UNIVERSITÉ DE YAOUNDÉ I

CENTRE DE RECHERCHE ET DE FORMATIO DOCTORALE EN SCIENCE DE LA VIE, SANTE ET ENVIRONNEMENT

UNITE DE RECHERCHE ET DE FORMATIONS DOCTORALE EN SCIENCES DE LA VIE

DEPARTEMENT DE BIOCHIMIE

THE UNIVERSITY OF YAOUNDE I

CENTRE FOR RESEARCH AND TRAINING IN GRADUATE STUDIES IN LIFE, HEALTH AND ENVIRONMENTAL SCIENCES

RESEARCH AND DOCTORATE TRAINING UNIT IN LIFE SCIENCES

DEPARTMENT OF BIOCHEMISTRY

DEPARTEMENT DE BIOCHIMIE
DEPARTMENT OF BIOCHEMISTRY

LABORATOIRE DE BIOLOGIE MOLECULAIRE - CIRCB
MOLECULAR BIOLOGY LABORATORY - CIRCB

Molecular Characterization of Killer Cell Immunoglobulin-like Receptors (KIRs) and Human Leucocyte Antigen (HLA) Class I Genes in Human Viral Types-Infected Individuals in Cameroon

THESIS

Submitted in partial fulfilment of the requirements for the award of the

Doctorate/Ph.D. in Biochemistry

Specialty: Immunogenetics/Infectious Diseases

By:

Yengo Clauvis Kunkeng

Matricule: 10R0800

Masters in Biochemistry

Supervisors:

Pr. Judith Ndongo Torimiro	**Pr. Barbara Atogho Tiedeu**	**Dr. Louis Marie Yindom**
Professor, UY1	***Associate Professor, UY1***	***Research Scientist, Oxon***

Academic Year: 2022 – 2023

REPUBLIQUE DU CAMEROUN
UNIVERSITE DE YAOUNDE I

FACULTE DES SCIENCES

DEPARTEMENT DE BIOCHIMIE

BP: 812 YAOUNDE

REPUBLIC OF CAMEROON
UNIVERSITY OF YAOUNDE I

FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY

PO BOX : 812 YAOUNDE

ATTESTATION DE CORRECTION

Nous, soussignés Pr. MBACHAM Wilfred FON, Pr. DJUIJE NGONOUE Marceline (MC), Dr. NCHINDA Godwin (Directeur de Recherche) et Pr. MOUNDIPA FEWOU respectivement Examinateurs et Président du jury de Thèse de Doctorat/Ph.D. en Biochimie option « Immunogenetics/Infectious Diseases », soutenue par Monsieur YENGO Clauvis KUNKENG (Matricule 10R0800) le 21 Décembre 2022 à 09 heures dans la Salle de conférence de l'annexe de la Faculté des Sciences de l'Université de Yaoundé I, sous le thème : " **Molecular Characterisation of Killer Cell Immunoglobulin-like Receptors (KIRs) and Human Leucocte Antigens (HLA) Class I Genes in Human Viral Types-infected Individuals in Cameroon**", attestons que le candidat a effectué les corrections conformément aux exigences du jury.

En foi de quoi, la présente attestation lui est établie pour servir et valoir ce que de droit.

Fait à Yaoundé, le 25 JAN 2023

Examinateurs

Marceline Djuidje-Ngounoue, Ph.D.
Associate Professor, University of Yaoundé I

Prof. Mbacham Wilfred Fon
MS, DS, ScD(Harvard), FASI, FCAS, FAA

Président du jury

Paul F. MOUNDIPA
Professor
Enzymology & Toxicology

UNIVERSITE DE YAOUNDE I
UNIVERSITY OF YAOUNDE I
Département de Biochimie
Department of Biochemistry
Le Chef du Département
The Head of Department

24-01-2023

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THE UNIVERSITY OF YAOUNDE I

Faculty of Science

Division of Programming and Follow-up of Academic Affaires

LISTE DES ENSEIGNANTS PERMANENTS **LIST OF PERMANENT TEACHING STAFF**

(Par Département et par Grade)

DATE D'ACTUALISATION Année 2021-2022

ADMINISTRATION

DOYEN : TCHOUANKEU Jean- Claude, *Maître de Conférences*

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2	FEKAM BOYOM Fabrice	Professeur	En poste
3	FOKOU Elie	Professeur	En poste
4	KANSCI Germain	Professeur	En poste
5	MBACHAM FON Wilfried	Professeur	En poste
6	**MOUNDIPA FEWOU Paul**	**Professeur**	**Chef de Département**
7	NGUEFACK Julienne	Professeur	En poste
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39	FOUPOUAPOUOGNIGNI Y	Assistant	En poste
40	MBOUCHE FANMOE Marceline Joëlle	Assistante	En poste

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7	FOMENA Abraham	Professeur	En Poste
8	KAMTCHOUING Pierre	Professeur	En poste
9	KEKEUNOU Sévilor	Professeur	En poste
10	NJAMEN Dieudonné	Professeur	En poste
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13	TAN Paul VERNYUY	Professeur	En poste
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27	DONFACK Mireille	Chargée de Cours	En poste
28	ETEME ENAMA Serge	Chargé de Cours	En poste
29	GOUNOUE KAMKUMO Raceline	Chargée de Cours	En poste

30	KANDEDA KAVAYE Antoine	Chargé de Cours	En poste
31	MAHOB Raymond Joseph	Chargé de Cours	En poste
32	MBENOUN MASSE Paul Serge	Chargé de Cours	En poste
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38	NOAH EWOTI Olive Vivien	Chargée de Cours	En poste
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40	TAMSA ARFAO Antoine	Chargé de Cours	En poste
41	YEDE	Chargé de Cours	En poste
42	BASSOCK BAYIHA Etienne Didier	Assistant	En poste
43	ESSAMA MBIDA Désirée Sandrine	Assistante	En poste
44	KOGA MANG DOBARA	Assistant	En poste
45	LEME BANOCK Lucie	Assistante	En poste
46	YOUNOUSSA LAME	Assistant	En poste

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3	DJOCGOUE Pierre François	Professeur	En poste
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5	MOSSEBO Dominique Claude	Professeur	En poste
6	YOUMBI Emmanuel	Professeur	Chef de Département
7	ZAPFACK Louis	Professeur	En poste

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22	NGALLE Hermine BILLE	Chargée de Cours	En poste
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26	ONANA JEAN MICHEL	Chargé de Cours	En poste
27	GODSWILL NTSOMBAH NTSEFONG	Assistant	En poste
28	KABELONG BANAHO Louis-Paul-Roger	Assistant	En poste
29	KONO Léon Dieudonné	Assistant	En poste

30	LIBALAH Moses BAKONCK	Assistant	En poste
31	LIKENG-LI-NGUE Benoit C	Assistant	En poste
32	TAEDOUNG Evariste Hermann	Assistant	En poste
33	TEMEGNE NONO Carine	Assistant	En poste

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26	MBEY Jean Aime	Chargé de Cours	En poste
27	NCHIMI NONO KATIA	Chargé de Cours	En poste
28	NEBA nee NDOSIRI Bridget NDOYE	Chargée de Cours	CT/ MINFEM
29	NYAMEN Linda Dyorisse	Chargée de Cours	En poste
30	PABOUDAM GBAMBIE A.	Chargée de Cours	En poste
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32	PATOUOSSA ISSOFA	Assistant	En poste
33	SIEWE Jean Mermoz	Assistant	En Poste

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18	NGO MBING Joséphine	Maître de Conférences	Sous/Direct. MINERESI
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25	TIH née NGO BILONG E. Anastasie	Maître de Conférences	En poste
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28	KAMTO Eutrophe Le Doux	Chargé de Cours	En poste
29	NGNINTEDO Dominique	Chargé de Cours	En poste
30	NGOMO Orléans	Chargée de Cours	En poste
31	OUAHOUO WACHE Blandine M.	Chargée de Cours	En poste
32	SIELINOU TEDJON Valérie	Chargé de Cours	En poste

33	MESSI Angélique Nicolas	Assistant	En poste
34	TSEMEUGNE Joseph	Assistant	En poste

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14	TAPAMO Hyppolite	Chargé de Cours	En poste
15	TINDO Gilbert	Chargé de Cours	En poste
16	TSOPZE Norbert	Chargé de Cours	En poste
17	WAKU KOUAMOU Jules	Chargé de Cours	En poste

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19	EKODECK Stéphane Gaël Raymond	Assistant	En poste
20	HAMZA Adamou	Assistant	En poste
21	JIOMEKONG AZANZI Fidel	Assistant	En poste
22	MAKEMBE. S . Oswald	Assistant	En poste
23	MESSI NGUELE Thomas	Assistant	En poste
24	MEYEMDOU Nadège Sylvianne	Assistante	En poste
25	NKONDOCK. MI. BAHANACK.N.	Assistant	En poste

7- DÉPARTEMENT DE MATHÉMATIQUES (MA) (30)

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4	MBANG Joseph	Maître de Conférences	En poste
5	MBEHOU Mohamed	Maître de Conférences	En poste
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8	NOUNDJEU Pierre	Maître de Conférences	*Chef service des programmes & Diplômes/FS/UYI*
9	TCHAPNDA NJABO Sophonie B.	Maître de Conférences	Directeur/AIMS Rwanda
10	TCHOUNDJA Edgar Landry	Maître de Conférences	En poste

11	AGHOUKENG JIOFACK Jean Gérard	Chargé de Cours	Chef Cellule MINPLAMAT
12	CHENDJOU Gilbert	Chargé de Cours	En poste
13	DJIADEU NGAHA Michel	Chargé de Cours	En poste
14	DOUANLA YONTA Herman	Chargé de Cours	En poste
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16	KIKI Maxime Armand	Chargé de Cours	En poste
17	MBAKOP Guy Merlin	Chargé de Cours	En poste
18	MENGUE MENGUE David Joe	Chargé de Cours	En poste
19	NGUEFACK Bernard	Chargé de Cours	En poste
20	NIMPA PEFOUKEU Romain	Chargée de Cours	En poste
21	POLA DOUNDOU Emmanuel	Chargé de Cours	En poste
22	TAKAM SOH Patrice	Chargé de Cours	En poste
23	TCHANGANG Roger Duclos	Chargé de Cours	En poste
24	TETSADJIO TCHILEPECK M. E.	Chargé de Cours	En poste
25	TIAYA TSAGUE N. Anne-Marie	Chargée de Cours	En poste
26	BITYE MVONDO Esther Claudine	Assistante	En poste
27	MBATAKOU Salomon Joseph	Assistant	En poste

28	MBIAKOP Hilaire George	Assistant	En poste
29	MEFENZA NOUNTU Thiery	Assistant	En poste
30	TCHEUTIA Daniel Duviol	Assistant	En poste

8- DÉPARTEMENT DE MICROBIOLOGIE (MIB) (18)

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5	BOYOMO ONANA	Maître de Conférences	En poste
6	RIWOM Sara Honorine	Maître de Conférences	En poste
7	SADO KAMDEM Sylvain Leroy	Maître de Conférences	En poste

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9	BOUGNOM Blaise Pascal	Chargé de Cours	En poste
10	ESSONO OBOUGOU Germain G.	Chargé de Cours	En poste
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12	TCHIKOUA Roger	Chargé de Cours	En poste
13	TOBOLBAÏ Richard	Chargé de cours	En poste
13	ESSONO Damien Marie	Assistant	En poste
14	LAMYE Glory MOH	Assistant	En poste
15	MEYIN A EBONG Solange	Assistante	En poste
16	NKOUDOU ZE Nardis	Assistant	En poste
17	SAKE NGANE Carole Stéphanie	Assistante	En poste

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4	ESSIMBI ZOBO Bernard	Professeur	En poste
5	KOFANE Timoléon Crépin	Professeur	En poste
6	NANA ENGO Serge Guy	Professeur	En poste
7	NANA NBENDJO Blaise	Professeur	En poste
8	NDJAKA Jean Marie Bienvenu	Professeur	Chef de Département
9	NJANDJOCK NOUCK Philippe	Professeur	En poste
10	NOUAYOU Robert	Professeur	En poste
11	PEMHA Elkana	Professeur	En poste
12	TABOD Charles TABOD	Professeur	Doyen FS Univ/Bda
13	TCHAWOUA Clément	Professeur	En poste
14	WOAFO Paul	Professeur	En poste
15	ZEKENG Serge Sylvain	Professeur	En poste

16	BIYA MOTTO Frédéric	Maître de Conférences	DG/HYDRO Mekin
17	BODO Bertrand	Maître de Conférences	En poste
18	ENYEGUE A NYAM épse BELINGA	Maître de Conférences	En poste
19	EYEBE FOUDA Jean sire	Maître de Conférences	En poste
20	FEWO Serge Ibraïd	Maître de Conférences	En poste
21	HONA Jacques	Maître de Conférences	En poste

22	MBANE BIOUELE César	Maître de Conférences	En poste
23	MBINACK Clément	Maître de Conférences	En poste
24	NDOP Joseph	Maître de Conférences	En poste
25	SAIDOU	Maître de Conférences	MINRESI
26	SIEWE SIEWE Martin	Maître de Conférences	En poste
27	SIMO Elie	Maître de Conférences	En poste
28	VONDOU Derbetini Appolinaire	Maître de Conférences	En poste
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DECLARATION

This thesis is my original work and has not been presented for any degree or other awards in any other university

Signature …… Date……..

Yengo Clauvis Kunkeng

DEDICATION

To God

and

To my family

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ABBREVIATIONS AND ACRONYMS

3TC	Lamivudine
Ab	Antibodies
ABC	: Abacavir
ADCC	: Antibody-Dependent Cellular Cytotoxicity
AF	: Allele Frequency
AIDS	: Acquired Immune Deficiency Syndrome
ALP	: Alkaline Phosphatase
ALT	: Alanine Aminotransferase
Anti-HBc	: Antibodies to Hepatitis B core antigen
APC	: Antigen Presenting Cell
ART	: Antiretroviral Therapy
ARV	: Antiretroviral
AST	: Aspartate Aminotransferase
ATV/r	: Boosted Atazanavir
AuAg	: Australian Antigen
AZT	: Azidothymidine
BCR	: B-Cell Receptor
BDT	: Big Dye Terminator
bp	: Base Pair
CAMPHIA	: Cameroon Population-based HIV Impact Assessment
cART	: Combination of Antiretroviral Therapy
cccDNA	: Covalently Closed Circular DNA
CCR	: C-C Chemokine Receptor
CCR5	: C-C Chemokine Receptor type 5
CD	: Cluster of Differentiation
CD4+	: Cluster of Differentiation 4
CDC	: Centres for Disease Control and Prevention

CHB : Chronic Hepatitis B

CIRCB : Chantal Biya International Reference Centre for Research on the Prevention and Management of HIV/AIDS

CLIP : Class II associated Invariant chain Peptide

CNV : Copy Number Variation

CRFs : Circulating Recombinant Forms

CSW : Commercial Sex Workers

CTL : Cytotoxic T Lymphocyte

CXCR4 : C-X-C Motif Chemokine Receptor 4

d4T : Stavudine

DAA : Direct Acting Antivirals

DC : Dendritic Cell

ddI : Didanosine

DLV : Delavirdine

DMA : Gene encoding the alpha chain of DM

DMB : Gene encoding the alpha chain of DM

DNA : Deoxyribonucleic Acid

dNTP : Deoxynucleoside Triphosphate

DO : HLA class II isoform O

DOA : Gene encoding the alpha chain of DO

DOB : Gene encoding the beta chain of DO

DP : HLA class II isoform P

DPA : Gene encoding the alpha chain of DP

DPA1 : Gene encoding the alpha chain number 1 of DP

DPA2 : Gene encoding the alpha chain number 2 of DP

DPB : Gene encoding the beta chain of DO

DPB1 : Gene encoding the beta chain number 1 of DP

DPB2 : Gene encoding the beta chain number 2 of DP

DQ : HLA class II isoform Q

DQA : Gene encoding the alpha chain of DQ

DQA1 : Gene encoding the alpha chain number 1 of DQ

DQA2 : Gene encoding the alpha chain number 2 of DQ

DQB" : Gene encoding the beta chain of DQ

DQB1 : Gene encoding the beta chain number 1 of DQ

DR : HLA class II isoform R

DRA : Gene encoding the alpha chain of DR

DRA1 : Gene encoding the alpha chain number 1 of DR

DRA2 : Gene encoding the alpha chain number 2 of DR

DRB1 : Gene encoding the beta chain number 1 of DB

DRiPs : Defective Ribosomal Products

DSV : Daclastasvir

DTG : Dolutegravir

EBR : Elbasvir

EDTA : Ethylene Diamine Tetra-acetate/acetic Acid

EFV : Efavirenz

EIs : Entry Inhibitors

ELISA : Enzyme-Linked Immunosorbent Assay

Env : HIV envelope protein

ER : Endoplasmic Reticulum

ESP : Exposed Seronegative Persons

ETR : Etravirine

ETV : Entecavir

EU : Exposed Uninfected

EVG : Elvitegravir

FDA : Food and Drug Administration

FTC : Emtricitabine

GF : Genotype Frequency

GLE : Glecaprevir

GRZ : Grazoprevir

HAART : Highly Active Antiretroviral Therapy

HBeAg : Hepatitis B envelope antigen

HBsAg :Hepatitis B surface antigen

HBV : Hepatitis B Virus

HCC : Hepatocellular Carcinoma

HCV : Hepatitis C Virus

HEPS : Highly Exposed Persistently Seronegative

HESN : Highly Exposed Seronegative

HIV : Human Immunodeficiency Virus

HIV-1 : Human Immunodeficiency Virus type 1

HIV-2 : Human Immunodeficiency Virus type 2

HLA : Human Leukocyte Antigen

HWE : Hardy-Weinberg Equilibrium

IDV : Indinavir

IFN- γ : Interferon gamma

Ig : Immunoglobulin

IgE : Immunoglobulin E

IgG : Immunoglobulin G

IgM : Immunoglobulin M

IL : Interleukin

ILT : Immunoglobulin-like Transcript

IN : Integrase

INI : Integrase Inhibitors

IQR : Interquartile Range

IRES : Internal Ribosomal Entry Site

ITAMs : Immunoreceptor Tyrosine-based Activating Motifs

ITIMs ` : Immunoreceptor Tyrosine-based Inhibitory Motifs

IVDU : Intravenous Drug Use

kb : Kilobase

kDa : Kilo Dalton

KIR : Killer cell Immunoglobulin-like Receptor

KIR2DS4-f : Full length functional KIR2DS4 alleles

KIR2DS4-v : Truncated non-functional KIR2DS4 alleles

KLR : Killer cell Lectin-like Receptors

LAIR : Leucocyte-Associated Inhibitory Receptors

LCR : Leucocyte Receptor Complex

LD : Linkage Disequilibrium

LDV : Ledispavir

LILR : Leukocyte Immunoglobulin-like Receptors

LILR : Leucocyte Immunoglobulin-Like Receptor

LPS : Lipopolysaccharide

LRC : Leucocyte Receptor Complex

LTNP : Long-Term Non-Progressor

LTR : Long Terminal Repeats

$MgCl_2$: Magnesium chloride

MHC : Major Histocompatibility Complex

MM : Master Mix

MTCT : Mother-To-Child Transmission

MTCT : Mother-To-Child-Transmission

NAs : Nucleotide Analogues

NCBI : National Centre for Biotechnology Information

NCR : Natural Cytotoxicity Receptors

NCR : Natural Cytotoxic Receptor

NK cell : Natural Killer cell

NKC : Natural Killer Complex

NNRTI : Non-nucleoside Reverse Transcriptase Inhibitors

NRTI : Nucleoside Reverse Transcriptase Inhibitors

NVP : Nevirapine

OBI : Occult HBV Infection

OBV : Ombitasvir

ORFs : Open Reading Frames

PBMC : Peripheral Blood Mononuclear Cells

PCR : Polymerase Chain Reaction

PEG-IFN : Pegylated Interferon

pgRNA : Pregenomic RNA

PIB : Pibrenstasvir

PIs : Protease Inhibitors

PMTCT : Prevention of Mother To Child Transmission

PreP : Pre-exposure Prophylaxis

PTV : Paritaprevir

RAL : Raltegravir

rcDNA : Recombinant DNA

Reg : HIV Regulatory protein

Rpm : Revolutions per minute

RPV : Rilpivirine

RT : Reverse Transcriptase

SBT : Sequence-Based Typing

sgRNA : Subgenomic RNA

SIV : Simian Immunodeficiency Virus

SIVcpz : Simian Immunodeficiency Virus of Chimpanzees

SIVgor : Simian Immunodeficiency Virus of Gorillas

SIVsmm : Simian Immunodeficiency Virus of Sooty Mangabeys

SLTs : Secondary Lymphoid Tissues

SMV : Simeprevir

SNP : Single Nucleotide Polymorphism

SOPs : Standard Operating Procedures

ssDNA : Single-Stranded Deoxyribonucleic Acid

SSP : Sequence Specific Primers

ssRNA : Single-Stranded Ribonucleic Acid

STIs : Sexually Transmitted Infections

TAF : Tenofovir Alafenamide

TAP : Transport protein associated with Antigen Processing

TCR : T Cell Receptor

TDF : Tenofovir Disoproxil Fumarate

TDR : Transmitted Drug Resistance

TLR : Toll Like Receptor

TM : Transmembrane region

TNF-α : Tumour Necrosis Factor alpha

TR : HIV-1 transmitting

TRAIL : TNF-Related Apoptosis-Inducing Ligand

UNAIDS : Joint United Nations Programme on HIV/AIDS

uNK : Uterine Natural Killer cell

URFs : Unique Recombinant Forms

UTR : Untranslated Region

VEL : Velpatasvir

VOX : Voxilaprevir

ZDV : Zidovudine

β2m : Beta-2 microglobulin

ABSTRACT

Killer cell Immunoglobulin-like Receptors (KIRs) interact with their cognate Human Leucocyte Antigen (HLA) ligands to modify natural killer and T-cell function in viral acquisition and disease progression, thereby determining the immunity and susceptibility of humans to viral infections and disease. In this thesis, we sought to determine the variation of KIR and HLA Class I (HLA-A and -C) genes in individuals infected with Human Immunodeficiency Virus type 1 (HIV-1), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV). After obtaining ethical approval from the Cameroon National Ethics Committee for Human Health Research to carry out this study, a total of 253 (84 HIV-1, 68 HCV, 38 HBV and 63 uninfected) -unrelated treatment-naïve adult participants were recruited. HLA-A and -C typing was done by Sequence-Based Techniques (SBT). KIR typing was carried out by Polymerase Chain Reaction–Sequence Specific Primer (PCR-SSP) techniques. Data analyses were done with SPSS version 25, STATA version 14 and Microsoft excel 2016. All the 15 KIR genes typed were present. We report high heterogeneity in allele and genotype frequencies in the study population, with 57 KIR genotypes, including 5 novel ones. Compared to uninfected healthy controls, the frequencies of *KIR2DL2* and *KIR3DS1* were significantly lower in the HBV+ group (58.1% vs 91.2% and 12.9% vs 64.7, $p = 0.003$ and $p < 0.001$, respectively). Conversely, *KIR3DS1* was significantly overrepresented in the HCV+ and HIV-1+ groups compared to controls (97.0% vs 64.7%, $p < 0.001$; 64.7% vs 20.2%, $p < 0.001$, respectively). Individuals carrying HLA-A*30:01 were six times more likely to be infected with hepatitis viruses than those without this allele (OR = 6.30, $p = 0.020$ (HBV); OR = 6.21, $p = 0.010$ (HCV)). Similarly, carriers of HLA-C*17:01 were overrepresented in the HBV-infected participants compared to the uninfected control group (21.9% vs. 6.4%, respectively), suggesting that this allele may play a role in the susceptibility to HBV infection. The compound genotypes *KIR2DL2/KIR2DL3/KIR2DS2* + C1C2 and *KIR2DL1/KIR2DS1* + C2C2, might increase the risk of individuals contracting an HCV or HBV infection respectively in this population.We observed that *KIR3DS1* carriers were less likely to be HBV-infected, but may be predisposed to HCV or HIV-1 infections. HLA-A*30:01 allele carriers had an increased risk of acquiring either HBV or HCV, while carriers of HLA-C*17:01 allele were more likely to be infected with HBV but not HCV. HLA-C*03:04 allele - is likely to offer some protection against infection with HBV.

Keywords: **Killer Cell Immunoglobulin-like Receptor genes, Human Leucocyte Antigen-A, Human Leucocyte Antigen-C, Hepatitis B Virus, Hepatitis C Virus, Human Immunodeficiency Virus-1..**

RÉSUMÉ

Les récepteurs de la famille « *Killer cell immunoglobulin-like Receptor (KIR)* » des cellules tueuses naturelles ou cellules NK *« (Natural Killer) »* interagissent avec leurs ligands, des groupes d'antigènes des leucocytaire humains (HLA) de classe I, pour modifier la de capacité tueuse naturelle et la fonction des cellules T dans l'acquisition virale et la progression de la maladie, déterminant ainsi l'immunité et la susceptibilité des humains aux infections et maladies virales. Dans cette thèse, nous avons cherché à déterminer la variation des gènes KIR et HLA de classe I (HLA-A et -C) chez les personnes infectées par le Virus de l'Immunodéficience Humaine de type I (VIH-1), le Virus de l'Hépatite B (VHB) ou le Virus de l'Hépatite C (VHC). Après avoir obtenu la clairance éthique du Comité National d'Ethique de la Recherche pour la Santé Humaine du Cameroun pour mener cette étude, un total de 253 participants adultes naïves de traitement antiviraux (84 VIH-1, 68 VHC, 38 VHB et 63 non infectés) et non liés ont été recrutés. Le génotypage des gènes HLA-A et -C a été effectué par des techniques basées sur des séquences (SBT). Le génotypage KIR a été réalisé par la PCR basée sur la séquence d'amorce spécifique. L'analyse des données a été effectuée avec SPSS version 25, STATA version 14 et Microsoft Excel 2016. Tous les 15 gènes KIR génotypés étaient présents. Nous rapportons une grande hétérogénéité dans les fréquences des allèles et des génotypes dans la population étudiée, avec 57 génotypes KIR, incluant 5 nouveaux. Comparativement aux témoins sains non infectés, les fréquences de KIR2DL2 et KIR3DS1 étaient significativement plus basses dans le groupe HBV+ (58.1% vs 91.2% and 12.9% vs 64.7,p = 0,003 et p <0,001, respectivement). À l'inverse, KIR3DS1 était significativement surreprésenté dans les groupes VHC+ et VIH-1+ par rapport aux témoins (97,0% vs 64,7%, p <0,001; 64,7% vs 20,2%, p <0,001, respectivement). Les individus porteurs de HLA-A*30:01 étaient six fois plus susceptibles d'être infectés par des virus de l'hépatite que ceux sans cet allèle (OR = 6,30, p = 0,020 (VHB); OR = 6,21, p = 0,010 (VHC), respectivement). De même, les porteurs de HLA-C*17: 01 étaient surreprésentés dans le groupe infecté par le VHB par rapport au groupe témoin non infecté (21,9% contre 6,4%, respectivement), ce qui suggère que cet allèle pourrait jouer un rôle dans la sensibilité à l'infection par le VHB. Les génotypes composés KIR2DL2/KIR2DL3/KIR2DS2 + C1C2 et KIR2DL1/KIR2DS1 + C2C2 pourraient augmenter le risque de contracter des infections par le VHC ou le VHB dans cette population. Nous avons observé que les porteurs de KIR3DS1 étaient moins susceptibles d'être infectés par le VHB, mais étaient prédisposés aux infections par le VHC ou le VIH-1. Les porteurs de l'allèle HLA-A*30:01 avaient un risque accru de contracter des virus de l'hépatite B ou de l'hépatite C, tandis que les porteurs de l'allèle HLA-C*17:01 étaient plus susceptibles d'être infectés par le VHB et non par le VHC. L'allèle HLA-C*03:04 - était susceptible d'offrir une certaine protection contre l'infection par le virus de l'hépatite B.

Mots clés: gène « ***Killer Cell Immunoglobuline-like Receptor*** **» ; Antigène leucocytaire humaine-A; Antigène leucocytaire humaine -C ; Hépatite B ; Hépatite C ; Virus d'Immunodéficience Humaine-1**

INTRODUCTION

Sub-Saharan Africa is the world's most affected region when it comes to viral infections. It is home to about 90% of the world's HIV-1 infected children, who have a 50% probability of dying by the age of 2 (Morgan and Whitworth, 2001). It also hosts 70% of the world's most affected adults (WHO, 2015), most of whom progress rapidly to AIDS if untreated. Similarly, viral hepatitis, caused by the Hepatitis B Virus (HBV) and the Hepatitis C Virus (HCV), accounts for more than 65% of deaths from cirrhosis and Hepatocellular carcinoma (HCC) in sub-Saharan Africa (Liaw and Chu, 2009).

The Human Immunodeficiency Virus (HIV) infection is still a global health problem. In 2021, there were about 38.4 million people living with HIV-1, 1.5 million new infections and about 650,000 deaths associated to HIV/AIDS in the world. About 5.0 million infected people were found in West and Central Africa, with about 190,000 new infections and 140,000 deaths associated to HIV/AIDS(UNAIDS, 2022). More than 1 million people die every year from HIV/AIDS in Africa alone. In Cameroon, in 2018, the prevalence of HIV among adults (15 – 49 years) was estimated at 2.9%. While an estimated 500,000 people were living with HIV, there were 15000 new infections and 13000 deaths (UNAIDS, 2022).

Viral Hepatitis caused by the Hepatitis B and C viruses is still endemic in some parts of the world, particularly in low-income countries like Cameroon. Globally, in 2015, it was estimated that 257 million people were living with chronic HBV infection and about 887, 000 deaths were associated with it (WHO, 2019). The prevalence of HBV in the West and Central African regions was reported to be >8% by Franco and colleagues (Franco *et al.*, 2012), and was estimated at 11 % in Cameroon (Bigna et al., 2017a). On the other hand, there were 71 million people infected worldwide with chronic Hepatitis C in 2015. Each year, 399,000 people die from HCV-associated diseases. In West and Central Africa, the prevalence was reported to range from 0.1 – 13.8%, and in Cameroon in particular, it was reported to be 6.5% among adults (Bigna et al., 2017b). Together, HIV and viral Hepatitis B and C represent major threats to public health in sub-Saharan Africa, and in Cameroon in particular.

Naturally occurring variations in host genetic and immunological factors have been associated with the differential clinical outcome of many infectious diseases such as HIV and viral Hepatitis B and C infections (de Wit et al., 2016; Yano et al., 2013; Zwolińska et al., 2016; Yindom et al., 2010b; Bashirova et al., 2011). The impact of these differences ranges from protection from HIV infection in HIV-exposed seronegative individuals, spontaneous control of HIV replication, to slower progression to AIDS (Miyazawa et al., 2009); protection against the HBV infection, protection of intravenous drug users against HCV, resolution of HCV infection, and protection against the development of HCC (Pan *et al.*, 2011; Kibar *et al.*, 2014; Tanimine and Ohdan, 2015; Yindom *et al.*, 2017a). Nevertheless, some host genetic factors have been associated with a faster rate of T-cell

decline in HIV-positive people, development of chronic HBV, HCV and HCC (Cariani et al., 2013; Carrington et al., 2008).

Among the immune factors that influence these different conditions are variants of genes encoding the receptors of Natural Killer (NK) cells and their ligands – the Human Leukocyte Antigen (HLA). NK cells are a critical component of the innate immune system and have great potential to mediate antiviral activity at the earliest stage of viral infections. The functional analysis of NK cells during HIV infection suggests a potential role in controlling virus proliferation (Alter and Altfeld, 2009). In this respect, these cells have the capacity to kill tumour- or virus-infected cells without prior sensitization, leading to an effective antiviral immune response (Caligiuri, 2008). Even though NK cells have numerous receptors on their cell surface, they mostly use Killer cell Immunoglobulin-like Receptors (KIRs) to distinguish disease targets from healthy cells (Rajalingam, 2011). Unlike T cells, NK cells lack antigen-specific receptors such as the T-cell receptor. However, they can recognize and preferentially kill abnormal cells that lack the expression of MHC-Class I molecules through a process referred to as the Missing Self-Hypothesis. This hypothesis, which was first proposed by Klaus Karre in 1986 (Karre, 2002), states that the absence of MHC-Class I molecules on a normal hematopoietic cell is enough to make it susceptible to NK-mediated killing. However, an immunogenetic analysis of different populations shows significant differences in terms of KIR and HLA gene/allele frequencies and haplotype distribution (Yindom et al., 2014). Hence, it is important to understand KIR and gene distribution in any population to assess their potential association with viral infections like HIV, HBV and HCV.

The KIR locus spans a region of about 150–200 kb on chromosome 19q13.4 and is part of the Leukocyte Receptor Complex (LRC). There are 15 KIR genes and two pseudogenes, twelve of which encode for receptors with two immunoglobulin domains (KIR2D genes) while 5 encode for receptors with three immunoglobulin domains (KIR3D genes) (Carrington et al., 2008). KIRs use HLA molecules as ligands and the interaction between them results in either an inhibiting or activating signal leading to either success or failure in lysing a target cell. KIRs are highly polymorphic as well as their HLA ligands both in the number of genes they expressed in an individual and the types of alleles present for each specific gene (Carrington et al., 2008).

KIR3DS1 is the most widely studied activating KIR so far. The first evidence of its role in viral infections came from genetic studies on HIV-infected individuals, where those homozygous for *KIR3DS1* and carrying HLA-Bw4 with an isoleucine at position 80 (HLA-Bw4 80I) progressed slower to AIDS compared to individuals carrying the HLA-Bw4 80T allotype. Interestingly, donors that were homozygous for *KIR3DS1* but lacking in HLA-Bw4 80I progressed faster to AIDS (Alter et al., 2009). Moreover, HLA-Bw4 80I individuals with increasing numbers of copies of *KIR3DS1*

due to copy number variation have been shown to have lower viral load set points (Pelak et al., 2011). NK cell function and KIR have not only been linked to HIV disease progression, but also to resistance to HIV infection in certain populations of exposed uninfected individuals (Boulet et al., 2008).

In a diverse human population like Cameroon with over 250 ethnic groups (Louis *et al.*, 1995), data on KIR/HLA frequency and diversity is lacking. Characterising these genes in HIV-1-, HBV- and HCV-infected patients will therefore help researchers and clinicians understand the immunogenetic variations of a given population and the role they play on disease outcome. Such knowledge may contribute to the development of better treatment options as well as effective therapeutic vaccines against these infections.

Research Questions

- What is the frequency of KIR and HLA class I (A and C) genes in HIV-1+, HBV+ and HCV+ Cameroonians?
- What is the association of HLA-A and HLA-C alleles with susceptibility to HIV-1, HBV and HCV infections?
- Is there any relationship between KIR/HLA profiles and HIV-1, HBV and HCV infections?

Hypothesis

Killer Cell immunoglobulin-like Receptors and Human Leucocyte Antigen genotypes influence susceptibility to HIV, HBV and HCV infections in the Cameroonian population

General Objective

- To perform the molecular characterisation of Killer cell Immunoglobulin-like Receptors and Human Leucocyte Antigen class I genes in HIV-1+, HBV+, HCV+ and uninfected individuals in Cameroon.

Specific Objectives

1) To determine the frequencies of KIR genes in HIV-1-, HBV, HCV-infected and uninfected participants using multiplex Sequence Specific Primer-PCR (SSP-PCR)
2) To assess the HLA class I (A and C) allele and genotype frequencies in HIV-1-, HBV, -HCV-infected and uninfected participants using sequence-based techniques
3) To demonstrate the associations between KIR and HLA compound genotypes and susceptibility to HIV-1, HBV and HCV.

LITERATURE REVIEW

1.1. The Biology of HIV

1.1.1. HIV Origin and Evolution

The Human Immunodeficiency Virus (HIV) is the etiologic agent of the Acquired Immunodeficiency Syndrome (AIDS), an advanced stage of HIV infection with severe loss of immunocompetence. It is documented that HIV-1 originated from Southern Cameroon and the Democratic Republic of Congo in the early 1920's from a single cross specie transmission of the simian lentivirus, Simian Immunodeficiency Virus (SIVcpz) from chimpanzees to humans through direct exposure to animal blood during hunting, butchering and or eating of raw or poorly cooked bush meat (Sharp and Hahn, 2011a; Véras *et al.*, 2011; D'arc *et al.*, 2015). The pandemic HIV strain (HIV-1 group M) infection in humans was first retrospectively confirmed in a plasma sample collected from a sickle cell male patient in 1959, in Kinshasa (then called Leopoldville), Democratic Republic of Congo (Motulsky, Vandepitte and Fraser, 1966; Nahmias *et al.*, 1986; Zhu *et al.*, 1998). From Central Africa, the Virus has adapted to humans and spread to different parts of the World, due to socio-economic factors, such as colonisation, urbanisation, building of new roads and rails which eased the movement of people and the concentration of younger people in urban areas, Changes in sexual behaviours, sex trade and the spread of other sexually transmitted diseases and mass vaccination campaigns contributed to the propagation of the virus (de Sousa *et al.*, 2010; Faria *et al.*, 2014).

HIV-related illness was first reported in 1981, in the United States, in Homosexual men and subsequently in intravenous drug users with a series of opportunistic infections and rare malignancies(Gottlieb *et al.*, 1981). A year later, it was termed as the Acquired Immunodeficiency Syndrome (AIDS) by the Centre for Disease Control (CDC) and the subsequent year, in 1983, the virus was isolated at the Pasteur Institute in France by Dr Luc Montagnier (Barré-Sinoussi *et al.*, 1983; Gallo *et al.*, 1983). It was identified as a retrovirus of the lentivirus group and initially classified as a Human T-lymphotropic virus III (HTLV-III), but was later renamed as the Human immunodeficiency Virus (HIV) by the international committee on taxonomy of viruses(CASE, 1986).

On the other hand, the Human Immunodeficiency Virus Type 2 (HIV-2) was first identified in individuals from West Africa. The first case was isolated from a healthy commercial sex worker in Senegal, in 1986, and named human T-lymphotropic virus IV (HTLV-IV) (Clavel *et al.*, 1986). Similar viruses were later isolated from two West African patients with AIDS and it was then renamed as HIV-2 (Clavel *et al.*, 1986). Phylogenetic analysis have shown that HIV-2 represents viruses similar to the SIV from the sooty mangabey (SIVsmm) and is closely related to SIV from Macaques with an AIDS like syndrome. HIV-2 group A is thought to have entered the human

population in around 1938 in Guinea-Bissau (Faria *et al.*, 2012). The current prevalence of HIV-2 is unknown, however, it is estimated that about 1- 2 million people are infected with HIV-2 (Gao *et al.*, 1994). HIV-2 testing is not routinely done in health structures outside of West Africa and it often requires a specialist laboratory for diagnosis, as such, HIV-2 data is relatively scarce.

1.1.2. HIV Classification and Diversity

Human immunodeficiency viruses are RNA viruses belonging to genus of lentiviruses and family of retroviruses. Retroviruses are viruses which reverse transcribe their RNA to DNA, while Lentiviruses are viruses with a long incubation period from time of infection to disease. Phylogenetic analysis of sequences similar to those of the simian immunodeficiency virus (SIV) isolated form primates classifies HIV into two major groups HIV types 1 and 2 (HIV-1 and HIV -2) (Sharp and Hahn, 2011b). Both HIVs result from independent multiple zoonotic transmissions of SIVs, which are naturally present in African primates (Hahn, 2000). HIV-1 represents viruses related to SIV from chimpanzee, Pan troglodytes (SIVcpz) and the gorilla (SIVgor), while HIV-2 represents viruses related to SIV from sooty managabey (SIVsmm) (Sharp and Hahn, 2011a; Kannangai, David and Sridharan, 2012). These viruses share a good number of similarities such as gene arrangement, intracellular replication pathways, modes of transmission and same opportunistic infections, but clinical findings from west Africa have shown that HIV-2 progresses more slowly to AIDS and is less easily transmissible compared to HIV-1 (Guyader *et al.*, 1987; Yindom *et al.*, 2010b; Nyamweya *et al.*, 2013). Unlike HIV-2 which is mainly in West African countries, HIV-1 is globally distributed and more diverse (Campbell-Yesufu and Gandhi, 2011). HIV-1 is classified into four main viral groups: M (main), N (non-M, non-O), O (outlier) and P (putative) (Figure 1). Group M accounts for most of the infections and is made up of several genetic variants, including 14 subtypes and many Circulating Recombinant Forms (CRFs) and Unique Recombinant Forms (URFs) with varying geographical distributions (Robertson *et al.*, 2000; Negedu-Momoh *et al.*, 2014; *HIV Sequence Database: Nomenclature Overview*, no date). HIV-2 has been classified into nine groups (A – I), but only subtypes A and B are prevalent and mostly restricted to the West African region (Smith *et al.*, 2008). In Cameroon, there are multiple subtypes and circulating recombinant forms, but the most prevalent circulating recombinant form is the CRF_02AG , contrary to most parts of the world in which pure subtypes are dominant (Konings *et al.*, 2006; Agyingi *et al.*, 2014; Abongwa *et al.*, 2019).

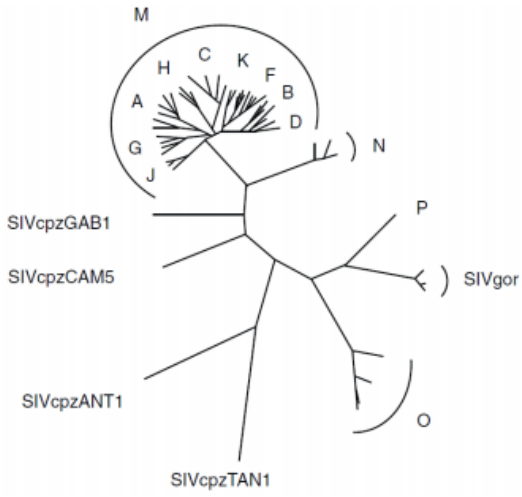
Phylogenetic tree of HIV & SIV	HIV-1	Geographic distribution
	A	Central Africa, sub-Saharan Africa
	B	South America, Brazil, United States, Thailand, Europe, Caribbean, India, Japan
	C	Brazil, India, South Africa
	D	Central Africa, sub-Saharan Africa
	F	Brazil, Romania, Democratic Republic of Congo (Zaire)
	G	Democratic Republic of Congo (Zaire), Gabon, Thailand, Russia, Central Africa
	H	Democratic Republic of Congo (Zaire), Gabon, Russia, Central Africa
	J	North, Central and West Africa, Caribbean
	K	Democratic Republic of Congo (Zaire), Cameroon

Figure 1: Phylogenetic tree of SIV and HIV with the geographic distribution of HIV-1 group M subtypes (Hemelaar, 2012).

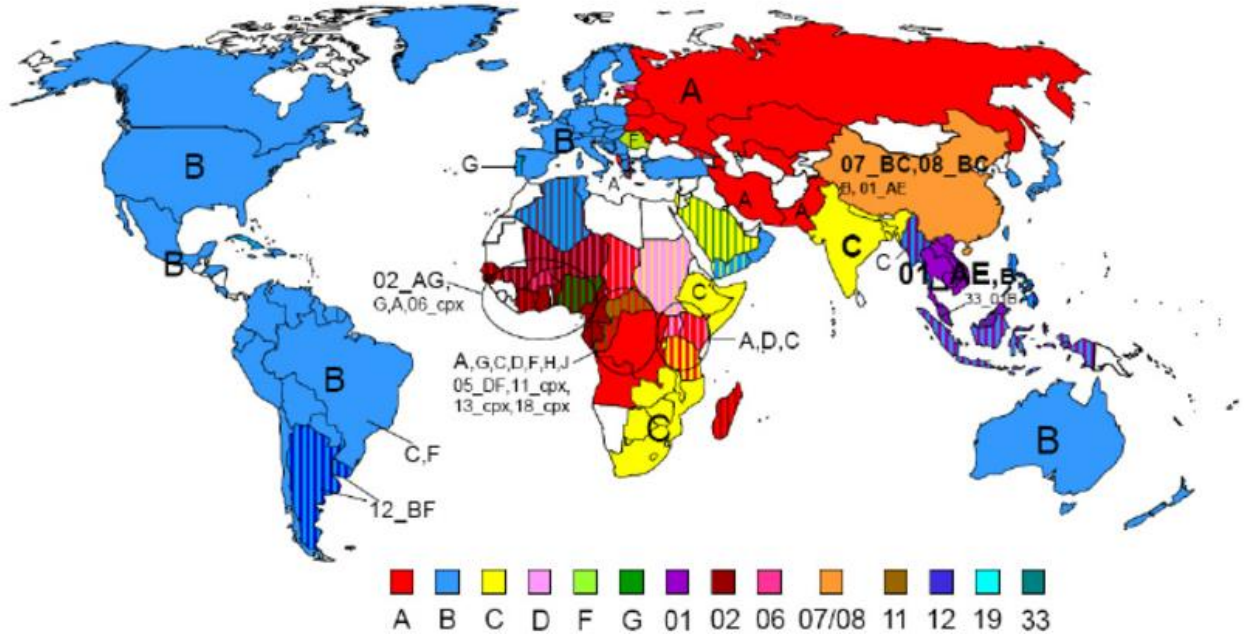


Figure 2: Global distribution of HIV-1 subtypes (adapted from WHO,2011)

1.1.3. Epidemiology of HIV

In the year 2018, about 37.9 Million people were living with HIV-1, with about 1.7 Million new infections and about 770,000 deaths associated to HIV/AIDS in the world. Of the 37.9 million HIV-infected people in the world, 5.0 Million people were in the west and central African regions, with about 280,000 new infections and 160,000 deaths associated to HIV/AIDS (UNAIDS 2019). More

than 1 million people die every year from HIV/AIDS in Africa alone. In Cameroon in 2018, the prevalence of HIV among those aged between 15 and 49 years in Cameroon was estimated at 3.6%, while an estimated 540,000 adults and children were living with the virus. There were 23,000 new infections, and 18,000 AIDS-related deaths (UNAIDS 2019). Women are disproportionally affected by HIV in Cameroon: of the 490 000 adults living with HIV, 330 000 (67.35%) were women. New HIV infections among young women aged 15–24 years were more than double those among young men: 5400 new infections among young women, compared to 2000 among young men (UNAIDS 2019). The prevalence of HIV among adults aged between 15 and 64 years in Cameroon varies by region, ranging from 6.3% in the South region to 1.5% in the Far North region. In Yaounde, where we collected the samples used in this study, the prevalence of HIV is estimated at 4.4% (CAMPHIA 2018) (Figure 3.

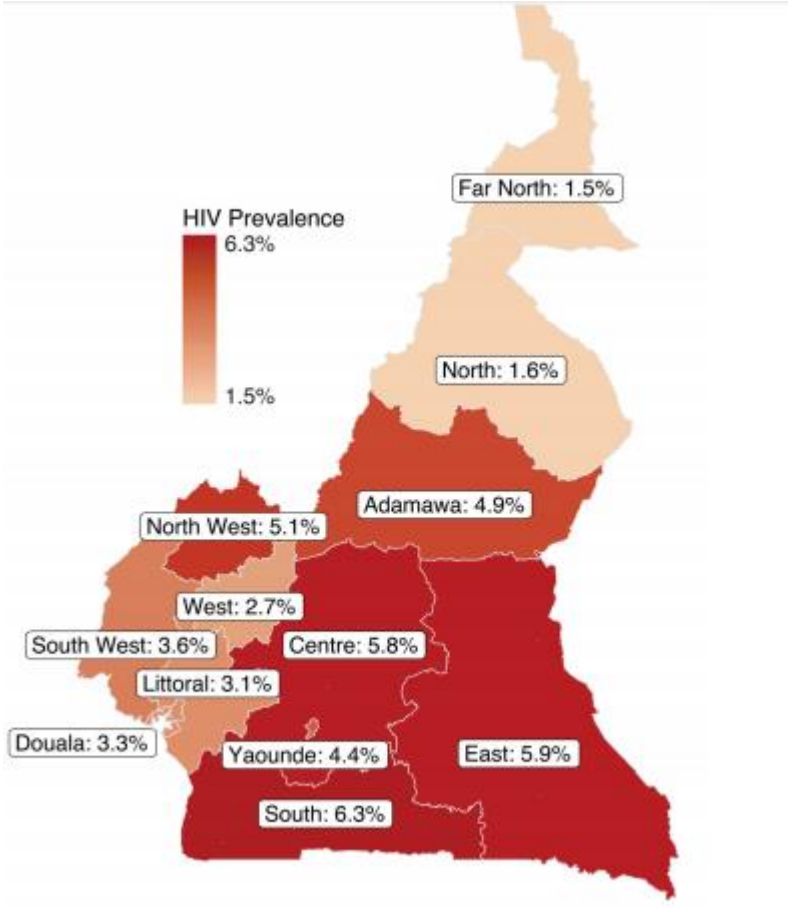


Figure 3: HIV Prevalence Among Adults, by Region in Cameroon (CAMPHIA, 2018)

1.1.4. HIV-1 Structure and Genome

The HIV-1 virion is about 120 nm in diameter and has a cone-shaped viral core covered with a lipid bilayer envelope obtained from host cells during its release. It contains two single-stranded RNAs

(ssRNAs) of approximately 9.2kb in length. It equally contains the following enzymes: reverse transcriptase, protease, integrase and ribonuclease, all enclosed in an outer lipid envelope. Its genome is made up of nine genes, with two regulatory genes (rev, tat), four accessory genes (vif, vpu, nef and vpr) and three structural genes (env, gag and pol) figure 3.

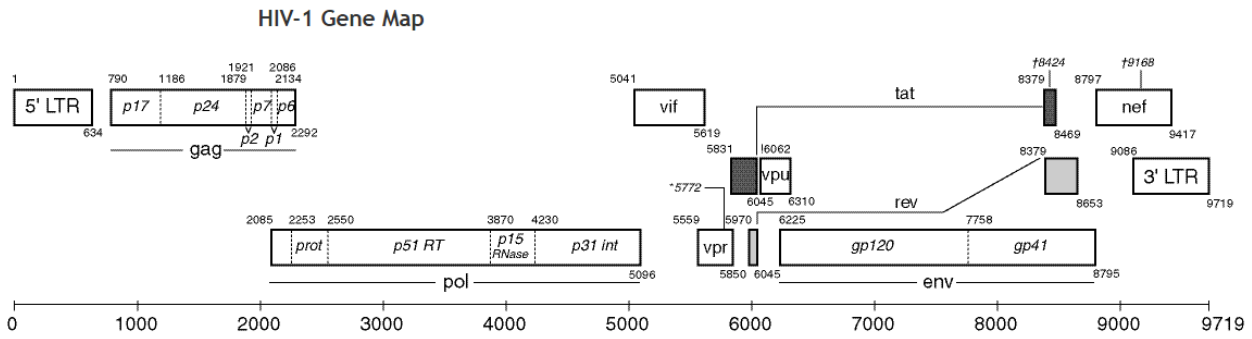


Figure 4: HIV-1 Gene Map showing the different HIV-1 proteins and the regions they cover on the HIV-1 genome (copied from https://www.hiv.lanl.gov.)

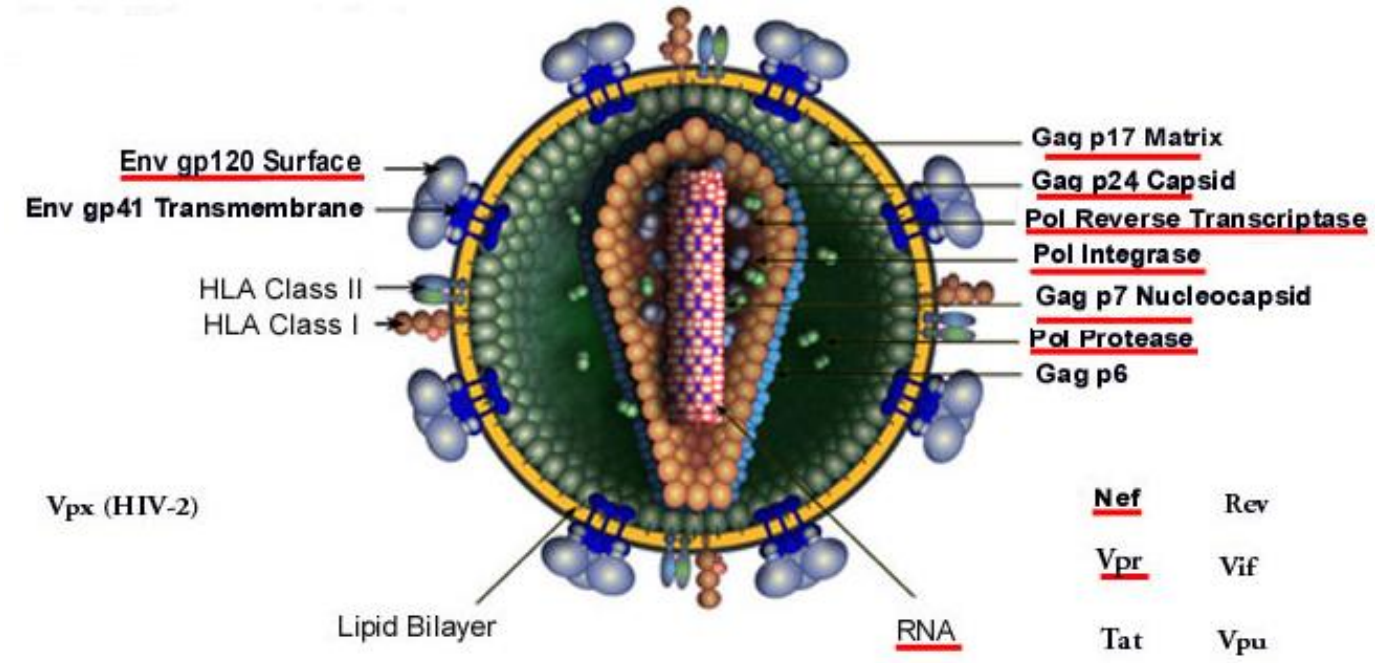


Figure 5: 3D structure of a mature HIV-1 virion (copied and adapted from https://www.hiv.lanl.gov accessed 14 April 2019.)

