P < 0.001

5.3.2. ***Variants not detected in the study cohort***

Similar to what has been found in healthy, HIV-1 uninfected South African Black

individuals (refer to Chapter 4), none of the mothers or infants was predicted to express functional FcγRIIc or possessed the FcγRIIb 2B.4 promoter haplotype (g.-386C/g.-120A) associated with increased surface expression and inhibitory capacity of FcγRIIb. Thus, in the present study population, the role of these variants in mother-to-child transmission could not be assessed.

5.3.3. **FCGR** *copy number variability*

FCGR copy number variability (CNV) display a gene dosage effect that correlates with the magnitude of FcγR-mediated effector functions and in some instances alter the cellular distribution of the inhibitory receptor, FcγRIIb. Overall, *FCGR3A* CNV was low, occurring in 13/217 (6%) of mothers and 9/214 (4.2%) infants. Conversely, *FCGR3B* CNV was observed frequently, occurring in 65/217 (30%) of mothers and 74/214 (34.6%) of infants. Figure 5.1 represents the distribution of *FCGR3A* and *FCGR3B* gene copies relative to mode of transmission/acquisition in mothers and infants.

Maternal FCGR3A and FCGR3B gene copy number did not differ significantly between non-transmitting and transmitting mothers (total, intrapartum, in utero, or in utero enriched; Figure 5.1A and B, Table 5.2). In the total infant group, however, carriage of a single FCGR3B gene copy was significantly associated with reduced odds of infection compared to infants with two FCGR3B gene copies as reference (adjusted odds ratio [AOR] 0.11, 95%CI: 0.01-0.87, P = 0.036; Figure 5.1C and D, Table 5.2). Interestingly, in the intrapartum infected group, possession of ≥3 FCGR3B gene copies associated with reduced odds of infection compared to two FCGR3B gene copies as reference (AOR 0.10, 95%CI: 0.01-0.83, P = 0.033).

5.3.4. FcγR variants and infectiousness of the transmitter/mother

To determine if FcγR variants associated with the infectiousness of the mother, HIV-1 transmission rates were assessed across maternal genotypes and allele carriage. Table 5.3 reports the observed genotype and allele frequencies together with adjusted AOR, 95% CI, and P-values for comparisons of transmitting pairs to non-transmitting pairs following adjustment for the confounding effect of maternal viral load, CD4+ T cell count, and nevirapine use as found to be significantly different in the univariate analysis.

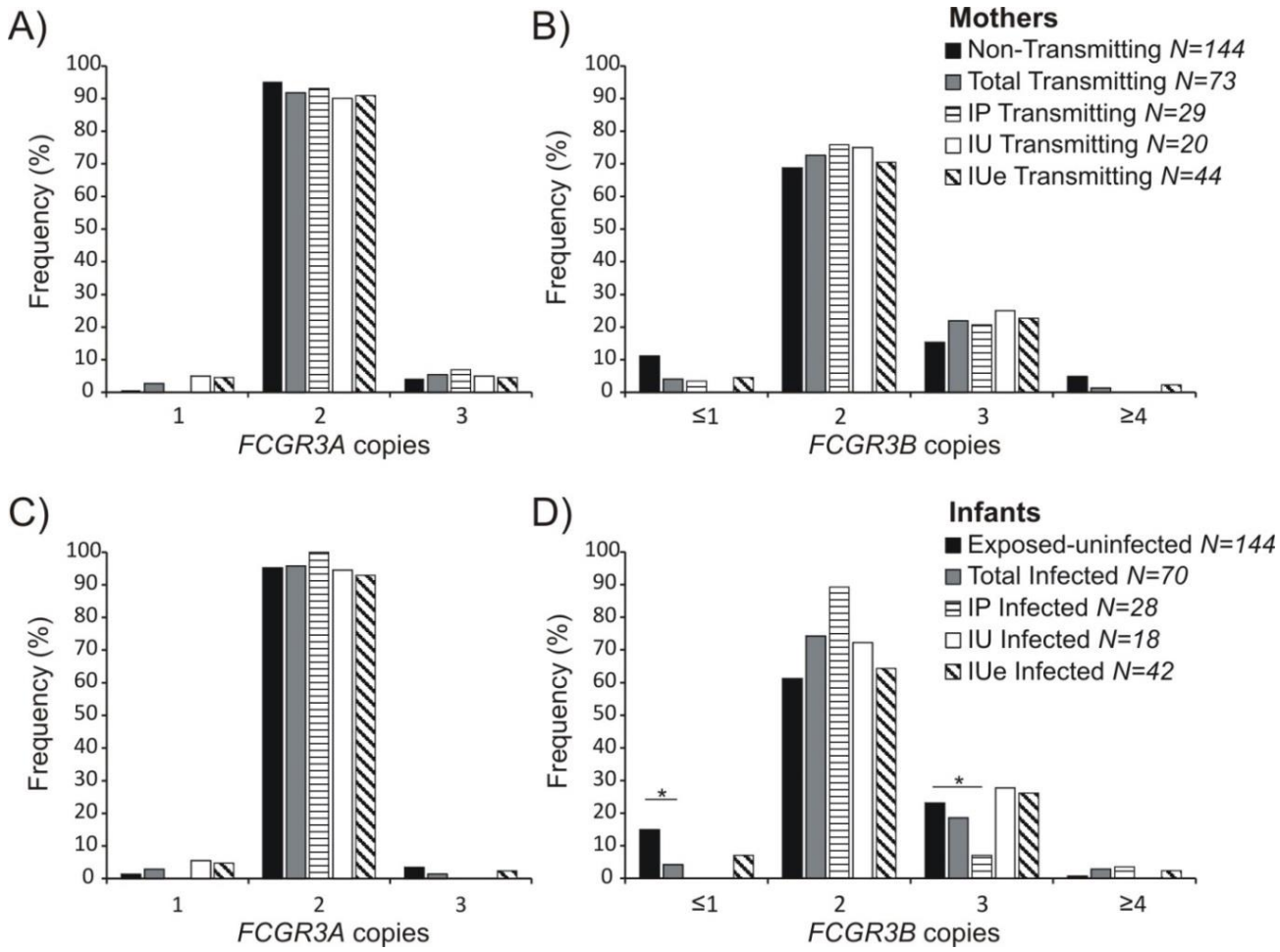


Figure 5.1. *FCGR* copy number variability in HIV-1 infected mothers and their infants. The distribution of *FCGR3A* and *FCGR3B* gene copy number in mothers (A and B, respectively) and infants (C and D, respectively). For comparisons of *FCGR3B* copy number variability, 2 gene copies were considered the reference copy number and compared against 1 gene copy and 3 gene copies, respectively. *, $P < 0.05$; IP, intrapartum; IU, *in utero*; IUe, *in utero* enriched.

Table 5.2. Association of *FCGR3A* and *FCGR3B* gene copy number with perinatal transmission of HIV-1

	Total Infected	**Intrapartum Infected**	***In utero* Infected**	**In utero enriched Infected**
	AOR (95% CI), P value	**AOR (95% CI), P value**	**AOR (95% CI), P value**	**AOR (95% CI), P value**
	Adjusted for mVL+mCD4	Adjusted for mVL+mNVP	Adjusted for mVL	Adjusted for mVL
Maternal *FCGR3A*				
2 copies vs 1 copy	Too few values	Too few values	4.20 (0.25-71.32), P=.320	5.90 (0.50-69.85), P=.159
2 copies vs 3 copies	1.69 (0.40-7.08), P=.472	1.72 (0.28-10.47), P=.555	0.96 (0.09-10.12), P=.973	1.20 (0.21-6.82), P=.839
Maternal *FCGR3B*				
2 copies vs 1 copy	0.32 (0.07-1.55), P=.157	0.38 (0.05-3.16), P=.369	Too few values	0.28 (0.04-2.32), P=.241
2 copies vs ≥3 copies	0.62 (0.26-1.48), P=.283	0.59 (0.17-2.03), P=.404	1.05 (0.31-3.56), P=.937	1.09 (0.46-2.62), P=.845
Infant *FCGR3A*				
2 copies vs 1 copy	4.35 (0.36-52.55), P=.248	Too few values	2.15 (0.18-25.54), P=.545	2.74 (0.36-20.87), P=.329
2 copies vs 3 copies	0.71 (0.07-6.99), P=.765	Too few values	Too few values	1.10 (0.11-11.05), P=.936
Infant *FCGR3B*				
2 copies vs 1 copy	0.11 (0.01-0.87), **P=.036**	Too few values	Too few values	0.18 (0.02-1.41), P=.102
2 copies vs ≥3 copies	0.51 (0.22-1.19), P.=121	0.10 (0.01-0.83), **P=.033**	0.73 (0.20-2.63), P=.626	0.92 (0.39-2.16), P=.853

In the unadjusted analysis, the FcγRIIIa-F158V and FcγRIIIb-HNA1a/b/c showed significant associations with transmission (Appendix C.1-4). For total transmitting pairs and the *in utero* enriched group, mothers homozygous for the FcγRIIIa-158V allele were less likely to transmit HIV-1 to their infants compared to mothers homozygous for the FcγRIIIa-158F allele (odds ration [OR] 0.35, 95%CI: 0.14-0.88, P = 0.026; and OR 0.32, 95%CI: 0.11-0.92, P = 0.034, respectively). Similarly, carriage of at least one FcγRIIIa-158V allele was significantly associated with reduced odds of transmission in both groups (OR 0.55, 95%CI: 0.31-0.97, P = 0.039; and OR 0.3, 95%CI: 0.15-0.61, P = 0.001, respectively). In the *in utero* enriched group, heterozygosity was also associated with reduced odds of transmission (OR 0.30, 95%CI: 0.14-0.65, P = 0.003). For the FcγRIIIb-HNA1a/b/c allotypes, mothers that carried a HNA1b/HNA1c combination were more likely to transmit HIV-1 compared to mothers that carried a HNA1a/HNA1b combination (predominant combination and thus selected as reference). This comparison was significant for total transmitting pairs (OR 2.84, 95%CI: 1.18-6.84, P = 0.020), *in utero* transmitting pairs (OR 6.79, 1.44-32.16, P = 0.016), and *in utero* enriched transmitting pairs (OR 3.45, 95%CI: 1.26-9.43, P = 0.016).

In the adjusted analysis, the association of the FcγRIIIa-F158V variant remained significant. For total transmitting pairs, homozygosity for the FcγRIIIa-158V allele was associated with a ~75% reduction in the odds of transmitting HIV-1 compared to homozygosity for the FcγRIIIa-158F allele (AOR 0.24, 95%CI: 0.08-0.74, P = 0.013), while carriage of at least one FcγRIIIa-158F allele was associated with a 3.36 times higher odds of HIV-1 transmission (AOR 3.36, 95%CI: 1.16-9.72, P = 0.025). While a similar association was observed for intrapartum and *in utero* transmitting pairs in the adjusted analysis, the comparisons were not significant (Table 5.3). For the *in utero* enriched transmitting pairs however, both homozygosity for the FcγRIIIa-158V allele and heterozygosity was associated with a >60% reduction in the odds of transmitting HIV-1 (AOR 0.25; 95%CI: 0.07-0.83, P = 0.023; and AOR 0.37, 95%CI: 0.16-0.85, P = 0.019, respectively), while carriage of at least one FcγRIIIa-158V allele was associated with a 67% reduction in the odds of transmission (AOR 0.33, 95%CI: 0.15-0.70, P = 0.004).

Unlike in the unadjusted analysis, the FcγRIIa-H131R variant significantly associated with transmission following adjustment for potential confounders. For total transmitting pairs, mothers homozygous for the FcγRIIa-131R allele were more likely to transmit HIV-1 to their infants compared to mothers homozygous for the FcγRIIa-131H allele (AOR 2.99, 95%CI: 1.09-8.24, P = 0.034), while carriage of at least one FcγRIIa-131H allele was associated with a 56% reduction in the odds of transmitting HIV-1 (AOR 0.44, 95%CI: 0.22-0.91, P = 0.026). This association was also significant for intrapartum transmitting pairs and *in utero* transmitting pairs for both FcγRIIa-131R homozygosity (AOR 4.21, 95%CI: 1.13-15.72, P = 0.032; and AOR 17.9, 95%CI: 1.86-172.72, P = 0.012, respectively) and carriage of at least one FcγRIIa-131H allele (AOR 0.27, 95%CI: 0.10-0.74, P = 0.011; and AOR 0.25, 95%CI: 0.08-0.78, P = 0.016, respectively). While not statistically significant, a similar association was observed for the *in utero* enriched transmitting pairs (Table 5.3).

In summary, the variants that affect the IgG subclass binding affinity of FcγRIIa and FcγRIIIa and consequently the effector functions mediated by these receptors were associated with the infectiousness of the mother. In particular, the alleles with decreased affinity for IgG were associated with an increased odds of HIV-1 transmission (FcγRIIa-131R and FcγRIIIA-158F).

Table 5.3. Genotypes and allele carriage in HIV-1 non-transmitting and transmitting mothers

	Non-Transmitting	Total Transmitting		Intrapartum Transmitting		*In Utero* Transmitting		*In Utero* Enriched Transmitting	
	N (%)	N (%)	AOR (95% CI), P value Adjusted for VL+CD4	N (%)	AOR (95% CI), P value Adjusted for VL+mNVP	N (%)	AOR (95% CI), P value Adjusted for VL	N (%)	AOR (95% CI), P value Adjusted for VL
FCGR2A (rs1801274)									
Genotype									
131HH (ref)	**38** *(26.4)*	**14** *(19.2)*	1	**6** *(20.7)*	1	**2** *(10.0)*	1	**8** *(18.2)*	1
131HR	**67** *(46.5)*	**32** *(43.8)*	1.48 (0.56-3.82), P=.427	**12** *(41.4)*	1.20 (0.35-4.17), P=.769	**9** *(45.0)*	6.93 (0.75-64.31), P=.890	**20** *(45.5)*	2.37 (0.78-7.18), P=.128
131RR	**39** *(27.1)*	**27** *(37.0)*	2.99 (1.09-8.24), **P=.034**	**11** *(37.9)*	4.21 (1.13-15.72), **P=.032**	**9** *(45.0)*	17.9 (1.86-172.72), **P=.012**	**16** *(36.4)*	3.58 (1.13-11.36), **P=.031**
Allele carriage									
≥1 131H[a] allele	**105** *(72.9)*	**46** *(63.0)*	0.44 (0.22-0.91), **P=.026**	**18** *(62.1)*	0.27 (0.10-0.74), **P=.011**	**11** *(55.0)*	0.25 (0.08-0.78), **P=.016**	**28** *(63.6)*	0.52 (0.24-1.13), P=.098
≥1 131R allele	**106** *(73.6)*	**59** *(80.8)*	1.95 (0.79-4.82), P=.147	**23** *(79.3)*	1.93 (0.62-5.96), P=.255	**18** *(90.0)*	10.21 (1.18-88.48), **P=.035**	**36** *(81.8)*	2.80 (0.98-8.04), **P=.055**
FCGR2B (rs1050501)									
Genotype									
232II (ref)	**68** *(47.2)*	**30** *(41.1)*	1	**11** *(37.9)*	1	**10** *(50.0)*	1	**19** *(43.2)*	1
232IT	**61** *(42.4)*	**33** *(45.2)*	1.29 (0.62-2.65), P=.494	**15** *(51.7)*	1.78 (0.67-4.70), P=.245	**6** *(30.0)*	0.78 (0.24-2.53), P=.677	**18** *(41.0)*	1.14 (0.51-2.54), P=.745
232TT	**15** *(10.4)*	**10** *(13.7)*	2.09 (0.73-5.93), P=.168	**3** *(10.3)*	1.77 (0.38-8.19), P=.463	**4** *(20.0)*	2.61 (0.54-12.54), P=.230	**7** *(15.9)*	1.93 (0.61-6.09), P=.262
Allele carriage									
≥1 232I allele	**129** *(89.6)*	**63** *(86.3)*	0.55 (0.21-1.44), P=.223	**26** *(89.7)*	0.77 (0.19-3.19), P=.716	**16** *(80.0)*	0.34 (0.78-1.52), P=.159	**37** *(84.1)*	0.55 (0.19-1.62), P=.281
≥1 232T allele	**76** *(52.8)*	**43** *(58.9)*	1.43 (0.73-2.84), P=.299	**18** *(62.1)*	1.78 (0.70-4.52), P=.227	**10** *(50.0)*	1.04 (0.36-3.00), P=.943	**25** *(56.8)*	1.29 (0.61-2.71), P=.510
FCGR3A (rs396991)									
Genotype									
158F/FF/FF (ref)	**50** *(34.7)*	**35** *(47.9)*	1	**7** *(24.1)*	1	**11** *(55.0)*	1	**28** *(63.6)*	1
158FV/FFV/FVV	**66** *(45.8)*	**31** *(42.5)*	0.64 (0.31-1.32), P=.224	**20** *(67.0)*	1.22 (0.45-3.34), P=.693	**8** *(40.0)*	0.69 (0.22-2.11), P=.513	**11** *(25.0)*	0.37 (0.16-0.85), **P=.019**
158V/VV	**28** *(19.4)*	**7** *(9.6)*	0.24 (0.08-0.77), **P=.016**	**2** *(6.9)*	0.15 (0.02-1.33), **P=.088**	**1** *(5.0)*	0.14 (0.02-1.29), **P=.083**	**5** *(11.4)*	0.25 (0.07-0.83), **P=.023**
Allele carriage									
≥1 158F allele	**116** *(80.6)*	**66** *(90.4)*	3.26 (1.10-9.65), **P=.033**	**27** *(93.1)*	7.75 (0.96-62.75), **P=.055**	**19** *(95.0)*	5.85 (0.69-49.97), P=.106	**39** *(88.6)*	2.66 (0.83-8.51), P=.100
≥1 158V allele	**94** *(65.3)*	**38** *(52.1)*	0.50 (0.25-1.00), **P=.051**	**22** *(75.9)*	0.88 (0.33-2.35), P=.792	**9** *(45.0)*	0.49(0.17-1.44), P=.194	**16** *(36.4)*	0.33 (0.15-0.70), **P=.004**
FCGR3B									
Genotype									
HNA1a+/1b-/1c-	**33** *(22.9)*	**13** *(17.8)*	0.59 (0.21-1.66), P=.316	**4** *(13.8)*	0.53 (0.13-2.07), P=.361	**6** *(30.0)*	1.70 (0.34-8.55), P=.520	**9** *(20.5)*	0.63 (0.21-1.86), P=.398
HNA1a-/1b+/1c-	**14** *(9.7)*	**7** *(9.6)*	1.85 (0.55-6.17), P=.317	**4** *(13.8)*	1.81 (0.41-8.05), P=.437	**1** *(5.0)*	2.73 (0.22-33.94), P=.435	**3** *(6.8)*	1.22 (0.28-5.30), P=.788
HNA1a-/1b-/1c+	**5** *(3.5)*	**0** *(0)*	-	**0** *(0)*	-	**0** *(0)*	-	**0** *(0)*	-
HNA1a+/1b+/1c- (ref)	**49** *(34.0)*	**23** *(31.5)*	1	**10** *(34.5)*	1	**3** *(15.0)*	1	**13** *(29.5)*	1
HNA1a+/1b-/1c+	**20** *(13.9)*	**11** *(15.1)*	0.96 (0.33-2.80), P=.944	**5** *(17.2)*	1.09 (0.26-4.54), P=.908	**4** *(20.0)*	2.33 (0.42-12.81), P=.331	**6** *(13.6)*	0.95 (0.29-3.04), P=.925
HNA1a-/1b+/1c+	**13** *(9.0)*	**16** *(21.9)*	1.95 (0.69-5.56), P=.210	**5** *(17.2)*	1.40 (0.34-5.77), P=.641	**5** *(25.0)*	4.53 (0.7925.93), P=.090	**11** *(25.0)*	2.10 (0.69-6.38), P=.189
HNA1a+/1b+/1c+	**9** *(6.3)*	**3** *(4.1)*	0.36 (0.21-1.66), P=.316	**1** *(3.4)*	0.31 (0.03-3.12), P=.321	**1** *(5.0)*	0.77 (0.06-9.33), P=.840	**1** *(2.3)*	0.43 (0.08-2.41), P=.336
Allele carriage									
≥1 HNA1a allotype	**111** *(77.1)*	**50** *(68.5)*	0.61 (0.29-1,27), P=.186	**20** *(69.0)*	0.66 (0.25-1.75), P=.409	**14** *(70.0)*	0.63 (0.16-2.11), P=.454	**30** *(68.2)*	0.65 (0.29-1.49), P=.309
≥1 HNA1b allotype	**85** *(59.0)*	**49** *(67.1)*	1.97 (0.95-4.07), **P=.067**	**20** *(69.0)*	1.83(0.70-4.81), P=.217	**10** *(50.0)*	1.07 (0.37-3.13), P=.902	**29** *(65.9)*	1.83 (0.82-4.04), P=.135
≥1 HNA1c allotype	**47** *(32.6)*	**30** *(41.1)*	1.02 (0.51-2.06), P=.946	**11** *(37.9)*	0.96 (0.37-2.47), P=.930	**10** *(50.0)*	1.52 (0.53-4.36), P=.441	**19** *(43.2)*	1.18 (0.55-2.51), P=.676

5.3.5. Linkage between the FcγRIIa and FcγRIIIa variants in the mothers

The genes that encode FcγRIIa and FcγRIIIa are contiguous on chromosome 1 and, thus, due to their close proximity it is possible that the variants, and their association with HIV-1 transmission, were linked. Over the entire cohort, the linkage disequilibrium was moderate between FcγRIIa-H131R and FcγRIIIa-F158V (D' = 0.434, r2= 0.143).

In particular, the variants that exhibited an association with increased odds of HIV-1 transmission, FcγRIIa-131RR genotype, FcγRIIIa-158FF genotype and carriage of at least one FcγRIIIa-158F allele, were in linkage disequilibrium (Table 5.4). The FcγRIIIa-158FF genotype occurred at a higher frequency in mothers bearing the FcγRIIa-131RR genotype (63.6%) compared to heterozygous mothers (23.8%) or those carrying the FcγRIIa-131HH genotype (28.8%). Moreover, 64/66 (96.9%) mothers bearing the FcγRIIa-131RR genotype carried at least one FcγRIIIa-158F allele. The association of the FcγRIIa-131RR/FcγRIIIa-158FF haplotype with HIV-1 transmission was not significant. However, the association of the FcγRIIa-131RR/FcγRIIIa-158FF(FV) haplotype with HIV-1 transmission was slightly stronger for the total and *in utero* transmitting group, but notably stronger for the intrapartum transmitting group compared to FcγRIIa-131RR alone (Table 5.5.).

The genotypes and alleles that associated with decreased odds of HIV-1 transmission, FcγRIIIa-158V homozygosity and carriage of at least one FcγRIIa-131H allele, were in linkage disequilibrium. The FcγRIIIa-158VV genotype was overrepresented in mothers bearing the FcγRIIa-131HH genotype (34.6%) and heterozygous mothers (28.6%) compared to those bearing the FcγRIIa-131RR genotype (3%), with 33/35 (93.5%) individuals bearing the FcγRIIIa-158VV genotype carrying at least one FcγRIIa-131H allele (Table 5.4).

Table 5.4. Association between the FcγRIIa-H131R and FcγRIIIa-F158V genotypes

	FcγRIIa genotype		
FcγRIIIa genotype	**131HH** N = 52	**131HR** N = 99	**131RR** N = 66
158F/FF/FFF	15 *(28.8)*	28 *(23.8)*	**42 *(63.6)***
158FV/FFV/FVV	19 *(36.5)*	56 *(47.6)*	**22 *(33.3)***
158V/VV	**18 *(34.6)***	**15 *(28.6)***	2 *(3.0)*

Table 5.5. Associations between HIV-1 perinatal transmission and the maternal FcγRIIa-131RR/FcγRIIIa-158F haplotype and FcγRIIa-131RR genotype alone

	Total transmitting	**Intrapartum transmitting**	***In utero* transmitting**	***In utero* enriched transmitting**
	AOR (95% CI), P value Adjusted for VL+CD4	**AOR (95% CI), P value** Adjusted for VL+mNVP	**AOR (95% CI), P value** Adjusted for VL	**AOR (95% CI), P value** Adjusted for VL
131RR alone	2.99 (1.09-8.24), **P=.034**	4.21 (1.13-15.72), **P=.032**	17.9 (1.86-172.72), **P=.012**	3.58 (1.13-11.36), **P=.031**
131RR-158F(*) haplotype	2.48 (1.20-5.12), **P=.014**	4.08 (1.47-11.36), **P=.007**	4.39 (1.40-13.75), **P=.011**	2.09 (0.95-4.56), **P=.066**
131RR-158FF haplotype	1.80 (0.81-3.99), P=.150	1.67 (0.52-5.34), P=.390	3.08 (0.93-10.18), **P=.065**	2.29 (0.99-5.33), **P=.054**

* The haplotype is for carriage of at least one FcγRIIIa-158F allele and thus represents both the 158FF and 158FV genotypes

5.3.6. FcγR variants and susceptibility of the recipient/infant

The role of FcγR variants in infant susceptibility to HIV-1 acquisition was subsequently assessed. Table 5.6 reports the observed genotype and allele frequencies together with AOR, 95% CI, and P-values for comparisons of infected infants to exposed-uninfected infants when controlled for the confounding effect of maternal factors as mentioned in the previous section.

In the unadjusted analysis, the FcγRIIb-I232T variant and FcγRIIIb-HNA1a/b/c allotypes associated with infant susceptibility to HIV-1 infection (Appendix C.5-8). For the *in utero* infected group, infants homozygous for the FcγRIIb-232T allele had a 3.42 higher odds of acquiring HIV-1 compared to infants that carried the FcγRIIb-232II genotype (OR 3.42, 95%CI: 1.03-11.39, P = 0.044). For the total infected group and intrapartum infected group, infants homozygous for the FcγRIIIb-HNA1a allotype were less likely to acquire HIV-1 compared to infants that carried the FcγRIIIb-HNA1a/HNA1b reference combination (OR 0.32, 95%CI: 0.12-0.82, P = 0.018; and OR 0.18, 95%CI: 0.04-0.87, P = 0.032, respectively). In the *in utero* enriched group, carriage of all three FcγRIIIb allotypes (HNA1a/b/c) were associated with a 5.63 higher odds of acquiring HIV-1 compared to infants that carried the FcγRIIIb-HNA1a/HNA1b reference combination (OR 5.63, 95%CI: 1.36-23.19, P = 0.017).

