Killer Immunoglobulin-like Receptors (KIR) frequencies in a rural community in North-Western Guinea-Bissau

Louis Marie Yindom
MRC The Gambia

Overview

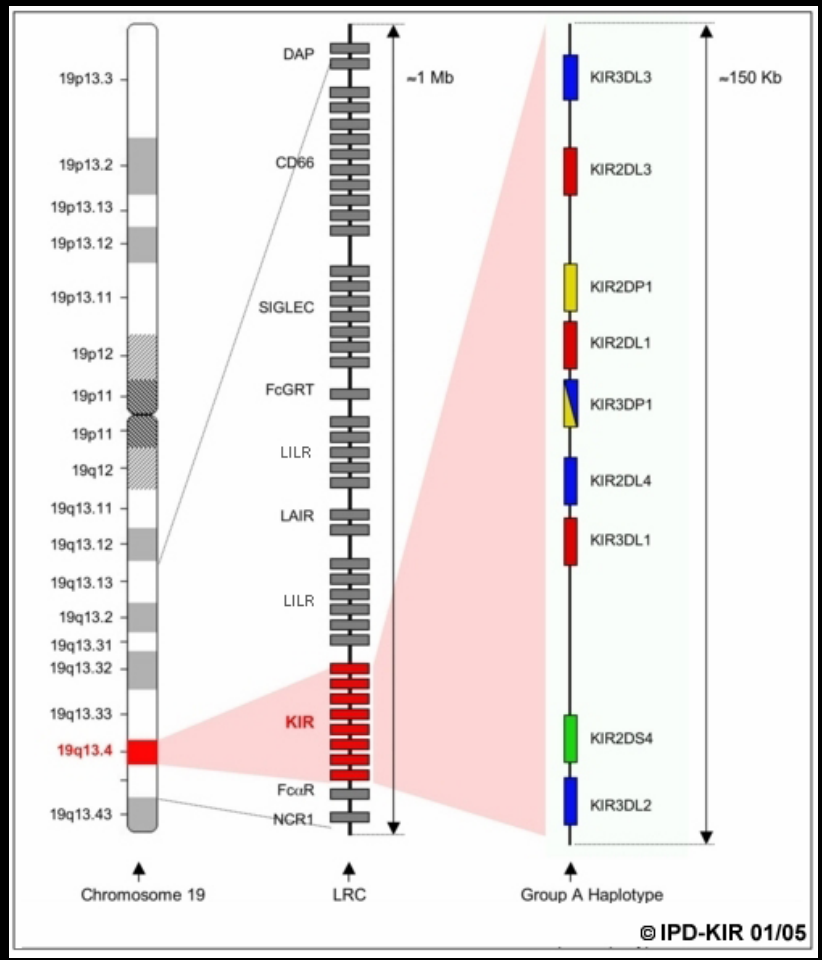
- Natural Killer (NK) cells and their receptors (KIR)
- KIR gene cluster
- KIR-HLA combinations and diseases (AIDS)
- The rationale for the main study
- Preliminary data analysis
- KIR gene profile diversity
- Summary
- Future work
- Acknowledgements

Natural Killer cells and the immune system

- NK cells are effector lymphocytes
- First line of defence against viral infections and transformed cells (tumours)
- Cells are killed by direct cytotoxicity and release of cytokines
- NK cell surface receptors are of two types
  - C type lectin - 12p13.1 (rodents)
  - KIR – LRC - 19q13.4 (humans)

