GENETIC POLYMORPHISMS OF INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1RA) GENE AMONG HIV INFECTED AND NON-INFECTED INDIVIDUALS IN NAIROBI

IRENE WANJIRU MUTHECA
BED (Sc)
I56/10016/07

A thesis submitted in partial fulfillment of the requirement for the award of the degree of Master of Science (Immunology) in the School of Pure and Applied Sciences of Kenyatta University

MAY 2012

DECLARATION

I hereby declare that this research is my original work and has not been submitted for degree or any other award in any other university.

IRENE WANJIRU MUTHECA (I56/10016/07)

Signature.....Date.....27/5/2012.....

This thesis has been submitted for examination with our approval as University Supervisors.

Dr. Michael M. Gicheru
School of Pure and Applied Sciences
Department of Zoological Sciences
Kenyatta University

.....
Signature

29/5/2012
.....
Date

Dr. Samoel Ashimosi Khamadi
Centre for Virus Research
Kenya Medical Research Institute

.....
Signature

27/5/2012
.....
Date

DEDICATION

I dedicate this thesis to my dear husband Martin who has been instrumental in my life and in pursuing my MSc degree course by praying for me and giving me moral and financial support and to all members of my family and friends who have been supportive and encouraged me through this work.

ACKNOWLEDGEMENTS

I would like to acknowledge the Almighty God for giving me the strength, knowledge and resilience to undertake this project. I would like to express my gratitude to my supervisor, Dr. Michael Gicheru who shared with me a lot of his expertise and research insight. His sage advice, insightful criticisms, and encouragement aided the writing of this thesis in innumerable ways. I would also like to express my gratitude to my other supervisor Dr. Samoel Khamadi of Kenya Medical Research Institute (KEMRI) Nairobi, whose steadfast support of this project and thoughtful advice was greatly needed and deeply appreciated. This work was carried out at the Kenya Medical Research Institute (KEMRI) Nairobi in HIV P3 laboratory and I thank Dr. Okoth, the director, Center for Virus Research (CVR) and Dr. Khamadi, head of HIV laboratory for granting permission to carry out my research work in HIV laboratory. I would like to express my gratitude to all those who gave me the possibility to complete this thesis. I also wish to thank all the staff of KEMRI HIV laboratory for their help, support, interest, suggestions and encouragement that helped me during the research and writing of this thesis. Lastly, I am grateful to my family and friends for the support they gave me during the course of my studies. Thank you so much and may God bless you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/ ACRONYMS

AIDS	Acquired immunodeficiency syndrome
Alleles	Alternative forms of the same gene
CD4	Cluster of differentiation antigen number 4
CD4 T cells	CD4 effector T lymphocytes
CD4+	CD4 positive effector T lymphocytes
CSF	Colony stimulating factor
CD 26	Cluster of differentiation twenty six
CDC	Centre for disease control
CRH	Corticotrophin releasing hormone
DNA	Deoxyribonucleic acid
Env	envelope
GM-CSF	Granulocyte macrophage- colony stimulating factor
Gag	Group specific antigen
Gp 41	A surface glycoprotein
Gp 120	A sugar containing protein called a glycoprotein of approximately 120,000 molecular weight
GRID	Gay related immune deficiency
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HCG	Human chorionic gonadotropin
IFN γ	Interferon gamma

IgG	Immunoglobulin gamma
IL-1	Interleukin 1
IL-4	Interleukin 4
IL-6	Interleukin 6
IL-1α	Interleukin 1 alpha molecule
IL-1β	Interleukin 1 beta molecule
IL-1RA	Interleukin 1 receptor antagonist molecule
IL-1RN	Interleukin 1 receptor antagonist gene
IL-IRN*1	Interleukin 1 receptor antagonist gene, allele 1
IL-1RN*2	Interleukin 1 receptor antagonist gene, allele 2
IcIL-1RA	Intracellular interleukin 1 receptor antagonist
KAIS	Kenya AIDS indicator survey
KEM	Kenya medical research institute
LTR	Long terminal repeat
MHC	Major histocompatibility complex
MOH	Ministry of health
mRNA	Messenger ribonucleic acid
MSH	Melanocyte stimulating hormone
NACC	National Aids control council
Nef	Negative regulatory factor
NRE	Negative regulatory element
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction

PCP	Pneumocystis pneumonia
PMTCT	Prevention of mother to child transmission
Primer	A short oligonucleotide sequence needed to start DNA sequencing
Pol	HIV-1 polymerase gene
RBC	Red blood cells
Rev	Regulator of virions
RNA	Ribonucleic acid
R1	Reverse primer
RP	Rapid progressors
sIL-1RA	Soluble interleukin 1 receptor antagonist
SNPs	Single nucleotide polymorphisms
TAR	Trans-acting responsive region
Tat	Trans-activator of transcription
TATAA	A region that determines proper initiation of transcription
TCR	T cell receptor
TNF	Tumour necrosis factor
TGF	Tumour growth factor
TH1&2	T helper 1 and 2 cells
Vif	Viral infectivity factor
Vpr	Viral protein regulatory
Vpu	Viral protein unknown
VNTR	Variable numbers of tandem repeat

ABSTRACT

Interleukin-1 (IL-1) and its endogenous antagonist IL-1 receptor antagonist (IL-1RA) play an important role in various inflammatory responses. They are both produced in chromosome two and their production is different between men and women. The Human Immunodeficiency Virus (HIV) utilizes IL-1 and viral tat protein to accelerate its replication and the synthesis of all HIV proteins. Interleukin-1 receptor antagonist gene has been shown to retard HIV disease progression by inhibiting HIV replication mediated by IL-1. Understanding the mechanisms that account for the lower viral loads in some individuals is important for designing of new antiviral drugs or vaccines which may be achieved by blocking the effects of IL-1. In intron 2 of the IL-1RA gene, a variable number of an 86-bp tandem repeat polymorphism leads to the existence of five different alleles. Distribution of the IL-1RA gene polymorphism within a population can be considered as a measure of genetic susceptibility of HIV infection and disease progression. This study sought to determine the frequency of IL-1RA gene polymorphisms in a population of HIV infected and non-infected men and women in Nairobi. Two hundred and forty samples were collected of which 170 samples were HIV positive and 70 samples were from HIV negative individuals. Peripheral blood mononuclear cells were extracted from whole blood. Genomic deoxyribonucleic acid was then extracted from PBMCs. The genotypes and alleles of IL-1RA were determined by polymerase chain reaction (PCR) using specific primers. All the PCR amplicons were analysed using 2% gel electrophoresis. Allele and genotype frequencies were calculated by direct counting. Chi square test was used in comparing frequency of alleles between infected and non-infected individuals. Four alleles were identified in the study group with allele 1 having the highest occurrence of 80.59%, allele 2 (4.71%), allele 3 (5.88%) and allele 4 (8.82%). In the control group, only two alleles were observed with allele 1 (74.3%) and allele 4 (25.7%). Several genotypes were observed and they were different between the study and control groups and also in men and women. The results showed that there was a gender difference in IL-1RA gene polymorphism expressed by a higher incidence of genotype 1/1 homozygotes in men (71.8%) and a higher occurrence of genotype 2/2 homozygotes in women (5.9%). Allele 1 and 4 were more frequent in men, while allele 2 and 3 were more prevalent in women. These data might hold the key to developing appropriate strategies for effective response of those who appear to be immune to HIV. Since the functional significance of this polymorphisms remains unclear, further investigations into a possible correlation between specific IL-1RA genotypes and alleles and HIV is necessary, with a view to a possible new therapeutical approach in treatment of HIV especially the use of protective IL-1RA alleles.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Acquired Immune Deficiency Syndrome (AIDS) is a collection of signs and symptoms of infections resulting from the depletion of the immune system. It is caused by the infection by Human Immunodeficiency Virus (HIV), which is a retrovirus belonging to the lentivirus family of the viruses that cause slow progressing diseases (Courgnaud *et al.*, 2001). The immune deficiency results from the loss of CD4+ (helper/inducer) T cells that are essential for both cell mediated immunity and humoral immunity. AIDS symptoms are mainly due to opportunistic infections that can be easily treated in healthy people (Coffin *et al.*, 1986). The disease has four stages: the first stage is asymptomatic; the second stage includes minor mucocutaneous manifestations and recurrent upper respiratory tract infections; chronic and pulmonary tuberculosis is seen in stage three; and stage four includes toxoplasmosis of the brain, candidiasis of the oesophagus, trachea, bronchi or lungs and Kaposi's sarcoma. Transmission routes of HIV are sexual, blood to blood products and vertical transmission of mother to child (Stewart *et al.*, 1985).