In the adjusted analysis, homozygosity for the FcγRIIIb-HNA1a allotype remained significantly associated with reduced odds of HIV-1 acquisition in the total infected infant

Table 5.6. Genotypes and allele carriage in HIV-1 exposed-uninfected and infected infants

	Exposed-Uninfected	Total Infected		Intrapartum Infected		*In Utero* Infected		*In Utero* Enriched Infected	
	N (%)	N (%)	AOR (95% CI), P value Adjusted for mVL+mCD4	N (%)	AOR (95% CI), P value Adjusted for mVL+mNVP	N (%)	AOR (95% CI), P value Adjusted for mVL	N (%)	AOR (95% CI), P value Adjusted for mVL
***FCGR2A* (rs1801274)**									
Genotype									
131HH (ref)	**30** *(20.8)*	**17** *(24.3)*	1	**7** *(25.0)*	1	**4** *(22.2)*	1	**10** *(23.8)*	1
131HR	**72** *(50.0)*	**33** *(47.1)*	0.99 (0.41-2.41), P=.983	**13** *(46.4)*	0.65 (0.20-2.10), P=.474	**6** *(33.3)*	0.66 (0.13-3.19), P=.602	**20** *(47.6)*	0.83 (0.31-2.21), P=.706
131RR	**42** *(29.2)*	**20** *(28.6)*	1.12 (0.43-2.95), P=.814	**8** *(28.6)*	1.04 (0.30-3.63), P=.949	**8** *(44.4)*	2.09 (0.45-9.60), P=.344	**12** *(28.6)*	0.96 (0.33-2.80), P=.944
Allele carriage									
≥1 131H[a] allele	**102** *(70.8)*	**50** *(71.4)*	0.88 (0.43-1.84), P=.742	**20** *(71.4)*	0.72 (0.27-1.93), P=.513	**10** *(55.6)*	0.36 (0.12-1.12), P=.077	**30** *(71.4)*	0.91 (0.40-2.05), P=.817
≥1 131R allele	**114** *(79.2)*	**53** *(75.7)*	1.04 (0.45-2.40), P=.929	**21** *(75.0)*	0.78 (0.27-2.30), P=.654	**14** *(77.8)*	1.13 (0.28-4.64), P=.862	**32** *(76.2)*	0.88 (0.35-2.21), P=.779
***FCGR2B* (rs1050501)**									
Genotype									
232II (ref)	**76** *(52.8)*	**31** *(44.3)*	1	**12** *(42.9)*	1	**7** *(38.9)*	1	**19** *(45.2)*	1
232IT	**49** *(34.0)*	**24** *(34.3)*	1.06 (0.49-2.30), P=.888	**11** *(39.3)*	2.18 (0.78-6.07), P=.135	**5** *(27.8)*	1.07 (0.28-4.16), P=.921	**13** *(32.0)*	1.21 (0.51-2.87), P=.660
232TT	**19** *(13.2)*	**15** *(21.4)*	2.18 (0.86-5.50), **P=.099**	**5** *(17.9)*	2.63 (0.70-9.92), P=.153	**6** *(33.3)*	3.49 (0.87-14.02), **P=.078**	**10** *(23.8)*	2.29 (0.83-6.32), P=.109
Allele carriage									
≥1 232I allele	**125** *(86.8)*	**55** *(78.6)*	0.47 (0.20-1.12), **P=.089**	**23** *(82.1)*	0.55 (0.16-1.83), P=.329	**12** *(66.7)*	0.29 (0.08-1.07), **P=.063**	**32** *(76.2)*	0.47 (0.18-1.22), P=.120
≥1 232T allele	**68** *(47.2)*	**39** *(55.7)*	1.37 (0.69-2.70), P=.369	**16** *(57.1)*	2.30 (0.89-5.94), **P=.085**	**11** *(61.1)*	1.74 (0.57-5.33), P=.332	**23** *(54.8)*	1.52 (0.71-3.24), P=.280
***FCGR3A* (rs396991)**									
Genotype									
158F/FF/FF (ref)	**52** *(36.1)*	**29** *(41.4)*	1	**10** *(35.7)*	1	**8** *(44.4)*	1	**19** *(45.2)*	1
158FV/FFV/FVV	**69** *(47.9)*	**35** *(50.0)*	0.81 (0.40-1.65), P=.564	**14** *(50.0)*	0.80 (0.30-2.12), P=.648	**8** *(44.4)*	0.62 (0.18-2.05), P=.430	**21** *(50.0)*	0.69 (0.31-1.53), P=.364
158V/VV	**23** *(16.0)*	**6** *(8.6)*	0.27 (0.07-1.06), **P=.061**	**4** *(14.3)*	0.19 (0.02-1.67), P=.133	**2** *(11.1)*	0.69 (0.12-3.87), P=.670	**2** *(4.8)*	0.26 (0.21-1.24), P=.090
Allele carriage									
≥1 158F allele	**121** *(84.0)*	**64** *(91.4)*	3.30 (0.89-12.24), **P=.074**	**24** *(93.1)*	4.67 (0.57-38.47), P=.152	**16** *(88.9)*	1.12 (0.22-5.67), P=.888	**40** *(95.2)*	3.20 (0.70-14.62), P=.133
≥1 158V allele	**92** *(63.9)*	**41** *(58.6)*	0.68 (0.34-1.35), P=.265	**18** *(75.9)*	0.64 (0.25-1.68), P=.368	**10** *(55.6)*	0.63(0.20-1.95), P=.425	**23** *(54.8)*	0.59 (0.27-1.26), P=.170
FCGR3B									
Genotype									
HNA1a+/1b-/1c-	**41** *(28.5)*	**7** *(10.0)*	0.27 (0.08-0.86), **P=.027**	**2** *(7.1)*	0.13 (0.02-0.69), **P=.017**	**2** *(11.1)*	0.32 (0.05-2.11), P=.237	**5** *(11.9)*	0.32 (0.09-1.13), **P=.076**
HNA1a-/1b+/1c-	**15** *(10.4)*	**6** *(8.6)*	0.80 (0.23-2.85), P=.731	**1** *(3.6)*	0.19 (0.02-1.90), P=.157	**1** *(5.6)*	0.74 (0.07-8.44), P=.812	**5** *(11.9)*	1.25 (0.35-4.47), P=.734
HNA1a-/1b-/1c+	**6** *(4.2)*	**4** *(5.7)*	0.98 (0.20-4.94), P=.983	**0** *(0)*	-	**1** *(5.6)*	1.03 (0.09-12.23), P=.983	**4** *(9.5)*	1.70 (0.34-8.43), P=.518
HNA1a+/1b+/1c- (ref)	**45** *(31.3)*	**24** *(34.3)*	1	**12** *(66.7)*	1	**5** *(27.8)*	1	**12** *(28.6)*	1
HNA1a+/1b-/1c+	**18** *(12.5)*	**10** *(14.3)*	0.95 (0.33-2.71), P=.922	**6** *(21.4)*	0.77 (0.21-2.80), P=.688	**2** *(11.1)*	0.99 (0.15-6.48), P=.989	**4** *(9.5)*	0.70 (0.19-2.58), P=.589
HNA1a-/1b+/1c+	**15** *(10.4)*	**13** *(18.6)*	1.42(0.49-4.06), P=.518	**7** *(25.0)*	1.23 (0.33-4.55), P=.758	**5** *(27.8)*	4.40 (0.78-24.90), **P=.094**	**6** *(14.3)*	1.43 (0.40-5.11), P=.577
HNA1a+/1b+/1c+	**4** *(2.8)*	**6** *(8.6)*	2.12 (0.46-9.63), P=.332	**0** *(0)*	-	**2** *(11.1)*	1.97 (0.20-19.10), P=.560	**6** *(14.3)*	3.94 (0.85-18.34), **P=.081**
Allele carriage									
≥1 HNA1a allotype	**108** *(75.0)*	**47** *(67.1)*	0.69 (0.33-1.44), P=.322	**20** *(71.4)*	0.92 (0.33-2.53), P=.869	**11** *(61.1)*	0.41 (0.12-1.36), P=.146	**27** *(64.3)*	0.57 (0.25-1.28), P=.173
≥1 HNA1b allotype	**79** *(54.9)*	**49** *(70.0)*	2.08 (1.02-4.24), **P=.045**	**20** *(71.4)*	2.49(0.93-6.65), **P=.068**	**13** *(72.2)*	2.65 (0.81-8.71), P=.109	**29** *(69.0)*	2.42 (1.08-5.46), **P=.033**
≥1 HNA1c allotype	**43** *(29.9)*	**33** *(47.1)*	1.87 (0.94-3.71), **P=.072**	**13** *(46.4)*	1.59 (0.63-4.03), P=.325	**10** *(55.6)*	2.88 (0.93-8.94), **P=.066**	**20** *(47.6)*	1.92 (0.90-4.12), **P=.093**

group (AOR 0.27, 95%CI: 0.08-0.86, P = 0.027) and the intrapartum infected infant group (AOR 0.13, 95%CI: 0.02-0.69, P = 0.017) compared to the FcγRIIIb-HNA1a/HNA1b reference combination. The protective effect of FcγRIIIb-HNA1a homozygosity was alsosignificant when compared to other allotype combinations (Table 5.7). When assessing allotype carriage, infants bearing at least one FcγRIIIb-HNA1b allotype were more likely to become infected compared to infants that did not possess an FcγRIIIb-HNA1b allotype. This association was significant for the total infected infant group (AOR 2.08, 95%CI: 1.02-4.24, P = 0.045) and the *in utero* enriched infected group (AOR 2.42, 95%CI: 1.08-5.46, P = 0.033).

In summary, following adjustment for the effect of potential confounders, the association between the FcγRIIb-I232T variant and HIV-1 acquisition was no longer significant. However, homozygosity for the FcγRIIIb-HNA1a allotype that confers enhanced phagocytic capacity and reactive oxygen species production by neutrophils remained significantly associated with protection from perinatal HIV-1 acquisition.

Table 5.7. Association of the FcγRIIIb-HNA1a homozygous genotype with perinatal acquisition of HIV-1 when compared to other combinations of FcγRIIIb-HNA allotypes

HNA1a homozygosity compared to:	**Total Infected**	**Intrapartum Infected**	***In utero* Infected**	***In utero* enriched Infected**
	AOR (95% CI), P value Adjusted for mVL+mCD4	**AOR (95% CI), P value** Adjusted for mVL+mNVP	**AOR (95% CI), P value** Adjusted for mVL	**AOR (95% CI), P value** Adjusted for mVL
HNA1a-/1b+/1c-	0.34 (0.08-1.47), P=.147	0.67 (0.05-9.01), P=.764	0.43 (0.03-6.09), P=.534	0.25 (0.06-1.15), **P=.075**
HNA1a-/1b-/1c+	0.27 (0.05-1.64), P=.158	-	0.31 (0.02-4.52), P=.394	0.19 (0.03-1.12), **P=.066**
HNA1a+/1b+/1c- (ref)	0.27 (0.08-0.86), **P=.027**	0.13 (0.02-0.69), **P=.017**	0.32 (0.05-2.11), P=.237	0.32 (0.09-1.13), **P=.076**
HNA1a+/1b-/1c+	0.28 (0.08-1.05), **P=.060**	0.17 (0.03-1.01), **P=.051**	0.33 (0.04-2.85), P=.311	0.46 (0.10-2.10), P=.313
HNA1a-/1b+/1c+	0.19 (0.05-0.70), **P=.013**	0.10 (0.02-0.65), **P=.015**	0.07 (0.01-0.60), **P=.015**	0.22 (0.05-1.00), **P=.050**
HNA1a+/1b+/1c+	0.13 (0.02-0.69), **P=.017**	-	0.16 (0.01-1.88), P=.147	0.08 (0.01-0.45), **P=.004**
All other combinations	0.25 (0.09-0.70), **P=.009**	0.19 (0.04-0.88), **P=.034**	0.24 (0.05-1.24), **P=.089**	0.26 (0.08-0.80), **P=.019**

5.3.7. Collective effect of FcγR variants on HIV-1 transmission and acquisition

Under patho-physiological conditions, immune complexes are likely to engage all FcγR isoforms expressed on a particular cell, and thus the collective effect of multilocus FcγR variability should be considered. To investigate the overall FcγR variability profile association with HIV-1 transmission/acquisition, mothers and infants were categorized into inhibitory, neutral and activatory profiles based on an allele scoring system.

Mothers. Maternal viral loads and $CD4^+$ T cell counts were comparable between mothers with inhibitory and activatory profiles (mean VL: 4.134 versus 4.251 $\log_{10}$ RNA copies/ml, P = 0.446; and mean $CD4^+$ T cell count: 424 versus 444 cells/mm^3, P = 0.605). However, HIV-1 transmission rates were notably different across the FcγR variability profiles: 16/63 (20.6%) mothers with activatory profiles transmitted HIV-1 to their infants, compared to 12/40 (30%) mothers with neutral profiles, and 48/114 (42%) mothers with inhibitory profiles. In the unadjusted analysis, mothers with an overall activatory profile were less likely to transmit HIV-1 to their infants compared to mothers with an overall inhibitory profile. This association was significant for total transmitting mothers (OR 0.36, 95%CI: 0.17-0.73, P = 0.005) and the *in utero* enriched transmitting group (OR 0.32, 95%CI: 0.13-0.79, P = 0.013). A similar trend was observed for the intrapartum transmitting group (OR 0.42, 95%CI: 0.16-1.12, P = 0.083). In the adjusted analysis (Table 5.8), the association remained significant for the total transmitting group (AOR 0.25, 95%CI: 0.10-0.61, P = 0.002) and *in utero* enriched transmitting group (AOR 0.24, 95%CI: 0.09-0.67, P = 0.006), but was also significant for the intrapartum transmitting group (AOR 0.25, 95%CI: 0.08-0.82, P = 0.023).

Infants. HIV-1 acquisition was comparable for the FcγR variability profiles. HIV-1 acquisition occurred in 14/44 (31.8%) infants with an overall activatory FcγR profile, 15/49 (30.6%) infants with a neutral FcγR profile, and 41/121 (33.9%) infants with an inhibitory profile. The overall FcγR variability profiles in the infants did not associate with acquisition of HIV-1 in neither the unadjusted nor the adjusted analysis (Table 5.8).

Table 5.8. The prevalence of the overall inhibitory, activatory, and neutral FcγR variability profiles in non-tranmsitting and transmitting mothers and HIV-1 exposed-uninfected and infected infants and the association thereof with HIV-1 transmission and acquisition.

	Non-transmitting/ Exposed-uninfected	Total Transmitted/Infected			Intrapartum Transmitted/Infected			*In Utero* Transmitted/Infected			*In Utero* Enriched Transmitted/Infected		
Overall FcγR variability profile	**N (%)**	**N (%)**	**AOR (95% CI), P value** Adjusted for mVL+mCD4		**N (%)**	**AOR (95% CI), P value** Adjusted for mVL+mNVP		**N (%)**	**AOR (95% CI), P value** Adjusted for mVL		**N (%)**	**AOR (95% CI), P value** Adjusted for mVL	
Mothers													
Inhibitory	**66** *(45.8)*	**48** *(65.7)*	4.06 (1.64-10.07)	**P=.002**	**19** *(65.5)*	3.97 (1.21-12.97)	**P=.023**	**13** *(65.0)*	2.98 (0.81-10.89)	**P=.099**	**29** *(65.9)*	4.18 (1.49-11.69)	**P=.006**
Neutral	**28** *(19.4)*	**12** *(16.4)*	-		**4** *(13.8)*	-		**3** *(15.0)*	-		**8** *(18.2)*	-	
Activatory	**50** *(34.7)*	**13** *(17.8)*	0.25 (0.10-0.61)		**6** *(21.4)*	0.25 (0.08-0.82)		**4** *(20.0)*	0.34 (0.09-1.23)		**7** *(15.9)*	0.24 (0.09-0.67)	
Infants													
Inhibitory	**80** *(55.6)*	**41** *(58.6)*	0.67 (0.25-1.76)	P=.413	**17** *(60.7)*	0.58 (0.17-2.05)	P=.399	**8** *(44.4)*	1.82 (0.51-6.57)	P=.358	**24** *(57.1)*	0.75 (0.26-2.17)	P=.599
Neutral	**34** *(23.6)*	**15** *(21.4)*	-		**4** *(14.3)*	-		**4** *(22.2)*	-		**11** *(26.2)*	-	
Activatory	**30** *(20.8)*	**14** *(20.0)*	1.50 (0.57-3.99)		**7** *(25.0)*	1.72 (0.49-6.04)		**6** *(33.3)*	0.55 (0.15-1.98)		**7** *(16.7)*	1.33 (0.46-3.84)	

5.4. DISCUSSION

The extent to which FcγR-mediated effector mechanisms contribute to the risk of HIV-1 transmission and acquisition is currently undefined. Through the study of FcγR functional variants we indirectly assessed the role for FcγR-mediated effector functions in modulating perinatal HIV-1 transmission and acquisition. Our findings indicate that the FcγR variants conferring different antibody binding affinity and functional capacity associate with infectiousness of the mother, while an FcγR variant conferring enhanced phagocytic capacity associated with reduced risk of HIV-1 acquisition by the infant.

The significance of FcγR-mediated effector functions in maintaining immune homeostasis is validated by the association of functionally significant FcγR variants with immune disorders. Low responder FcγR variants (FcγRIIa-131R, FcγRIIIa-158F, FcγRIIIb-HNA1b and low *FCGR* copy number) are associated usually with autoimmune pathologies characterized by the presence of circulating IgG complexes, whereas the high responder variants (FcγRIIa-131H, FcγRIIIa-158V, FcγRIIIb-HNA1a and high *FCGR* copy number) have been linked to chronic inflammatory conditions characterized by excessive or inappropriate leukocyte activation (Gillis et al 2014). Here we describe an association between the low responder FcγRIIa and FcγRIIIa alleles and enhanced maternal infectiousness in perinatal transmission of HIV-1. In particular, mothers bearing the FcγRIIa-131RR genotype had a ~4 times higher odds of transmitting HIV-1 to their infants compared to mothers bearing the FcγRIIa-131HH genotype, while carriage of at least one FcγRIIa-131H allele associated with a 56% reduction in odds of HIV-1 transmission. The FcγRIIa-H131R variant affects the receptor's affinity for IgG subclasses, such that FcγRIIa-131H exhibit enhanced binding of IgG2 over FcγRIIa-131R (Forthal et al 2007b, Salmon et al 1992, Warmerdam et al 1991). IgG2 is a component of HIV-1-specific immune responses. Since 18-67% (median 48%) of infectious and non-infectious HIV-1 virions occur as immune complexes in peripheral blood (Liu et al 2011), the FcγRIIa-H131R variant may modulate clearance of infectious virus from the circulation. Indeed, it has been demonstrated that monocytes from FcγRIIa-131RR donors exhibit reduced uptake of HIV-IgG immune complexes *in vitro* (Forthal et al 2007b). Taken together, the impaired uptake of insufficiently neutralized virions by FcγRIIa-131RR leukocytes may enhance the mother's infectiousness.

Similar to that observed for FcγRIIa, carriage of the FcγRIIIa low responder variant by the mother was associated with ~3 times higher odds of HIV-1 transmission, whereas homozygosity for the high responder allele associated with a >60% reduction in the odds of transmission. The significant association thereof in the *in utero* transmission group, but not intrapartum group, suggests that the underlying mechanism may be more pronounced at the maternofoetal interface. Importantly, FcγRIIIa-bearing leukocytes including natural killer cells, macrophages and γδ T lymphocytes are readily recruited to the decidua where they likely contribute to eliminating cell-associated HIV-1 through ADCC (Ditzian-Kadanoff et al 1993, Williams et al 2009). While decidual NK (dNK) cells are primarily FcγRIIIa negative during a healthy pregnancy, they have been demonstrated to upregulate FcγRIIIa expression in the presence of a pathogen (Siewiera et al 2013). Since cell-associated HIV-1 is thought to be more infectious *in utero* compared to cell-free virus (Milligan & Overbaugh 2014), ADCC-mediated killing of HIV-1 infected cells may contribute to protective immunity at the maternofetal interface. Of consequence, the FcγRIIIa-F158V variant impacts on ADCC capacity, such that the FcγRIIIa-158V allele exhibits enhanced IgG binding and ADCC capacity compared to the FcγRIIIa-158F allele (Bruhns et al 2009, Wu et al 1997). The decreased *in utero* transmission risk associated with homozygosity for the high responder FcγRIIIa-158V allele suggests that the enhanced ADCC capacity conferred by this variant may potentiate elimination of cell-associated HIV-1 and reduce the odds of HIV-1 crossing the placenta through cell-cell interactions.

The genes that encode the low affinity FcγRs are clustered on the long arm of chromosome 1. Due to their close proximity genotypic variants are likely to be co-inherited and potentially exhibit a combined effect. Indeed, linkage disequilibrium has been described for the FcγRIIa-H131R and FcγRIIIa-F158V variants in Caucasian individuals and African Americans (Lejeune et al 2008). In concordance, we observed moderate linkage disequilibrium between these loci, with the low responder FcγRIIa-131R and FcγRIIIa-158F alleles often occurring together and the high responder variants FcγRIIa-131H and FcγRIIIa-158V frequently occurring together. Since the low responder alleles of both FcγRIIa and FcγRIIIa associated with increased odds of HIV-1 transmission, we investigated their combined effect on HIV-1 transmission. Interestingly, the FcγRIIa-131RR/FcγRIIIa-158FF haplotype did not significantly associate with HIV-1 transmission, although a trend remained for the *in utero*

transmission groups. This is likely attributable to loss of statistical power. Alternatively, these variants do not act synergistically and are independently associated with HIV-1 transmission. Interestingly, carriage of the FcγRIIa-131RR genotype and at least one FcγRIIIa-158F allele strengthened the association of the FcγRIIa-131RR genotype with HIV-1 transmission, in particular for the intrapartum transmitting group. Viewed differently, removing high responder FcγRIIIa-158VV individuals from the analysis strengthened the association of the low responder FcγRIIa-131RR with increased risk of HIV-1 transmission. This raises the question as to whether the high responder variants at one FcγR locus may compensate for the low FcγR variants at a different locus. To assess the collective effect of multilocus FcγR variants, a scoring system was employed where the balance of low responder and high responder alleles were calculated. Interestingly, HIV-1 transmission rates differed for the maternal FcγR variability profiles in the rank order of inhibitory > neutral > activatory, with mothers bearing an overall inhibitory profile having a ~4 times higher odds of transmitting HIV-1 to their infants compared to mothers with an overall activatory profile. These findings suggest that while independent FcγR loci associate with risk of transmission, the collective effect of multilocus FcγR variants may also be a contributing factor.

The variants and overall FcγR variability profile that associated with risk of HIV-1 transmission in the mother did not associate with HIV-1 acquisition in the infant. Rather an association between the infant FcγRIIIb allotypes and HIV-1 acquisition was observed. The different FcγRIIIb allotypes arise from multiple amino acid substitutions that do not alter antibody binding affinity, but affect the glycosylation and tertiary structure of the receptor (Bruhns et al 2009, Bux et al 1997, Ory et al 1989a, Ravetch & Perussia 1989). The allotypes are functionally significant. Neutrophils from FcγRIIIb-HNA1a homozygous donors have an enhanced phagocytic and respiratory burst capacity compared to neutrophils from FcγRIIIb-HNA1b homozygous donors (Bredius et al 1994b, Salmon et al 1990). In the present study, homozygosity for the FcγRIIIb-HNA1a allotype in the infant associated with ~75% reduced odds of HIV-1 acquisition compared to other allotype combinations, whereas carriage of at least one FcγRIIIb-HNA1b allotype associated with ~2 times higher odds of HIV-1 acquisition. The association of increased *FCGR3B* copy number with reduced risk of intrapartum transmission complements the association of the FcγRIIIb-HNA1a allele as both are high responder variants that confer enhanced FcγRIIIb-mediated effector functions

(Willcocks et al 2008). Since expression of FcγRIIIb is largely restricted to neutrophils, these findings suggest a potential role for neutrophil-mediated FcγR effector functions in protection from perinatal HIV-1 acquisition. The underlying mechanism may also involve basophils as FcγRIIIb is detected at low levels on a subset of this cell population, although its function here is unknown.

Mother-to-child transmission is an attractive model in which to study the role of antibodies and their effector functions in HIV-1 protective immunity. It represents a natural situation where the individual at risk is passively immunized with HIV-1-specific antibodies through transplacental transfer of IgG (Aldrovandi & Kuhn 2010, Braibant & Barin 2013). Moreover, it affords the opportunity to study both members of the transmitting dyad, thus allowing the assessment of factors contributing to the infectiousness of the transmitter (mother) as well as the susceptibility of the recipient (infant). Thus, the findings of this study not only highlight additional immunological factors associated with risk of MTCT, but further support a role for FcγR-mediated effector functions in HIV-1 protective immunity. In particular, it highlights a potential involvement of neutrophils in protection from HIV-1 transmission and suggests a possible role of FcγR-mediated effector functions in modulating the infectiousness of an HIV-1 infected individual. The significance of these findings in the context of sexual transmission will need to be determined.

In conclusion, the findings of this study suggest a role for FcγR-mediated effector functions in perinatal HIV-1 transmission. Moreover, we demonstrate through an allele scoring system that the combined effect of multilocus FcγR variants modulates HIV-1 transmission risk. A limitation of the scoring system employed, however, is that it is based on the contribution of FcγR variants to cell activation and effector function in healthy individuals and may not reflect that of HIV-1 infected individuals. In addition, it does not take into account a greater or lesser role of a given variable compared to another and does not consider the effect of FcγRIIIb-HNA1c as its functional role has not been determined. Another limitation of this study may be that there was reduced statistical power for some of the multivariate logistic regression analyses due to the low frequencies of some of the genotypes. Further studies are required to elucidate the functional capacity of FcγR variants in HIV-1 infected individuals.

CHAPTER 6

FCGR VARIABILITY ASSOCIATES WITH MARKERS OF HIV-1 DISEASE SEVERITY: A CROSS-SECTIONAL STUDY

6.1. INTRODUCTION

The crystalizable fragment (Fc) of IgG has the capacity to recruit potent effector functions through engagement with Fcγ receptors (FcγR), which are widely expressed throughout the haematopoietic system. These interactions can contribute to antiviral immunity by inhibiting virus replication, clearing virions by antibody-dependent cellular phagocytosis (ADCP), orchestrating the homing of effector cells, and killing virus-infected cells by antibody-dependent cellular cytotoxicity (ADCC).

Accumulating data suggest that FcγR-mediated effector functions contribute to HIV-1 protective immunity and modulate post-infection control of viraemia (Lewis 2014). In particular, numerous studies have demonstrated a correlation between ADCC titres and disease outcome (Ahmad et al 1994, Ahmad et al 2001, Broliden et al 1993, Forthal et al 2001, Lambotte et al 2013, Ljunggren et al 1987, Sawyer et al 1990). However, the relevance of these studies is limited, since the *in vitro* ADCC assays employed used effector cells from healthy donors, and thus do not fully mimic the natural situation in HIV-1 infected individuals. Moreover, only a single FcγR-mediated mechanism is measured, while under patho-physiological conditions it is likely an accumulation of diverse FcγR-dependent processes, mediated by the full complement of FcγR-bearing effector cells, which modulate viral replication. At present, data on the involvement of other FcγR-mediated mechanisms in disease progression are limited. While there is support for phagocytosis (Dugast et al 2011, Holl et al 2006a, Holl et al 2006b), further studies are required to define its contribution to HIV-1 protective immunity.