1.1.5. HIV-1 Lifecycle

The HIV-1 lifecycle is made up of 7 successive steps namely: fusion, entry, integration, reverse transcription, replication, assembly and budding (Figure 6 (Chan and Kim, 1998). An infection is usually initiated upon entry across either genital, rectal, mucosal or the oral tract (Coffin and

Swanstrom, 2013). It uses glycoproteins, gp120 and gp41, on its envelope to fuse with target cells, CD4 cells, macrophages, monocytes and dendritic cells. These glycoproteins undergo conformational changes permitting gp120 to interact with either and α- or CXC-chemokine or β- or

CC -chemokine coreceptor, CXCR4 or CCR5, respectively, based on viral tropism. T- cell tropic isolates and macrophage-tropic isolates use CXCR4 and CCR5, respectively, for entry (Gorry and Ancuta, 2011). Upon engagement of the chemokine coreceptor, more conformational changes are initiated to expose the binding domain of gp41. This results in a stable fusion between the HIV-1 and the host cell membrane, providing a pore for entry of the viral core into the host cell cytoplasm (Simon, Ho and Abdool Karim, 2006).

When the virus gets into the host cell, its core disassembles and the viral RNA is reverse transcribed into complementary DNA. The single stranded DNA is then used to synthesize double stranded DNA (Moss, 2013). The high mutation and recombination rates observed in HIV-1 is due to the error prone nature of the reverse transcriptase enzyme which lacks proof reading capacity. This nature permits the HIV-1 to evolve very rapidly (Preston, Poiesz and Loeb, 1988). Once the double stranded viral DNA is synthesized, it then uses the viral enzyme integrase to integrate the host's chromosomal DNA. The proviral DNA is then transcribed into messenger RNA, spliced and results in the translation of viral proteins (Tat, Rev, and Nef). The singly spliced and unspliced RNA transcripts are then transported to the cytoplasm, where mRNA is translated into structural HIV-1 proteins. The viral particles are then assembled and budding is initiated. The assembled virions then undergo subsequent processing into mature virions, capable of infecting other cells. Viral budding from macrophages and monocytes sometimes result in intracellular vacuoles which result in latent reservoirs of HIV-1 (Fanales-Belasio *et al.*, 2010). This is one of the greatest roadblocks in the fight against HIV-1 infection, as Anti-Retroviral Therapies (ARTs) are unable to eliminate viral reservoirs.

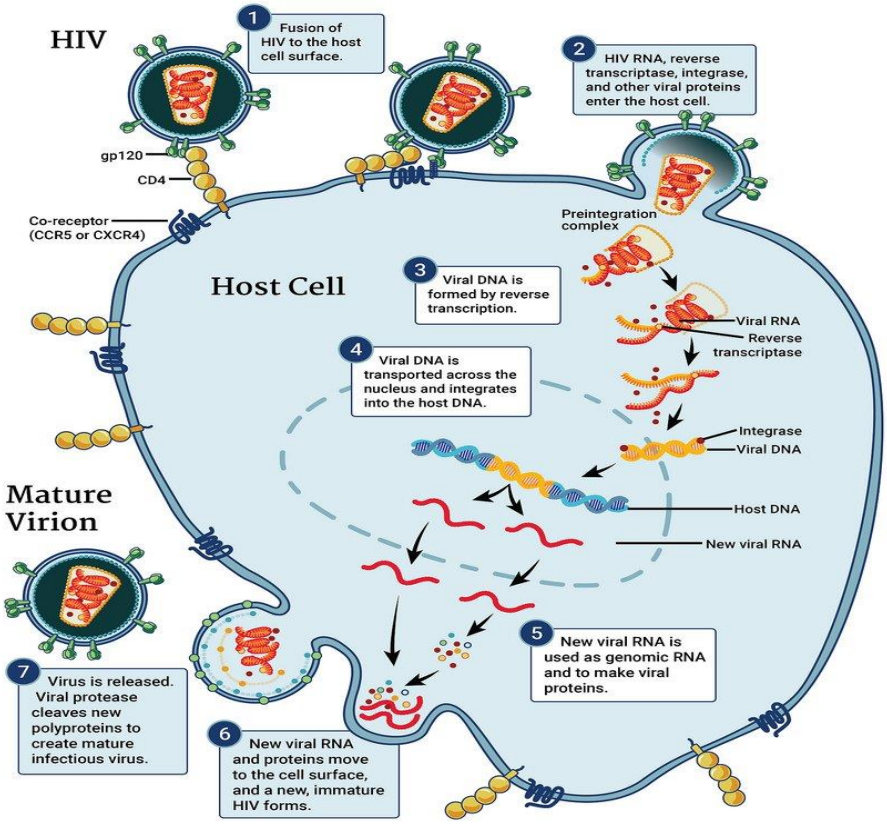


Figure 6: HIV-1 Replication cycle

Source : https://www.niaid.nih.gov/diseases-conditions/hiv-replication-cycle, accessed 20 June 2018

1.1.6. HIV-1 Transmission Routes

HIV can be transmitted horizontally, that is through sexual contact across mucosal surfaces, by blood transfusion, or through contact with contaminated hypodermic needles and syringes. It can also be transmitted vertically by maternal-infant exposure, either prenatally during pregnancy, perinatally during childbirth or postnatally through breastfeeding. Globally, sexual transmission across the mucosal surface accounts for most of the cases of HIV-1 infection. The risk of transmission is increased by factors such as, high viral load, male uncircumcision, anal vs vaginal intercourse. Moreover, the presence of other sexually transmitted diseases increases the risk of sexual transmission, due to the presence of large amounts of viral particles in genital fluids of those infected with syphilis, gonorrhoea and genital herpes (Moss, 2013). Nevertheless, there must be exchange of fluids with either a cell-free or cell-associated virus for transmission to occur.

1.1.7. HIV-1 Disease Course: From Infection to AIDS

Clinically, The HIV-1 infection is usually initiated by a single virus infecting a target cell. The subsequent course of infection is made up of three stages (figure 3): acute, chronic and AIDS as follows. During the first stage of the acute phase, eclipse phase (1-2wks) of infection, the virus is replicating and spreading from the infection site to other tissues and organs of the host, viremia is at an undetectable level, and neither symptoms of infection or immune response are visible (Cohen *et al.*, 2010; Coffin and Swanstrom, 2013). Then comes the acute (primary) phase (2 – 4wks), characterised by relatively high levels of viremia (up to 107 or more copies of viral RNA per millilitre of blood), and large amounts of infected CD4+ T cells in lymph nodes and blood. This phase is often accompanied by fever, "flu-like" symptoms, enlarged lymph nodes, fatigue, muscle aches, nausea and/or diarrhoea. The immune response begins to appear in the form of antibodies against viral proteins (seroconversion), and a CD8+ T-cell response against HIV-1 antigens expressed on infected cells (Coffin and Swanstrom, 2013). HIV antibody tests are usually negative in the first three weeks of infection, but HIV-1 can be detected in blood at this stage using the p24 antigen test (Klatt *et al.*, 2013). When the acute phase is over, viremia drops sharply (100-fold or more) as a result of exhaustion of activated target cells and viral control by the immune system. This phase is also accompanied by a decline in the number of CD4+ T-cells in the blood. Then comes the chronic phase or "clinical latency" (1 – 20yrs). This is usually characterized by a slowly increasing level of viremia, 1 – 100,000 copies/mL, and steady or gradually falling levels of CD4+ T-cells (Bashirova, Thomas and Carrington, 2011a). Patients in this phase are asymptomatic and mostly unaware of their status. Finally, the number of CD4+ T cells declines to approximately 200 cells/uL or less than 14% of CD4%, immune control can no longer be maintained, and opportunistic infections with common pathogens like cytomegalovirus, candidiasis and herpes simplex virus start to take control (Coffin and Swanstrom, 2013). The level of viremia rises during the AIDS phase, culminating in death of the host. Most of the patients who progress to AIDS in the absence of ART die within two years (Poorolajal *et al.*, 2016). The main difference between HIV-1 and HIV-2 resides in the duration taken to progress to AIDS. In a comparative study of the two viruses, it was found that the time taken to progress to AIDS was 6 years for HIV-1 versus 14 years for HIV-2 and the rate of CD4+ T cell decline was 0.9% versus 0.4% per year for HIV-1 and HIV-2, respectively, (Esbjörnsson *et al.*, 2018).

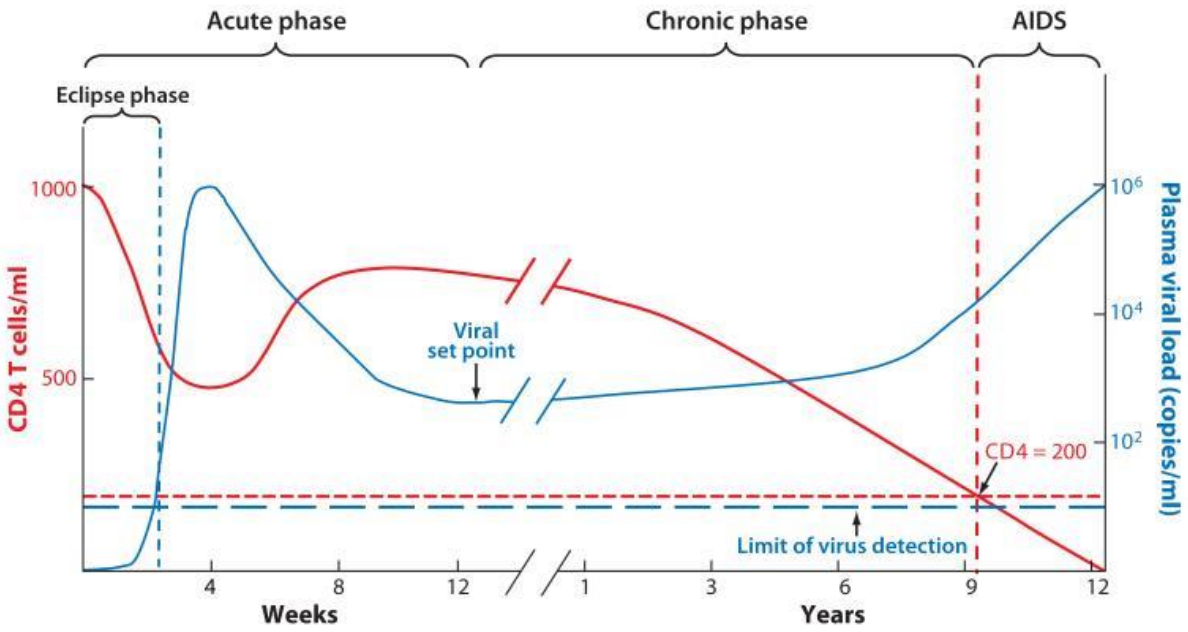


Figure 7: The clinical course of HIV-1 infection showing the dynamics of peripheral blood CD4 T cells and plasma viral load (Bashirova, Thomas and Carrington, 2011b)

1.1.8. HIV Treatment

Since the discovery of HIV-1 in the 80s, a lot has been done to combat the virus and make HIV/AIDS a manageable disease in the fields of biology, pharmacology and clinical care. Nevertheless, it is still not curative. However, Highly Active Antiretroviral therapy has as goal to reduce HIV-associated morbidity, prolong survival and prevent HIV transmission. To date, there are six classes of antiviral agents: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (IIs), fusion and entry inhibitors.

Nucleoside and nucleotide reverse transcriptase inhibitors (N (t)RTIs)

They were the first antiviral agents developed for the treatment of HIV. Examples include. Zidovudine (ZDV or AZT), Didanosine (ddI), Zalcitabine (ddC), Stavudine (d4T), Lamivudine (3TC), Abacavir (ABC), and emtricitabine (FTC) and the nucleotide reverse transcriptase inhibitor Tenoforvir disoproxil fumarate, (TDF). They are inactive in their parent form, but become active upon phosphorylation by host cell kinases and phosphotransferases. They are DNA chain terminators, structurally similar to endogenous DNA nucleoside bases. They compete with the endogenous nucleoside bases and become efficiently incorporated into the viral DNA at the 3’-end as a NRTI monophosphate. Once incorporated, they inhibit the elongation of the viral DNA chain

due to the lack of a 3'hydroxyl group or an altered sugar moiety that prevents the incorporation of incoming nucleotides(Iyidogan and Anderson, 2014).

Non -nucleoside reverse transcriptase inhibitors (NNRTIs)

Contrarily to NRTIs and NtRTIs, they are active in their parent form and do not require phosphorylation. They act by binding to the allosteric hydrophobic site of the reverse transcriptase enzyme, preventing the HIV RNA from converting to DNA. They are non-competitive inhibitors. Examples include: Nivaripine (NVP), delavirdine (DLV), efavirenz (EFV) and entravirine (ETR) (De Clercq, 2013; Iyidogan and Anderson, 2014).

Protease inhibitors

They act by binding to the active site of the protease as competitive inhibitors and hence inhibit the production of individual functional proteins needed for viral maturation. Examples include: saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir, duarunavir.

Integrase inhibitors

They prevent the integration of viral genetic material into DNA by displacing the 3' end of the Viral DNA from the active site and chelating the divalent cation (Mg2+ or Mn2+) that is needed for integrase enzymatic activity. Examples include: Raltegravir (RAL), Elvitegravir (EVG), Dolutegravir (DTG).

Entry and co-receptor inhibitors.

These are new classes of drugs which act by interfering with the virus ability to bind to the host cell. The co-receptor inhibitors block the interaction between the HIV-1 envelop and CCR5 or CXCR4, while the attachment inhibitors block the interaction between the HIV-1 envelope and CD4 cells (Iyidogan and Anderson, 2014).

1.1.9. Eradication of HIV

Currently available antiretroviral therapy cannot eradicate HIV due to the pool of latently infected T-cells which develop at the early stages of the infection. There is also a problem of drug resistance, the ability of the HIV virus to mutate and continue reproducing itself in the presence of HIV drugs. This drug resistance is due to the high replicative capacity of the HIV virus and the error prone nature of the viral polymerase, which leads to the production of diverse quasi species. HIV can be eliminated either by curing all those already infected or by preventing new infections by the use of a vaccine.

So far, two people so far ("The Berlin patient" and "The London patient") have been reported to attain full remission and long-term control of HIV without ART. The London patient was reported last in 2019 while the Berlin patient was reported in 2007. These patients underwent haematopoietic stem cell transplant from an HLA match donor with homozygous CCR5 Δ32/Δ32 alleles. The patients' viral loads remained undetectable without antiretroviral therapy (Brown, 2015; Jilg and Li, 2019). This was clear proof that eliminating HIV from primary target cells might prevent viral rebound providing further evidence that advances in science will surely eliminate HIV someday. The difficulty in using this method lies on the possibility of finding an HLA match with the CCR5 Δ32/Δ32 allele. This allele has been reported to be present in only about 1% of Caucasians (Jilg and Li, 2019). Nevertheless, research is expanding towards new therapeutic approaches aiming at eliminating the HIV reservoir, which may lead to a cure. These research activities are geared on latency reversing agents to activate the HIV viral reservoir, innate immunity activators and effector antibodies, gene therapies and therapeutic vaccines to induce an effective immune control or to eliminate the viral reservoir. Hopefully, these research activities will bring forth significant progress in the fight against HIV.

1.1.10. HIV Treatment in Cameroon

Combinational antiretroviral therapy (cART) has significantly slowed the AIDS pandemic and reduced the incidence of HIV infections (UNAIDS, 2020). In Cameroon, effective access to cART began in 2004 after the WHO/UNAIDS "3 by 5" initiative to provide three million people living with HIV (PLHIV) in low and middle-income countries with life-prolonging cART by the end of 2005 (WHO, 2003). Before 2004, only a few generic antiretroviral drugs [lamivudine (3TC), zidovudine (ZDV), stavudine (d4T), and nevirapine (NVP)] were available at a very low scale in main cities in Cameroon such as the capital Yaoundé (Bourgeois *et al.*, 2005; Aghokeng *et al.*, 2011). cART usage in Cameroon expanded in June 2007 with free access to cART for eligible patients based on CD4 count guidelines, and from 2016 cART has been accessible to all HIV-infected individuals following the implementation of the "test and treat" UNAIDS's initiative (WHO, 2005; National AIDS Control Committee, 2015). The scale-up of cART considerably improved the lives of PLHIV in Cameroon. Cameroon and other West-Central African countries mainly use a triple cocktail of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and one non-NRTIs (NNRTIs) as first-line treatment supported by PIs in the second line (WHO, 2015).

Cameroon has opted for the "Treat All" strategy at the national level. The commitment of the NSP for the year 2023 is that 92% of PLHIV adults, adolescents and children who know their status are actually receiving antiretroviral treatment by the end of 2023. To achieve this, various strategies are planned, these include Improving the link between testing and ARV treatment services; The

implementation of the policy of decentralization of HIV care; Improving the retention of patients on ARVs in care; Community intervention to improve adherence and compliance('PSN-2021-2023-version-anglaise_23052021-4.pdf', no date).

1.2. The Biology of HBV

1.2.1. HBV Origin and Diversity

The Human Hepatitis B virus is a small, enveloped, partially double stranded DNA virus that belongs to the Hepadnaviridae family (Grimm, Thimme and Blum, 2011a). The Hepadnaviridae were initially divided into two genera: the orthohepadnaviridae (infects mammals) and Avihepadnaviridae (infects birds), but of recent, putative hepadnaviruses have been discovered that infect fish (Hahn *et al.*, 2015). HBV was first discovered by the noble prize winner Baruch Blumberg as the Australian Antigen (AuAg) in Serum samples of Australian Aborigines in 1963 (Blumberg and Alter, 1965; London, Sutnick and Blumberg, 1969). It was later identified as the Hepatitis B Virus Surface antigen (İnan and Tabak, 2015).

The Hepatitis B virus is very diverse, based on nucleotide sequence divergence of >7.5%, the HBV can be classified into nine (09) known genotypes (A-I) and a 10^{th} putative genotype (J) with specific geographical distributions (Lin and Kao, 2015; Velkov *et al.*, 2018) (figure 6). These genotypes can be further divided into subtypes based on genomic variabilities of >4% (Kramvis, 2014; Pourkarim *et al.*, 2014a). Genotypes A – H had been long identified and accepted as individual genotypes, while genotypes I and J are new genotypes (Tran, Trinh and Abe, 2008; Tatematsu *et al.*, 2009a). Genotype I was first isolated in Vietnam and Laos and it is a recombination of genotypes A, C and G (Tran, Trinh and Abe, 2008), while the most recent genotype J was first identified in Japan, and based on phylogeny it resembles gibbon rather than Human HBV and might constitute a recombination with Human genotype C (Tatematsu *et al.*, 2009b; Locarnini *et al.*, 2013). HBV genotypes are distributed distinctively around the world. Genotype A is highly prevalent in Sub Saharan Africa, Western Africa and Northern Europe, B and C are common in Asia pacific regions, D is dominant in Africa, Europe, the Mediterranean region and India, E is restricted to west Africa, F is found in central and south America, G has been reported in France, Germany and the United States, H is found in central and south America, I has been reported in Vietnam and Laos and J has been reported in the Ryukyu islands in Japan (Tatematsu *et al.*, 2009c; Kramvis, 2014; Pourkarim *et al.*, 2014b; Sunbul, 2014; Mahmood *et al.*, 2016). In Cameroon, three genotypes (A, E and D) have been reported to be prevalent in Cameroonian patients, with genotypes A and E being the most prevalent (Kurbanov *et al.*, 2005; Kramvis and Kew, 2007; Ducancelle *et al.*, 2013). In addition, according to serological

reactivities of the Hepatitis B surface Antigen (HBsAg), the Hepatitis B virus can also be classified into nine serotypes, called subtypes - adw2, adw4, adrq+, adrq, ayw1, ayw2, ayw3, ayw4 and ayr with distinct geographical distributions (Thedja *et al.*, 2015).

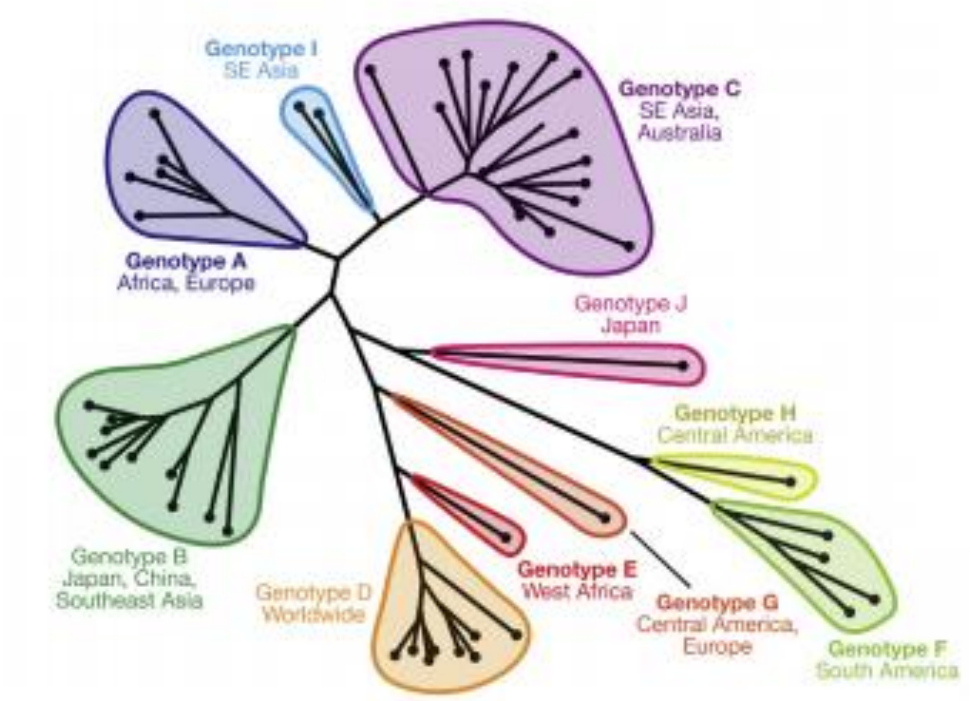


Figure 8: Phylogenetic tree showing relationship between HBV genotypes and subtypes with geographic distribution (McNaughton *et al.*, 2019)

1.2.2. HBV Epidemiology

The World health organization estimates that in 2015, there were 257 million people living with chronic hepatitis B in the world and there were 887,000 deaths associated with viral hepatitis B, mostly due to cirrhosis and hepatocellular carcinoma. The prevalence of HBV is highest in the WHO western pacific and African regions, with 6.2% and 6.1% of the adult population of these regions infected respectively (WHO 2019). In the west and central African region, the prevalence of hepatitis B is reported to be > 8% (Franco *et al.*, 2012) and in Cameroon, the prevalence of chronic hepatitis B has been reported to range from 6 – 16% (Frambo *et al.*, 2014; Noubiap *et al.*, 2015; Ayano *et al.*, 2018).

1.2.3. HBV Structure and Genome

HBV is a small partially double stranded circular DNA virus, with a diameter of about 40 – 42nm and a lipoprotein envelope. The two DNA strands are made up of a longer negative sense complete strand responsible for the coding of viral mRNA and the transcription of pre-genomic viral RNA,

and a shorter plus strand, variable in length, with two direct repeats (DR1 and DR2) at the 5'end responsible for strand specific DNA synthesis during replication. A short cohesive overlap region at the 5'end of both strands stabilizes the circular structure of the genome. The HBV genome is approximately 3.2kb in length with four overlapping open reading frames (ORFs), each responsible for coding one of the four HBV genes (Polymerase (P), Surface (S; pre-S1 and pre-s2), Precore/core (C), and the X-protein) (Tiollais, Pourcel and Dejean, 1985; Liang, 2009).

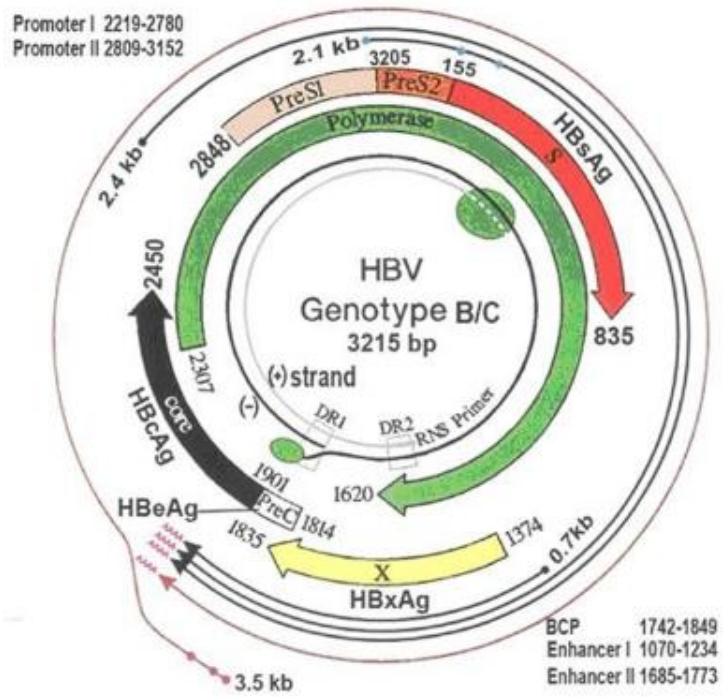


Figure 9: HBV Genomic Structure (Zhang and Cao, 2011)

1.2.4. HBV Lifecycle

The HBV lifecycle starts with the entry of the virus into hepatocytes. This is the first interaction between the viral particle and the host. It plays an important role in hepatocellular tropism and species specificity. It is equally a target for host neutralizing antibodies for vaccine development. This is done firstly through low affinity, reversible attachment of hepatocyte specific preS1 receptors on the surface of mature Dane particles to heparan sulphate proteoglycans on hepatotcyes, followed by the specific and irreversible binding of the preS1 region on the envelope of the virion to the hepatocellular sodium taurocholate cotransporting polypeptide (NTCP) (Revill and Locarnini, 2016). After attachment to the receptors, the virus enters the hepatocyte through two potential mechanisms: endocytosis followed by the release of the nucleocapsids from endocytic vesicles and the fusion of the viral envelope with the endoplasmic reticulum. Once the virion is released into the cytoplasm, uncoating takes place, and the virion is transported to the nucleus via HBV endocytosis. The capsid transports the rcDNA to the nucleus via the nuclear pore complex (NPC), once in the NPC, the

disassembly of the capsid is completed and the rcDNA is released into the nucleoplasm. The plus strand of the rcDNA is then completed by the viral polymerase present at the 5'end of the negative chain. The short RNA primer is used for the` synthesis of the DNA positive strand. The RNA primers and the viral polymerase involved in the plus strand synthesis are removed by host cellular enzymes such as proteinases. When the positive strand is complete, it ligates covalently with the negative strand at both ends to form a circular supercoiled molecule, covalently closed circular DNA (cccDNA). This cccDNA is highly resistant to antiviral therapy and is hence responsible for viral persistence. The cccDNA molecule is organised as a chromatin-like structure (microchromosome) and serves as the template for the transcription of all the viral mRNAs (Grimm, Thimme and Blum, 2011b; Watashi *et al.*, 2014). The cccDNA uses the cellular transcription machinery to produce all the viral RNAs needed for protein synthesis and viral replication. The process is regulated by host transcription factors and viral proteins, which may modulate viral gene expression by interacting with the viral promoters of the four main open reading frames (ORFs): the precore/core gene, coding for the nucleocapsid protein and the non-structural, secreted, precore protein, the HBeAg; the polymerase gene coding for the reverse trancriptase, RNase H and terminal protein domains; the L-,M-, and S- gene, coding for three envelope proteins, and the X-gene coding for the small regulatory X-protein. All four major mRNAs use a single common polyadenylation signal, nevertheless, processing of viral RNAs, nuclear export as well as stabilization of viral RNAs seem to be mediated by host factors (Pollicino *et al.*, 2006; İnan and Tabak, 2015).

The cccDNA is transcribed into pregenomic RNA (pgRNA) and subgenomic (sgRNA). The pgRNA is then translated to the core protein and the polymerase, while the sgRNA is translated to the three viral envelope proteins and the X-protein. Self-assembly of the RNA-containing viral nucleocapsid takes place by a complex formation of the pgRNA with the polymerase and the core protein. Maturation of RNA-containing nucleocapsids to DNA containing nucleocapsids takes place in the cytoplasm by reverse transcription of pgRNA (Pollicino *et al.*, 2006). Due to the lack of a proofreading capacity by the viral reverse transcriptase, so many genetically distinct viral species (quasispecies) are produced in infected persons. The DNA containing nucleocapsids are either enveloped and released through the edoplasmic reticulum or re-imported to the nucleus for cccDNA amplification. cccDNA acts as a template for all viral mRNAs, as such, it is a stable source for new viruses from the nucleus of infected hepatocytes. It therefore plays an important role in disease reactivation after immunosuppression and drug resistance. Unfortunately, available regimens used for the treatment of HBV do not eliminate cccDNA. After budding, when they are released into the lumen of the endoplasmic reticulum, the envelope proteins are secreted either as infectious virions of 42 nm in diameter (Dane particles) or as non-infectious subviral filamentous or spherical particles

(SVPs) of 22nm in diameter. The SVPs lack a nucleocapsid and are hence non-infectious (Urban *et al.*, 2010).

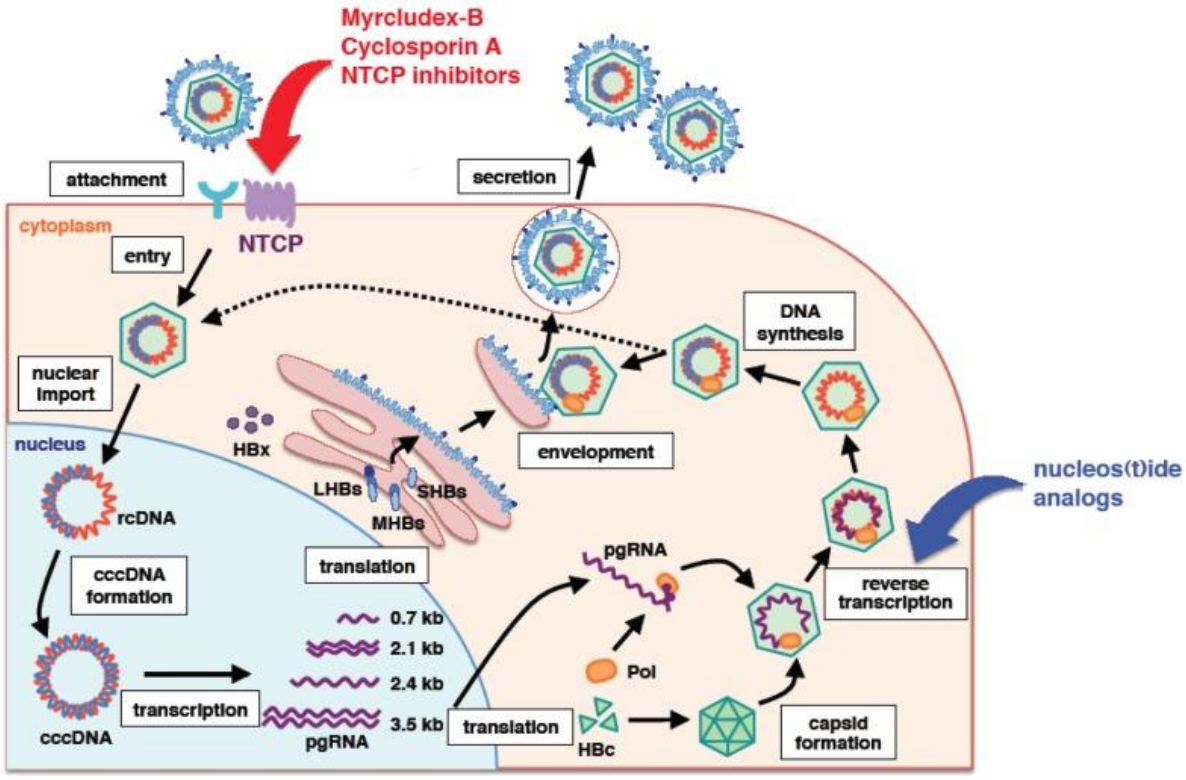


Figure 10: Schematic Representation of the HBV Lifecycle (Watashi *et al.*, 2014)

1.2.5. HBV Pathogenesis

In HBV endemic areas, the Hepatitis B virus is mostly transmitted perinatally (mother to child) or horizontally (exposure to infected blood). The development of chronic Hepatitis B is common in 90% of children who get infected perinatally. HBV can also be acquired percutaneously or through mucosal exposure to infected blood and various body fluids such as vaginal, seminal, saliva and menstrual fluids (Zuckerman, 1996). The incubation period of HBV is averagely 75days, but it can vary from 30 to 180 days. The natural course of the HBV infection varies among persons, host characteristic, viral strains, and host – viral interaction. About 65% of acutely infected persons develop a subclinical asymptomatic infection characterised by the appearance of one or more viral antibodies in their blood, while 25% develop an acute resolving infection and the remaining 10% develop a chronic infection which might lead to cirrhosis and HCC. Symptomatic HBV infected patients present with an inflammation of the liver, called hepatitis and associated nausea, jaundice, vomiting and abdominal pain (Lamontagne, Bagga and Bouchard, 2016). However, most HBV infected persons are usually asymptomatic.

The rate of progression of HBV infection depends on the age when first infected (McMahon *et al.*, 1985). About 90% of children perinatally infected with HBV and about 25 – 50% of infants infected between the ages 1 – 5 years will develop chronic hepatitis. About 25% of these HBV infected infants and infected children older than 6 years later develop HBV related cirrhosis and HCC (Shepard *et al.*, 2006). While in adults, less than 10% develop a chronic infection (Locarnini *et al.*, 2015). Moreover, other factors such as gender, HBV genotypes and variants, coinfections (with HCV, HIV, HDV), lifestyle (smoking, drinking), carcinogenic substances, host genetics and other comorbidities (obesity, diabetes) influence the natural history of the HBV infection.

The clinical course of a chronic HBV infection (HBsAg persistently present in blood for more than six months) can be divided into five stages: "immune tolerant", "immune active", "immune control", "immune escape" and "reactivation".

Phase 1. Immune tolerant

The first phase is usually longer in children infected at birth or at a very young age. During this phase, the HBV is undergoing incubation, characterised by normal Alanine aminotransferase (ALT) levels, HBV replication, the presence of HBV DNA, HBeAg, HBsAg in the serum and high viral loads (Liaw and Chu, 2009). The individual is usually asymptomatic. Antibodies to hepatitis B core Antigen (anti-HBc) (Immunoglobulin M[IgM], followed by immunoglobulin G [IgG]) are produced in small quantities, however, these antibodies are unable to eliminate the infection (Aspinall *et al.*, 2011). Treatment is generally not advised at this stage, but constant monitoring is necessary.

Phase 2. Immune active

The mechanism that switches the immune system from the immune tolerance to the immnune active phase is not yet fully understood. However, this phase is characterised by higher levels of serum ALT, HBeAg, accompanied by moderate to severe liver necroinflammation and rapid progression to fibrosis (EASL, 2012). Chronically infected patients may take more than 10 years to develop liver cirrhosis, immune clearance or HCC. During this phase, the level of HBV replication reduces and HBsAg and HBeAg are being cleared by the immune system. HBeAg Antibody develops and HBeAg is seroconverted (Inoue and Tanaka, 2016). Treatment may be initiated during this phase.

Phase 3. Immune control

After the seroconversion of HBeAg comes the inactive chronic hepatitis phase, which is characterized by low or undetectable serum HBV DNA levels, clearance of HBeAg and normal serum aminotransferase levels (EASL, 2012). Most of the patients have a better outcome, thanks to immune control of the HBV infection. During this phase, the HBsAg is still present in serum, but its clearance

and the production of Anti-HBsAg might occur in about 1 – 3% of cases (Martinot-Peignoux *et al.*, 2002). Treatment may not be recommended, but constant monitoring is required to check for reactivation and HCC.

Phase 4: immune escape phase

This phase is also known as the HBeAg negative chronic hepatitis phase, it comes immediately after the development of Anti-HBeAg and the clearance of HBeAg, or directly from the immune active phase. Persons in this phase have a higher risk of progressive liver disease, cirrhosis and HCC (EASL, 2012). Treatment may be recommended in this phase.

Phase 5: Reactivation

Reactivation may be stimulated spontaneously or by immunosuppressive therapies or chemotherapy. Occult HBV infection (OBI) occurs when the HBsAg cannot be detected in blood but the individual has persistent HBV DNA in the liver. Patients positive for anti-HBc , but who have cleared HBsAg and are negative for serum HBV DNA, are liable to reactivation, when placed on immunosuppressive therapy (Inoue and Tanaka, no date). A better outcome is observed when the HBsAg is lost before the onset of cirrhosis, but if cirrhosis should develop before treatment induced clearance or natural clearance of HBsAg, the patients will be at a higher risk of developing HCC. Treatment is often recommended during this phase.

1.2.6. HBV Treatment

Despite the presence of an effective vaccine against HBV, Hepatitis B virus infection still remains a public health threat. Current treatment strategies are aimed at preventing the progression of the disease to cirrhosis, hepatic failure and Hepatocellular carcinoma. The current drugs used for Chronic Hepatitis B (CHB) treatment are nucleoside/nucleotide analogues (NAs) and pegylated interferon (PEG-IFN).

Pegylated interferon

This is the first treatment that was approved for CHB. It's mechanism of action involves interference with the HBV lifecycle. PEG-IFN therapy induces long-term immunological control with a finite duration of treatment with higher rates of HBeAg and HBsAg loss compared to NAs. Nonetheless, PEG-IFN therapy is less effective in the suppression of viral replication and needs to be administered through subcutaneous injections with adverse effects including flu-like symptoms, myelosuppression, worsening of underlying mood disorders and exacerbation of underlying autoimmune conditions. It is not recommended in patients with pregnancy, decompensated cirrhosis. It is rarely used because of these disadvantages.

Nucleoside/nucleotide analogues

NAs used for the treatment of CHB act by inhibiting viral polymerase/reverse transcriptase, hence suppressing viral replication and decreasing the risk of development of cirrhosis and HCC. CHB treatment is lifelong, it is not curative. FDA currently available approved nucleotide analogues include: tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), and entecavir (ETV). TAF is a prodrug of TDF with less side effects, it was approved in November 2016 by the FDA.

However, the ultimate treatment goal of CHB patients is to achieve cure. What has been achieved so far is "functional" cure, characterized by an undetectable HBV DNA and loss of HBsAg, with or without Anti -HBs. Unfortunately, patients with functional cure still risk HBV reaction due to the presence of cccDNA and viral sequences within the host chromosome. Nonetheless, more research efforts are being directed towards new antiviral agents that target specific viral gene products (direct-acting antiviral agents [DAAs]) and host immune modulatory response (indirect-acting antiviral agent [IDAAs]).

1.2.7. Treatment of HBV in Cameroon

The management of HBV infection in Cameroon turns out to be very much of a challenge and this is partly because it may require long term clinical follow up (by a health specialist) in order to identify those who need treatment. Despite being an HBV endemic country, Cameroon does not yet have a national algorithm or guideline for the management and treatment of HBV infected patients. According to the World Health Organisation (WHO), the following group of people should be treated: (1) Everybody with an Aspartate aminotransferase/platelet ratio (APRI) score >2 (evidence of cirrhosis) irrespective of ALT and HBV DNA levels. (2) Chronically infected adults > 30 years of age with APRI ≤2, persistently abnormal ALT levels and HBV DNA >20 000 IU/mL regardless of HBeAg status. However, the control of viral replication in Cameroon is achieved either with direct acting antiviral therapy (lamivudine, telbivudine, tenofovir, emtricitabine) or indirectly using interferon (IFN) to stimulate immune control(Tufon *et al.*, 2018; Liégeois *et al.*, 2020).

1.3 The Biology of HCV

1.3.1 HCV Origin and Diversity

The hepatitis C virus (HCV) is an enveloped virus, belonging to the Hepacivirus genus in the Flaviviridae family. It belongs to the same family with the yellow fever and dengue viruses. HCV is classed in the Hepacivirus genus with other animal homologs such as the GB virus B (GBV-B), a virus found in housed tamarins (Stapleton *et al.*, 2011). Many other GB viruses (GBV -A, GBV-C

and GBV-D) do not cause hepatitis and are referred to as pegi viruses (Smith *et al.*, 1997; Simmonds, 2001). The HCV was first fully identified in 1975 by Feinstone et al, when they found that most transfusion-associated cases of hepatitis were not as a result of hepatitis A or B, it was then referred to as non-A and non-B hepatitis (NANBH) (Feinstone *et al.*, 1975). The exact origin of the HCV in humans is unclear. However, it is suspected that HCV originated from zoonotic transmissions like HIV-1 from chimpanzees, but as of now, no supporting evidence has been published to support this theory (Simmonds, 2013).

The Hepatitis C virus is extremely diverse, and has been classified into seven main genotypes (1-7) with a nucleotide difference of about 30 - 35% and 67 recognised subtypes, 20 provisional subtypes and 21 unassigned subtypes that differ between 15 and 25% at the nucleotide level over the complete genome (Simmonds *et al.*, 2005; Smith *et al.*, 2014). HCV genotypes are distinctly geographically distributed around the world. Genotypes 1, 2 and 3 are globally circulating, though varying with geographic regions (Messina *et al.*, 2015). Genotypes 1 and 2 are dominant in West Africa, 3 in south Asia, while 4 is prevalent in the central Africa and the middle east and 5 is common in South Africa, and 6 in South East Asia (Smith *et al.*, 1997; Nakano *et al.*, 2004; Pybus *et al.*, 2005). So far, only one genotype 7 has been isolated in Canada from a Central African immigrant (Tanaka *et al.*, 2002). The global distribution of the HCV has greatly been influenced by human migration. In Cameroon, genotypes 1, 2 and 4 have been reported to be prevalent, with genotype 1 being the most frequent (Ndjomou *et al.*, 2002; Ndjomou, Pybus and Matz, 2003; Pasquier *et al.*, 2005).

1.3.2 HCV Epidemiology

Globally, the WHO estimated that there were 71 million people living with chronic hepatitis C in 2016 and 399,000 people died as a result of Hepatitis C related illnesses [mostly cirrhosis and hepatocellular carcinoma (WHO 2019)]. In West and Central Africa, the prevalence of HCV is reported to range from 0.1 – 13.8%, and in Cameroon, the prevalence among adults is reported to be 4.9% but varies among populations and different age groups with the elderly (> 55years) being the most affected with an estimated prevalence of 7.6% (Bigna et al., 2017a; Njouom et al., 2018).

1.3.3 HCV Genome and Structure

HCV is an enveloped virus with a diameter of about 40 – 50nm and contains a genome of approximately 9.6 kilo base (kb) in length, positive-sense single-stranded RNA (+ssRNA) with a single open reading frame (ORF) flanked by two untranslated regions (5'UTR and 3'UTR) that encodes a polyprotein of about 3,000 amino acid, containing three structural (Core, E1 and E2) and seven non-structural(P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins (Figure 11 (Scheel and Rice, 2013).

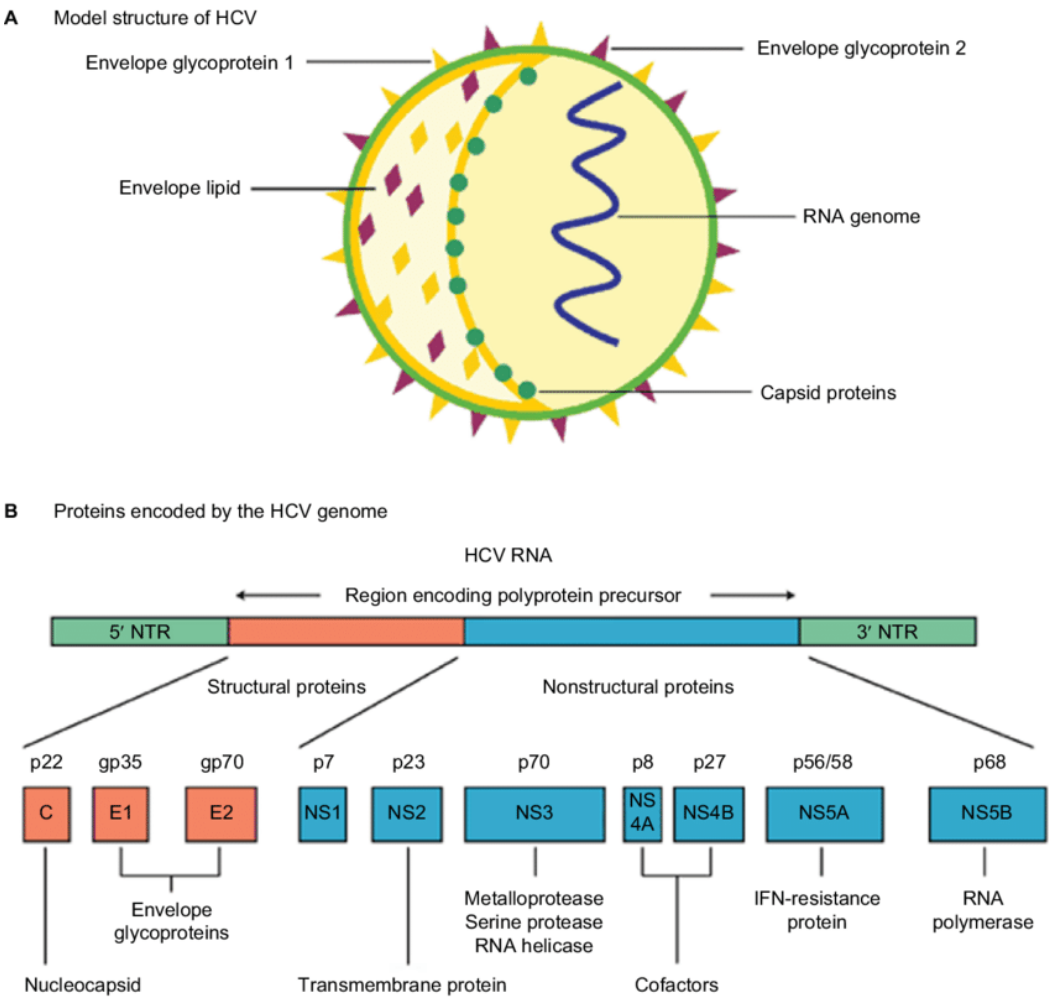


Figure 11: HCV model structure and genomic organisation (Elgharably *et al.*, 2016)

1.3.4 HCV Lifecycle

HCV infects its host in the form of a lipoviral particle via low density and very low-density lipoproteins (LDL and VLDL). The entry of the virus into the cell involves a series of multistep complex processes, involving different host receptors. LDL receptors and glycosaminoglycans are responsible for the low affinity binding, before the interaction and formation of bonds between E1 - E2 with scavenger receptor class B member 1 (SR-B1) and CD81. Claudin, a protein which induces clatherin-mediated endocytosis and Occuldin, a protein whose precise role is unknown are also needed for HCV viral entry. This subsequently leads to target cell entry and the release of the HCV RNA genome into the host cytoplasm through a pH-dependent and clathrin-mediated endocytosis (Blanchard *et al.*, 2006; Tscherne *et al.*, 2006; Alvisi, Madan and Bartenschlager, 2011). So far, some stages of HCV uptake, fusion with the host cell and viral uncoating have not yet been fully elucidated. Once the RNA genome is released, the HCV RNA positive strand is then used to translate the HCV polyprotein in the rough Endoplasmic reticulum. This translation is initiated in a cap-independent manner through the Internal Ribosome Entry Site (IRES) in the 5'NTR. The resulting polyprotein of about 3000 amino acids is then processed by cellular (signalase and signal peptide peptidase) and

viral (NS2-NS3 cysteine protease, NS3-NS4a serine protease) proteases to generate 10 individual viral proteins (core and envelop glycoproteins, E1 and E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Alvisi, Madan and Bartenschlager, 2011; Kim and Chang, 2013). Viral helicase encoded by NS3 facilitates viral RNA processing. The key component in viral replication is the RNA-dependent RNA polymerase (NS5B), in charge of the synthesis of viral RNA. This polymerase lacks proof reading capacity and as such is responsible for the high variability observed in the HCV genome. The other structural proteins also play key roles in HCV replication: NS4B is responsible for the organisation of the viral replication complex, inducing the formation of the Endoplasmic reticulum derived vesicles and NS5A is a regulator of viral replication. A good number of cellular factors have been reported to be involved in HCV replication, such as Cyclophilin A and phosphatidylinositol 4 kinase IIIα. Once the viral proteins are ready the virus is then assembled and released. The viral assembly and release mechanism have not yet been fully understood. Nevertheless, it seems to be closely linked to lipid metabolism. it is likely that viral proteins play a major role in the assembly process, centred around lipid droplets, where assembly is initiated in the membranous lipid rich environment by structural HCV proteins (core, E1, E2, p7 and NS2) and the replication complex (Kim and Chang, 2013).

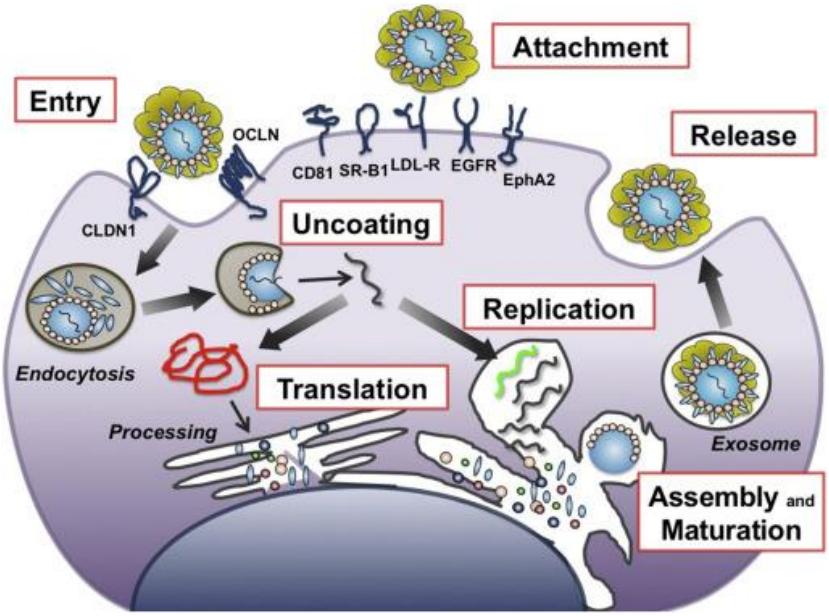


Figure 12: HCV Replication Cycle (Dustin *et al.*, 2016)

1.3.5 HCV Transmission

The Hepatitis C virus is a bloodborne virus and is mostly transmitted through the parenteral route. it is commonly transmitted through infectious blood, blood products, organ and tissue transplants, intravenous drug use (IVDU), tattooing, acupuncture, body piercings, health care exposure (needle stick injury) and it can also be transmitted sexually and perinatally. In the developed world, HCV is

mostly transmitted via intravenous drug use, while in the developing world, poor health standards are still a major cause of HCV transmission. In the past, there have been two significant HCV transmission events, that have led to the spread of a single strain outbreak: one in Ireland and Eastern Germany which resulted from the injection of >2500 women with contaminated anti-D immunoglobulin and the second case was in Egypt where during a schistomiasis treatment campaign where potassium antimony tartarate was administered to people using syringes and needles that were reused without proper sterilisation. This lead to a genotype 4 outbreak in Egypt. Sexual transmission is not seen as a major risk factor of HCV transmission, except in immunocompromised patients. Mother to Child transmission is estimated at 5% with a higher risk in HIV infected women.

1.3.6 Clinical Course of HCV

The HCV virus can cause an acute and/or a chronic infection. Acute infections are scarcely observed and is mostly asymptomatic. in about 20% of cases, symptoms such as fatigue, nausea, fever, abdominal pain, dark urine, pale faeces and jaundice may occur. HCV RNA can be detected within seven to ten days after exposure, followed by HCV specific antibodies, which are detectable within 8 – 20 weeks after exposure. Upon infection, only 15 – 45% of people will spontaneously resolve the infection, while the remainder will move to the chronic stage. Of those that fail to control their initial infection, approximately 20 – 30% will progress to cirrhosis (Lingala and Ghany, 2015). The clinical course of Chronic hepatitis C is greatly influenced by a series of host (age at infection, gender, race, obesity, insulin resistance, diabetes, genetics, ALT levels and exercise), viral (genotype, viral load, co-infections with HBV and/or HIV) and environmental factors (alcohol, smoking, cannabis use, caffeine) (Lingala and Ghany, 2015). Progression of liver disease has been shown to be faster in persons who get infected at an older age (> 40 years) compared to those infected at a younger age (Poynard, Bedossa and Opolon, 1997; Minola *et al.*, 2002; Wright *et al.*, 2003; Pradat *et al.*, 2007).