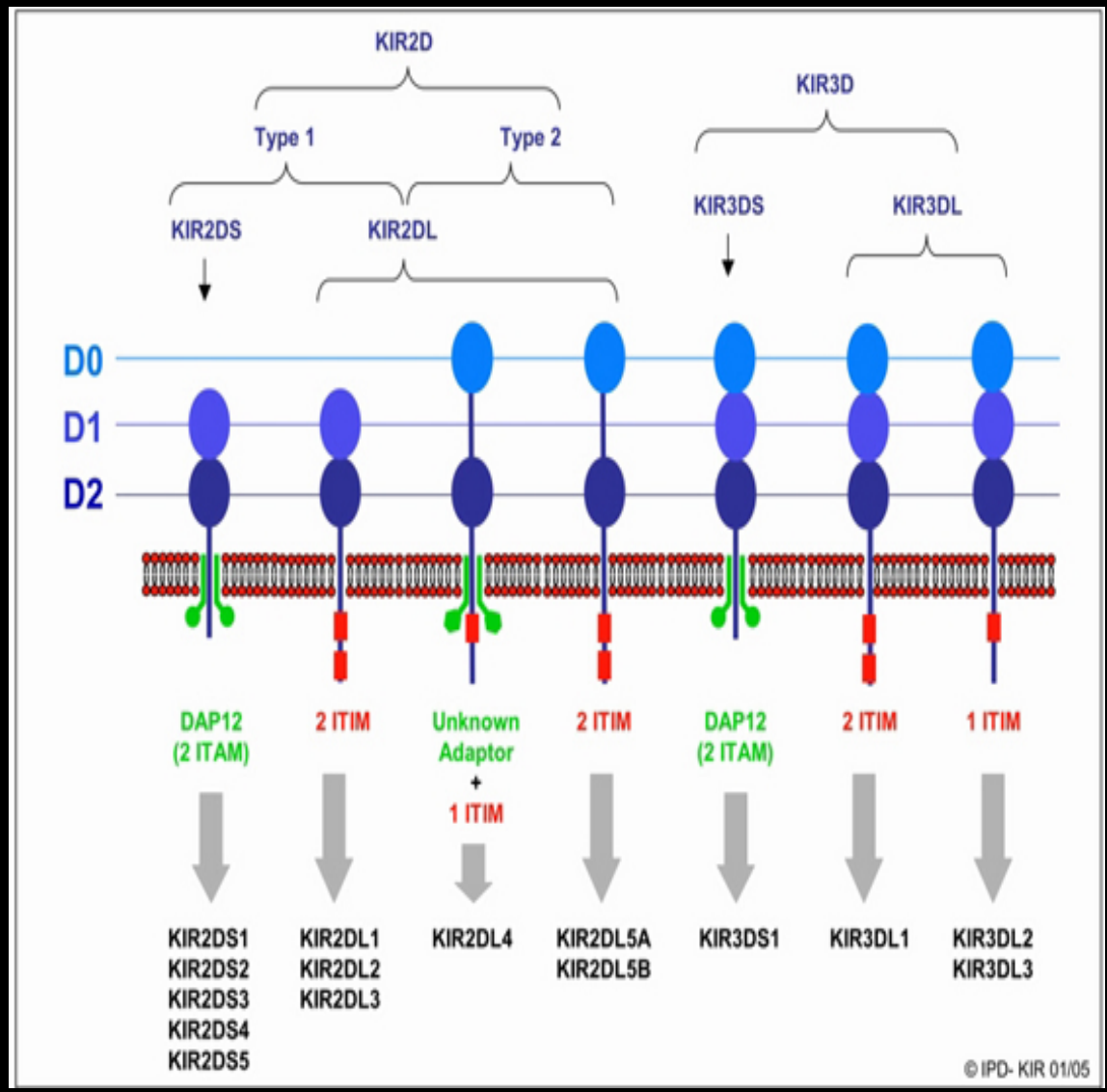
Killer immunoglobulin-like receptors (KIR)

- KIRs are type I transmembrane glycoproteins with 2-3 extracellular domains
- A group of regulatory molecules expressed by NK cells, a subpopulation of $\gamma\delta$ T cells, and some memory $\alpha\beta$ T cells
- They were first identified by their ability to impart some specificity on natural killer cytolysis
- They are specific for allelic forms of HLA class I molecules
- Their interaction with HLA class I molecules modulate the cytolytic activity of NK cells
- Different populations have different KIR patterns