The role of FcγR-mediated effector functions in HIV-1 disease progression can be indirectly assessed through studying FcγR variants that affect the magnitude of responses. These include genotypic variants that alter receptor IgG subclass binding affinity (FcγRIIa-H131R and FcγRIIIa-F158V), subcellular localization (FcγRIIb-I232T), post-translational modification (FcγRIIIb-HNA1a/b/c), expression of an otherwise pseudogene (FcγRIIc), and surface density (gene copy number variability and promoter haplotypes). To date, FcγRIIa-H131R has been implicated in HIV-1 disease progression. In particular, homozygosity for the FcγRIIa-131R allele and corresponding impaired uptake of HIV-1 immune complexes have been associated with a faster rate of $CD4^+$ T cell decline in HIV-1 infected adults (Forthal et al 2007b). Conversely, the FcγRIIIa-F158V variant did not associate with disease course (Forthal et al 2007b). At present,

data on FcγRIIb- and FcγRIIIb-mediated effector functions in HIV-1 disease progression are limited. Similar to what has been described for FcγRIIa, insights into their role may be gained through studying their functional variants in a natural infection cohort. This cross-sectional study investigates all known functional FcγR variants in a cohort of treatment naïve, HIV-1 infected South African Black women, and thus indirectly assesses the association of FcγR-mediated effector functions with markers of disease progression, plasma viral load and $CD4^+$ T cell counts (Fahey et al 1990, Mellors et al 1996).

6.2. MATERIALS AND METHODS

6.2.1. Study populations

Drug naïve, HIV-1 infected mothers recruited as part of a perinatal HIV-1 transmission cohort were selected to investigate the association of FcγR variability with cross-sectional markers of HIV-1 disease progression. Refer to Chapter 2, Materials and Methods section 2.1 for a detailed description of the cohort. HIV-1 viral loads and $CD4^+$ T cell counts were measured after childbirth as described in Chapter 2, Materials and Methods section 2.2.

6.2.2. **FCGR** *gene copy number variability and nucleotide variant detection*

Gene copy number and nucleotide variants within the low-affinity *FCGR* genes were determined using the *FCGR*-specific multiplex ligation-dependent probe amplification (MLPA) assay as described in Chapter 2, Materials and Methods sections 2.3 and 2.5. Discrimination of *FCGR2B/C* promoter variants were achieved through gene specific PCR amplification and nucleotide sequencing as described in Chapter 2, Materials and Methods section 2.6 and 2.10.

6.2.3. Overall FcγR variability profile: Allele scoring system

To assess the effect of the overall FcγR variability profile, individuals were categorized as possessing an overall inhibitory profile, neutral profile, or activatory profile. Refer to Chapter 2, Materials and Methods section 2.26. In brief, the high responder FcγR variants (FcγRIIa-131H, FcγRIIb-232T, FcγRIIIa-158V, FcγRIIIb-HNA1a, and high copy number) were each assigned a +1 value, whereas the low responder variants (FcγRIIa-131R, FcγRIIb-232I, FcγRIIIa-158F, FcγRIIIb-HNA1b, and low copy

number) were each assigned a -1 value. The sum of the allele scores were determined for each individual, which were subsequently categorized as possessing an overall inhibitor profile (total score ≤ -1), neutral profile (total score = 0), and activatory profile (total score ≥ 1).

6.2.4. Statistical analysis

Statistical analysis was performed as described in Chapter 2, Materials and Methods section 2.17.

6.3. RESULTS

6.3.1. Cohort

The goal of this study was to investigate the potential association of *FCGR* variability with markers of HIV-1 disease progression i.e. plasma viral load and $CD4^+$ T cell counts. For this purpose, 197 HIV-1 infected, treatment naïve South African Black women part of an existing perinatal cohort were selected and genotyped. The mean age of the study participants was 27 years (range: 16 – 42 years).

The study participants had a broad range of plasma viral loads and $CD4^+$ T cell counts, which is likely representative of HIV-1 infected women in the clinical latency phase and those with more progressive infection and AIDS (Figure 6.1A and B). Plasma viral loads were available for 192 women, $CD4^+$ T cell counts for 176 women, and both measurements for 171 women. The mean plasma viral load was 4.129 $\log_{10}$ RNA copies per ml (range: 2.601 – 5.875) and the mean $CD4^+$ T cell count was 486 cells/mm^3 (range: 16 – 1655 cells/mm^3). HIV-1 RNA concentrations were significantly correlated ($P < 0.0001$; Spearman's $R = -0.442$) with the corresponding $CD4^+$ T cell counts. However, a broad range of plasma viral loads were observed within narrow ranges of $CD4^+$ T cell counts (Figure 6.1C), which likely represents differences in the immunological control of HIV-1 replication between individuals. To investigate whether FcγR variability contributes to this diversity, all study participants were genotyped for known FcγR functional variants.

6.3.2. **FCGR** *copy number variability*

FCGR copy number variability (CNV) display a gene dosage effect that correlates with

the magnitude of FcγR-mediated effector functions and in some instances alter the cellular distribution of the inhibitory receptor, FcγRIIb. Figure 6.2 represents the distribution of *FCGR3A* and *FCGR3B* gene copies relative to plasma viral loads and CD4⁺ T cell counts. Overall, *FCGR3A* CNV was low, occurring in 12/197 (6.1%) of women, and did not show an association with viral load or CD4⁺ T cell count (Figure 6.2A). Conversely, *FCGR3B* CNV was more prevalent, occurring in 58/197 (29.4%) of women and was significantly associated with viral load. Compared to women bearing two *FCGR3B* copies, those that possessed three or more *FCGR3B* copies had significantly higher viral loads (median: 4.143 vs. 4.529 $\log_{10}$ copies/ml; P = 0.028, Figure 6.2B). Figure 6.2C illustrates the relative distribution of ≥3 *FCGR3B* copy number across the entire study with respect to paired viral loads and CD4⁺ T cell counts. Fourteen out of 39 (35.9%) women possessing ≥3 *FCGR3B* copies had viral loads below the study mean (4.129 $\log_{10}$ RNA copies/ml), and only 13/36 (36.1%) women bearing ≥3 *FCGR3B* copies had CD4⁺ T cell counts above the study mean (486 cells/mm^3).

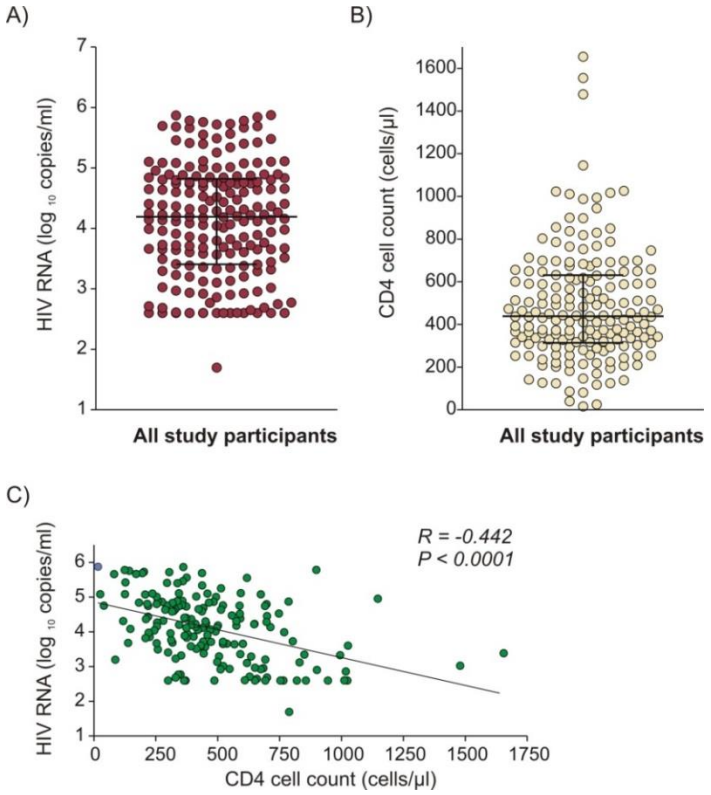


Figure 6.1. Plasma HIV-1 viral load and CD4⁺ T cell distributions of the study participants. The distribution of A) plasma viral load and B) CD4⁺ T cell counts in the cohort of drug naïve, HIV-1 infected South African Black women, and C) Spearman's correlation between plasma viral load and CD4⁺ T cell counts.

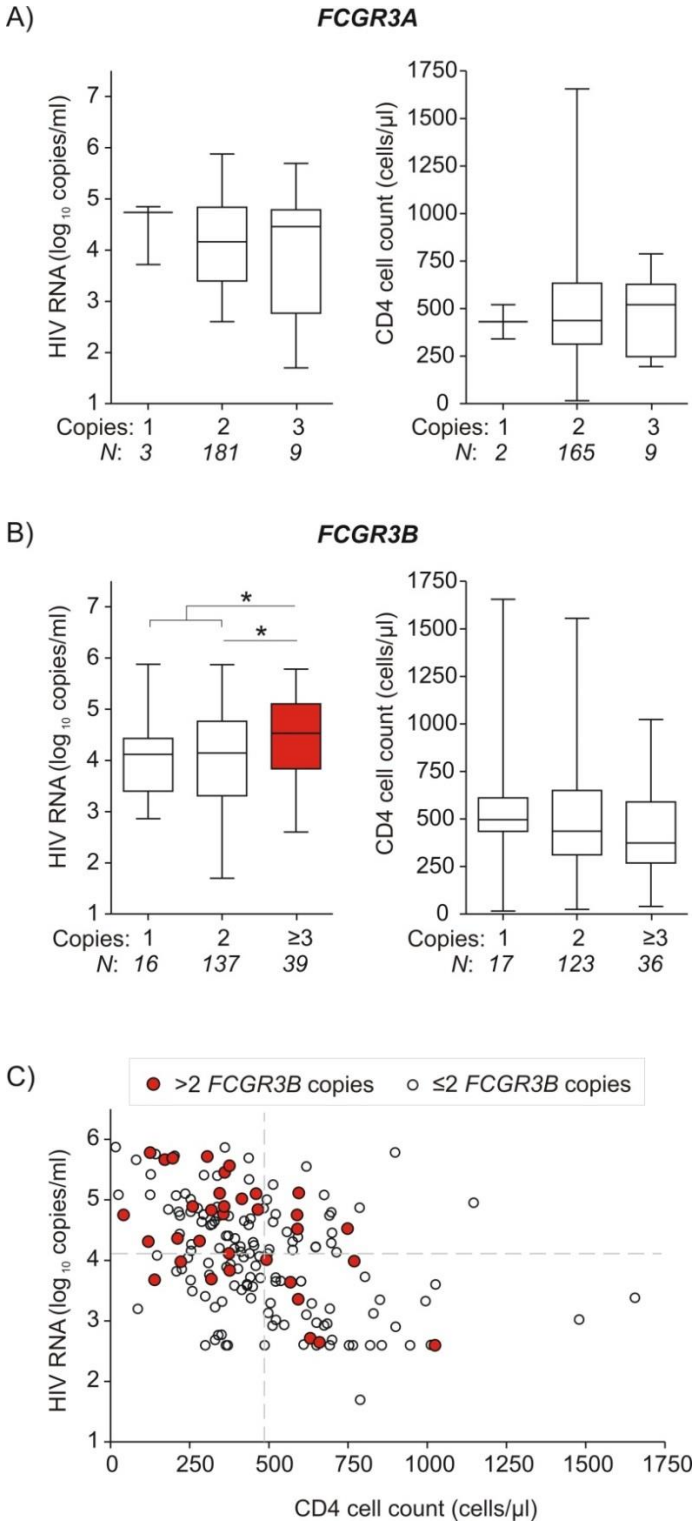


Figure 6.2. *FCGR* gene copy number variation and markers of HIV-1 disease progression. Plasma HIV-1 viral loads and CD4⁺ T cell counts according to *FCGR3A* (A) and *FCGR3B* (B) gene copy number of treatment-naïve, HIV-1 infected South African Black women. (C) represents the distribution of ≥3 *FCGR3B* gene copies relative to the study mean viral load and mean CD4⁺ T cell count. *N* - Viral loads and CD4⁺ T cell counts were not available for all participants. *, $P < 0.05$.

6.3.3. FcγR variants and prognostic markers of HIV-1 infection

To determine if FcγR functional variants associated with prognostic markers of HIV-1 infection, the cross-sectional viral loads and $CD4^+$ T cell counts were compared across FcγR genotypes and alleles. To adjust for the possible confounding effect of *FCGR3B* gene copy number, only women with ≤2 *FCGR3B* gene copies were considered in the HIV-1 viral load analysis.

HIV-1 viral loads and $CD4^+$ T cell counts did not differ significantly across FcγRIIa, FcγRIIb and FcγRIIIa genotypes or alleles (Figure 6.3), but varied considerably across FcγRIIIb genotypes (Figure 6.4A and B). Women homozygous for the FcγRIIIb-HNA1b allotype had the best clinical presentation, with the lowest mean viral loads (VL) and highest mean $CD4^+$ T cell counts. This comparison was significant for HNA1a homozygosity (VL: P = 0.036; $CD4^+$: P = 0.013) and HNA1c homozygosity (only $CD4^+$: P = 0.017) as well as for heterozygous combinations i.e. HNA1a/HNA1b (only $CD4^+$: P = 0.036), HNA1a/HNA1c (VL: P = 0.039, $CD4^+$: P = 0.001), and HNA1b/HNA1c (VL: P = 0.007, $CD4^+$: P = 0.030). Figure 6.3C illustrates the relative distribution of the FcγRIIIb-HNA1b homozygous genotype across the entire study with respect to paired viral loads and $CD4^+$ T cell counts. For women that possessed this genotype, 14/20 (70%) had viral loads below the study mean (4.129 $\log_{10}$ RNA copies/ml) and 17/20 (85%) had $CD4^+$ T cell counts above the study mean (486 cells/mm^3).

For allotype carriage (Figure 6.4D and E), women bearing at least one FcγRIIIb-HNA1b allotype had significantly lower viral loads compared to women that did not possess this allotype (mean: 3.910 *versus* 4.250 $\log_{10}$ RNA copies/ml; P = 0.025). Conversely, women bearing at least one FcγRIIIb-HNA1c allotype had significantly higher viral loads and lower $CD4^+$ T cell counts compared to those that did not possess this allotype (mean VL: 4.288 *versus* 3.958 $\log_{10}$ RNA copies/ml; P = 0.043; and mean $CD4^+$: 410 *versus* 514 cells/mm^3; P = 0.013). Figure 6.3F illustrates the relative distribution of FcγRIIIb-HNA1c allotype carriage across the entire study with respect to paired viral loads and $CD4^+$ T cell counts. For women possessing this allotype, only 16/47 (34%) had viral loads below the study mean (4.129 $\log_{10}$ RNA copies/ml), and only 12/42 (28.6%) had $CD4^+$ T cell counts above the study mean (486 cells/mm^3).

In summary, the FcγRIIIb-HNA1b allotype and homozygous genotype associated with favourable disease progression markers, whereas the FcγRIIIb-HNA1c allotype

associated with poor disease progression markers.

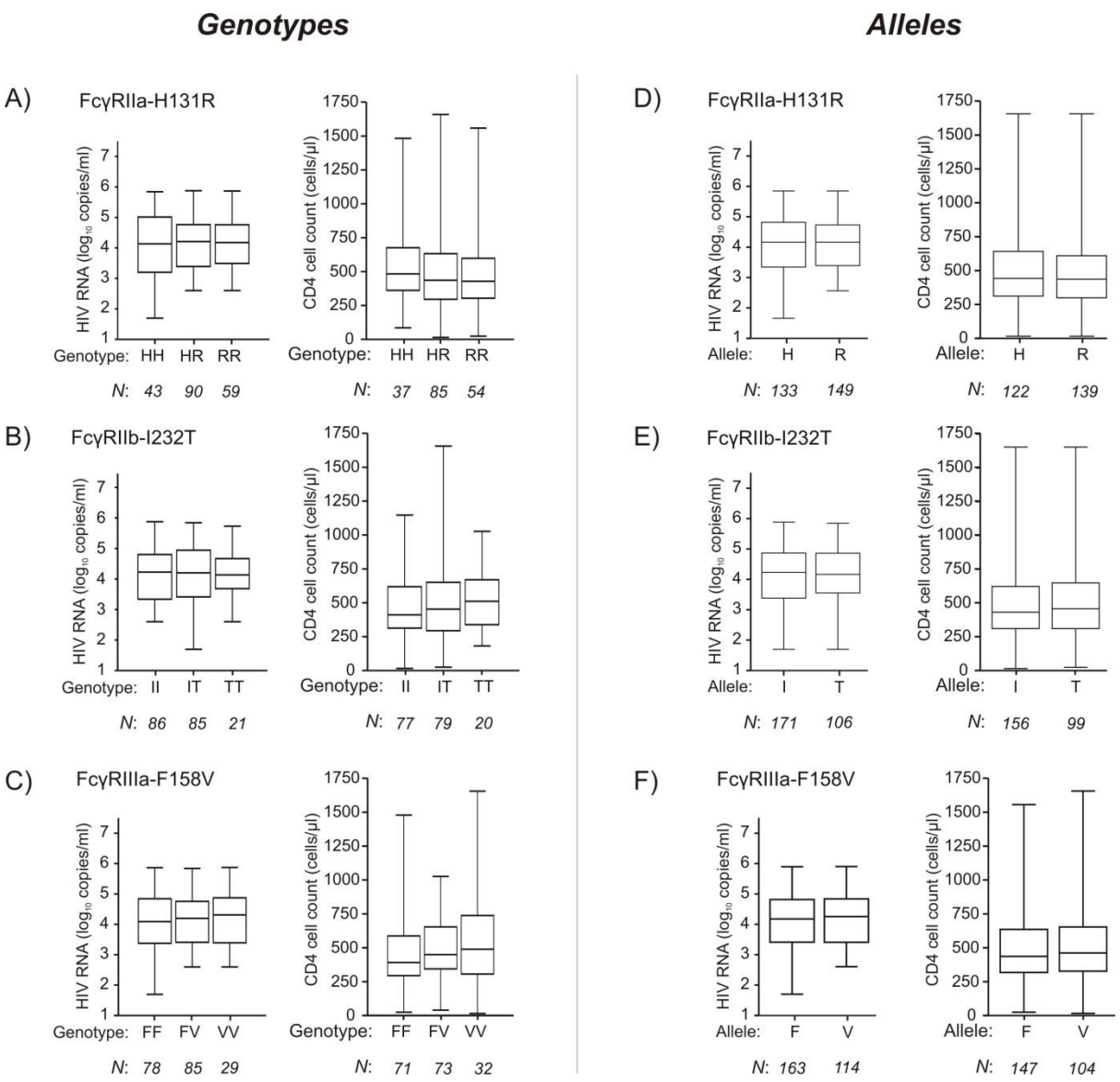


Figure 6.3. FcγRIIa, FcγRIIb, and FcγRIIIa allelic variants and markers of HIV-1 disease progression. Plasma HIV-1 viral loads and CD4⁺ T cell counts according to FcγRIIa-H131R, FcγRIIb-I232T, and FcγRIIIa-F158V genotypes (A - C) and alleles (D - F) of treatment-naïve, HIV-1 infected South African Black women. *N* - Viral loads and CD4⁺ T cell counts were not available for all participants.

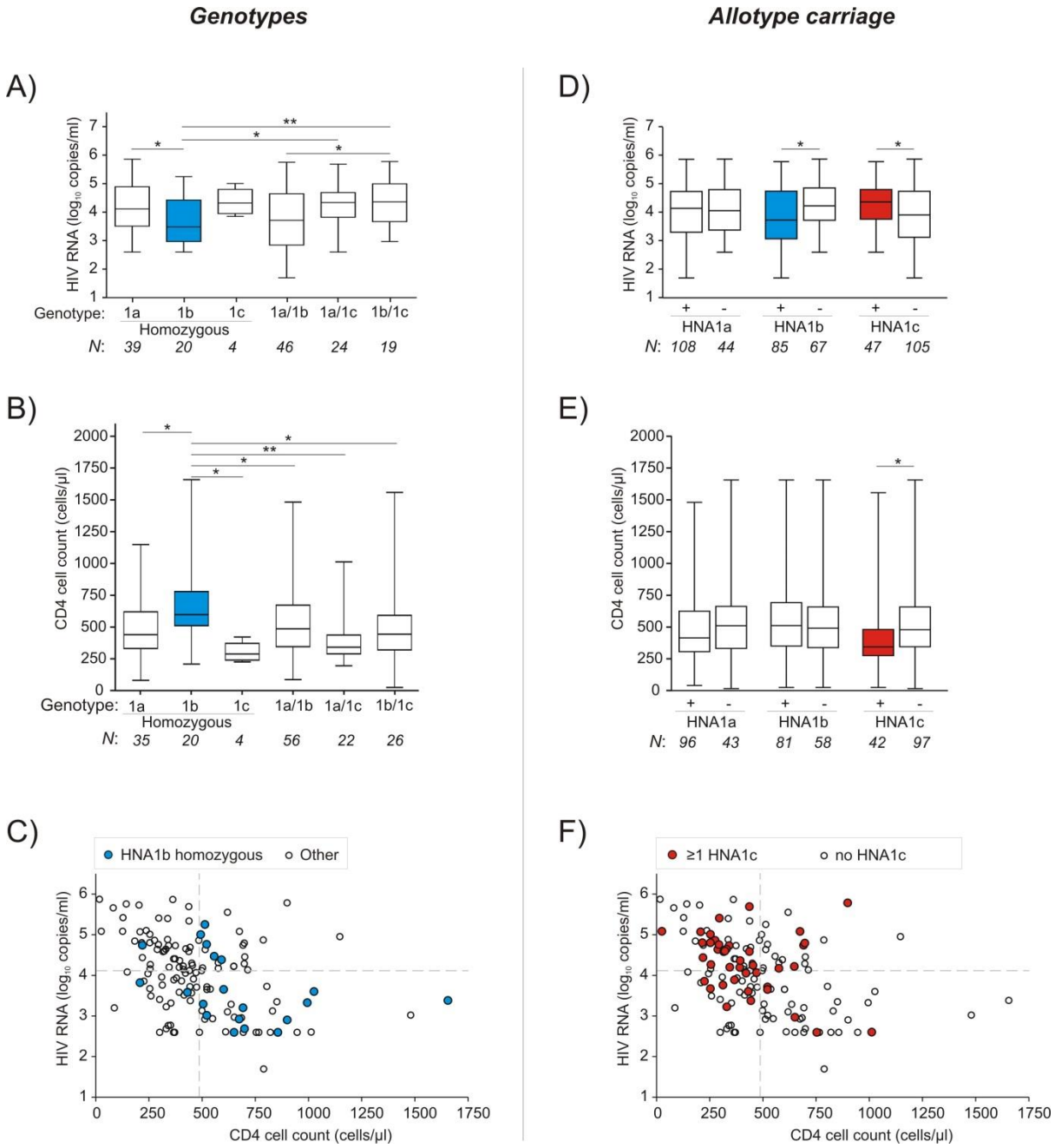


Figure 6.4. FcγRIIIb allotypes and markers of HIV-1 disease progression. Plasma HIV-1 viral loads and CD4⁺ T cell counts according to FcγRIIIb genotypes (A and B) and allotype carriage (D and E) of treatment-naïve, HIV-1 infected South African Black women. For women where both viral load and CD4⁺ T cell count was measured, figures C and F represents the distribution of the protective FcγRIIIb-HNA1b/1b genotype (C) and deleterious FcγRIIIb-HNA1c allotype (F) relative to the study mean viral load and mean CD4⁺ T cell counts (indicated as grey dashed lines). For the viral load analysis, only women with ≤2 *FCGR3B* gene copies were included to adjust for the possible confounding effect of ≥3 *FCGR3B* gene copies, as it displayed a significant association with increased viral loads. *N* - Viral loads and CD4⁺ T cell counts were not available for all participants. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

6.3.4. Collective effect of FcγR variants on markers of HIV-1 disease progression

To investigate whether the overall FcγR variability profile of an individual associated with markers of HIV-1 disease progression, the participants in this study were categorized into inhibitory, activatory and neutral profiles according to the overrepresentation of FcγR variants that confer reduced cell activation or low responder variants (FcγRIIa-131R, FcγRIIb-232I, FcγRIIIa-158F, FcγRIIIb-HNA1b, and low gene copy number), the overrepresentation of variants that enhance cell activation or high responder variants (FcγRIIa-131H, FcγRIIb-232T, FcγRIIIa-158V, FcγRIIIb-HNA1a, and high gene copy number), and a balance between the different variants, respectively.

Study participants with an overall 'neutral' or balanced FcγR variability profile had lower viral loads and higher $CD4^+$ T cell counts compared to individuals with either an overrepresentation of low or high responder variants (Figure 6.5). While only a trend was observed for $CD4^+$ T cell counts, the comparisons were significant for viral loads: neutral *versus* inhibitory (mean: 3.738 *versus* 4.167 $\log_{10}$ RNA copies/ml; P = 0.017) and neutral *versus* activatory (mean: 3.738 *versus* 4.313 $\log_{10}$ RNA copies/ml; P = 0.006).

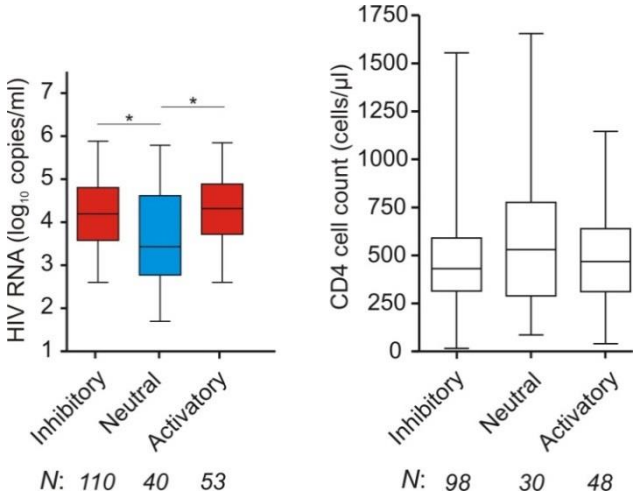


Figure 6.5. Overall FcγR variability profile and markers of HIV-1 disease progression. Plasma viral loads and $CD4^+$ T cell counts of women that had an overall inhibitory, neutral or activatory FcγR variability profile.

6.4. DISCUSSION

Through studying FcγR functional variants in a natural HIV-1 infection cohort, this study describes, for the first time, a potential *in vivo* role for FcγRIIIb-mediated effector functions in modulating HIV-1 disease severity. Furthermore, the findings of this study indicate that perturbations in the overall balance of FcγR-mediated activation/inhibition associate with viral load levels.

In the present study, homozygosity for the FcγRIIIb-HNA1b allotype associated with lower viral loads and higher $CD4^+$ T cell counts compare to all other allotype combinations, while carriage of at least one FcγRIIIb-HNA1c allotype associated with higher viral loads and lower $CD4^+$ T cell counts. Since FcγRIIIb expression is restricted to neutrophils and a subset of basophils, these associations implicate a potential role for these cell types in modifying HIV-1 disease severity. The function of FcγRIIIb on basophils is unknown. However, on neutrophils – the most abundant leukocyte subset in peripheral blood – FcγRIIIb is involved in phagocytosis, respiratory burst, degranulation, and ADCC (Hunt et al 2003, Kushner & Cheung 1992, Marois et al 2011, Nagarajan et al 2000). The FcγRIIIb-HNA1a/b allotypes differentially affect phagocytosis and respiratory burst responses. Their significance for other neutrophil functions remains to be investigated. Resting neutrophils from homozygous FcγRIIIb-HNA1b donors exhibit ~20% reduced phagocytic capacity and a decreased oxidative burst response compared to neutrophils from homozygous FcγRIIIb-HNA1a donors (Bredius et al 1994b, Salmon et al 1990, Urbaczek et al 2014). Furthermore, neutrophils pre-activated with a combination of granulocyte colony stimulating factor (G-CSF) and interferon γ (IFNγ) exhibit shedding of surface FcγRIIIb and induced expression of the inhibitory FcγRIIb occurs in an allotype-dependent way. In particular, pre-activated neutrophils from FcγRIIIb-HNA1b donors shed significantly less FcγRIIIb and have near undetectable levels of FcγRIIb compared to pre-activated neutrophils from FcγRIIIb-HNA1a homozygous and heterozygous donors (van der Heijden et al 2014). Taken together, the mechanism underlying the association of FcγRIIIb-HNA1b homozygositiy may involve reduced functional capacity of resting neutrophils and/or the distinct FcγR phenotypic profile of activated neutrophils.