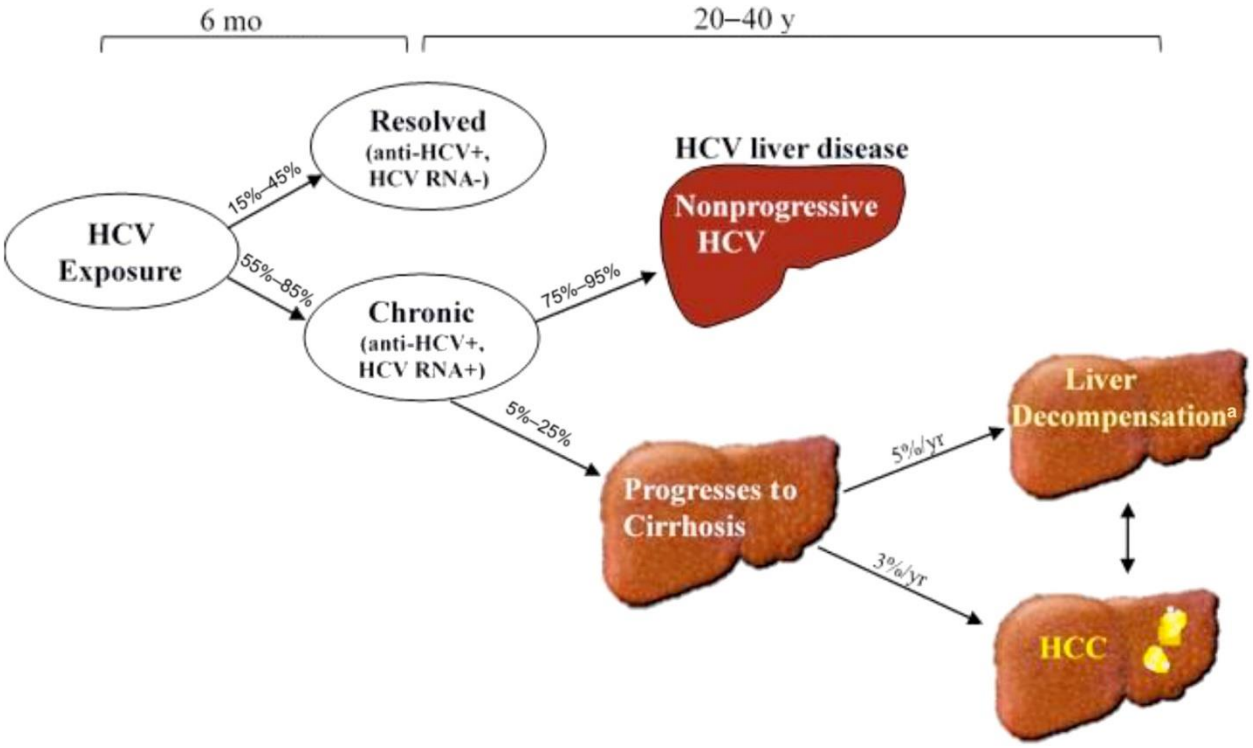


Figure 13: Natural History and Clinical Course of HCV Infection. [a] Liver Decompensation includes: hepatic encephalopathy, esophageal varies, ascites. (Reid, Price and Tien, 2017)

The effect of HCV co-infection on HIV progression remains controversial, HIV infection clearly has an impact on HCV induced disease progression. Firstly, HIV co-infection during acute HCV infection is associated with low rates of HCV clearance. Secondly, HIV-infected individuals have higher levels of viremia. Thirdly, progression to liver fibrosis, cirrhosis and end-stage liver disease is faster in co-infected individuals, liver cirrhosis can decompensate with development of jaundice, ascetism coagulopathy, variceal bleeding and hepatic encephalopathy. A major threat is the development of HCC in patients with cirrhosis due to HCV infection (the incidence of which – 4% per year). Similarly, decompensated liver cirrhosis and HCC are currently the major indications for liver transplantation in Western Europe and the USA. Liver transplantation is an effective treatment for decompensated cirrhosis and for small HCCs. However, hepatitis C recurrence due to graft re-infection is common after transplantation. Although death as a result of end stage liver disease or HCC due to chronic hepatitis occurs in probably less than 30% of all HCV-infected patients, the worldwide epidemic leads to a mortality rate of approximately 350,000 deaths per year. The incidence of HCC and the mortality due to HCV infection will probably increase in the coming decades (Brown, 2005).

1.3.7 HCV Treatment

The Hepatitis C virus infection is currently being treated with Direct-acting antivirals agents (DAAs). These effective, well-tolerated, oral HCV regimens cure HCV in about 95% of HCV-monoinfected persons within a short period of time (about 8 – 12 weeks). The Hepatis C virus treatment is genotype specific and the drugs are often used to combination. Currently, there is no vaccine for the Hepatitis C virus. So far, there are 13 FDA approved HCV drugs, which can be grouped into four classes:

NS5B nucleoside/nucleotide polymerase inhibitors (NS5B-NI): They act by impairing viral replication, through the provision of structurally similar 'false' substrates for the polymerase, resulting in premature chain termination. Sofosbuvir is the only currently used pan-genotypic NS5B-NI, with high efficacy, tolerability and resistance barrier.

NS5B non-nucleoside/nucleotide polymerase inhibitors (NS5B-NNI): They act by binding outside the active site, hence inhibiting the activity of the NS5B polymerase. They are currently used to treat genotype 1, for example dasabuvir (DSV) is specific to HCV genotype 1.

NS5A inhibitors: There are currently six approved NS5A inhibitors: daclastasvir (DCV), ledispavir (LDV), elbasvir (EBR), ombitasvir (OBV), velpatasvir (VEL) and pibrenstasvir (PIB). They are pan-genotypic and show little drug – drug interaction.

NS3/4A protease inhibitors: They were the first DAAs to be approved against HCV. Some are pan-genotypic: voxilaprevir (VOX) and glecaprevir (GLE), while others are specific to particular genotypes, for example simeprevir (SMV), Paritaprevir (PTV), and grazoprevir (GRZ) are specific to genotypes 1 and 4 (Schlabe and Rockstroh, 2018).

Despite the presence of these effective drugs against Chronic Hepatitis C virus infection, there are still areas with very high HCV endemicity. This could be seen as an implementation problem, because most of the people infected with HCV are unaware of their status and being unaware, they continually infect others through sexual intercourse and intravenous drug use. Moreover, the cost of treatment in low-income countries is not affordable to all. However, large scale screening of the general population, particularly in low-income countries and the development of low-cost effective medications can greatly help in the elimination of the Hepatitis C virus in low-income countries like Cameroon.

1.3.8. HCV Treatment in Cameroon

Treatment for chronic HCV infection in Cameroon has undergone significant transformation in the past years with the introduction of direct acting antiviral drugs (DAA). Prior to January 2016, HCV treatment in Cameroon was interferon based. However, the treatment was expensive, and this led to

a low uptake. The Ministry of Public Health and the Cameroon Society of Gastroenterology and Hepatology went into an agreed with a pharmaceutical firm regarding lowering cost with the hope of improving access to screening and treatment. As a result of this agreement, Peg-interferon was subsequently sold at 67% of the market price while ribavirin was provided free of charge. Two treatment centres were created and eligibility criteria for HCV treatment were set(Luma *et al.*, 2018).

Generic genotype specific DAA which have high efficacy, limited side effects and short duration of treatment became available in Cameroon in January 2016. Sofosbuvir is a DAA used for the treatment of chronic hepatitis C, genotypes 1, 2, and 4, circulating in Cameroon, usually in combination with other medications depending on the specific genotype. For the treatment of genotypes 1, and 4 hepatitis C infections, sofosbuvir is used in combination with the viral NS5A inhibitor ledipasvir. For the treatment of other genotypes, sofosbuvir is used in combination with weight-based ribavirin alone in genotype 2 HCV infections. Compared to previous treatments, sofosbuvir-based regimens provide a higher cure rate, fewer side effects, and a two- to four-fold reduced duration of therapy. Sofosbuvir allows most people to be treated successfully without the use of peg-interferon, an injectable drug with severe side effects that is a key component of older drug combinations for the treatment of hepatitis C virus in Cameroon('EASL Recommendations on Treatment of Hepatitis C 2016', 2017; Luma *et al.*, 2018).

1.4 The Immune System

The human immune system has developed over the years to adapt, protect and defend the host against constantly evolving hostile pathogens in the environment. The immune system equally helps eliminate toxins and/or allergens that get into the body through mucosal surfaces. It uses its power to distinguish "self" from "non-self" and possesses effector mechanisms to eliminate pathogens, toxins and allergens from the host (Gonzalez *et al.*, 2011). The immune system from a structural point of view is made up of molecules, cells, tissues, organs and a circulatory system (Charles A Janeway *et al.*, 2001). It can basically be divided into two collaborative arms: the innate and the adaptive immune system, both using the self-nonself discriminative mechanisms to identify and eradicate a plethora of pathogens. The innate immune response provides the first line of rapid defence and is known to have three functions: host defence in early stages of infection through non-specific recognition of pathogens, induction of an adaptive immune response and the determination of the type of immune response; while the adaptive immune system is responsible for immunological memory and provides a stronger and more specific response (Janeway and Medzhitov, 2002). Nevertheless, aberrant

immune reactions also occur and are responsible for auto immune diseases, immunopathologies, chronic inflammations and allergic reactions.

1.4.1 Innate Immunity

The innate immune response is the first line of defence against pathogens and is always active and ready to initiate an immune response, it recognizes pathogen-associated molecular patterns (PAMPs), using their pathogen recognition receptors (PRRs) expressed mostly by innate immune cells (Koenderman, Buurman and Daha, 2014). This system works in a non-specific manner without any memory to help prevent the entry of pathogens into the body through physical and chemical barriers, cytotoxic and phagocytic mechanisms, and the complement system. It also activates the adaptive immune system through the synthesis of cytokines and antigen presentation to T and B cells (Charles A Janeway *et al.*, 2001; Carrillo *et al.*, 2017).

The innate immune response can be grouped into two parts, the physical and the bloodborne barrier. The physical barrier consists of the skin, the epithelial cells lining the gastrointestinal, respiratory and genitourinary tracts, as well as lysozyme in tears, saliva and other body secretions. While the bloodborne barrier is made up of hematopoietic cells such as macrophages, monocytes, neutrophils, eosinophils, dendritic cells, mast cells, natural killer cells and natural killer T cells. The cells can be grouped based on their functions into phagocytic cells (macrophages, monocytes, and neutrophils) which engulf foreign particles, cytotoxic cells (basophils, mast cells and eosinophils) which release inflammatory mediators (chemokines and cytokines) charged with recruiting immune cells to infected sites, and natural killer (NK) cells which are capable of mediating cytolysis with the release of perforins and granzymes which initiate apoptosis. In addition to these cellular defences, the innate immune system also has a humoral component which includes complement proteins, C-reactive protein, lipopolysaccharide (LPS) binding proteins, pentraxins, collectins and other antimicrobial peptides including defensins which accelerates the clearance of the infection (Turvey and Broide, 2010). The innate immune system is fast and usually acts within hours, and when it fails to eliminate the pathogen, the adaptive immune system is called upon.

1.4.2 Adaptive Immunity

The adaptive or acquired immune response is antigen-dependent and specific, it generates a highly efficient and specific immune response when the innate immune response is ineffective in clearing the causal pathogens. Unlike the innate immune response, it takes days to weeks to become established, and it has the ability to produce memory cells which permits the host effectuate a rapid immune response when it encounters the pathogen again. Adaptive responses are based primarily on B and T lymphocytes that express highly diverse receptors adapted for the recognition of a large

array of pathogens. The antigen-specific receptors on T and B lymphocytes are encoded by genes which are assembled by somatic rearrangements of germ-line gene elements to constitute complete T cell receptor (TCR) and B cell Receptor (immunoglobulin) genes (Bonilla and Oettgen, 2010). Some components of the innate immune system participate in the activation of the adaptive immune response. Moreover, the antigen-specific cells augment their response by employing innate effector mechanisms to help in the eradication of pathogens. As such, the two systems function in synergy for an efficient clearance of microbes. Adaptive immune response can be grouped into two: humoral and cell-mediated responses. The cells of the adaptive immune system include: Antigen specific T cells, which are activated through the action of antigen presenting cells (APCs) and B cells which differentiate into plasma cells to produce antibodies (Warrington *et al.*, 2011).

The humoral response involves antibody-producing cells, B cells, which are derived from hematopoietic stem cells in the bone marrow and, upon maturation, leave the marrow expressing unique B cell antigen-binding receptors (BCR) that are secreted upon antigen binding and activation of B cell. B lymphocytes make up about 15% of peripheral blood lymphocytes. BCR are dimers of heavy and light immunoglobulin chains, whose major function is to produce antibodies against antigens. B cells can recognize free antigens directly without the help of APCs (Bonilla and Oettgen, 2010; Warrington *et al.*, 2011). Once activated by foreign antigens, B cells proliferate and differentiate into antibody – secreting plasma cells or memory B cells. Plasma cells do not express antigen-binding receptors, while memory B cells continue to express antigen-binding receptors and serve as rapid responders during re-exposure. Plasma cells have a short lifespan and are eliminated via apoptosis once the pathogen is cleared. Five types of antibodies are produced by B cells: immunoglobulins A(IgA1-2), IgD, IgE, IgG1-4, and IgM. IgG is the most abundant and constitutes 80 – 85% of immunoglobulins in the blood and responsible for most of the protection against infections. IgM is the largest immunoglobulin and first antibody to be produced during an infection. It is usually synthesized early in neonates and exists as a pentamer, stabilised by a J chain. IgD primarily serves as an antigen receptor on the surface of B cells, and is found in low concentrations in blood. IgE is common in persons suffering from allergic reactions or from a parasitic infection. Each of these antibodies have distinctive functions and are capable of recognizing and eliminating specific antigens. Antibodies play a key role in containing viral proliferation during an acute infection, but once the infection has established, they are unable to eliminate the virus (Bonilla and Oettgen, 2010; Marshall *et al.*, 2018).

T cells (lymphocytes) are generated from hematopoietic stems cells in the bone marrow, and following migration, mature in the thymus. They express a unique antigen-binding receptor on their membranes, known as the T-cell receptor (TCR), and need the action of APCs (dendritic cells,

macrophages, fibroblast and epithelial cells) for specific antigen recognition. APCs express a group of cell-surface proteins known as the major histocompatibility complex (MHC) on their surfaces. MHC are classified into Class I (also known as human leucocyte antigen [HLA] A, B and C) present on all nucleated cells, or class II (HLA DP, DQ, and DR), found on some cells of the immune system (macrophages, dendritic cells and B cells). MHC class I and II molecules display endogenous and exogenous peptides, respectively. The MHC proteins display fragments of antigens (peptides) when a cell is infected with an intracellular pathogen, like a virus or has phagocytised foreign organisms or proteins. T cells that react with self-antigens undergo negative selections and are eliminated by apoptosis (Warrington *et al.*, 2011). T cells can be grouped into cytotoxic CD8+ T lymphocytes (CTL) cells and CD4+ T helper (Th) cells. When mature T cells come in contact with an antigen, they are activated through the interaction of their TCRs with antigenic peptides bound to its MHC molecules. The circulation of T cells throughout the body via the lymphatic system and the blood stream and their accumulation in lymph nodes increases the chances for the right T cells to meet with an APC carrying the appropriate peptide MHC complex. Once the T cells are activated, they secret cytokines which further control the immune response. When T-cells are presented with antigens, they differentiate into either cytotoxic CD8+ cells or Th cells (CD4+) cells.

CD8+ cells are primarily implicated in the destruction of infected cells. Clonal expansion of cytotoxic T cells produces effector cells which release perforin and granzyme which cause lysis of target cells, and granulysin which induces apoptosis of target cells (Marshall *et al.*, 2018). Once the infection is resolved, most effector cells die and are cleared by phagocytosis. Nevertheless, some of these cells are retained to serve as memory cells during subsequent encounters with the same antigen.

Th cells are not cytotoxic, phagocytic and cannot directly kill infected cells or clear pathogens. However, they serve as a mediator of the immune response, by directing other cells to act. Th cells are activated via TCR recognition of antigen bound to HLA class II molecules. Upon activation, they release cytokines that mediate the activity of many cell types, including the APCs that activate them. APC can induce a series of Th cell responses with the most prominent being Th1, Th2 and Th17. Th1 response is characterized by the production of Interferon gamma INF-γ which initiates the bactericidal activities of macrophages and enhances anti-viral and intracellular immunity to pathogens (Bonilla and Oettgen, 2010). Th1- derived cytokines also contribute to the differentiation of B cells to make opsonizing antibodies that enhances the efficacy of phagocytes. Th2 responses are associated with the release of cytokines (IL-4, 5 and 13), which are implicated in the development of Immunoglobulin E (IgE) antibody producing B cells, alongside the development and recruitment of eosinophils and mast cells which are needed for effective responses against many parasites. IgE antibodies are also associated with allergic reactions. Th 17 cells have been associated with the

production of cytokines of the IL17 family. Once the infection is cleared, the Th cells die, and a few are retained to serve as memory Th cells. Another set of CD4+ T cell, termed regulatory T cells (T reg), participates in the immune response by limiting and suppressing immune responses, and as such may serve to control aberrant responses to self-antigens and autoimmune diseases (Warrington *et al.*, 2011; Marshall *et al.*, 2018).

1.4.3 Natural Killer (NK) Cells

Natural killer cells are derived from the bone marrow and secondary lymphoid tissues (SLTs) including spleen, tonsils and lymph nodes (Scoville, Freud and Caligiuri, 2017). They are effector lymphocytes of the innate immune system and constitute about 5 - 20% of peripheral blood mononuclear cells and are also present in many tissues such as the placenta, peritoneal cavity, liver and uterine mucosa in humans, making them the 3rd largest group of lymphocytes after B and T cells (Langers *et al.*, 2012). Human NK cells can be divided into two functional subgroups based on their surface expression of CD56; CD56(bright) immune-regulatory cells and CD56(dim) cytotoxic cells (Farag and Caligiuri, 2006). They differ from B and T cells in that they do not express receptors that require somatic gene rearrangements to generate specific and diverse receptors, they are rather controlled by a diverse array of germline-encoded inhibitory and activating receptors, most of which are expressed in a stochastic, variegated pattern, resulting in many subsets of functionally different NK cells (Orr and Lanier, 2010).

The activation of NK cells and subsequent lysis of a target cell occurs when the activating signals outweigh the inhibitory signals. The first control is the expression of HLA class I molecules, in fact, downregulation of MHC-I is seen during viral infections or tumour transformation and prevents the binding of inhibitory receptors to the target cell. Parallelly, HLA class I ligands must be expressed on activating receptors for NK cell cytotoxicity to be initiated. NK cells are known to act via one of the following mechanisms: direct release of perforin and granzymes onto target cells, secretion of cytokines (INF-γ and Tumour Necrosis Factor (TNF), inhibition of viral replication and expression of apoptotic ligands (Fas ligand and TNF-related apoptosis-inducing ligand, TRAIL) that engage death receptors on infected cells (Sowrirajan and Barker, 2011; Langers *et al.*, 2012). Moreover, NK cells, through their CD16 receptor (FcγRIIIa, a low-affinity receptor for the Fc portion of Ig-G), also joins with antibody-coated cells to initiate the antibody-dependent cellular cytotoxicity (ADCC) response. NK cells also express TLRs (TLR2, TLR3, TLR7/8 and TLR9), which can recognise pathogen-associated molecular patterns (Sivori *et al.*, 2004).

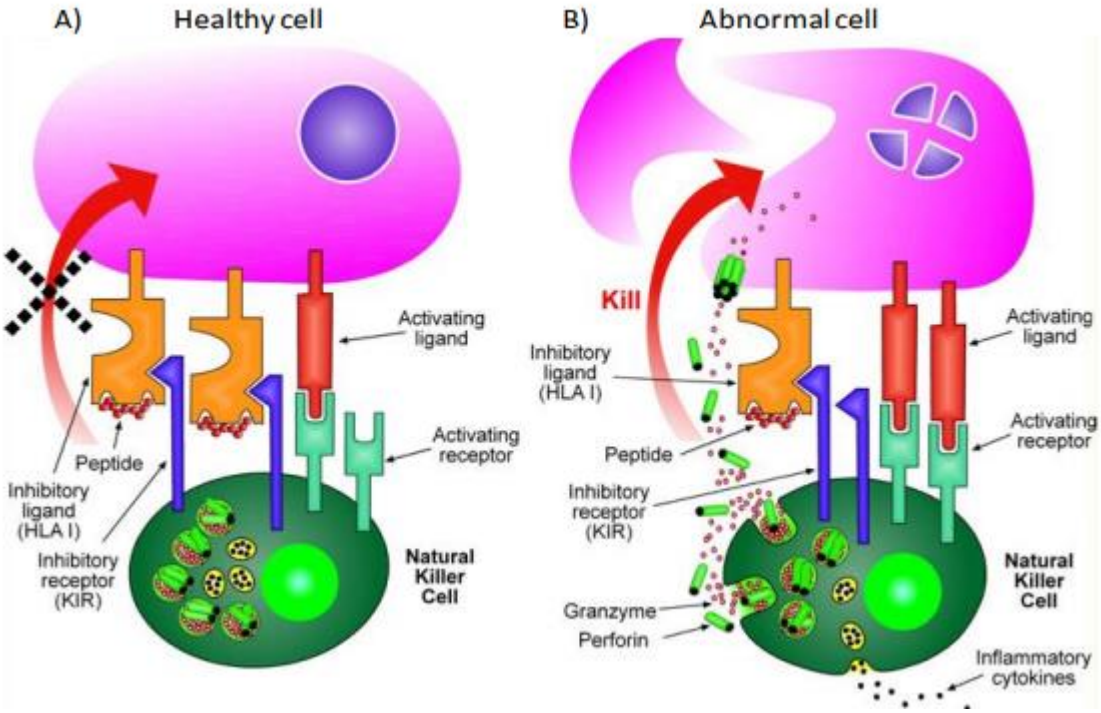


Figure 14: NK cell activity on abnormal and healthy cells. Cells expressing normal levels of HLA class I ligands are resistant to NK cell activity (A), while cells with abnormal levels of HLA class I ligand molecules due to viral infections or tumour transformations are vulnerable to NK cell activity (B) (https://www.immunopaedia.org/, accessed 15 June 2018)

Based on flow cytometry, peripheral NK cells have been defined as being CD3-CD56+, which may be subdivided into three subsets with different phenotypic and functional properties; CD56 bright, CD56 dim, and CD56- NK cells. About 90%, accessed 15 June 2017 of NK cells in healthy persons is made up of CD56 dim NK cells, which express CD16 and KIRs (Caligiuri, 2008). These NK cells have strong cytotoxic activity but a limited ability to secret cytokines. While the CD56bright NK cells which do not express CD16 and KIRs are dominant in the lymph nodes, this subset has a high proliferation capacity and the ability to secret large amounts of proinflammatory cytokines, hence might be involved in immunoregulation. Moreover, an expanded subset of NK cells (CD56-CD16+) has been reported in chronic viral infections like HIV (Mavilio *et al.*, 2003). These NK cells do not react to stimulation with HLA class I -lacking target cells, despite the presence of KIRs, indicating that they represent an exhausted/anergic subset of NK cells. Another special set of NK cells is found in the uterus, known as uterine NK (uNK) cells, they represent about 70% of the leucocyte present in the first trimester of pregnancy (Mavilio *et al.*, 2005). They have been defined as CD56 super bright and CD16- cells, which do not express KIRs, NKG2c and NKG2E (Jost and Altfeld, 2013).

1.4.4 NK Cell Receptors

The activity of NK cells is strictly regulated by a sophisticated array of germline encoded activating and inhibitory receptors. Genes encoding NK cell receptors are clustered within two main gene complexes: the natural killer complex (NKC) encoding C-type lectin-like molecules and the Leucocyte receptor complex (LRC), encoding the immunoglobulin-like receptors (Orr and Lanier, 2010; Li and Mariuzza, 2014). The NK cell receptors include: killer immunoglobulin-liker receptors (KIRs), Killer cell lectin-like receptors (KLRs) such as CD94/NKG2, leucocyte immunoglobulin-like receptors (LILR and natural cytotoxic receptors (NCR) such as NKp46, NKp44, NKP30 and 2B4 (Rajalingam, 2012). These receptors have been reported to identify both viral and cellular ligands, including MHC, MHC-like and non-MHC molecules. The action of NK cells is mediated by a balance between the signals generated by these receptors when they come in contact with a target cell. A dominant activating signal induces cell lysis while a dominant inhibitory signal impedes cell lysis. Healthy cells usually express a large number of inhibitory ligands (HLA class I) and are hence resistant to NK cell attack. When HLA class I expression is downregulated in virally infected or transformed cells, the inhibitory signal on the NK cells is reduced and the NK cell is activated leading to elimination of the target cells. Moreover, NK cell lysis may also be induced by the up-regulation of stress-induced proteins, MHC class I chain-related molecules (MICA and MICB) as a result of a viral infection or tumour transformation, this is known as "induced self", or when HLA class I molecules present foreign peptides, termed "altered self" recognition (Rajalingam, 2012). In addition, the "missing-self" hypothesis failed to fully explain the absence of NK cell autoreactivity in MHC class I deficient cells. Some studies reported the failure of NK cells to kill MHC -I deficient cells and their self-tolerance. Hence the "licencing" hypothesis was proposed, in which NK cells need to be educated to obtain their full effector function (Raulet and Vance, 2006). In this hypothesis, NK cells had to come in contact with self-MHC in order to be responsive to subsequent signals received through activating receptors, thus tuning their optimal NK cell reactivity threshold. However, if self-MHC is absent, NK cells would stay unlicensed and hence hyporesponsive. This mechanism was further adapted to the "arming/disarming" model which suggested that the major factor in the adaptation of NK cell reactivity was in the duration of stimulation. Based on this model, an acute downregulation of MHC-I would lead to the chronic activation of NK cells and induce their hyporeactivity (Jaeger and Vivier, 2012). Nonetheless, more studies are still needed to unravel the diversity of the mechanisms regulating NK cell activity. Individuals inherit a full complement of these receptors, but not all are expressed on every NK cell. Moreover, due to the variability in the affinity and number of self MHC-I inhibitory receptors expressed, the strength of the educating signal varies from one cell to another (Brodin *et al.*, 2009; Sternberg-Simon *et al.*, 2013). KIRs are the most

polymorphic of NK cells receptors and are key to the control and development of NK cell activity (Rajalingam, 2012). KIRs recognize a diverse array of polymorphic HLA class I ligands that have rapidly evolved to mount immune responses against evolving viruses.

1.4.5 Killer Cell Immunoglobulin-like Receptors (KIRs)

KIR molecules are glycoproteins belonging to the immunoglobulin superfamily. They are expressed on NK cells and some T lymphocytes (Kuśnierczyk, 2013). KIR genes span a region of 100 – 200 kb and are arranged in a "head-to-tail" cluster within the Leucocyte Receptor Complex (LRC) on chromosome 19 (19q13.4) (Trowsdale *et al.*, 2001). So far, 15 distinct KIR gene loci (KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1/S1, KIR3DL2, KIR3DL3) and two pseudogenes (KIR2DP1 and KIR3DP1) have been identified. KIR genes can be functionally grouped into two, activating (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KRI2DS5, and KIR3DS1) and inhibitory receptors (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, 3DL1, 3DL2 and 3DL3) with respect to the number of extracellular domains, the length of their cytoplasmic tail and the composition of their transmembrane region. Activating KIR molecules are made up of two or three extracellular domains (2D or 3D), a short (S) cytoplasmic tail and a positively charged residue in the transmembrane region. On the other hand, inhibitory receptors are composed of two or three extracellular domains (2D or 3D), a long (L) cytoplasmic tail with at least one immune-tyrosine-based inhibitory motif (ITIM).

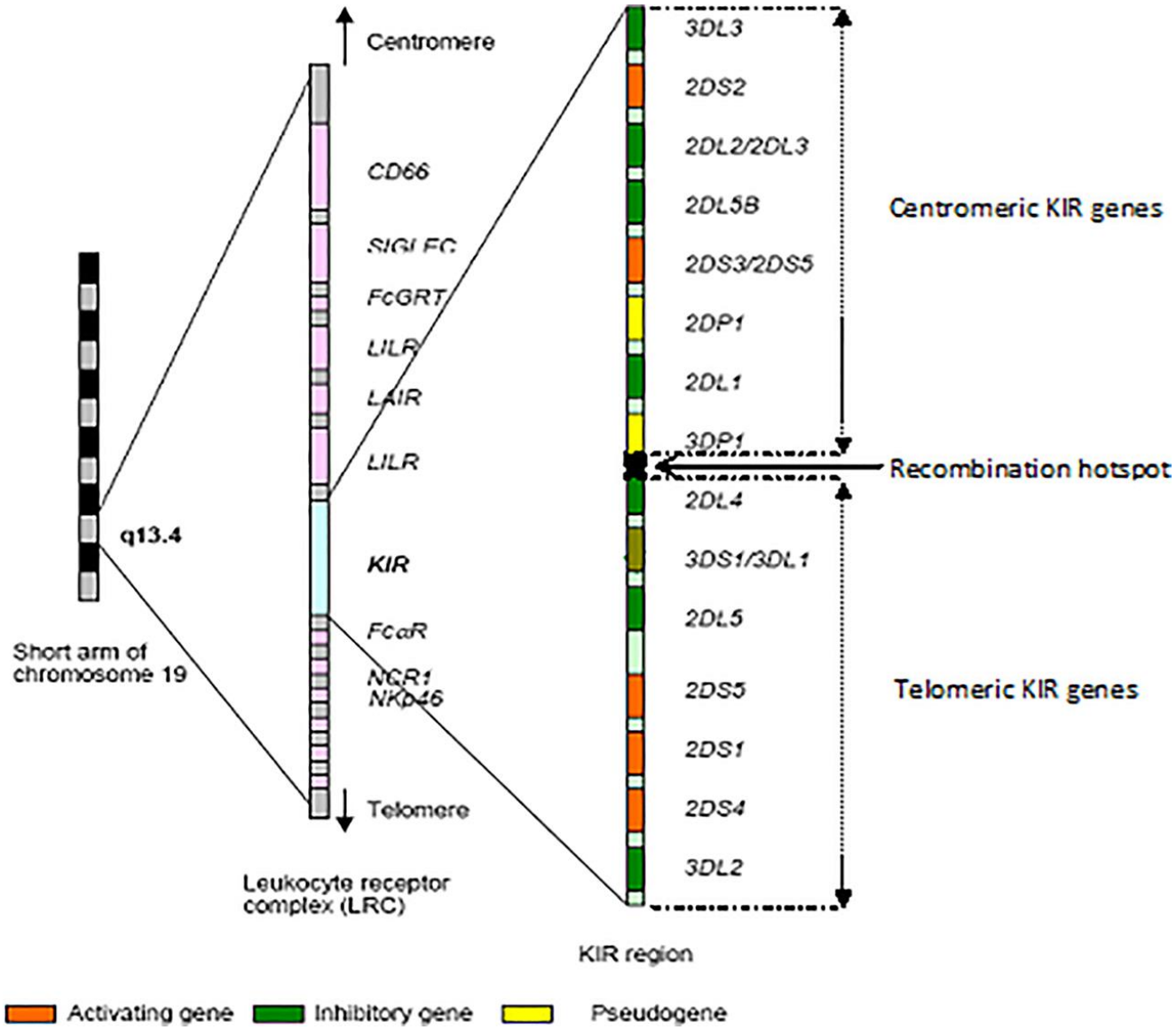


Figure 15: KIR genes within the leucocyte receptor complex (Yindom *et al.*, 2017b)

1.4.6 KIR Nomenclature

KIR genes are named according to the structures of the molecules they encode. Each name has five main compartments. The KIR acronym which represents "Killer-Cell Immunoglobulin-like Receptor); the first digit following the KIR acronym stands for the number of immunoglobulin-like domains in the molecule; 'D' stands for 'domain"; After the D comes either an 'L', denoting a 'Long' cytoplasmic tail, or an 'S' denoting a 'Short' cytoplasmic tail or a 'P' for a 'pseudogene' (Vilches and Parham, 2002). The last digit represents the number of the genes encoding a protein with this structure. When two or more genes have very similar structures and very similar sequences, they may be given the same number but distinguished with a final letter, for example *KIR2DL5A* and *KIR2DL5B* genes (Middleton and Gonzelez, 2010).

KIR alleles are named in a similar manner to HLA alleles. Immediately after the gene name is an asterisk "*" used as a separator, then comes a numerical designation. The first three digits of this numerical designation are used to represent alleles that differ in the sequences of proteins encoded; the next two digits are used to differentiate alleles that differ by synonymous differences within the exons. Two last digits are then added to distinguish between alleles that differ by nucleotide substitutions in the non-coding regions. Example KIR2D*0030202

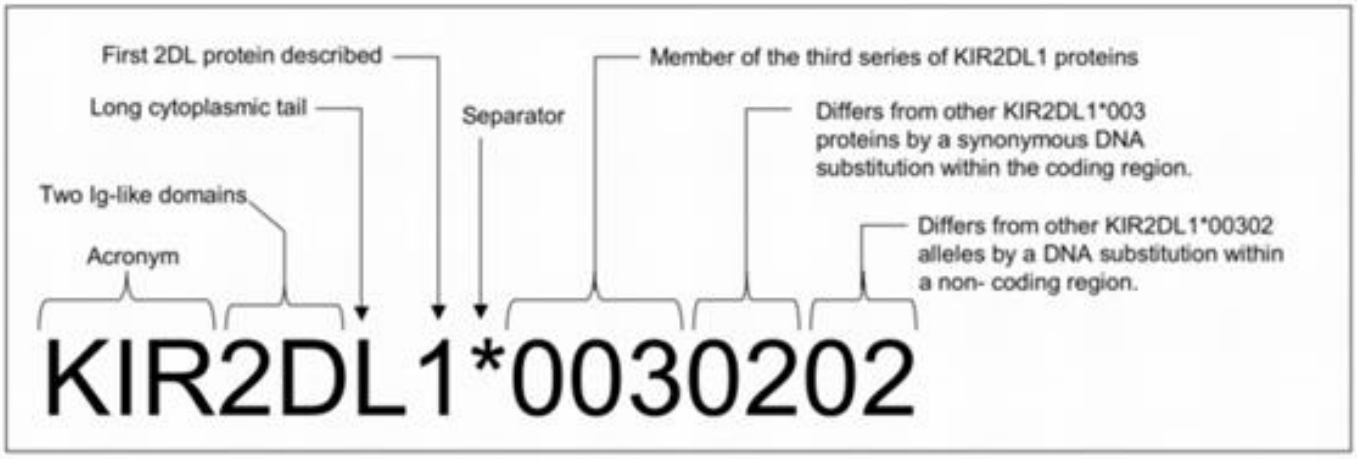


Figure 16:KIR Allele Nomenclature (https://www.ebi.ac.uk/ipd/kir/alleles.html)

1.4.7 Structure of KIR Genes

KIR genes contain between four to nine exons, and can be structurally divided into two basic groups with two (KIR2D) or three (KIR3D) extracellular immunoglobulin-like domains. KIR2D genes scan be further divided into type I KIR2D, that encode for receptors with two (KIR2D) extracellular immunoglobulin-like domains with D1 and D2 conformations and type II KIR2D genes, which encode for two extracellular domain proteins with a D0 and D2 conformation. While the KIR3D genes encode for three extracellular Ig-like domains (D0, D1 and D2). Type I KIR2D genes (*KIR2DL1 – 3*, *KIR2DS1 - 5* and *KIR2DP1*) are made up of eight exons and also a pseudoexon 3. Type II KIR2D genes (*KIR2DL4* and *KIR2DL5*), have a translated exon 3 and a deleted exon 4. *KIR2DL4* has an exon 1 which is 6 nucleotides longer than that of *KIR2DL5* and it contains an initiation codon which is different from those of the other KIR genes. KIR3D genes (*KIR3DL1*, *3DS1* and *3DP1*) have nine exons but vary in the length of exon 9. *KIR3DL3* does not contain exon 6, while *KIR3DP1* lacks exons 6 to 9, at times exon 2 (Rajalingam, 2012).

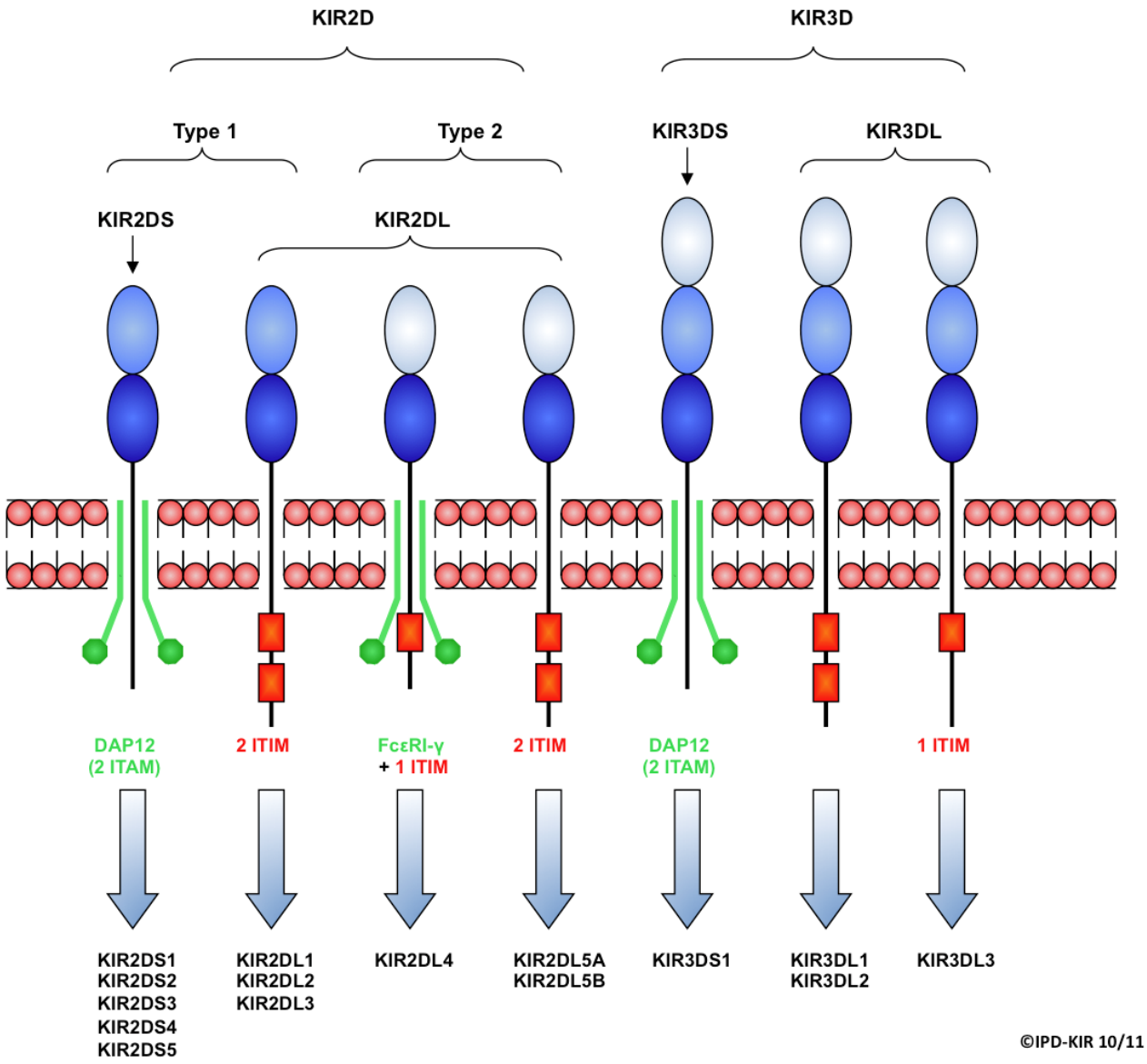


Figure 17: Structures of KIR Proteins with two and three immunoglobulin like domains corresponding to various activating and inhibitory KIRs (https://www.ebi.ac.uk/ipd/kir/alleles.html)

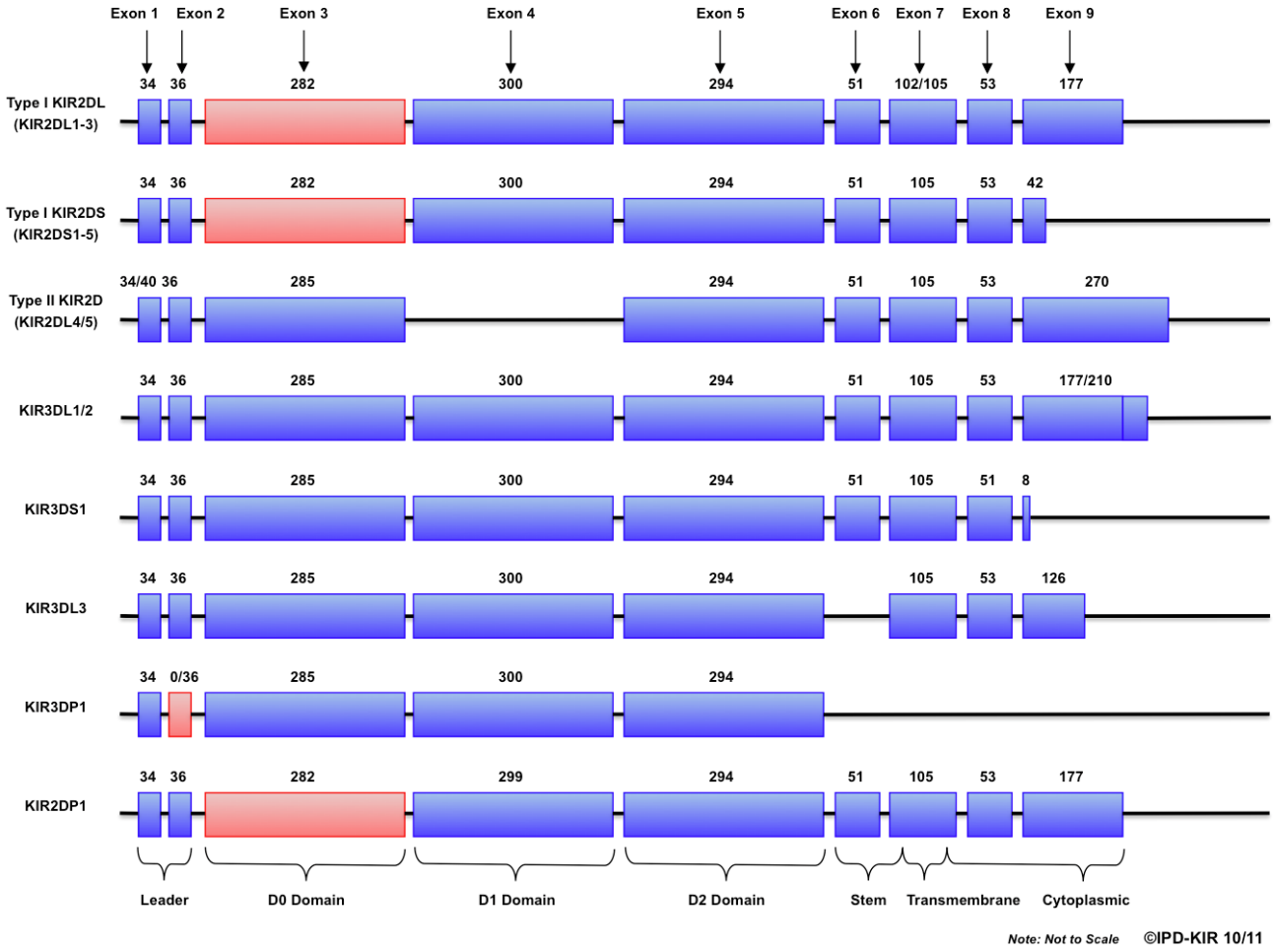


Figure 18: Genetic organisation of KIR genes with the coding regions of the exons in blue and the pseudoexon 3 and the deleted exon 2 of *KIR3DP1* in pink (https://www.ebi.ac.uk/ipd/kir/alleles.html)

1.4.8 KIR Gene Diversity

KIR genes are classified into two broad haplotypes, known as haplotype A and B, with variations in number and type of KIR genes present. Each haplotype contains four framework genes (*KIR2DL4*, *KIR3DL2*, *KIR3DL3* and *KIR3DP1*), which are present in almost all individuals. Group A haplotypes are made up of nine genes, all four framework genes plus *KIR2DL1*, *KIR2DL3*, *KIR3DL1*, *KIR2DS4* and *KIR3DP1*. Group A haplotypes are generally non-variable, while the Group B haplotypes show a lot of variation in the number and combination of KIR genes present, over 40 distinct B haplotypes have been identified so far. Group B haplotype consist of several activating KIR genes and inhibitory genes. Hence, individuals can be grouped into A/A genotype (homozygous for A haplotypes) or A/B genotype (heterozygous). Every KIR haplotype is a combination of a centromeric and a telomeric KIR gene motifs. The entire KIR region can be divided into two: a centromeric and a telomeric half, based on the framework KIR genes present in all haplotypes (Pyo et al., 2010). *KIR3DL3* and

KIR3DP1 mark the centromeric region, while *KIR2DL4* and *KIR3DL2* mark the telomeric region (Cooley *et al.*, 2010a).

Within the centromeric half, *KIR2DL2* and *KIR2DL3* segregates as alleles of a single locus, can be designated as *KIR2DL2/3*, while *KIR3DL1* and *KIR3DS1* (*KIR3DL1/S1*) behave as alleles of the same locus within the telomeric half. Virtually all haplotypes will contain either *KIR2DL2* or *KIR2DL3*, and *KIR3DL1* or *KIR3DS1* within the KIR genome. *KIR2DL1, 2DL2, 2DL3* and *2DS2* are unique to the telomeric half, while, *KIR3DL1, KIR3DS1, KIR2DS1* and *KIR2DS4* are specific to the telomeric half, and *KIR2DL5*, *2DS3*, and *2DS5* are present in both halves. The KIR genes show significant linkage disequilibrium within each half, but much less for genes in the two different halves. Group A haplotype diversity is basically associated with allelic polymorphism, while the group B haplotype show greater diversity in gene content exhibiting moderate allelic polymorphism (Rajalingam, 2012).

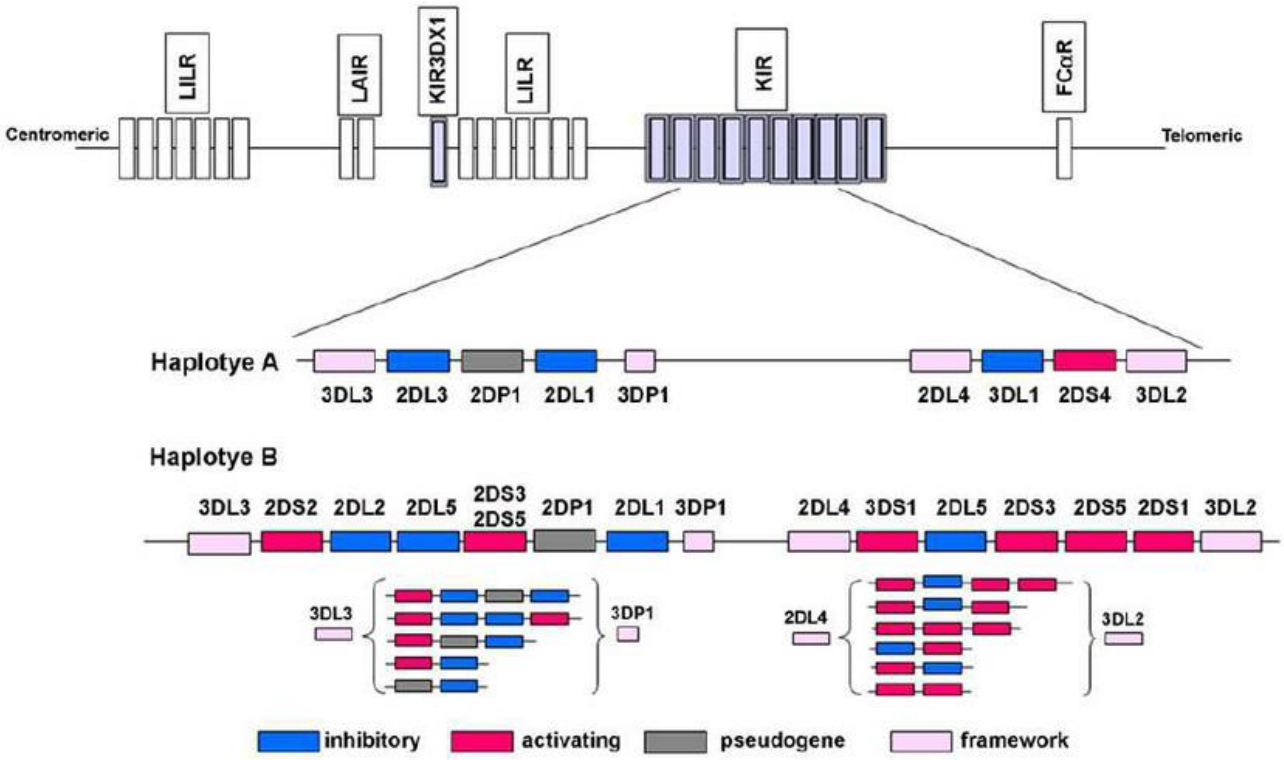


Figure 19: Organisation of KIR genes showing the centromeric and the telomeric motifs separated by the recombination hotspot. (Kulkarni, Martin and Carrington, 2008a)

In addition to allelic and haplotypic diversity, the number of copies of each KIR gene carried by an individual may also vary. Copy number variation (CNV) is likely to influence mRNA transcription. CNV of *KIR3DL1* and *KIR3DS1* have been reported to be immunologically advantageous. In the case of HIV-1, Individuals with higher copy numbers of *KIR3DS1* have been reported to have higher levels of expression of KIR3DS1, and correlated with the inhibition of viral replication. Whereas in

the case of HCV, individuals with two copies of *KIR2DL3* have been associated with an increase in HCV clearance compared to those with one or no copy. As of the 16th of December 2020, 1110 KIR alleles and 660 different KIR genotypes have been recorded on the IPD-KIR Database (http://www.ebi.ac.uk/ipd/kir/stats.html) (release 2.10.0) and the Allele Frequencies KIR database (http://www.allelefrequencies.net/kir6001a.asp), respectively.

1.5 Human Leucocyte Antigen (HLA) Complex

In humans, the major histocompatibility complex (MHC) proteins are referred to as Human Leucocyte Antigens (HLA). The MHC system was first observed in mice that rejected transplanted organs, and later described in human patients who presented antibodies against antigens expressed on leucocytes after receiving multiple blood transfusions in 1954 by Jean Dausset and Jan Van Rood and were referred to as the human leucocyte antigens (Thorsby, 2009). This complex governs the expression of cell surface proteins. The HLA genes are located on the short arm of chromosome 6, namely at position 6p21.31. They are one of the most polymorphic genes known to man. In the past, serological methods were used to characterise MHC antigens in humans and mice, but with the development of molecular biology, affordable DNA based techniques are currently being used to identify MHC genes. This has led to an exponential increase in the number of HLA alleles identified in different populations and ethnic groups in the World.

The MHC is divided into three regions based on the structure and function of their gene products: class I, class II and class III, containing several genes implicated in immunity. The HLA class I regions consist of genes encoding for classical (HLA-A, -B, and -C) genes, whose main function is the presentation of endogenous peptides to CD8+ T cells, and non-classical (HLA-E, -F, and -G) genes. The class II region contains genes coding for HLA-DRA, DRB, DQA1, DQB1, DPA1, DMA, DMB and DOB molecules, whose function is to process exogenous peptides for presentation to CD4+ helper T cells and lastly, the class III genes are not related to HLA class I and II genes, but contains genes which code for proteins implicated in immune regulation such as Tumor Necrosis Factor (TNF), complement factors C3, C4, C5 and heat shock proteins (Beck and Trowsdale, 2000).

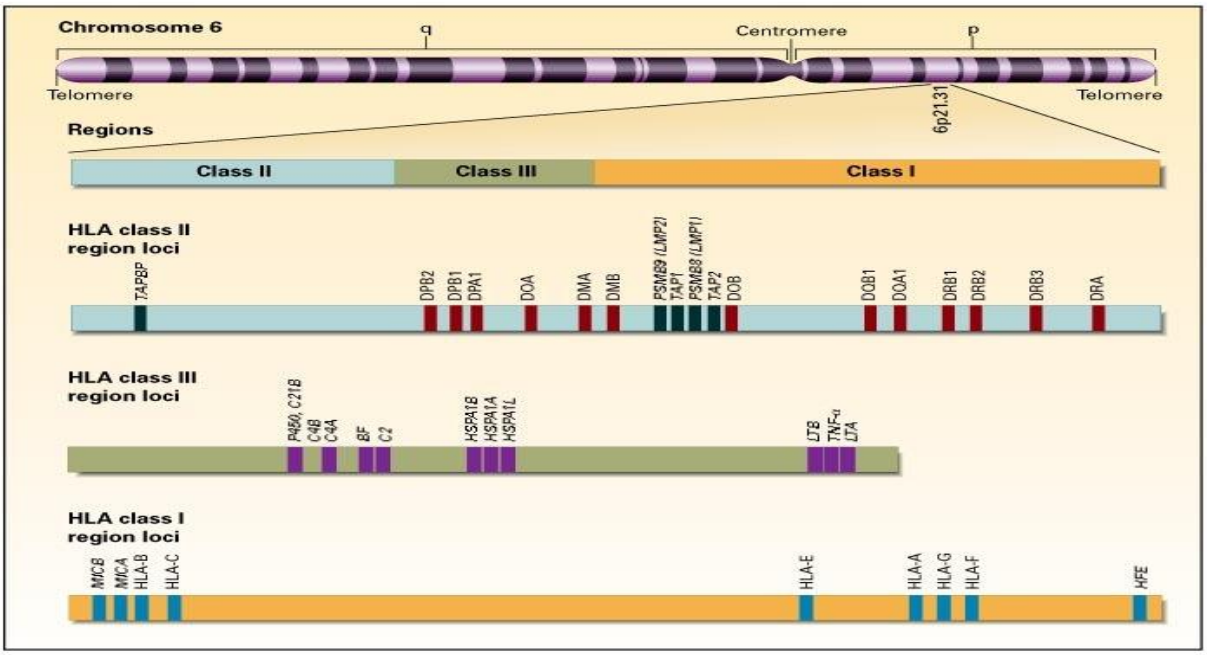


Figure 20: The HLA Region on Chromosome 6p21.31. HLA Class I genes are found at the telomeric end while the class II genes are at the centromeric end and in-between the class I and II genes are the class III genes. (Fernando and Vyse, 2011)

HLA class I and II antigens are differentially expressed on different subsets of human cells as shown on Table 2 below. HLA class I antigens are expressed on all nucleated cells and platelets (apart from those of the central nervous system), while the class II antigens are expressed on antigen presenting cells (APC) like B lymphocytes, macrophages, monocytes, dendritic cells, endothelial cells, Langerhans cells, and thymic epithelial cell (Abbas et al., 2012).