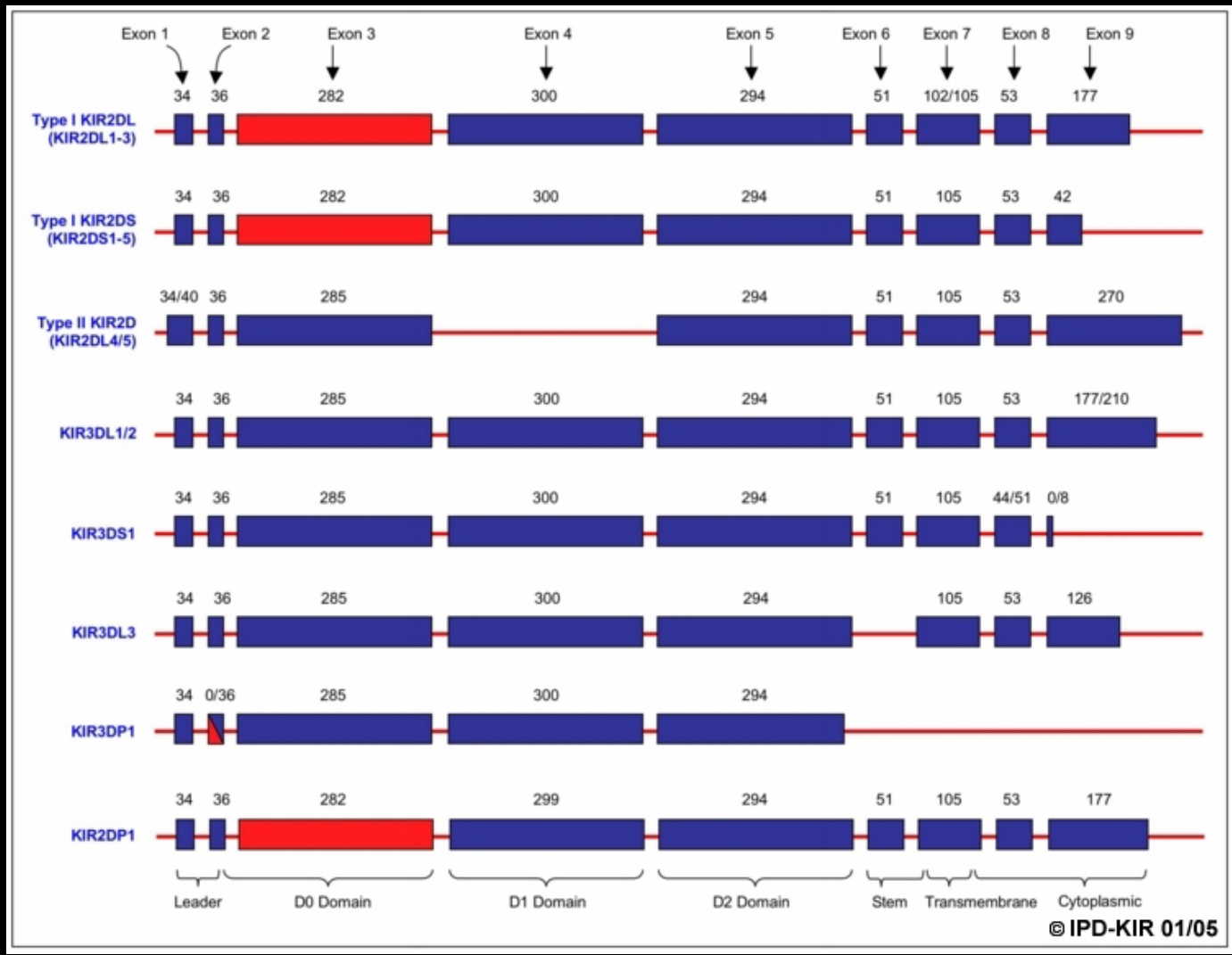
KIR structure

- Functionally KIR molecules have been classified as either inhibitory or activating receptors
- Activating KIR have short (S) cytoplasmic tail with a positively charged residue in the transmembrane region
- Inhibitory KIR have a long (L) cytoplasmic tail containing $ITIM^S$
- Their transmembrane and cytoplasmic regions are functionally relevant as they define the type of signal transduced to NK cells
- KIR proteins possess Ig-like domains (D0, D1 & D2) which interact with HLA class I ligands to initiate or inhibit NK cell activity