The functional consequence of the FcγRIIIb allotypes may differentially impact on HIV-1 infection during the initial response to the virus and during chronic infection. IgG-

HIV-1 immune complexes are present during both acute and chronic HIV-1 infection (Liu et al 2011). The initial disappearance of these immune complexes in acute infection, ~3 weeks after HIV-1 RNA first become detectable, suggests a role for phagocytosis of FcγR-bearing leukocytes (Tomaras et al 2008). Reduced phagocytic capacity and concomitant neutrophil activation of FcγRIIIb-HNA1b homozygous individuals may decrease the initial pro-inflammatory cytokine production by these cells and limit their contribution to immune activation during early HIV-1 infection. Since immune activation levels during early HIV-1 infection predicts $CD4^+$ T cell loss over time (Deeks et al 2004), the impaired FcγRIIIb-HNA1b-mediated neutrophil activation may contribute to a lower immunologic activation set point and subsequent favourable disease outcome.

As HIV-1 disseminates through the body and viral loads rise, systemic immune activation occurs. Consequently, neutrophils from HIV-1 infected individuals exhibit enhanced activation through all stages of infection (Elbim et al 1994). Thus, the FcγRIIIb allotype-specific changes of activated neutrophils may play a role here. *In vitro* activated neutrophils shed FcγRIIIb, which contributes to the pool of soluble FcγRIIIb (sFcγRIIIb) (Huizinga et al 1990a). In concordance, neutrophils from HIV-1 infected individuals display reduced surface expression of FcγRIIIb and increased sFcγRIIIb levels *in vivo* (Boros et al 1990, Khayat et al 1990). Soluble FcγRIIIb has the ability to potentiate immune activation and HIV-1 replication by stimulating production of pro-inflammatory cytokines IL-6 and IL-8 by neutrophils and monocytes through binding complement receptor 3 and 4 (Galon et al 1996, Kedzierska & Crowe 2001). Since activated neutrophils from FcγRIIIb-HNA1b homozygous donors exhibit reduced FcγRIIIb shedding compared to neutrophils from FcγRIIIb-HNA1a homozygous or heterozygous donors (van der Heijden et al 2014), FcγRIIIb-HNA1b homozygous HIV-1 infected individuals may have lower sFcγRIIIb levels and concomitant reduced sFcγRIIIb-mediated cellular activation. This hypothesis is further supported by the association of ≥3 *FCGR3B* gene copies with increased viral load levels, since *FCGR3B* copy number correlates with protein expression and sFcγRIIIb levels (Willcocks et al 2008).

While the functional consequences of FcγRIIIb-HNA1a and -HNA1b have been described to a limited extent, the functional significance of the FcγRIIIb-HNA1c allotype

is unknown. The single amino acid substitution distinguishes FcγRIIIb-HNA1b from -HNA1c. This residue is reportedly under positive selective pressure of which a potential candidate is malaria (Machado et al 2012), since the FcγRIIIa-HNA1c allotype is more prevalent in malaria endemic areas than other regions in the world (allotype carriage of ~30% *versus* 6%). Due to the minor difference between FcγRIIIb-HNA1b and -HNA1c, it is thought that the two allotypes are linked. However, a significant association of FcγRIIIb-HNA1c with protection against clinical malaria, but contrasting association of FcγRIIIb-HNA1b with susceptibility to clinical malaria, suggests that these allotypes may not be that closely related in terms of function (Adu et al 2012). Moreover, the present study also describes contrasting associations for FcγRIIIb-HNA1b and FcγRIIIb-HNA1c with markers of HIV-1 disease progression. Homozygosity for the FcγRIIIb-HNA1b allotype associated with high $CD4^+$ T cell counts and low viral loads, whereas carriage of at least one FcγRIIIb-HNA1c allotype associated with low $CD4^+$ T cell counts and high viral loads. Thus, the FcγRIIIb-HNA1c allotype that associates with protection against clinical malaria associated a more severe clinical HIV-1 disease, while the FcγRIIIb-HNA1b allotype that associates with susceptibility to clinical malaria associated with good HIV-1 disease progression markers.

Our results may appear to be in conflict with an earlier study which did not observe an association between HIV-1 viral load and FcγRIIIb allotypes in a Tanzanian and Ethiopian cohort (Machado et al 2013). However, the clinical and demographic characteristics of study participants as well as FcγRIIIb genotyping were very different from the present study. Clinically, participants in the Machado et al. (2013) study were more representative of the advanced stages of HIV-1 disease (mean CD4 count of 92 = cells/mm^3), where FcγRIIIb variability was assessed across viral loads measured at the onset of immunological AIDS (CD4+ cell count of <200 cells/mm^3). In contrast, the study participants in the present study are more representative of different stages of HIV-1 infection with a broad range of $CD4^+$ T cell counts and viral loads. Moreover, Machado et al. (2013) did not distinguish between FcγRIIIb-HNA1b and -HNA1c, the two allotypes that showed significant but opposing associations in the present study.

In addition, our data are not in agreement with a study that observed an association between the FcγRIIa-H131R variant and $CD4^+$ T cell count decline (Forthal et al 2007b), which is also likely attributable to study design. The study by Forthal et al. (2007)

measured the longitudinal $CD4^+$ T cell decline in a cohort of men of which 86% were Caucasian individuals, whereas in the present study cross-sectional data from a cohort of South African Black women were assessed according to genotypes. Our findings are, however, in concordance with a recent study in Kenyan women that did not observe an association between this variant and $CD4^+$ T cell decline or HIV-1 disease progression (Weis et al 2015). The association between FcγRIIa-H131R and $CD4^+$ T cell decline may thus be ethnically confined.

Since the different FcγR isoforms often have overlapping cellular distributions and IgG subclass binding affinities and are likely co-ligated under patho-physiological conditions, the combination of FcγR functional variants may have a collective effect. Through employing an allele scoring system it was demonstrated that HIV-1 infected women with an overrepresentation of either low or high responder FcγR variants had higher viral loads compared to HIV-1 infected women with a balance between high and low responder variants. In non-communicable pathologies, low responder FcγR variants (FcγRIIa-131R, FcγRIIIa-158F, FcγRIIIb-HNA1b and low *FCGR* copy number) are linked to chronic inflammatory conditions characterized by the presence of circulating IgG complexes, whereas the high responder variants (FcγRIIa-131H, FcγRIIIa-158V, FcγRIIIb-HNA1a and high FCGR copy number) have been associated with chronic inflammatory conditions characterized by excessive or inappropriate leukocyte activation [reviewed by (Gillis et al 2014)]. Thus, an overrepresentation of both high and low FcγR variants and their associated effect on cellular activation appears to contribute to enhanced HIV-1 replication *in vivo*.

In conclusion, this study describes a novel association of FcγRIIIb allotypes and, by inference, a potential role for neutrophils in modifying HIV-1 disease severity. Moreover, these findings underscore the need to study the full complement of FcγR-bearing leukocytes and effector functions in the context of HIV-1 infection. A limitation of this study is that the findings are based on cross-sectional analysis, which is much less sensitive than a longitudinal study of time-to-AIDS as viral load and $CD4^+$ T cell measurements fluctuate and individuals with low viral loads and high $CD4^+$ T cell counts are known to progress to AIDS somewhat unpredictably. Future studies on longitudinal data are required to investigate the importance of these associations with HIV-1 disease progression. However, this study has highlighted important new insights that provide a

basis for further investigations into the mechanisms that underlie differential control of HIV-1 infection.

CHAPTER 7

SUMMARIZING DISCUSSIONS AND CONCLUSION

FcγR-mediated biological responses are potent, complex, and comprise both activating and inhibitory effects. These responses are diverse and through several mechanisms activate, regulate and modulate immunity (Nimmerjahn & Ravetch 2008b). The significance of these receptors in maintaining a well-balanced immune response is validated by the association of aberrant FcγR expression or the presence of functional allelic FcγR variants with the pathogenesis of a variety of autoimmune diseases.

While FcγRs and their allelic variants have been extensively studied over the past 25 years and great advances have been made towards elucidating their function, our understanding of these complex molecules are limited and a number of questions remain regarding their biological functions. Firstly, the cellular distribution and function of each FcγR isoform is not clearly defined. Studies as recent as 2009 and 2013 have discovered the presence of FcγRIIIb on basophils and FcγRIIc on B lymphocytes (Li et al 2013, Meknache et al 2009). While the functionality of the latter has been addressed, the role of FcγRIIIb on basophils is unknown. Since the responses induced by IgG-FcγR interactions are cell type-specific rather than FcγR isoform-specific, the FcγR-mediated function on one cell type cannot necessarily be extrapolated to another.

Defining cell-specific FcγR-mediated effector functions are imperative to understanding the role of FcγRs in immune homeostasis and disease, in particular if these receptors and their functions are to be targeted for therapeutic interventions (Hogarth & Pietersz 2012). Secondly, the functional consequence for all FcγR variants has not been investigated, in particular for FcγRIIIb-HNA1c. Based on the findings of this thesis and other studies, this variant is of clinical significance, and thus understanding its functionality may be of value for future interventions. Thirdly, FcγRs are often co-expressed on the same cell, either in the steady-state or induced by inflammation. Yet, the collective functional consequence of allelic variants carried by co-expressed FcγR isoforms is largely undefined. It was recently demonstrated that FcγRIIIb functional variants modulate neutrophil function in a manner dependent on the FcγRIIa functional variants and that functional differences observed between different FcγRIIIb/FcγRIIa haplotypes expressed on resting neutrophils disappeared once these neutrophils were pre-activated. This suggests that the *in vitro* functional assays employed to delineate the FcγR-mediated responses using effector cells in the steady state are of limited relevance, since they do not speak to the *in vivo* function

where inflammation may modulate FcγR expression and interaction. For these reasons, inferences made from association studies investigating FcγR variability are at best speculative. Future studies investigating the functionality of FcγR variants expressed on leukocytes isolated from individuals with the disease of interest will contribute to our understanding of the *in vivo* significance of these variants.

Genetic association studies of FcγR functional variability are impeded by the complexity of the *FCGR* locus, which is attributable to high nucleotide sequence identity between *FCGR* genes and gene copy number variation. As for all copy number variable regions, nucleotide variants located in *FCGR* genes subject to copy number variation (*FCGR2C*, *FCGR3A*, and *FCGR3B*) has poor coverage in genome-wide association studies (GWAS) as they fail Hardy-Weinberg and mendelian inheritance checks (Khor et al 2011, McCarroll & Altshuler 2007). As a result, GWAS do not evaluate variants within these regions and investigations thereof are limited to candidate gene studies. However, candidate gene studies using nucleotide sequencing or sequence-specific primer PCR (SSP-PCR) assays often fail to accurately quantitate allele carriage of *FCGR2C*, *FCGR3A*, and *FCGR3B* variants. Similarly, in the present study, assessing the prevalence of, and linkage disequilibrium between, recently described nucleotide variants (Thai haplotype in *FCGR2C* and *FCGR3A* intragenic haplotype) through nucleotide sequencing and SSP-PCR had to be limited to individuals with one or two gene copies due to technical limitations of these methods. A technology that overcomes these limitations is multiplex ligation-dependent probe amplification (MLPA), which allows the qualitative and quantitative assessment of allelic variants. An *FCGR*-specific MLPA assay has been described and is commercially available (Breunis et al 2008). This assay is specific for known functional *FCGR* variants but also allows the addition of new targets, making this an important and powerful tool to study *FCGR* variability. For this reason, this assay was employed to characterize known functional *FCGR* variants in healthy South African individuals and assess the association of *FCGR* variability with HIV-1 transmission and disease progression.

Infectious diseases have, in part, driven the diversity at the *FCGR* locus (Machado et al 2012). In Africa, malaria has been the evolutionary driving force behind several human phenotypes (Kwiatkowski 2005) and may also have shaped *FCGR* variability in Africans. In particular, the FcγRIIIb-HNA1c that associates with protection against clinical

malaria is significantly more prevalent in malaria endemic populations than in non-endemic populations, while the opposite is observed for the FcγRIIIb-HNA1b allotype that associates with susceptibility to clinical malaria. In this thesis, the inverse was observed for the association of these variants with HIV-1 disease progression in South African Black women. Taken together, these findings suggest that the evolutionary driving force (malaria or other), which selected for FcγRIIIb variants that confer resistance to malaria in Africans potentially predisposed to more severe HIV-1 disease. It also suggests these FcγRIIIb variants may be host genetic risk factors contributing to the rapid HIV-1 disease progression observed in South African Black women, where ~50% of women progress to <350 $CD4^+$ T cells within two years of infection (Mlisana et al 2014). Although the associations of FcγRIIIb allotypes with HIV-1 disease progression were identified in a cross-sectional study, and therefore lack the statistical power afforded to a longitudinal study, the associations were strong enough to stand out and warrant further investigation. It should be noted, however, that the FcγRIIIb allotypes implicated in modifying HIV-1 disease course is subject to significant ethnic diversity, such that the FcγRIIIb-HNA1c allotype is largely absent from populations outside of Africa, and thus the observed associations may be ethnically confined. Nevertheless, the FcγRIIIb-HNA1a and -HNA1b allotypes, that are both highly prevalent in other regions of the world, do still show significant differences in our population and should be investigated further.

The mechanism underlying the association of FcγRIIIb allotypes with HIV-1 disease progression is not obvious. We proposed that it may involve modulation of immune activation through modifying the levels of soluble FcγRIIIb. However, further studies are required to delineate the biological processes involved. Future studies that investigate the correlation between the FcγRIIIb allotypes, serum levels of sFcγRIIIb, and immune activation in HIV-1 infected individuals may be of value. It is also not clear when these FcγRIIIb allotypes impact on HIV-1 disease course, whether it modifies infection by reducing peak viraemia and viral set point during acute infection or maintain efficient immune homeostasis during chronic infection. This is of consequence should these FcγRIIIb-mediated mechanisms ever be harnessed for therapeutic interventions. This may potentially be addressed by a longitudinal study that assesses FcγRIIIb allotypes in relation to viral load levels and $CD4^+$ T cell counts monitored

throughout an HIV-1 infection, from the time when plasma RNA levels are first detectable during acute infection to AIDS.

An unexpected, but relevant finding was the contrasting association of the same FcγRIIIb allotype with HIV-1 disease progression and perinatal HIV-1 acquisition. The allotype that conferred protection against perinatal HIV-1 acquisition associated with that are linked to poor disease outcome (high viral load, low $CD4^+$ T cell counts), while those that associated with a favourable disease course were linked to risk of perinatal HIV-1 acquisition. These findings suggest that immune correlates of protection for HIV-1 acquisition in the presence of HIV-1-specific IgG may not necessarily be the same as for post-infection control of viraemia and maintenance of immune integrity, and *vice versa*. This brings into question the relevance of elite controller cohorts to identify immune correlates of protection to complement vaccine design. However, protective mechanisms identified in these cohorts are relevant to the design of a functional cure. It is here where the association of the FcγRIIIb-HNA1b allotype with a favourable disease course may be of significance.

Investigating immune correlates of protection against HIV-1 acquisition in the mother-to-child transmission model may provide important insights into the antibody responses that would be valuable for vaccine development, since it represents a natural situation where the person at risk is passively immunized with HIV-1-specific IgG. The association of the FcγRIIIb variants with protection from perinatal HIV-1 acquisition suggests a role for neutrophils in mediating anti-HIV-1 immunity in immunized individuals. Neutrophils are not only efficient at antibody-mediated killing of HIV-1 infected cells and phagocytosis of opsonized virions (Smalls-Mantey et al 2013), they are recognized as instructors of the immune system (Amulic et al 2012, Mantovani et al 2011). Due to the experimentally intractable nature of neutrophils few studies have investigated neutrophil antibody-mediated effector functions in HIV-1 specific immunity. However, the findings of this study provide incentive for further investigations. Future studies investigating the FcγRIIIb variants according to the risk of HIV-1 acquisition in vaccine recipients may provide further insight into the associations observed in this study.

HIV-1 transmission risk is determined by the complex interplay between the infectiousness of the transmitter and the susceptibility of the recipient. The

infectiousness of an HIV-1 infected individual is largely defined by biological factors that influence the levels of HIV-1 in the blood and genital secretions and these involve viral factors, host immune factors and host genetic factors. We describe, for the first time, an association between FcγR variants and infectiousness of an HIV-1 infected individual, after adjustment for the confounding effect of viral load. While the association was described in the context of mother to child transmission, it may be of relevance to sexual transmission. It has recently been demonstrated that the neonatal Fc receptor has the ability to trancytose infectious IgG-HIV-1 immune complexes across epithelial cells (Gupta et al 2013). This receptor is expressed at the maternofoetal interface but also on genital epithelia where it may provide a route for infection (Gupta et al 2013). We proposed that the mechanism underlying the association of low responder FcγR variants with increased infectiousness involves poor uptake and clearance of IgG-HIV-1 immune complexes and inefficient killing of cell-associated virus. Future studies in serodiscordant couples that investigate FcγR variability, levels of infectious HIV-1 (opsonized or cell-associated) in semen and cervicovaginal secretions, relative to the likelihood of becoming infected will be of value.

To conclude, the characterization of FcγR variability in healthy South Africans Black individuals and Caucasian individuals emphasized the extent of ethnic diversity at this locus and has provided important baseline data for any future association studies. Through investigating functionally significant FcγR variants in the context of HIV-1 transmission and disease progression, we provided indirect support of the role of FcγR-mediated effector functions in HIV-1 protective immunity *in vivo*. A caveat of this study, however, is that the antibody component was not evaluated. Since FcγR-mediated effector functions involve an appropriate antibody and a functional effector cell, these components should ideally be investigated together.

REFERENCES

1000_Genomes_Project_Consortium. 2010. A map of human genome variation from population-scale sequencing. *Nature* 467: 1061-73

Abrahams MR, Anderson JA, Giorgi EE, Seoighe C, Mlisana K, et al. 2009. Quantitating the multiplicity of infection with human immunodeficiency virus type 1 subtype C reveals a non-poisson distribution of transmitted variants. *J Virol* 83: 3556-67

Adu B, Dodoo D, Adukpo S, Hedley PL, Arthur FK, et al. 2012. Fc gamma receptor IIIB (FcgammaRIIIB) polymorphisms are associated with clinical malaria in Ghanaian children. *PLoS One* 7: e46197

Ahmad A, Morisset R, Thomas R, Menezes J. 1994. Evidence for a defect of antibody-dependent cellular cytotoxic (ADCC) effector function and anti-HIV gp120/41-specific ADCC-mediating antibody titres in HIV-infected individuals. *J Acquir Immune Defic Syndr* 7: 428-37

Ahmad R, Sindhu ST, Toma E, Morisset R, Vincelette J, et al. 2001. Evidence for a correlation between antibody-dependent cellular cytotoxicity-mediating anti-HIV-1 antibodies and prognostic predictors of HIV infection. *J Clin Immunol* 21: 227-33

Aitman TJ, Dong R, Vyse TJ, Norsworthy PJ, Johnson MD, et al. 2006. Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. *Nature* 439: 851-5

Alberobello AT, Congedo V, Liu H, Cochran C, Skarulis MC, et al. 2011. An intronic SNP in the thyroid hormone receptor beta gene is associated with pituitary cell-specific over-expression of a mutant thyroid hormone receptor beta2 (R338W) in the index case of pituitary-selective resistance to thyroid hormone. *J Transl Med* 9: 144

Aldrovandi GM, Kuhn L. 2010. What infants and breasts can teach us about natural protection from HIV infection. *J Infect Dis* 202 Suppl 3: S366-70

Aman MJ, Tosello-Trampont AC, Ravichandran K. 2001. Fc gamma RIIB1/SHIP-mediated inhibitory signaling in B cells involves lipid rafts. *J Biol Chem* 276: 46371-8

Amigorena S, Bonnerot C, Drake JR, Choquet D, Hunziker W, et al. 1992. Cytoplasmic domain heterogeneity and functions of IgG Fc receptors in B lymphocytes. *Science* 256: 1808-12

Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. 2012. Neutrophil function: from mechanisms to disease. *Annu Rev Immunol* 30: 459-89

Anderson CL, Shen L, Eicher DM, Wewers MD, Gill JK. 1990. Phagocytosis mediated by three distinct Fc gamma receptor classes on human leukocytes. *J Exp Med* 171: 1333-45

Anderson DJ, Politch JA, Nadolski AM, Blaskewicz CD, Pudney J, Mayer KH. 2010. Targeting Trojan Horse leukocytes for HIV prevention. *Aids* 24: 163-87

Antczak AJ, Vieth JA, Singh N, Worth RG. 2011. Internalization of IgG-coated targets results in activation and secretion of soluble CD40 ligand and RANTES by human platelets. *Clin Vaccine Immunol* 18: 210-6

Ayouba A, Cannou C, Nugeyre MT, Barre-Sinoussi F, Menu E. 2008. Distinct efficacy of HIV-1 entry inhibitors to prevent cell-to-cell transfer of R5 and X4 viruses across a human placental trophoblast barrier in a reconstitution model in vitro. *Retrovirology* 5: 31

Babior BM. 1984. The respiratory burst of phagocytes. *J Clin Invest* 73: 599-601

Barabe F, Rollet-Labelle E, Gilbert C, Fernandes MJ, Naccache SN, Naccache PH. 2002. Early events in the activation of Fc gamma RIIA in human neutrophils: stimulated insolubilization, translocation to detergent-resistant domains, and degradation of Fc gamma RIIA. *J Immunol* 168: 4042-9

Barouch DH, Liu J, Li H, Maxfield LF, Abbink P, et al. 2012. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. *Nature* 482: 89-93

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-5

Barron P, Pillay Y, Doherty T, Sherman G, Jackson D, et al. 2013. Eliminating mother-to-child HIV transmission in South Africa. *Bull World Health Organ* 91: 70-4

Baum LL, Cassutt KJ, Knigge K, Khattri R, Margolick J, et al. 1996. HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. *J Immunol* 157: 2168-73

Behnen M, Leschczyk C, Moller S, Batel T, Klinger M, et al. 2014. Immobilized immune complexes induce neutrophil extracellular trap release by human neutrophil granulocytes via FcgammaRIIIB and Mac-1. *J Immunol* 193: 1954-65

Bjorkstrom NK, Gonzalez VD, Malmberg KJ, Falconer K, Alaeus A, et al. 2008. Elevated numbers of Fc gamma RIIIA+ (CD16+) effector CD8 T cells with NK cell-like function in chronic hepatitis C virus infection. *J Immunol* 181: 4219-28

Boily MC, Baggaley RF, Wang L, Masse B, White RG, et al. 2009. Heterosexual risk of HIV-1 infection per sexual act: systematic review and meta-analysis of observational studies. *Lancet Infect Dis* 9: 118-29

Bonsignori M, Pollara J, Moody MA, Alpert MD, Chen X, et al. 2012. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. *J Virol* 86: 11521-32

Boros P, Gardos E, Bekesi GJ, Unkeless JC. 1990. Change in expression of Fc gamma RIII (CD16) on neutrophils from human immunodeficiency virus-infected individuals. *Clin Immunol Immunopathol* 54: 281-9

Boruchov AM, Heller G, Veri MC, Bonvini E, Ravetch JV, Young JW. 2005. Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J Clin Invest* 115: 2914-23

Bournazos S, Hart SP, Chamberlain LH, Glennie MJ, Dransfield I. 2009a. Association of FcgammaRIIa (CD32a) with lipid rafts regulates ligand binding activity. *J Immunol* 182: 8026-36

Bournazos S, Woof JM, Hart SP, Dransfield I. 2009b. Functional and clinical consequences of Fc receptor polymorphic and copy number variants. *Clin Exp Immunol* 157: 244-54

Bournazos S, Klein F, Pietzsch J, Seaman MS, Nussenzweig MC, Ravetch JV. 2014. Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for in vivo activity. *Cell* 158: 1243-53

Bowerman B, Brown PO, Bishop JM, Varmus HE. 1989. A nucleoprotein complex mediates the integration of retroviral DNA. *Genes Dev* 3: 469-78

Braakman E, van de Winkel JG, van Krimpen BA, Jansze M, Bolhuis RL. 1992. CD16 on human gamma delta T lymphocytes: expression, function, and specificity for mouse IgG isotypes. *Cell Immunol* 143: 97-107

Braibant M, Barin F. 2013. The role of neutralizing antibodies in prevention of HIV-1 infection: what can we learn from the mother-to-child transmission context? *Retrovirology* 10: 103

Bredius RG, Derkx BH, Fijen CA, de Wit TP, de Haas M, et al. 1994a. Fc gamma receptor IIa (CD32) polymorphism in fulminant meningococcal septic shock in children. *J Infect Dis* 170: 848-53

Bredius RG, Fijen CA, De Haas M, Kuijper EJ, Weening RS, et al. 1994b. Role of neutrophil Fc gamma RIIa (CD32) and Fc gamma RIIIb (CD16) polymorphic forms in phagocytosis of human IgG1- and IgG3-opsonized bacteria and erythrocytes. *Immunology* 83: 624-30

Breunis WB, van Mirre E, Bruin M, Geissler J, de Boer M, et al. 2008. Copy number variation of the activating FCGR2C gene predisposes to idiopathic thrombocytopenic purpura. *Blood* 111: 1029-38

Breunis WB, van Mirre E, Geissler J, Laddach N, Wolbink G, et al. 2009. Copy number variation at the FCGR locus includes FCGR3A, FCGR2C and FCGR3B but not FCGR2A and FCGR2B. *Hum Mutat* 30: E640-50

Broliden K, Sievers E, Tovo PA, Moschese V, Scarlatti G, et al. 1993. Antibody-dependent cellular cytotoxicity and neutralizing activity in sera of HIV-1-infected mothers and their children. *Clin Exp Immunol* 93: 56-64

Broliden PA, Morfeldt-Mansson L, Rosen J, Jondal M, Wahren B. 1989. Fine specificity of IgG subclass response to group antigens in HIV-1-infected patients. *Clin Exp Immunol* 76: 216-21

Bronner IM, Hoogendijk JE, De Visser M, Van De Vlekkert J, Badrising UA, et al. 2009. Association of the leukocyte immunoglobulin G (Fcγ) receptor IIIa-158V/F polymorphism with inflammatory myopathies in Dutch patients. *Tissue Antigens* 73: 586-89

Brooks DG, Qiu WQ, Luster AD, Ravetch JV. 1989. Structure and expression of human IgG FcRII(CD32). Functional heterogeneity is encoded by the alternatively spliced products of multiple genes. *J Exp Med* 170: 1369-85

Brouwer KC, Lal RB, Mirel LB, Yang C, van Eijk AM, et al. 2004. Polymorphism of Fc receptor IIa for IgG in infants is associated with susceptibility to perinatal HIV-1 infection. *Aids* 18: 1187-94

Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, et al. 2009. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. *Blood* 113: 3716-25

Burton DR, Hessell AJ, Keele BF, Klasse PJ, Ketas TA, et al. 2011. Limited or no protection by weakly or nonneutralizing antibodies against vaginal SHIV challenge of macaques compared with a strongly neutralizing antibody. *Proc Natl Acad Sci U S A* 108: 11181-6

Bux J, Stein EL, Bierling P, Fromont P, Clay M, et al. 1997. Characterization of a new alloantigen (SH) on the human neutrophil Fc gamma receptor IIIb. *Blood* 89: 1027-34

Carter PJ. 2006. Potent antibody therapeutics by design. *Nat Rev Immunol* 6: 343-57

Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, et al. 2002. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. *Blood* 99: 754-8

Cassel DL, Keller MA, Surrey S, Schwartz E, Schreiber AD, et al. 1993. Differential expression of Fc gamma RIIA, Fc gamma RIIB and Fc gamma RIIC in hematopoietic cells: analysis of transcripts. *Mol Immunol* 30: 451-60

Chauhan AK, Moore TL. 2012. Immune complexes and late complement proteins trigger activation of Syk tyrosine kinase in human CD4(+) T cells. *Clin Exp Immunol* 167: 235-45

Chu ZT, Tsuchiya N, Kyogoku C, Ohashi J, Qian YP, et al. 2004. Association of Fcgamma receptor IIb polymorphism with susceptibility to systemic lupus erythematosus in Chinese: a common susceptibility gene in the Asian populations. *Tissue Antigens* 63: 21-7

Chuachoowong R, Shaffer N, Siriwasin W, Chaisilwattana P, Young NL, et al. 2000. Short-course antenatal zidovudine reduces both cervicovaginal human

immunodeficiency virus type 1 RNA levels and risk of perinatal transmission. Bangkok Collaborative Perinatal HIV Transmission Study Group. *J Infect Dis* 181: 99-106

Chuang FY, Sassaroli M, Unkeless JC. 2000. Convergence of Fc gamma receptor IIA and Fc gamma receptor IIIB signaling pathways in human neutrophils. *J Immunol* 164: 350-60

Clark MR, Clarkson SB, Ory PA, Stollman N, Goldstein IM. 1989. Molecular basis for a polymorphism involving Fc receptor II on human monocytes. *J Immunol* 143: 1731-4

Clatworthy MR, Willcocks L, Urban B, Langhorne J, Williams TN, et al. 2007. Systemic lupus erythematosus-associated defects in the inhibitory receptor FcgammaRIIb reduce susceptibility to malaria. *Proc Natl Acad Sci U S A* 104: 7169-74

Clemenceau B, Vivien R, Berthome M, Robillard N, Garand R, et al. 2008. Effector memory alphabeta T lymphocytes can express FcgammaRIIIa and mediate antibody-dependent cellular cytotoxicity. *J Immunol* 180: 5327-34

Clynes RA, Towers TL, Presta LG, Ravetch JV. 2000. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. *Nat Med* 6: 443-46

Collin M, Shannon O, Bjorck L. 2008. IgG glycan hydrolysis by a bacterial enzyme as a therapy against autoimmune conditions. *Proc Natl Acad Sci U S A* 105: 4265-70

Congy-Jolivet N, Bolzec A, Ternant D, Ohresser M, Watier H, Thibault G. 2008. Fc gamma RIIIa expression is not increased on natural killer cells expressing the Fc gamma RIIIa-158V allotype. *Cancer Res* 68: 976-80

Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, et al. 1994. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N Engl J Med* 331: 1173-80

Cunningham AL, Harman AN, Nasr N. 2013. Initial HIV mucosal infection and dendritic cells. *EMBO Mol Med* 5: 658-60

Daeron M. 1997. Fc receptor biology. *Annu Rev Immunol* 15: 203-34

de Andres B, Hagen M, Sandor M, Verbeek S, Rokhlin O, Lynch RG. 1999. A regulatory role for Fc gamma receptors (CD16 and CD32) in hematopoiesis. *Immunol Lett* 68: 109-13

de Haas M, Koene HR, Kleijer M, de Vries E, Simsek S, et al. 1996. A triallelic Fc gamma receptor type IIIA polymorphism influences the binding of human IgG by NK cell Fc gamma RIIIa. *J Immunol* 156: 2948-55

De Souza TL, De Souza Campos Fernandes RC, Medina-Acosta E. 2012. HIV-1 control in battleground: important host genetic variations for HIV-1 mother-to-child

transmission and progression to clinical pediatric AIDS. *Future Virology* 7: 659-78

Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, et al. 2004. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood* 104: 942-7

Demberg T, Brocca-Cofano E, Kuate S, Aladi S, Vargas-Inchaustegui DA, et al. 2013. Impact of antibody quality and anamnestic response on viremia control post-challenge in a combined Tat/Env vaccine regimen in rhesus macaques. *Virology* 440: 210-21

Didigu CA, Doms RW. 2012. Novel approaches to inhibit HIV entry. *Viruses* 4: 309-24

Dimou NL, Nikolopoulos GK, Hamodrakas SJ, Bagos PG. 2010. Fcgamma receptor polymorphisms and their association with periodontal disease: a meta-analysis. *J Clin Periodontol* 37: 255-65

Ditzian-Kadanoff R, Garon J, Verp MS, Zilberstein M. 1993. Gamma delta T cells in human decidua. *Am J Obstet Gynecol* 168: 831-6

Domingo P, Muniz-Diaz E, Baraldes MA, Arilla M, Barquet N, et al. 2002. Associations between Fc gamma receptor IIA polymorphisms and the risk and prognosis of meningococcal disease. *Am J Med* 112: 19-25

Dugast AS, Tonelli A, Berger CT, Ackerman ME, Sciaranghella G, et al. 2011. Decreased Fc receptor expression on innate immune cells is associated with impaired antibody-mediated cellular phagocytic activity in chronically HIV-1 infected individuals. *Virology* 415: 160-7

Elbim C, Prevot MH, Bouscarat F, Franzini E, Chollet-Martin S, et al. 1994. Polymorphonuclear neutrophils from human immunodeficiency virus-infected patients show enhanced activation, diminished fMLP-induced L-selectin shedding, and an impaired oxidative burst after cytokine priming. *Blood* 84: 2759-66

Ellery PJ, Tippett E, Chiu YL, Paukovics G, Cameron PU, et al. 2007. The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo. *J Immunol* 178: 6581-9

Embretson J, Zupancic M, Ribas JL, Burke A, Racz P, et al. 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* 362: 359-62

Ernst LK, Metes D, Herberman RB, Morel PA. 2002. Allelic polymorphisms in the FcgammaRIIC gene can influence its function on normal human natural killer cells. *J Mol Med (Berl)* 80: 248-57

Fahey JL, Taylor JM, Detels R, Hofmann B, Melmed R, et al. 1990. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N Engl J Med* 322: 166-72

Falasca M, Logan SK, Lehto VP, Baccante G, Lemmon MA, Schlessinger J. 1998. Activation of phospholipase C gamma by PI 3-kinase-induced PH domain-mediated membrane targeting. *EMBO J* 17: 414-22

Faria NR, Rambaut A, Suchard MA, Baele G, Bedford T, et al. 2014. HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. *Science* 346: 56-61

Ferguson KM, Lemmon MA, Schlessinger J, Sigler PB. 1995. Structure of the high affinity complex of inositol trisphosphate with a phospholipase C pleckstrin homology domain. *Cell* 83: 1037-46

Fernandes MJ, Rollet-Labelle E, Pare G, Marois S, Tremblay ML, et al. 2006. CD16b associates with high-density, detergent-resistant membranes in human neutrophils. *Biochem J* 393: 351-9

Ferrante A, Beard LJ, Feldman RG. 1990. IgG subclass distribution of antibodies to bacterial and viral antigens. *Pediatr Infect Dis J* 9: S16-24

Ferrari G, Pollara J, Kozink D, Harms T, Drinker M, et al. 2011. An HIV-1 gp120 envelope human monoclonal antibody that recognizes a C1 conformational epitope mediates potent antibody-dependent cellular cytotoxicity (ADCC) activity and defines a common ADCC epitope in human HIV-1 serum. *J Virol* 85: 7029-36

Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, et al. 2003. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *Aids* 17: 1871-9

Florese RH, Demberg T, Xiao P, Kuller L, Larsen K, et al. 2009. Contribution of nonneutralizing vaccine-elicited antibody activities to improved protective efficacy in rhesus macaques immunized with Tat/Env compared with multigenic vaccines. *J Immunol* 182: 3718-27

Floto RA, Clatworthy MR, Heilbronn KR, Rosner DR, MacAry PA, et al. 2005. Loss of function of a lupus-associated FcgammaRIIb polymorphism through exclusion from lipid rafts. *Nat Med* 11: 1056-8

Forbes JC, Alimenti AM, Singer J, Brophy JC, Bitnun A, et al. 2012. A national review of vertical HIV transmission. *Aids* 26: 757-63

Forthal DN, Gabriel EE, Wang A, Landucci G, Phan TB. 2012. Association of Fcgamma receptor IIIa genotype with the rate of HIV infection after gp120 vaccination. *Blood* 120: 2836-42

Forthal DN, Gilbert PB, Landucci G, Phan T. 2007a. Recombinant gp120 vaccine-induced antibodies inhibit clinical strains of HIV-1 in the presence of Fc receptor-bearing effector cells and correlate inversely with HIV infection rate. *J Immunol* 178: 6596-603

Forthal DN, Landucci G, Bream J, Jacobson LP, Phan TB, Montoya B. 2007b. FcgammaRIIa genotype predicts progression of HIV infection. *J Immunol* 179: 7916-23

Forthal DN, Landucci G, Keenan B. 2001. Relationship between antibody-dependent cellular cytotoxicity, plasma HIV type 1 RNA, and CD4+ lymphocyte count. *AIDS Res Hum Retroviruses* 17: 553-61

Freed EO, Martin MA. 2013. Human immunodeficiency viruses: Replication In *Field's Virology*, ed. DM Knipe, PM Howley, pp. 1502-60. Philadelphia USA: Lippincott Williams & Wilkins

Galandrini R, Tassi I, Mattia G, Lenti L, Piccoli M, et al. 2002. SH2-containing inositol phosphatase (SHIP-1) transiently translocates to raft domains and modulates CD16-mediated cytotoxicity in human NK cells. *Blood* 100: 4581-9

Galon J, Gauchat JF, Mazieres N, Spagnoli R, Storkus W, et al. 1996. Soluble Fcgamma receptor type III (FcgammaRIII, CD16) triggers cell activation through interaction with complement receptors. *J Immunol* 157: 1184-92

Garcia-Garcia E, Nieto-Castaneda G, Ruiz-Saldana M, Mora N, Rosales C. 2009. FcgammaRIIA and FcgammaRIIIB mediate nuclear factor activation through separate signaling pathways in human neutrophils. *J Immunol* 182: 4547-56

Garcia PM, Kalish LA, Pitt J, Minkoff H, Quinn TC, et al. 1999. Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. Women and Infants Transmission Study Group. *N Engl J Med* 341: 394-402

Gessner JE, Grussenmeyer T, Dumbsky M, Schmidt RE. 1996. Separate promoters from proximal and medial control regions contribute to the natural killer cell-specific transcription of the human FcgammaRIII-A (CD16-A) receptor gene. *J Biol Chem* 271: 30755-64

Gessner JE, Grussenmeyer T, Kolanus W, Schmidt RE. 1995a. The human low affinity immunoglobulin G Fc receptor III-A and III-B genes. Molecular characterization of the promoter regions. *J Biol Chem* 270: 1350-61

Gessner JE, Grussenmeyer T, Schmidt RE. 1995b. Differentially regulated expression of human IgG Fc receptor class III genes. *Immunobiology* 193: 341-55

Gilbert PB, McKeague IW, Eisen G, Mullins C, Gueye NA, et al. 2003. Comparison of HIV-1 and HIV-2 infectivity from a prospective cohort study in Senegal. *Stat Med* 22: 573-93

Gillis C, Gouel-Cheron A, Jonsson F, Bruhns P. 2014. Contribution of Human FcgammaRs to Disease with Evidence from Human Polymorphisms and Transgenic Animal Studies. *Front Immunol* 5: 254

Gomez-Roman VR, Patterson LJ, Venzon D, Liewehr D, Aldrich K, et al. 2005. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated

with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. *J Immunol* 174: 2185-9

Gonda MA, Wong-Staal F, Gallo RC, Clements JE, Narayan O, Gilden RV. 1985. Sequence homology and morphologic similarity of HTLV-III and visna virus, a pathogenic lentivirus. *Science* 227: 173-7

Graf SW, Lester S, Nossent JC, Hill CL, Proudman SM, et al. 2012. Low copy number of the FCGR3B gene and rheumatoid arthritis: a case-control study and meta-analysis. *Arthritis Res Ther* 14: R28

Gray ES, Madiga MC, Moore PL, Mlisana K, Abdool Karim SS, et al. 2009. Broad neutralization of human immunodeficiency virus type 1 mediated by plasma antibodies against the gp41 membrane proximal external region. *J Virol* 83: 11265-74

Gray ES, Moore PL, Choge IA, Decker JM, Bibollet-Ruche F, et al. 2007. Neutralizing antibody responses in acute human immunodeficiency virus type 1 subtype C infection. *J Virol* 81: 6187-96

Gray GE, Urban M, Chersich MF, Bolton C, van Niekerk R, et al. 2005. A randomized trial of two postexposure prophylaxis regimens to reduce mother-to-child HIV-1 transmission in infants of untreated mothers. *Aids* 19: 1289-97

Guay LA, Musoke P, Fleming T, Bagenda D, Allen M, et al. 1999. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. *Lancet* 354: 795-802

Gupta S, Gach JS, Becerra JC, Phan TB, Pudney J, et al. 2013. The Neonatal Fc receptor (FcRn) enhances human immunodeficiency virus type 1 (HIV-1) transcytosis across epithelial cells. *PLoS Pathog* 9: e1003776

Haldane JBS. 1954. An exact test for randomness of mating. *Journal of Genetics* 52: 631-35

Hatjiharissi E, Xu L, Santos DD, Hunter ZR, Ciccarelli BT, et al. 2007. Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the Fc{gamma}RIIIa-158 V/V and V/F polymorphism. *Blood* 110: 2561-4

Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, et al. 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* 366: 1275-86

Hessell AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, et al. 2007. Fc receptor but not complement binding is important in antibody protection against HIV. *Nature* 449: 101-4

Hogarth PM. 2002. Fc receptors are major mediators of antibody based inflammation in autoimmunity. *Curr Opin Immunol* 14: 798-802

Hogarth PM, Pietersz GA. 2012. Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. *Nat Rev Drug Discov* 11: 311-31

Holl V, Peressin M, Decoville T, Schmidt S, Zolla-Pazner S, et al. 2006a. Nonneutralizing antibodies are able to inhibit human immunodeficiency virus type 1 replication in macrophages and immature dendritic cells. *J Virol* 80: 6177-81

Holl V, Peressin M, Schmidt S, Decoville T, Zolla-Pazner S, et al. 2006b. Efficient inhibition of HIV-1 replication in human immature monocyte-derived dendritic cells by purified anti-HIV-1 IgG without induction of maturation. *Blood* 107: 4466-74

Horton RE, Vidarsson G. 2013. Antibodies and their receptors: different potential roles in mucosal defense. *Front Immunol* 4: 200

Huizinga TW, de Haas M, Kleijer M, Nuijens JH, Roos D, von dem Borne AE. 1990a. Soluble Fc gamma receptor III in human plasma originates from release by neutrophils. *J Clin Invest* 86: 416-23

Huizinga TW, Kuijpers RW, Kleijer M, Schulpen TW, Cuypers HT, et al. 1990b. Maternal genomic neutrophil FcRIII deficiency leading to neonatal isoimmune neutropenia. *Blood* 76: 1927-32

Hulett MD, Hogarth PM. 1994. Molecular basis of Fc receptor function. *Adv Immunol* 57: 1-127

Hundt M, Schmidt RE. 1992. The glycosylphosphatidylinositol-linked Fc gamma receptor III represents the dominant receptor structure for immune complex activation of neutrophils. *Eur J Immunol* 22: 811-6

Hunt GM, Papathanasopoulos MA, Gray GE, Tiemessen CT. 2003. Characterisation of near-full length genome sequences of three South African human immunodeficiency virus type 1 subtype C isolates. *Virus Genes* 26: 49-56

Jakubik JJ, Saifuddin M, Takefman DM, Spear GT. 2000. Immune complexes containing human immunodeficiency virus type 1 primary isolates bind to lymphoid tissue B lymphocytes and are infectious for T lymphocytes. *J Virol* 74: 552-5

John GC, Nduati RW, Mbori-Ngacha DA, Richardson BA, Panteleeff D, et al. 2001. Correlates of mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission: association with maternal plasma HIV-1 RNA load, genital HIV-1 DNA shedding, and breast infections. *J Infect Dis* 183: 206-12

Kaneko Y, Nimmerjahn F, Ravetch JV. 2006. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 313: 670-3

Karassa FB, Trikalinos TA, Ioannidis JP, Fcgamma R-SLEM-AI. 2002. Role of the Fcgamma receptor IIa polymorphism in susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. *Arthritis Rheum* 46: 1563-71

Karnasuta C, Paris RM, Cox JH, Nitayaphan S, Pitisuttithum P, et al. 2005. Antibody-dependent cell-mediated cytotoxic responses in participants enrolled in a phase I/II ALVAC-HIV/AIDSVAX B/E prime-boost HIV-1 vaccine trial in Thailand. *Vaccine* 23: 2522-9

Kaset C, Leetrakool N, Intharanut K, Nathalang O. 2013. Frequency of FCGR3B alleles in Thai blood donors. *Ann Lab Med* 33: 426-30

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30: 772-80

Kedzierska K, Crowe SM. 2001. Cytokines and HIV-1: interactions and clinical implications. *Antivir Chem Chemother* 12: 133-50

Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, et al. 2008. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A* 105: 7552-7

Khayat D, Soubrane C, Andrieu JM, Visonneau S, Eme D, et al. 1990. Changes of soluble CD16 levels in serum of HIV-infected patients: correlation with clinical and biologic prognostic factors. *J Infect Dis* 161: 430-5

Khor CC, Davila S, Breunis WB, Lee YC, Shimizu C, et al. 2011. Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. *Nat Genet* 43: 1241-6

Klasse J, Blomberg J. 1987. Patterns of antibodies to human immunodeficiency virus proteins in different subclasses of IgG. *J Infect Dis* 156: 1026-30

Kobayashi T, Westerdaal NA, Miyazaki A, van der Pol WL, Suzuki T, et al. 1997. Relevance of immunoglobulin G Fc receptor polymorphism to recurrence of adult periodontitis in Japanese patients. *Infect Immun* 65: 3556-60

Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de Haas M. 1997. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. *Blood* 90: 1109-14

Koene HR, Kleijer M, Roos D, de Haas M, Von dem Borne AE. 1998. Fc gamma RIIIB gene duplication: evidence for presence and expression of three distinct Fc gamma RIIIB genes in NA(1+,2+)SH(+) individuals. *Blood* 91: 673-9

Kondadasula SV, Roda JM, Parihar R, Yu J, Lehman A, et al. 2008. Colocalization of the IL-12 receptor and FcgammaRIIIa to natural killer cell lipid rafts leads to activation of ERK and enhanced production of interferon-gamma. *Blood* 111: 4173-83

Kono H, Kyogoku C, Suzuki T, Tsuchiya N, Honda H, et al. 2005. FcgammaRIIB Ile232Thr transmembrane polymorphism associated with human systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. *Hum Mol Genet* 14: 2881-92

Korbet SM, Schwartz MM, Evans J, Lewis EJ, Collaborative Study G. 2007. Severe lupus nephritis: racial differences in presentation and outcome. *J Am Soc Nephrol* 18: 244-54

Kourtis AP, Bulterys M. 2010. Mother-to-child transmission of HIV: pathogenesis, mechanisms and pathways. *Clin Perinatol* 37: 721-37, vii

Kuhn L, Meddows-Taylor S, Gray G, Trabattoni D, Clerici M, et al. 2001. Reduced HIV-stimulated T-helper cell reactivity in cord blood with short-course antiretroviral treatment for prevention of maternal-infant transmission. *Clin Exp Immunol* 123: 443-50

Kuhn L, Schramm DB, Donninger S, Meddows-Taylor S, Coovadia AH, et al. 2007. African infants' CCL3 gene copies influence perinatal HIV transmission in the absence of maternal nevirapine. *Aids* 21: 1753-61

Kurosaki T, Ravetch JV. 1989. A single amino acid in the glycosyl phosphatidylinositol attachment domain determines the membrane topology of Fc gamma RIII. *Nature* 342: 805-7

Kushner BH, Cheung NK. 1992. Absolute requirement of CD11/CD18 adhesion molecules, FcRII and the phosphatidylinositol-linked FcRIII for monoclonal antibody-mediated neutrophil antihuman tumor cytotoxicity. *Blood* 79: 1484-90

Kwiatkowski DP. 2005. How malaria has affected the human genome and what human genetics can teach us about malaria. *Am J Hum Genet* 77: 171-92

Lambotte O, Ferrari G, Moog C, Yates NL, Liao HX, et al. 2009. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. *Aids* 23: 897-906

Lambotte O, Pollara J, Boufassa F, Moog C, Venet A, et al. 2013. High antibody-dependent cellular cytotoxicity responses are correlated with strong CD8 T cell viral suppressive activity but not with B57 status in HIV-1 elite controllers. *PLoS One* 8: e74855

Lanier LL, Kipps TJ, Phillips JH. 1985. Functional properties of a unique subset of cytotoxic CD3+ T lymphocytes that express Fc receptors for IgG (CD16/Leu-11 antigen). *J Exp Med* 162: 2089-106

Lanier LL, Yu G, Phillips JH. 1991. Analysis of Fc gamma RIII (CD16) membrane expression and association with CD3 zeta and Fc epsilon RI-gamma by site-directed mutation. *J Immunol* 146: 1571-6

Lassauniere R, Shalekoff S, Tiemessen CT. 2013. A novel FCGR3A intragenic haplotype is associated with increased FcgammaRIIIa/CD16a cell surface density and population differences. *Hum Immunol* 74: 627-34

Lau CS, Yin G, Mok MY. 2006. Ethnic and geographical differences in systemic lupus erythematosus: an overview. *Lupus* 15: 715-9

Lee FK, Scinicariello F, Ou CY. 2004. Partially reverse transcribed HIV genome in uninfected HIV-exposed infants*. Presented at 11th Conference on Retroviruses and Opportunistic Infections*, San Francisco

Lee Y, Ji J, Song G. 2009. Fcγ receptor IIB and IIIB polymorphisms and susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. *Lupus* 18: 727-34

Lee YH, Ji JD, Song GG. 2008. Associations between FCGR3A polymorphisms and susceptibility to rheumatoid arthritis: a metaanalysis. *J Rheumatol* 35: 2129-35

Lehman DA, Farquhar C. 2007. Biological mechanisms of vertical human immunodeficiency virus (HIV-1) transmission. *Rev Med Virol* 17: 381-403

Lejeune J, Thibault G, Ternant D, Cartron G, Watier H, Ohresser M. 2008. Evidence for linkage disequilibrium between Fcgamma RIIIa-V158F and Fcgamma RIIa-H131R polymorphisms in white patients, and for an Fcgamma RIIIa-restricted influence on the response to therapeutic antibodies. *J Clin Oncol* 26: 5489-91; author reply 91-2

Lewis GK. 2014. Role of Fc-mediated antibody function in protective immunity against HIV-1. *Immunology* 142: 46-57

Li LH, Yuan H, Pan HF, Li WX, Li XP, Ye DQ. 2010. Role of the Fcgamma receptor IIIA-V/F158 polymorphism in susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. *Scand J Rheumatol* 39: 148-54

Li SS, Gilbert PB, Tomaras GD, Kijak G, Ferrari G, et al. 2014. FCGR2C polymorphisms associate with HIV-1 vaccine protection in RV144 trial. *J Clin Invest* 124: 3879-90

Li X, Wu J, Carter RH, Edberg JC, Su K, et al. 2003. A novel polymorphism in the Fcgamma receptor IIB (CD32B) transmembrane region alters receptor signaling. *Arthritis Rheum* 48: 3242-52

Li X, Wu J, Ptacek T, Redden DT, Brown EE, et al. 2013. Allelic-dependent expression of an activating Fc receptor on B cells enhances humoral immune responses. *Sci Transl Med* 5: 216ra175

Liu P, Overman RG, Yates NL, Alam SM, Vandergrift N, et al. 2011. Dynamic antibody specificities and virion concentrations in circulating immune complexes in acute to chronic HIV-1 infection. *J Virol* 85: 11196-207

Liu Q, Sun Y, Rihn S, Nolting A, Tsoukas PN, et al. 2009. Matrix metalloprotease inhibitors restore impaired NK cell-mediated antibody-dependent cellular cytotoxicity in human immunodeficiency virus type 1 infection. *J Virol* 83: 8705-12

Ljunggren K, Bottiger B, Biberfeld G, Karlson A, Fenyo EM, Jondal M. 1987. Antibody-dependent cellular cytotoxicity-inducing antibodies against human immunodeficiency virus. Presence at different clinical stages. *J Immunol* 139: 2263-7

Ljunggren K, Broliden PA, Morfeldt-Manson L, Jondal M, Wahren B. 1988. IgG subclass response to HIV in relation to antibody-dependent cellular cytotoxicity at different clinical stages. *Clin Exp Immunol* 73: 343-7

Löfgren R, Serrander L, Forsberg M, Wilsson Å, Wasteson Å, Stendahl O. 1999. CR3, FcγRIIA and FcγRIIIB induce activation of the respiratory burst in human neutrophils: the role of intracellular Ca2+, phospholipase D and tyrosine phosphorylation. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1452: 46-59

Mabuka J, Nduati R, Odem-Davis K, Peterson D, Overbaugh J. 2012. HIV-specific antibodies capable of ADCC are common in breastmilk and are associated with reduced risk of transmission in women with high viral loads. *PLoS Pathog* 8: e1002739

Machado LR, Bowdrey J, Ngaimisi E, Habtewold A, Minzi O, et al. 2013. Copy number variation of Fc gamma receptor genes in HIV-infected and HIV-tuberculosis co-infected individuals in sub-Saharan Africa. *PLoS One* 8: e78165

Machado LR, Hardwick RJ, Bowdrey J, Bogle H, Knowles TJ, et al. 2012. Evolutionary history of copy-number-variable locus for the low-affinity Fcgamma receptor: mutation rate, autoimmune disease, and the legacy of helminth infection. *Am J Hum Genet* 90: 973-85

Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA, Axel R. 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* 47: 333-48

Mantovani A, Cassatella MA, Costantini C, Jaillon S. 2011. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol* 11: 519-31

Marois L, Pare G, Vaillancourt M, Rollet-Labelle E, Naccache PH. 2011. Fc gammaRIIIb triggers raft-dependent calcium influx in IgG-mediated responses in human neutrophils. *J Biol Chem* 286: 3509-19

Matsuhashi M, Tsuno NH, Kawabata M, Mishima Y, Okochi N, et al. 2012. The frequencies of human neutrophil alloantigens among the Japanese population. *Tissue Antigens* 80: 336-40