Table 1: HLA class I and II molecules with differential expressions on human cells

Cell type	HLA class I	HLA class II
B cells	+++	+++
T cells	+++	+/-
Neutrophils	+++	-
Macrophages	+++	++
Other APCs	+++	+++
Thymic epithelial cells	+	+++
Brain cells	+	-
Hepatocytes	+	-
Kidney cells	+	-
Red blood cells	-	-

1.5.1 HLA Class I Molecules

HLA class I molecules are present on nucleated cell surfaces, where they present diverse intracellular peptides to receptors expressed on circulating antigen-specific CD8+ T lymphocytes. The HLA class I molecule is a heterodimer, made up of two non-covalently linked polypeptide chains: a highly polymorphic heavy α-chain, encoded by the classical class I genes and one stabilizing light β2-microglobulin chain, a protein encoded by a non-HLA gene located on chromosome 15.

The heavy α-chain (44 to 47kDa) is made up of five distinct domains: the extracellular domains (α1, α2, α3), the transmembrane region containing hydrophobic amino acids which the molecule uses to anchor itself to the cell and a cytoplasmic tail. Two extracellular domains α1 and α2 fold in a special manner to form the peptide binding cleft, which captures and holds a short peptide fragment (pathogen or self) of about 8 – 10 amino acids for presentation to cytotoxic T lymphocytes. Different domains of the HLA class I α-chain are encoded by different exons. The leader sequence is encoded by exon 1, the extracellular domains (α1, α2, α3) are encoded by exon 2, 3, and 4 respectively, the transmembrane region by exon 5, the cytoplasmic tail by exon 6 and 7, and the 3'untraslated region by exon 8. The highly polymorphic amino acid residues clustering with the HLA class I peptide binding cleft of the α-chain are encoded by exons 2 and 3. Mutations in these amino acids at the peptide binding cleft influence the ability of peptide binding and T cell recognition.

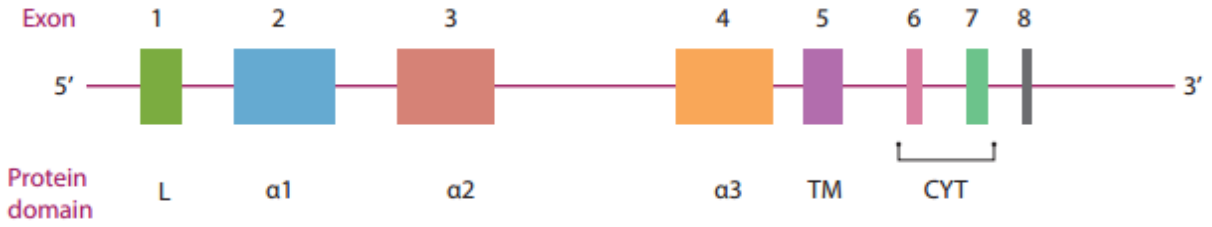


Figure 21:HLA class I Exon-intron organisation. Each domain is encoded by a different exon. Leader sequence (L); transmembrane region (TM), cytoplasmic tail (CYT). (Cruz-Tapias, Castiblanco and Anaya, 2013)

The light (β2-microglobulin) chain is a water-soluble protein weighing about 12kDa, encoded by a monomorphic gene on chromosome 15 and as such is not considered as a member of the HLA family because it is encoded by a gene out of the MHC region. Though not polymorphic as HLA genes, it has been reported to be a good marker of immune activation, with abnormal levels in the plasma and serum of person infected with chronic viral infections such as HIV, HBV, and Cytomegalovirus etc.

Only three (HLA-A, -B and –C) of the over 20 ubiquitously expressed genes within the HLA class I region are reported highly polymorphic. Mutations in exon 2 and 3 are the basis of the polymorphism observed in HLA class I genes. The other non-classical HLA-class I (HLA-E, -F and –G) molecules are less polymorphic.

1.5.2 HLA Class II Molecules

Unlike HLA class I molecules which are ubiquitously expressed on nucleated cells, HLA class II molecules are expressed only on some subsets of immune cells such as on the surfaces of antigen presenting cells (APCs), B cells, activated T-cells, macrophages and dendritic cells. They present extracellular peptides from bacteria, fungi and toxins to circulating CD4+ T cells. The HLA class II heterodimer consist of two subunits encoded by two polymorphic HLA genes, with the α-chain more polymorphic than the β-chain. The two heavy chains are comprised of approximately a 35kDa α chain and a 28kDa β chain, each made up of four domains: Two extracellular domains an α1 and β1 domain, which make up the peptide binding grove and an α2 and a β2 responsible for binding the CD4 coreceptor for the α and β chains, respectively, a transmembrane region and a cytoplasmic tail. The peptide binding grove is capable of accommodating longer peptides of about 14 amino acids. The organisation of exon and introns in the HLA class II gene is similar to that of the class I genes with different exons encoding for domains of the protein. The leader sequence in the alpha and beta chains are encoded by exon 1; α1 and β1 domains are encoded by exon 2 while α2 and β2 domains are encoded by exon 3; the transmembrane region and cytoplasmic tail of the α chains are encoded by exon 4, while for the β chains the transmembrane region is encoded by exon 4 while the cytoplasmic tail is encoded by exons 5 and 6.

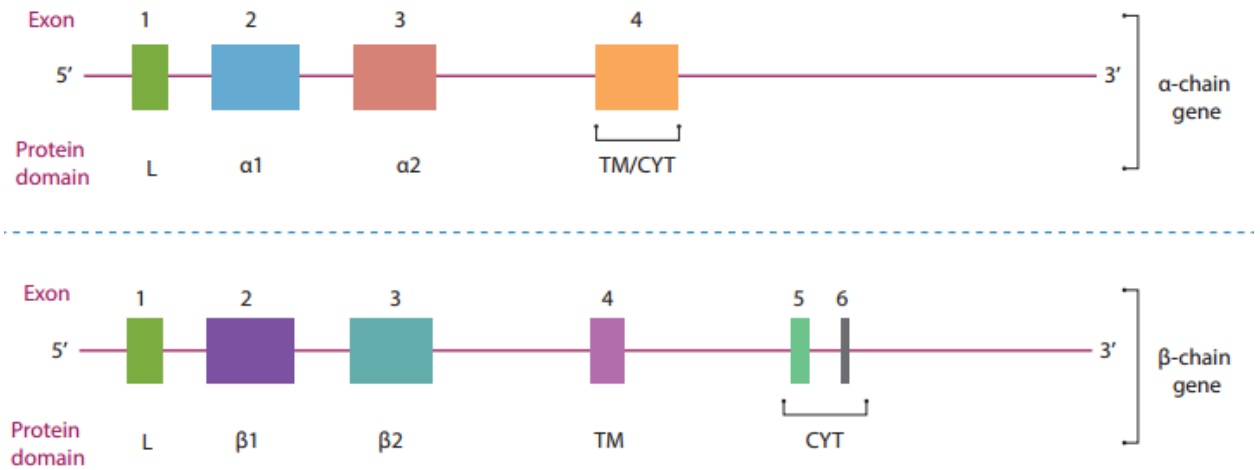


Figure 22: HLA class II chains exon-intron organisation. L: leader sequence, TM: Transmembrane, CYT: Cytoplasmic tail (Cruz-Tapias, Castiblanco and Anaya, 2013)

HLA class II molecules are made up of five isotypes (HLA-DM, -DO, -DP, -DQ and –DR) with each type having a gene that encodes an α chain termed A (HLA-DRA, DQA etc) and one or more genes encoding a β chain termed B (HLA-DQB, DRB etc). The first letter of the HLA class II gene after the HLA for example (D) stands for the class, the second (M, O, P, Q or R) indicates the family and the third letter (A or B) stands for α or β, respectively.

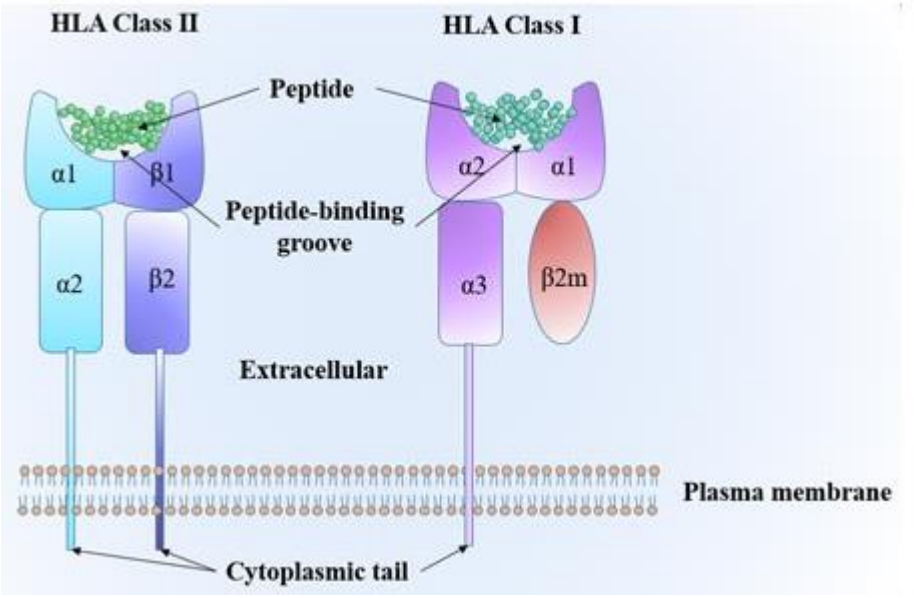


Figure 23: Structure of HLA class I and II molecules (source: https://www.cusabio.com/c-20782.html)

1.5.3 HLA Nomenclature

HLA genes are named and standardized by the World Health Organisation Nomenclature committee for Factors of the HLA system. The nomenclature is based on the typing method used. When the antigens are identified by serology, the HLA name begins with the denomination of the HLA gene locus (e.g. HLA-A, HLA-DR), followed by the numerical identification of the antigen (e.g. HLA-A1, HLA-DR1). The letter 'w' is added when denoting the C locus (HLA-Cw1, HLA-Cw2), to differentiate it from the complement system.

On the other hand, the nomenclature of HLA alleles defined by molecular biology depends on the HLA class and for the class I genes it begins with the acronym 'HLA' followed by the gene name separated by '-' and then followed by an asterisk to defined the method as a molecular biology method, then one to four sets of digits separated by colons. The length of the gene name depends on the allele and its closest relative. The digits before the first colon correspond to the type (allele - family), the second set of digits represent the subtype (the specific protein encoded), the third set of digits is used to indicate a synonymous DNA substitution within the coding region, the last set of

digits is used to show differences in a non-coding region and lastly a suffix may be used to denote changes in gene expression (http://www.ebi.ac.uk/imgt/hla). For example, figure 24 below.

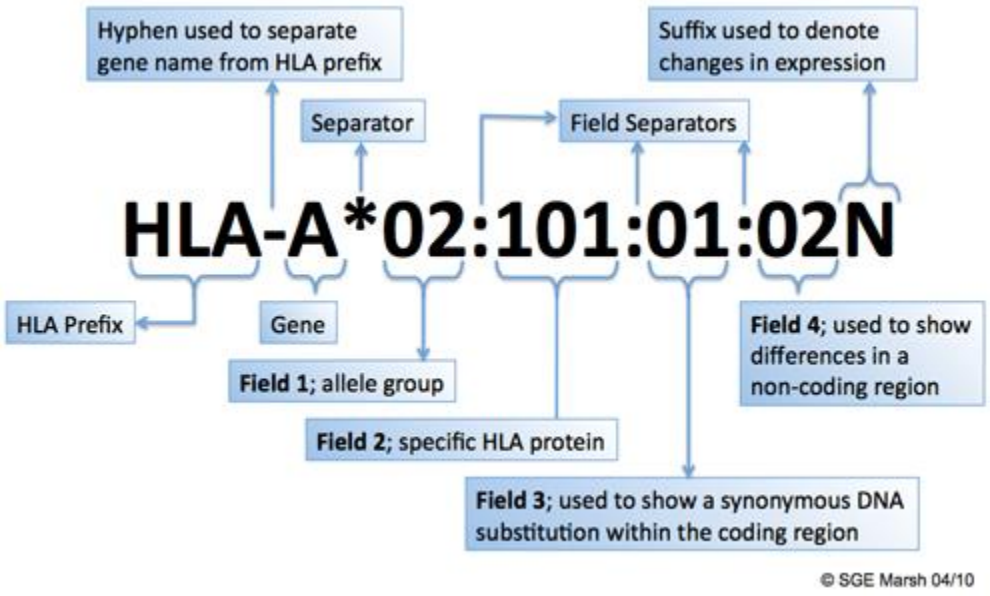


Figure 24: HLA Allele nomenclature (*HLA Nomenclature @ hla.alleles.org*, no date)

When naming the HLA class II genes, after the designation of the locus, the letter 'A' or 'B' is added to indicate the polymorphic α or β chains, respectively of HLA-DR, HLA-DQ and HLA-DP (e.g. HLA-DQA, HLA-DRB, HLA-DPB). Regions with several α and β chains receive a corresponding number e.g. HLA-DRB1, followed by four to eight digits after an asterisk as with the HLA class I genes (e.g. HLA-DRB1*02:01). Other suffixes may be added to indicate the expression status of the allele.

1.5.4 HLA Antigen Processing and Presentation

Antigen processing and presentation are key to immune recognition of self, malignant and infected cells by T cells. Two major pathways have been described for antigen processing and presentation namely: the HLA class I and II pathways.

In the HLA class I pathway, intracellular proteins presented by class I molecules are derived from viruses, mature proteins which have completed their life cycles destined for degradation, proteins synthesized in the cytosol, defective ribosomal products (DRiPs) derived from poorly folded nascent proteins including newly translated products of living cells. These proteins are degraded to small peptides and tagged for degradation by the proteasome; a cytosolic protease that generates peptides of about 7 – 9 amino acids and determines the C-terminus of HLA-I bound peptides (Pamer and Cresswell, 1998; Cascio *et al.*, 2001). The resulting peptides are translocated to the endoplasmic reticulum (ER) by the adenosine triphosphate-dependent transporters associated with antigen

processing (TAP). Once in the ER they can bind to the HLA class I heavy chain and beta 2 macroglobulin (β2m). Once the peptides are loaded in the MHC class I peptide binding groove, they stabilize the MHC complex. Prior to the loading of the peptide, the complex is stabilized by ER chaperones such as Calreticulin, a thiol oxidoreductase (Erp57), and Tapasin. When the peptide bound complex is loaded onto an MHC class I molecule, the chaperones are released. Tapasin interacts with TAP, thereby associating peptide transportation into the ER with peptide loading onto the MHC class I molecules. Once stably assembled, the HLA class I-peptide complex is trafficked to the cell surface through the ER and Golgi network for recognition by specific TCR on antigen-specific CD8+ T cells. HLA class I molecules bind short peptides of about 8 – 10 amino acids, different HLA class I alleles have distinct HLA binding specificities. Peptides and MHC class I molecules that do not take part in the formation of the complex in the ER are relocated to the cytosol for degradation (Neefjes *et al.*, 2011).

Unlike HLA class I molecules, HLA class II molecules are primarily expressed on specialized APCs, such as dendritic cells, B cells and Macrophages. The MHC class II complex presents extracellular peptides to the TCR of CD4+ T cells. The MHC class II α and β chains are assembled in the ER, where they combine with the invariant chain (Ii) to form the MHC class II complex. This complex is translocated to the MHC class II compartment (MIIC), where Ii is digested to give a residual class II-associated peptide (CLIP) in the peptide binding groove of the MHC class II molecule. A chaperone, HLA-DM serves as a facilitator for the exchange of CLIP fragments for antigenic peptides derived from the degradation of proteins in the endosomal pathway. The peptide binding groove of MHC class II molecules is open at both ends permitting it to accommodate longer peptides ranging from 12 to 25 amino acids (Neefjes *et al.*, 2011). Once the peptide is exchanged, MHC class II complex is then transferred to the plasma membrane for presentation to the TCR of specific CD4+ T cells. Moreover, some antigen presenting cells are able to cross-present exogenous peptides on MHC class I molecules. This property is not common, but is serves for the initiation of immune responses against viruses that do not infect APCs (Neefjes *et al.*, 2011).

1.5.5 HLA Polymorphism

The Human Leucocyte Antigen system is the most polymorphic gene cluster known to man. The polymorphism of HLA genes is mostly restricted to the antigen binding groove. Alterations in this groove alter the specificity of HLA molecules. HLA alleles are differentially distributed in different populations and ethnic groups in the world. To date, A total of 28,320 (20,597 HLA class I and 7,723 HLA class II) as shown in Table 2 below have been registered in the IPD/IMGT HLA (IMGT/HLA) database (release 3.42.0of 15th October 2020, https://www.ebi.ac.uk/ipd/imgt/hla.

Table 2: Number of HLA class I and II alleles and proteins identified

Class	Genes	Number of alleles	Number of proteins
I	A	6291	3896
	B	7562	4803
	C	6223	3618
	E	256	110
	F	45	6
	G	82	22
II	DRA	29	2
	DRB	3526	2476
	DQA1	264	114
	DQA2	38	11
	DQB1	1930	1273
	DPA1	216	80
	DPA2	5	0
	DPB1	1654	1064
	DPB2	6	0
	DMA	7	4
	DMB	13	7
	DOA	12	3
	DOB	13	5

The diverse polymorphism observed in different populations might be due to selection pressures exerted by infectious diseases, which lead to the expression of HLA alleles with specific peptide-binding properties. The presence of a variety of HLA alleles in a population, ensures that individuals are able to recognise antigens produced by a large variety of pathogens for immune action.

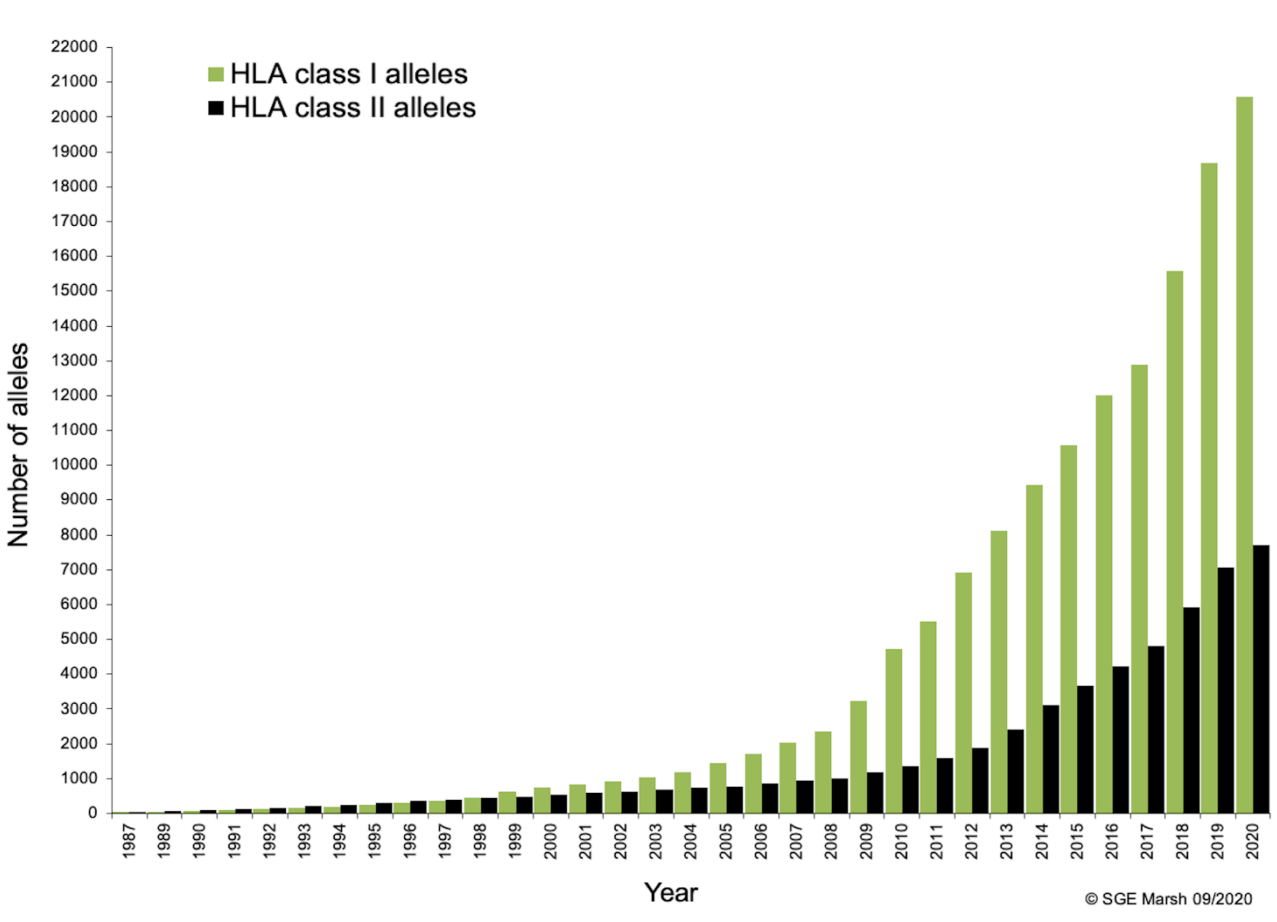


Figure 25: Number of HLA class I (green), class II (black) allele sequences deposited annually in the IMGT/HLA Database (http://hla.alleles.org/nomenclature/index.html). The slope of the line shows the rate of acquisition, which has accelerated in recent years (Robinson *et al.*, 2015).

1.5.6 HLA Class I Ligands

So far, only HLA class I molecules have been identified as ligands for KIR. KIR2DL1, KIR2DL2 and KIR2DL3 recognize HLA-C ligands. HLA-C allotypes are grouped into two (HLA-C1 and HLA-C2) based on a dimorphism at position 80 in the alpha helix. HLA-C1 possesses an asparagine, while HLA-C2 possesses a lysine at position 80. Position 44 in the KIR D1 domain seems to determine the ability of KIRs to discriminate between the two HLA-C allotypes. KIR2DL2/2DL3 bind the HLA-C1 allotypes, while KIR2DL1 binds the HLA-C2 allotype (Winter and Long, 1997). But recent reports show that KIR2DL2/2DL3 weakly bind to HLA-C2 invitro (Moesta *et al.*, 2008). KIR3DL1 binds the HLA-B allotypes with a Bw4 motif (Gumperz *et al.*, 1995), but some low affinity binding with the Bw6 allotype has been reported (Carr, Pando and Parham, 2005). HLA-Bw4 with an isoleucine at position 80 (HLA-Bw4-80I) generally exhibit stronger inhibition through the KIR3DL1, whereas Bw4 allotypes containing threonine at position 80 (HLA-Bw4-80T) seem to be better ligands for some KIR3DL1 subtypes (Luque *et al.*, 1996). KIR2DL4 binds specifically to HLA-G and

KIR3DL2 binds to HLA-A3 and A11. Since the activating ligands KIR2DS1, KIR2DS2 and KIR3DS1 share sequence similarities in their extracellular domains, they are also thought to share HLA ligand binding specificities. KIR2DS1 has been reported to bind weakly with HLA-C2 allotypes, while KIR2DS2 may bind weakly with HLA -C1 ligands. KIR2DS4 interacts with HLA-Cw4 alleles. Some of the ligands have not yet been identified (Kulkarni, Martin and Carrington, 2008b). Interactions of independently segregating KIR and HLA loci are important for NK cell recognition of target cells and 'licencing'. The KIR and the HLA genes are found on chromosomes 19 and 6, respectively. Their presence on different chromosomes makes their inheritance and expression completely independent of one another. Therefore, it is possible for certain KIR, its ligands or both to be absent in a given individual, each of which results to dysfunctionality of the NK cells. Several studies have reported associations between KIR and HLA genes that influence the susceptibility and/or pathogenesis of infectious diseases, autoimmune/inflammatory diseases, cancer and reproduction.

Table 3: KIR genes and their cognate HLA class I ligands

KIR genes	HLA Ligands	Function
2DL1	HLA-C2 alleles	Inhibitory
2DL2 and 2DL3	HLA-C1 alleles	Inhibitory
	Some HLA-C2 alleles (C*02:02, c*04:01 and C*05:01)	
	Some HLA-B alleles (B*46:01, B*73:01)	
3DL1	HLA-A and HLA-B alleles with the Bw4 epitope	Inhibitory
	Some HLA-B alleles (B*08, B*27, B*57 and B*58)	
	Some HLLA-A alleles (A*23, A*24 and A*32)	
3DL2	Some HLA-A alleles (A*03 and A*11)	Inhibitory
2DL4	HLA-G	Activating?
2DS1	Putatively HLA-C2 alleles	Activating
2DS2	Putatively HLA-C1 alleles	Activating
2DS4	Some HLA-C1 alleles (C*01:02, C*14:02 and C*16:01)	Activating
	Some HLA-C2 alleles (C*02:02, C*04:01 and C*05:01)	
	Come HLA-A alleles (A*11:01 and A*11:02)	
3DS1	Putatively HLA-Bw4?	Activating

Ligands for KIR2DL5, KIR2DS3, KIR2DS5, and KIR3DL3 have not yet been determined (Jamil and Khakoo, 2011a; Parham *et al.*, 2012; Wang *et al.*, 2016)

1.6 KIR/HLA Association with Disease

Interactions between KIR and HLA genes have been reported to greatly affect immune response to pathogens. The KIR and HLA loci are highly heterogenous, as such, individuals in various populations display a substantially diverse KIR and HLA repertoire. This diversity gives room for many KIR-HLA combinations, which may potentially endow natural killer cells with variegated abilities to recognize and respond to abnormal cells. A variety of KIR and HLA genotypes have been associated with the susceptibility, resistance, disease progression and clinical outcome of various diseases such viral infections, autoimmune diseases, inflammatory disorders, cancers and reproduction. Some compound genotypes with stronger activation signals have been linked to an increased risk of deleterious effects such as autoimmune diseases and some forms of cancers, and also with stronger antiviral responses leading beneficial effects like slower AIDS progression, protection against HCC in HCV. Whilst some strong inhibitory haplotypes have been associated with an increased risk of detrimental effects like melanoma and preeclampsia and also with some beneficial effects such as the protection and spontaneous resolution of the Hepatitis C virus infection (Kulkarni, Martin and Carrington, 2008c). However, the impact of KIR and HLA genotypes on diseases depend on a fine tune between activating and inhibitory signals.

1.6.1 KIR/HLA in HIV-1 Infection.

KIR/HLA ligands have been shown to demonstrate a series of beneficial and detrimental effects against HIV-1 infection and disease progression. HIV is one of the earliest infections to have shown an association with KIRs. In a study by Martin et al, The activating KIR3DS1 receptor in association with HLA-Bw4 ligand with an isoleucine at position 80 (HLA-Bw480I) was shown to protect against a rapid decline in CD4 T cell count and hence slowing down the development of AIDS (Martin *et al.*, 2002a). Other investigators have demonstrated that this same combination is associated with a delayed onset of opportunistic infections and a slightly lower viral load in HIV infected adults (Martin *et al.*, 2002b; Qi *et al.*, 2006; Jason D. Barbour *et al.*, 2007). Generally, this association has been linked to an enhanced NK cell activity and, subsequently a better antiviral immune response.

Moreover, some viruses down regulate HLA class I expression on the surfaces of their infected cells. Upon entry, they cease the host cell's machinery. This is the case with the HIV-1 Nef protein which has been reported to downregulate the expression of HLA-A and HLA-B, by triggering their retention in the golgi apparatus, in order to escape lysis by CD8+ T cells and NK cells. However, HLA-C whose ligands are the inhibitory KIR2D and CD94/NKG2A are not downregulated (Cohen *et al.*, 1999; Martin and Carrington, 2013). Additionally, in a study by Boulet et al, involving a cohort of seronegative injection drug users, the prevalence of KIR3DS1 was found to be relatively higher

when compared to a matched seropositive cohort, indicating a protective role of KIR3DS1 against HIV in these individuals (Boulet *et al.*, 2008a). Even so,, they did not find any association with HLA-B alleles in their cohort. Yet, Alter an al. showed a significant invitro inhibition of HIV-1 replication in HIV infected HLA-Bw4-80I – positive T cells when cultured with NK cells expressing KIR3DS1 compared to NK cells that did not express KIR3DS1 (Alter *et al.*, 2007). Findings from a study by Long et al, have shown that HIV-1 infected persons with KIR3DS1 demonstrate higher levels of INFγ production, CD107a upregulation, and a diminished CD8+ T cell activation, which are beneficial to the HIV-1 infected persons (Long *et al.*, 2008). The KIR3DL1 allele which segregates from the same locus with the KIR3DS1 has been reported to have a more protective effect against HIV when associated with HLA-B*57 (a HLA-B Bw4-80I allele) by martin and al (Martin *et al.*, 2007a).

Studies have shown that HLA class II alleles have very little effects compared to the HLA class I alleles on the HIV-1 infection. HLA-B alleles have been the most implicated in HIV infections, however, genome wide association studies have implicated the HLA-C in defining HIV "elite controllers" (International HIV Controllers Study *et al.*, 2010). HLA-B*57, B*27, B*58:01, B*51, B*13, and B*81:01 are among the most reported alleles with a protective effect on HIV. Conversely, HLA-B*35Px, B*18:01, B*45:01 and HLA-B*58:02 have been associated with an increased rate of HIV disease progression (Adland *et al.*, 2015; Naranbhai and Carrington, 2017). Moreover, some of the alleles have intermediate effects. While some of the allelic effects have been reproduced across ethic groups, there is probably an effect of age, as proposed by comparing adults vs. children (Adland *et al.*, 2015).

Table 4: Some HLA: KIR genotypes and their effect on HIV

Gene	**Effect**	**Reference**
KIR3DS1	Slower rate of $CD4^+$ T cell decline	(Jason D Barbour *et al.*, 2007)
	Increased INF-ϒ production	(Long *et al.*, 2008)
	Homozygosity- resistance to HIV infection	(Boulet *et al.*, 2008b)
KIR3DS1 + HLA-B Bw4-80I	Slower disease progression	(Martin *et al.*, 2002b)
	Slower progression to opportunistic infection but not malignancy	(Qi *et al.*, 2006)
	Lower viral load	(Alter *et al.*, 2007)
	Inhibition of HIV-1 replication	
	Slower rate of $CD4^+$ T cell decline but faster disease progression	(Gaudieri *et al.*, 2005a)

KIR2DS2/ KIR2DL2	Faster rate of $CD4^+$ T cell decline	(Gaudieri *et al.*, 2005b)
KIR3DL1*h +Bw4-80I	Slower disease progression, lower viral load	(Martin *et al.*, 2007b)
KIR3DL1 +B*57s	Protection against progression to AIDS	(A. López-Vázquez *et al.*, 2005)
HLA-A, HLA-B, HLA-C homozygosity	Faster rate of disease progression	(Carrington *et al.*, 1999; Tang *et al.*, 1999)
HLA-B*35Px	Accelerate disease progression	(Gao *et al.*, 2001)
B*57, B*27, Bw4	Slower disease progression	(Migueles *et al.*, 2000; Flores-Villanueva *et al.*, 2001; Martin *et al.*, 2002c; Gao *et al.*, 2005)
HLA-G*0105N	Protection from HIV-1 infection	(Matte *et al.*, 2004)
HLA-G*010108	Increased risk of infection	
HLA-E*0103	Decreased risk of HIV-1 infection	(Lajoie *et al.*, 2006)
HLA-C*16:01 + KIR2DL3	Higher viral load and lower $CD4^+$ T cell count Rapid progression to AIDS	(Mori *et al.*, 2019)
Maternal KIR2DL2, KIR2DL5, KIR2DS5, KIR2DS2	Reduction of HIV-1 transmission from mother to child	(Omosun *et al.*, 2018)

1.6.2 KIR and HLA in HBV Infection

Natural Killer cells are activated at the earliest stages of viral Hepatitis B virus infection. Increasing evidence have suggested the role of KIR and HLA ligands in HBV infection (Gao *et al.*, 2010). However, very few large cohort HBV studies have been carried out so far to study the impact of KIR and HLA genes in viral Hepatitis B infections. Reports from the studies done so far warrant more studies on the impact of KIR/HLA in HBV infection. However, there is a suggestion that inhibitory KIR may have a protective role against HBV infection (Di Bona *et al.*, 2017). In a study carried out by Lu et al., they found a high frequency of the B haplotype in Hepatitis exposed patients compared to healthy controls, suggesting that the B haplotype may have a susceptible effect to HBV (Lu *et al.*, 2008). Another study done by Gao et al. reported similar results. The KIR2DL3 or 2DL3 homozygote and HLA-C1 homozygosity was reported to be protective, while the KIR2DL1:HLA-C2 was linked with susceptibility to HBV infection in a Chinese population (Gao *et al.*, 2010). Zhi-ming et al, studied a Chinese population and reported that the KIR2DS2, KIR2DS3 genes are

associated with Hepatitis B chronicity, and the KIR2DS1, KIR3DS1 and KIR2DS2 are protective genes accelerating HBV viral clearance (Zhi-ming *et al.*, 2007). Moreover, a study carried out on a Turkish cohort by Kibar et al. demonstrated that the KIR2DL3 and KIR3DS1 genes might also be protector genes for HBV infection (Kibar *et al.*, 2014). A study in West Africa, on Gambians demonstrated that carriers of KIR3DS1 gene had a greater risk of being HBeAg positive and also of having high HBV viral loads, while carriers of the KIR2DL3 gene were associated with lower viral loads. In the same study, it was reported that homozygosity for KIR group A gene-content haplotype was associated with HBsAg carriage, while the telomeric A genotype was associated with reduced risk of e antigenaemia and lower viral loads (Yindom *et al.*, 2017a). In addition, a study done in Bukina Fasso by Sohgo et al, showed that the KIR3DL1, KIR3DL2, KIR2DS1 and KIR2DP1 genes may be associated with immunity against HBV infection, while in the same study, KIR2DL2, KIR2DL3 and KIR2DS2 were associated with HBV chronicity (Sorgho *et al.*, 2018). A study in the South reported that KIR2DS2:C1, 3DS1:Bw4 and 3DL1:HLA-Bw4-I80 compound genotypes are associated with a high-risk of an asymptomatic HBV infection (Kalyanaraman, Thayumanavan and Jayalakshmi, 2016). Furthermore, in a study evaluating the therapeutic outcome of HBV patients, it was shown that HBeAg positive chronic HBV patients with the KIR3DS1:HLA-B Bw480I compound genotype had a better treatment outcome with Interferon alpha (Li *et al.*, 2017).

1.6.3 KIR/HLA in HCV Infection

Many recent substantial research evidences have shown the relevance of interactions between KIR and HLA genes in the outcome of HCV infection. Studies have demonstrated that, in the early stages of the HCV infection, specific KIR and HLA-C pairs are associated with spontaneous clearance of the HCV infection (Khakoo *et al.*, 2004; Cheent and Khakoo, 2011). The compound KIR:HLA genotype KIR2DL3 and its HLA-C group 1 ligands (HLA-C1) alleles have been associated with resolution of the HCV infection and a sustained viral response to antiviral therapy. This was observed in persons homozygous for both KIR2DL3 and HLA-C1, and also individuals exposed to very low inoculate of the virus such as in intravenous drug users compared to those exposed through large amounts of infected blood products (Jamil and Khakoo, 2011b). This observation was also found in a study by Romero et al. involving 160 intravenous drug users as the risk factor of infection (Romero *et al.*, 2008). KIR2DL3 has a low avidity for HLA-C1 than KIR2DL1 or KIR2DL2 receptors. This low avidity of the KIR2DL3:HLA-C1 interaction causes reduced NK cell inhibition, permitting a greater capacity of NK cells to control the infection. Zúñiga et al. also demonstrated that inhibitory receptors in the presence of HLA-C1 and the KIR2DS4 gene confer protection from HCV infection (Zúñiga *et al.*, 2009). Moreover, some studies have reported that individuals with KIR2DL3:HLA-C1 are more responsive to Interferon-α-based therapy as compared to those without. Hence this compound genotype has portrayed several beneficial effects over different cases of HCV exposure

and infection. Studies comparing the patients with spontaneous clearance and chronicity of HCV infections have linked the KIR2DS3 gene with HCV chronicity. The KIR2DL3 and KIR2DS4 genes are found on the "A" haplotype, which has been associated with protective effects against chronic HCV infection. Likewise, the B group of haplotypes are marked with KIR2DL5, and have been associated with a poor response to HCV treatment (Jamil and Khakoo, 2011b). In a study by Vasconcelos et al., the KIR2DS3 alongside KIR2DL2 and KIR2DS2 genes were associated with a chronic HCV infection. In another study by Kysneirczyk et al. it was observed that patients with KIR2DS3+/KIR2DS5- had HCV viremia levels 2.6 times lower than in patients with other KIR genotypes (Kuśnierczyk *et al.*, 2015). This contradicts reports from Podhorzer et al. who showed that KIR2DS3 gene expression was associated with high levels of viral load (Podhorzer *et al.*, 2018). The impact of the KIR2DS3 gene in chronic HCV infection needs to be evaluated in larger ethnically diverse populations. In addition, with respect to disease progression and the development of hepatocellular carcinoma and lymphoproliferative disease, a study on Italian chronic HCV infected patients showed that the KIR2DS3 and the KIR2DS5 genes are protective against disease progression. An increased risk of hepatocellular carcinoma was associated with a decrease of HLA-Bw4 + KIR3DS1 compound genotype in the same cohort (Boyington and Sun, 2002) . Moreover, the HLA-C1 alleles and KIR3DS1 have been reported to be independently protective against the development of Hepatocellular carcinoma in chronic HCV infections (Antonio López-Vázquez *et al.*, 2005). Another study conducted by Podhorzer et al, in Japan showed associations between the KIR2DL2-HLA-C1 and KIR2DS2-HLA-C1 were significantly common in younger patients with HCC. Furthermore, HLA-A*03, B*27, DRB1*01:01, DRB1*04:01 have been strongly associated with HCV spontaneous clearance (Podhorzer *et al.*, 2018).

MATERIALS AND METHODS

2.1. Study Design

This was a cross-sectional study on the molecular characterisation of KIR and HLA genes in HIV-1-, HBV- and HCV-infected individuals in Cameroon with the main aim of providing the KIR and HLA profile in association with the disease status of this sample of people living in Cameroon using multiplex Polymerase Chain Reaction Sequence-Specific Primer (PCR-SSP) and Sequence-Based Techniques (SBT).

2.2. Study Site

This study was carried out in five sites: the Yaounde Central Hospital Day Care Unit for the collection of HIV-1 positive and uninfected samples; the Yaounde University Teaching Hospital for the collection of HCV samples, the Douala General Hospital for the collection of HBV and HCV samples; the Molecular Biology Laboratory of the Chantal Biya International Reference Center for Research on the Prevention and Management of HIV/AIDS (CIRCB), Yaounde, where sample treatment, storage and KIR typing were done; and the Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Oxford, where HLA Class I typing and analysis were done.

At the recruitment sites (the Yaounde Central Hospital Day care unit, the Douala General Hospital and the Yaounde University Teaching hospital), Research students presented the study with an information sheet (Appendix 1) to adult patients consecutively visiting for routine daily consultations. Those who accepted to participate in the study and who fulfilled the inclusion criteria, were requested to sign an informed consent form (Appendix 2). A well-structured questionnaire (Appendix 3) was then used to collect demographic information (age, sex, marital status, level of education and occupation) and some clinical information (viral load, ALAT, ASAT) from the patients records. Once recruited, the patients were directed to a state registered nurse for sample collection.

2.3. Study Period

This study was carried out over a period of 26 months from January 2017 to June 2019.
Sample collection lasted for 6 months, laboratory and data analysis lasted for 2 years

2.4. Study Population

The target population for this study was treatment-naive HIV-1-, HBV- and HCV- infected persons visiting the above-mentioned hospitals in Cameroon.

2.5. Sample Size

No data was found on the frequency of KIR genes in Cameroon. So, the sample size was calculated using the frequency of the *KIR3DS1* and *KIR2DS1* genes from a study done in neighbouring Gabon by Wauquier et al (2010), as well as the frequencies of HLA class I (*A*23:01* and *C*04:01*) from a study done by Torimiro et al (2006) in Cameroon, using the formula for estimating the population proportion p of a large population as stated below:

$$n = \frac{Z^2_{\alpha/2}\hat{P}(1-\hat{P})}{\varepsilon^2}$$

where,

$Z_{\alpha/2}$, normal distribution value = 1.96

$\hat{P}$, relative proportion of gene

ε , precision (sampling error) = 0.05

This gave the sample sizes presented on the table below.

Using the proportion of KIR genes in Gabon				
KIR gene	$\hat{P}$ ***in Gabon***	$Z_{\alpha/2}$,	ε	**Sample size**
3DS1	0.074	1.96	0.05	105.2 ≈105
2DS1	0.185	1.96	0.05	231.6 ≈ 232
Using the proportion of HLA Class I genes in Cameroon				
A*23:01	0.128	1.96	0.05	161.2 ≈161
C*04:01	0.166	1.96	0.05	212.7 ≈213

Given that this was a genetic study involving multiple genes, no appropriate sample size calculation could fit the study properly, as such, based on the calculations, we decided to recruit at least 250 persons to participate in the study. A total of 257 (84 HIV-1+, 68 HCV+, 42 HBV+ and 63 uninfected controls) participants were recruited.

2.6. Ethical Considerations

2.6.1. Administrative Approval

Administrative Approval no 016-1066 UY1/VREPDTIC/DAAC/DEPE/SPD/CRFDSVSE of 13th December 2016 (Appendix 4) was obtained from the University of Yaounde I delivered by the Rector, and Approval No. 1233/017L/CIRCB/DIR/SAA of 7th July 2017 (Appendix 5) from CIRCB delivered by the Director.

2.6.2. Ethical Approval

Ethical clearance was obtained from the Cameroon National Ethics Committee for Human Health Research, No 2017/05/903/CE/CNERSH/SP of 15th May 2017: (Appendix6). Ethical and/or administrative approvals were provided by each of the study sites. Written informed consent was obtained from each of the participants at the time of enrolment.

Participants were recruited in accordance with the declarations of Helsinki in 1964 by the World Medical Association (WMA)(World Medical Association, 2013).

BENEFITS, RISKS, AND DISCOMFORT

Participants were informed that Blood will be taken from them as part of this study. This could cause pain and the participant could get a small wound but no disease will be contracted at the place where blood will be taken. Bleeding could also occur from the place where blood will be taken. The amount of blood taken could not lead to any disease. To ensure the safety of participants, blood samples were collected by trained medical personnel.

There was no special gain for participants in this study. Eligible individuals were informed that taking part in this study will help us to gain more knowledge on the genetic factors that influence susceptibility to HIV, HBV and HCV infections in Cameroon. Nevertheless, the tests were done free of charge.

PRIVACY AND CONFIDENTIALITY

Participants were given anonymous codes, which were used to label the specimens, all personal data collected from the study participants were stored in an electronically password locked excel sheet, that could only be accessed by the lead investigators in the study.

Eligible Participants were informed that they had the right to terminate their participation in this study at any time, and that if they decided to leave, it would not affect their care at the hospital.

2.7. Flowchart of Study

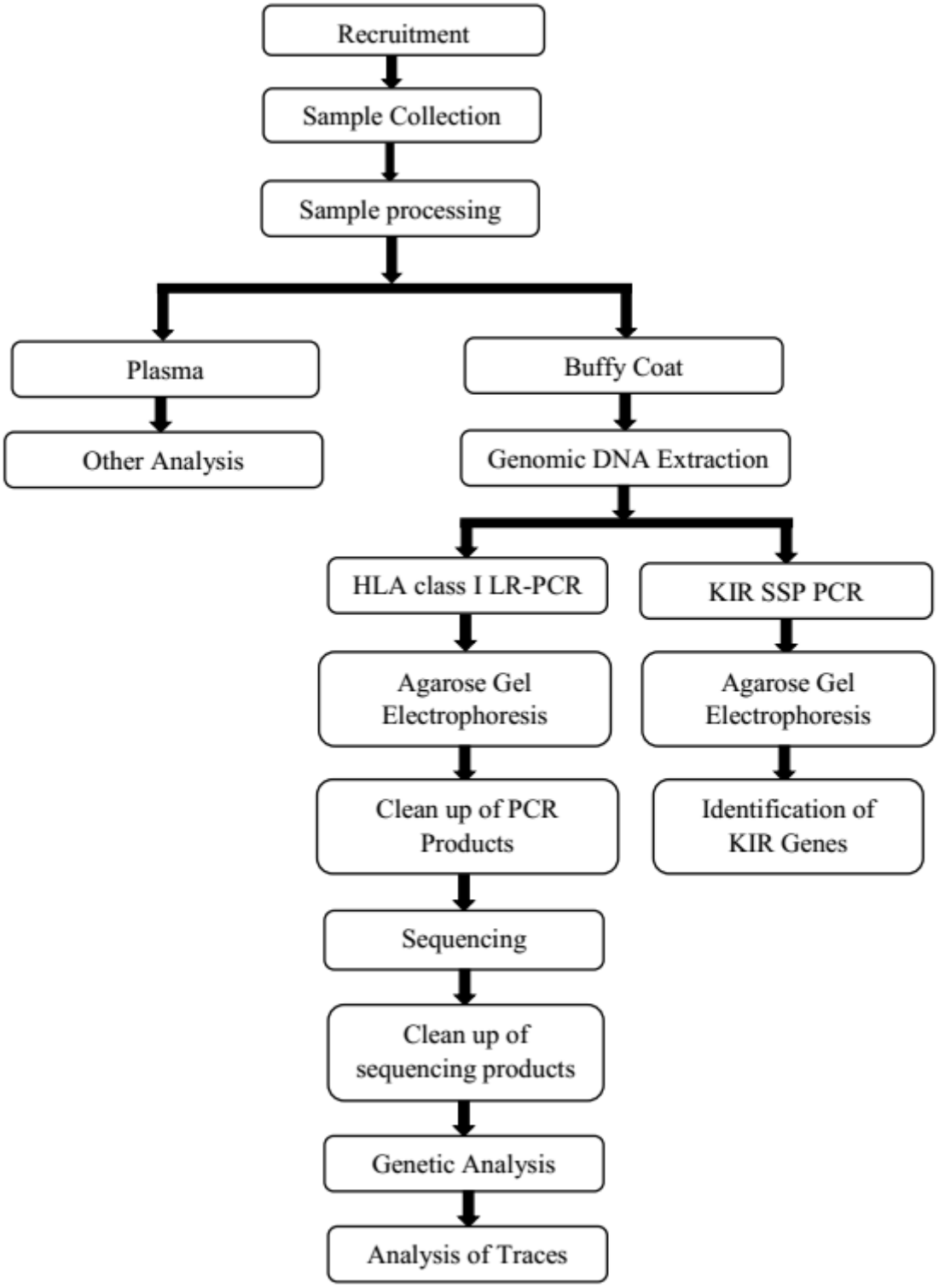


Figure 26: Schematic Representation of the Study

2.8. Selection Criteria

2.8.1. Inclusion Criteria

- Newly diagnosed HIV-1-infected persons (>21 years) who gave written consent
- Newly diagnosed HBV-infected persons (>21 years) who gave written consent
- Newly diagnosed HCV-infected persons (>21 years) who gave written consent

2.8.2. Non-inclusion Criteria

- HIV and HBV/HCV co-infected persons
- Tuberculosis co-infected persons
- Persons below 21 years of age
- Severely ill patients
- Prior exposure to antiretroviral drugs
- Refusal to continue in the study

2.9. Experimental Procedures

The experimental procedures that were used were according to the Standard Operating Procedures (SOPs) of the Molecular Biology Laboratory at CIRCB and the Target Discovery Institute of the Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.

2.9.1. Sample Collection and Treatment

Ten millilitres of venous blood were collected in uniquely coded Ethylene Diamine Tetraacetic Acid (EDTA) tubes. The samples were then tested for HIV, HBV and HCV infections using Rapid Diagnostic Tests (RDTs – Determine™ HIV-1/2 SET, Alere Medical Co, Ltd, 357 Matsuhida, Matsudo-Shi, Chiba, Japan and OraQuick® HIV 1/2 Rapid Antibody Test, manufactured in Thailand for OraSure Technologies, Inc.; OnSite HBsAg Combo Rapid Test, San Diego, CA, USA; OnSite HCV Ab Plus Combo Rapid Test, CTK BIOTECH, San Diego, CA, USA). Confirmation was done with Enzyme-Linked Immunosorbent Assay (ELISA) kits (DiaSorin S.p.A. Via Crescentino 13040 Saluggia (VC) – Italy) and PCR. After testing, the samples collected in Douala were immediately separated into plasma and buffy coat, stored at -20^{o}C for later transportation to the CIRCB Molecular Biology Laboratory in Yaounde, while the samples collected in Yaounde were stored in an ice box and later transported within a period of 4 hours to the CIRCB Molecular Biology Laboratory for processing and storage. Once there, the samples were tested for the different components of the whole blood sample (plasma and buffy coat), separated by centrifugation using an ultracentrifuge, and stored at -20 ^{o}C for subsequent treatment and analysis.

2.9.2. Genomic DNA Extraction

Genomic DNA (gDNA) was extracted from the 257 buffy coat samples using the QIAamp DNA Blood extraction kit, following an in-house protocol adapted from the manufacturer's instructions. DNA concentrations and quality were determined using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, MA). The DNA samples obtained were then stored at -20 ^{o}C for subsequent analysis.

2.10. KIR Gene Typing by SSP-PCR

KIR genes were typed for in 182 samples (84 HIV-1+, 31 HBV+, 33 HCV+ and 34 uninfected controls) using the Sequence-Specific Primer-Polymerase Chain Reaction (SSP-PCR) technique. For this, two sets of primers were used to amplify 30 different fragments with different sizes of KIR genes, found on different exons of the KIR locus. As such, a total of 60 primers (see Appendix 7) were used to type 14 KIR genes (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1) and 1 pseudogene (KIR2DP1). Two sets of primers were used per gene to ensure that the desired gene is not missed due to mutations on one of the target exons. A set of internal control primers was also added to the primer mixes to amplify a 796 bp fragment of the third intron of the HLA-DRB1 gene. This was to ascertain that the sample was human genomic DNA.

2.10.1. Preparation of Primer Mix and Master Mix (MM)

The primers were lyophilized, reconstituted to 100 µM with molecular grade water using an in-house protocol, then used to prepare the primer mixes with the internal control as described below.

The Master Mix (MM) for 15 reactions was prepared by successively adding the following reagents into a labelled 1 ml Eppendorf vial.

Table 5: PCR SSP Master Mix

Reagent	Volume (µl)
αQH_2O	135
10X buffer	21.2
$MgCl_2$ (50 mM)	6.4
dNTPs (25 mM)	1.7
DNA polymerase	0.9
DNA sample	1.5

The vial was immediately placed on ice.

2.10.2. PCR Amplification

Principle

PCR (Polymerase chain reaction) is a revolutionary technique developed revolutionary method developed by Kary Mullis in the 1980s. PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the initial template strand. DNA polymerase can add a nucleotide only onto a pre-existing 3'-OH group, it needs a primer to which it can add the first nucleotide. This requirement makes it possible to map out a specific region of template sequence to amplify. At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies (amplicons)(Hue-Roye and Vege, 2008).

Procedure

Two microliters (2 µl) of each primer mix were added into the corresponding well of a 96-well plate as indicated on the template in Figure 27 below. Ten microliters of master mix with sample were also then added into their corresponding wells. The plate was sealed and centrifuged briefly at 1500 rpm for 1 minute.

	1	2	3	4	5	6	7	8	9	10	11	12
A	KM1	KM2	KM1	KM2	KM1	KM2	KM1	KM2	KM1	KM2	KM1	KM2
B	KM3	KM4	KM3	KM4	KM3	KM4	KM3	KM4	KM3	KM4	KM3	KM4
C	KM5	KM6	KM5	KM6	KM5	KM6	KM5	KM6	KM5	KM6	KM5	KM6
D	KM7	KM8	KM7	KM8	KM7	KM8	KM7	KM8	KM7	KM8	KM7	KM8
E	KM8	KM10	KM8	KM10	KM8	KM10	KM8	KM10	KM8	KM10	KM8	KM10
F	KM11	KM12	KM11	KM12	KM11	KM12	KM11	KM12	KM11	KM12	KM11	KM12
G	KM13	KM14	KM13	KM14	KM13	KM14	KM13	KM14	KM13	KM14	KM13	KM14
H												

Figure 27: A 96- well plate labelled for six samples

Legend:

For Rows A to G: Columns 1 and 2 – Sample 1; Columns 3 and 4 – Sample 2; Columns 5 and 6 – Sample 3; Columns 7 and 8 – Sample 4; Columns 9 and 10 – Sample 5; Columns 11 and 12 – Sample 6KM1 – KM14 : Primer mixes for the 6 samples

The sealed plate was then placed in the thermal cycler, the lid closed and the "KIR-SSP" program was run under the following amplification conditions. At the end, the plate was taken out of the thermal cycler and centrifuged briefly.