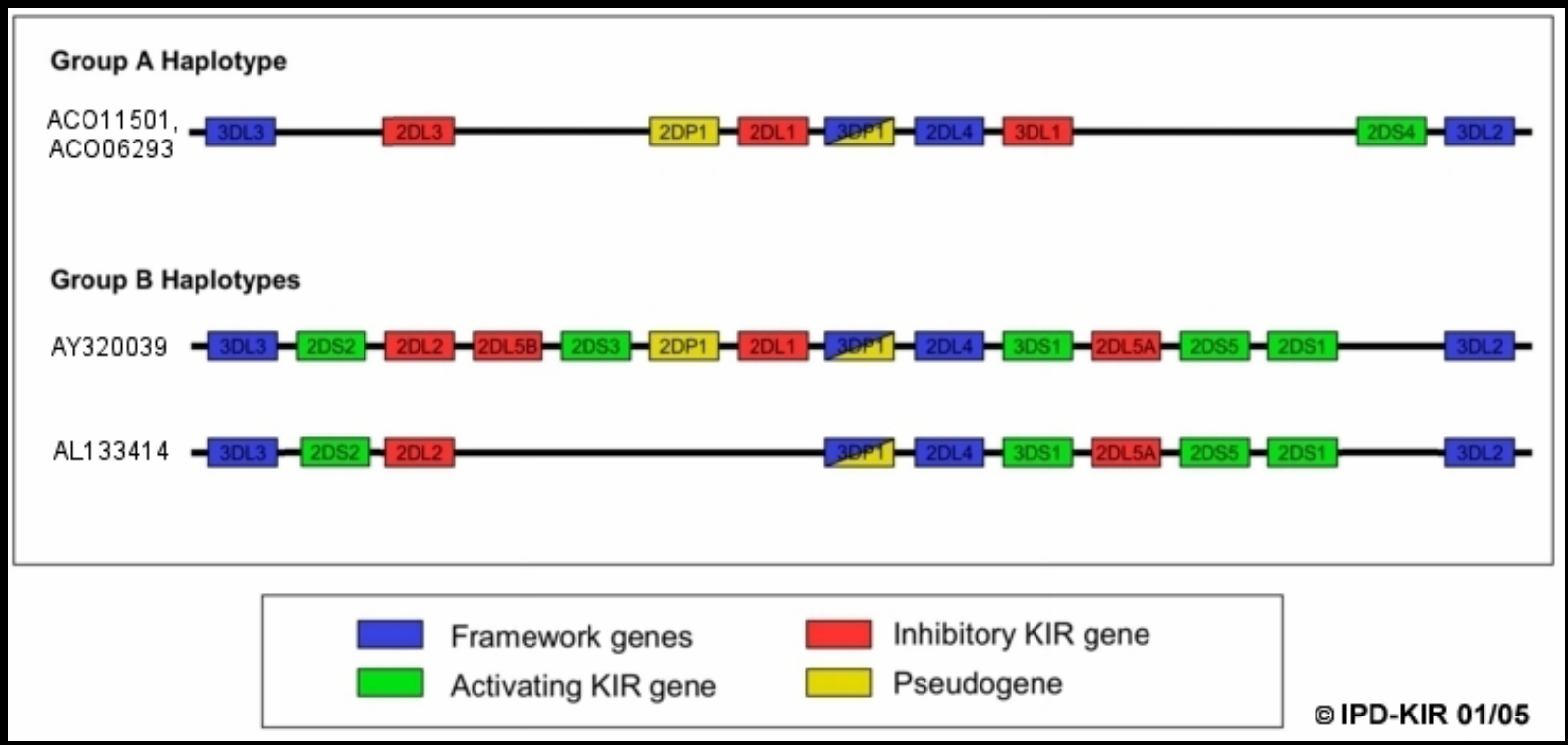
KIR genes

- Type I KIR2D genes possess eight exons and a pseudoexon 3 sequence
- Type II KIR2D genes lack exon 4 and possess a translated exon 3
- KIR3D genes possess nine exons

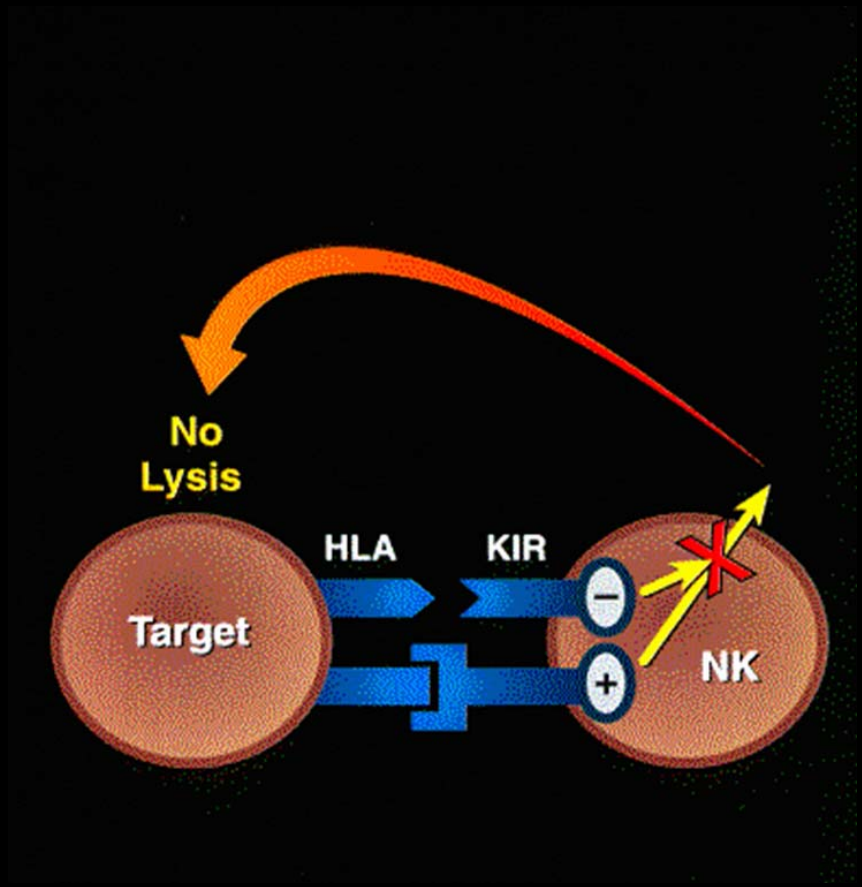


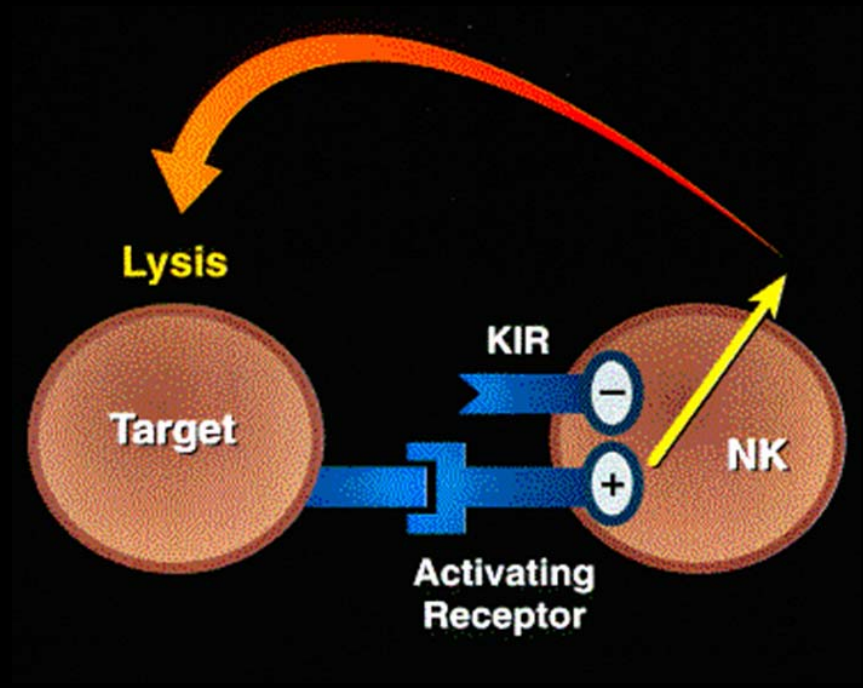
KIR haplotypes

- Two main haplotypes (A and B)
  - A: inhibitory genes (except KIR2DS4)
  - B: both activating and inhibitory genes