Maxwell KF, Powell MS, Hulett MD, Barton PA, McKenzie IF, et al. 1999. Crystal structure of the human leukocyte Fc receptor, Fc gammaRIIa. *Nat Struct Biol* 6: 437-42

McCarroll SA, Altshuler DM. 2007. Copy-number variation and association studies of human disease. *Nat Genet* 39: S37-42

McCarroll SA, Kuruvilla FG, Korn JM, Cawley S, Nemesh J, et al. 2008. Integrated detection and population-genetic analysis of SNPs and copy number variation. *Nat Genet* 40: 1166-74

McDougal JS, Kennedy MS, Nicholson JK, Spira TJ, Jaffe HW, et al. 1987. Antibody response to human immunodeficiency virus in homosexual men. Relation of

antibody specificity, titer, and isotype to clinical status, severity of immunodeficiency, and disease progression. *J Clin Invest* 80: 316-24

McKinney C, Broen JC, Vonk MC, Beretta L, Hesselstrand R, et al. 2012. Evidence that deletion at FCGR3B is a risk factor for systemic sclerosis. *Genes Immun* 13: 458-60

Meknache N, Jonsson F, Laurent J, Guinnepain MT, Daeron M. 2009. Human basophils express the glycosylphosphatidylinositol-anchored low-affinity IgG receptor FcgammaRIIIB (CD16B). *J Immunol* 182: 2542-50

Mellors JW, Rinaldo CR, Jr., Gupta P, White RM, Todd JA, Kingsley LA. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 272: 1167-70

Mergener K, Enzensberger W, Rubsamen-Waigmann H, von Briesen H, Doerr HW. 1987. Immunoglobulin class- and subclass-specific HIV antibody detection in serum and CSF specimens by ELISA and Western blot. *Infection* 15: 317-22

Metes D, Ernst LK, Chambers WH, Sulica A, Herberman RB, Morel PA. 1998. Expression of functional CD32 molecules on human NK cells is determined by an allelic polymorphism of the FcgammaRIIC gene. *Blood* 91: 2369-80

Metes D, Manciulea M, Pretrusca D, Rabinowich H, Ernst LK, et al. 1999. Ligand binding specificities and signal transduction pathways of Fc gamma receptor IIc isoforms: the CD32 isoforms expressed by human NK cells. *Eur J Immunol* 29: 2842-52

Milligan C, Overbaugh J. 2014. The role of cell-associated virus in mother-to-child HIV transmission. *J Infect Dis* 210 Suppl 3: S631-40

Mlisana K, Werner L, Garrett NJ, McKinnon LR, van Loggerenberg F, et al. 2014. Rapid disease progression in HIV-1 subtype C-infected South African women. *Clin Infect Dis* 59: 1322-31

Montano M, Russell M, Gilbert P, Thior I, Lockman S, et al. 2003. Comparative prediction of perinatal human immunodeficiency virus type 1 transmission, using multiple virus load markers. *J Infect Dis* 188: 406-13

Moog C, Dereuddre-Bosquet N, Teillaud JL, Biedma ME, Holl V, et al. 2014. Protective effect of vaginal application of neutralizing and nonneutralizing inhibitory antibodies against vaginal SHIV challenge in macaques. *Mucosal Immunol* 7: 46-56

Morgan AW, Griffiths B, Ponchel F, Montague BM, Ali M, et al. 2000. Fcgamma receptor type IIIA is associated with rheumatoid arthritis in two distinct ethnic groups. *Arthritis Rheum* 43: 2328-34

Mueller M, Barros P, Witherden AS, Roberts AL, Zhang Z, et al. 2013. Genomic pathology of SLE-associated copy-number variation at the FCGR2C/FCGR3B/FCGR2B locus. *Am J Hum Genet* 92: 28-40

Muta T, Kurosaki T, Misulovin Z, Sanchez M, Nussenzweig MC, Ravetch JV. 1994. A 13-amino-acid motif in the cytoplasmic domain of Fc gamma RIIB modulates B-cell receptor signalling. *Nature* 368: 70-3

Nagarajan S, Venkiteswaran K, Anderson M, Sayed U, Zhu C, Selvaraj P. 2000. Cell-specific, activation-dependent regulation of neutrophil CD32A ligand-binding function. *Blood* 95: 1069-77

Ngo-Giang-Huong N, Candotti D, Goubar A, Autran B, Maynart M, et al. 2001. HIV type 1-specific IgG2 antibodies: markers of helper T cell type 1 response and prognostic marker of long-term nonprogression. *AIDS Res Hum Retroviruses* 17: 1435-46

Niederer HA, Willcocks LC, Rayner TF, Yang W, Lau YL, et al. 2010. Copy number, linkage disequilibrium and disease association in the FCGR locus. *Hum Mol Genet* 19: 3282-94

Nielsen KR, Koelbaek MD, Varming K, Baech J, Steffensen R. 2012. Frequencies of HNA-1, HNA-3, HNA-4, and HNA-5 in the Danish and Zambian populations determined using a novel TaqMan real time polymerase chain reaction method. *Tissue Antigens* 80: 249-53

Nimmerjahn F, Anthony RM, Ravetch JV. 2007. Agalactosylated IgG antibodies depend on cellular Fc receptors for in vivo activity. *Proc Natl Acad Sci U S A* 104: 8433-7

Nimmerjahn F, Ravetch JV. 2007. Fc-receptors as regulators of immunity. *Adv Immunol* 96: 179-204

Nimmerjahn F, Ravetch JV. 2008a. Anti-inflammatory actions of intravenous immunoglobulin. *Annu Rev Immunol* 26: 513-33

Nimmerjahn F, Ravetch JV. 2008b. Fcgamma receptors as regulators of immune responses. *Nat Rev Immunol* 8: 34-47

O'Brien SJ, Hendrickson SL. 2013. Host genomic influences on HIV/AIDS. *Genome Biol* 14: 201

Ogonda LA, Orago AS, Otieno MF, Adhiambo C, Otieno W, Stoute JA. 2010. The levels of CD16/Fc gamma receptor IIIA on CD14+ CD16+ monocytes are higher in children with severe Plasmodium falciparum anemia than in children with cerebral or uncomplicated malaria. *Infect Immun* 78: 2173-81

Ono M, Bolland S, Tempst P, Ravetch JV. 1996. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. *Nature* 383: 263-6

Onouchi Y, Ozaki K, Burns JC, Shimizu C, Terai M, et al. 2012. A genome-wide association study identifies three new risk loci for Kawasaki disease. *Nat Genet* 44: 517-21

Ory PA, Clark MR, Kwoh EE, Clarkson SB, Goldstein IM. 1989a. Sequences of complementary DNAs that encode the NA1 and NA2 forms of Fc receptor III on human neutrophils. *J Clin Invest* 84: 1688-91

Ory PA, Goldstein IM, Kwoh EE, Clarkson SB. 1989b. Characterization of polymorphic forms of Fc receptor III on human neutrophils. *J Clin Invest* 83: 1676-81

Osborne JM, Chacko GW, Brandt JT, Anderson CL. 1994. Ethnic variation in frequency of an allelic polymorphism of human Fc gamma RIIA determined with allele specific oligonucleotide probes. *J Immunol Methods* 173: 207-17

Pantaleo G, Fauci AS. 1996. Immunopathogenesis of HIV infection. *Annu Rev Microbiol* 50: 825-54

Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, et al. 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 362: 355-8

Panther LA, Tucker L, Xu C, Tuomala RE, Mullins JI, Anderson DJ. 2000. Genital tract human immunodeficiency virus type 1 (HIV-1) shedding and inflammation and HIV-1 env diversity in perinatal HIV-1 transmission. *J Infect Dis* 181: 555-63

Petra Study Team. 2002. Efficacy of three short-course regimens of zidovudine and lamivudine in preventing early and late transmission of HIV-1 from mother to child in Tanzania, South Africa, and Uganda (Petra study): a randomised, double-blind, placebo-controlled trial. *Lancet* 359: 1178-86

Platonov AE, Shipulin GA, Vershinina IV, Dankert J, van de Winkel JG, Kuijper EJ. 1998. Association of human Fc gamma RIIa (CD32) polymorphism with susceptibility to and severity of meningococcal disease. *Clin Infect Dis* 27: 746-50

Pollara J, Hart L, Brewer F, Pickeral J, Packard BZ, et al. 2011. High-throughput quantitative analysis of HIV-1 and SIV-specific ADCC-mediating antibody responses. *Cytometry A* 79: 603-12

Powell MS, Barnes NC, Bradford TM, Musgrave IF, Wines BD, et al. 2006. Alteration of the Fc gamma RIIa dimer interface affects receptor signaling but not ligand binding. *J Immunol* 176: 7489-94

Qiu WQ, de Bruin D, Brownstein BH, Pearse R, Ravetch JV. 1990. Organization of the human and mouse low-affinity Fc gamma R genes: duplication and recombination. *Science* 248: 732-5

Ra C, Jouvin MH, Blank U, Kinet JP. 1989. A macrophage Fc gamma receptor and the mast cell receptor for IgE share an identical subunit. *Nature* 341: 752-4

Rambaut A, Posada D, Crandall KA, Holmes EC. 2004. The causes and consequences of HIV evolution. *Nat Rev Genet* 5: 52-61

Ramsland PA, Farrugia W, Bradford TM, Sardjono CT, Esparon S, et al. 2011. Structural basis for Fc gammaRIIa recognition of human IgG and formation of inflammatory signaling complexes. *J Immunol* 187: 3208-17

Rappaport EF, Cassel DL, Walterhouse DO, McKenzie SE, Surrey S, et al. 1993. A soluble form of the human Fc receptor Fc gamma RIIA: cloning, transcript analysis and detection. *Exp Hematol* 21: 689-96

Ravetch JV, Bolland S. 2001. IgG Fc receptors. *Annu Rev Immunol* 19: 275-90

Ravetch JV, Perussia B. 1989. Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. *J Exp Med* 170: 481-97

Read JS, Newell MK. 2005. Efficacy and safety of cesarean delivery for prevention of mother-to-child transmission of HIV-1. *Cochrane Database Syst Rev*: CD005479

Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, et al. 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* 361: 2209-20

Richman DD, Wrin T, Little SJ, Petropoulos CJ. 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc Natl Acad Sci U S A* 100: 4144-9

Rosales C, Brown EJ. 1992. Signal transduction by neutrophil immunoglobulin G Fc receptors. Dissociation of intracytoplasmic calcium concentration rise from inositol 1,4,5-trisphosphate. *J Biol Chem* 267: 5265-71

Rousseau CM, Nduati RW, Richardson BA, John-Stewart GC, Mbori-Ngacha DA, et al. 2004. Association of levels of HIV-1-infected breast milk cells and risk of mother-to-child transmission. *J Infect Dis* 190: 1880-8

Rouzioux C, Costagliola D, Burgard M, Blanche S, Mayaux MJ, et al. 1995. Estimated timing of mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission by use of a Markov model. The HIV Infection in Newborns French Collaborative Study Group. *Am J Epidemiol* 142: 1330-7

Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, et al. 2009. Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. *J Exp Med* 206: 1273-89

Salmon JE, Brogle NL, Edberg JC, Kimberly RP. 1991. Fc gamma receptor III induces actin polymerization in human neutrophils and primes phagocytosis mediated by Fc gamma receptor II. *J Immunol* 146: 997-1004

Salmon JE, Edberg JC, Brogle NL, Kimberly RP. 1992. Allelic polymorphisms of human Fc gamma receptor IIA and Fc gamma receptor IIIB. Independent mechanisms for differences in human phagocyte function. *J Clin Invest* 89: 1274-81

Salmon JE, Edberg JC, Kimberly RP. 1990. Fc gamma receptor III on human neutrophils. Allelic variants have functionally distinct capacities. *J Clin Invest* 85: 1287-95

Salmon JE, Millard SS, Brogle NL, Kimberly RP. 1995. Fc gamma receptor IIIb enhances Fc gamma receptor IIa function in an oxidant-dependent and allele-sensitive manner. *J Clin Invest* 95: 2877-85

Sanders LA, Feldman RG, Voorhorst-Ogink MM, de Haas M, Rijkers GT, et al. 1995. Human immunoglobulin G (IgG) Fc receptor IIA (CD32) polymorphism and IgG2-mediated bacterial phagocytosis by neutrophils. *Infect Immun* 63: 73-81

Sawyer LA, Katzenstein DA, Hendry RM, Boone EJ, Vujcic LK, et al. 1990. Possible beneficial effects of neutralizing antibodies and antibody-dependent, cell-mediated cytotoxicity in human immunodeficiency virus infection. *AIDS Res Hum Retroviruses* 6: 341-56

Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. 2002. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res* 30: e57

Schramm DB, Kuhn L, Gray GE, Tiemessen CT. 2006. In vivo effects of HIV-1 exposure in the presence and absence of single-dose nevirapine on cellular plasma activation markers of infants born to HIV-1-seropositive mothers. *J Acquir Immune Defic Syndr* 42: 545-53

Shields RL, Lai J, Keck R, O'Connell LY, Hong K, et al. 2002. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. *J Biol Chem* 277: 26733-40

Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, et al. 2003. The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. *J Biol Chem* 278: 3466-73

Shrestha S, Wiener H, Shendre A, Kaslow RA, Wu J, et al. 2012. Role of activating FcgammaR gene polymorphisms in Kawasaki disease susceptibility and intravenous immunoglobulin response. *Circ Cardiovasc Genet* 5: 309-16

Siber GR, Schur PH, Aisenberg AC, Weitzman SA, Schiffman G. 1980. Correlation between serum IgG-2 concentrations and the antibody response to bacterial polysaccharide antigens. *N Engl J Med* 303: 178-82

Siewiera J, El Costa H, Tabiasco J, Berrebi A, Cartron G, et al. 2013. Human cytomegalovirus infection elicits new decidual natural killer cell effector functions. *PLoS Pathog* 9: e1003257

Simister NE. 2003. Placental transport of immunoglobulin G. *Vaccine* 21: 3365-9

Siriboonrit U, Tsuchiya N, Sirikong M, Kyogoku C, Bejrachandra S, et al. 2003. Association of Fcgamma receptor IIb and IIIb polymorphisms with susceptibility to systemic lupus erythematosus in Thais. *Tissue Antigens* 61: 374-83

Sirugo G, Hennig BJ, Adeyemo AA, Matimba A, Newport MJ, et al. 2008. Genetic studies of African populations: an overview on disease susceptibility and response to vaccines and therapeutics. *Hum Genet* 123: 557-98

Smalls-Mantey A, Connors M, Sattentau QJ. 2013. Comparative efficiency of HIV-1-infected T cell killing by NK cells, monocytes and neutrophils. *PLoS One* 8: e74858

Smith DK, Grohskopf LA, Black RJ, Auerbach JD, Veronese F, et al. 2005. Antiretroviral postexposure prophylaxis after sexual, injection-drug use, or other nonoccupational exposure to HIV in the United States: recommendations from the U.S. Department of Health and Human Services. *MMWR Recomm Rep* 54: 1-20

Sodora DL, Gettie A, Miller CJ, Marx PA. 1998. Vaginal transmission of SIV: assessing infectivity and hormonal influences in macaques inoculated with cell-free and cell-associated viral stocks. *AIDS Res Hum Retroviruses* 14 Suppl 1: S119-23

Sondermann P, Huber R, Oosthuizen V, Jacob U. 2000. The 3.2-A crystal structure of the human IgG1 Fc fragment-Fc gammaRIII complex. *Nature* 406: 267-73

Stamatatos L, Morris L, Burton DR, Mascola JR. 2009. Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? *Nat Med* 15: 866-70

Stewart-Akers AM, Cunningham A, Wasko MC, Morel PA. 2004. Fc gamma R expression on NK cells influences disease severity in rheumatoid arthritis. *Genes Immun* 5: 521-9

Su K, Li X, Edberg JC, Wu J, Ferguson P, Kimberly RP. 2004a. A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcgammaRIIb alters receptor expression and associates with autoimmunity. II. Differential binding of GATA4 and Yin-Yang1 transcription factors and correlated receptor expression and function. *J Immunol* 172: 7192-9

Su K, Wu J, Edberg JC, Li X, Ferguson P, et al. 2004b. A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcgammaRIIb alters receptor expression and associates with autoimmunity. I. Regulatory FCGR2B polymorphisms and their association with systemic lupus erythematosus. *J Immunol* 172: 7186-91

Sundqvist VA, Linde A, Kurth R, Werner A, Helm EB, et al. 1986. Restricted IgG subclass responses to HTLV-III/LAV and to cytomegalovirus in patients with AIDS and lymphadenopathy syndrome. *J Infect Dis* 153: 970-3

Takai T. 2002. Roles of Fc receptors in autoimmunity. *Nat Rev Immunol* 2: 580-92

Tamm A, Schmidt RE. 1996. The binding epitopes of human CD16 (Fc gamma RIII) monoclonal antibodies. Implications for ligand binding. *J Immunol* 157: 1576-81

Tiemessen CT, Kuhn L. 2006. Immune pathogenesis of pediatric HIV-1 infection. *Curr HIV/AIDS Rep* 3: 13-9

Tishkoff SA, Reed FA, Friedlaender FR, Ehret C, Ranciaro A, et al. 2009. The genetic structure and history of Africans and African Americans. *Science* 324: 1035-44

Tishkoff SA, Williams SM. 2002. Genetic analysis of African populations: human evolution and complex disease. *Nat Rev Genet* 3: 611-21

Tomaras GD, Ferrari G, Shen X, Alam SM, Liao HX, et al. 2013. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. *Proc Natl Acad Sci U S A* 110: 9019-24

Tomaras GD, Yates NL, Liu P, Qin L, Fouda GG, et al. 2008. Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. *J Virol* 82: 12449-63

Tong Y, Jin J, Yan L, Neppert J, Marget M, Flesch BK. 2003. FCGR3B gene frequencies and FCGR3 variants in a Chinese population from Zhejiang Province. *Ann Hematol* 82: 574-8

Townsend CL, Cortina-Borja M, Peckham CS, de Ruiter A, Lyall H, Tookey PA. 2008. Low rates of mother-to-child transmission of HIV following effective pregnancy interventions in the United Kingdom and Ireland, 2000-2006. *Aids* 22: 973-81

Trist HM, Tan PS, Wines BD, Ramsland PA, Orlowski E, et al. 2014. Polymorphisms and interspecies differences of the activating and inhibitory FcgammaRII of Macaca nemestrina influence the binding of human IgG subclasses. *J Immunol* 192: 792-803

Tuomala RE, O'Driscoll PT, Bremer JW, Jennings C, Xu C, et al. 2003. Cell-associated genital tract virus and vertical transmission of human immunodeficiency virus type 1 in antiretroviral-experienced women. *J Infect Dis* 187: 375-84

Tzeng SJ, Bolland S, Inabe K, Kurosaki T, Pierce SK. 2005. The B cell inhibitory Fc receptor triggers apoptosis by a novel c-Abl family kinase-dependent pathway. *J Biol Chem* 280: 35247-54

UNAIDS. 2014a. Fact Sheet. Joint United Nations Programme on HIV/AIDS (UNAIDS)

UNAIDS. 2014b. The Gap Report. Joint United Nations Programme on HIV/AIDS (UNAIDS)

Urbaczek AC, Toller-Kawahisa JE, Fonseca LM, Costa PI, Faria CM, et al. 2014. Influence of FcgammaRIIIb polymorphism on its ability to cooperate with FcgammaRIIa and CR3 in mediating the oxidative burst of human neutrophils. *Hum Immunol* 75: 785-90

van de Winkel JG, Boonen GJ, Janssen PL, Vlug A, Hogg N, Tax WJ. 1989. Activity of two types of Fc receptors, Fc gamma RI and Fc gamma RII, in human monocyte cytotoxicity to sensitized erythrocytes. *Scand J Immunol* 29: 23-31

Van den Herik-Oudijk IE, Capel PJ, van der Bruggen T, Van de Winkel JG. 1995. Identification of signaling motifs within human Fc gamma RIIa and Fc gamma RIIb isoforms. *Blood* 85: 2202-11

van der Heijden J, Breunis WB, Geissler J, de Boer M, van den Berg TK, Kuijpers TW. 2012. Phenotypic variation in IgG receptors by nonclassical FCGR2C alleles. *J Immunol* 188: 1318-24

van der Heijden J, Nagelkerke S, Zhao X, Geissler J, Rispens T, et al. 2014. Haplotypes of FcgammaRIIa and FcgammaRIIIb polymorphic variants influence IgG-mediated responses in neutrophils. *J Immunol* 192: 2715-21

van Sorge NM, van der Pol WL, Jansen MD, Geleijns KP, Kalmijn S, et al. 2005. Severity of Guillain-Barre syndrome is associated with Fc gamma Receptor III polymorphisms. *J Neuroimmunol* 162: 157-64

Vance BA, Huizinga TW, Wardwell K, Guyre PM. 1993. Binding of monomeric human IgG defines an expression polymorphism of Fc gamma RIII on large granular lymphocyte/natural killer cells. *J Immunol* 151: 6429-39

Vestrheim AC, Moen A, Egge-Jacobsen W, Reubsaet L, Halvorsen TG, et al. 2014. A pilot study showing differences in glycosylation patterns of IgG subclasses induced by pneumococcal, meningococcal, and two types of influenza vaccines. *Immun Inflamm Dis* 2: 76-91

Vidarsson G, Dekkers G, Rispens T. 2014. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol* 5: 520

Warmerdam PA, Nabben NM, van de Graaf SA, van de Winkel JG, Capel PJ. 1993. The human low affinity immunoglobulin G Fc receptor IIC gene is a result of an unequal crossover event. *J Biol Chem* 268: 7346-9

Warmerdam PA, van de Winkel JG, Gosselin EJ, Capel PJ. 1990. Molecular basis for a polymorphism of human Fc gamma receptor II (CD32). *J Exp Med* 172: 19-25

Warmerdam PA, van de Winkel JG, Vlug A, Westerdaal NA, Capel PJ. 1991. A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. *J Immunol* 147: 1338-43

Wei X, Decker JM, Wang S, Hui H, Kappes JC, et al. 2003. Antibody neutralization and escape by HIV-1. *Nature* 422: 307-12

Weis JF, McClelland RS, Jaoko W, Mandaliya KN, Overbaugh J, Graham SM. 2015. Short communication: Fc gamma receptors IIa and IIIa genetic polymorphisms do not predict HIV-1 disease progression in Kenyan women. *AIDS Res Hum Retroviruses* 31: 288-92

Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, et al. 2014. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature* 512: 74-7

Willcocks LC, Carr EJ, Niederer HA, Rayner TF, Williams TN, et al. 2010. A defunctioning polymorphism in FCGR2B is associated with protection against malaria but susceptibility to systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 107: 7881-5

Willcocks LC, Lyons PA, Clatworthy MR, Robinson JI, Yang W, et al. 2008. Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake. *J Exp Med* 205: 1573-82

Williams PJ, Searle RF, Robson SC, Innes BA, Bulmer JN. 2009. Decidual leucocyte populations in early to late gestation normal human pregnancy. *J Reprod Immunol* 82: 24-31

Wu J, Edberg JC, Redecha PB, Bansal V, Guyre PM, et al. 1997. A novel polymorphism of FcgammaRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J Clin Invest* 100: 1059-70

Xiao P, Zhao J, Patterson LJ, Brocca-Cofano E, Venzon D, et al. 2010. Multiple vaccine-elicited nonneutralizing antienvelope antibody activities contribute to protective efficacy by reducing both acute and chronic viremia following simian/human immunodeficiency virus SHIV89.6P challenge in rhesus macaques. *J Virol* 84: 7161-73

Yasuda K, Sugita N, Kobayashi T, Yamamoto K, Yoshie H. 2003. FcgammaRIIB gene polymorphisms in Japanese periodontitis patients. *Genes Immun* 4: 541-6

Yates NL, Lucas JT, Nolen TL, Vandergrift NA, Soderberg KA, et al. 2011. Multiple HIV-1-specific IgG3 responses decline during acute HIV-1: implications for detection of incident HIV infection. *Aids* 25: 2089-97

Yee AM, Phan HM, Zuniga R, Salmon JE, Musher DM. 2000. Association between FcgammaRIIa-R131 allotype and bacteremic pneumococcal pneumonia. *Clin Infect Dis* 30: 25-8

Youinou P, Durand V, Renaudineau Y, Pennec YL, Saraux A, Jamin C. 2002. Pathogenic effects of anti-Fc gamma receptor IIIb (CD16) on polymorphonuclear neutrophils in non-organ-specific autoimmune diseases. *Autoimmun Rev* 1: 13-9

Zhao J, Ma L, Chen S, Xie Y, Xie L, et al. 2014. Association between Fc-gamma receptor IIa (CD32) gene polymorphism and malaria susceptibility: a meta-analysis based on 6928 subjects. *Infect Genet Evol* 23: 169-75

Zhou M, Todd RF, 3rd, van de Winkel JG, Petty HR. 1993. Cocapping of the leukoadhesin molecules complement receptor type 3 and lymphocyte function-associated antigen-1 with Fc gamma receptor III on human neutrophils. Possible role of lectin-like interactions. *J Immunol* 150: 3030-41

Zhou XJ, Lv JC, Bu DF, Yu L, Yang YR, et al. 2010. Copy number variation of FCGR3A rather than FCGR3B and FCGR2B is associated with susceptibility to anti-GBM disease. *Int Immunol* 22: 45-51

Zhu T, Korber BT, Nahmias AJ, Hooper E, Sharp PM, Ho DD. 1998. An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. *Nature* 391: 594-97

APPENDIX A

Appendix A.1. Ethics clearance certificate

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130871

NAME: (Principal Investigator)	Ms Maria M Lassauniere
DEPARTMENT:	Centre for HIV and STIs/Virology National Institute for Communicable Diseases
PROJECT TITLE:	FcγReceptorIIIA/CD16a Gene Variation: Expression and Function in Human Immunodeficiency Virus Infection
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof C Tiemessen
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 04/09/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature M130871Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix A.2. Description of the four perinatal HIV-1 transmission cohorts from which mother-infant pairs were selected for the nested-case control study

	Cohort 1	**Cohort 2**	**Cohort 3**	**Cohort 4**
Cohort name	PETRA	PEP/DART	Bara-PIPE	Coro-PIPE
References	(Kuhn et al 2001, Petra Study 2002)	(Gray et al 2005, Schramm et al 2006)	(Kuhn et al 2007)	(Kuhn et al 2007)
Site	Chris Hani Baragwanath	Chris Hani Baragwanath Coronation Hospital	Chris Hani Baragwanath	Coronation Hospital
Description	Mothers recruited at 35 weeks' gestation. Received one of four regimens: A) AZT + 3TC @ 36wks, oral intrapartum dosing, 7 days postpartum dosing of mother and infant B) As regimen A without prepartum component C) Intrapartum AZT + 3TC only D) Placebo	Group 1: Maternal HIV status was unknown at delivery. Only diagnosed after birth. Did not receive ARVs before or during delivery. Group 2: Small number of women received sdNVP as part of a demonstration of antiretroviral therapy initiative.	Postpartum drug naïve women and women that received sdNVP as part of routine PMTCT services.	Women already enrolled in PMTCT services. Women were recruited at 6 weeks postpartum.
Enrolment period	1996-2000	2000-2002	After 2002	After 2002
***N* in complete cohort**	31	202	284	332
Infant HIV testing	At birth and 6 weeks of age	At birth and 6 weeks of age	At birth and 6 weeks of age	At 6 weeks of age, no birth sample
Maternal VL+CD4 testing time point	Not available	24 hours after deliver	Soon after birth, before discharge	6 weeks postpartum
Maternal drug regimen	As above	None or sdNVP (smaller *N*)	sdNVP	Either sdNVP or triple-drug combination therapy (smaller *N*)
Infant drug regimen	As above	Zidovudine or sdNVP Group 1 within 24 h Group 2 within 72 h	sdNVP (all) Within 72 h	sdNVP Within 72 h