Table 6: Cycling conditions for KIR-SSP PCR

1 cycle	94 °C	3:00				
5 cycles	94 °C	0:15	65 °C	0:15	72 °C	0:30
21 cycles	94 °C	0:15	60 °C	0:15	72 °C	0:30
5 cycles	94 °C	0:15	55 °C	1:00	72 °C	2:00
1 cycle	72 °C	7:00	(4 °C∞ optional)			

2.10.3. Agarose Gel Electrophoresis

Agarose Gel electrophoresis separates DNA fragments by size in a solid support medium (agarose gel). Sample (DNA) are pipetted into the sample wells, followed by the application of an electric current which causes the negatively-charged DNA to migrate (electrophorese) towards the anodal, positive (+ve) end. The rate of migration is proportional to size: smaller fragments move more quickly and wind up at the bottom of the gel.

DNA is visualized by including in the gel an intercalating dye, ethidium bromide. DNA fragments take up the dye as they migrate through the gel. Illumination with ultraviolet light causes the intercalated dye to fluoresce.

2.10.3.1. Gel Preparation (2%)

Four grams (4 g) of Agarose powder were weighed and poured into a conical flask. The flask was then filled up to 200 ml with 1XTAE (Tris-Acetate-Ethylene Diamine Tetra-Acetate) buffer and the contents mixed to dissolve. The mixture was brought to boil in a microwave oven while cautiously checking to avoid overflow. The flask was then removed and rapidly cooled in a jar of water to about 65 °C. Four microliters (4 µl) of ethidium bromide (10mg/ml) were added to the conical flask and mixed by swirling with caution to avoid the formation of air bubbles. The mixture was then poured into a previously prepared casting tray placed on a flat surface, combs were then put in place and the gel allowed to set for 15 minutes.

2.10.3.2. Gel Electrophoresis

When the amplification was complete, gel electrophoresis was then carried out. Five microliters (5 µl) of Loading Buffer were added into each well of the ELISA plate containing the PCR products and the plate was centrifuged again briefly. The combs were carefully removed from the gel and the

casting tapes from both ends of the tray. A good quantity of TAE buffer enough to immerse the gel was then poured into the electrophoresis tank and the gel was placed in it while paying attention to avoid air bubbles from being trapped beneath the gel tray. The whole mixture from each well (17 µl) was then loaded into the corresponding well of the Agarose gel. The gel tank was then connected to the Power Pack and ran at 350 V for 15 minutes.

2.10.3.3. Gel Visualization and Photography

After 15 minutes, the gel was removed from the electrophoresis tank, placed on a UV trans-illuminator and a picture (electrophoregram) taken with a camera attached to a computer. Each electronic copy of the electrophoregram was then pasted onto an Excel template (KIR Interpretation Sheet) for data analysis.

The protocol used for KIR genotyping is found in Appendix 8

2.10.4. KIR Data Analysis

2.10.4.1. KIR Identification

A Low DNA Mass Ladder was used to identify the different bands. The gel pictures obtained were adjusted for the bands to correspond to the size of the lanes on the template. Two specific lanes were scored for each KIR gene. They were scored as (1) if the specific band was present and (0) if it was absent (Figure 28). Samples with discordant results were repeated, with particular attention paid to KIR3DS1 which is reported to be less frequent in Africans. If one of the genes remained present after repeating, it was considered as present.

2.10.4.2. KIR Haplotype and Genotype Determination

Based on the presence or absence of each gene, they were grouped into haplotypes A and B. Group A haplotypes were defined by the presence of the following KIR genes: KIR3DL3, KIR2DL3, KIR2DL1, KIR2DP1, KIR2DL4, KIR3DL1, KIR2DS4, and KIR3DL2. A fixed- gene content characteristic of group A haplotypes was considered to carry 2 copies of group-A haplotypes (AA genotypes). The Group B haplotypes were defined by the presence of one or more of the following genes: KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, and KIR3DS1. These were collectively denoted as Bx since they constitute a mixture of AB and BB haplotypes. KIR Genotype Identification (GID) and haplotypes were assigned using the online Allele Frequency Net Database (Gonzalez-Galarza *et al.*, 2011). These genes were also grouped into Centromeric (Cen) and Telomeric (Tel) motifs based on the presence or absence of specific genes separated by the 3DP1-2DL4 recombination hotspot as described by Cooley et al (Cooley *et al.*, 2010b) and Pyo et al (Pyo *et al.*, 2010).

2.11. HLA Class I Sequence-Based Typing

HLA typing was done using a SBT technique. In this case, locus-specific primers were used to amplify exons 2 and 3 of HLA-A, -B and –C loci. After the amplification, sequence-specific primers were used to sequence the PCR products from both directions as described below.

2.11.1. HLA Class I Amplification

HLA class I amplification was done using locus-specific primers flanking exons 2 and 3 and the enzyme PrimerStar GXL, following an SOP. The master mix was prepared with respect to the number of samples to be amplified alongside a positive and a negative control.

2.11.1.1. Preparation of Master Mix

This was prepared by adding each of the following items into a 1ml labelled Eppendorf tube.

2.11.1.2. Master Mix Preparation

The master mix for HLA Class I (-A and -C) long range PCR amplification was prepared by adding the following reagents respectively as presented in Table 6 below.

Table 7: Master Mix for HLA Class I (-A and -C) Long Range PCR Amplification

Reagent	**Volume (µl)**
PCR grade water (sterile, RNA- and DNA-free)	12.5
5X buffer	5
Forward primer at 10ng/µl	1.5
Reverse primer at 10ng/µl	1.5
PrimeStar GXL (enzyme)	0.5
50 ng/µl of genomic DNA sample	2.0
Total	**23**

2.11.2. DNA Amplification

This was done in a thermal cycler using the amplification conditions described below.

Table 8: Cycling conditions for HLA-A and -C Amplification

Cycling conditions for HLA-A				
35 cycles	98 °C	0:10	68 °C	3:00
Store	8 °C for ever			
Cycling conditions for HLA -B and -C:				
35 cycles	98 °C	0:10	68 °C	4:00
Store	8 °C for ever			

The product was stored at 8°C until use. After the amplification, 3µl of the PCR products were separated via gel electrophoresis on a 2% Agarose gel as described above to check for amplification products.

2.11.3. Clean up and Sequencing of PCR Products

Samples containing the products were identified by the presence of an appropriately sized PCR product. Successful reactions were cleaned using a mixture of Shrimp Alkaline Phosphatase (SAP) and Exonuclease I (ExoI) (New England Biolabs): 0.1µl ExoI (0.5U), 0.5µl SAP (0.25U) and 1.4 µl QH_2O were combined with 22 µl of the PCR product and the clean-up reaction was performed at 37 °C for 45 minutes, followed by an inactivation step at 80 °C for 15 minutes. The product was stored at 4°C until use.

2.11.3.1. HLA Class I Sequencing

Principle

In Sanger sequencing technique, 2′,3′-dideoxynucleotides are used for DNA synthesis. In the absence of 3′-hydroxyl group in 2′,3′-dideoxynucleotides, DNA cannot be synthesized further as no phosphodiester bond can be formed with the next dNTP, and the chain terminates.

Procedure

Purified products were sequenced in both directions using exon 2 and 3 specific primers and a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster city, CA) in an ABI - 3130XL DNA Analyser (Applied Biosystems). Six primers were used for the sequencing of HLA-A, four for HLA-B and four for HLA-C.

The master mix for sequencing was prepared by adding the following items.

Table 9: Master Mix for HLA Class I Sequencing

Reagent	**Volume (µL)**
5x buffer	2.075
BigDye Terminator version 3.1	0.5
Molecular grade H_2O	5.355
Primer (10 µM)	0.32
PCR product	2.0
Total	**10.25**

The reaction was then run through

1 cycle	96 °C					
32 cycles	96 °C	0:10	50 °C	0:05	60 °C	4:00

The product was stored at 8 °C until use.

2.11.4. Clean up of Sequencing Products and DNA Analysis

The sequenced products were cleaned by ethanol precipitation and submitted to DNA analysis with an Applied Biosystems (ABI)-3730 genetic analyser.

Five microliters of 125 mM EDTA were added to each sample well, followed by 40 µl of 100 % ethanol. The plate was sealed and homogenised briefly for proper mixing, then incubated at room temperature for 15 minutes. The plate was centrifuged at 3400 revolutions per minute (rpm) for 30 minutes, the seal was removed and the plate was covered with a pad of tissue. These covered plates were then inverted and briefly centrifuged again at 100 xg. The wet pad of tissue was discarded and 40 µl of 70 % ethanol were added into each well. The plate was sealed, homogenised and then centrifuged anew but this time at 1700 xg for 15 minutes. Again, the seal was replaced with a clean pad of tissue, inverted and centrifuged at 200 xg for 1 minute. The wet pad of tissue was once more discarded and 10 µl of Hi-Di Formamide were added into each well. The plate was sealed one last time and submitted for loading into the 3730XL ABI Sequence Analyser.

The protocols for HLA long range amplification and sequencing are found in Appendix 9.

Sequence traces were analysed with Assign 400 software (Conexio Genomics, Western Australia).

2.12. Statistical Analyses

Statistical analyses were done using Microsoft Excel 2013, SPSS version 25 (IBM Corp., Armonk, NY) and Stata, v14.1 (Stata Corp., College Station, TX). Frequencies of genes, alleles, genotypes and haplotypes were determined using direct counting and confirmed with Microsoft Excel 2013. The frequencies were then compared between groups (HIV-1+, HBV+ and HCV+ with uninfected controls) using the Chi-Square Test, Fisher Exact Test, and Independent Sample t-test, as deemed necessary. A p value <0.05 was considered to be statistically significant after Bonferonni correction for multiplicity testing.

Linkage disequilibrium (LD) describes the non-random association of alleles at different loci in a given population. Loci are said to be in linkage disequilibrium when the frequency of association of their different alleles is higher or lower than expected if the loci were independent and associated

randomly (Slatkin, 2008). LD is used to understand evolutionary and demographic events, map genes that are associated with inherited diseases and quantitative characters and to understand the evolution of sets of genes that are linked. The coefficient of LD, DAB, is used to quantify the level of LD between A and B.

It is defined as $D_{AB} = p^{AB} - p^{A}p^{B}$,

where DAB is the coefficient of LD between two alleles, A and B; p^{AB} is the frequency of haplotypes with these alleles; p^{A} and p^{B} are the allele frequencies. LD is usually reported with D', the ratio of D to its maximum possible absolute value, given the allele frequencies. Absolute LD is reported as D' = 1.0. When alleles exist at a low frequency, D cores can be biased towards high scores. In this case, the r^2 is preferable for evaluating LD.

$$r^2 = \frac{D^2}{pA(1-pA)pB(1-pB)}$$

D can be affected by evolutionary forces like random mutation, genetic drift, gene flow and selective pressure acting at the population level. Pairwise LD analysis between KIR genes was calculated using Cramer's V statistic D` and Cochran test on SPSS (Pandey et al., 2015).

The Hardy–Weinberg Equilibrium (HWE) theory states that allele and genotype frequencies in a population will remain constant from generation to generation provided evolutionary influences such as genetic drift, mate choice, assortative mating, natural selection, sexual selection, mutation, gene flow, meiotic drive, genetic hitchhiking, population bottleneck, founder effect and inbreeding are absent (Ryckman and Williams, 2008). This theorem describes an ideal condition against which the effects of these influences can be analysed. In real populations, these evolutionary influences are always present. Hence, in this study, we assessed deviations from the HWE with the Pypop version 0.7.0. software. All p-values less than 0.05 were considered statistically significant.

RESULTS

3.1. Socio-demographic Characteristics of the Study Population

A total of 257 persons participated in this study. They were recruited from two urban regions of Cameroon namely Yaounde (the political capital of the nation) and Douala (the economic hub of the nation). Based on disease status, each participant could be classified into four groups: 84 were chronically HIV-1 infected (HIV-1+), 42 were infected with hepatitis B virus (HBV+), 68 were hepatitis infected (HCV+) and 63 were healthy and uninfected with any of the three viruses under investigation (uninfected controls).

In the HIV-1 positive group, demographic data (age and sex) and clinical information (viral load and CD4+ count) were not systematically collected from all the participants. However, analysis of the demographic data available showed that the mean age was 33.1 years and there were no significant differences between the ages and sex of the participants in the HIV-1 infected and the uninfected control group (Table 5)..

In the HBV+, HCV+ and Uninfected Control Groups, this as well as some clinical information was systematically collected using well-structured questionnaires. Males were significantly overrepresented in the HBV+ group compared to the HCV-infected and the Uninfected Control Group. The median age was significantly different between HBV-infected, HCV-infected and the Uninfected Control Groups (Table 5).

HCV-infected participants were significantly older (mean = 61.6 years) than their HBV+ counterparts and Uninfected Control Groups, with mean ages of 39.0 and 31.9 years, respectively (Table 5). A significant proportion of the HCV-infected patients were either married or living with a partner, in contrast to the HBV+ group in which a similar number of married and single participants were infected. In the HBV+ group, all the 38 participants had records of viral loads, while in the HCV+ group this data was missing for six participants.

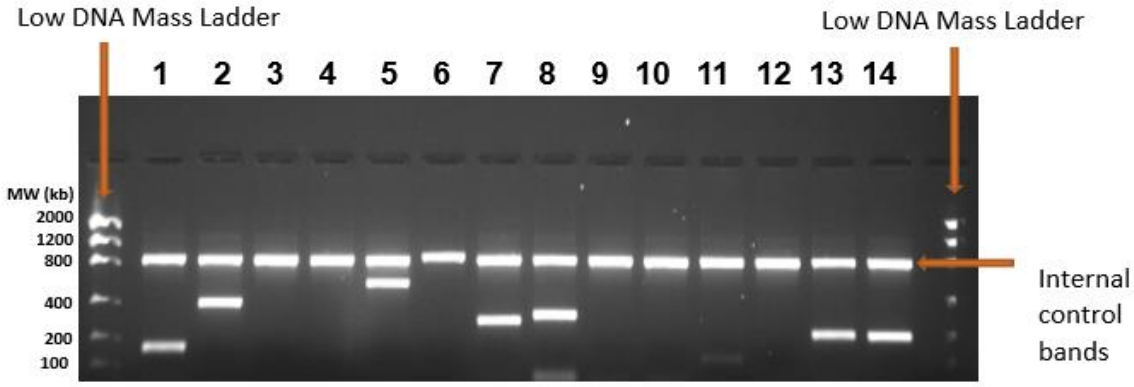
Table 10: Demographic characteristics of the study groups

	HIV+ (%)	**Controls[a] (%)**	**HBV+ (%)**	**HCV (%)**	**P[a]**	**p[b]**	**p[c]**
Number	34	63	38	68			
Male	-16	33 (52.4)	33 (86.8)	24 (35.3)		**<0.001**	**0.049**
Female	-18	30 (47.6)	5 (13.2)	44 (64.7)	1.000		
Age (mean ± SD)	33.1 ± 7.2	31.9 ± 7.2	39.0 ± 10.7	61.6 ± 12.5	0.429	**0.003**	**<0.001**

Controls: Participants who tested negative for HBV, HCV and HIV; p^a p-values comparing HIV+ and Uninfected Controls, p^b: p-values comparing HBV+ and Uninfected Controls; p^c: p-values comparing HCV+ and Uninfected Controls; d: comparison of age by disease status (Bonferroni); e: HCV viral load data was missing for six participants in the HCV group.

3.2 Distribution of KIR Genes

All the 14 typed KIR genes (*3DL1*, *2DL1*, *2DL3*, *2DS4*, *2DL2*, *2DL5*, *3DS1*, *2DS1*, *2DS2*, *2DS3*, *2DS5*, *2DL4*, *3DL2*, *3DL3*) and one pseudogene (*2DP1*) were present in the study population. Most inhibitory KIR genes were present in more than 90% of the study population with the exception of *KIR2DL2* (67.58%), and the least common was *KIR2DL5* (59.89%). Meanwhile most of the activating genes were present in less than 65% of the participants, but for the ubiquitous *KIR2DS4* that had a high frequency of 97.80%. *KIR3DS1* (41.21%) was the least frequent activating KIR genes (Figure 29).



Gene	Score	Lane	Size
2DL1	1	1	146
	1	2	330
2DL2	0	3	173
	0	4	151
2DL3	1	5	550
	0	6	800
2DL4	1	7	254
	1	8	288
2DS2	0	9	175
	0	10	240
2DS3	1	11	242
	0	12	190
2DS4	1	13	204
	1	14	197/219

Figure 28: Sample Electrophoregram and KIR scoring table

Legend:

MW (kb): Molecular Weight in Kilobase

1 and 2: KIR2DL1; 3 and 4: KIR2DL2; 5 and 6: KIR2DL3; 7 and 8: KIR2DL4; 9 and 10: KIR2DS2; 11 and 12: KIR2DS3; 13 and 14: KIR2DS4

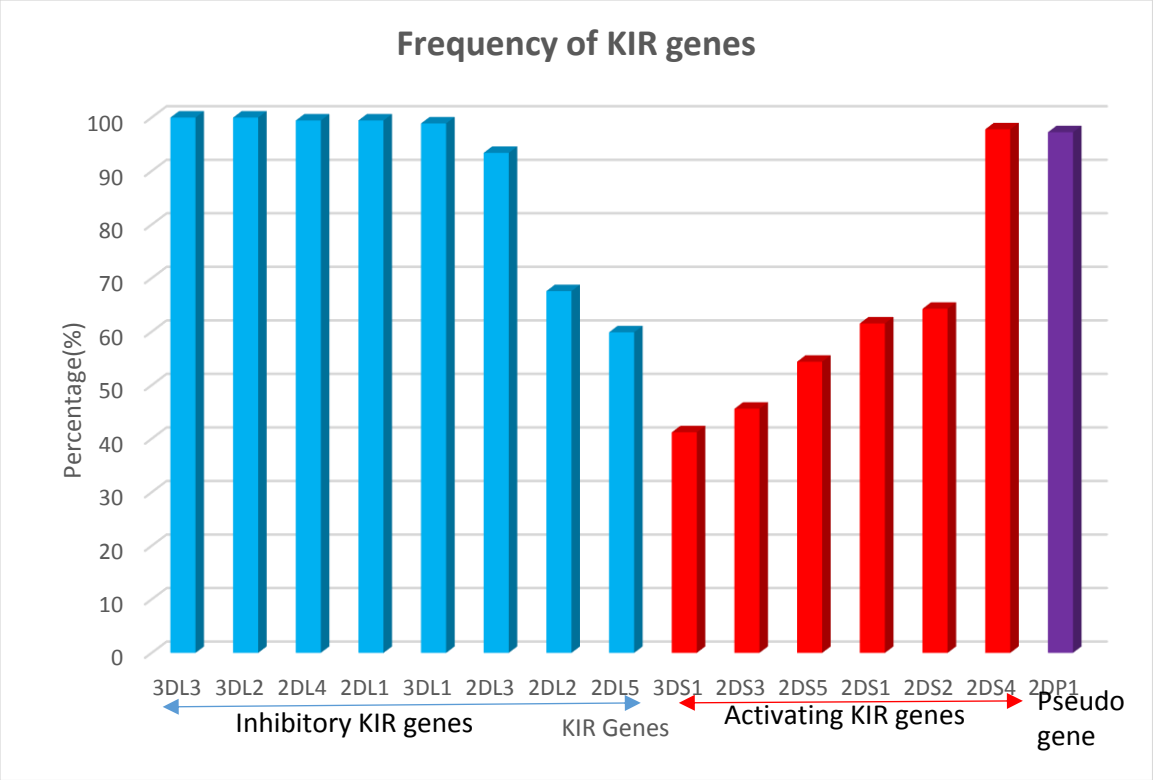


Figure 29: Frequency of KIR genes in the overall population

All three framework genes investigated (*KIR3DL2, KIR3DL3 and KIR2DL4*) were present in all samples, with the exception of KIR2DL4 that was absent in one Uninfected Control participant. The frequency of various KIR genes in this sample of a Cameroonian population was compared with those from other populations in Africa, Europe and Asia reported in the Allele Frequency Net Database (González-Galarza *et al.*, 2015). No data from Cameroon was found, implying to our knowledge that this is the first study of *KIR* gene analysis in this population. Apart from the ubiquitous KIR2DS4 gene, the frequency of all the other activating KIR genes were higher in our study population compared to data reported from other African countries. Strikingly, the frequency of *KIR3DS1* (41.2%), which has been reported to be very low in Africans, was relatively high in our study population, and was similar to frequencies obtained in Europe and Asia (Table 6). The *KIR2DS1*(61.5%) also had a relatively high frequency in this study population compared to reports from other African countries, Asia and Europe. Its frequency in other African populations was generally below 30%. The frequencies of the inhibitory genes were similar to those reported in other African populations as well as those of the framework genes.

Table 11: Frequency of KIR Genes in Some Regions of the World

Genes	Cameroon (n=182)	Equatorial Guinea (n=95)	Gabon (n=54)	Congo Kinshasa (n=38)	Central African Republic (n=69)	Nigeria (n=75)	England (n=136)	France (n=102)	China Eastern Mainland Han (n=106)	India North (n=512)
2DL1	99.5	95.8	100	94.4	97.1	100.0	91.0	97.0	99.0	85.7
2DL2	67.6	57.3	64.8	65.7	58	38.4	49.0	58.0	25.0	74.8
2DL3	93.4	80.2	79.6	61.8	80.3	92.0	92.0	89.2	99.0	71.5
2DL4	99.5	99.0	100	NA	NA	NA	100	100	100	100
2DL5	59.9	52.1	70.4	NA	NA	NA	NA	57.8	45.0	73.8
2DP1	97.3	96.9	100	NA	NA	NA	NA	98.0	99.0	90.2
2DS1	61.5	19.8	18.5	27.8	1.4	14.7	45.0	45.1	42.0	53.7
2DS2	64.3	47.9	57.4	65.7	56.5	34.7	51.0	57.8	27.0	53.9
2DS3	45.6	26.0	35.2	NA	NA	NA	24.0	32.4	22.0	41.0
2DS4	97.8	96.9	100	NA	NA	NA	96.0	93.1	95.0	83.2
2DS5	54.4	34.4	33.3	NA	NA	NA	32.0	36.3	94.0	57.8
3DL1	98.9	99.0	100	97.2	100	98.7	97.0	93.1	100.0	81.6
3DL2	100	100	100	NA	NA	NA	100	100	100	100
3DL3	100	100	100	NA	NA	NA	NA	100	100	100
3DS1	41.2	5.2	7.4	11.1	2.9	12.0	42.0	45.1	41.0	51.0

3.2. KIR Genes/Profiles in Cases and Controls

All the 15 KIR genes typed were present at various frequencies (Figure 4.2). Interestingly, *KIR2DL2* was significantly less frequent in the HIV-1+ and HBV+ groups compared to their HCV-infected counterparts and Uninfected Controls (50%, 58.1%, 97.0%, and 91.2%), respectively.

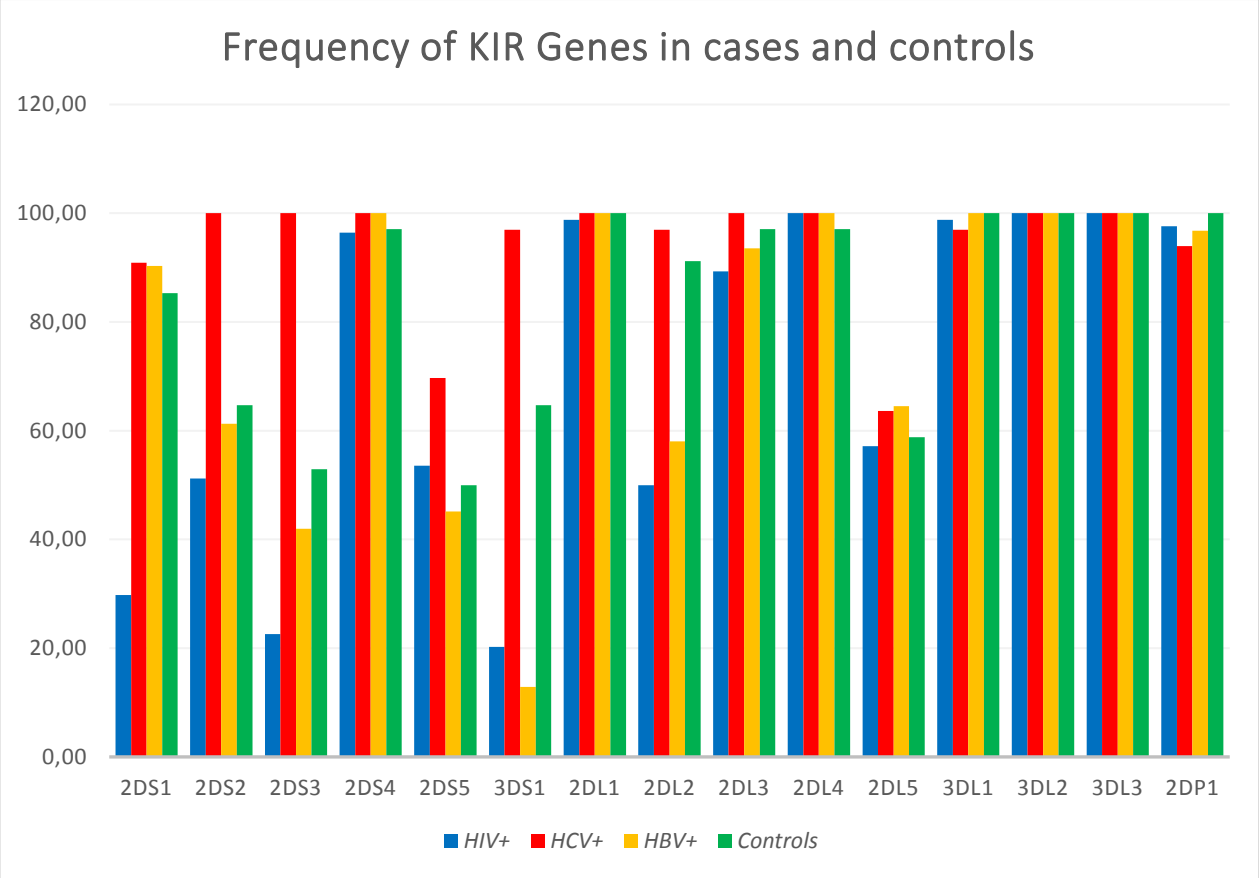


Figure 30: Frequency of KIR genes in Cases and Uninfected (HIV-,HBV- and HCV-) Controls

3.3.1. KIR Association with HIV-1 Infection

We compared *KIR* gene frequencies between groups to evaluate the association between KIR genes and HIV-1 infection. All 15 KIR genes were detected in the HIV infected group (Table 7). The three framework genes (*KIR3DL2, KIR3DL3 and KIR2DL4*) were also present in all HIV-1 positive participants, while the others were present at varying percentages. The frequency of each inhibitory gene was higher compared to that of their activating counterpart. Moreover, comparisons between the individual genes present in HIV-1 infected participants and in controls showed that three activating *KIR* genes – *KIR2DS1, KIR2DS3, KIR3DS1*– and an inhibitory *KIR* gene – *KIR2DL2* – were significantly overrepresented in controls compared to the HIV-1 infected groups (Table 7). This indicates that these genes may play a protective role against HIV-1 infection in this Cameroonian population.

Table 12: Association of KIR genes with HIV-1 infection

KIR Genes	HIV+ (84)	Controls (34)	OR (95%CI)	p
Activating				
2DS1	25(29.8)	29(85.3)	13.69 (4.75 - 39.43)	<0.001
2DS2	43(51.2)	22(64.7)	1.75 (0.77 - 3.98)	0.181
2DS3	19(22.6)	18(52.9)	3.85 (1.65 - 8.96)	0.001
2DS4	81(96.4)	33(97.1)	1.22 (0.12 - 12.18)	0.864
2DS5	45(53.6)	17(50.0)	0.87 (0.39 - 1.92)	0.725
3DS1	17(20.2)	22(64.7)	7.23 (2.99 - 17.46)	<0.001
Inhibitory				
2DL1	83(98.8)	34(100)		0.523
2DL2	42(50.0)	31(91.2)	10.33 (2.93 - 36.43)	<0.001
2DL3	75(89.3)	33(97.1)	3.96 (0.48 - 32.54)	0.17
2DL4	84(100)	33(97.1)	1.03 (0.97 - 1.09)	0.114
2DL5	48(57.1)	20(58.8)	1.07 (0.48 - 2.40)	0.867
3DL1	83(98.8)	34(100)	-	0.523
3DL2	84(100)	34(100)	-	-
3DL3	84(100)	34(100)	-	-
Pseudogene				
2DP1	82(97.6)	34(100)	-	0.364

OR: Odds ratio, p: p value comparing the control group with the HIV group. 95%CI: 95% Confidence Interval

3.3.2. KIR Association with Viral Hepatitis B and C

Univariate analyses comparing observed KIR frequencies between Uninfected Controls and HBV-infected (Table 8) and HCV-infected (Table 9) participants revealed that *KIR3DS1* is strongly but differentially associated with the two disease models. *KIR3DS1* carriers were significantly less likely to be HBV-infected compared to Uninfected Controls (12.9% vs 64.7%, OR= 0.08, p=2.4 x 10-5). Conversely, a significant proportion of HCV-infected participants were KIR3DS1 carriers (97.0% vs 64.7%, OR = 17.45, p = 9.2 x 10-4,), as depicted in Table 8. This suggests that this three-domain KIR activating gene may predispose its carriers to HCV infection. Carriers of two other activating KIR genes (*KIR2DS2 and KIR2DS3*) were also all infected with HCV (Table 8). An inhibitory *KIR* gene (*KIR2DL1*) was also significantly enriched (p = 0.0021; OR 0.13; 95%CI 0.02 – 0.61) in the control group compared to the HBV+ group, indicating that it might also have a protective effect on HBV infection in this population.

Table 13: KIR association with HBV infection

	Control n (%)	**HBV+ n (%)**	**OR (95% CI)**	**P**	**Pc**
Number	34	31			
KIR 2DL2	31 (91.2)	18 (58.1)	0.13 (0.02 - 0.61)	0.0021	0.0147
KIR 2DL3	33 (97.1)	29 (93.6)	0.44 (0.03 - 5.25)	0.5038	3.5266
KIR 2DS1	29 (85.3)	28 (90.3)	1.61 (0.35 - 7.50)	0.5408	3.7856
KIR 2DS2	22 (64.7)	19 (61.3)	0.86 (0.31 - 2.39)	0.7773	5.4411
KIR 2DS3	18 (52.9)	13 (41.9)	0.64 (0.23 - 1.74)	0.3786	2.6502
KIR 2DS5	17 (50.0)	14 (45.2)	0.82 (0.30 - 2.20)	0.6987	4.8909
KIR 3DS1	22 (64.7)	4 (12.9)	0.08 (0.02 - 0.36)	2.39 x 10-5	1.67 x 10-4

Only KIR genes with apparent variability between groups are shown, P: uncorrected p-values, Pc: p-values corrected for multiple comparisons by the Bonferroni method. 95%CI: 95% Confidence Interval

Table 14: KIR Association with HCV infection

	Control n (%)	HCV+ n (%)	OR (95% CI) or Chi-squared	P	Pc
Number	34	33			
KIR2DL2	31 (91.2)	32 (97.0)	3.09 (0.29 - 32.52)	0.3207	2.5656
KIR 2DL5	20 (58.8)	21 (63.6)	1.23 (0.45 - 3.31)	0.6883	5.5064
KIR 2DS1	29 (85.3)	30 (90.9)	1.72 (0.37 - 8.02)	0.4819	3.8552
KIR 2DS2	22 (64.7)	33 (100.0)	13.98	1.90 x 10-4	0.0016
KIR 2DS3	18 (52.9)	33 (100.0)	20.10	7.36 x 10-6	5.89x 10-5
KIR 2DS5	17 (50.0)	23 (69.7)	2.30 (0.82 - 6.44)	0.1029	0.8232
KIR 3DS1	22 (64.7)	32 (97.0)	17.45 (1.70 - 179.31)	9.21 x 10-4	0.0072
KIR 2DP1	34 (100.0)	31 (94.0)	2.09	0.148	1.184

Only KIR genes with apparent variability between groups are shown, P: uncorrected p-values, Pc: p-values corrected for multiple comparisons by the Bonferroni method. 95%CI: 95% Confidence Interval

3.4. Effect of Centromeric, Telomeric Motifs and KIR Genotype on HIV-1, Hepatitis B and C Infections

We subdivided the KIR cluster into centromeric and telomeric motifs in each study participant based on KIR genes present at their centromeric and telomeric loci as previously reported (Cooley *et al.*, 2010b; Re *et al.*, 2015; Mhandire *et al.*, 2016). We identified seven different centromeric (Cen 1 – 7), eight telomeric (Tel 1 – 6, 9 and 10) and eight centromeric/telomeric (Cent/Tel 1 -8) gene motifs. Comparisons of the frequencies of these haplotypes in the HIV-1+, HBV+, or HCV+ versus uninfected groups helped to find possible associations between cases and controls. The frequency of Centromeric1 (Cen1), which carries *KIR2DL3* and lacks *KIR2DL2*, was significantly enriched in the HIV-1+ and HBV+ groups compared to the uninfected control group (41.7% and 38.7% vs 5.9%, p <0.001 and p = 0.001, respectively). Thus participants with Cen1 were more likely to be HIV-1+ or HBV+ than those without it. Likewise, Cen2 and Cen3, which both carry the *2DL2* and *2DL3* allelic variants, were significantly higher in the HIV-1+ and HCV+ groups compared to the uninfected

control groups, thus associating these motifs with HIV+ or HCV+ infection status. Analyses of the telomeric motif showed that carriers of Telomeric 1 (Tel 1) were 1.6 times more likely to be HIV-1 infected compared to those without Tel 1. Similarly, participants with Tel 2 were more likely to be HCV-infected. In contrast, HIV-1+ and HCV+ participants carrying the Tel 2 haplotype were 11.9 and 10.9 times, respectively, less likely to be HIV-1 or HCV–infected when compared to the Uninfected Controls. Lastly, the frequency of haplotype Cen 1/Tel 1 was higher in the HCV+, associating it with an increased risk for HCV infection, while that of Cen 1/Tel 6 motif was significantly higher in the Uninfected Control group compared to the HCV+ group, indicating that it might have a protective effect. In the same light, Cen 1/Tel 4 was associated with a protective effect against HIV-1 infection (Table 10).

Table 15: Centromeric and Telomeric KIR Locus

ID	KIR GENES				HIV+ n (%)	HBV+ n (%)	HCV+ n (%)		HIV+ vs Control		HBV+ vs Control		HCV+ vs Control	
	2DS2	*2DL2*	*2DL3*	*2DL1*				Controls n (%)	OR (95%CI)	Pa	OR (95%CI)	pb	OR (95%CI)	pc
Ce1					35(41.7)	12(38.7)	0(0.0)	2(5.9)	0.09(0.02 - 0.39)	**<0.001**	0.10(0.02 - 4.91)	**0.001**		0.493
Ce2					28(33.3)	16(51.6)	32(97.0)	20(58.8)	2.86(1.26 - 6.49)	0.01	1.54(0.50 - 3.57)		0.045(0.055 - 0.37)	**<0.001**
Ce3					5(6.0)	0(0.0)	0(0.0)	10(29.4)	6.59(2.05 - 21.14)	**0.001**				**0.001**
Ce4					7(8.3)	1(3.2)	1(3.0)	1(2.9)	0.33(0.04 - 2.82)	0.291	0.91(0.05 - 15.18)		0.97(0.058 - 16.17)	0.983
Ce5					1(1.2)	0(0.0)	0(0.0)	0(0.0)	0.99(0.97 - 1.01)	0.523				
Ce6					7(8.3)	2(6.5)	0(0.0)	1(2.9)	0.33(0.04 - 2.82)	0.291	0.44(0.04 - 5.10)			0.321
Ce7					1(1.2)	0(0.0)	0(0.0)	0(0.0)	0.99(0.97 - 1.01)	0.523				
Total					84(100)	31(100)	33(100)	34(100)						
ID	*3DS1*	*2DS1*	*3DL1*	*2DS4*										
Te1					54(64.3)	3(9.7)	0(0.0)	5(14.7)	0.09(0.03 - 0.27)	**<0.001**	1.61(0.35 - 7.34)	0.54		0.053
Te2					10(11.9)	4(12.9)	28(84.8)	21(61.8)	11.95(4.59 - 31.10)	**<0.001**	10.90(3.10 - 38.34)	**<0.001**	0.29(0.09 - 0.94)	0.03
Te3					12(14.3)	24(77.4)	1(3.0)	7(20.6)	1.56(0.55 - 4.37)	0.399	0.08(0.02 - 0.25)	**<0.001**	8.29(0.96 - 71.72)	0.027
Te4					5(6.0)	0(0.0)	3(9.1)	0(0.0)		0.146				0.114
Te5					0(0.0)	0(0.0)	1(3.0)	0(0.0)						0.306
Te6					1(1.2)	0(0.0)	0(0.0)	0(0.0)	0.99(0.97 - 1.01)					
Te9					1(1.2)	0(0.0)	0(0.0)	0(0.0)	0.99(0.97 - 1.01)	0.52				
Te10					1(1.2)	0(0.0)	0(0.0)	1(2.9)	2.52(0.15 - 41.40)	0.51		0.336		0.321

Centromeric and Telomeric KIR Locus *(continued)*

	2DL5	2DS3	2DS5								
Total				84(100)	31(100)	33(100)	34(100)						
Ce/Te1	■	■	■	10(11.9)	7(22.6)	19(57.6)	6(17.6)	1.59(0.53 - 4.77)	0.409	0.74(0.22 - 2.49)	0.619	0.16(0.052 - 0.48)	**0.001**
Ce/Te2	■		■	30(35.7)	6(19.4)	0(0.0)	8(23.5)	0.55(0.22 - 1.38)	0.2	1.28(0.39 - 4.22)	0.683		**0.005**
Ce/Te3	■	■		8(9.5)	6(19.4)	2(6.1)	6(17.6)	2.04(0.65 - 6.39)	0.22	0.89(0.26 - 3.13)	0.859	3.32(0.62 - 17.82)	0.259
Ce/Te4		■		1(1.2)	0(0.0)	8(24.2)	5(14.7)	14.3(1.60 - 127.64)	**0.002**		0.026	0.54(016 - 1.86)	0.324
Ce/Te5	■			0(0.0)	1(3.2)	0(0.0)	0(0.0)				0.291		
Ce/Te6				30(35.7)	10(32.3)	0(0.0)	6(17.6)	0.39(0.14 - 1.04)	0.05	0.45(0.14 - 1.44)	0.172	1.21(1.04 - 1.42)	0.025
CenTe7			■	5(6.0)	1(3.2)	0(0.0)	2(5.9)	0.99(0.18 - 5.35)	0.99	1.88(0.16 - 21.76)	0.61	1.03(0.98 - 1.16)	0.493
Ce/Te8		■	■	0(0.0)	0(0.0)	4(12.1)	1(2.9)		0.11		0.336	0.22(0.02 - 2.08)	0.197
Total				84(100)	31(100)	33(100)	34(100)						

KIR gene profiles grouped into 9 centromeric, 8 telomeric and 6 cent/tel motifs made up of genes that can be present in the centromeric and telomeric regions. The green boxes indicate the presence of specific KIR genes while the unshaded boxes indicate their absence.

Legend

Ce = Centromere; Te = Telomer. Significant p values are in bold. pa = p values comparing HIV-1+ and Uninfected Controls; pb = p values comparing HBV+ and uninfected controls; pc = p values comparing HCV+ and Uninfected Controls. 95%CI: 95% Confidence Interval

3.5. Frequency of KIR Genotypes and Profiles Stratified by Disease Group

Based on the presence or absence of KIR genes and with the application of the allele frequency net database (González-Galarza *et al.*, 2015), 57 KIR genotypes in 182 individuals were detected, 5 of which have hitherto not been reported in the Allele Frequency Net Database (Table 11). The six most common genotypes identified in the study population were Bx6 (15.9%), AA1 (12.1%), Bx 15 (4.9%), Bx 9 (4.4%), Bx 11 and Bx 233 (3.8%). Worthy of note is the fact that Genotype 6 belongs to the Bx haplogroup with all 15 KIR genes present as illustrated in Table 11. Genotype AA1 was overrepresented in the HIV-1 infected group compared to the HBV+, HCV+ and Uninfected Control Groups (23.8%, 3.23%, 0.00% and 2.94%, respectively), suggesting that participants with this genotype might be more susceptible to HIV-1 infection than non-carriers.

Genotype 6 was overrepresented in the HCV-infected group compared to the Uninfected Controls, the HBV- and HIV-1 positive participants (51.5%, 17.7%, 6.5%, and 4.76%, respectively, p=0.005). In a univariate analysis comparing HCV-positive individuals to uninfected controls, carriers of Genotype 6 were five times more likely to be HCV-infected than those without this genotype, suggesting that its presence may increase the risk of a person being infected with Hepatitis C. Similarly, Genotype 15 was found to be present only in the HBV-infected and HIV-1 positive groups (22.6% and 2.38%, respectively) suggesting its role in the susceptibility to HBV. It shared nine genes in common with Genotype 6 but lacked *KIR2DL2, KIR2DS2, KIR2DS3, KIR2DL5, KIR2DS5,* and *KIR3DS1* (Table 11).

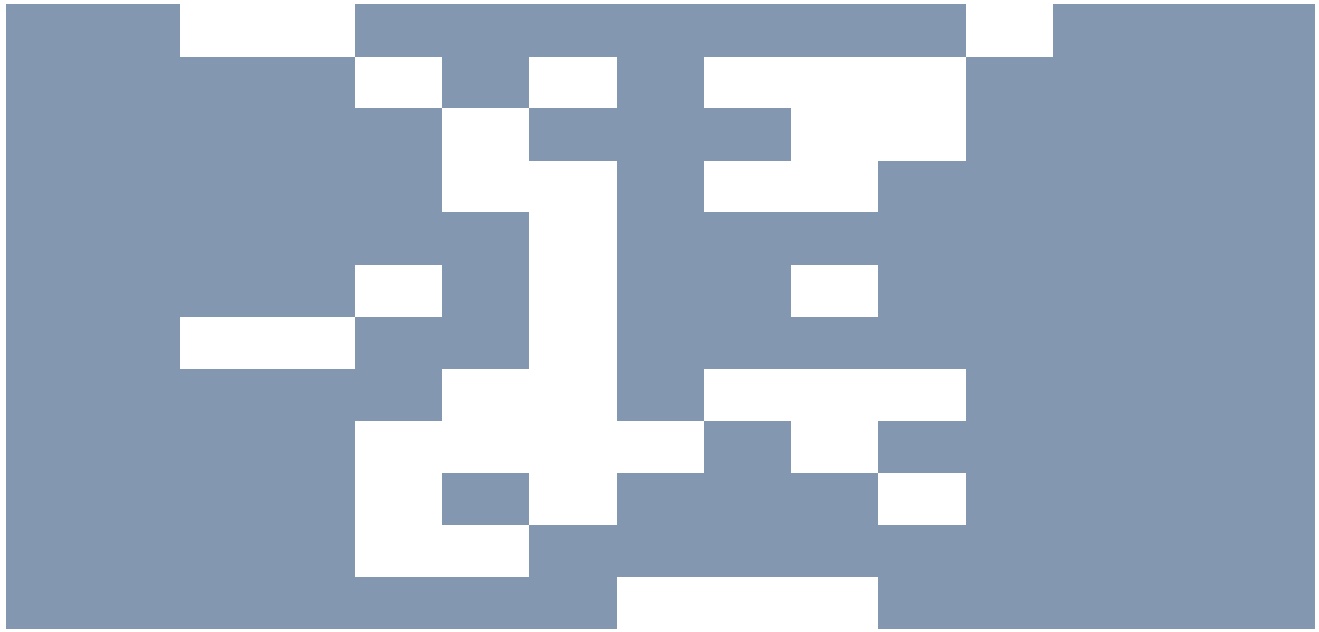
Table 16: Frequency of KIR genotypes and profiles stratified by disease group

HID	GID	*3DL1*	*2DL1*	*2DL3*	*2DS4*	*2DL2*	*2DL5*	*3DS1*	*2DS1*	*2DS2*	*2DS3*	*2DS5*	*2DL4*	*3DL2*	*3DL3*	2DP1	n(%)	HIV+ n(%)	HBV+ n(%)	HCV+ n(%)	Control n(%)
		Common Genotypes																			
AA	1	■	■	■	■								■	■	■		22(12.1)	20(23.8)	1(3.23)	0(0.00)	1(2.94)
Bx	2	■	■	■	■		■	■	■	■		■	■	■	■		3(1.6)	2(2.38)	1(3.23)	0(0.00)	0(0.00)
Bx	3	■	■	■	■	■	■	■	■			■	■	■	■		6(3.3)	2(2.38)	1(3.23)	0(0.00)	3(8.82)
Bx	4	■	■	■	■		■						■	■	■		3(1.6)	2(2.38)	1(3.23)	0(0.00)	0(0.00)
Bx	5	■	■	■	■		■				■		■	■	■		6(3.3)	5(5.95)	0(0.00)	0(0.00)	1(2.94)
Bx	6	■	■	■	■		■	■	■	■	■	■	■	■	■		29(15.9)	4(4.76)	2(6.45)	17(51.5)	6(17.65)
Bx	7	■	■	■	■		■	■	■	■	■		■	■	■		4(2.2)	0(0.00)	0(0.00)	1(3.03)	3(8.82)
Bx	9	■	■	■	■		■		■	■	■	■	■	■	■		8(4.4(	3(3.57)	3(9.68)	0(0.00)	2(5.88)
Bx	10	■	■	■	■					■			■	■	■		2(1.1)	2(2.38)	0(0.00)	0(0.00)	0(0.00)
Bx	11	■	■	■	■	■	■		■	■	■		■	■	■		7(3.8)	0(0.00)	5(16.13)	1(3.03)	1(2.94)
Bx	12	■	■	■	■		■	■	■	■		■	■	■	■		2(1.1)	1(1.19)	0(0.00)	0(0.00)	1(2.94)
Bx	15	■	■	■	■				■				■	■	■		9(4.9)	2(2.38)	7(22.58)	0(0.00)	0(0.00)
Bx	16	■	■	■	■			■	■				■	■	■		1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	17	■	■	■	■		■	■				■	■	■	■		1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	18	■	■	■	■	■	■	■	■			■	■	■	■		2(1.1)	0(0.00)	0(0.00)	0(0.00)	2(5.88)
Bx	19	■	■	■	■		■						■	■	■		2(1.1)	1(1.19)	0(0.00)	0(0.00)	1(2.94)
Bx	20	■	■	■	■		■					■	■	■	■		1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	21	■	■	■	■		■			■		■	■	■	■		5(2.7)	5(5.95)	0(0.00)	0(0.00)	0(0.00)

Frequency of KIR genotypes and profiles stratified by disease group *(continued)*

Bx	22	1(0.5)	0(0.00)	0(0.00)	1(3.03)	0(0.00)
Bx	23	5(2.7)	4(4.76)	1(3.23)	0(0.00)	0(0.00)
Bx	24	2(1.1)	2(2.38)	0(0.00)	0(0.00)	0(0.00)
Bx	30	1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	32	3(1.6)	3(3.57)	0(0.00)	0(0.00)	0(0.00)
Bx	41	1(0.5)	0(0.00)	0(0.00)	1(3.03)	0(0.00)
Bx	48	3(1.6)	3(3.57)	0(0.00)	0(0.00)	0(0.00)
Bx	62	2(1.1)	1(1.19)	0(0.00)	0(0.00)	1(2.94)
Bx	68	1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	71	1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	89	1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	91	2(1.1)	1(1.19)	1(3.23)	0(0.00)	0(0.00)
Bx	106	1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	112	1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	113	1(0.5)	0(0.00)	1(3.23)	0(0.00)	0(0.00)
Bx	175	1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	192	1(0.5)	0(0.00)	1(3.23)	0(0.00)	0(0.00)
Bx	228	1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	233	7(3.8)	0(0.00)	0(0.00)	6(18.18)	1(2.94)
Bx	260	1(|0.5)	0(0.00)	0(0.00)	0(0.00)	1(2.94)
Bx	281	1(|0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	319	4(2.2)	0(0.00)	0(0.00)	3(9.09)	1(2.94)

Frequency of KIR genotypes and profiles stratified by disease group *(continued)*



Bx	325																2(1.1)	1(1.19)	0(0.00)	0(0.00)	1(2.94)
Bx	326																1(0.5)	0(0.00)	1(3.23)	0(0.00)	0(0.00)
Bx	328																1(0.5)	0(0.00)	0(0.00)	0(0.00)	1(2.94)
Bx	371																2(1.1)	0(0.00)	0(0.00)	0(0.00)	2(5.88)
Bx	382																5(2.7)	2(2.38)	3(9.68)	0(0.00)	0(0.00)
Bx	393																1(0.5)	0(0.00)	1(3.23)	0(0.00)	0(0.00)
Bx	467																1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	472																3(1.6)	1(1.19)	0(0.00)	0(0.00(	2(5.88)
Bx	567																1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	586																1(0.5)	1(1.19)	0(0.00)	0(0.00)	0(0.00)
Bx	591																1(0.5)	0(0.00)	0(0.00)	1(3.03)	0(0.00)
Bx	680																2(1.1)	2(2.38)	0(0.00)	0(0.00)	0(0.00)
		New Genotypes																			
Bx	Na																1(0.5)	0(0.00)	1(3.23)	0(0.00)	0(0.00)
Bx	Nb																1(0.5)	0(0.00)	0(0.00)	1(3.03)	0(0.00)
Bx	Nc																1(0.5)	0(0.00)	0(0.00)	1(3.03)	0(0.00)
Bx	Nd																2(1.1)	0(0.00)	0(0.00)	0(0.00)	2(5.88)
Bx	Ne																1(0.5)	0(0.00)	0(0.00)	0(0.00)	1(2.94)
N		55	56	47	54	39	35	22	32	36	24	32	56	57	57		182	84	31	33	34
Phe%		96.5	98.2	82.5	94.7	68.4	61.4	38.6	56.1	63.2	42	56.1	98.2	100	100	93	100	100	100	100	100

GID: Genotype Identity, HID: Haplotype Identity, N: number, Phe%, Phenotype Percentage, AA: genotype AA, Bx: Genotype Bx,

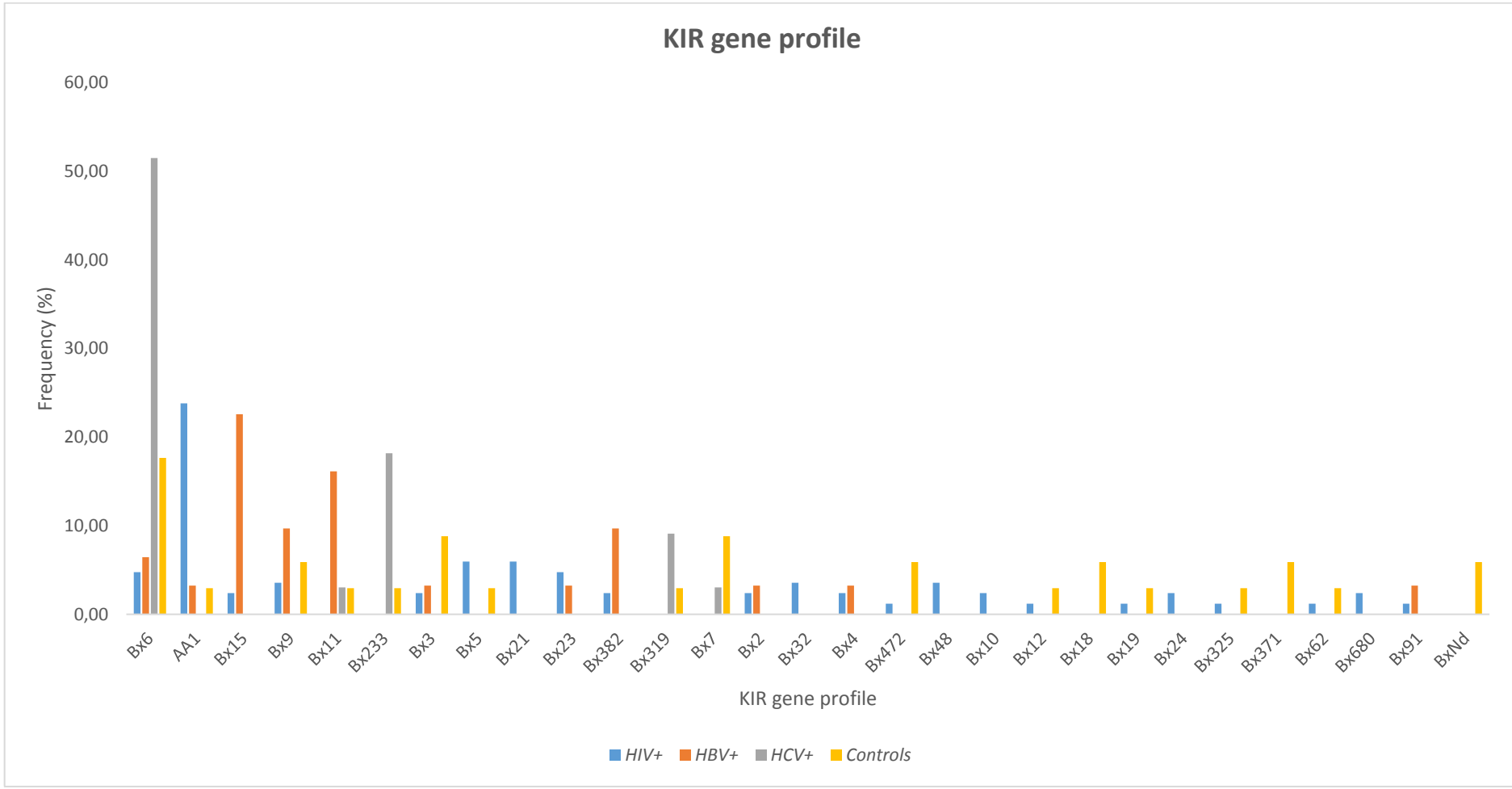


Figure 31: Common KIR gene profiles

3.6. Pairwise Linkage Disequilibrium (LD) Between KIR Genes

Pairwise LD between KIR genes (excluding framework genes) was calculated in the HIV-1+, HBV+, HCV+ and uninfected control groups separately to determine patterns of co-inheritance in the KIR locus. Seven pairs showed significant LD while one pair (2DS2-2DL5) was in strong LD in the HBV+ and uninfected control groups. Three other pairs of genes (2DL1/2DP1, 2DL2/2DS2, 2DL5/2DS5) were in strong LD in the HIV-1+ group (Table 12), while 2DS3/2DL5, 3DL1/2DP1, 2DS4/2DL3 each showed similar characteristics but, in the HBV,+ (Table 13), HCV+ (Table 14) and uninfected control groups respectively.