KIR and NK cell function





NK cells kill targets that do not express HLA class I

KIR3DS1/Bw4-80I in HIV infection

a) protects against AIDS progression (Nat Gen 2005 31: 429)

b) protects against OI independent of $CD4^+$ T cell decline (PLoS Pathog 2006 2(8): 741)

c) associates with lower virus load set point (PLoS Pathog 2006 2(8): 741)

Why this project?

- The burden of HIV epidemic rest in Sub-Sahara Africa (2/3 of all affected people)
- An effective vaccine is yet to be found
- Mechanism(s) of protective immunity poorly understood
- HIV-2 (West Africa) is a naturally attenuated form of HIV
  - Less transmissible and less pathogenic
  - Offers opportunity for longitudinal studies
- There is a paucity of information from HIV-2 research
- Understanding the reason why most HIV-2 infected people do not develop immune deficiency could provide unique insights into protective immunity in HIV infection and pave the way for design of vaccines against the virus

Specific objectives

- To determine the KIR genes pool in a community based cohort in Caio (Guinea-Bissau)
- To study the effect of individual KIR genes and haplotypes on susceptibility or resistance to HIV-2 infection
- To determine the haplotype diversity and its role in long-term non-progression status observed in HIV-2 infection

Methods (1)

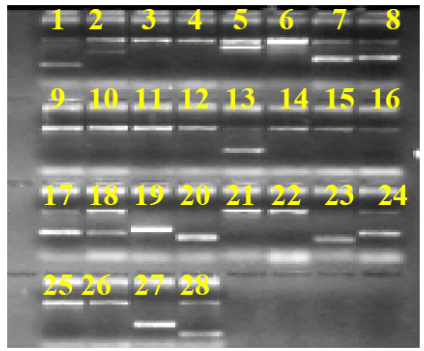
- DNA was extracted by the salting-out technique from 150 HIV-2 infected, 35 HIV-1&2, and 328 uninfected subjects from a Manjago community in Guinea-Bissau
- KIR specific primers were used to design a phototype of 28 PCR reactions to detect the presence or absence of KIR genes

Methods (2)

Each reaction (except lane 6) had an internal control that amplified a highly conserved 796bp fragment in the APC gene.

KIR GENOTYPING SHEET (PCR-SSP)

Cohort	CAIO
Study	EDCTP
ID Number	**90110076**
DNA Number	**N004370**
TDMD Batch	KT1
Date	23.11.2006



Gene	Result	Lane	Size
2DL1	+	*1*	*122*
	+	*2*	*330*
2DL2	-	*3*	*175*
	-	*4*	*150*
2DL3	+	*5*	*550*
	+	*6*	*800*
2DL4	+	*7*	*254*
	+	*8*	*288*
2DS2	-	*9*	*173*
	-	*10*	*240*
2DS3	-	*11*	*242*
	-	*12*	*190*
2DS4	+	*13*	*204*
	-	*14*	*197/219*
2DS5	-	*15*	*125*
2DS1	-	*16*	*102*
3DL1	+	*17*	*197*
	+	*18*	*181*
3DL2	+	*19*	*300*
	+	*20*	*179*
3DS1	-	*21*	*245*
	-	*22*	*130*
3DL3	+	*23*	*112*
	+	*24*	*190*
2DL5	-	*25*	*214*
	-	*26*	*194*
2DP1	+	*27*	*205*
	+	*28*	*90*

Table 1: Study population stratified by HIV status

HIV status	Freq (%)	Diagnosed Since 1989	2006	
			Alive	Died
HIV-negative	328 (63.94)	-	-	-
HIV-2	150 (29.24)	64	58	6
HIV-1&2	35 (6.82)	10	9	1
Total	513	74	67	7