1.2 Origin and discovery of HIV/AIDS

The AIDS epidemic was first discovered in 1981, when the U.S. Centers for Disease Control (CDC) reported a cluster of *Pneumocystis pneumonia* caused by a form of *Pneumocystis carinii*, now recognized as a distinct species of *Pneumocystis jirovecii,* in five homosexual men in Los Angeles (CDC, 1981). The disease was originally dubbed gay related immune deficiency (GRID), but health authorities soon realized that nearly half of the people identified with the syndrome were not homosexual men. In 1982, the

CDC introduced the term AIDS to describe the newly recognized syndrome, though it was still casually referred to as GRID.

Human immunodeficiency virus was classified as a member of the genus lentivirus (Mayo and Haenni, 2006), part of the family of retroviridae (Hull, 2001). Many species are infected by lentiviruses, which are characteristically responsible for long duration illnesses with a long incubation period (Levy, 1993). Lentiviruses are transmitted as single stranded, positive-sense, enveloped RNA viruses (Fauci, 1988). Upon entry to the target cell, the viral RNA genome is converted to double stranded DNA by a virally encoded reverse transcriptase that is present in the virus particle. The viral DNA is then integrated into the cellular DNA by a virally encoded integrase so that the genome can be transcribed. Once the virus has infected the cell, two pathways are possible: either the virus becomes latent and the infected cell continues to function, or the virus becomes active and replicates, and a large number of virus particles are liberated that can then infect other cells. Two species of HIV infect humans: HIV-1 and HIV-2 (Gao *et al.*, 1996). HIV-1 is more virulent and more easily transmitted and is responsible for the majority of HIV infections globally. HIV-2 is less easily transmitted and is at the moment largely confined to West Africa (Reeves and Doms, 2002).

1.3 Impact of HIV/AIDS on global health

Human immunodeficiency virus is one of the biggest threats to global health and stability. Out of the 42 million people infected with HIV, 29 million are from sub-Saharan Africa accounting for 70% of all infections, where transmission is mainly by

heterosexual contact. Half of all infected adults are women of child bearing age (Brouwer *et al.,* 2005). Globally, the leading mode of transmission is by heterosexual contact. About 96% of the people with HIV live in the developing world, mostly in the sub-Saharan Africa and the Caribbean (UNAIDS/WHO, 2005b). In adults, the mechanisms underlying delayed HIV-1 disease progression are heterogeneous and include viral attenuation, immunological factors and host genetic determinants. Each of these factors alone or in combination could determine susceptibility to infection and subsequent rate of progression towards AIDS. It has been shown that the distribution of these genetic polymorphisms and their role in the course of disease varies between racial, ethnic and risk groups (Wasik *et al.,* 2005). This study looks at cytokine gene polymorphisms of interleukin-1 receptor antagonist (IL-1RA) which has been shown to influence the outcome of HIV.

1.4 Cytokines and HIV-1

Cytokines are proteins produced by cells of both innate and adaptive immunity. In the activation phase, they play a role in growth and differentiation of lymphocytes while in the effector phase they activate different cells which function to eliminate the pathogen. Infection with HIV results in dysregulation of the cytokine profile *in vivo* and *in vitro* (Warley *et al.,* 2003). Replication of HIV is under the control of both viral and host factors (Roberts *et al.,* 2010). Among the latter, the regulatory network of cytokines has been shown to affect virtually every step of the virus life cycle, from cell entry to budding of new progeny virions. During the course of HIV-1 infection secretion of T-helper type 1 (Th1) cytokines, such as interleukin 2 (IL-2) and antiviral interferon (IFN)-

gamma, is generally decreased, whereas production of T helper type 2 (Th2) cytokines, IL-4, IL-10, pro-inflammatory cytokines (IL-1, IL-6, IL-8) and tumour necrosis factor (TNF)-alpha, is increased (Kedzierska and Crowe, 2001). Such abnormal cytokine production contributes to the pathogenesis of the disease by impairing cell-mediated immunity.