Appendix A.3. Primer pairs used for PCR amplification of the complete ~9.4 kilobase *FCGR3A* gene

Primer Name	Sequence 5'-3'
Fragment 1	
*FCGR3A*Set1-4364F	AGG TTT CCC AGA TAA GCA TCC
*FCGR3A*Set1-6276R	CAC AAG AAA GGG TAG AAA TTG AAA AT
Fragment 2	
First round	
*FCGR3A*Set2-5793F	TAG CTG TGG ATT GAG CTC CT
*FCGR3A*Set2-9485R	CGT GTG TTG GTC ATG ATT CTC TAC
Nested	
*FCGR3A*Set2-6046F	TAG CTG TGG ATT GAG CTC CT
*FCGR3A*Set2-8926R	GAT CAA GAC CAT CCT GGC TAA C
Fragment 3	
First round	
*FCGR3A*Set3-8157F	GTC CCT ACA ATC TTA CCA CAT AGG
*FCGR3A*Set3-12025R	AAA ATG ACC AGA ATA GTT TAA TCT CGT
Nested	
*FCGR3A*Set3-8382F	TTC ACT CTC CAG AGC TAC AAG AAG A
*FCGR3A*Set3-11679R	TGG GAT CAT AGG ATA TTA GTG CTT G
Fragment 4	
*FCGR3A*Set4-10763F	CAC ATA TTT ACA GAA TGG CAA AG**G***
*FCGR3A*Set4-13968R	ATT TAT ATG AGT TGT GGT GAG ATG G**T***

* 3' Locked nucleic acid (LNA) base used for improved discrimination between *FCGR3A* and *FCGR3B* genes

APPENDIX B

Appendix B.1. Comparison between genotypes and carriage of at least one allele between different population groups

Overall H131R genotype distribution (Chi-square)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = .097	-	-	-	-
YRI	P = .703	P = .202	-	-	-
SA Cauc	P = .014	P = .415	P = .036	-	-
EUR	P = .280	P = .483	P = .543	P = .080	-
Dutch Cauc	P = .107	P = .557	P = .389	P = .250	P = .482

HH vs. HR (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = .100	-	-	-	-
YRI	P = 1	P = .109	-	-	-
SA Cauc	P = .037	P = .498	P = .033	-	-
EUR	P = .308	P = .279	P = .309	P = .095	-
Dutch Cauc	P = .414	P = .410	P = .375	P = .171	P = 1

HH vs. RR (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = .046	-	-	-	-
YRI	P = .688	P = .218	-	-	-
SA Cauc	P = .009	P = .277	P = .051	-	-
EUR	P = .121	P = .359	P = .497	P = .056	-
Dutch Cauc	P = .058	P = 1	P = .205	P = .277	P = .336

HH vs. at least one R (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = .044	-	-	-	-
YRI	P = 1	P = .095	-	-	-
SA Cauc	P = .007	P = .285	P = .018	-	-
EUR	P = .194	P = .250	P = .339	P = .038	-
Dutch Cauc	P = .160	P = .641	P = .240	P = .127	P = .702

H vs. R (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = .046	-	-	-	-
YRI	P = .559	P = .211	-	-	-
SA Cauc	P = .005	P = .191	P = .028	-	-
EUR	P = .110	P = .305	P = .497	P = .031	-
Dutch Cauc	P = .039	P = 1	P = .215	P = .193	P = .314

Overall I232T genotype distribution (Chi-square)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = .482	-	-	-	-
YRI	P = .039	P = .043	-	-	-
SA Cauc	P = .002	P = .001	P = .094	-	-
EUR	P < .0001	P < .0001	P = .002	P = .874	-
Dutch Cauc*	P < .0001	P < .0001	P = .003	P = .983	P = .505

II vs. IT (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = .490	-	-	-	-
YRI	P = .117	P = .025	-	-	-
SA Cauc	P = .0009	P = .0003	P = .042	-	-
EUR	P < .0001	P < .0001	P = .0008	P = .654	-
Dutch Cauc*	P < .0001	P < .0001	P = .001	P = 1	P = .384

II vs. TT (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = .587	-	-	-	-
YRI	P = .038	P = .241	-	-	-
SA Cauc	P = .171	P = .406	P = 1	-	-
EUR	P = .0004	P = .036	P = 1	P = 1	-
Dutch Cauc*	P = .030	P = .280	P = 1	P = 1	P = .743

II vs. at least one T (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = .687	-	-	-	-
YRI	P = .038	P = .028	-	-	-
SA Cauc	P = .0006	P = .0002	P = .032	-	-
EUR	P < .0001	P < .0001	P = .002	P = .825	-
Dutch Cauc*	P < .0001	P < .0001	P = .003	P = 1	P = .285

I vs. T (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc	EUR
LWK	P = 1	-	-	-	-
YRI	P = .016	P = .024	-	-	-
SA Cauc	P = .001	P = .002	P = .127	-	-
EUR	P < .0001	P < .0001	P = .005	P = .754	-
Dutch Cauc*	P < .0001	P < .0001	P = .023	P = 1	P = .767

Overall F158V genotype distribution (Chi-square)

	SA Black individuals	LWK	YRI	SA Cauc*	EUR*
LWK	P < .0001	-	-	-	-
YRI	P = .026	P = .001	-	-	-
SA Cauc*	P = .056	P = .001	P = .001	-	-
EUR*	P = .001	P = .001	P = .025	P = .102	-
Dutch	P = .195	P = .001	P = .303	P = .057	P = .264

FF vs. FV (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc*	EUR*
LWK	P < .0001	-	-	-	-
YRI	P = .121	P = .0002	-	-	-
SA Cauc*	P = .125	P = .065	P = .651	-	-
EUR*	P = .0002	P = .002	P = .175	P = 1	-
Dutch	P = .124	P = .0003	P = 1	P = .654	P = .185

FF vs. VV (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc*	EUR*
LWK	P = .008	-	-	-	-
YRI	P = .022	P = 1	-	-	-
SA Cauc*	P = .381	P = .002	P = .003	-	-
EUR*	P = .336	P = .030	P = .059	P = .063	-
Dutch	P = .327	P = .200	P = .172	P = .057	P = .684

FF(F) vs. at least one V (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc*	EUR*
LWK	P < .0001	-	-	-	-
YRI	P = .052	P = .0007	-	-	-
SA Cauc*	P = .687	P = .001	P = .536	-	-
EUR*	P = .0005	P = .0003	P = .553	P = .199	-
Dutch	P = .108	P = .0001	P = .663	P = .685	P = .255

V vs. F (Fisher's exact)

	SA Black individuals	LWK	YRI	SA Cauc*	EUR*
LWK	P < .0001	-	-	-	-
YRI	P = .012	P = .008	-	-	-
SA Cauc*	P = 1	P < .0001	P = .079	-	-
EUR*	P = .003	P = .0001	P = .605	P = .068	-
Dutch	P = .091	P = .0003	P = .417	P = .222	P = .598

*Population frequencies not in Hardy-Weinberg equilibrium – associations with these populations are shaded in grey

EUR – European ancestry super group; YRI – Yoruba in Ibadan, Nigeria; LWK – Luhya in Webuye, Kenya; SA – South African; Cauc - Caucasian

Appendix B.2. Low affinity *FCGR* genotypes and FcγRIIIa surface density for South African Caucasian participants.

Caucasian donor ID	FcγRIIa-R131H	FcγRIIIa CNV	FcγRIIIa-intragenic haplotype (IH)	FcγRIIIa-F158V	FcγRIIc CNV	FcγRIIc -386G>C/ -120T>A	FcγRIIc Express/Pseudogene (Pseudo) Genotype	FcγRIIb -386G>C/ -120T>A	FcγRIIb-I232T	FcγRIIIa surface density (antibodies bound per cell) CD56dim NK cells	FcγRIIIa surface density (antibodies bound per cell) CD14dim monocytes
CCR07	**HH**	2	**IH**/wt	**VV**	2	**C**G/TT	**Express**/Pseudo	**C**G/**A**T	II	90142	72873
CCR60	HR	2	**IH**/wt	F**V**	2	**C**G/TT	**Express**/Pseudo	GG/TT	II	83856	70074
CCR21	RR	2	**IH**/wt	F**V**	2	**C**G/TT	**Express**/Pseudo	**C**G/**A**T	II	68046	74639
CCR06	**HH**	3	**IH**/wt/wt	F**VV**	3*	**C**GG/TTT	**Express**/Pseudo/Pseudo	GG/TT	II	61372	57590
CCR01	HR	2	**IH**/wt	F**V**	3	**C**GG/TTT	**Express**/Pseudo/Pseudo	**C**G/**A**T	II	61153	73168
CCR12	**HH**	2	**IH**/wt	**VV**	1	**C**/T	**Express**	**C**G/**A**T	II	57355	69038
CCR15	HR	2	**IH**/wt	**VV**	2	**C**G/TT	**Express**/Pseudo	**C**G/**A**T	II	55477	40392
CCR13	**HH**	2	**IH**/wt	F**V**	2	**CC**/TT	**Express**/Pseudo	**C**G/**A**T	II	53498	64317
CCR58	**HH**	2	**IH**/wt	F**V**	2	**C**G/TT	**Express**/Pseudo	GG/TT	II	49386	65251
CCR14	RR	3	**IH**/wt/wt	FFF	3*	GGG/ATT	**Express**/Pseudo/Pseudo	**C**G/**A**T	II	49375	72380
CCR59	HR	2	**IH**/wt	F**V**	2	**C**G/TT	**Express**/Pseudo	**C**G/**A**T	II	45789	42144
CCR23	**HH**	2	**IH**/wt	F**V**	2	**C**G/TT	**Express**/Pseudo	**C**G/**A**T	IT	41575	42600
CCR04	**HH**	2	**IH**/wt	**VV**	2	**C**G/TT	**Express**/Pseudo	**C**G/**A**T	II	40518	39458
CCR05	**HH**	2	**IH/IH**	**VV**	2	**CC**/TT	**Express/Express**	**CC**/**AA**	II	36395	30443
CCR57	**HH**	2	**IH**/wt	**VV**	2	**C**G/TT	**Express**/Pseudo	GG/TT	II	35833	36315
CCR51	**HH**	2	**IH/IH**	**VV**	2	**CC**/TT	**Express/Express**	**CC**/**A**T	TT	23206	32201
CCR48	**HH**	2	wt/wt	F**V**	2	GG/TT	Pseudo/Pseudo	GG/TT	II	85563	54076
CCR50	HR	3	wt/wt/wt	FF**V**	3*	GGG/TTT	Pseudo/Pseudo/Pseudo	GG/TT	II	39733	30160
CCR11	RR	2	wt/wt	FF	5	GGGGG/TTTTT	Pseudo(x5)	GG/TT	II	36595	37236
CCR02	HR	2	wt/wt	FF	2	GG/TT	Pseudo/Pseudo	GG/TT	II	35700	67902
CCR55	HR	2	wt/wt	FF	3	GGG/TTT	Pseudo(x3)	**C**G/**A**T	IT	34371	46533
CCR19	**HH**	2	wt/wt	FF	1	G/T	Pseudo	GG/TT	II	32759	45647
CCR20	HR	2	wt/wt	FF	2	GG/TT	Pseudo/Pseudo	GG/TT	IT	31875	40284
CCR52	HR	2	wt/wt	FF	2	GG/TT	Pseudo/Pseudo	GG/TT	II	31313	38551
CCR16	RR	2	wt/wt	FF	2	**C**G/TT	**Express**/Pseudo	GG/TT	IT	26630	37813
CCR56	HR	2	wt/wt	FF	4	GGGG/TTTT	Pseudo(x4)	GG/TT	II	25648	42923
CCR54	HR	2	wt/wt	FF	2	GG/TT	Pseudo/Pseudo	GG/TT	II	23026	28505
CCR53	RR	2	wt/wt	FF	2	GG/TT	Pseudo/Pseudo	GG/TT	IT	18944	34925
CCR08	HR	2	wt/wt	FF	3	GGG/TTT	Pseudo(x3)	GG/TT	II	18868	31008
CCR17	HR	2	wt/wt	FF	1	G/T	Pseudo	GG/TT	II	16724	20549
CCR10	**HH**	2	wt/wt	FF	2	GG/TT	Pseudo/Pseudo	GG/TT	II	15304	18456
CCR18	**HH**	2	wt/wt	FF	2	GG/TT	Pseudo/Pseudo	GG/TT	II	13500	22086

N/G – Not Genotyped

*Duplication of CNR2 region, thus one *FCGR2C* gene copy lacks exon 7

APPENDIX C

Appendix C.1. Associations from univariate and multivariate analyses of ***maternal*** FcγR genotypes and allele carriage with HIV-1 transmission across the total cohort

Genotype/allele	Univariate			Multivariate analysis - Adjusted for the following:											
				Viral Load			CD4 count			Maternal sdNVP			Viral Load + sdNVP		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
FcγRIIa															
131HH (Ref)	1			1			1			1			1		
131HR	1.30	0.62-2.73	0.494	1.70	0.71-4.09	0.234	1.04	0.46-2.34	0.929	1.30	0.62-2.74	0.488	1.71	0.71-4.10	0.232
131RR	1.88	0.86-4.12	0.115	3.22	1.28-8.10	**0.013**	1.69	0.72-3.97	0.232	1.87	0.85-4.10	0.118	3.15	1.25-7.99	**0.015**
≥1 131H allele	0.63	0.35-1.15	0.135	0.45	0.23-0.88	**0.020**	0.61	0.32-1.16	0.132	0.64	0.35-1.16	0.143	0.46	0.23-0.91	**0.025**
≥1 131R allele	1.51	0.76-3.013	0.242	2.21	0.97-5.02	**0.059**	1.27	0.59-2.70	0.54	1.51	0.76-3.02	0.241	2.18	0.96-4.97	**0.063**
FcγRIIb															
232II	1			1			1			1			1		
232IT	1.23	0.67-2.24	0.508	1.32	0.67-2.59	0.417	1.21	0.63-2.33	0.558	1.23	0.67-2.24	0.509	1.32	0.62-2.59	0.420
232TT	1.51	0.61-3.75	0.373	1.83	0.67-4.98	0.239	1.62	0.61-4.28	0.328	1.49	0.60-3.72	0.387	1.79	0.66-4.87	0.257
≥1 232I allele	0.73	0.31-1.72	0.476	0.63	0.25-1.61	0.335	0.68	0.27-1.69	0.407	0.74	0.31-1.75	0.492	0.64	0.25-1.65	0.358
≥1 232T allele	1.28	0.73-2.27	0.392	1.42	0.75-2.67	0.282	1.29	0.70-2.39	0.413	1.28	0.72-2.26	0.398	1.41	0.75-2.66	0.291
FcγRIIIa															
158F/FF/FFF (Ref)	1			1			1			1			1		
158FV/FFV/FVV	0.63	0.34-1.16	0.138	0.66	0.34-1.29	0.226	0.63	0.33-1.23	0.176	0.63	0.34-1.17	0.143	0.67	0.34-1.34	0.263
158VV	0.35	0.14-0.88	**0.026**	0.24	0.08-0.72	**0.011**	0.40	0.15-1.04	**0.061**	0.35	0.14-0.89	**0.027**	0.24	0.08-0.74	**0.013**
≥1 158F allele	2.28	0.94-5.50	**0.068**	3.42	1.19-9.84	**0.023**	2.00	0.81-4.94	0.135	2.26	0.94-5.47	**0.070**	3.36	1.16-9.72	**0.025**
≥1 158V allele	0.55	0.31-0.97	**0.039**	0.53	0.28-1.00	**0.048**	0.56	0.30-1.04	**0.067**	0.55	0.31-0.98	**0.042**	0.54	0.28-1.03	**0.063**
FcγRIIIb															
NA1+/NA2-/SH-	0.91	0.41-2.03	0.814	0.65	0.26-1.63	0.354	0.82	0.33-2.02	0.663	0.92	0.41-2.06	0.834	0.66	0.26-1.66	0.374
NA1-/NA2+/SH-	1.15	0.41-3.23	0.788	1.52	0.50-4.68	0.462	1.62	0.54-4.85	0.387	1.19	0.423.37	0.742	1.66	0.53-5.18	0.382
NA1-/NA2-/SH+		-			-			-			-			-	
NA1+/NA2+/SH- (ref)	1			1			1			1			1		
NA1+/NA2-/SH+	1.27	0.52-3.07	0.599	1.01	0.38-2.72	0.977	1.20	0.46-.12	0.712	1.24	0.51-3.02	0.632	0.99	0.37-2.64	0.979
NA1-/NA2+/SH+	2.84	1.18-6.84	**0.020**	1.83	0.68-4.88	0.230	2.87	1.11-7.43	**0.030**	2.87	1.19-6.94	**0.019**	1.84	0.69-4.93	0.224
NA1+/NA2+/SH+	0.77	0.19-3.10	0.711	0.40	0.09-1.74	0.220	0.65	1.16-2.72	0.557	0.78	0.19-3.15	0.727	0.40	0.09-1.75	0.222
≥1 HNA1a	0.65	0.34-1.21	0.173	0.66	0.33-1.32	0.237	0.58	0.30-1.15	0.120	0.64	0.34-1.20	0.162	0.64	0.32-1.29	0.212
≥1 HNA1b	1.42	0.79-2.56	0.247	1.73	0.89-3.36	0.104	1.65	0.86-3.16	0.132	1.43	0.79-2.59	0.234	1.76	0.91-3.43	**0.096**
≥1 HNA1c	0.65	0.34-1.21	0.173	1.06	0.55-2.03	0.864	1.32	0.70-2.47	0.395	1.43	0.80-2.57	0.228	1.04	0.54-1.99	0.912

Appendix C.2. Associations from univariate and multivariate analyses of ***maternal*** FcγR genotypes and allele carriage with HIV-1 transmission across the group of intrapartum transmitting mothers

Genotype/allele	Univariate			Multivariate analysis - Adjusted for the following:											
				Viral Load			CD4 count			Maternal sdNVP			Viral Load + sdNVP		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
FcγRIIa															
131HH (Ref)	1			1			1			1			1		
131HR	1.13	0.39-3.27	0.815	1.24	0.37-4.19	0.730	0.83	0.25-2.70	0.755	1.17	0.40-3.43	0.769	1.2	0.35-4.17	0.769
131RR	1.79	0.60-5.32	0.297	3.58	1.00-12.83	**0.050**	1.75	0.53-5.77	0.357	2.01	0.66-6.11	0.219	4.21	1.13-15.72	**0.032**
≥1 131H allele	0.61	0.26-1.40	0.243	0.32	0.12-0.85	**0.021**	0.50	0.20-1.25	0.139	0.55	0.24-1.30	0.174	0.27	0.10-0.74	**0.011**
≥1 131R allele	1.37	0.52-3.63	0.521	1.87	.61-5.69	0.272	1.15	0.39-3.36	0.805	1.46	0.55-3.92	0.448	1.93	0.62-5.96	0.255
FcγRIIb															
232II	1			1			1			1			1		
232IT	1.52	0.65-3.56	0.335	1.80	0.69-4.70	0.227	1.37	0.54-3.48	0.503	1.53	0.65-3.63	0.332	1.78	0.67-4.70	0.245
232TT	1.24	0.31-4.98	0.765	1.74	0.40-7.67	0.461	1.35	0.32-5.64	0.681	1.39	0.34-5.75	0.645	1.77	0.38-8.19	0.463
≥1 232I allele	1.01	0.27-3.73	0.991	0.79	0.20-3.10	0.733	0.87	0.23-3.32	0.804	0.9	0.24-3.40	0.874	0.77	0.19-3.19	0.716
≥1 232T allele	1.46	0.65-3.32	0.361	1.79	0.72-4.49	0.213	1.37	0.57-3.31	0.486	1.51	0.66-3.36	0.332	1.78	0.70-4.52	0.227
FcγRIIIa															
158F/FF/FFF (Ref)	1			1			1			1			1		
158FV/FFV/FVV	1.80	0.73-4.44	0.203	1.35	0.51-3.58	0.551	1.51	0.58-3.9	0.400	1.78	0.71-4.45	0.217	1.22	0.45-3.34	0.693
158VV	0.45	0.09-2.25	0.328	0.18	0.02-1.57	0.120	0.43	0.08-2.24	0.318	0.39	0.08-2.00	0.259	0.15	0.016-1.33	0.088
≥1 158F allele	3.26	0.73-14.52	0.121	6.87	0.85-55.31	**0.070**	2.95	0.64-13.57	0.165	3.72	0.82-16.86	**0.089**	7.75	0.96-62.75	**0.055**
≥1 158V allele	1.40	0.58-3.38	0.459	0.99	0.38-2.58	0.983	1.17	0.46-2.95	0.738	1.34	0.55-3.29	0.516	0.88	0.33-2.35	0.792
FcγRIIIIb															
NA1+/NA2-/SH-	0.64	0.19-2.21	0.484	0.58	0.15-2.25	0.429	0.83	0.22-3.05	0.774	0.54	0.15-1.91	0.342	0.53	0.13-2.07	0.361
NA1-/NA2+/SH-	1.51	0.41-5.56	0.532	2.22	0.52-9.57	0.283	2.41	0.59-9.90	0.222	1.27	0.34-4.79	0.719	1.81	0.41-8.05	0.437
NA1-/NA2-/SH+		-			-			-			-			-	
NA1+/NA2+/SH- (ref)	1			1			1			1			1		
NA1+/NA2-/SH+	1.33	0.40-4.36	0.643	1.01	0.25-4.04	0.984	1.08	0.28-4.14	0.915	1.56	0.46-5.29	0.477	1.09	0.26-4.54	0.908
NA1-/NA2+/SH+	2.04	0.59-7.00	0.258	1.59	0.40-6.23	0.510	2.18	0.54-8.77	0.272	1.73	0.49-6.08	0.392	1.4	0.34-5.77	0.641
NA1+/NA2+/SH+	0.59	0.07-5.18	0.633	0.37	0.04-3.50	0.386	0.52	0.06-4.85	0.568	0.53	0.06-4.72	0.566	0.31	0.03-3.12	0.321
≥1 HNA1a	0.66	0.27-1.59	0.354	0.61	0.24-1.59	0.317	0.60	0.23-1.55	0.290	0.72	0.30-1.76	0.473	0.66	0.25-175	0.409
≥1 HNA1b	1.54	0.66-3.62	0.320	1.94	0.75-5.03	0.172	1.68	0.66-4.29	0.275	1.48	0.63-3.52	0.371	1.83	0.70-4.81	0.217
≥1 HNA1c	1.26	0.55-2.88	0.582	0.94	0.38-2.34	0.900	0.96	0.38-2.40	0.932	1.37	0.59-3.19	0.463	0.96	0.37-2.47	0.930

Appendix C.3. Associations from univariate and multivariate analyses of ***maternal*** FcγR genotypes and allele carriage with HIV-1 transmission across the group of *in utero* transmitting mothers

				Multivariate analysis - Adjusted for the following:											
	Univariate			Viral Load			CD4 count			Maternal sdNVP			Viral Load + sdNVP		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
FcγRIIa															
131HH (Ref)	1			1			1			1			1		
131HR	2.55	0.52-12.43	0.246	6.93	0.75-64.31	0.890	3.08	0.36-26.45	0.305	2.56	0.53-12.49	0.244	6.91	0.75-64.00	**0.089**
131RR	4.38	0.89-21.63	**0.069**	17.9	1.86-172.72	**0.013**	6.08	0.70-52.51	0.101	4.47	0.89-22.30	**0.068**	18.84	1.88-188.26	**0.012**
≥1 131H allele	0.45	0.17-1.18	0.105	0.25	0.08-0.78	**0.016**	0.40	0.13-1.20	0.102	0.45	0.17-1.18	0.105	0.24	0.07-0.79	**0.019**
≥1 131R allele	3.23	0.715-14.56	0.128	10.21	1.18-88.48	**0.035**	4.12	0.52-32.87	0.181	3.23	0.71-14.63	0.128	10.07	1.16-87.71	**0.036**
FcγRIIb															
232II	1			1			1			1			1		
232IT	0.67	0.23-1.95	0.461	0.78	0.239-2.53	0.677	0.79	0.23-2.65	0.699	0.67	0.23-1.95	0.461	0.78	0.24-2.55	0.683
232TT	1.81	0.50-6.57	0.365	2.61	0.54-12.54	0.230	2.27	0.50-10.41	0.290	1.82	0.50-6.63	0.366	2.60	0.54-12.46	0.233
≥1 232I allele	0.47	0.14-1.57	0.218	0.34	0.78-1.52	0.159	0.40	0.09-1.65	0.203	0.46	0.14-1.58	0.221	0.35	.08-1.53	0.161
≥1 232T allele	0.89	0.35-2.28	0.816	1.04	0.36-3.00	0.943	1.04	0.35-3.06	0.944	0.89	0.35-2.28	0.813	1.04	0.36-3.01	0.940
FcγRIIIa															
158F/FF/FFF (Ref)	1			1			1			1			1		
158FV/FFV/FVV	0.55	0.21-1.47	0.234	0.69	0.22-2.11	0.513	0.81	0.26-2.49	0.709	0.55	0.21-1.47	0.231	0.70	0.22-2.19	0.538
158VV	0.16	0.02-1.32	**0.090**	0.14	0.02-1.29	**0.083**	0.24	0.03-2.12	0.200	0.16	0.02-1.31	**0.088**	0.14	0.02-1.34	**0.089**
≥1 158F allele	4.59	0.59-35.72	0.146	5.85	0.69-49.97	0.106	3.67	.46-29.6	0.222	4.65	0.59-36.50	0.144	5.73	0.66-49.42	0.112
≥1 158V allele	0.44	0.17-1.12	**0.085**	0.49	0.17-1.44	0.194	0.63	0.21-1.85	0.399	0.43	0.17-1.12	**0.085**	0.51	0.17-1.52	0.225
FcγRIIIIb															
NA1+/NA2-/SH-	3.21	0.75-13.73	0.115	1.70	0.34-8.55	0.520	2.60	0.41-16.87	0.313	3.28	0.76-14.18	0.111	1.65	0.32-8.43	0.544
NA1-/NA2+/SH-	1.26	0.12-13.08	0.845	2.73	0.22-33.94	0.435	2.38	0.18-30.33	0.505	1.31	0.12-13.87	0.822	3.10	0.24-39.46	0.384
NA1-/NA2-/SH+		-			-			-			-				
NA1+/NA2+/SH- (ref)	1			1			1			1			1		
NA1+/NA2-/SH+	3.53	0.73-17.20	0.118	2.33	0.42-12.81	0.331	4.81	0.79-29.10	**0.087**	3.48	0.71-17.01	0.124	2.21	0.41-12.03	0.359
NA1-/NA2+/SH+	6.79	1.44-32.16	**0.016**	4.53	0.7925.93	**0.090**	8.22	1.32-51.02	**0.024**	6.91	1.45-32.94	**0.015**	4.51	0.79-25.55	**0.089**
NA1+/NA2+/SH+	1.96	0.18-21.02	0.577	0.77	0.06-9.33	0.840	2.21	0.18-27.70	0.537	1.99	0.19-21.35	0.570	0.75	0.06-9.19	0.820
≥1 HNA1a	0.69	0.25-1.95	0.487	0.63	0.19-2.11	0.454	0.57	0.18-1.83	0.348	0.69	0.24-1.94	0.480	0.62	0.18-2.07	0.434
≥1 HNA1b	0.69	0.27-1.77	0.445	1.07	0.37-3.13	0.902	0.84	0.28-2.48	0.749	0.70	0.27-1.78	0.447	1.10	0.37-3.23	0.869
≥1 HNA1c	2.06	0.80-5.30	0.132	1.52	0.53-4.36	0.441	2.68	0.87-8.27	**0.087**	2.07	0.80-5.36	0.133	1.50	0.52-4.33	0.449