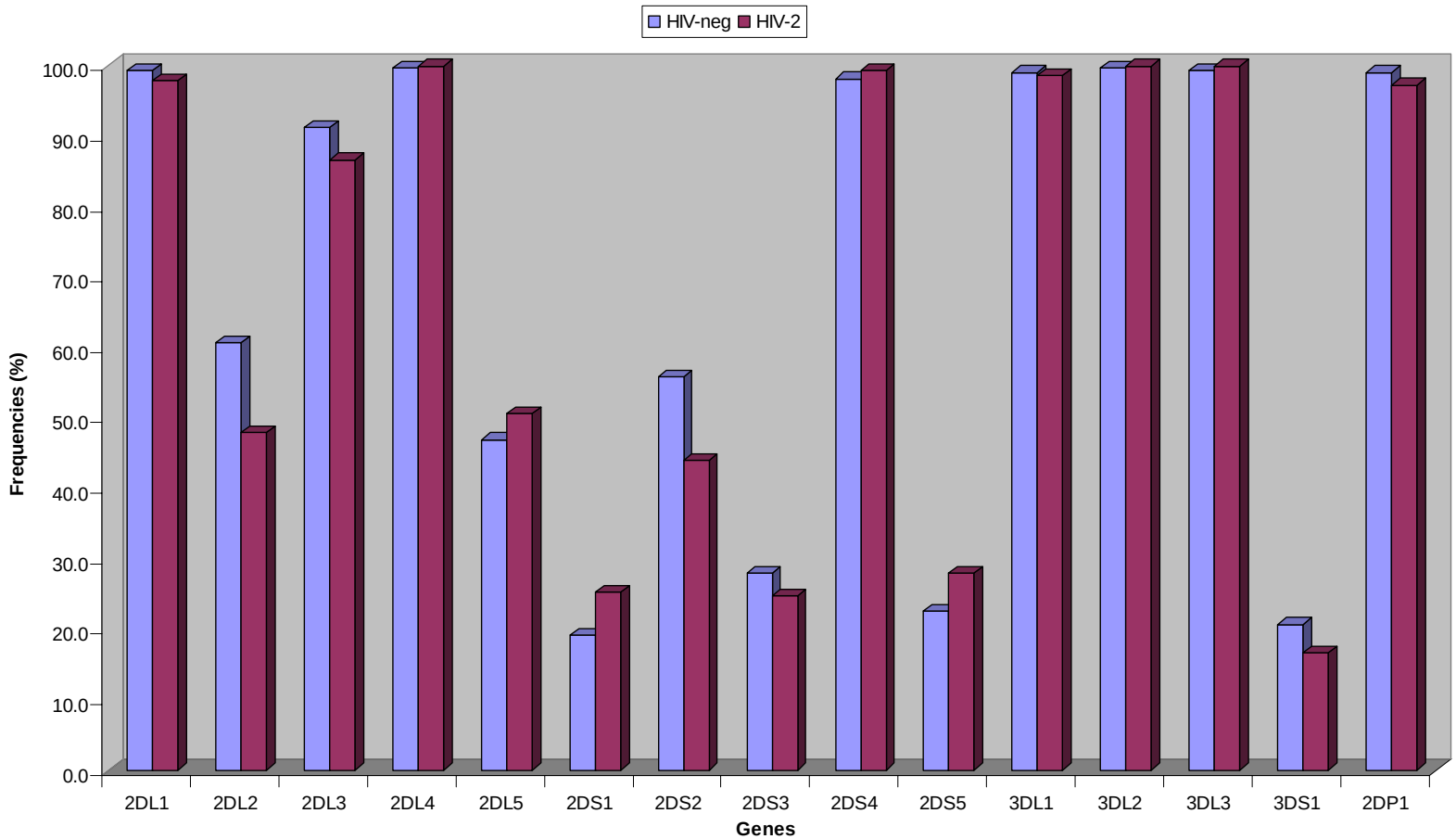
Table 2: HIV-2 incidence in the control group since 1989

HIV status	Freq	Percent	Dead by 2006 (%)
HIV-negative	177	80.82	12 (5.5)
HIV-2	30	13.70	1 (0.5)
HIV-1&2	12	5.48	1 (0.5)
Total	219	100.00	14 (6.4)

Table 3: KIR gene frequencies in the Manjago population

Genes	Manjago (n = 513)	%	HIV-0 (n = 328)	%	HIV-2 (n = 150)	%	Fisher's	χ^2	OR	95% CI
2DL1	508	99.0	326	99.4	147	98.0	0.181	-	0.30	0.05 - 1.81
2DL2	291	56.7	199	**60.7**	72	**48.0**	**-**	**0.009**	**0.60**	**0.40 - 0.88**
2DL3	461	89.9	299	91.2	130	86.7	-	0.233	0.63	0.34 - 1.15
2DL4	512	99.8	327	99.7	150	100.0	1.000	-	-	-
2DL5	247	48.2	154	47.0	76	50.7	-	0.451	1.16	0.79 - 1.71
2DS1	107	20.9	63	19.2	38	25.3	-	0.123	1.43	0.90 - 2.26
2DS2	267	52.1	183	**55.8**	66	**44.0**	**-**	**0.017**	**0.62**	**0.42 - 0.92**
2DS3	140	27.3	92	28.0	37	24.7	-	0.440	0.84	0.54 - 1.31
2DS4	506	98.6	322	98.2	149	99.3	0.442	-	2.78	0.33 - 23.27
2DS5	125	24.4	74	22.6	42	28.0	-	0.198	1.33	0.86 - 2.10
3DL1	508	99.0	325	99.1	148	98.7	0.651	-	0.68	0.11 - 4.13
3DL2	512	99.8	327	99.7	150	100.0	1.000	-	-	-
3DL3	511	99.6	326	99.4	150	100.0	1.000	-	-	-
3DS1	95	18.5	68	20.7	25	16.7	-	0.297	0.76	0.46 - 1.27
2DP1	506	98.6	325	99.1	146	97.3	0.213	-	0.34	0.07 - 1.52