The cytokine network is constitutively activated in most HIV-infected individuals, as demonstrated by recent analysis of intracellular signaling molecules such as the Janus kinase/signal transducer and activator of transcription pathway (Shannon *et al.*, 2001). Cytokines have already shown their potential use as pharmacological agents able to restore at least some of the compromised immune functions in infected individuals, as exemplified by the potent enhancing effect of IL-2 on the number of circulating $CD4^+$ T lymphocytes. This review outlines the interactions between cytokines and HIV-1, and presents clinical applications of cytokine therapy combined with highly active antiretroviral therapy or vaccines (Price *et al.*, 2004).

1.5 IL-1RA gene polymorphism and HIV

Interleukin-1 receptor antagonist (IL-1RA) is an endogenous receptor and anti-inflammatory cytokine. It is able to block the action of IL-1α and IL-1β by modulating their biological effects and preventing signal transduction (Dinarello, 2009). Interleukin-1 and Interleukin-1 receptor antagonist balance plays an important role in the normal physiology of body organs and tissues. It is found on the long arm of chromosome two where there are also genes for IL-1 receptors (Dinarello *et al.*, 2003). In the second intron

of the IL-1RA gene, there exists a tandem repeat sequence of eighty six base pairs in length. The number of times this sequence is repeated in different persons varies from two to six. The frequency of the individual alleles varies among different ethnic or geographic populations, but allele 1 (IL1RN*1), containing 4 repeats, is always more common than allele 2 (IL1RN*2), containing 2 repeats. The remaining alleles, representing 3, 5, and 6 repeats, occur in less than 1% of most populations. In every population studied to date, most persons are either homozygous for allele 1(IL1RN*1) or heterozygous for allele 1and 2 (IL1RN*1/IL1RN*2). The prevalence of allele 2 (IL1RN*2) homozygotes is typically less than10% (Rider *et al.*, 2000). In black Africans and African American persons, the frequency of allele 2 (IL1RN*2) homozygotes is considerably lower than in the white population (Mwantembe *et al.*, 2001).

There are several reported associations between HIV and AIDS and IL-1RA serum levels and also with IL-1RA polymorphisms. A study involving HIV infected African women reported that plasma IL-1RA levels were elevated in asymptomatic HIV positive women whereas levels in AIDS patients were not different from those in the controls (Witkin *et al.*, 2001). Findings from a study that analysed human promonocytic cell line indicated that both TNF and IL-1 were up regulated by HIV infection *in vitro* and *in vivo*. IL-1 induced expression of HIV in the cell line was inhibited by IL-1RA (Walter, 2010). These findings imply that IL-1/IL-1RA balance is an important factor in host resistance to HIV infection. IL-1/IL-1RA balance is on the other hand affected by IL-1RN gene polymorphisms with most studies reporting the highest IL-1RA plasma levels in individuals who are IL-1RN*2 homozygous (Mwantembe *et al.*, 2001). It has been

suggested that possession of the genotype may be beneficial in the immune defence against HIV replication. It was reported from a study among HIV positive Brazilian women at the same disease stage that IL-1RN*2 homozygotes had significantly lower plasma viraemia than did women with other IL-1RN genotypes (Witkin *et al.*, 2001). This genotype perhaps confers this relative advantage in the immune defence against infection by promoting prolonged Th1 cell mediated immune response. It is of significance that a positive correlation was observed between antiretroviral treatment and IL-1RA levels (Roberts *et al.*, 2010).

1.6 Problem statement

HIV is still one of the biggest threats to global health and stability and it's still mysterious since its first discovery over twenty years ago, there is still no cure. There is considerable variation in rates of HIV/AIDS disease progression among HIV infected individuals. Although the majority of patients develop AIDS within 10 years of infection, 5% of long-term infected individuals remain clinically and immunologically stable for more than a decade, and are referred to as non-progressors. The precise mechanisms responsible for non-progression remain unclear. Virological, host, genetic and immunological factors have all been implicated especially polymorphisms in certain genes. The polymorphisms differ on their influence on disease in different individuals and also among different geographic populations. This study looks at one of the polymorphism which is IL-1RA that has been shown to retard HIV disease progression by inhibiting HIV replication mediated by IL-1.