Appendix C.4. Associations from univariate and multivariate analyses of ***maternal*** FcγR genotypes and allele carriage with HIV-1 transmission across the group of *in utero* enriched transmitting mothers

	Univariate			Multivariate analysis - Adjusted for the following: Viral Load			CD4 count			Maternal sdNVP			Viral Load + sdNVP		
	OR	95% CI	P	OR	95% CI	P	4.21	95% CI	P	OR	95% CI	P	OR	95% CI	P
FcγRIIa															
131HH (Ref)	1			1			1			1			1		
131HR	1.42	0.57-3.53	0.453	2.37	0.78-7.18	0.128	1.18	0.44-3.16	0.738	1.48	0.59-3.74	0.405	2.46	0.80-7.60	0.117
131RR	1.95	0.75-5.08	0.173	3.58	1.13-11.36	**0.031**	1.74	0.62-4.89	0.297	1.84	0.70-4.86	0.219	2.93	0.90-9.52	**0.074**
≥1 131H allele	0.65	0.32-1.33	0.238	0.52	0.24-1.13	**0.098**	0.65	0.30-1.39	0.267	0.71	0.34-1.46	0.352	0.65	0.28-1.45	0.291
≥1 131R allele	1.61	0.69-3.78	0.271	2.80	.98-8.04	**0.055**	1.38	0.55-3.45	0.495	1.62	0.68-3.84	0.272	2.65	0.91-7.70	**0.074**
FcγRIIb															
232II	1			1			1			1			1		
232IT	1.06	0.51-2.19	0.884	1.14	0.51-2.54	0.745	1.14	0.52-2.50	0.734	1.04	0.50-2.18	0.920	1.11	0.49-2.51	0.803
232TT	1.67	0.60-4.68	0.330	1.93	0.61-6.09	0.262	1.81	0.59-5.56	0.301	1.50	0.52-4.27	0.451	1.69	0.52-5.44	0.380
≥1 232I allele	0.61	0.23-1.62	0.325	0.55	0.19-1.62	0.281	0.59	0.21-1.69	0.327	0.68	0.25-1.82	0.444	0.62	0.21-1.87	0.399
≥1 232T allele	1.18	0.60-2.32	0.638	1.29	0.61-2.71	0.510	1.27	0.61-2.63	0.527	1.13	0.57-2.26	0.722	1.22	0.57-2.63	0.605
FcγRIIIa															
158F/FF/FFF (Ref)	1			1			1			1			1		
158FV/FFV/FVV	0.30	0.14-0.65	**0.003**	0.37	0.16-0.85	**0.019**	0.34	0.15-0.78	**0.011**	0.31	0.14-0.68	**0.004**	0.44	0.19-1.05	**0.065**
158VV	0.32	0.11-0.92	**0.034**	0.25	0.07-0.83	**0.023**	0.37	0.13-1.20	**0.074**	0.35	0.12-1.04	**0.058**	0.30	0.86-1.03	**0.056**
≥1 158F allele	1.88	0.68-5.21	0.223	2.66	0.83-8.51	0.100	1.70	0.60-4.82	0.316	1.72	0.61-4.84	0.300	2.38	0.72-7.86	0.156
≥1 158V allele	0.30	0.15-0.61	**0.001**	0.33	0.15-0.70	**0.004**	0.35	0.17-0.73	**0.005**	0.32	0.16-0.65	**0.002**	0.40	0.18-0.87	**0.021**
FcγRIIIIb															
NA1+/NA2-/SH-	1.11	0.43-2.89	0.828	0.63	0.21-1.86	0.398	0.85	0.28-2.52	0.764	1.23	0.46-3.25	0.681	0.68	0.22-2.13	0.512
NA1-/NA2+/SH-	0.87	0.22-3.50	0.849	1.22	0.28-5.30	0.788	1.14	0.27-4.88	0.858	1.08	0.26-4.46	0.916	1.89	0.41-8.79	0.417
NA1-/NA2-/SH+		-			-			-			-			-	
NA1+/NA2+/SH- (ref)	1			1			1			1			1		
NA1+/NA2-/SH+	1.22	0.41-3.66	0.719	0.95	0.29-3.04	0.925	1.26	0.40-3.94	0.688	1.14	0.37-3.45	0.823	0.91	0.28-2.93	0.871
NA1-/NA2+/SH+	3.45	1.26-9.43	**0.016**	2.10	0.69-6.38	0.189	3.40	1.17-9.85	**0.024**	3.73	1.33-10.40	**0.012**	2.20	0.70-6.89	0.177
NA1+/NA2+/SH+	0.91	0.17-4.71	0.907	0.43	0.08-2.41	0.336	0.76	0.14-4.05	0.746	1.00	0.19-5.27	0.999	0.42	0.07-2.49	0.337
≥1 HNA1a	0.64	0.30-1.34	0.235	0.65	0.29-1.49	0.309	0.58	0.26-1.29	0.181	0.59	0.28-1.27	0.176	0.59	0.25-1.39	0.227
≥1 HNA1b	1.34	0.66-2.72	0.414	1.83	0.83-4.04	0.135	1.62	0.75-3.52	0.223	1.39	0.68-2.85	0.365	1.87	0.83-4.20	0.132
≥1 HNA1c	1.57	0.79-3.13	0.202	1.18	0.55-2.51	0.676	1.58	0.75-3.33	0.228	1.49	0.74-3.00	0.264	1.09	0.50-2.36	0.825

Appendix C.5. Associations from univariate and multivariate analyses of ***infant*** FcγR genotypes and allele carriage with HIV-1 acquisition across the total cohort

Genotype/allele	Univariate			Multivariate analysis - Adjusted for the following:											
				Maternal Viral Load			Maternal CD4 count			Maternal sdNVP			Viral Load + sdNVP		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
FcγRIIa															
131HH (Ref)	1			1			1			1			1		
131HR	0.81	0.39-1.67	0.566	0.81	0.36-1.84	0.623	0.84	0.38-1.85	0.664	0.82	0.39-1.69	0.586	0.82	0.36-1.86	0.636
131RR	0.84	0.38-1.87	0.669	0.98	0.40-2.38	0.964	0.87	0.37-2.07	0.757	0.85	0.38-1.88	0.682	0.97	0.40-2.37	0.955
≥1 131H allele	1.03	0.55-1.93	0.928	0.88	0.45-1.76	0.726	1.01	0.52-1.99	0.966	1.03	0.55-1.94	0.927	0.89	0.45-1.78	0.750
≥1 131R allele	0.82	0.42-1.62	0.567	0.87	0.41-1.88	0.730	0.85	0.4-1.79	0.671	0.83	0.42-1.64	0.587	0.88	0.41-1.89	0.735
FcγRIIb															
232II	1			1			1			1			1		
232IT	1.20	0.63-2.28	0.577	1.50	0.74-3.06	0.263	0.87	0.43-1.75	0.691	1.20	0.63-2.28	0.582	1.49	0.73-3.04	0.274
232TT	1.94	0.87-4.29	0.104	2.29	0.95-5.54	**0.066**	1.71	0.73-3.99	0.217	1.92	0.87-4.27	0.108	2.24	0.92-5.45	**0.075**
≥1 232I allele	0.56	0.26-1.18	0.125	0.52	0.23-1.18	0.119	0.55	0.25-1.24	0.151	0.56	0.26-1.18	0.130	0.53	0.23-1.21	0.133
≥1 232T allele	1.40	0.79-2.50	0.245	1.73	0.91-3.28	0.096	1.09	0.59-2.02	0.774	1.40	0.79-2.49	0.251	1.70	0.89-3.25	0.106
FcγRIIIa															
158F/FF/FFF (Ref)	1			1			1			1			1		
158FV/FFV/FVV	0.91	0.49-1.67	0.761	0.82	0.42-1.60	0.558	0.96	0.50-1.85	0.911	0.91	0.50-1.68	0.773	0.84	0.43-1.64	0.604
158VV	0.47	0.17-1.28	0.139	0.23	0.06-0.89	**0.033**	0.63	0.22-1.77	0.377	0.47	0.17-1.29	0.143	0.24	0.06-0.92	**0.037**
≥1 158F allele	2.03	0.79-5.23	0.144	3.82	1.06-13.75	**0.040**	1.57	0.59-4.18	0.369	2.02	0.78-5.22	0.146	3.75	1.04-13.53	**0.043**
≥1 158V allele	0.80	0.45-1.43	0.452	0.67	0.35-1.28	0.225	0.88	0.47-1.65	0.696	0.80	0.45-1.44	0.464	0.69	0.36-1.33	0.264
FcγRIIIIb															
NA1+/NA2-/SH-	0.32	0.12-0.82	**0.018**	0.24	0.08-0.69	**0.008**	0.35	0.13-0.96	**0.042**	0.32	0.12-0.82	**0.018**	0.24	0.08-0.69	**0.008**
NA1-/NA2+/SH-	0.75	0.26-2.18	0.598	0.73	0.23-2.32	0.592	0.82	0.25-2.64	0.740	0.77	0.26-2.26	0.637	0.73	0.23-2.35	0.600
NA1-/NA2-/SH+	1.25	0.32-4.86	0.748	0.86	0.18-4.09	0.852	1.54	0.37-6.47	0.555	1.23	0.32-4.81	0.762	0.85	0.18-4.05	0.839
NA1+/NA2+/SH- (ref)	1			1			1			1			1		
NA1+/NA2-/SH+	1.04	0.42-2.61	0.931	0.86	0.32-2.30	0.760	1.05	0.39-2.84	0.922	1.07	0.43-2.71	0.879	0.88	0.33-2.36	0.797
NA1-/NA2+/SH+	1.63	0.67-3.97	0.286	1.41	0.52-3.84	0.499	1.58	0.61-4.11	0.346	1.68	0.68-4.13	0.260	1.47	0.53-4.02	0.458
NA1+/NA2+/SH+	2.81	0.72-10.94	0.136	1.97	0.44-8.83	0.375	2.87	0.72-11.39	0.134	2.87	0.73-11.19	0.130	1.95	0.44-8.60	0.376
≥1 HNA1a	0.68	0.36-1.27	0.229	0.69	0.34-1.38	0.294	0.66	0.34-1.30	0.232	0.67	0.36-1.26	0.218	0.68	0.34-1.37	0.286
≥1 HNA1b	1.92	1.04-3.52	**0.035**	2.28	1.16-2.48	**0.016**	1.82	0.94-3.49	**0.073**	1.93	1.05-3.54	**0.035**	2.29	1.17-4.49	**0.016**
≥1 HNA1c	2.09	1.16-3.78	**0.014**	1.85	0.97-3.53	**0.061**	2.08	1.11-3.91	**0.023**	2.13	1.18-3.87	**0.012**	1.89	0.99-3.60	**0.054**

Appendix C.6. Associations from univariate and multivariate analyses of ***infant*** FcγR genotypes and allele carriage with HIV-1 acquisition across the group of intrapartum infected infants

Genotype/allele	Univariate			Multivariate analysis - Adjusted for the following:											
				Maternal Viral Load			Maternal CD4 count			Maternal sdNVP			Viral Load + sdNVP		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
FcγRIIa															
131HH (Ref)	1			1			1			1			1		
131HR	0.77	0.28-2.13	0.620	0.77	0.25-2.38	0.644	0.70	0.23-2.12	0.528	0.66	0.23-1.86	0.430	0.65	0.20-2.10	0.474
131RR	0.82	0.27-2.50	0.722	1.05	0.31-3.55	0.942	0.70	0.20-2.40	0.571	0.78	0.25-2.42	0.664	1.04	0.30-3.63	0.949
≥1 131H allele	1.03	0.42-2.52	0.949	0.80	0.30-2.09	0.645	1.11	0.42-2.96	0.829	0.97	0.39-2.40	0.942	0.72	0.27-1.93	0.513
≥1 131R allele	0.79	0.31-2.03	0.624	0.86	0.30-2.47	0.783	0.70	0.25-1.98	0.501	0.70	0.27-1.84	0.471	0.78	0.27-2.30	0.654
FcγRIIb															
232II	1			1			1			1			1		
232IT	1.42	0.58-3.47	0.440	1.99	0.73-5.39	0.177	0.88	0.33-2.36	0.795	1.48	0.60-3.67	0.397	2.18	0.78-6.07	0.135
232TT	1.67	0.52-5.31	0.387	2.26	0.64-8.04	0.207	1.31	0.37-4.62	0.678	1.91	0.58-6.25	0.285	2.63	0.70-9.92	0.153
≥1 232I allele	0.70	0.24-2.01	0.516	0.61	0.19-1.93	0.400	0.73	0.22-2.42	0.602	0.62	0.21-1.88	0.400	0.55	0.16-1.83	0.329
≥1 232T allele	1.49	0.667-3.37	0.338	2.07	0.82-5.19	0.123	0.99	0.41-2.40	0.983	1.60	0.69-3.66	0.273	2.30	0.89-5.94	**0.085**
FcγRIIIa															
158F/FF/FFF (Ref)	1			1			1			1			1		
158FV/FFV/FVV	1.06	0.43-2.56	0.906	1.01	0.40-2.58	0.979	1.05	0.40-2.76	0.923	0.96	0.39-2.38	0.932	0.80	0.30-2.12	0.648
158VV	0.90	0.26-3.18	0.876	0.22	0.03-1.90	0.169	1.31	0.35-4.88	0.690	0.88	0.24-3.14	0.839	0.19	0.02-1.67	0.133
≥1 158F allele	1.14	0.36-3.60	0.822	4.57	0.57-36.87	0.153	0.79	0.24-2.61	0.694	1.12	0.35-3.58	0.854	4.67	0.57-38.47	0.152
≥1 158V allele	1.02	0.44-2.37	0.968	0.81	0.33-2.03	0.660	1.11	0.44-2.75	0.828	0.94	0.40-2.22	0.889	0.64	0.25-1.68	0.368
FcγRIIIIb															
NA1+/NA2-/SH-	0.18	0.04-0.87	**0.032**	0.14	0.03-0.72	**0.019**	0.22	0.05-1.06	**0.059**	0.17	0.04-0.83	**0.029**	0.13	0.02-0.69	**0.017**
NA1-/NA2+/SH-	0.25	0.03-2.09	0.200	0.22	0.02-2.04	0.181		-		0.21	0.02-1.80	0.155	0.19	0.02-1.90	0.157
NA1-/NA2-/SH+		-			-			-			-			-	
NA1+/NA2+/SH- (ref)	1			1			1			1			1		
NA1+/NA2-/SH+	1.25	0.41-3.84	0.697	0.93	0.27-3.19	0.909	1.12	0.33-3.83	0.858	1.04	0.33-3.28	0.950	0.77	0.21-2.80	0.688
NA1-/NA2+/SH+	1.75	0.58-5.26	0.319	1.43	0.40-5.15	0.581	1.56	0.48-5.06	0.456	1.50	0.49-4.64	0.479	1.23	0.33-4.55	0.758
NA1+/NA2+/SH+		-			-			-			-			-	
≥1 HNA1a	0.83	0.34-2.05	0.692	0.90	0.33-2.43	0.831	0.97	0.35-2.69	0.954	0.92	0.37-2.29	0.852	0.92	0.33-2.53	0.869
≥1 HNA1b	2.09	0.85-4.97	0.109	2.37	0.91-6.2	**0.078**	1.94	0.75-5.07	0.174	2.12	0.87-5.20	**0.099**	2.49	0.93-6.65	**0.068**
≥1 HNA1c	2.04	0.89-4.64	**0.091**	1.78	0.72-4.38	0.209	1.80	0.73-4.43	0.200	1.82	0.79-4.22	0.159	1.59	0.63-4.03	0.325

Appendix C.7. Associations from univariate and multivariate analyses of ***infant*** FcγR genotypes and allele carriage with HIV-1 acquisition across the group of *in utero* infected infants

	Univariate			Multivariate analysis - Adjusted for the following:											
				Maternal Viral Load			Maternal CD4 count			Maternal sdNVP			Viral Load + sdNVP		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
FcγRIIa															
131HH (Ref)	1			1			1			1			1		
131HR	0.63	0.16-2.37	0.490	0.66	0.13-3.19	0.602	0.77	0.13-4.52	0.773	0.61	0.16-2.35	0.477	0.66	0.13-3.20	0.602
131RR	1.43	0.39-5.18	0.587	2.09	0.45-9.60	0.344	2.61	0.51-13.48	0.252	1.43	0.39-5.17	0.589	2.10	0.45-9.73	0.345
≥1 131H allele	0.51	0.19-1.39	0.192	0.36	0.12-1.12	**0.077**	0.32	0.10-0.99	**0.048**	0.51	0.19-1.39	0.189	0.36	0.11-1.13	**0.079**
≥1 131R allele	0.92	0.28-3.00	0.892	1.13	0.28-4.64	0.862	1.45	0.30-6.96	0.644	0.92	0.28-3.01	0.888	1.13	0.27-4.61	0.870
FcγRIIb															
232II	1			1			1			1			1		
232IT	1.11	0.33-3.69	0.867	1.07	0.28-4.16	0.921	0.69	0.16-2.96	0.620	1.12	0.34-3.75	0.851	1.07	0.28-4.17	0.920
232TT	3.42	1.03-11.39	**0.044**	3.49	0.87-14.02	**0.078**	3.98	1.02-15.51	**0.046**	3.59	1.05-12.25	**0.041**	3.58	0.86-14.86	**0.078**
≥1 232I allele	0.30	0.10-0.91	**0.033**	0.29	0.08-1.07	**0.063**	0.22	0.06-0.79	**0.020**	0.29	0.10-0.89	**0.031**	0.29	0.08-1.08	**0.065**
≥1 232T allele	1.76	0.64-4.79	0.271	1.74	0.57-5.33	0.332	1.44	0.47-4.43	0.520	1.77	0.65-4.87	0.266	1.73	0.56-5.33	0.339
FcγRIIIa															
158F/FF/FFF (Ref)	1			1			1			1			1		
158FV/FFV/FVV	0.08	0.27-2.14	0.595	0.62	0.18-2.05	0.430	0.81	0.24-2.68	0.727	0.75	0.26-2.13	0.592	0.62	0.18-2.12	0.443
158VV	0.57	0.11-2.87	0.491	0.69	0.12-3.87	0.670	0.86	0.16-4.69	0.863	0.56	0.11-2.87	0.487	0.69	0.12-3.93	0.674
≥1 158F allele	1.52	0.33-7.07	0.593	1.12	0.22-5.67	0.888	1.04	0.21-5.06	0.963	1.53	0.33-7.11	0.590	1.12	0.22-5.65	0.893
≥1 158V allele	0.71	0.26-1.90	0.492	0.63	0.20-1.95	0.425	0.82	0.27-2.52	0.730	0.70	0.26-1.90	0.488	0.63	0.20-2.01	0.439
FcγRIIIIb															
NA1+/NA2-/SH-	0.44	0.08-2.39	0.341	0.32	0.05-2.11	0.237	0.42	0.04-4.23	0.459	0.44	0.08-2.42	0.349	0.32	0.05-2.12	0.240
NA1-/NA2+/SH-	0.60	0.06-5.55	0.653	0.74	0.07-8.44	0.812	1.24	0.12-13.38	0.857	0.63	0.07-5.95	0.683	0.80	0.07-9.24	0.856
NA1-/NA2-/SH+	1.50	0.15-15.11	0.731	1.03	0.09-12.23	0.983	2.36	0.20-27.85	0.496	1.53	0.15-15.49	0.719	1.03	0.09-12.32	0.980
NA1+/NA2+/SH- (ref)	1			1			1			1			1		
NA1+/NA2-/SH+	1.00	0.18-5.63	1	0.99	0.15-6.48	0.989	1.60	0.24-10.73	0.626	1.03	0.18-5.86	0.977	1.01	0.15-6.61	0.994
NA1-/NA2+/SH+	3.00	0.76-11.81	0.116	4.40	0.78-24.90	0.094	4.41	0.84-23.01	**0.079**	3.10	0.77-12.51	0.112	4.45	0.78-25.35	**0.093**
NA1+/NA2+/SH+	4.50	0.65-31.08	0.127	1.97	0.20-19.10	0.560	6.26	0.79-49.79	**0.083**	4.69	0.66-33.47	0.123	2.23	0.21-23.86	0.507
≥1 HNA1a	0.52	0.19-1.45	0.214	0.41	0.12-1.36	0.146	0.40	0.13-1.27	0.121	0.52	0.18-1.46	0.212	0.41	0.12-1.36	0.146
≥1 HNA1b	2.14	0.72-6.31	0.169	2.65	0.81-8.71	0.109	2.05	0.60-6.94	0.251	2.14	0.72-6.31	0.169	2.68	0.81-8.85	0.106
≥1 HNA1c	2.94	1.08-7.95	**0.034**	2.88	0.93-8.94	**0.066**	3.93	1.22-12.61	**0.021**	2.99	1.09-8.18	**0.033**	2.92	0.94-9.10	**0.064**

Appendix C.8. Associations from univariate and multivariate analyses of ***infant*** FcγR genotypes and allele carriage with HIV-1 acquisition across the group of *in utero* enriched infected infants

	Univariate			Multivariate analysis - Adjusted for the following:											
				Maternal Viral Load			Maternal CD4 count			Maternal sdNVP			Viral Load + sdNVP		
	OR	95% CI	P	OR	95% CI	P	4.21	95% CI	P	OR	95% CI	P	OR	95% CI	P
FcγRIIa															
131HH (Ref)	1			1			1			1			1		
131HR	0.83	0.35-1.99	0.681	0.83	0.31-2.21	0.706	0.92	0.35-2.38	0.856	0.92	0.37-2.19	0.819	0.82	0.30-2.23	0.698
131RR	0.86	0.33-2.24	0.753	0.96	0.33-2.80	0.944	1.00	0.36-2.82	0.996	0.90	0.33-2.35	0.806	0.88	0.30-2.61	0.815
≥1 131H allele	1.03	0.48-2.20	0.940	0.91	0.40-2.05	0.817	0.94	0.43-2.067	0.872	1.05	0.49-2.27	0.893	0.99	0.43-2.29	0.983
≥1 131R allele	0.84	0.37-1.90	0.680	0.88	0.35-2.21	0.779	0.95	0.39-2.32	0.907	0.91	0.39-2.09	0.828	0.84	0.33-2.15	0.719
FcγRIIb															
232II	1			1			1			1			1		
232IT	1.06	0.48-2.34	0.883	1.21	0.51-2.87	0.660	0.86	0.37-2.01	0.726	1.00	0.45-2.24	0.991	1.13	0.47-2.74	0.783
232TT	2.11	0.84-5.26	0.111	2.29	0.83-6.32	0.109	2.09	0.79-5.54	0.138	1.90	0.75-4.80	0.174	1.87	0.66-5.32	0.238
≥1 232I allele	0.49	0.21-1.15	0.100	0.47	0.18-1.22	0.120	0.45	0.18-1.14	**0.091**	0.53	0.22-1.25	0.148	0.56	0.21-1.49	0.245
≥1 232T allele	1.35	0.68-2.70	0.391	1.52	0.71-3.24	0.280	1.18	0.57-2.44	0.658	1.26	0.63-2.54	0.514	1.35	0.62-2.95	0.447
FcγRIIIa															
158F/FF/FFF (Ref)	1			1			1			1			1		
158FV/FFV/FVV	0.83	0.41-1.71	0.618	0.69	0.31-1.53	0.364	0.91	0.43-1.95	0.810	0.86	0.41-1.77	0.675	0.82	0.36-1.85	0.635
158VV	0.24	0.05-1.11	**0.067**	0.26	0.21-1.24	**0.090**	0.30	0.06-1.42	0.128	0.26	0.06-1.22	**0.087**	0.32	0.06-1.56	0.158
≥1 158F allele	3.80	0.86-16.84	**0.079**	3.20	0.70-14.62	0.133	3.20	0.71-14.41	0.131	3.55	0.79-15.90	**0.097**	2.84	0.61-13.27	0.183
≥1 158V allele	0.68	0.34-1.37	0.285	0.59	0.27-1.26	0.170	0.76	0.36-1.59	0.464	0.71	0.35-1.44	0.344	0.70	0.32-1.54	0.381
FcγRIIIIb															
NA1+/NA2-/SH-	0.46	0.15-1.41	0.173	0.32	0.09-1.13	0.076	0.49	0.14-1.73	0.270	0.47	0.15-1.49	0.201	0.34	0.09-1.25	0.105
NA1-/NA2+/SH-	1.25	0.38-4.13	0.715	1.25	0.35-4.47	0.734	1.71	0.49-6.01	0.402	1.64	0.48-5.64	0.435	1.53	0.40-5.79	0.531
NA1-/NA2-/SH+	2.50	0.61-10.31	0.205	1.70	0.34-8.43	0.518	3.28	0.73-14.67	0.121	2.62	0.62-11.15	0.193	1.89	0.369.83	0.448
NA1+/NA2+/SH- (ref)	1			1			1			1			1		
NA1+/NA2-/SH+	0.83	0.24-2.93	0.776	0.70	0.19-2.58	0.589	0.97	0.26-3.62	0.961	0.97	0.27-3.48	0.957	0.75	0.20-2.85	0.669
NA1-/NA2+/SH+	1.50	0.48-4.69	0.486	1.43	0.40-5.11	0.577	1.59	0.46-5.53	0.468	1.95	0.60-6.35	0.270	1.70	0.45-6.49	0.437
NA1+/NA2+/SH+	5.63	1.36-23.19	**0.017**	3.94	0.85-18.34	**0.081**	6.09	1.43-25.93	**0.014**	7.29	1.65-32.14	**0.009**	4.70	1.01-21.75	**0.048**
≥1 HNA1a	0.60	0.29-1.25	0.173	0.57	0.25-1.28	0.173	0.53	0.24-1.17	0.115	0.52	0.24-1.11	**0.092**	0.52	0.22-1.21	0.129
≥1 HNA1b	1.84	0.88-3.82	0.104	2.42	1.08-5.46	**0.033**	1.73	0.79-3.75	0.167	1.90	0.91-4.00	**0.089**	2.45	1.07-5.62	**0.035**
≥1 HNA1c	2.14	1.06-4.31	**0.034**	1.92	0.90-4.12	**0.093**	2.24	1.06-4.71	**0.034**	2.38	1.15-4.9	**0.019**	2.05	0.93-4.50	**0.073**