Fig.1: KIR genes in cases and controls



* p<0.05

Table 4: KIR genes in other populations

Genes	Manjago (n=513)	Senegalese* (n= 118)	South African ♣ (n= 50)	English¤ (n= 136)	French* (n= 108)	Indian§ (n= 72)	South Korean¥ (n= 154)
2DL1	99	100	96	91	97	88	99
2DL2	57	55	72	49	50	79	14
2DL3	90	90	64	92	91	65	99
2DL4	100	100	100	100	100	100	100
2DL5	48	52	82	N/A	47	79	38
2DS1	21	13	40	45	36	54	38
2DS2	52	42	64	51	51	62	17
2DS3	27	24	38	24	31	43	16
2DS4	99	100	100	96	96	81	94
2DS5	24	30	62	32	27	47	27
3DL1	99	99	100	97	96	88	94
3DL2	100	100	100	100	100	100	100
3DL3	100	100	100	N/A	100	100	100
3DS1	19	4	N/A	N/A	44	N/A	N/A
2DP1	99	100	98	N/A	97	N/A	N/A

*Denis L et al. 2005 Tissue Antigens 66, 267-76; ♣Williams F et al. 2004 Human Immunology 65(9-10),1084-1085; ¤Norman P et la. 2004 Immunogenetics; §Rajalingam R et al. 2002 Immunogenetics 53,1009-19; ¥Whang, D. H.. 2005 Human Immunology 66,146

Fig. 2: Most frequent haplotypes in Cases and Controls

Hap	3DL3	2DS2	2DL2	2DL3	2DL5	2DS3	2DP1	2DL1	2DL4	3DL1	3DS1	2DS5	2DS1	2DS4	3DL2	Total	All (%)	HIV-0	HIV-2
YA1	■			■			■	■	■	■				■	■	145	30.3	27.4	36.7
YB1	■	■	■	■	■	■	■	■	■	■				■	■	54	11.3	11.9	10.0
YB2	■	■	■	■			■	■	■	■				■	■	35	7.3	8.8	4.0
YA2	■		■	■			■	■	■	■				■	■	16	3.3	4.0	2.0
YB3	■			■	■		■	■	■	■	■	■	■	■	■	15	3.1	2.4	4.7
YB4	■			■	■	■	■	■	■	■				■	■	11	2.3	1.5	4.0
YB5	■		■	■	■		■	■	■	■		■		■	■	11	2.3	1.8	3.3
YB6	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	10	2.1	2.7	0.7
YB7	■	■	■	■	■		■	■	■	■			■	■	■	10	2.0	1.5	3.3
YB8	■	■	■	■			■	■	■	■	■			■	■	9	1.9	2.7	0.0
YB9	■	■	■	■	■		■	■	■	■		■		■	■	9	1.9	2.1	1.3
YB10	■	■	■	■	■		■	■	■	■				■	■	9	1.9	2.4	0.7
YB11	■	■	■	■	■		■	■	■	■		■	■	■	■	8	1.6	1.2	2.7
YB12	■	■	■		■	■	■	■	■	■		■		■	■	8	1.6	1.2	2.7
YB13	■	■	■		■	■	■	■	■	■	■	■	■	■	■	8	1.6	1.5	2.0
YB14	■	■	■	■	■		■	■	■	■	■	■	■	■	■	7	1.4	1.2	2.0
YB15	■	■		■			■	■	■	■				■	■	7	1.4	1.5	1.3
YB16	■			■			■	■	■	■			■	■	■	7	1.4	0.6	3.3
YB17	■	■	■		■	■	■	■	■	■				■	■	6	1.2	1.5	0.7
YB18	■			■			■	■	■	■	■			■	■	5	1.0	1.5	0.0