1.7 Justification

Many host genetic factors are now known to affect disease progression rates, especially polymorphisms in certain genes. The effects of different natural human polymorphisms on HIV-1 disease variants influence the susceptibility to HIV-1 infection and/or subsequent rates of disease progression towards AIDS. Interleukin-1 receptor antagonist plays an important role in inhibiting HIV replication. It has been linked with the control of HIV viraemia in patients receiving highly active antiretroviral therapy. Interleukin-1 receptor antagonist has been given to both experimental animals and humans as a safe antagonist of excessive or inappropriate production of IL-1. Homozygosity for allele 2 is associated with prolonged T helper 1 immune responses that retard HIV and the individuals had a lower viral load compared with the other genotypes. The status of IL-1RA gene polymorphisms in Kenya remains unknown and yet it plays an important role in HIV transmission. There is need to identify and determine the frequency of occurrence of these polymorphisms so as to understand its distribution and their effect on HIV prevalence.

1.8 Research questions

a) What is proportion of individuals with allele 2 in a population of HIV positive and negative males and females in Nairobi

b) What different polymorphic alleles of IL-1RA gene are found in males and females in Nairobi

c) What are the differences in the prevalence of IL-1RA gene polymorphisms between HIV positive and negative males and females in Nairobi

1.9 Null hypothesis

There is no difference in the prevalence of the IL-1RA allelic variants between HIV positive and negative males and females in Nairobi.

1.10 Study objectives

1.10.1 General objective

To determine the frequency of IL-1RA gene polymorphisms amongst HIV positive and HIV negative individuals in Nairobi.

1.10.2 Specific objectives

a) To determine the presence of allele 2 in a population of HIV positive and negative males and females in Nairobi
b) To determine the different polymorphic alleles of IL-1RA gene in males and females in Nairobi
c) To compare the frequency of IL-1RA alleles between HIV positive and negative males and females in Nairobi

1.11 Significance of the study

It is hoped that the findings of this study will help in shedding light on existence of the various IL-1RA polymorphisms and the differences existing between HIV positive and negative males and females in Nairobi County. This will in turn form a basis for further studies to determine the effects of the IL-1RA alleles on HIV/AIDS disease progression and severity. Understanding the mechanisms that inhibit HIV replication will be

important for designing new antiviral drugs which may be achieved by blocking the effects of IL-1. This information will have important implications on the ongoing therapeutic approach for HIV and AIDS especially the use of protective or recombinant interleukin-1 receptor antagonist.

CHAPTER TWO: LITERATURE REVIEW

2.1 Epidemiology of HIV

United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO) estimate that AIDS has killed more than 25 million people since it was first recognized in 1981, making it one of the most destructive pandemics in recorded history. Despite recent improved access to antiretroviral treatment and care in many regions of the world, the AIDS pandemic claimed an estimated 2.8 million (between 2.4 and 3.3 million) lives in 2005 of which more than half a million (570,000) were children (UNAIDS/WHO, 2005b). Globally, approximately 4.1 million people were newly infected and between 2.4 and 3.3 million people with AIDS died, an increase from 2004 and the highest number since 1981 (UNAIDS/WHO, 2005b).

Sub-Saharan Africa remains by far the worst affected region, with an estimated 21.6 to 27.4 million people currently living with HIV. Two million (1.5-3.0 million) of them are children younger than 15 years of age. More than 64% of all the people living with HIV are in sub-Saharan Africa, as are more than three quarters of all women living with HIV. In 2005, there were 12.0 million (10.6-13.6 million) AIDS orphans living in sub-Saharan Africa (UNAIDS, 2005a). South and South East Asia are the second worst affected with 15% of the total. AIDS accounts for the deaths of 500,000 children in this region. Two thirds of HIV/AIDS infections in Asia occur in India, with an estimated 5.7 million infections surpassing South Africa's estimated 5.5 million, making India the country with the highest number of HIV infections in the world (UNAIDS, 2006a). In the thirty five

African nations with the highest prevalence, average life expectancy is 48.3 years, 6.5 years less than it would be without the disease.

The latest evaluation report of the World Bank's Operation Evaluation Department assesses the effectiveness of the World Bank's country level HIV/AIDS assistance defined as policy dialogue, analytic work and lending with the explicit objective of reducing the scope or impact of the AIDS pandemic (Bank, 2005). This is the first comprehensive evaluation of the World Bank's HIV/AIDS support to countries, from the beginning of the epidemic through mid-2004. Because the bank aims to assist in the implementation of national government programmes, their experience provides important insights on how national AIDS programmes can be more effective.