Table 17: Linkage disequilibrium analysis of HIV-1 positive and uninfected control participants

	3DL1	2DL1	2DL3	2DS4	2DL2	2DL5	3DS1	2DS1	2DS2	2DS3	2DS5
3DL1	D’										
	r2										
2DL1	0.012										
	0.912										
2DL3	0.038	0.317		1.000	0.054	0.146	0.129	0.072	0.129	0.164	0.174
	0.727	0.004		0.000	0.752	0.396	0.453	0.673	0.453	0.339	0.310
2DS4	0.570	0.021	0.348		0.054	0.146	0.129	0.072	0.121	0.164	0.174
	0.000	0.846	0.001		0.752	0.396	0.453	0.673	0.453	0.339	0.310
2DL2	0.110	0.110	0.346	0.192		0.161	0.204	0.456	0.204	0.122	0.104
	0.314	0.314	0.001	0.078		0.347	0.234	0.008	0.234	0.476	0.543
2DL5	0.095	0.095	0.222	0.167	0.577		0.383	0.328	0.633	0.169	0.478
	0.384	0.384	0.042	0.127	0.000		0.026	0.056	0.000	0.324	0.005
3DS1	0.218	0.055	0.079	0.222	0.207	0.376		0.562	0.356	0.290	0.246
	0.046	0.612	0.471	0.042	0.057	0.001		0.001	0.038	0.091	0.151
2DS1	0.169	0.169	0.111	0.296	0.234	0.353	0.450		0.215	0.059	0.415
	0.122	0.122	0.308	0.007	0.032	0.001	0.000		0.211	0.732	0.015
2DS2	0.107	0.107	0.261	0.188	0.691	0.550	0.136	0.271		0.413	0.246
	0.326	0.326	0.017	0.085	0.000	0.000	0.454	0.013		0.016	0.151
2DS3	0.059	0.059	0.273	0.203	0.427	0.411	0.082	0.270	0.471		0.236
	0.587	0.587	0.012	0.063	0.000	0.000	0.454	0.013	0.000		0.169
2DS5	0.102	0.102	0.168	0.179	0.358	0.689	0.410	0.345	0.333	0.010	
	0.349	0.349	0.123	0.101	0.001	0.000	0.000	0.002	0.002	0.926	
2DP1	0.017	0.703	0.198	0.030	0.156	0.135	0.079	0.069	0.152	0.084	0.145
	0.875	0.000	0.069	0.783	0.152	0.213	0.471	0.526	0.162	0.439	0.183

D’ the coefficient of linkage disequilibrium, r^2: the square of the correlation coefficient between two genes.

Table 18: Linkage disequilibrium analysis of the KIR genes of HBV+ participants

	2DL2	2DL5	3DS1	2DS1	2DS2	2DS3	2DS5	2DP1
2DL2	D’							
	r2							
2DL3	0.223	0.195	0.101	0.086	0.209	0.309	0.026	0.048
	0.214	0.278	0.574	0.632	0.245	0.085	0.887	0.790
2DL2			0.132	0.164	0.935	0.590	0.245	0.155
			0.462	0.361	0.000	0.001	0.171	0.388
2DL5			0.285	0.441	0.656	0.630	0.538	0.135
			0.112	0.014	0.000	0.000	0.003	0.451
3DS1				0.126	0.108	0.063	0.424	0.070
				0.483	0.546	0.726	0.018	0.696
2DS1					0.188	0.278	0.078	0.060
					0.296	0.121	0.665	0.739
2DS2						0.541	0.322	0.145
						0.003	0.073	0.419
2DS3							0.148	0.155
							0.409	0.388
2DS5								0.201
								0.263

D’ the coefficient of linkage disequilibrium, r^2: the square of the correlation coefficient between two genes.

Table 19: Linkage disequilibrium analysis of the KIR genes of HCV+ participants

	3DL1	2DL2	2DL5	3DS1	2DS1	2DP1
3DL1	D’					
	r2					
2DL1	0.031					
	0.858					
2DL5	0.134	0.234				
	0.443	0.179				
3DS1	0.031	0.031	0.134			
	0.858	0.858	0.443			
2DS1	0.056	0.056	0.199	0.056		
	0.748	0.748	0.252	0.748		
2DS5	0.117	0.117	0.598	0.250	0.250	
	0.503	0.503	0.001	0.151	0.151	
2DP1	0.696	0.043	0.072	0.361	0.360	0.109
	0.000	0.796	0.679	0.038	0.038	0.532

D’ the coefficient of linkage disequilibrium, r^2: the square of the correlation coefficient between two genes.

3.7 HLA Class I Alleles

HLA Class I typing was done by Sanger sequencing using the BigDye Terminator version 3.1 (Applied Biosystems, USA). The sequences were analysed with the Assign 400 software (Conexio Genomic, Western Australia). Genes in 184 participants were successfully typed for HLA class I (HLA-A and -C). Due to limitation of funds, typing could not be done for HLA-B. HLA-A and HLA-C data were available for 156 and 165 individuals, respectively. Table 12 below gives a summary of all the HLA class I (HLA-A and -C) 4-digit alleles found in this sample of Cameroonian population. Individual alleles were computed as a proportion of the total number of alleles determined per locus, while the genotype frequency of a particular allele was calculated as the proportion of individuals with a particular allele compared to the total number of individuals with a genotype result assigned for that locus. A total of 27 HLA-A and 28 HLA-C 4-digit alleles were identified in this cohort. The most frequent HLA-A allele group was the HLA-A*23 (19.23%) while the most common HLA-C one was the C*07 (27.57) (Figure 32). The number of individuals with homozygous alleles (two copies with the same allele) at the A locus were 22 (14.10%), while at the C locus there were 18 (10.91%). No two-locus (A-C) homozygosity was found in the study population (Table 13).

Table 20: HLA-A and -C allele frequencies in the study population

HLA-A Alleles	Frequency (n = 312)	HLA-C Allele	Frequency (n = 330)
A*01:01	0.006(2)	C*01:02	0.003(1)
A*02:01	0.077(24)	C*02:02	0.015(5)
A*02:02	0.061(19)	C*02:10	0.052(17)
A*02:05	0.016(5)	C*02:27	0.003(1)
A*03:01	0.087(27)	C*03:02	0.006(2)
A*23:01	0.186(58)	C*03:03	0.003(1)
A*23:02	0.003(1)	C*03:04	0.036(12)
A*23:14	0.003(1)	C*04:01	0.167(55)
A*24:02	0.006(2)	C*04:07	0.012(4)
A*26:01	0.016(5)	C*05:01	0.027(9)
A*29:01	0.010(3)	C*06:02	0.115(38)
A*29:02	0.074(23)	C*07:01	0.173(57)
A*30:01	0.061(19)	C*07:02	0.085(28)
A*30:02	0.087(27)	C*07:04	0.003(1)
A*30:04	0.006(2)	C*07:05	0.003(1)
A*31:01	0.010(3)	C*07:27	0.012(4)
A*32:01	0.019(6)	C*08:02	0.061(20)
A*33:01	0.010(3)	C*08:04	0.006(2)
A*33:03	0.022(7)	C*12:03	0.015(5)
A*34:02	0.013(4)	C*14:02	0.012(4)
A*36:01	0.026(8)	C*14:03	0.027(9)
A*66:01	0.051(16)	C*14:05	0.003(1)
A*66:02	0.013(4)	C*14:20	0.003(1)
A*66:03	0.003(1)	C*15:05	0.012(4)
A*68:01	0.022(7)	C*16:01	0.042(14)
A*68:02	0.074(23)	C*16:07	0.003(1)
A*74:01/02	0.038(12)	C*17:01	0.052(17)
		C*18:01	0.048(16)

n: total number of alleles for a particular locus; alleles that could not be discriminated based on sequences of exons 2 and 3 (A*74 = A*74:01/02). Common HLA alleles with a frequency >5% are in bold.

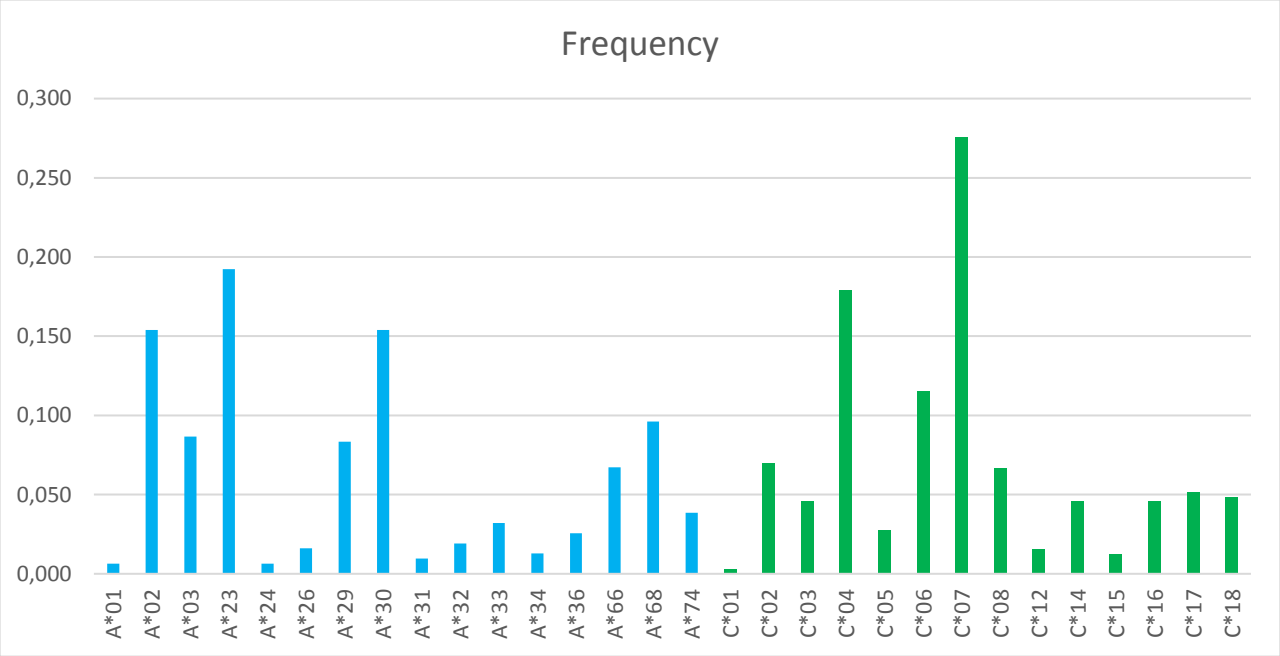


Figure 32: HLA -A and -C allele groups

Table 21: Zygosity of HLA-A and -C Loci

Locus	Frequency Homozygous(n)	p^a	Frequency Heterozygous(n)	P^b
A(n=156)	22 (14.10)	**0.0087**	134 (85.90)	0.4353
C(n=165)	18 (10.91)	0.5633	147 (89.09)	0.8513
A-C(n=137)	0 (0.00)		137 (100.00)	

n: number of genotypes; p^a and p^b: p value for the HWE test of homozygosity of HLA-A and HLA-C alleles respectively

There were no significant deviations from the HWE in the HLA-A and HLA-C alleles observed in this study using the Guo and Thompson Hardy Weinberg output (mcmc) test.

3.8. HLA-A Alleles and Association with Viral Hepatitis B and C

Twenty-seven distinct 4-digit HLA-A alleles were identified in 156 samples. The most common one was the A*23:01 (18.6%), followed by the A*30:02(8.7%) and A*03:01(8.7%). Five other HLA-A alleles had frequencies greater than 5% (A*02:01, A*02:02, A*29:02, A*66:01, A*68:02). We were unable to unambiguously distinguish between A*74:01 and A*74:02 with the sequences from Exons

2 and 3 because their nucleotide and amino acid sequences are similar there, but differ at Exon 1 by one nucleotide at Position 67 (Codon 23). At that point, A*74:01 has an Adenine (A) while A*74:02 has a Thymine (T), hence changing their amino acid at Codon 23 from Arginine (R) in A*74:01 to Tryptophan (W) in A*74:02. Due to financial constraints once more, we were unable to sequence Exon 1 to discriminate between these two alleles. As such, A*74:01 and A*74:02 were denoted as A*74:01/02. The cumulative frequency of the nine most common HLA-A alleles with a frequency of at least 5% in this study population was 75.6%. We had four very diverse allele groups (A*02, A*23, A*30 and A*66) with three alleles each and varying frequencies.

Furthermore, we determined the population Genotype Frequencies (GFs) of all the four-digit HLA-A alleles found in our study population (Figure 33). We carried out univariate analysis comparing the HLA-A GFs between the cases (HBV+ and HCV+) and the uninfected controls (HIV-1-, HBV- and HCV-negative) as shown in Table 17 below.

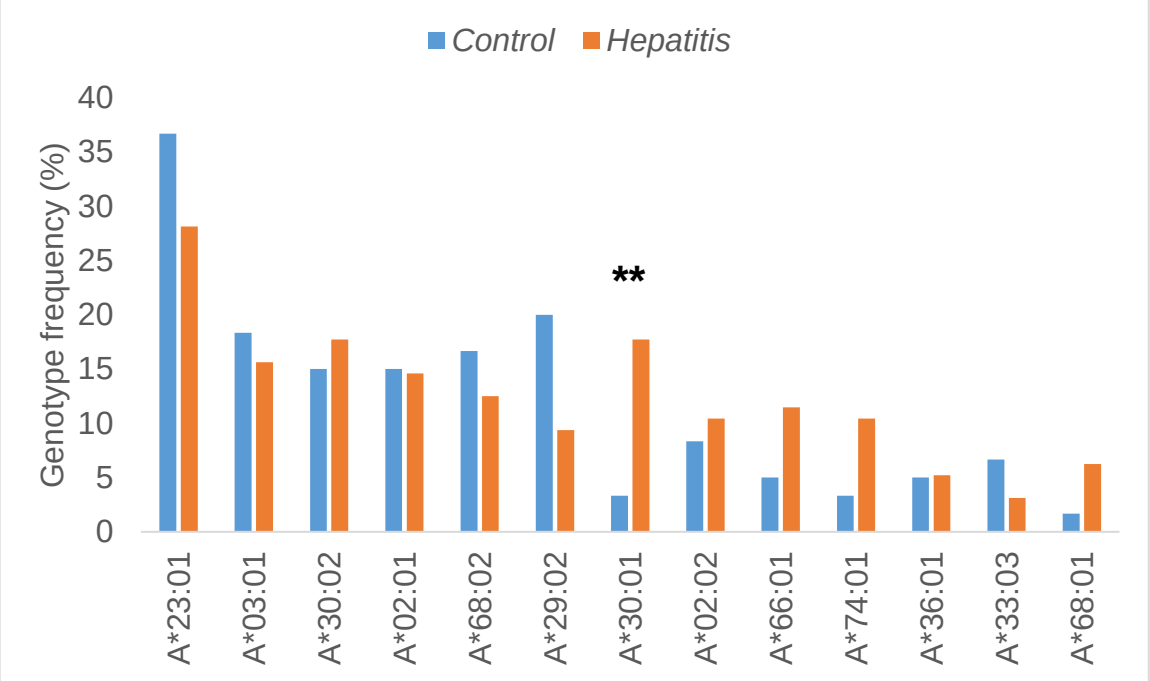


Figure 33: HLA-A genotype distribution between cases (with hepatitis) and controls (uninfected).

Only HLA-A alleles present in at least 4 % of the study populations are represented. Table 17 below shows the distribution of all 27 alleles stratified by disease status.

We observed that the frequency of HLA-A*30:01 was significantly higher in the Cases (combined Hepatitis group) than in the uninfected control group (17.71 vs 3.33, OR: 6.24 (1.33 – 29.24), p = 0.008), suggesting that the presence of this allele in an individual might increase the risk of contracting viral Hepatitis B and or C compared to individuals without this allele

Table 22: HLA-A Genotype Frequency Distribution Between Cases and Controls

HLA-A alleles		All		Control		Hepatitis			
	N=156	(%)	N=60	(%)	N=96	(%)	OR	P	95% CI
A*23:01	49	31.4	22	36.7	27	28.1	0.68	0.265	0.34 - 1.35
A*03:01	26	16.7	11	18.3	15	15.6	0.82	0.660	0.35 - 1.95
A*30:02	26	16.7	9	15.0	17	17.7	1.22	0.660	0.50 - 2.95
A*02:01	23	14.7	9	15.0	14	14.6	0.97	0.943	0.39 - 2.40
A*68:02	22	14.1	10	16.7	12	12.5	0.71	0.468	0.29 - 1.78
A*29:02	21	13.5	12	20.0	9	9.4	0.41	0.059	0.16 - 1.07
A*30:01	**19**	**12.2**	**2**	**3.3**	**17**	**17.7**	**6.24**	**0.008**	**1.33 - 29.24**
A*02:02	15	9.6	5	8.3	10	10.4	1.28	0.669	0.41 - 3.96
A*66:01	14	9.0	3	5.0	11	11.5	2.46	0.171	0.65 - 9.32
A*74:01	12	7.7	2	3.3	10	10.4	3.37	0.107	0.70 - 16.25
A*36:01	8	5.1	3	5.0	5	5.2	1.04	0.954	0.24 - 4.56
A*33:03	7	4.5	4	6.7	3	3.1	0.45	0.300	0.10 - 2.11
A*68:01	7	4.5	1	1.7	6	6.3	3.93	0.180	0.45 - 34.17
A*32:01	6	3.9	3	5.0	3	3.1	0.61	0.555	0.12 - 3.16
A*02:05	5	3.2	3	5.0	2	2.1	0.40	0.316	0.06 - 2.52
A*26:01	5	3.2	3	5.0	2	2.1	0.40	0.316	0.06 - 2.52
A*66:02	4	2.6	3	5.0	1	1.0	0.20	0.129	0.02 - 2.02
A*34:02	3	1.9	2	3.3	1	1.0	0.31	0.312	0.03 - 3.50
A*29:01	3	1.9	0	0.0	3	3.1	-	-	-
A*31:01	3	1.9	1	1.7	2	2.1	1.26	0.854	0.11 - 14.27
A*33:01	3	1.9	2	3.3	1	1.0	0.31	0.312	0.03 - 3.50
A*01:01	2	1.3	1	1.7	1	1.0	0.62	0.737	0.04 - 10.22
A*24:02	2	1.3	1	1.7	1	1.0	0.62	0.737	0.04 - 10.22
A*30:04	2	1.3	1	1.7	1	1.0	0.62	0.737	0.04 - 10.22
A*23:02	1	0.6	0	0.0	1	1.0	-	-	-
A*23:14	1	0.6	0	0.0	1	1.0	-	-	-
A*66:03	1	0.6	0	0.0	1	1.0	-	-	-

N: number of individuals, OR: Odds ratio: P value comparing the control group with the hepatitis group. 95%CI: 95% confidence interval.

Comparing the GFs in the individual disease status (HBV+ or HCV+) with the uninfected control group, HLA-A*30:01 which was overrepresented in the combined cases was still over-represented in the Hepatitis groups (HBV+ and HCV+) (17.9 vs 3.3, OR: 6.30 (1.07 – 37.22), p = 0.020 and 3.3 vs 17.7, OR: 6.21 (1.27 – 30.46), p = 0.010 respectively). This supports the idea that this allele might render persons susceptible to viral Hepatitis B and C infections compared to persons without it.

Table 23: HLA-A Genotype Frequency in the Study Population Stratified by Disease Status

HLA	Control	HBV	HCV	HBV			HCV		
	N=60	N=28	N=68	OR	P	95% CI	OR	P	95% CI
A*23:01	22 (36.7)	6 (21.4)	21 (30.9)	0.47	0.155	0.16 - 1.36	0.77	0.491	0.37 - 1.62
A*03:01	11 (18.3)	4 (14.3)	11 (16.2)	0.74	0.640	0.21 - 2.60	0.86	0.748	0.34 - 2.16
A*30:02	9 (15.0)	4 (14.3)	13 (19.1)	0.94	0.930	0.26 - 3.40	1.34	0.539	0.53 - 3.42
A*02:01	9 (15.0)	4 (14.3)	10 (14.7)	0.94	0.930	0.26 - 3.40	0.98	0.963	0.37 - 2.60
A*29:02	12 (20.0)	2 (7.1)	7 (10.3)	0.31	0.127	0.06 - 1.53	0.46	0.125	0.17 - 1.27
A*68:02	10 (16.7)	5 (17.9)	7 (10.3)	1.09	0.891	0.33 - 3.57	0.57	0.291	0.20 - 1.63
A*02:02	5 (8.3)	2 (7.1)	8 (11.8)	0.85	0.848	0.15 - 4.70	1.47	0.523	0.45 - 4.78
A*30:01	2 (3.3)	5 (17.9)	12 (17.7)	**6.30**	**0.020**	**1.07 - 37.22**	**6.21**	**0.010**	**1.27 - 30.46**
A*66:01	3 (5.0)	1 (3.6)	10 (14.7)	0.70	0.766	0.07 - 7.19	3.28	0.071	0.84 - 12.81
A*74:01	2 (3.3)	2 (7.1)	8 (11.8)	2.23	0.427	0.29 - 17.03	3.87	0.077	0.77 - 19.48
A*36:01	3 (5.0)	4 (14.3)	1 (1.5)	3.17	0.136	0.64 - 15.70	0.28	0.254	0.03 - 2.86
A*33:03	4 (6.7)	1 (3.6)	2 (2.9)	0.52	0.561	0.05 - 4.95	0.42	0.322	0.07 - 2.44
A*68:01	1 (1.7)	3 (10.7)	3 (4.4)	7.08	0.059	0.66 - 75.99	2.72	0.375	0.27 - 27.34

Only HLA-A alleles present in at least four percent of the study populations are represented.

N: number, p: p value comparing the control and the HBV and HCV groups, 95%CI: 95% Confidence Interval

The HLA-A alleles were then grouped into Bw4/Bw4, Bw4/Bw6 and Bw6/Bw6. Comparative analyses between the frequencies of these ligand groups showed that the Bw4/Bw6 were significantly overrepresented in the control group compared to the HCV+ infected group (p = 0.004, OR (95%CI): 0.13(0.03 – 0.614) (Table 18). This implies that the Bw4/Bw6 ligands might be associated with protection against HCV infections.

Table 24: Association of HLA-A Bw4 and non-Bw4 with viral Hepatitis B and C infections

	HBV+	**HCV**	**Control**	**HBV+ vs Control**		**HCV+ vs Control**	
HLA-A type	**n(%)**	**n(%)**	**n(%)**	**p**	**OR(95%CI)**	**p**	**OR(95%CI)**
Bw4/Bw4	4(14.29)	5(7.25)	3(5.00)	0.72	1.50(0.35 - 659)	0.202	3.17(0.66 - 15.23)
Bw4/Bw6	2(7.14)	20(29.41)	22(36.67)	0.45	0.72(0.34 - 1.50)	0.004	0.13(0.03 - 0.614)
Bw6/Bw6	22(78.57)	43(63.24)	35(58.33)	0.59	1.23(0.60 - 2.50)	0.093	2.619(0.93 - 7.39)

95%CI: 95% Confidence Interval, OR: Odds ratio, p: p value comparing the control and the HBV and HCV groups: n(%): number(percentage)

3.9. HLA–C Alleles

Similarly to the HLA-A locus, we detected 28 HLA-C alleles at the HLA-C locus from 140 samples. The most frequent one was the C*07:01 (17.1%) followed by C*04:01 (15.1%), C*06:02 (11.4%) and C*07:02 (9.1%). Four other common alleles had frequencies of at least 5% (C*02:10, C*08:02, C*17:01 and C*18:01) (Table 19). Comparisons of the GFs of these alleles between the cases and the controls (Figure 34) showed that HLA-C*03:04 was significantly enriched in the uninfected control group (12.8 vs 2.2, OR: 0.15(0.02 – 0.81), p = 0.0109), indicating that its presence might reduce the risk of acquiring viral Hepatitis B and C.

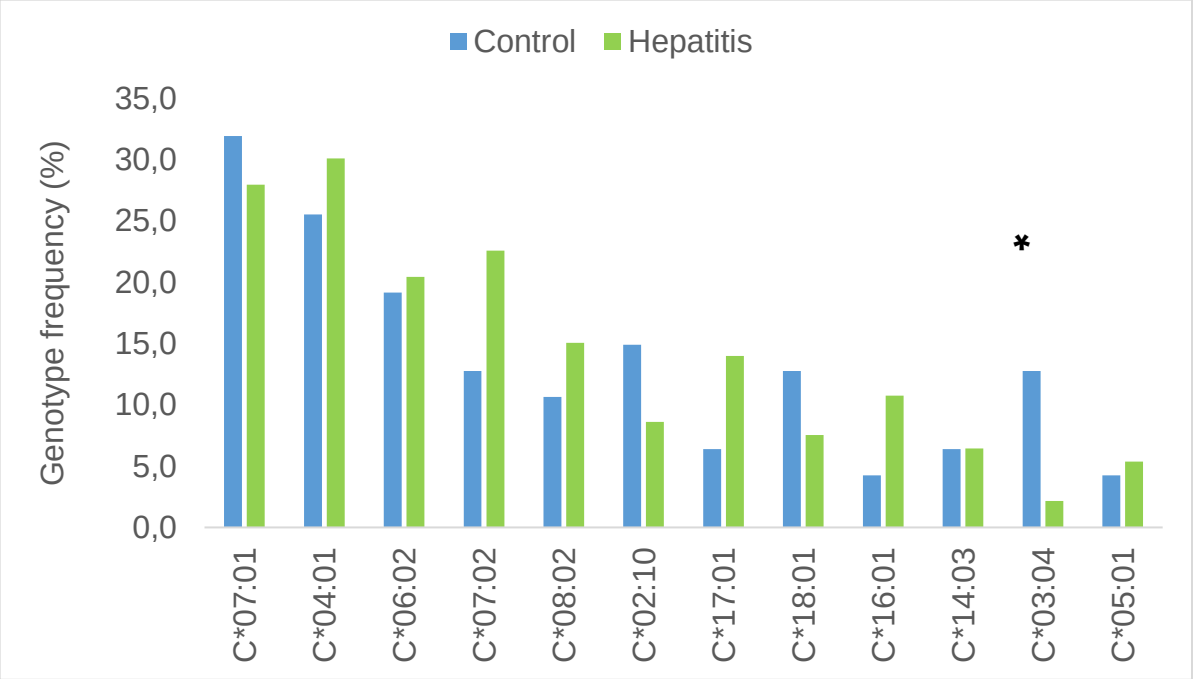


Figure 34: HLA-C genotype distribution between cases and controls

HLA-C alleles present in at least 5 % of the study population are represented.

Three HLA-C*03 subtypes were represented in our study population with two of them (HLA-C*03:02 and -C*03:03) at minor allele frequencies (< 5%) (Table 21). HLA-C*03:04, however, was overrepresented in the uninfected control group compared to individuals with chronic hepatitis B and C (12.8% vs. 2.2%, P=0.010) suggesting that this allele may be protective against hepatitis.

Table 25: HLA-C Genotype Frequency Distribution Between Cases and Controls

HLA-C alleles	N=140	All (%)	N=47	Control (%)	N=93	Hepatitis (%)	OR	P	95% CI
C*07:01	41	29.3	15	31.9	26	28.0	0.83	0.628	0.38 - 1.78
C*04:01	40	28.6	12	25.5	28	30.1	1.26	0.573	0.57 - 2.78
C*06:02	28	20.0	9	19.1	19	20.4	1.08	0.859	0.45 - 2.63
C*07:02	27	19.3	6	12.8	21	22.6	1.99	0.166	0.74 - 5.39
C*08:02	19	13.6	5	10.6	14	15.1	1.49	0.473	0.50 - 4.44
C*02:10	15	10.7	7	14.9	8	8.6	0.54	0.257	0.18 - 1.60
C*17:01	16	11.4	3	6.4	13	14.0	2.38	0.184	0.64 - 8.93
C*18:01	13	9.3	6	12.8	7	7.5	0.56	0.315	0.17 - 1.78
C*16:01	12	8.6	2	4.3	10	10.8	2.71	0.196	0.56 - 13.11
C*14:03	9	6.4	3	6.4	6	6.5	1.01	0.988	0.24 - 4.26
C*03:04	**8**	**5.7**	**6**	**12.8**	**2**	**2.2**	**0.15**	**0.011**	0.03 - 0.81
C*05:01	7	5.0	2	4.3	5	5.4	1.28	0.775	0.24 - 6.90
C*02:02	5	3.6	2	4.3	3	3.2	0.75	0.757	0.12 - 4.68
C*04:07	4	2.9	1	2.1	3	3.2	1.53	0.714	0.15 - 15.30
C*15:05	4	2.9	1	2.1	3	3.2	1.53	0.714	0.15 - 15.30
C*07:27	3	2.1	1	2.1	2	2.2	1.01	0.993	0.09 - 11.54
C*14:02	3	2.1	1	2.1	2	2.2	1.01	0.993	0.09 - 11.54
C*08:04	2	1.4	0	0.0	2	2.2	-	-	-
C*12:03	2	1.4	0	0.0	2	2.2	-	-	-
C*01:02	1	0.7	0	0.0	1	1.1	-	-	-
C*02:27	1	0.7	1	2.1	0	0.0	-	-	-
C*03:02	1	0.7	1	2.1	0	0.0	-	-	-
C*03:03	1	0.7	0	0.0	1	1.1	-	-	-
C*07:04	1	0.7	1	2.1	0	0.0	-	-	-
C*07:05	1	0.7	0	0.0	1	1.1	-	-	-
C*14:05	1	0.7	0	0.0	1	1.1	-	-	-
C*16:07	1	0.7	0	0.0	1	1.1	-	-	-

N: number of individuals, OR: Odds ratio: P value comparing the control group with the hepatitis group. 95%CI: 95% confidence interval.

Moreover, we performed a comparative analysis of the GFs of the individual alleles present in the various infected groups (HBV+ and HCV+) and the uninfected control group (Table 19). The HLA-C*17:01 was found to be significantly enriched in the HBV+ group as compared to the uninfected control group (21.9 vs 6.4, OR: 4.11(0.93 – 18.18), p = 0.043), suggesting that allele might be a HBV-susceptible allele. This was not the case with the HCV+ group whose GFs showed no statistically significant difference from the uninfected control group. The HLA-C*03:04 allele which was significantly different in the combined Hepatitis group showing no statistically significant difference in the various groups.

Table 26:HLA-C genotype frequency in the study population by disease status

HLA-C alleles	Control N=47	HBV N=32	HCV N=61	HBV			HCV		
				OR	P	95% CI	OR	P	95% CI
C*07:01	15 (31.9)	8 (25.0)	18 (29.5)	0.71	0.509	0.26 - 1.97	0.89	0.789	0.39 - 2.04
C*04:01	12 (25.5)	12 (37.5)	16 (26.2)	1.75	0.259	0.65 - 4.69	1.04	0.935	0.43 - 2.48
C*06:02	9 (19.1)	6 (18.8)	13 (21.3)	0.97	0.965	0.31 - 3.09	1.14	0.783	0.44 - 2.97
C*07:02	6 (12.8)	8 (25.0)	13 (21.3)	2.28	0.165	0.69 - 7.52	1.85	0.250	0.64 - 5.37
C*08:02	5 (10.6)	2 (6.3)	12 (19.7)	0.56	0.503	0.10 - 3.13	2.06	0.203	0.66 - 6.40
C*02:10	7 (14.9)	3 (9.4)	5 (8.2)	0.59	0.472	0.14 - 2.52	0.51	0.275	0.15 - 1.75
C*17:01	3 (6.4)	7 (21.9)	6 (9.8)	**4.11**	**0.043**	0.93 - 18.18	1.60	0.522	0.37 - 6.83
C*18:01	6 (12.8)	3 (9.4)	4 (6.6)	0.71	0.644	0.16 - 3.09	0.48	0.272	0.13 - 1.83
C*16:01	2 (4.3)	3 (9.4)	7 (11.5)	2.33	0.362	0.36 - 15.12	2.92	0.180	0.56 - 15.06
C*14:03	3 (6.4)	0 (0.0)	6 (9.8)	-	-	-	1.60	0.522	0.37 - 6.83
C*03:04	6 (12.8)	0 (0.0)	2 (3.3)	-	-	-	0.23	0.063	0.04 - 1.25
C*05:01	2 (4.3)	4 (12.5)	1 (1.6)	3.21	0.177	0.53 - 19.33	0.38	0.414	0.03 - 4.35

Only HLA-C alleles present in at least four percent of the study populations are represented

N: number of individuals, OR: Odds ratio: P value comparing the control group with the hepatitis group. 95%CI: 95% confidence interval.

HLA-C alleles were grouped into two mutually exclusive categories, C1 and C2, based on the amino acid present at position 80. Alleles with Asparagine at position 80 were classified as C1 whereas those with Lysine at position 80 were classified as C2. This grouping is essential because certain KIRs on the surfaces of NK cells specifically bind to group C1 alleles while others bind to C2 alleles as their ligands in order to modulate cell activity. We determined the frequency of C1 and C2 alleles in the cases and controls and tested their effect on disease status.

Table 27: Association of HLA-C1 and -C2 groups of alleles with viral Hepatitis B and C

	HBV+	**HCV+**	**Healthy Controls**				
	n = 32	**n = 61**	**n = 56**				
HLA-C type	**(%)**	**(%)**	**(%)**	**ORa (95% CI)**	**Pa**	**ORb (95% CI)**	**Pb**
C1C1	15.6	31.1	30.4	2.35(0.0.78 – 7.15)	0.2	0.96(0.44 – 2.12)	1
C1C2	46.9	49.2	41.1	0.79(0.33 – 1.89)	0.66	0.72(0.35 – 1.49)	0.46
C2C2	37.5	19.7	28.6	0.67(0.27 – 1.68)	0.48	1.63(0.69 – 3.85)	0.28

n: number of individuals, OR: Odds ratio: P value comparing the control group with the hepatitis group. 95%CI: 95% confidence interval.

The heterozygous (C1/C2) allele group was the most common in the overall population with a frequency of (45.7%), followed by the homozygous C2 (C2/C2) (28.6%) and then the homozygous C1 (C1/C1) (25.7%). There were no statistically significant differences between the frequencies of the various categories (C1/C1, C1/C2, C2/C2) between the cases and the control groups. This suggest that this HLA-C group of alleles may not have an impact on disease status in this population.

3.10. HLA-A and -C Haplotypes

A total number of 128 possible two-loci haplotypes were identified, 24 of which had a frequency of at least 1% in our cohort. A*23:01–C*05:01 was the most prevalent haplotype with a frequency 3.7% followed by A*23:01–C*03:04 (3.3%) (Table 28)

Table 28: Common HLA Class I two-locus (-A-C) haplotypes in the study population

Haplotype	F	Haplotype	F	Haplotype	F	Haplotype	F	Haplotype	F
A*23:01-C*05:01	0.037	A*30:01-C*02:10	0.009	A*68:01-C*04:07	0.006	A*03:01-C*06:02	0.003	A*33:03-C*06:02	0.003
A*23:01-C*03:04	0.033	A*30:01-C*04:01	0.009	A*68:02-C*06:02	0.006	A*23:01-C*04:01	0.003	A*33:03-C*12:03	0.003
A*23:01-C*07:01	0.024	A*30:02-C*08:02	0.009	A*74:01-C*05:01	0.006	A*23:01-C*06:02	0.003	A*33:03-C*16:01	0.003
A*23:01-C*07:02	0.021	A*33:03-C*04:07	0.009	A*02:02-C*17:01	0.006	A*23:14-C*16:01	0.003	A*34:02-C*06:02	0.003
A*03:01-C*02:10	0.021	A*36:01-C*04:01	0.009	A*02:02-C*04:01	0.005	A*24:02-C*07:01	0.003	A*34:02-C*07:01	0.003
A*23:01-C*16:01	0.021	A*36:01-C*07:01	0.009	A*02:01-C*03:04	0.005	A*26:01-C*02:10	0.003	A*36:01-C*14:03	0.003
A*03:01-C*07:02	0.019	A*68:01-C*04:01	0.009	A*02:01-C*07:01	0.004	A*26:01-C*16:01	0.003	A*66:01-C*02:10	0.003
A*29:02-C*02:10	0.018	A*68:02-C*07:02	0.009	A*03:01-C*07:01	0.004	A*29:01-C*07:02	0.003	A*66:01-C*07:27	0.003
A*02:01-C*07:02	0.017	A*74:01-C*07:27	0.009	A*23:01-C*17:01	0.004	A*29:01-C*17:01	0.003	A*66:01-C*14:02	0.003
A*29:02-C*07:02	0.017	A*74:01-C*17:01	0.009	A*23:01-C*08:02	0.004	A*29:02-C*04:01	0.003	A*66:01-C*14:03	0.003
A*02:01-C*14:02	0.016	A*30:02-C*04:01	0.008	A*24:02-C*16:01	0.004	A*29:02-C*16:07	0.003	A*66:02-C*17:01	0.003
A*68:02-C*12:03	0.015	A*29:02-C*06:02	0.008	A*02:02-C*08:02	0.003	A*29:02-C*18:01	0.003	A*66:03-C*08:02	0.003
A*30:02-C*05:01	0.014	A*30:01-C*07:05	0.007	A*66:01-C*07:02	0.003	A*30:01-C*08:04	0.003	A*68:01-C*15:05	0.003
A*30:01-C*06:02	0.014	A*30:04-C*07:01	0.007	A*02:02-C*07:02	0.003	A*30:01-C*17:01	0.003	A*68:02-C*03:04	0.003
A*23:01-C*18:01	0.012	A*03:01-C*16:01	0.006	A*01:01-C*01:02	0.003	A*30:02-C*02:10	0.003	A*68:02-C*05:01	0.003
A*23:02-C*08:02	0.012	A*68:02-C*07:01	0.006	A*01:01-C*07:04	0.003	A*30:02-C*07:01	0.003	A*68:02-C*14:02	0.003
A*30:02-C*02:02	0.012	A*02:01-C*06:02	0.006	A*02:01-C*02:10	0.003	A*30:02-C*18:01	0.003	A*68:02-C*14:03	0.003
A*66:01-C*02:02	0.012	A*02:02-C*07:01	0.006	A*02:01-C*14:05	0.003	A*30:04-C*14:03	0.003	A*68:02-C*15:05	0.003
A*66:01-C*07:01	0.012	A*02:02-C*14:03	0.006	A*02:01-C*16:01	0.003	A*31:01-C*08:04	0.003	A*74:01-C*02:10	0.003
A*68:02-C*02:10	0.012	A*02:02-C*16:01	0.006	A*02:02-C*02:02	0.003	A*31:01-C*17:01	0.003	A*74:01-C*03:04	0.003
A*03:01-C*04:07	0.011	A*26:01-C*08:02	0.006	A*02:02-C*02:10	0.003	A*31:01-C*18:01	0.003	A*74:01-C*06:02	0.003
A*29:02-C*07:01	0.011	A*30:02-C*14:03	0.006	A*02:02-C*18:01	0.003	A*32:01-C*02:02	0.003	A*74:01-C*08:02	0.003
A*03:01-C*08:02	0.010	A*30:02-C*15:05	0.006	A*02:05-C*02:02	0.003	A*32:01-C*03:04	0.003	A*03:01-C*04:01	0.003
A*30:02-C*07:02	0.010	A*32:01-C*18:01	0.006	A*02:05-C*03:04	0.003	A*32:01-C*05:01	0.003	A*66:01-C*06:02	0.002
A*02:01-C*18:01	0.009	A*33:01-C*07:02	0.006	A*02:05-C*07:01	0.003	A*32:01-C*07:01	0.003		
A*02:02-C*06:02	0.009	A*66:02-C*04:01	0.006	A*02:05-C*07:02	0.003	A*33:01-C*04:01	0.003		

F: frequency

3.11 KIRs and their Corresponding HLA Ligands

Interactions between KIRs and their cognate HLA ligands determine the type of signal transmitted to the NK cell with the particular KIR on its surface. Individuals were grouped based on the presence or absence of particular KIR genes and their cognate ligands and assessed whether KIR/HLA pairs correlated with disease status as shown in Table 22 below. It was observed that none of the HBV+, HIV-1+ and HCV+ participants had the *2DL2/2DL3* + C1C1 compound genotype while 6 (20%) of the Uninfected Controls had it (p = 0.025, 0.024 and 0.305 respectively). This indicates that this KIR: HLA combination may have a protective effect against viral Hepatitis B and C. Contrarily, the 2DL2/2DL3/2DS2 +C1C2 compound genotype was found to be highly enriched in the HCV-infected group compared to the uninfected controls (58.62% vs 20.0%, p = 0.003), indicating that its presence might increase the risk of acquiring HCV infection. In the same light, the 2DL3 + C1C2 and the 2DL1 +C1C2 KIR: HLA combinations were significantly overrepresented in the HIV-1+ positive group (33.3% vs 0%; p = 0.009 and 55.5% vs 3.3%; p = 0.001 respectively), while the incidence of 2DL1/2DS1 + C2C2 KIR:HLA combination was significantly higher in the HBV-positive group compared to the uninfected group (46% vs 0.0%; p = 0.02), suggesting that it might increase the risk of acquiring HBV infection (Table 29).

Table 29: Distribution of KIR-HLA combinations among cases (HIV-1+, HBV+, HCV+) and Uninfected Controls

	HBV+ N = 26	HCV+ N= 29	HIV+ N = 9	Control N = 30	HBV+ vs control		HCV+ vs control		HIV+ vs Control	
KIR: HLA Ligand	N (%)	N (%)	N (%)	N (%)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)
2DL2/2DL3/2DS2 + C1C1	1(3.85)	8(27.59)	1(11.11)	4(13.33)	0.358	3.84(0.40 - 36.82)	0.209	0.40(0.11 - 1.52)	1	1.23(0.13 - 12.65)
2DL2/2DL3 + C1C1	0(0.00)	0(0.00)	0(0.00)	6(20.00)	**0.025**	1.250(1.05 - 1.49)	**0.024**		0.305	
2DL2/2DS2 + C1C1	0(0.00)	0(0.00)	0(0.00)	1(3.33)	1		1		1	
2DL3 + C1C1	3(11.54)	0(0.00)	1(11.11)	0(0.00)	0.094				0.231	
2DL2/2DL3/2DS2 + C1C2	8(30.77)	17(58.62)	3(33.33)	6(20.00)	0.375	0.56(0.17 - 1.91)	**0.003**	0.18 (0.06 - 0.56)	0.406	0.50(0.09 - 2.60)
2DL2/2DS2 + C1C2	1(3.85)	0(0.00)	0(0.00)	0(0.00)	0.464				1	
2DL2/2DL3 + C1C2	0(0.00)	0(0.00)	0(0.00)	2(6.7)	0.494		0.492			
2DL3/2DS2 + C1C2	0(0.00)	1(3.45)	0(0.00)	1(3.33)	1		1		1	
2DL2 + C1C2	0(0.00)	0(0.00)	1(11.11)	0(0.00)					0.231	
2DL3 + C1C2	3(11.54)	0(0.00)	3(33.33)	0(0.00)	0.094				**0.009**	
2DL1/2DS1 + C1C2	12(46.15)	15(51.72)	2(22.22)	9(30.00)	0.273	0.50(0.17 - 1.49)			1	1.50 (0.26 - 8.67)
2DL1 + C1C2	0(0.00)	3(10.34)	5(55.56)	1(3.33)	1		0.115	0.40(0.19 - 1.12)	**0.001**	0.03 (0.003 - 0.30)
2DL1/2DS1 + C2C2	12(46.15)	3(10.34)	0(0.00)	0(0.00)	**0.022**	0.23(0.07 - 0.79)	0.353	0.29(0.03 - 3.05)	0.318	
2DL1 + C2C2	1(3.85)	0(0.00)	0(0.00)	3(10.00)	0.615	2.78(0.27 - 28.42)	0.706	1.73(0.37 - 8.03)	1	

N: number of individuals, OR: Odds ratio: P value comparing the control group with the hepatitis and HIV group. 95%CI: 95% confidence interval.

DISCUSSION

The mechanisms of pathogenesis used by HIV and Hepatitis B and C viruses to evade immune surveillance and establish lifelong persistence in humans are still not fully understood. Nonetheless, host immune factors alongside viral and environmental factors are major players that modulate progression of most viral diseases. Several studies have implicated KIR and HLA class I as well as KIR-HLA compound genotype diversity at the population level with clearance or persistence of HIV and viral hepatitis infections. The interplay between KIR/HLA and other regulatory cells and molecules to modulate the outcomes of HIV, HBV or HCV infection is likely multifactorial and involves a cascade of immune responses and may also be population specific. In this study, we determined the frequencies of *KIR* and *HLA* class I (*HLA-A* and *–C*) genes in a cohort of adult Cameroonians infected with HIV-1, hepatitis B or hepatitis C viruses. Specifically, we sought to describe the heterogeneity at their KIR and HLA class I (HLA-A and -C) loci and to find out if the presence or absence of specific *KIR* genes and/or *HLA-A* and *–C* alleles is associated with susceptibility or resistance to HIV-1, HBV and HCV infections. We also aimed to study the association between KIR and HLA compound genotypes with HIV-1, HBV and HCV. In this study, we enrolled 169 participants with complete demographic information, 90 males (53.26%) and 79 females (46.74%). Looking more closely at the demographics however, we observed that significantly, there were more females in the HCV-infected group compared to the controls (64.7% versus 35.3%), which could be attributed to the fact that females visit hospitals more than men, and as such get more diagnosis. As a result of this, the difference in the gender between the HCV-infected and the Control Group was slightly statistically significant ($p = 0.049$). In the HBV-infected counterparts, the male sex was significantly overrepresented (88.1% versus 11.9%, $p<0.001$). This is in accordance with previous reports from other studies, which showed that the male sex is a risk factor for HBV infection (Baig, 2009; Ayano *et al.*, 2018). This significant disparity in gender can be associated with the fact that females often develop a more intense, effective and prolonged immune response. This can be associated with the difference in the expression levels of Toll-Like Receptors (TLRs) which are responsible for recognition of viral components (such as DNA, dsRNA, ssRNA and viral proteins), and the initiation of an immune response, as well as some monocytes, dendritic cells and macrophages. Moreover, females have higher numbers of CD4+ T cells compared to males, stronger cytotoxic cell activity and overexpression of pro-inflammatory and antiviral genes (Liu and Liu, 2014). These make females less prone to viral infections than males, however, they often develop more severe symptoms as a

result of the overexpression of inflammatory responses. Furthermore, the expression of sex steroid hormones on immune cells such as lymphocytes, monocytes and dendritic cells affects the release of cytokines and chemokines which mediate the differentiation, maturation and proliferation of immune cells. The synergistic action of different sex hormones secreted by males and females and the immune system is also a contributing factor to the differences observed. Estrogens have been reported to be immune stimulating and androgens immune suppressing (Ruggieri, Gagliardi and Anticoli, 2018).

We observed that HBV-and HCV-infected participants were significantly older compared to their uninfected counterparts in the control group ($p = 0.003$ and $p < 0.001$ respectively). The mean age in the HCV-infected group, was 61.6 years. This late age could be one of the reasons why the female sex was dominant in this group. This can be linked to the decreasing levels of sex hormones expressed by women as they get older, especially post menopause.

All the 15 KIR genes typed were present in our study population. A comparison of our results to data obtained from neighbouring Central African countries indicated that the frequencies of the framework genes and inhibitory genes were similar to those reported in Equatorial Guinea and Gabon. However, the frequencies of our activating genes were relatively higher compared to those reported in neighbouring African countries, but rather similar to those reported in England and China Eastern main land.

The frequency of the *KIR3DS1* gene in our study population was similar to that of European and Asian countries. Previous studies showed that it had a very low frequency generally less than 20% in African populations. A study by Jennes et al. (2006) reported a frequency of 16% in Ivory Coast, and another study by Mhandire et al. (2016a) in Zimbabwe reported a score of 18%. Some mixed African populations have, however, reported high frequencies of this gene, like the South African mixed ancestry with 36% (Gentle *et al.*, 2017). There was a difference in the frequency of KIR3DS1 observed in our study from that in neighbouring African countries like the Central African Republic (2.9%), Equatorial Guinea (5.2%), Gabon (7.4%), and Congo Kinshasa (11.1%) (Single *et al.*, 2007; Wauquier *et al.*, 2010). This might be associated with factors like the sample size, ethnicity and environmental factors.

Studies from sub-Saharan Africa have also shown that activating *KIR* genes are relatively uncommon there compared to other populations. We found three activating (*KIR2DS1, KIR2DS3,*

KIR3DS1) and one inhibitory KIR gene (*KIR2DL2*) to be significantly enriched in the Uninfected Control Group compared to the HIV-1-infected group, suggesting that they might offer some protection against HIV-1 infection. This concords with reports from other studies which have associated the *KIR3DS1* gene with a reduced risk of HIV-1 infection, and protective effect in serodiscordant couples. However, it is worth noting that this particular three-domain activating KIR gene is low in people of African descent though we had a strikingly higher frequency which was similar to those found in Europeans and Asians. Similarly, the *2DS3* and the *2DL2* genes have also been reported to play a protective role against HIV-1 infection. Numerous studies identified an association between KIR genes and HIV-1 susceptibility/protection and disease progression, but some of these results differed according to specific populations. This may be due to the ethnic diversity of the subjects and could also be associated to the limited sample size used.

Though the frequency of the *KIR3DS1* gene was generally high in our study population compared to other studies in sub-Saharan Africa, it was very low in the HBV-infected group. This is in accordance with reports from other studies (Norman et al. 2007; Yindom et al. 2010) and might indicate that the gene offers protection against HBV infection. We also observed that the *2DL2* gene might have a protective effect against HBV acquisition, though to a lesser extent than the *KIR3DS1* gene. These results are in accordance with those of Zhi–ming et al. (2007) on a Chinese Han population who reported that the *KIR3DS1, KIR2DS1* and *KIR2DL5* genes may be protective against chronic HBV infections. In this same study, they equally reported that the *KIR2DS2* and *KIR2DS3* genes might be chronic HBV-susceptible. This is equally in accord with a study report from Kibar et al. (2014), on a Turkish cohort which showed that the *KIR3DS1* and *KIR2DL3* genes may be HBV-protector genes. However, other studies from the Gambia associated KIR3DS1 with HBeAg (HBV) positivity and a high viral load, but associated the *KIR2DL3* gene with a low viral load (Yindom et al., 2017).

On the other hand, *KIR3DS1* was seen to be strongly associated with susceptibility to HCV infection. We observed that all but one HCV infected individual had KIR3DS1 compared with 2/3 of the Uninfected Control group (97.0% vs 64.7%, respectively, $p = 9.21 \times 10^{-4}$). Two other activating genes were present in all HCV-positive individuals compared to about half (*KIR2DS3*) and 2/3 (*KIR2DS3*) in the Uninfected Control group, and were associated with susceptibility to HCV infection. These results are in agreement with those of Re et al. (2015a) who found the

KIR2DS3 gene to be associated with chronic HCV infection. Studies on a larger population would be needed to confirm the effect of these genes on viral Hepatitis B and C transmission and disease susceptibility.

Our HLA data revealed the genetic diversity of this population. These results are similar to those obtained in some sub-Saharan African countries (Tshabalala, Mellet and Pepper, 2015). Few studies have looked at the HLA Class I genetic diversity in Cameroon. The HLA-A*23:01 allele which has been reported to be frequent in Africans was the most prevalent (AF= 18.6%) in this study and this was very similar to results from previous studies conducted in Cameroon by Ellis et al. (2000) and Torimiro et al. (2006). They found HLA-A*23:01 as the most prevalent allele, with frequencies of 18.7 and 12.8% respectively. Torimiro et al. in that same study reported the HLA-Cw*04:01 (AF=16.6%) allele as the most frequent HLA-C allele. In the present study, we found the C*07:01 allele to be the most prevalent (AF=17.1%), followed by C*04:01 (AF=15.1%). These results match other previous reports from Cameroon which showed that the C*07 and the C*04:01 alleles were prevalent (Spínola *et al.*, 2011).