Fig.3: Haplotype frequency in cases and controls

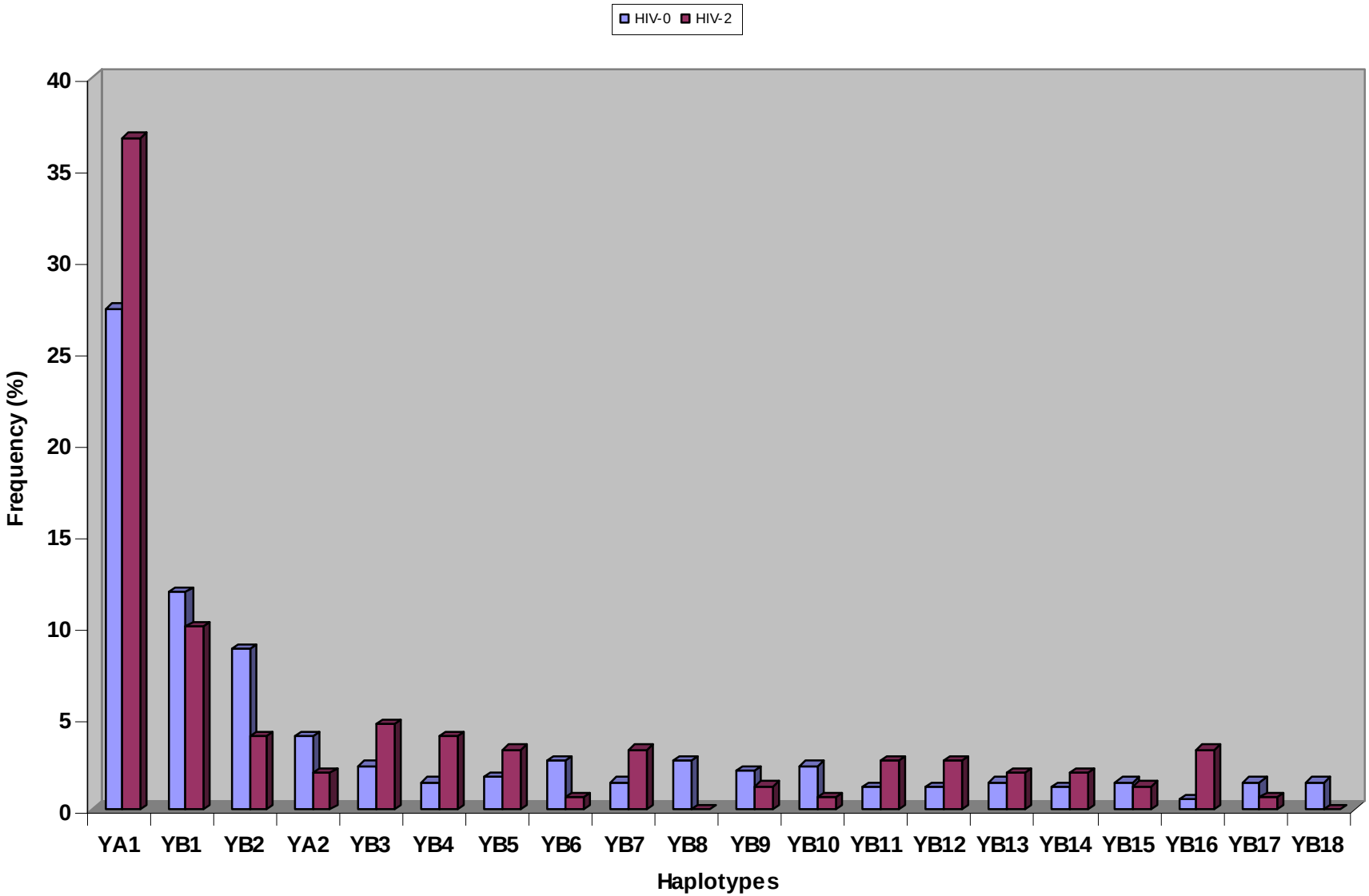


Table 5: Frequencies of A and B haplotypes

Haplotype	Proportion of HIV negative with haplotype		Proportion of HIV-2 positive with haplotype	
	N	%	N	%
A	5	6.3	2	2.5
B	74	93.7	77	97.5

Fisher's exact p = 0.003

Summary (1)

- 15 KIR genes were detected in this population
- HIV-2 incidence was 14% among the uninfected group seen in 1989 with only 0.5% dead compared to 5.5% amongst the persistently uninfected (177) since 1989
- Activating genes were the least frequent
- KIR2DS2 and KIR2DL2 frequencies were significantly higher in the control group than cases

Summary (2)

- KIR3DS1 gene is frequent in the study population compared other African populations
- Other activating KIR genes frequencies in this Manjago population were lower compared to a genetically distant South African population
- 79 distinct haplotypes were present in our samples (5 A and 74 B) with an A haplotype (YA1) predominating
- 3/5 A haplotypes were absent among cases

Future directions

- Detail analysis of Caio KIR data
- KIR and HLA sequencing (Caio cohort)
- KIR typing (Fajara cohort)
- HLA and KIR sequencing (Fajara cohort)
- Complete data analysis
- Functional assays to check the level of expression of genes of interest
- Development of new HLA and KIR typing techniques (SNPlex platform)
- Thesis and paper write-ups and publications in peer-reviewed journals

Acknowledgements

MRC Fajara

Robert Walton
Sarah Rowland-Jones
Aleksandra Leligdowicz
Akram Zaman
Carla Van Tienen
Maarten Schim v. d. Loeff
Giorgio Sirugo
Assan Jaye
Hilton Whittle
Shabbar Jaffar
Matthew Cotten
Sarah Crozier
Paul Snell
Abraham Alabi

Akum Aveika Awasana
Victor Nuvor
Ramu Sarge-Njie
Mathurin Diatta
Biobank team
Bakary Sanneh
Ramatoulie Drammeh
Kwekam F. Yindom
Serology lab team
Viral Diseases staff
Genetics group
GUM clinic staff
Study participants

Directors–MRC Gambia

CAIO

Tim Vincent
Field Workers
Study participants

LGD/NCI Frederick, MD

Mary Carrington
Maureen P. Martin
Xiaojian Gao
Arman Bashirova
Amy Poisson
Darlene Marti

Sponsors

EDCTP

MRC