2.2 HIV/AIDS in Kenya

Kenya has a severe, generalized HIV epidemic, but in recent years, the country has experienced a notable decline in HIV prevalence, attributed in part to significant behavioral change and increased access to ART. National adult HIV prevalence is estimated to have fallen from 10 percent in the late 1990s to about 6.1 percent in 2005 (UNAIDS 2006b). According to new statistics, Kenya's HIV prevalence was 5.1% in 2006, down from 5.9% in 2005 and 6.1% in 2004 (Kenya Aids Indicator Survey, 2007). However, the most recent estimates of HIV incidence released in 2008 have shown that the prevalence of HIV has risen to 7.4% nationally (Kenya Aids Indicator Survey, 2008). This is attributed in part to the longer life expectancy of HIV positive people due to the

increased uptake of HAART. The best indicator of the problem is incidence which shows number of new infections.

HIV prevalence in urban areas is about 8.3%, compared with 4% in rural areas. In addition, deaths from AIDS-related causes decreased from 120,000 in 2003 to 85,000 in 2006. The decrease in AIDS related deaths is attributed to increased access to antiretroviral drugs, which have prevented about 57,000 deaths from access to no-cost antiretrovirals to HIV positive people (Kenya Aids Indicator Survey, 2008). Women face considerably higher risk of HIV infection than men, and also experience a shorter life expectancy due to HIV/AIDS. The 7th edition of AIDS in Kenya reports an HIV prevalence rate of 8% in adult women and four percent in adult men (Ministry of Health, 2005). Populations in Kenya especially at risk include injecting drug users and commercial sex workers, whose prevalence rates are estimated at 53% and 27%, respectively.

2.3 Economic impact of HIV/AIDS

Human Immunodeficiency Virus (HIV) retards economic growth by destroying human capital. UNAIDS has predicted outcomes for sub-Saharan Africa to the year 2025. These range from a plateau and eventual decline in deaths beginning around 2012 to a catastrophic continual growth in the death rate with potentially 90 million cases of infection (UNAIDS, 2005a). Without proper nutrition, health care and medicine that is available in developed countries, large numbers of people in these countries are falling victims to AIDS. They will not only be unable to work, but will also require significant

medical care. The forecast is that this will likely cause a collapse of economics and societies in the region. In some heavily infected areas, the epidemic has left behind many orphans cared for by elderly grandparents (UNAIDS, 2005a).

The increased mortality in this region will result in a smaller skilled population and labor force (Papadopoulos *et al.*, 2004). This smaller labor force will be predominantly young people, with reduced knowledge and work experience leading to reduced productivity. An increase in workers time off to look after sick family members or on sick leave will also lower productivity. Increased mortality will also weaken the mechanisms that generate human capital and investment in people, through loss of income and the death of parents. By killing off mainly young adults, AIDS seriously weakens the taxable population, reducing the resources available for public expenditure such as education and health services resulting in increasing pressure for the state's finances and slower growth of the economy. This results in slower growth of the tax base, an effect that will be reinforced if there is a growing expenditure on treating the sick, training (to replace sick workers), sick pay and caring for AIDS orphans (UNAIDS, 2005a). This is especially true if the sharp increase in adult mortality shifts the responsibility and blame from the family to the government in caring for these orphans. On the level of the household, AIDS result in both the loss of income and increased spending on the healthcare by the household. The income effects of this lead to spending reduction as well as substitution effect away from education and towards health care and funeral spending. A study in Cote d'Ivoire showed that households with an HIV/AIDS patient spent twice as much on medical expenses as other households (Cohen, 1994).

2.4 HIV genetic structure

To fully understand the significance of the interaction of HIV with the human genome, it is important first to review the genetic structure of HIV. Although a detailed examination of HIV gene structure is beyond the scope of this thesis, a general understanding of HIV structure is helpful in illustrating the methods employed by HIV to utilize human IL-1 and glucocorticoid hormones for its own benefit. The HIV particle (Fig. 2.1) consists of an RNA-protein core surrounded by a lipid membrane containing glycoproteins (gp) 41 and 120 that interact with CD4 antigens on the surface of immune cell.