There is a paucity of data on the association of HLA Class I genes and Hepatitis infection and liver diseases in Cameroon and several countries in SSA. While working on a cohort of individuals with Hepatitis B in South Africa, Matthews and colleagues (2016) demonstrated that HLA-A was a significant predictor of HBeAg status, confirming the predicted dominant role of HLA-A locus in the overall host immune responses against DNA viruses (Hertz *et al.*, 2011). In the present study, we report for the first time that HLA-A*30:01 may play a significant role in the acquisition of both Hepatitis viruses (HBV and HCV). Carriers of this allele were six times more likely to be HBV+ or HCV+ than non-carriers. In contrast, HLA-C*03:04 was found to be significantly more prevalent in controls compared to cases, showing that it might have a protective effect against viral Hepatitis. A growing body of reports have documented that different HLA Class I alleles including HLA-A*02, HLA-A*03 and HLA-A*31 are differentially associated with either a positive or negative effect in the control of viral Hepatitis (Nitschke *et al.*, 2016). None of the C1/C1, C1/C2 or C2/C2 allele groups had an effect on the susceptibility or resistance to Hepatitis B or C in this sample of Cameroonians.

Several studies have associated KIR-HLA compound genotype diversity at the population level with the clearance or persistence of viral infections. In this study, we found the *2DL2/2DL3* +

C1C1 compound genotype completely absent in infected groups (HIV-1+, HBV+ and HCV+) in this cohort. This could probably imply that this compound genotype offers some protection against these viral infections. However, the *2DL2/2DL3/2DS2* + C1C2 compound genotype was overrepresented in the HCV infected group, indicating that it might increase susceptibility to HCV infection in this study population.

CONCLUSION, RECOMMENDATIONS AND PERSPECTIVES

5.1. Conclusion

Genetic epidemiological studies have shown that host genetic variations significantly modulate viral infections. In relation our general objective, which was to perform the molecular characterisation of Killer cell Immunoglobulin-like Receptors and Human Leucocyte Antigen class I genes in HIV-1+, HBV+ and HCV+ individuals in Cameroon.

1. . Although with a small sample size, this study is the first to report on the frequencies and profiles of KIR genes in this country. Five potentially novel KIR genotypes were found which contribute to the growing number of KIR haplogroups being reported. This therefore lays the grounds for further *in vitro* studies on the mechanisms of action of host factors against hepatitis B and C infections and could provide useful information for the development of better treatment and vaccines.

2. Furthermore, in a bid to determine the HLA Class I profile of HIV-1, HBV and HCV patients, our data supports the potential role of HLA-A*30:01 and HLA-C*17:01 in the predisposition to viral hepatitis caused by HBV and HCV in the studied population. These findings contribute to the current knowledge of the role of HLA-A and HLA-C loci in the control of HBV and HCV infections in SSA and to the available data on HLA Class I (HLA-A and –C) diversity in Cameroon. Although this study is of great importance, the small sample size does not allow for firm interpretations of these findings.

3. In studying the associations between KIR genes and HIV-1, HBV and HCV disease, 27 HLA-A and 28 HLA-C 4-digit alleles were detected from 156 and 165 samples, respectively. HLA-C*03:04 was overrepresented in the uninfected control group compared to individuals with chronic Hepatitis B and C (12.8% vs. 2.2%, P=0.010), suggesting that this allele may be protective against chronic Hepatitis. The number of individuals carrying either HLA-A*30:01 or HLA-C*17:01 was shown to be significantly higher in the groups infected with either HBV or HCV, respectively, suggesting that these alleles may predispose their carriers to acquiring these viruses. In fact, carriers of HLA-A*30:01 had equal risk of acquiring either Hepatitis B or Hepatitis C viruses while carriers of HLA-C*17:01 were more likely to be infected with HBV but not HCV. Conversely, the analyses suggested that one of three HLA-C*03 subtypes (HLA-C*03:04) is likely to offer some protection against infection with the Hepatitis B virus.

5.2. Recommendations

We recommend that the KIR genes and their HLA ligands be typed in a larger population and in different regions of Cameroon in order to have a representative database of the different KIR and HLA alleles present in Cameroon. This database can then be used to study the impact of these genes on different diseases plaguing the country like HIV/AIDS, Hepatitis, Malaria, Typhoid, etc.

We recommend that the Ministry of Public Health should improve on the surveillance of viral Hepatitis B and C, which seem to be neglected.

5.3. Perspectives

Data from this study, though of a limited sample size lays down groundwork for further immunogenetic studies. Further studies should be conducted on larger sample sizes from different geographic and anthropologic settings, so as to generate more clinical data necessary to confirm our results. We therefore hope to:

- Investigate the KIR and HLA profiles in a larger population of HCV-infected patients in Cameroon, paying particular attention to the *KIR3DS1* gene which was strikingly over represented in the HCV-infected group of this pilot study.
- Sequence all the samples used in this study for HLA -B and also to re-sequence all the samples that we failed to amplify in this project.
- Deposit all the new KIR and HLA alleles in public databases for exploitation by the scientific community at large.

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APPENDICES

APPENDIX 1

INVESTIGATOR'S STATEMENT

……

…….

Participant code ……. Date of enrolment …….. Telephone ……

Title of study: studies on clinical outcome and antiviral drug resistance profile of individuals infected with HIV Type 1, HBV and HCV

INVESTIGATORS :

- Professor Judith N. Torimiro, Investigator Principal, CIRCB, Telephone: 677 729 270
- Professor Oudou Njoya, Co-Investigator, Telephone 699 811 717
- Professor Henry N. Luma, Co-Investigator, University of Yaounde I, Telephone: 677 707 416
- Dr. Charles Kouanfack, Co-Investigator, HCY, Telephone: 699 950 743
- Professor Lazare Kaptue, Chair of National Ethics committee, Telephone : 243 – 762 – 114

PURPOSE

You are being asked to join this study because you have been tested positive to HIV and/or HBV and/or HCV. These diseases cause serious public health problems worldwide and are likely to develop resistance to antiviral treatment. No study exists in Cameroon on the level of drug resistance of common antiviral drugs that are used for HIV/AIDS and Hepatitis B treatment in Cameroon. There is the need to carefully observe to carefully observe those factors that lead to drug resistance within patients at a population level to be able to predict the outcome of treatment of theses viral diseases. The aim of this study is to determine the genetic (HLA and KIR) profile as well as the profile of mutations of HIV-1, HBV and HCV and their impacts on clinical outcome

in patients who are infected with one or more of these viruses irrespective of if they have been placed under treatment or not within 96 weeks in selected hospitals in Cameroon. We hope that the results obtained in this study will be forwarded to the Ministry of Public Health for the better understanding of the mutation profiles of HIV-1, HBV, HCV and management of persons infected.

PROCEDURES

If you are okay with these explanations, we shall take about 30ml (6 teaspoons) of blood from your arm before putting you in treatment and each time the clinician will ask you about virological and biological analysis. We shall use a clean needle to collect the blood. This sample of blood will be used to carryout tests for HIV, HBV and HCV with the means of different analyses. You will return in two weeks for your results which will be explained to you by a specialist.

RISKS, STRESS AND DISCOMFORT

Blood will be taken from you as part of this study. This can cause pain and you can get a small wound but no disease will be contracted at the place where blood will be taken. Bleeding may also occur from the place where blood will be taken. The amount of blood taken cannot lead to any disease.

BENEFITS AND COMPENSATION

There is no special gain if you participate in this study. If you join this study, it will help us to gain more knowledge on the mutation profile of HIV, HBV and HCV and how it affects treatment in Cameroon. Nevertheless, these tests will be done free of charge.

PRIVACY AND CONFIDENTIALITY

We shall not tell anyone that you took part in this study. Your name will not be on the specimen of blood we take. No information about you will be given to people who are not working in the project except with you permission. At the end of the study, all findings will be used for teaching and given to other people in similar fields to enable them learn more about the mutations profile

of HIV, HBV and HCV, and the level of drug resistance of common antiviral drugs that are used for treatment in Cameroon.

You may decide to terminate your participation in this study at any time. If you decide to do so, it will not affect your care at this Hospital. We will give you a copy of this paper to keep.

APPENDICE 1

NOTICE D'INFORMATION

Code du participant ……..……Date d'enregistrement……. Téléphone ……

Titre de l'étude : **Etude sur les profils cliniques et les résistances aux médicaments antiviraux chez les individus infectes par le VIH-1, le VHB et le VHC**

INVESTIGATEURS :

- Professeur Judith N. Torimiro, Investigateur Principal, CIRCB, Téléphone : 677 729 270
- Professeur Oudou Njoya, Co-Investigateur, 699 811 717
- Professeur Henry N. Luma, Co-Investigateur, Université de Yaoundé I, Téléphone : 677 707 416
- Dr. Charles Kouanfack, Co-Investigateur, HCY, Téléphone : 699 950 743
- Professeur Lazare Kaptue, Président du Comité National D'Ethique de la Recherche au Cameroun, Telephone : 243 – 762 – 114

INTRODUCTION

Il vous a été demandé de vous joindre à cette étude parce que vous avez été diagnostique positif au virus du VIH et/ou VHB et/ou VHC. Ces maladies causent des problèmes graves de sante publique dans le monde entier et sont susceptibles de causer des résistances aux traitements antiviraux. Aucune étude n'existe concernant la résistance aux médicaments antiviraux communément utilises dans le traitement du VIH/SIDA et l'hépatite B au Cameroun. Il est nécessaire d'observer attentivement les facteurs qui conduisent à la résistance aux médicaments chez les patients au sein de la population afin d'être en mesure de prédire le résultat du traitement de ces maladies virales. Le but de cette étude est de déterminer le profil génétiques (HLA et KIR) ainsi que les mutations du VIH-1, du VHB, VHC et leur impact sur les résultats cliniques chez les

patients qui sont infectes par un ou deux, voire la totalité de ces virus indépendamment de s'ils ont été place sous traitement ou non pendant 96 semaines dans les hôpitaux sélectionnés au Cameroun.

Nous souhaitons que les résultats que nous obtiendrons de cette étude soient suggérer au Ministre de la Sante Publique pour la meilleure compréhension du profil de mutations du VIH-1, VHB, VHC et la meilleure prise en charge des personnes infectées soient faites,

PROCEDURE

Si vous êtes d'accord par rapport a ces explications, nous allons prélever environ 30ml (6 cuillères à soup) de votre sang au niveau de votre bras avant votre mise sous traitement et chaque fois qu'il vous sera demande des analyses biologiques après votre mise sous traitement. Nous allons à cet effet utiliser une aiguille propre pour le prélèvement. Cet échantillon sera utilisé pour effectuer les tests du VIH, VHB et VHC par moyen des analyses spécifiques. Vous reviendrez après 2 semaines pour le retrait de vos résultats avec des explications a l'appui d'un spécialiste.

RISQUES, STRESS ET MALAISES

Il vous sera prélevé du sang dans le déroulement de cette étude. Cet exercice peut être douloureux et vous pourrez avoir une petite blessure a l'endroit ou le sang a été prélevé, mais vous ne pourrez pas contracter de maladie. Il pourrait également y avoir saignement au niveau de ce site de prélèvement. La quantité de sang prélevée ne pourra entrainer aucune maladie.

BENEFICE ET COMPENSATION

Il n'y aura aucun profit particulier si vous participez à l'étude. Si vous participez à cette étude, cela nous aidera à acquérir de nouvelles connaissances sur le profil de mutation du VIH, VHB, VHC et comment ça influence le traitement au Cameroun. Toutefois, ces examens vous seront effectués gratuitement.

CONFIDENTIALITE

Nous ne dirons à personne que vous avez pris part à cette étude. Votre nom n'en sera pas inscrit sur l'échantillon de sang que nous allons prélever. Aucune information a propos de vous, ne sera divulguée aux personnes qui ne sont pas impliques dans le projet, sauf sous votre permission. A la fin de l'étude, toute connaissance acquise sera utilisée dans l'enseignement et transmis a d'autre

personnes afin d'en savoir plus sur le profil de mutations du VIH, VHB et le niveau de résistance antiviraux qui sont prescrits dans le traitement au Cameroun.

À tout moment, vous pouvez décider d'interrompre votre participation a cette étude. Si vous le décidez ainsi, cela n'affectera en rien votre suivi médical dans cet hôpital. Nous vous remettrons une copie de ce document pour conservation.

APPENDIX 2

CONSENT FORM

I, the undersigned, Mr/Mrs/Miss

……..……

After having been invited to participate in the research study entitled, "**Studies on Clinical outcome and Antiviral Drug Resistance of Individuals infected with HIV Type 1, HBV and HCV"**

- I have understood the Investigator's statement which was given in relation to the study
- Or it has been read to me and the Investigator's statement with respect to the study explained.
- I have fully understood the aim and objectives of this study
- I got all the answers to the questions I asked
- The risks and benefits have been presented and explained to me
- I have fully understood that I am free to agree or refuse to participate
- My consent does not discharge the research investigators of their responsibilities, I reserve my rights such as provided by the law.

I freely agree to participate in this study under the conditions provided in the Investigator's statement which are:

- To respond to enquires
- To communicate medical results
- To donate 30ml of blood

I give permission for my specimens to be used for other research.

(please, tick one. If YES, sign below)

…… YES

……... NO

Done in …….. the ……..

Principal Investigator Participant

APPENDICE 2

FORMULAIRE DE CONSENTEMENT

Je soussigne, M, Mme, Mlle

……

Avoir été invite à participer au travail de recherche intitule « Etude sur les profils cliniques et les résistances aux médicaments antiviraux chez les individus infectes par le VIH-1, le VHB et le VHC »

- J'ai bien compris la notice d'information qui m'a été remise concernant cette étude
- Ou bien on m'a lu et expliqué la notice d'information relative a cette étude
- J'ai bien compris le but et les objectifs de cette étude
- Les risques et bénéfices m'ont été présentes et expliques
- J'ai bien compris que je suis libre d'accepter ou de refuser d'y participer
- Mon consentement ne décharge pas les investigateurs de la recherche de leurs responsabilités, je conserve tous mes droits garantis par la loi

J'accepte librement de participer à cette étude dans les conditions précises dans la notice de l'information, c'est – à – dire

- De répondre aux questions d'enquêté
- De communiquer les informations médicales
- De donner 30 ml de mon sang

Je donne mon accord pour que le reste des échantillons prélevées pour cette étude soient utilises dans les études ultérieures.

(Bien vouloir, choisir une réponse, si oui, signer ci – dessous)

…….. OUI

…….. NON

Fait a …….. Le …….

Investigateur Principal Participant

APPENDIX 3

QUESTIONNAIRE

Title of the Project: **"Studies on clinical outcome and Antiviral Drug Resistance of Individuals infected with HIV Type 1, HBV and HCV"**

I – Identification

Name of the patient: ……

ID number: ……..

Age: …….

Sex: M ☐ ☐

Marital Status:

Married ☐ Singl☐ Divor☐ Fia☐ Widow☐

When were you diagnosed as HBV+ HIV+ HCV+

HBV+ / /

HIV+ / /

HCV+ / /

Are you vaccinated against HBV? Ye☐ N☐

When have you been vaccinated against HBV / /

When did you start treatment : _____ _____

HBV+: / /

HIV+: / /

HCV+: / /

Occupation: ……Contact: ……

APPENDICE 3

QUESTIONNAIRE

Titre du projet : Etude sur les profils cliniques et les cliniques aux médicaments antiviraux chez les individus infectes par le VIH-1, le VHB et le VHC.

I- Identification

Nom du patient :…….

Code du patient : ……..……

Âge : ……..

Sexe : M ☐ F ☐

Marié(e) ☐ célibataire ☐ divorcé(s) ☐ Fiancé(e) ☐ Veu(f/ve) ☐

Quand aviez-vous été diagnostique(e)s positif(ve) au VHB, VIH et VHC ?

VHB+ : / /

VIH+ : / /

VHC+ : / /

Êtes-vous vacciné(e) contre le VHB ? OUI ☐ NON ☐

Quand aviez-vous fait ce vaccin contre le VHB ? / /

Date du début du traitement : _____ _____

VHB+ : / /

VIH+ : / /

VHC+ : / /

VHB/VIH+ : / /

Occupation : …… contact : …….

REPUBLIQUE DU CAMEROUN
Paix - Travail – Patrie

UNIVERSITE DE YAOUNDE I

B.P. 337 Yaoundé
Tél/Fax: 222 22 13 20
e-mail : rect_cab@uy1.uninet.cm
Direction des Affaires Académiques et de la Coopération

REPUBLIC OF CAMEROON
Peace – Work – Fatherland

THE UNIVERSITY OF YAOUNDE I

P.O.Box 337 Yaoundé
Phone number/Fax: 222 22 13 20
e-mail : rect_cab@uy1.uninet.cm
Department of Acadmic Affairs and Cooperation

Décision N° 0165 /UYI/VREPDTIC/DAAC/DEPE/SPD/CRFDSVSE du 13 DEC 2016 portant sélection des candidats au cycle de doctorat PhD dans le centre de recherche et de formation doctorale de l'Université de Yaoundé I au titre de l'année académique 2016-2017.

Les étudiants dont les noms suivent sont autorisés à s'inscrire en cycle de Doctorat/Ph.D au titre de l'année académique 2016-2017.

Unité de Recherche et de Formation Doctorale en Sciences de la Vie, Santé et Environnement

Biochimie

N°	Noms et prénoms	Matricule	Statut	Directeur / Co-directeur	Sujet de Thèses
1	AMBASSA AXEL CYRIAQUE	10R0832	Etudiant	PENLAP BENG VERONIQUE (PR) DJUIDJE NGOUNOUE MARCELINE (CC)	INVESTIGATION DES COINFECTIONS VIH VHB TB AU CAMEROUN: HEPATOTOXICITE CARACTERISTIQUES ET ANTIGENIQUES CHEZ LES POPULATIONS A RISQUES
2	ANDOSEH GENEVIEVE	10R0040	Etudiant	PENLAP BENG VERONIQUE,(PR)	CHARACTERISATION OF MTBC STRAINS AMONGST PULMONARY TUBERCULOSIS PATIENTS USING BIOLOGICAL AND IMMUNOLOGICAL INDICATORS OF VIRULENCE
3	DJUKOUO LARISSA GERTRUDE	06R214	Etudiant	BIGOGA JUDE (MC) NCHINDA GODWIN (CR)	THE EFFECT OF VIRAL VACCINE VECTOR ON THE PROTEOME OF MONOCYTES AND NK CELLS FROM HIV-1 INFECTED PEOPLE
4	DOUMANI DJONABAYE	012Q1300	Etudiant	MOUNDIPA FEWOU PAUL (PR)	ETUDE DES MODIFICATIONS EPIGENETIQUES LIEES A LA TOXICITE DES MEDICAMENTS CHEZ LES PATIENTS HYPERTENDUQ ET DIABETIQUES
5	FANGNANG NDEYONG TATIANA	09R1259	Etudiant	PENLAP BENG VERONIQUE (PR) NCHINDA GODWIN (CR)	EVOLTION OF IMMUNE BIOMARKERS DURING TREATEMENT IN DUAL HIV1/TUBERCULOSIS INFECTED PEOPLE
6	GUIATEU TAMO IDA MARLENE	10R0275	Etudiant	MOUNDIPA FEWOU PAUL (PR) DJUIDJE NGOUNOUE MARCELINE (CC)	CARACTERISATION MOLECLAIRE DU VHC ET TYPAGE HLA CHEZ LES PERSONNES SEROPOSITIVES A VIH SOUS TRAITEMENT ANTIRETROVIRAL AU CAMEROUN
7	KABEYENE NVOMO STEPHANE BERTINE	14Z4680	Etudiant	PENLAP BENG VERONIQUE (PR) ASSAM ASSAM JEAN PAUL	EPIDEMIOLOGIE MOLECULAIRE DE LA TUBERCULOSE PULMONAIRE DANS LES ZONES DE FORTE ENDEMICITE AU CAMEROUN: ADAMAOUA ET LITTORAL
8	KETCHA NYONTA ARTHUR STEPHANE	06R424	Etudiant	MOUNDIPA FEWOU PAUL (PR)	ETUDE DES PROTEINES MARQUEURS DE LA PRESENCE DE SPZ CHEZ LES PATEINTS AZOSPERMIQUE
9	MBASSA NNOUMA GREGOIRE	07R457	Etudiant	BENG PENLAP VERONIQUE (PR) MAURO MODESTIE (CC)	ROLE DES PARALOGUES DE RAD51 ET LIENS AVEC LE CANCER
10	MBEKOU KANKO MICHELE INFS	10R0948	Etudiant	FEKAM BOYOM FABRICE (PR)	ACTIVATION OF MICROORGANISMS GENE FOR THE PRODUCTION OF ANTIMICROBIAL METABOLITES
11	MBOINDI MFOPOU OUSMANE	09R0092	Etudiant	OBEN ENYONG JULUS (PR)	DOUBLE FARDEAU DE LA MALNUUTRITION CHEZ LES ADULTES BAMOUNS RESIDANT DANS LE DEPARTEMENT DU NOUN

12	NGOUMBE HAMED BECHIR	09R0900	Etudiant	MOUNDIPA PAUL (PR) NJAYOU FREDERIC NICO (CC)	ETUDE LE L'EFFET DE KG25 SUR L'HETEROL AUUTOHEPATOPROTECTION DUU PARACETAMOL (APAR)
13	NJANKOUO NDAM YOUCHAOU	09R0808	Etudiant	NYEGUE MAXILILLIENNE ASCENSION (MC) MOUNJOUENPOU PAULINE (MR)	LA SCOPOLETINE DE MANIOC: PROPIETES BIOLOGIQUES ET APPLICATION DANS LA CONSERVATION DU MAIS
14	NJANPA NGANSOP CYRILLE A	10R0594	Etudiant	FEKAM BOYOM FABRICE (PR)	RECHERCHE DE SUBSTANCES BIOACTIVES CONTRE L'AGENT CAUSAL DE LA LETSHMANIOSE
15	NKEUMACHA IDA PATRICK		Etudiant	PR MOUNDIPA FEWOU PAUL (PR) KOANGA MOGTOMO MARTIN LUTHER (CC)	LES PERTUUBATEURS ENDOCRINIENS ET ALTERATIONS HORMONALES DANS LE CANCER DU SEIN DE LA FEMME
16	NNANGA LEILA SANDRA	10R0618	Etudiant	NGONDI JUDITH LAURE (MC)	POTENTIEL ANTI ATHEROGENE CARDIOPROTECTEUR ET ANTIDIABETIQUE DE QUELQUES PLANTES ALIMENTAIRES CAMERONAISE
17	SAAGUE KEMEWELE PETER		Etudiant	PIEME CONSTANT ANATOLE (MC)	Etude des propriétés antifatigue, anti stress antioxydant immunomodulatrice et dopante de quelques plantes
18	TATFO KEUTCHATANG FABRICE DE PAUL	10R1045	Etudiant	KANSCI GERMAIN (MC) MEDOUA NAMA GABRIEL (MR)	EVALUATION DES RISQUES LIES A L'UTILISATION DES MEDICAMENTS VETERINAIRES DANS L'ELEVAGE DE POULETS AUU CAMEROUN
19	TCHUIGOUA ARIANE CLARISSE	09R0732	Etudiant	KANSCI GERMAIN (MC) MEZAJOUG KENFACK L.B (CC)	EFFETS NUTRITIONNEL DES PROTEINES DES OLEAGINEUX NON CONVENTIONNELS SUR LE METABOLISME INVIVO DES RATS ADULTES
20	VANESSA TCHAWE TSASSE	10R0780	Etudiant	NGONDI JUDITH LAURE (MC)	EFFETS ANTIOXYDANT ET ENTIHYPERLIPIDEMIANT DE QUELQUES PLANTES ALIMENTAIRES CAMEROUNAISES DANS UN MODELE EXPERIMENTAL D'HYPERLIPIDEMIE
21	WOTCHOKO SIAKAM JUSTIN		Etudiant	MOUNDIPA PAUL (PR) DJUIDJE NGOUNOUE MARCELINE (CC) KOANGA MOGTOMO MARTIN LUTHER (CC)	INVESTIGATIONS IMMNOLOGIQUE?? VIROLOGIQUE ET BIOCHIMIQUE DES MARQUEURS PRO ET ANTI INFLAMMATOIRES CHEZ LES PVVIH ET PROFIL DES GAMMA GLOBULINES
22	YENGO CLAUVIS KUNKENG	10R0803	Etudiant	JUDITH NDONGO TORIMIRO (MC) LOUIS-MARIE YINDOM (CC) TIEDEU ATOGHO B (CC)	MOLECULAR CHARACTERISATION OF KILLER CELL IMMUNOGLOBULIN -LIKE RECEPTORS AND HUMAN LEUCOCYTE ANTIGENS IN HIV INFECTED INDIVIDALS IN CAMEROON
23	THELMA PETER NGWA NIBA		Bousier MARCAD	MBACHAM WILFRED. (PR) MICHAEL AIFRANGIO (PR)	MOLECULAR EPIDEMIOLOGY OF ANTIMALARIA DRUG RESISTANCE AND INFLAMMATION IN CAMEROON

LE RECTEUR DE L'UNIVERSITE DE YAOUNDE I

REPUBLIQUE DU CAMEROUN
Paix –Travail – Patrie

REPUBLIC OF CAMEROON
Peace – Work - Fatherland

CIRCB
CENTRE INTERNATIONAL DE RÉFÉRENCE "CHANTAL BIYA"
POUR LA RECHERCHE SUR LA PRÉVENTION ET LA PRISE EN CHARGE DU VIH/SIDA

La Direction

N°1233/017/L/CIRCB/DIR/SAA

Yaoundé, le 07 JUIL 2017

Le Directeur du CIRCB
A
Monsieur YENGO Clauvis
Tél: 676 72 15 32
FS/Université de Yaoundé I

YAOUNDE

Objet: Autorisation pour travaux de recherche.

Monsieur,

Dans le cadre de vos travaux de recherche relatifs à la thèse intitulée "**Caractérisation des facteurs génétiques influençant la progression de l'hépatite B et C**",

J'ai l'honneur de marquer mon accord pour la réalisation desdits travaux au Laboratoire de Biologie Moléculaire du CIRCB, pendant la période allant du **07 juillet 2017 au 05 janvier 2018**.

A cet effet, je vous prie de bien vouloir prendre attache avec le Chef dudit laboratoire en vue des modalités pratiques et du planning de ces travaux.

Veuillez agréer, **Monsieur**, l'assurance de ma parfaite considération./-

Le Directeur

CENTRE INTERNATIONAL DE REFERENCE CHANTAL BIYA POUR LA RECHERCHE SUR LA PREVENTION ET LA PRISE EN CHARGE DU VIH/SIDA

Prof. Ndjolo Alexis

Siège : BP / P.O BOX : 3077 Yaoundé - Messa Tél. : (237) 222 31 54 50 / Fax : (237) 222 31 54 56 E-mail : contacts@circb-cameroun.org
Site web : www.circb-cameroun.org Décret présidentiel N° 2012/249 du 31 mai 2012

COMITE NATIONAL D'ETHIQUE DE LA RECHERCHE POUR LA SANTE HUMAINE

Arrêté N° 0977/A/MINSANTE/SESP/SG/DROS/ du 18 avril 2012 portant création, organisation et fonctionnement des comités d'éthique de la recherche pour la santé humaine au sein des structures relevant du Ministère en charge de la santé publique

N° 2017/05/903/CE/CNERSH/SP

Yaoundé, le 15 mai 2017

Cnethique_minsante@yahoo.fr

CLAIRANCE ETHIQUE

Le Comité National d'Ethique de la Recherche pour la Santé Humaine (CNERSH), en sa session ordinaire du 04 mars 2016, a examiné le projet de recherche intitulé : «**Studies on clinical outcome and antiviral drug resistance profile of individuals infected with HIV type 1, HBV and HCV**» soumis par le **Docteur Judith Ndongo TORIMIRO**, Investigateur Principal, Centre International de Référence Chantal Biya pour la Recherche sur la Prévention et la prise en charge du VIH/SIDA.

Le projet est d'un grand intérêt scientifique et social. Le but de cette étude est de déterminer le profil des mutations du VIH-1, du VHB et du VHC après 96 semaines de traitement chez les patients mono infectés ou co-infectés, naïfs aux traitements dans des hôpitaux sélectionnés au Cameroun et leur impact sur le résultat clinique. La procédure de l'étude est bien documentée et claire. Les risques liés au prélèvement de sang sont précisés dans le document. Les mesures prises pour garantir la confidentialité des données collectées sont présentes dans le document. Les CVs des investigateurs les décrivent comme des personnes compétentes, capables de mener à bien cette étude. Pour toutes ces raisons, le Comité National d'Ethique approuve pour une durée d'un an, la mise en œuvre de la présente version du protocole.

Les investigateurs sont responsables du respect scrupuleux du protocole approuvé et ne devraient y apporter aucun amendement aussi mineur soit-il, sans avis favorable du CNERSH. Les investigateurs sont appelés à collaborer pour toute descente du CNERSH pour le suivi de la mise en œuvre du protocole approuvé. Le rapport final du projet devra être soumis au CNERSH et aux autorités sanitaires du Cameroun.

La présente clairance peut être retirée en cas de non respect de la réglementation en vigueur et des recommandations susmentionnées.

En foi de quoi, la présente clairance éthique est délivrée pour servir et valoir ce que de droit.

Ampliations

- MINSANTE

Le Président

Pr Nicolas KAPTUE

N.B : cette clairance éthique ne vous dispense pas de l'autorisation administrative de recherche (AAR), exigée pour mener cette étude sur le territoire camerounais. Cette dernière vous sera délivrée par le Ministère de la Santé Publique.

KIR Primers

Primer Code	Sequence
K1	GTTGGTCAGATGTCATGTTTGAA
K2	GGTCCCTGCCAGGTCTTGCG
K3	TGGACCAAGAGTCTGCAGGA
K4	TGTTGTCTCCCTAGAAGACG
K5	CTGGCCCACCCAGGTCG
K6	GGACCGATGGAGAAGTTGGCT
K7	GAGGGGGAGGCCCATGAAT
K8	TCGAGTTTGACCACTCGTAT
K9	CTTCATCGCTGGTGCTG
K10	AGGCTCTTGGTCCATTACAA
K11	TCCTTCATCGCTGGTGCTG
K12	GGCAGGAGACAACTTTGGATCA
K13	CAGGACAAGCCCTTCTGC
K14	CTGGGTGCCGACCACT
K15	ACCTTCGCTTACAGCCCG
K16	CCTCACCTGTGACAGAAACAG
K17	TTCTGCACAGAGAGGGGAAGTA
K18	GGGTCACTGGGAGCTGACAA
K19	CGGGCCCCACGGTTT
K20	GGTCACTCGAGTTTGACCACTCA
K21	TGGCCCACCCAGGTCG
K22	TGAAAACTGATAGGGGGAGTGAGG
K23	CTATGACATGTACCATCTATCCAC
K24	AAGCAGTGGGTCACTTGAC
K25	CTGGCCCTCCCAGGTCA
K26	TCTGTAGGTTCCTGCAAGGACAG

K27	GTTCAGGCAGGAGAGAAT
K28	GTTTGACCACTCGTAGGGAGC
K29	TGATGGGGTCTCCAAGGG
K30	TCCAGAGGGTCACTGGGC
K31	CTTCTCCATCAGTCGCATGAA
K32	CTTCTCCATCAGTCGCATGAG
K33	AGAGGGTCACTGGGAGCTGAC
K34	CGCTGTGGTGCCTCGA
K35	GGTGTGAACCCCGACATG
K36	CCCTGGTGAAATCAGGAGAGAG
K37	TGTAGGTCCCTGCAAGGGCAA
K38	CAAACCCTTCCTGTCTGCCC
K39	GTGCCGACCACCCAGTGA
K40	CCCATGAACGTAGGCTCCG
K41	CACACGCAGGGCAGGG
K42	AGCCTGCAGGGAACAGAAG
K43	GCCTGACTGTGGTGCTCG
K44	CCTGGTGAAATCAGGAGAGAG
K45	GTCCCTGCAAGGGCAC
K50	GCGCTGTGGTGCCTCG
K51	GACCACTCAATGGGGGAGC
K52	TGCAGCTCCAGGAGCTCA
K53	GGGTCTGACCACTCATAGGGT
K54	GTCTGCCTGGCCCAGCT
K55	GTGTGAACCCCGACATCTGTAC
K56	CCATCGGTCCCATGATGG
K57	CACTGGGAGCTGACAACTGATG
K58	ACAGAGAGGGGACGTTTAACC

K59	ATGTCCAGAGGGTCACTGGG
K60	GTCAGGACAAGCCCTTCCTC
K61	GAGTGTGGGTGTGAACTGCA
K62	TTCTGCACAGAGAGGGGATCA
K63	GAGCCGACAACTCATAGGGTA
K64	CTTCTCCATCAGTCGCATGAR
C1	TGCCAAGTGGAGCACCCAA
C2	GCATCTTGCTCTGTGCAGAT

KIR Typing – PCR-SSP method

Master mix (MM)

	µl/sample	**µl/plate (96-well)**	**µl (384-well) + subtypes**
αQH_2O	270	**810**	**3258**
10X buffer	42.3	**127**	**511**
$MgCl_2$ (50mM)	12.7	**38.1**	**153.6**
dNTPs (25mM)	3.4	**10.2**	**40.8**
Taq Platinum	1.8	**5.4**	**21.6**

1. Take the primer mixes out of the fridge, vortex each tube briefly and place on the bench to warm-up to room temperature
2. Prepare the MM as above, mix and place immediately on ice
3. Add 2µl of each primer mix into the corresponding well of a 96-well or 384-well plate as indicated on the template below
4. Add 3µl of DNA (50ng/µl) into lebelled eppendorf vials (0.5 ml eppendorf)
   - For 96-well plate, add 330 µl of MM into the 0.5 ml vial containing 150 ng of DNA
   - For 384-well plate format, add 330 ul of MM into the 0.5 ml vial containing 150 ng of DNA
5. Vortex briefly and add
   a. 10µl of the mixture into the corresponding well of a labelled 96-well plate
   b. 8µl of the mixture into the corresponding well of a labelled 384-well plate
6. Seal the plate and centrifuge briefly at 1500 rpm (1 minute)
7. Place the plate in the thermal cycler, close the lid and run the program “KIR-SSP”

Amplification conditions

1 cycle	94 ºC	3:00				
5 cycles	94 ºC	0:10	65 ºC	0:15	72 ºC	0:30
21 cycles	94 ºC	0:10	60 ºC	0:15	72 ºC	0:30
5 cycles	94 ºC	0:10	55 ºC	0:30	72 ºC	1:00
1 cycle	72 ºC	7:00				
(4 ºC∞ optional)						

Agarose gel preparation

	For big tray	for small tray
Agarose powder	8g	2 g
1 X TAE buffer	400 ml	100 ml

a. Weigh 8g or 2g of agarose and add in a conical flask containing TAE
b. Add 400 ml or 100 ml of 1 X TAE into a flask and mix to dissolve
c. Place in a microwave and bring to boil (**caution:** check for overflow)
d. Remove the flask and cool rapidly in a jar of water to 65 ºC.
e. Add 8 µl of ethidium bromide (10gm/ml) and mix by swirling (**caution:** Avoid air-bubble formation)
f. Prepare the casting tray by sealing both ends with brown adhesive tape
g. Place the tray on a flat area and pour the mixture into the tray
h. Place the combs in place and allow to set for 10-15 minutes

Gel electrophoresis

1. Take the plate out of the thermal cycler and centrifuge briefly
2. Add 5 µl of loading buffer into each well containing the PCR products and centrifuge again briefly
3. Gently remove the combs from the gel, then the brown adhesive tape from both ends of the tray
4. Add enough TAE into the tank and place the gel into the tank avoiding trapping air-bubbles beneath the gel tray.
5. Load all of the mixture (or 15 µl) into the corresponding well of the agarose gel.
6. Connect the gel tank to the power pack and run at 300 V for 25 minutes or 80 V for 50 minutes.

Gel photography

a. Cut the gel to size and place on the UV light box.
b. Take the gel picture with the camera attached to a computer.
c. Save the electronic copy of the gel picture and print a copy for the lab book.
d. Copy and paste each sample's picture onto the excel template (KIR interpretation sheet) for subsequent analysis.

Gel interpretation

1. Adjust the gel picture to size the lanes on the template.
2. Enter the sample details as indicated
3. Score (1) if the specific band is present and (0) if it is absent.

Data entry

Upload the Excel file results sheet into an Access database (or any other suitable database) for subsequent statistical manipulations.

Plate format_96 Well

	1	2	3	4	5	6	7	8	9	10	11	12
A	KM1	KM9	KM17	KM25	KM1	KM9	KM17	KM25	KM1	KM9	KM17	KM25
B	KM2	KM10	KM18	KM26	KM2	KM10	KM18	KM26	KM2	KM10	KM18	KM26
C	KM3	KM11	KM19	KM27	KM3	KM11	KM19	KM27	KM3	KM11	KM19	KM27
D	KM4	KM12	KM20	KM28	KM4	KM12	KM20	KM28	KM4	KM12	KM20	KM28
E	KM5	KM13	KM21	KM29	KM5	KM13	KM21	KM29	KM5	KM13	KM21	KM29
F	KM6	KM14	KM22	KM30	KM6	KM14	KM22	KM30	KM6	KM14	KM22	KM30
G	KM7	KM15	KM23	KM31	KM7	KM15	KM23	KM31	KM7	KM15	KM23	KM31
H	KM8	KM16	KM24	KM32	KM8	KM16	KM24	KM32	KM8	KM16	KM24	KM32

Sample 1 Sample 2 Sample 3

Plate format_384 Well

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	KM1	KM17	KM1	KM17	KM1	KM17	KM1	KM17	KM1	KM17	KM1	KM17	KM1	KM17	KM1	KM17	KM1	KM17	KM1	KM17	KM1	KM17	KM1	KM17
B	KM9	KM25	KM9	KM25	KM9	KM25	KM9	KM25	KM9	KM25	KM9	KM25	KM9	KM25	KM9	KM25	KM9	KM25	KM9	KM25	KM9	KM25	KM9	KM25
C	KM2	KM18	KM2	KM18	KM2	KM18	KM2	KM18	KM2	KM18	KM2	KM18	KM2	KM18	KM2	KM18	KM2	KM18	KM2	KM18	KM2	KM18	KM2	KM18
D	KM10	KM26	KM10	KM26	KM10	KM26	KM10	KM26	KM10	KM26	KM10	KM26	KM10	KM26	KM10	KM26	KM10	KM26	KM10	KM26	KM10	KM26	KM10	KM26
E	KM3	KM19	KM3	KM19	KM3	KM19	KM3	KM19	KM3	KM19	KM3	KM19	KM3	KM19	KM3	KM19	KM3	KM19	KM3	KM19	KM3	KM19	KM3	KM19
F	KM11	KM27	KM11	KM27	KM11	KM27	KM11	KM27	KM11	KM27	KM11	KM27	KM11	KM27	KM11	KM27	KM11	KM27	KM11	KM27	KM11	KM27	KM11	KM27
G	KM4	KM20	KM4	KM20	KM4	KM20	KM4	KM20	KM4	KM20	KM4	KM20	KM4	KM20	KM4	KM20	KM4	KM20	KM4	KM20	KM4	KM20	KM4	KM20
H	KM12	KM28	KM12	KM28	KM12	KM28	KM12	KM28	KM12	KM28	KM12	KM28	KM12	KM28	KM12	KM28	KM12	KM28	KM12	KM28	KM12	KM28	KM12	KM28
I	KM5	KM21	KM5	KM21	KM5	KM21	KM5	KM21	KM5	KM21	KM5	KM21	KM5	KM21	KM5	KM21	KM5	KM21	KM5	KM21	KM5	KM21	KM5	KM21
J	KM13	KM19	KM13	KM19	KM13	KM19	KM13	KM19	KM13	KM19	KM13	KM19	KM13	KM19	KM13	KM19	KM13	KM19	KM13	KM19	KM13	KM19	KM13	KM19
K	KM6	KM22	KM6	KM22	KM6	KM22	KM6	KM22	KM6	KM22	KM6	KM22	KM6	KM22	KM6	KM22	KM6	KM22	KM6	KM22	KM6	KM22	KM6	KM22
L	KM14	KM30	KM14	KM30	KM14	KM30	KM14	KM30	KM14	KM30	KM14	KM30	KM14	KM30	KM14	KM30	KM14	KM30	KM14	KM30	KM14	KM30	KM14	KM30
M	KM7	KM23	KM7	KM23	KM7	KM23	KM7	KM23	KM7	KM23	KM7	KM23	KM7	KM23	KM7	KM23	KM7	KM23	KM7	KM23	KM7	KM23	KM7	KM23
N	KM15	KM31	KM15	KM31	KM15	KM31	KM15	KM31	KM15	KM31	KM15	KM31	KM15	KM31	KM15	KM31	KM15	KM31	KM15	KM31	KM15	KM31	KM15	KM31
O	KM8	KM24	KM8	KM24	KM8	KM24	KM8	KM24	KM8	KM24	KM8	KM24	KM8	KM24	KM8	KM24	KM8	KM24	KM8	KM24	KM8	KM24	KM8	KM24
P	KM16	KM32	KM16	KM32	KM16	KM32	KM16	KM32	KM16	KM32	KM16	KM32	KM16	KM32	KM16	KM32	KM16	KM32	KM16	KM32	KM16	KM32	KM16	KM32

Sample 1 Sample 2 Sample 3 Sample 4 Sample 5 Sample 6 Sample 7 Sample 8 Sample 9 Sample 10 Sample 11 Sample 12

LR-PCR amplification for HLA class I sequencing using PrimeSTAR GXL enzyme

Reaction mix

	ul/rxn
Water	12.5
5x buffer	5
dNTPs	2
Primer 1	1.5
Primer 2	1.5
Enzyme	0.5
DNA	2.0
Total	25

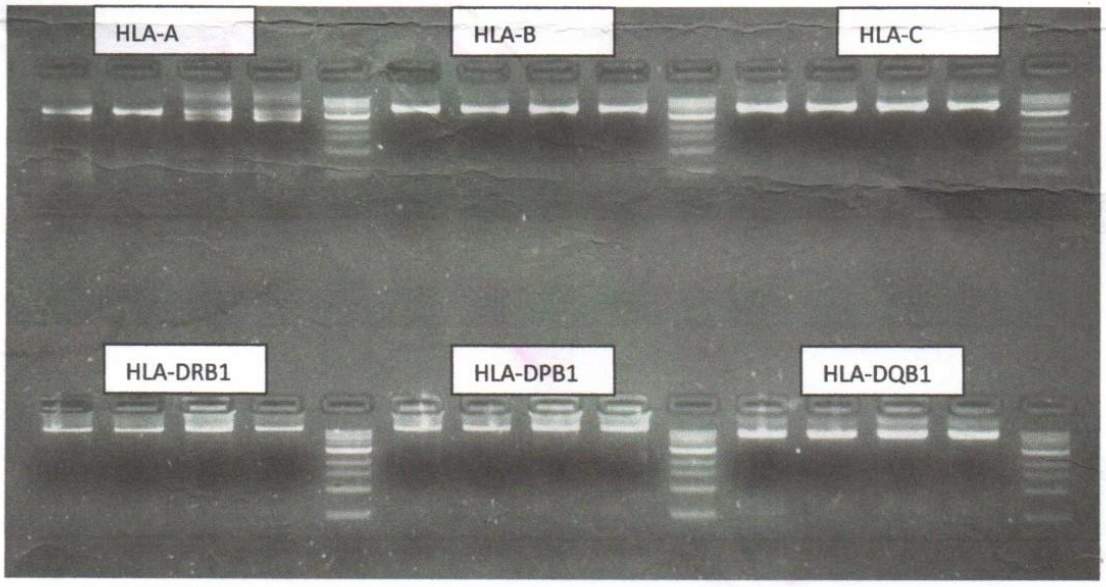
Cycling condition (HLA-A)

35 cycles 98°C 0:10 68 °C 3:00
Store 8 °C for ever

Cycling condition (HLA-B and -C)

35 cycles 98°C 0:10 68 °C 4:00
Store 8 °C for ever

Gel pictures



Comment: They all worked well.
Action: Design internal primers for sequencing

PCR amplification for HLA class I sequencing

10X PCR buffer (Class I buffer)

Trizma base	4.1 g
Ammonium Sulfate	1.1 g (Sigma Cat No A4418-100G)

- Dissolve Trizma base in 40 ml of sterile distilled water and adjust the pH to 8.8 with concentrated HCl
- Add ammonium sulfate into the solution and mix well to dissolve
- Make up the volume to 50 ml by adding sterile distilled water
- Pass through a 22 µm filter into a clean sterile bottle
- Make aliquots, label and store in -20 °C until needed

HLA PCR

Master mix	µl/rxn
Class I buffer	2.0
dNTPs (10 uM)	0.4
$MgCl_2$ (25 mM)	1.6
Primer (F)	0.4
Primer (R)	0.4
Platinum taq	0.2
Total	5.0
DNA (150ng) + D/w	15.0
Final rxn vol	20

Primers

A1: ccc aga cgc cga gga tgr csg	(F)
A4: gca ggg cgg aac ctc aga gtc act ctc t	(R)
B1: tcc cag ttc taa agt ccc cac g	(F)
B4: tcc att caa ggg agg gcg ac	(R)
C1: agc gag gkg ccc gcc cgg cga	(F)
C4: gga gat ggg gaa ggc tcc cca ct	(R)

Amplification conditions

HLA A

1 cycle	95 °C	3:00				
5 cycles	95 °C	0:25	70 °C	0:45	72 °C	0:45
26 cycles	95 °C	0:25	65 °C	0:50	72 °C	0:45
4 cycles	95 °C	0:25	55 °C	1:00	72 °C	2:00
1 cycle	72 °C	5:00				
1 cycle	4 °C	∞				

HLA B

1 cycle	96 °C	2:00				
40 cycles	96 °C	0:15	58 °C	0:15	72 °C	1:00
1 cycle	72 °C	7:00				
1 cycle	4 °C	∞				

HLA C

1 cycle	96 °C	2:00				
31 cycles	96 °C	0:25	70 °C	0:25	72 °C	1:00
1 cycle	72 °C	5:00				
1 cycle	4 °C	∞				

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Research article

Variation of HLA class I (-A and -C) genes in individuals infected with hepatitis B or hepatitis C virus in Cameroon

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ABSTRACT

The Human Leucocyte Antigens (HLA) work in concert with other immune factors to modulate immunity to viral infections. Extensive variation has been reported in the genetic sequences and functions of classical HLA class I genes in many (mostly Western) populations, and several HLA associations with infectious disease outcomes have been reported. Little is known about their role in the susceptibility or resistance to hepatitis viruses in Central African populations. The aim of this study was to determine variants of two HLA class I genes (HLA-A and -C) in adults infected with hepatitis B (HBV)- or -C (HCV) virus in Cameroon.

In this case-control study, a total of 169 unrelated adults comprising 68 HCV-infected, 38 HBV-infected and 63 uninfected (controls) individuals participated. Each consented participant was screened for HBV, HCV, and HIV infections and willingly donated a single blood sample for genomic DNA isolation and some clinical laboratory tests. HLA-A and HLA-C were genotyped using previously described sequence-based techniques (SBT).

A total of 54 HLA alleles were identified in the study population (27 HLA-A and 27 HLA-C). HLA-A*23:01 and HLA-C*07:01 were the most common alleles with genotype frequencies of 31.4% and 29.3%, respectively. Hepatitis individuals were six times more likely to be HLA-A*30:01 carriers than uninfected controls (OR = 6.30, p = 0.020 (HBV); OR = 6.21, p = 0.010 (HCV), respectively). Similarly, carriers of HLA-C*17:01 were over-represented in the HBV-infected compared to the uninfected control group (21.9% vs. 6.4%, respectively) suggesting that this allele could play a role in the susceptibility to HBV infection.

These findings demonstrate that carriers of HLA-A*30:01 were over-represented in the hepatitis group compared to uninfected controls while HLA-C*17:01 was completely absent in the HCV + group.

1. Introduction

Globally, infection with the hepatitis B (HBV) and hepatitis C (HCV) viruses are the leading causes of liver-related morbidity and mortality (Mohd Hanafiah et al., 2013; Mortality & Causes of Death, 2016; Rao et al., 2015). In 2015, it was estimated that 257 million and 71 million people were living with chronic HBV and HCV infections, respectively (WHO, 2017), the majority of whom are in resource limited regions such as the sub-Saharan Africa (SSA). Little is known about the burden of these infections in the Central Africa sub region and even less so in Cameroon. However, some recent reports have shown that the prevalence of HBV amongst pregnant women in some regions of Cameroon ranges from 5.7% to 7.7% (Eyong et al., 2019; Fomulu et al., 2013; Torimiro et al., 2006), while that of HCV amongst young adults and the elderly ranges from 0.8% to 6.5 % (Bigna et al., 2017; Njouom et al., 2018; Rodgers et al., 2019). Although more than 90% of children in the developing world will become exposed to the hepatitis B virus at some stage of their lives, only about 5% will remain chronically infected in adulthood.

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Factors associated with chronicity following HBV infection are not fully understood but available data show that chronic hepatitis B infection can lead to irreversible end organ damage with complications such as cirrhosis and hepatocellular carcinoma, commonly observed among those who acquire infection during childhood (Alward et al., 1985; McMahon et al., 1985). On the other hand, approximately 70–80% of HCV-infected persons develop chronic infection and 15–30% of them are likely to end up with serious debilitating complications including liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC) or liver failure (Amin et al., 2006; Micallef et al., 2006; WHO, 2017). Of the many factors previously implicated with the clearance or chronicity of viral Hepatitis B and C (Aisyah et al., 2018; Akuta et al., 2019; Alric et al., 2000; Bulteel et al., 2016; Chang et al., 1989; Ferreira et al., 2014; Grebely et al., 2007; Isaguliants and Ozeretskovskaya, 2003; Kong et al., 2014; Micallef et al., 2006; Morsica et al., 2019; O'Brien et al., 2019; Perez-Cano et al., 2002; Singh et al., 2007; Thomas et al., 2009; Wang et al., 2007; Yan and Wang, 2017) and disease progression, the host immunogenetic factors affecting components of the innate and adaptive immune system that modulate host-viral interactions are key players (Alric et al., 2000; Isaguliants and Ozeretskovskaya, 2003; Kummee et al., 2007; Singh et al., 2007). However, these factors including those of the Human Leucocyte Antigen (HLA) system are yet to be fully elucidated in African cohorts, where both the burden of infection and HLA diversity are greatest.

HLA in humans comprises a complex set of hyper polymorphic genes, located within an approximately 3.6Mbp region of the short arm of human chromosome 6 (6p21.31) (Beck and Trowsdale, 2000) that encodes cell-surface molecules making up part of the innate and adaptive immune response system. The HLA molecules interact with the Killer Cell immunoglobulin-like Receptor (KIR) molecules on the surface of Natural Killer (NK) cells to modulate both innate and adaptive immune responses to self and non-self-peptides (like those derived from microorganisms). They also play a key role in presenting epitope peptides from intracellular organisms to Cytotoxic T lymphocytes (CTL). The HLA genes are divided into three classes –I, II and III – with classes I and II known as the "classical HLA genes" which are naturally highly polymorphic. To date, there are 26,214 (19,031 HLA class I and 7,183 HLA class II) alleles in the Immuno-Polymorphism (IPD-IMGT/HLA) Database (release 3.39.0 of January 20th 2020) (Robinson et al., 2020). The HLA class I region is made up of 19 gene loci but only three are classical genes (HLA –A, -B and –C) with 8 exons each. Exons 2 and 3 are highly polymorphic and encode the peptide binding groove (Shiina et al., 2009).

Recent studies have demonstrated the heterogeneity of HLA class I genes in Cameroonian populations (Ellis et al., 2000; Spinola et al., 2011; Torimiro et al., 2006) consistent with their diverse ethnic backgrounds. Although a number of studies have associated HLA class I genes with both beneficial and deleterious effects on viral infection including HBV and HCV (Khakoo et al., 2004; Singh et al., 2007; Thio et al., 2003; Valenzuela-Ponce et al., 2018; Yindom et al., 2010), there is a paucity of data from African populations with regards to the role of HLA diversity in chronic hepatitis B or C infections. The objectives of this study were to determine and describe the level of HLA-A and -C diversity in a sample of Cameroonian population chronically infected with hepatitis B or C viruses. The authors also wanted to compare the frequency of specific HLA allotype with that observed in the general population recruited in the same setting as the hepatitis cases. The overall aim was to investigate any relationship between HLA alleles and chronic hepatitis caused by HBV and HCV in adult Cameroonians.

2. Materials and methods

In this cross-sectional case-control study, 169 unrelated adults consented to participate and were recruited by convenience sampling from two hospitals in Douala and Yaoundé in Cameroon. Each participant donated a single blood sample that was tested for HBV, HCV and HIV infections and genomic DNA extraction. Those that tested positive for either the HBsAg or HCV Antibodies but negative for HIV were recruited as cases, while those that tested negative for all three viruses accepted to participate as population controls. HIV-positive and hepatitis dually infected individuals were excluded from the study. This study was approved by the Cameroon National Ethics Committee for Human Health Research. A structured questionnaire was used to collect participants demographic information including age, sex, marital status and profession.