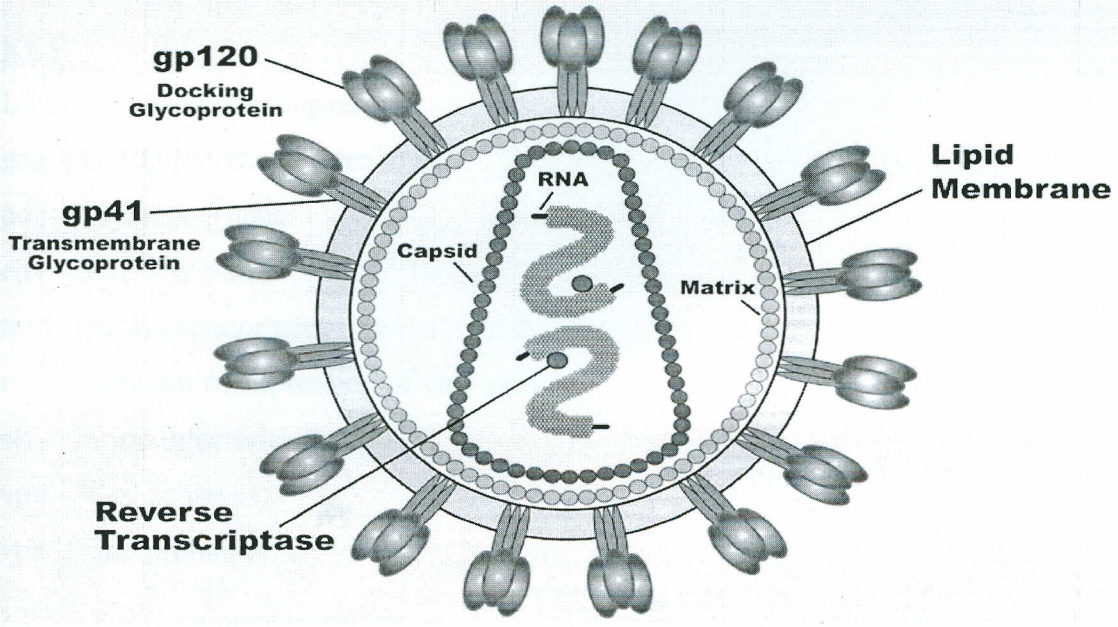


Figure 2.1 An illustration of HIV particle (http://www.idealibrary.com).

Closer examination of the RNA core (Fig. 2.2) reveals that HIV-1 consists of 9 major genes flanked by long terminal repeats (LTR) at either end. Closer examination of the LTR is shown in Figure 2.3.

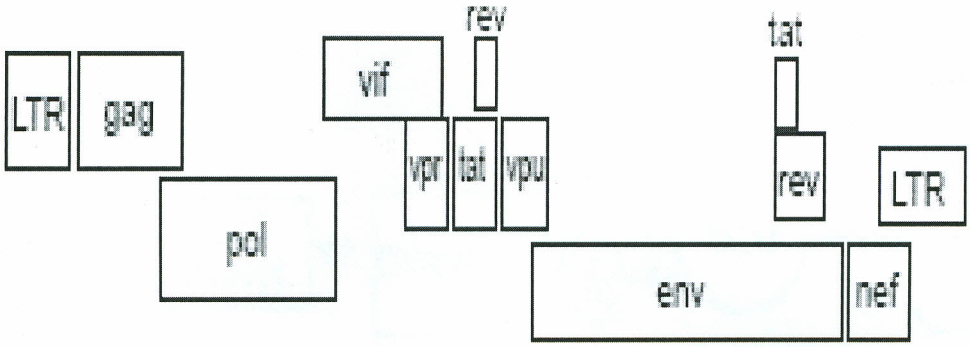


Figure 2.2 Genomic structure of HIV-1

KEY

LTR - Long terminal repeat

gag - Group specific antigen gene

pol - Polymerase gene

env - envelope gene;