Screening for HIV, HBV and HCV were done using rapid diagnostic test kits [Determine HIV-1/2 SET (Alere Medical Co, Ltd, Japan) and OnSite HBsAg and OnSite HCV Combo kits (CTK BIOTECH, CA, USA)]. Serological detection of the Hepatitis B envelope Antigen and Antibodies (HBeAg and Anti-HBe) was done using a commercial ELISA kit (Biomerieux Clinical Diagnostics, Geneva, Switzerland).

Plasma load of HBV DNA and HCV RNA were determined using the Abbott Real Time PCR (Abbott Molecular Diagnostics, Wiesbaden, Germany). Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. HLA-A and HLA-C sequencing was performed using locus specific primers and the Big Dye Terminator Version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) in ABI 3130XL DNA Analyser (Applied Biosystems, Foster City, CA) as previously described (Yindom et al., 2010).

Allele and genotype frequencies were computed using IBM SPSS Statistics for windows, v25 (IBM Corp., Amonk, NY), and Stata, v14.1 (Stata Corp., College Station, TX). Comparisons of allele and genotype frequencies between groups were performed using either Chi -square test, Fisher exact test, or independent samples t-test as may be appropriate and $p < 0.05$ was considered statistically significant after Bonferroni correction for multiplicity testing. Hardy-Weinberg Equilibrium (HWE) and haplotype analysis were performed using the Arlequin Software version 3.5.2.2 (Excoffier and Schneider, 2005).

3. Results

3.1. Participant characteristics

Table 1 shows the demographic characteristics of the study participants. Thirty-eight participants tested positive for HBsAg and 68 for HCV antibodies and the remainder 63 tested negative for all three viruses (Controls). Males were overrepresented in the HBV infected group 33/38 (86.8%) while the majority of HCV infected participants were females 44/68 (64.7%). In the control group, however, the proportion of male to female was similar: 33 males (52.4%) and 31 females (47.6%). HCV infected participants were significantly older compared to the HBV-infected and HIV-, HBV- and HCV- uninfected control individuals. Histological data was not available for any of the participants but a combination of routine clinical and laboratory data including Fibro test results was used to classify HBV infected participants according to the American Association for the Study of Liver Diseases (AASLD) as illustrated in Table 2. Eighteen (47.4%) were in the inactive chronic hepatitis phase while 19/38 (50.0%) were in the immune reactivation phase.

3.2. HLA distribution and association with hepatitis B or C virus infections

A total of 54 HLA alleles (27 for each locus) were identified in the study population. The most frequent was HLA-A*23:01 (31.4%) (Table 3), followed by HLA-C*07:01 and HLA-C*04:01 at 29.3% and 28.6%, respectively, (Table 4). HLA-A*30:01 was significantly overrepresented in people with hepatitis compared to uninfected controls (Figure 1). Interestingly, stratifying analysis by disease status revealed carriers of this allele were six times more likely to be in the HBsAg-positive or HCV antibody-positive groups compared to uninfected controls (Table 5), suggesting that this might be a susceptibility allele for hepatitis B or C virus infection.

Of the 27 HLA-C alleles, twelve had genotype frequencies ranging from 5.0-29.3% (Figure 2). Three HLA-C*03 subtypes were represented

Table 1. Demographic characteristics of the study groups.

	Control[a] (%)	HBV (%)	HCV (%)	p[b]	p[c]
Number (N = 169)	63	38	68		
Male	33 (52.4)	33 (86.8)	24 (35.3)	<0.001	0.049
Female	30 (47.6)	5 (13.2)	44 (64.7)		
Age (mean ± SD)	31.9 ± 7.2	39.0 ± 10.7	61.6 ± 12.5	<0.001	<0.001
Single	-	20 (52.6)	9 (13.2)		
Married	-	18 (47.4)	37 (54.4)		
Widowed/divorced	-	-	22 (32.4)		
Log HBV viral load (mean ± SD)	-	3.9 ± 1.8	-		
Log HCV viral load (mean ± SD)[d]	-	-	5.7 ± 1.4		

[a] Participants who tested negative for HBV, HCV and HIV.
[b] p-values comparing HBV+ and uninfected controls.
[c] p-values comparing HCV+ and uninfected controls.
[d] HCV viral load data was missing for six participants in the HCV group.

Table 2. Hepatitis B patients' classification according to the American Association for the Study of Liver Diseases.

Phases	ALT	HBV DNA (IU/mL)	HBeAg	Fibro test	Interpretation	n (%)
Inactive Chronic Hepatitis B	Normal	<2,000	Negative	F1 – F4	Minimal necroinflammation but variable fibrosis	18 (47.4)
Immune Reactivation	Elevated	2,000 - < 20,000	Negative	F1 – F4	Moderate to severe inflammation or fibrosis	19 (50.0)
Immune Active	Elevated	≥20,000	Positive	-	Moderate to severe inflammation or fibrosis	0 (0.0)
Immune Tolerant	Normal	>1 million	Positive	F3	Minimal inflammation and fibrosis	1 (2.6)

n: number of participants; ATL: Alanine aminotransferase; HBeAg: Hepatitis B e-antigen.

Table 3. HLA-A genotype frequency distribution between cases and controls.

HLA-A alleles	N = 156	All (%)	N = 60	Control (%)	N = 96	Hepatitis (%)	OR	P	95% CI
A*23:01	49	31.4	22	36.7	27	28.1	0.68	0.265	0.34–1.35
A*03:01	26	16.7	11	18.3	15	15.6	0.82	0.660	0.35–1.95
A*30:02	26	16.7	9	15.0	17	17.7	1.22	0.660	0.50–2.95
A*02:01	23	14.7	9	15.0	14	14.6	0.97	0.943	0.39–2.40
A*68:02	22	14.1	10	16.7	12	12.5	0.71	0.468	0.29–1.78
A*29:02	21	13.5	12	20.0	9	9.4	0.41	0.059	0.16–1.07
A*30:01	**19**	**12.2**	**2**	**3.3**	**17**	**17.7**	**6.24**	**0.008**	**1.33–29.24**
A*02:02	15	9.6	5	8.3	10	10.4	1.28	0.669	0.41–3.96
A*66:01	14	9.0	3	5.0	11	11.5	2.46	0.171	0.65–9.32
A*74:01	12	7.7	2	3.3	10	10.4	3.37	0.107	0.70–16.25
A*36:01	8	5.1	3	5.0	5	5.2	1.04	0.954	0.24–4.56
A*33:03	7	4.5	4	6.7	3	3.1	0.45	0.300	0.10–2.11
A*68:01	7	4.5	1	1.7	6	6.3	3.93	0.180	0.45–34.17
A*32:01	6	3.9	3	5.0	3	3.1	0.61	0.555	0.12–3.16
A*02:05	5	3.2	3	5.0	2	2.1	0.40	0.316	0.06–2.52
A*26:01	5	3.2	3	5.0	2	2.1	0.40	0.316	0.06–2.52
A*66:02	4	2.6	3	5.0	1	1.0	0.20	0.129	0.02–2.02
A*34:02	3	1.9	2	3.3	1	1.0	0.31	0.312	0.03–3.50
A*29:01	3	1.9	0	0.0	3	3.1	-	-	-
A*31:01	3	1.9	1	1.7	2	2.1	1.26	0.854	0.11–14.27
A*33:01	3	1.9	2	3.3	1	1.0	0.31	0.312	0.03–3.50
A*01:01	2	1.3	1	1.7	1	1.0	0.62	0.737	0.04–10.22
A*24:02	2	1.3	1	1.7	1	1.0	0.62	0.737	0.04–10.22
A*30:04	2	1.3	1	1.7	1	1.0	0.62	0.737	0.04–10.22
A*23:02	1	0.6	0	0.0	1	1.0	-	-	-
A*23:14	1	0.6	0	0.0	1	1.0	-	-	-
A*66:03	1	0.6	0	0.0	1	1.0	-	-	-

Bold indicates HLA allele with significant difference between groups. N: number of individual, OR: Odds ratio: P value comparing the control group with the hepatitis group. 95%CI: 95% confidence interval.

in the study population with two (HLA-C*03:02 and -C*03:03) at minor allele frequencies (<5%) (Table 4). HLA-C*03:04, however, was over-

Table 4. HLA-C genotype frequency distribution between cases and controls.

HLA-C alleles	N = 140	All (%)	N = 47	Control (%)	N = 93	Hepatitis (%)	OR	P	95% CI
C*07:01	41	29.3	15	31.9	26	28.0	0.83	0.628	0.38–1.78
C*04:01	40	28.6	12	25.5	28	30.1	1.26	0.573	0.57–2.78
C*06:02	28	20.0	9	19.1	19	20.4	1.08	0.859	0.45–2.63
C*07:02	27	19.3	6	12.8	21	22.6	1.99	0.166	0.74–5.39
C*08:02	19	13.6	5	10.6	14	15.1	1.49	0.473	0.50–4.44
C*02:10	15	10.7	7	14.9	8	8.6	0.54	0.257	0.18–1.60
C*17:01	16	11.4	3	6.4	13	14.0	2.38	0.184	0.64–8.93
C*18:01	13	9.3	6	12.8	7	7.5	0.56	0.315	0.17–1.78
C*16:01	12	8.6	2	4.3	10	10.8	2.71	0.196	0.56–13.11
C*14:03	9	6.4	3	6.4	6	6.5	1.01	0.988	0.24–4.26
C*03:04	**8**	**5.7**	**6**	**12.8**	**2**	**2.2**	**0.15**	**0.011**	0.03–0.81
C*05:01	7	5.0	2	4.3	5	5.4	1.28	0.775	0.24–6.90
C*02:02	5	3.6	2	4.3	3	3.2	0.75	0.757	0.12–4.68
C*04:07	4	2.9	1	2.1	3	3.2	1.53	0.714	0.15–15.30
C*15:05	4	2.9	1	2.1	3	3.2	1.53	0.714	0.15–15.30
C*07:27	3	2.1	1	2.1	2	2.2	1.01	0.993	0.09–11.54
C*14:02	3	2.1	1	2.1	2	2.2	1.01	0.993	0.09–11.54
C*08:04	2	1.4	0	0.0	2	2.2	-	-	-
C*12:03	2	1.4	0	0.0	2	2.2	-	-	-
C*01:02	1	0.7	0	0.0	1	1.1	-	-	-
C*02:27	1	0.7	1	2.1	0	0.0	-	-	-
C*03:02	1	0.7	1	2.1	0	0.0	-	-	-
C*03:03	1	0.7	0	0.0	1	1.1	-	-	-
C*07:04	1	0.7	1	2.1	0	0.0	-	-	-
C*07:05	1	0.7	0	0.0	1	1.1	-	-	-
C*14:05	1	0.7	0	0.0	1	1.1	-	-	-
C*16:07	1	0.7	0	0.0	1	1.1	-	-	-

Bold indicates HLA allele with significant difference between groups. N: number of individuals, OR: Odds ratio: P value comparing the control group with the hepatitis group. 95%CI: 95% confidence interval.

represented in the uninfected control group compared to individuals with chronic hepatitis B and C (12.8% vs. 2.2%, P = 0.010) suggesting that this allele may be protective against hepatitis. Further analysis revealed that individuals with HLA-C*17:01 were four times more likely to be in the hepatitis B infected group compared to the uninfected control group (OR = 4.11, P = 0.043, 95% CI: 0.93–18.18, Table 6). This wasn't the case in the HCV + group. Each locus was tested for Hardy-Weinberg (H–W) equilibrium using Arlequin software version 3.5.2.2. Both loci were in equilibrium (p values: 0.075 and 0.445 for HLA-A and -C loci, respectively) (Table 7). A total number of 128 possible two-loci haplotypes were identified, 24 of which had a frequency of at least 1% in this cohort. A*23:01–C*05:01 was the most prevalent haplotype with a frequency of 3.7% followed by A*23:01–C*03:04 (3.3%) (Table 8).

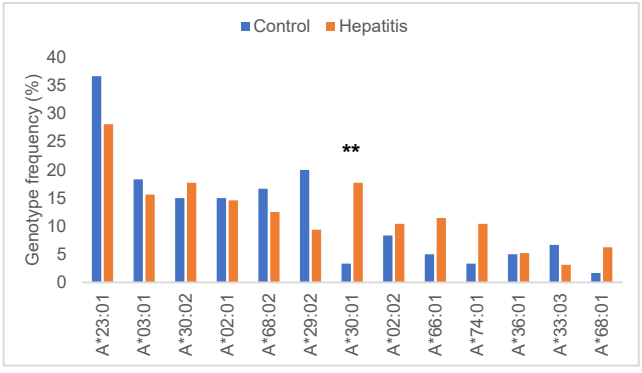

Figure 1. HLA-A genotype distribution between cases (participants with hepatitis) and controls (uninfected participants). Only HLA-A alleles present in at least four percent of the study populations are represented. Table 3 shows the distribution of all 27 alleles stratified by disease status.

4. Discussion

Viral hepatitis caused by HBV and HCV is the main driver of chronic liver diseases including cirrhosis and hepatocellular carcinoma (HCC). The mechanisms of pathogenesis employed by these viruses to evade immune surveillance and establish lifelong persistence in humans are still not fully understood. However, host immunogenetic factors together with viral and environmental factors are key players that modulate progression of most viral diseases. Several studies have implicated HLA class I and II diversity at the population level with clearance or persistence of hepatitis virus infection (reviewed in (Crux and Elahi, 2017; Singh et al., 2007)). The interplay between HLA and other immune regulatory cells and molecules to modulate the outcomes of HBV or HCV infection (viral clearance or persistence) is likely to be multifactorial involving a cascade of immune responses and may also be population specific. In this study, authors sought to determine the diversity of HLA class I molecules focussing on HLA-A and HLA-C loci in a sample of adults living in Cameroon with or without hepatitis B or C virus. Those with dual infection (HBV and HCV) or coinfected with HIV were excluded from the study.

Fifty four (54) HLA alleles were identified (27 HLA-A and 27 HLA-C) in this study population of 169 participants, 106 (62.7%) of whom were infected with either HBV or HCV. The most frequent alleles were HLA-A*23:01 (31.4%), HLA-C*07:01 (29.3%) and HLA-C*04:01 (28.6%). These common HLA types have been previously described in other populations in sub Saharan Africa (SSA), and in Cameroon, HLA-A*23:01, HLA-C*04:01 and HLA-C*07, have been reported to be very

Table 5. HLA-A genotype frequency in the study population by disease status.

HLA	Control N = 60	HBV N = 28	HCV N = 68	HBV			HCV		
				OR	P	95% CI	OR	P	95% CI
A*23:01	22 (36.7)	6 (21.4)	21 (30.9)	0.47	0.155	0.16–1.36	0.77	0.491	0.37–1.62
A*03:01	11 (18.3)	4 (14.3)	11 (16.2)	0.74	0.640	0.21–2.60	0.86	0.748	0.34–2.16
A*30:02	9 (15.0)	4 (14.3)	13 (19.1)	0.94	0.930	0.26–3.40	1.34	0.539	0.53–3.42
A*02:01	9 (15.0)	4 (14.3)	10 (14.7)	0.94	0.930	0.26–3.40	0.98	0.963	0.37–2.60
A*29:02	12 (20.0)	2 (7.1)	7 (10.3)	0.31	0.127	0.06–1.53	0.46	0.125	0.17–1.27
A*68:02	10 (16.7)	5 (17.9)	7 (10.3)	1.09	0.891	0.33–3.57	0.57	0.291	0.20–1.63
A*02:02	5 (8.3)	2 (7.1)	8 (11.8)	0.85	0.848	0.15–4.70	1.47	0.523	0.45–4.78
A*30:01	2 (3.3)	5 (17.9)	12 (17.7)	**6.30**	**0.020**	**1.07–37.22**	**6.21**	**0.010**	**1.27–30.46**
A*66:01	3 (5.0)	1 (3.6)	10 (14.7)	0.70	0.766	0.07–7.19	3.28	0.071	0.84–12.81
A*74:01	2 (3.3)	2 (7.1)	8 (11.8)	2.23	0.427	0.29–17.03	3.87	0.077	0.77–19.48
A*36:01	3 (5.0)	4 (14.3)	1 (1.5)	3.17	0.136	0.64–15.70	0.28	0.254	0.03–2.86
A*33:03	4 (6.7)	1 (3.6)	2 (2.9)	0.52	0.561	0.05–4.95	0.42	0.322	0.07–2.44
A*68:01	1 (1.7)	3 (10.7)	3 (4.4)	7.08	0.059	0.66–75.99	2.72	0.375	0.27–27.34

Only HLA-A alleles present in at least four percent of the study populations are represented. Bold indicates HLA allele with significant difference between groups.

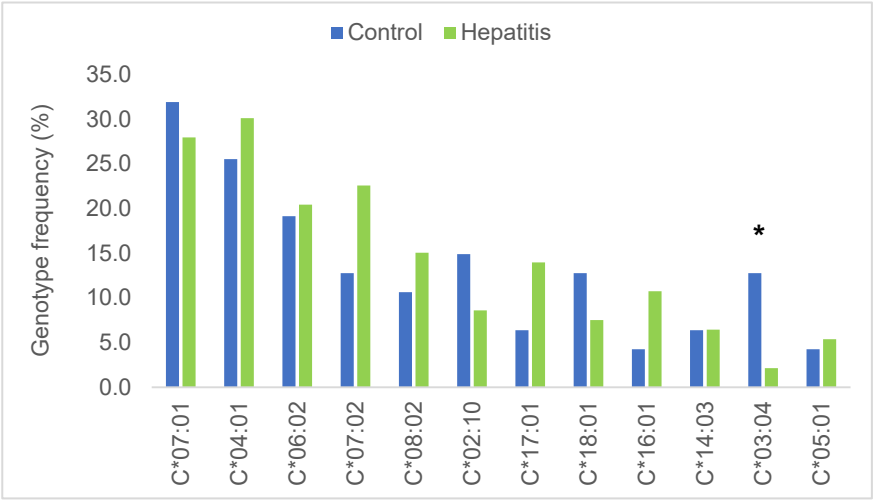

Figure 2. HLA-C genotype distribution between cases and controls. Only HLA-C alleles present in at least four percent of the study populations are represented. Table 4 shows the distribution of all 27 alleles found in the study population.

Table 6. HLA-C genotype frequency in the study population by disease status.

HLA-C alleles	Control N = 47	HBV N = 32	HCV N = 61	HBV			HCV		
				OR	P	95% CI	OR	P	95% CI
C*07:01	15 (31.9)	8 (25.0)	18 (29.5)	0.71	0.509	0.26–1.97	0.89	0.789	0.39–2.04
C*04:01	12 (25.5)	12 (37.5)	16 (26.2)	1.75	0.259	0.65–4.69	1.04	0.935	0.43–2.48
C*06:02	9 (19.1)	6 (18.8)	13 (21.3)	0.97	0.965	0.31–3.09	1.14	0.783	0.44–2.97
C*07:02	6 (12.8)	8 (25.0)	13 (21.3)	2.28	0.165	0.69–7.52	1.85	0.250	0.64–5.37
C*08:02	5 (10.6)	2 (6.3)	12 (19.7)	0.56	0.503	0.10–3.13	2.06	0.203	0.66–6.40
C*02:10	7 (14.9)	3 (9.4)	5 (8.2)	0.59	0.472	0.14–2.52	0.51	0.275	0.15–1.75
C*17:01	3 (6.4)	7 (21.9)	6 (9.8)	**4.11**	**0.043**	0.93–18.18	1.60	0.522	0.37–6.83
C*18:01	6 (12.8)	3 (9.4)	4 (6.6)	0.71	0.644	0.16–3.09	0.48	0.272	0.13–1.83
C*16:01	2 (4.3)	3 (9.4)	7 (11.5)	2.33	0.362	0.36–15.12	2.92	0.180	0.56–15.06
C*14:03	3 (6.4)	0 (0.0)	6 (9.8)	-	-	-	1.60	0.522	0.37–6.83
C*03:04	6 (12.8)	0 (0.0)	2 (3.3)	-	-	-	0.23	0.063	0.04–1.25
C*05:01	2 (4.3)	4 (12.5)	1 (1.6)	3.21	0.177	0.53–19.33	0.38	0.414	0.03–4.35

Only HLA-C alleles present in at least four percent of the study populations are represented. Bold indicates HLA allele with significant difference between groups.

common (Ellis et al., 2000; Shepherd et al., 2015; Spinola et al., 2008; Torimiro et al., 2006; Tshabalala et al., 2018; Yindom et al., 2010). In the present study, the number of individuals carrying either HLA-A*30:01 or HLA-C*17:01 was significantly higher in the groups infected with either

Table 7. Hardy-Weinberg equilibrium test results.

Locus	Obs.Het.	Exp.Het.	P-value
HLA-A	0.85897	0.9218	0.075
HLA-C	0.9	0.91083	0.445

Obs.Het.: Observed heterozygosity, Exp.Het.: Expected heterozygosity.

Table 8. Two-loci haplotypes in this study population.

Haplotype	F	Haplotype	F	Haplotype	F	Haplotype	F	Haplotype	F
A*23:01-C*05:01	0.037	A*30:01-C*02:10	0.009	A*68:01-C*04:07	0.006	A*03:01-C*06:02	0.003	A*33:03-C*06:02	0.003
A*23:01-C*03:04	0.033	A*30:01-C*04:01	0.009	A*68:02-C*06:02	0.006	A*23:01-C*04:01	0.003	A*33:03-C*12:03	0.003
A*23:01-C*07:01	0.024	A*30:02-C*08:02	0.009	A*74:01-C*05:01	0.006	A*23:01-C*06:02	0.003	A*33:03-C*16:01	0.003
A*23:01-C*07:02	0.021	A*33:03-C*04:07	0.009	A*02:02-C*17:01	0.006	A*23:14-C*16:01	0.003	A*34:02-C*06:02	0.003
A*03:01-C*02:10	0.021	A*36:01-C*04:01	0.009	A*02:02-C*04:01	0.005	A*24:02-C*07:01	0.003	A*34:02-C*07:01	0.003
A*23:01-C*16:01	0.021	A*36:01-C*07:01	0.009	A*02:01-C*03:04	0.005	A*26:01-C*02:10	0.003	A*36:01-C*14:03	0.003
A*03:01-C*07:02	0.019	A*68:01-C*04:01	0.009	A*02:01-C*07:01	0.004	A*26:01-C*16:01	0.003	A*66:01-C*02:10	0.003
A*29:02-C*02:10	0.018	A*68:02-C*07:02	0.009	A*03:01-C*07:01	0.004	A*29:01-C*07:02	0.003	A*66:01-C*07:27	0.003
A*02:01-C*07:02	0.017	A*74:01-C*07:27	0.009	A*23:01-C*17:01	0.004	A*29:01-C*17:01	0.003	A*66:01-C*14:02	0.003
A*29:02-C*07:02	0.017	A*74:01-C*17:01	0.009	A*23:01-C*08:02	0.004	A*29:02-C*04:01	0.003	A*66:01-C*14:03	0.003
A*02:01-C*14:02	0.016	A*30:02-C*04:01	0.008	A*24:02-C*16:01	0.004	A*29:02-C*16:07	0.003	A*66:02-C*17:01	0.003
A*68:02-C*12:03	0.015	A*29:02-C*06:02	0.008	A*02:02-C*08:02	0.003	A*29:02-C*18:01	0.003	A*66:03-C*08:02	0.003
A*30:02-C*05:01	0.014	A*30:01-C*07:05	0.007	A*66:01-C*07:02	0.003	A*30:01-C*08:04	0.003	A*68:01-C*15:05	0.003
A*30:01-C*06:02	0.014	A*30:04-C*07:01	0.007	A*02:02-C*07:02	0.003	A*30:01-C*17:01	0.003	A*68:02-C*03:04	0.003
A*23:01-C*18:01	0.012	A*03:01-C*16:01	0.006	A*01:01-C*01:02	0.003	A*30:02-C*02:10	0.003	A*68:02-C*05:01	0.003
A*23:02-C*08:02	0.012	A*68:02-C*07:01	0.006	A*01:01-C*07:04	0.003	A*30:02-C*07:01	0.003	A*68:02-C*14:02	0.003
A*30:02-C*02:02	0.012	A*02:01-C*06:02	0.006	A*02:01-C*02:10	0.003	A*30:02-C*18:01	0.003	A*68:02-C*14:03	0.003
A*66:01-C*02:02	0.012	A*02:02-C*07:01	0.006	A*02:01-C*14:05	0.003	A*30:04-C*14:03	0.003	A*68:02-C*15:05	0.003
A*66:01-C*07:01	0.012	A*02:02-C*14:03	0.006	A*02:01-C*16:01	0.003	A*31:01-C*08:04	0.003	A*74:01-C*02:10	0.003
A*68:02-C*02:10	0.012	A*02:02-C*16:01	0.006	A*02:02-C*02:02	0.003	A*31:01-C*17:01	0.003	A*74:01-C*03:04	0.003
A*03:01-C*04:07	0.011	A*26:01-C*08:02	0.006	A*02:02-C*02:10	0.003	A*31:01-C*18:01	0.003	A*74:01-C*06:02	0.003
A*29:02-C*07:01	0.011	A*30:02-C*14:03	0.006	A*02:02-C*18:01	0.003	A*32:01-C*02:02	0.003	A*74:01-C*08:02	0.003
A*03:01-C*08:02	0.010	A*30:02-C*15:05	0.006	A*02:05-C*02:02	0.003	A*32:01-C*03:04	0.003	A*03:01-C*04:01	0.003
A*30:02-C*07:02	0.010	A*32:01-C*18:01	0.006	A*02:05-C*03:04	0.003	A*32:01-C*05:01	0.003	A*66:01-C*06:02	0.002
A*02:01-C*18:01	0.009	A*33:01-C*07:02	0.006	A*02:05-C*07:01	0.003	A*32:01-C*07:01	0.003		
A*02:02-C*06:02	0.009	A*66:02-C*04:01	0.006	A*02:05-C*07:02	0.003	A*33:01-C*04:01	0.003		

F: frequency.

HBV or HCV, respectively, suggesting that these alleles may predispose their carriers to acquiring these hepatitis viruses. In fact, carriers of HLA-A*30:01 had equal risk of acquiring either hepatitis B or hepatitis C viruses while carriers of HLA-C*17:01 were more likely to be infected with HBV and not HCV. Conversely, further analyses suggest that one of three HLA-C*03 subtypes - HLA-C*03:04 - is likely to offer some protection against carriage of hepatitis B virus. Thus, whilst certain HLA alleles may protect from infection with hepatitis viruses, others may actually predispose their carriers to HBV and/or HCV infections.

There is a paucity of data on the association of HLA class I genes, hepatitis infection and liver diseases in Cameroon and several countries in SSA. These results confirm the predicted dominant role of the HLA-A locus in the overall host immune responses against DNA viruses (Hertz et al., 2011; Thio et al., 2003). This study reports for the first time that HLA-A*30:01 may have a significant role to play in the acquisition of both hepatitis viruses (HBV and HCV). Hepatitis B and C patients when compared to healthy participants are six times more likely to be carriers of this allele. In contrast, HLA-C*03:04 was found to be significantly more prevalent in controls compared to cases, showing that it might have a protective effect against viral hepatitis. A growing body of reports have documented that different HLA class I alleles including HLA-A*02, HLA-A*03 and HLA-A*31 are differentially associated with either a good or bad effect in the control of viral hepatitis (Crux and Elahi, 2017).

5. Conclusion

These data support the potential role of HLA-A*30:01 and HLA-C*17:01 in the predisposition to viral hepatitis caused by HBV and HCV in this sample of Cameroonian population. These findings add to the current knowledge of the role of HLA-A and HLA-C loci in the control of HBV and HCV infections in SSA and to the available data on HLA class I (HLA-A and –C) diversity in Cameroon. Although this study is of great importance, the authors acknowledge that it had its own limitations including the fact that information on the ethnicity of the study participants was not collected, the use of healthy (uninfected) individuals as controls and the small sample size. The problem of using healthy control subject in this type of study is that about 80% of people when exposed to HCV for the first time will develop chronicity while the remainder

spontaneously clears the virus. To overcome this inconstancy in future studies, participants with spontaneous viral clearance should be compared to those with persistent infection (El-Bendary et al., 2019; Fakhir et al., 2018; Neamatallah et al., 2020). Despite the shortcomings of the present study, the analyses of HLA polymorphisms reported herein will provide valuable guide in the design of future studies to: (1) describe the genomic variation of the HLA class I loci and (2) examine whether variations at those loci are associated with chronicity of hepatitis in this and other populations in the sub region. Future studies should consider using a much larger sample sizes from this and other populations in SSA.

Declarations

Author contribution statement

C. Yengo and L. Yindom: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

J. Torimiro: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper. M. Kowo, B. Tiedeu, H. Luma, O. Njoya and S. Rowland-Jones: Contributed reagents, materials, analysis tools or data; Wrote the paper. P. Lebon: Performed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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Research Article

Killer Cell Immunoglobulin-Like Receptor Genotypes and Haplotypes Contribute to Susceptibility to Hepatitis B Virus and Hepatitis C Virus Infection in Cameroon

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Abstract

Over 325 million people worldwide are living with hepatitis B and C viral infections and are at greater risk of developing hepatocellular carcinoma. The interactions between killer cell immunoglobulin-like receptors (KIRs) and their cognate ligands, human leukocyte antigens, modulate both infection processes and disease progression. We report here (1) genotype and haplotype variations in *KIR* genes in Cameroon and (2) their impact on susceptibility to hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. In 98 unrelated individuals (33 HCV+, 31 HBV+, and 34 uninfected healthy controls), we determined the presence of 15 *KIR* genes by polymerase chain reaction–sequence-specific primer techniques. One pseudogene and all 14 *KIR* genes were present. We identified 36 KIR genotypes, 5 of which have not been previously reported in public databases. Two inhibitory (*KIR2DL1* and *KIR2DL3*) and three activating (*KIR2DS4*, *KIR2DS2*, and *KIR2DS3*) genes were present in all HCV-infected individuals. Similarly, *KIR3DL1*, *KIR2DL1*, and *KIR2DS4* were present at 100% in the HBV+ group. Compared with uninfected healthy controls, the frequencies of *KIR2DL2* and *KIR3DS1* were significantly lower in the HBV+ group ($p = 0.003$ and $p < 0.001$, respectively). Conversely, *KIR3DS1* was significantly overrepresented in the HCV+ group compared with controls (97.0% vs. 64.7%, respectively, $p < 0.001$). These results may imply that *KIR3DS1* carriers were less likely to be HBV infected, but may be predisposed to HCV infection compared with uninfected controls, indicating their important role in transmission of these viruses. However, phenotypic, functional, and genomic studies to elucidate the role of these KIR genotypes and haplotypes in infection with HBV and HCV are important.

Keywords: *KIR* genes, hepatitis B, hepatitis C, liver cancer, association study, Cameroon

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) account for the majority of cases of hepatocellular carcinoma worldwide. It is estimated that 325 million people are living with hepatitis B and C infections in 2018 (WHO 2019). In highly endemic areas, HBV can be transmitted through different routes, including perinatally from mother to child, sexually, or through contact with contaminated body fluids. HBV and HCV infections are endemic in Cameroon, with about 11% chronically infected with HBV and about 6.5% with HCV (Bigna et al., 2017a, 2017b).

Host genetic factors have been associated with differential outcomes of many infectious diseases (de Wit et al., 2016; Yano et al., 2013; Yindom et al., 2010; Zwolińska, 2009).

Among the immune factors, variants of genes encoding the killer cell immunoglobulin-like receptors (KIRs) and human leukocyte antigens (HLAs) are strong correlates of susceptibility or resistance to viral infections, including HBV and HCV infections (Araujo et al., 2014; Martin and Carrington, 2013; Shepherd et al., 2015). KIRs are a family of activating and inhibitory type I transmembrane glycoproteins with two to three extracellular domains (Campbell and Purdy, 2011) and are highly polymorphic. They serve as key regulators of

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natural killer (NK) cell function and are expressed by NK cells and some T cell lymphocytes (Caligiuri, 2008). The KIR locus resides within the leukocyte receptor complex and spans a region of about 150–200 kb on the human chromosome 19q13.4 (Wende et al., 1999).

There are 15 *KIR* genes (*2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5A*, *2DL5B*, *2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5*, *3DL1*, *3DL2*, *3DL3*, and *3DS1*) and 2 pseudogenes (*2DP1* and *3DP1*). More than 20 KIR haplotypes and at least 40 KIR genotypes have been reported from different geographic populations. Recently, it was demonstrated that *KIR* genes modulate susceptibility to hepatitis B infection (Yindom et al., 2017).

KIR genes and their cognate HLA ligands have been associated with differential outcomes of hepatitis B and C disease progression, but there is very little data on their mechanism of action. The compound genotype, KIR3DS1/HLA Bw4-80I, is associated with protection from rapid progression to AIDS (Hens et al., 2016; Qi et al., 2006), while KIR2DL3/HLA-C1 directly influences clearance of HCV (Khakoo et al., 2004).

Furthermore, some *KIR* genes, including *KIR2DL3* and *KIR3DS1*, have a protective role against HBV infection (Di Bona et al., 2017; Kibar et al., 2014). Several studies have reported that the *KIR3DS1* gene is less frequent in African populations (<25%) compared with Caucasians (>50%) and Asians (<30%) (Hollenbach et al., 2012). *KIR* genes have not been fully mapped in Cameroon with over 250 ethnic groups (Louis et al., 1995). In this study, we identified variations of KIR genes in unrelated individuals infected with HBV, HCV, and uninfected (healthy) controls in Cameroon and evaluated their association with susceptibility to infection.

Materials and Methods

Study participants

A total of 98 unrelated individuals were recruited from two main cities (Douala and Yaoundé) in Cameroon between January 2016 and December 2018 and each gave written informed consent to participate in this study. They were all screened for HBV, HCV, and HIV infections using the rapid tests, Determine HIV (Alere), HBsAg (ABON), anti-HCV (ABON), and ELISA (Murex), respectively, following manufacturers' instructions. Thirty-one (31.6%) were HBV infected, 33 (33.7%) HCV infected, and 34 (34.7%) tested negative for all three viruses. Questionnaires were administered to collect demographic characteristics (Table 1).

Ten milliliters of blood was collected from each participant and processed within 4 h to obtain plasma and buffy coat, which were stored at −20°C until analyses. Genomic DNA was extracted from the buffy coat using the QIAamp DNA mini extraction kit (Qiagen) following manufacturer's instructions. This study was approved by the Cameroon National Ethics Committee on Human Health Research.

KIR typing

Genomic DNA samples were typed by the polymerase chain reaction–sequence-specific primer (PCR-SSP) techniques as previously described (Martin and Carrington, 2008) to detect the presence of 14 *KIR* genes (*2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5*, *2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5*, *3DL1*, *3DL2*, *3DL3*, and *3DS1*) and 1 pseudogene (*2DP1*). Two KIR-specific primer pairs were used to amplify segments of different sizes from the same *KIR* gene. The amplicons were visualized on a 2% agarose gel by electrophoresis under a UV transilluminator. Discordant results (one band present and the other absent for the same gene) were repeated and if one of the bands was consistently positive, it was then considered positive. A pair of internal control primers to check for PCR efficiency was included in each reaction that amplifies the *HLA-DRB1* gene.

Data analyses

Statistical analyses were done using IBM SPSS Statistics for Windows, v25 (IBM Corp., Armonk, NY), and Stata, v14.1 (Stata Corp., College Station, TX). The observed frequency for each *KIR* gene was obtained by direct counting and verified using Microsoft Excel 2013. These frequencies were compared between groups (HBV+ and HCV+ and uninfected controls) using the chi-square, Fisher exact test, and independent samples *t*-test, as appropriate. $p < 0.05$ was considered statistically significant after Bonferroni correction for multiplicity testing. We assigned KIR haplotypes and genotypes identification based on reported IDs available in public databases, including the Allele Frequency Net Database (www.allelefrequencies.net).

Table 1. Demographic Characteristics of the Study Population

	Control,[a] n *(%)*	*HBV+,* n *(%)*	*HCV+,* n *(%)*	*Frequency (%)*	p
No. of participants	34 (34.7)	31 (31.6)	33 (33.7)		
Sex					
Male	16 (28.0)	27 (47.4)	14 (24.6)		<0.001
Female	18 (43.9)	4 (9.8)	19 (46.3)		
Age, years					
Median (IQR)	29 (27–35)	36 (29–43)	62 (57–66)		
Mean (SD)	30.6 (5.7)	37.1 (11.0)	59.2 (12.9)	69.8	<0.001
Marital status					
Single		16 (51.6)	5 (15.2)		<0.001
Married		15 (48.4)	21 (63.6)		
Widowed		0 (0.0)	7 (21.2)		

[a]Control individuals tested negative for HBV, HCV, and HIV. *p*: uncorrected *p*-value. Between groups, age differences were compared using analysis of variance, the differences remained significant after Bonferroni correction, as shown below: (a) Control versus (HBV+) $p1 = 0.039$; (b) Control versus (HCV+) $p2 = 0.000$; (c) (HBV+) versus (HCV+) $p3 = 0.000$.

HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range; SD, standard deviation.

Table 2. Killer Cell Immunoglobulin-Like Receptor Frequency Distribution by Disease Status

KIR gene	*Control,* n *(%)*	*HBV+,* n *(%)*	*HCV+,* n *(%)*	χ^2	p	p_c
2DL1	34 (100.0)	31 (100.0)	33 (100.0)			
2DL2	31 (91.2)	18 (58.1)	32 (97.0)	**8.31**	**0.0157**	0.1256
2DL3	33 (97.1)	29 (93.6)	33 (100.0)	0.20	0.9058	ns
2DL4	33 (97.1)	31 (100.0)	33 (100.0)	0.06	0.9719	ns
2DL5	20 (58.8)	20 (64.5)	21 (63.6)	0.19	0.9118	ns
2DS1	29 (85.3)	28 (90.3)	30 (90.9)	0.19	0.9098	ns
2DS2	22 (64.7)	19 (61.3)	33 (100.0)	**8.92**	**0.0116**	0.0928
2DS3	18 (52.9)	13 (41.9)	33 (100.0)	**18.37**	**<0.0001**	$\mathbf{8\times10^{-4}}$
2DS4	33 (97.1)	31 (100.0)	33 (100.0)	0.06	0.9719	ns
2DS5	17 (50.0)	14 (45.2)	23 (69.7)	3.26	0.1959	ns
3DL1	34 (100.0)	31 (100.0)	32 (97.0)	0.06	0.9706	ns
3DL2	34 (100.0)	31 (100.0)	33 (100.0)			
3DL3	34 (100.0)	31 (100.0)	33 (100.0)			
3DS1	22 (64.7)	4 (12.9)	32 (97.0)	**34.02**	**<0.0001**	$\mathbf{8\times10^{-4}}$
2DP1	34 (100.0)	30 (97.0)	31 (94.0)	0.18	0.9125	ns

Number of participants per group: 31 HBV+, 33 HCV+, and 34 uninfected controls; χ^2: chi-squared with two degrees of freedom from the Kruskal–Wallis test of equality of populations; *p*: uncorrected *p*-values; p_c: *p*-values corrected for multiplicity testing using the Bonferroni method are shown below:
KIR2DL2: (a) Control versus (HBV+) $p1=0.005$; (b) Control versus (HCV+) $p2=0.62$; and (c) (HBV+) versus (HCV+) $p3=0.0005$.
KIR2DS2: (a) Control versus (HBV+) $p1=0.97$; (b) Control versus (HCV+) $p2=0.0005$; and (c) (HBV+) versus (HCV+) $p3=0.0002$.
KIR2DS3:(a) Control versus (HBV+) $p1=0.52$; (b) Control versus (HCV+) $p2=0.00000$; and (c) (HBV+) versus (HCV+) $p3=0.0000$.
KIR3DS1: (a) Control versus (HBV+) $p1=0.52$; (b) Control versus (HCV+) $p2=0.000$; and (c) (HBV+) versus (HCV+) $p3=0.000$.
KIR, killer cell immunoglobulin-like receptor.

Results

Demographic and clinical characteristics of the study population

Of the 98 participants recruited, 57 were males (58.2%) and significantly overrepresented in the HBV-infected group (47.4%) compared with the HCV-infected group (24.6%) and uninfected controls (28.0%). Median age was significantly different between groups (Table 1). Participants infected with HCV were significantly older (median 62 years) than their HBV+ counterparts and uninfected control groups (36 and 29 years, respectively, $p<0.001$). A significant proportion of those infected with HCV (63.6%) were either married or living with a partner. This is, however, not the case with the HBV+ individuals as similar numbers of married and single participants were affected.

Distribution of KIR *genes and association with hepatitis infection*

We investigated three framework genes (*KIR3DL2*, *KIR3DL3*, and *KIR2DL4*) and they were present in all samples, with the exception of *KIR2DL4* that was absent in one uninfected control participant (Table 2). Most inhibitory *KIR* genes were present in more than 90% of the study population, with the exception of *KIR2DL2* (82.7% overall frequency), and the least common was *KIR2DL5* (62.2%). Interestingly, KIR2DL2 was significantly less frequent in the HBV+ group compared with their HCV-infected counterparts and uninfected controls (58.1%, 97.0%, and 91.2%), respectively. Univariate analyses comparing observed KIR frequencies between uninfected controls and HBV-infected (Table 3) or HCV-infected (Table 4) participants revealed that *KIR3DS1* is strongly, but differentially, associated with both disease models.

KIR3DS1 carriers were significantly less likely to be HBV infected compared with uninfected controls (12.9%vs. 64.7%, odds ratio [OR] = 0.08, $p=2.4\times10^{-5}$). Conversely, a significant proportion of HCV-infected participants were KIR3DS1 carriers (97.0% vs. 64.7%, OR = 17.45, $p=9.2\times10^{-4}$; Table 4), suggesting that this three-domain *KIR* activating gene may predispose its carriers to HCV infection in the study population in Cameroon. Carriers of

Table 3. Killer Cell Immunoglobulin-Like Receptor Association with Hepatitis B Virus Infection

	Control, n *(%)*	*HBV+,* n *(%)*	*OR (95% CI)*	p
Number	34	31		
KIR 2DL2	31 (91.2)	18 (58.1)	**0.13 (0.02–0.61)**	**0.0021**
KIR 2DL3	33 (97.1)	29 (93.6)	0.44 (0.03–5.25)	0.5038
KIR 2DS1	29 (85.3)	28 (90.3)	1.61 (0.35–7.50)	0.5408
KIR 2DS2	22 (64.7)	19 (61.3)	0.86 (0.31–2.39)	0.7773
KIR 2DS3	18 (52.9)	13 (41.9)	0.64 (0.23–1.74)	0.3786
KIR 2DS5	17 (50.0)	14 (45.2)	0.82 (0.30–2.20)	0.6987
KIR 3DS1	22 (64.7)	4 (12.9)	**0.08 (0.02–0.36)**	$\mathbf{2.39\times10^{-5}}$

Only KIR genes with apparent variability between groups are shown, *p*: uncorrected *p*-values.

Table 4. Killer Cell Immunoglobulin-Like Receptor Association with Hepatitis C Virus Infection

	Control, n *(%)*	*HCV+,* n *(%)*	*OR (95% CI) or Chi-squared*	p
Number	34	33		
KIR2DL2	31 (91.2)	32 (97.0)	3.09 (0.29–32.52)	0.3207
KIR 2DL5	20 (58.8)	21 (63.6)	1.23 (0.45–3.31)	0.6883
KIR 2DS1	29 (85.3)	30 (90.9)	1.72 (0.37–8.02)	0.4819
KIR 2DS2	22 (64.7)	33 (100.0)	**0.667**	**0.141**
KIR 2DS3	18 (52.9)	33 (100.0)	—	—
KIR 2DS5	17 (50.0)	23 (69.7)	2.30 (0.82–6.44)	0.1029
KIR 3DS1	22 (64.7)	32 (97.0)	**17.45 (1.70–179.31)**	$\mathbf{9.21\times10^{-4}}$
KIR 2DP1	34 (100.0)	31 (94.0)	2.09	0.148

Only KIR genes with apparent variability between groups are shown, *p*: uncorrected *p*-values.

two other activating *KIR* genes (*KIR2DS2* and *KIR2DS3*) were also all infected with HCV (Table 4).

Thirty-six KIR genotypes, 5 of which have not been previously reported, were found in 98 individuals (Table 5; Supplementary Tables S1 and S2). The most frequent genotypes we identified in the study population include genotypes 6 (25.5%), 11 (7.1%), 15 (7.1%), 233 (7.1), and 9 (5.1%). Of note, genotype 6 belongs to the Bx haplogroup with all 15 *KIR* genes present, as illustrated in Table 5. We found genotype 6 to be overrepresented in the HCV-infected group compared with uninfected controls and HBV-positive participants (51.5%, 17.7%, and 6.5%, respectively, $p = 0.005$).

In a univariate analysis comparing HCV-positive individuals with uninfected controls, carriers of genotype 6 were five times more likely to be HCV infected than people without this genotype. Similarly, we found genotype 15 to be present only in the HBV-infected group (22.6%), suggesting its role in the susceptibility to HBV. It shares nine genes in common with genotype 6, but lacks *KIR2DL2*, *KIR2DS2*, *KIR2DS3*, *KIR2DL5*, *KIR2DS5*, and *KIR3DS1* (Table 5).

Effects of centromeric and telomeric motifs and KIR genotype on hepatitis B and C

We identified individual KIR motifs in each study participant based on *KIR* genes present at their centromeric and telomeric loci, as previously reported (Cooley et al., 2010). Comparisons of the groups of genes present at the telomeric and centromeric loci were performed between the HBV-positive versus uninfected groups and the HCV-positive versus uninfected groups. It was observed that the centromeric A (c-A)/A genotype was significantly more frequent in the HBV-infected group compared with the uninfected group (38.7% vs. 5.9%, $p = 0.002$, OR = 0.099, 95% confidence interval [CI]: 0.020–0.491), while the c-A/B genotype was more frequent in the uninfected group compared with the HBV-positive group ($p = 0.001$, OR = 8.51, 95% CI = 2.141–33.83) (Table 6).

Discussion

KIRs, alongside their cognate HLA class I ligands, have been associated with several viral infections and autoimmune diseases. In this study, *KIR* genes and genotypes were characterized in HBV- and HCV-infected and uninfected individuals recruited from two major cities in Cameroon. We investigated the presence of 15 *KIR* genes using well-defined techniques and observed that all 15 *KIR* genes were present in our study population.

Reports from sub-Saharan Africa have shown that activating *KIR* genes are relatively infrequent compared with other populations. In this study, it was observed that the *KIR3DS1* gene was least frequent in the hepatitis B-infected group compared with other groups, which is coherent with other reports from sub-Saharan Africa (Norman et al., 2007; Yindom et al., 2010). One plausible interpretation is that *KIR3DS1* may offer protection against HBV infection (although in this small sample) of the Cameroonian population. In addition, the inhibitory *KIR2DL2* gene may also offer protection against acquisition of HBV, although to a lesser degree than *KIR3DS1* in the studied population. This is consistent with data published by Zhi-ming et al. (2008), which showed that *KIR3DS1*, *2DS2*, and *2DL5* are protective genes that facilitate HBV clearance.

Table 5. Frequencies of Common Killer Cell Immunoglobulin-Like Receptor Genotypes and Profiles Stratified by Disease Groups

HID[a]	GID[b]	KIR genes[c]															HBV+, n (%)	HCV+, n (%)	Control, n (%)	χ^2	p
		3DL1	*2DL1*	*2DL3*	*2DS4*	*2DL2*	*2DL5*	*3DS1*	*2DS1*	*2DS2*	*2DS3*	*2DS5*	*2DL4*	*3DL2*	*3DL3*	*2DP1*					
Bx	6	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2 (6.5)	17 (51.5)	6 (17.7)	10.596	0.0050
Bx	11	■	■	■	■	■	■		■	■	■		■	■	■	■	5 (16.1)	1 (3.0)	1 (2.9)	1.087	0.5806
Bx	15	■	■	■	■				■				■	■	■	■	7 (22.6)	0 (0.0)	0 (0.0)	3.209	0.2010
Bx	233	■	■	■	■	■		■	■	■	■		■	■	■	■	0 (0.0)	6 (18.2)	1 (2.9)	1.842	0.3981
Bx	9	■	■	■	■	■	■		■	■		■	■	■	■	■	3 (9.7)	0 (0.0)	2 (5.9)	0.454	0.7969
Bx	3	■	■	■	■	■	■	■	■	■		■	■	■	■	■	1 (3.2)	0 (0.0)	3 (8.8)	0.397	0.8199
Bx	7	■	■	■	■	■	■	■	■	■	■		■	■	■	■	0 (0.0)	1 (3.0)	3 (8.8)	0.391	0.8223
Bx	319	■	■	■	■	■		■	■	■	■	■	■	■	■	■	0 (0.0)	3 (9.1)	1 (2.9)	0.412	0.8137

a: HID; b: GID as previously described in the Allele Frequency Net Database; www.allelefrequencies.net; c: Inhibitory (DL), activating (DS), and pseudo (DP) *KIR* genes, the dark box indicates the presence of the corresponding *KIR* gene, while its absence is indicated by a white box. *n*, number of individuals carrying the genotype/profile of interest; χ^2: chi-squared with two degrees of freedom from the Kruskal–Wallis test of equality of populations; *p*: uncorrected *p*-values. Only KIR profiles present in at least two of the study groups or with an overall frequency >5% are shown. All KIR genotypes/profiles are presented in Supplementary Table S1.

Bx6: (a) Control versus (HBV+) $p1 = 0.3$; (b) Control versus (HCV+) $p2 = 0.007$; and (c) (HBV+) versus (HCV+) $p3 = 0.0002$.

GID, genotype identity; HID, haplotype identity.

Table 6. Frequencies of Centromeric, Telomeric, and Killer Cell Immunoglobulin-Like Receptor Motifs Stratified by Disease Status

	HBV+, n *(%)*	*HCV+,* n *(%)*	*Control,* n *(%)*	OR^b *(95% CI)*	p_b	OR^c *(95% CI)*	p_c
Centromeric motif							
c-A/A	12 (38.7)	0 (0.0)	2 (5.9)	0.1 (0.0-0.5)	0.002*	1.0 (0.1–7.3)	1.000
c-A/B	17 (54.8)	33 (100.0)	31 (91.2)	8.5 (2.1–33.8)	0.001*	1.0 (0.2–5.5)	1.000
c-B/B	2 (6.5)	0 (0.0)	1 (2.9)	0.4 (0.3–5.1)	0.602	1.0 (0.6–16.2)	1.000
Telomeric motif							
t-A/A	3 (9.7)	0 (0.0)	5 (14.7)	1.6 (0.4–7.4)	0.711	—	—
t-A/B	28 (90.3)	32 (96.9)	28 (82.3)	0.5 (0.1–2.2)	0.480	0.1 (0.0–1.3)	1.020
t-B/B	0 (0.0)	1 (3.0)	1 (2.9)		1.000	1.0 (0.6–16.2)	1.000
KIR haplotype							
AA	1 (3.2)	0 (0.0)	1 (2.9)	0.9 (0.5–15.2)	1.000	—	—
Bx	30 (96.7)	33 (100.0)	33 (97.0)	1.1 (0.1–18.4)	1.000	—	—

Thirty-one participants were HBV+, 33 were HCV+, and 34 uninfected controls tested negative for HIV and both hepatitis viruses; p_b: *p*-value of comparison between HBV+ and uninfected control groups; p_c: *p*-value of comparison between HCV+ and uninfected controls. c-A, centromeric A. **p*-Value is significant.

Conversely, *KIR3DS1* is strongly associated with susceptibility to infection with hepatitis C virus. We found that all but one HCV-positive individual were *KIR3DS1* carriers compared with 2/3 of the uninfected group (97.0% vs. 64.7%, respectively, $p = 9.21 \times 10^{-4}$). Two other activating genes (*KIR2DS2* and *KIR2DS3*) were associated with susceptibility to HCV infection. Both genes were present in all hepatitis C-infected individuals compared with about half (*KIR2DS3*) or 2/3 (*KIR2DS2*) of the uninfected group. Other studies with bigger sample sizes are needed to highlight the effects of these genes in HCV and HBV transmission and disease susceptibility.

Thirty-six KIR genotypes were identified with five previously unreported, which might be specific to the Cameroonian population. Haplotype B profiles were the most frequent in our study population. Genotype 6 that possesses all 15 *KIR* genes was present in a quarter of our sample (25.5%) and appears to significantly predispose its carriers to HCV acquisition.

At the telomeric and centromeric levels, homozygosity for c-A motif (c-A/A) was overrepresented in the hepatitis B individuals, while the heterozygous motif c-A/B was present in all HCV-infected participants. Telomeric motifs, however, were similarly distributed across groups.

Conclusion

Genetic epidemiological studies have shown that host genetic variations significantly modulate viral infections (López-Vázquez et al., 2007; Yindom et al., 2017). However, no study has evaluated the role of *KIR* genes in viral hepatitis in Cameroon. Although with a small sample size, this study is one of the few to report on the *KIR* gene frequencies and profiles from Cameroon. We found five potentially novel *KIR* genotypes, which will be deposited in public databases to contribute to the growing number of KIR haplogroups reported. These data therefore lay the ground for further *in vitro* studies on the mechanisms of action of host factors against hepatitis B and C infections and could provide useful information for development of better treatments and vaccines.

Author Disclosure Statement

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Supplementary Material

Supplementary Table S1
Supplementary Table S2

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Abbreviations Used

AIDS = acquired immunodeficiency syndrome
c-A = centromeric A
CI = confidence interval
HBV = hepatitis B virus
HCV = hepatitis C virus
HLA = human leukocyte antigen
KIR = killer cell immunoglobulin-like receptor
NK = natural killer
OR = odds ratio
PCR = polymerase chain reaction
SPSS = Statistical Package for the Social Sciences
SSP = sequence-specific primer
WHO = World Health Organization