tat – Trans-activator gene

rev - Regulator of expression of viral protein

vif - Virion infectivity factor

vpu - Viral protein U

vpr - Viral protein R

NRE - Negative regulator element

TAR - Trans-acting responsive region

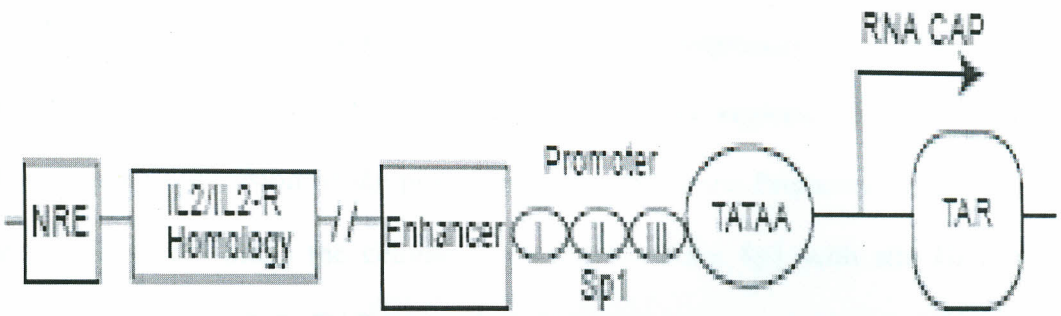


Figure 2.3 Genomic structure of HIV-1 Long Terminal Repeat

(http://www.idealibrary.com)

In summary, the nine major genes of HIV-1 include: Gag, which codes for the precursor of the internal structural proteins of the virus (including p7, p9, p15, p17, and p24). Pol gene codes for the viral replicative proteins protease, reverse transcriptase, integrase and ribonuclease. Vif gene produces virion infectivity factor that increases viral infectivity while Vpr produces viral protein R that accelerates replication. Tat which is a trans-activator gene is essential to HIV replication and increases the rate of its own synthesis as well as that of all viral proteins. Rev gene regulates the accumulation of unspliced or partially spliced mRNA while Vpu produces a protein that enhances viral release from the cell. Env which is the envelope gene codes for envelope glycoproteins gp41 and gp120 while Nef produces a protein that appears to suppress HIV infectivity. In its absence, infectivity appears to be enhanced suggesting a negative regulatory role (Terwilliger *et al.,* 1986).

The major genomic region of the HIV-1 long terminal repeat consists of NRE, a negative regulatory element whose deletion leads to an increase in HIV-1 LTR gene expression. The IL2/IL2-R homology region consists of two sequences that share significant homology with the IL-2 and IL-2 receptor regulatory regions. The enhancer region enhances gene expression of the promoter region while the Promoter, region containing three tandem sites binds the cellular transcription factor Sp1 with site III having the greatest affinity for Sp1. TATAA region determines proper initiation of transcription while TAR the trans-acting responsive region serves to suppress viral RNA expression and prevents RNA exit from the nucleus except in the presence of Tat protein. Tat protein binds directly to TAR RNA. It is apparent that the HIV genome closely depends on the human cellular production of IL-1 and glucocorticoid hormones to stimulate and maintain its life cycle. This observation may lead to vulnerable targets for therapeutic intervention that are not subject to mutation and/or the elusive genetic diversity of the virus (Corley, 2000).

2.5 Factors produced on chromosome 2 that facilitate Tat-TAR transactivation

As previously mentioned, there are several products of the long arm of chromosome 2 that play a role in HIV pathogenesis, and are the required factor for Tat-mediated transactivation of HIV replication that occurs via the TAR region of the LTR (Campbell *et al.,* 2004). This includes IL-1, IL-1RA, glucocorticoid hormone and CD 26. Any combination of the above products theoretically facilitates AIDS pathogenesis. It has been observed that there is an increase in HIV replication in the presence of IL-1 as well as the observed increase in IL-1 production in the presence of HIV (Goletti *et al.,* 1996).

IL-1 is stimulated by HIV through the blocking of melanocyte stimulating hormone (MSH), which is an IL-1 inhibitor, and this occurs via the interaction of the peptide T sub region of gp120 with MSH receptors. Additionally, the induction of glucocorticoid hormones by IL-1 further stimulates HIV infectivity via the virion infectivity factor (Vif) region (Kawa *et al.,* 1993). HIV infectivity is also stimulated in presence of CD26. CD26 (also known as dipeptidyl peptidase IV) serves as a co-receptor with CD4 that interacts with the V3 loop in gp120 of HIV, and may be the key to active infection. IL-1 also increases HIV infectivity by stimulating CD26 (Fujiwara *et al.,* 1994).

Finally IL-1RA is also involved in HIV infection and AIDS pathogenesis. This is based on the finding that antiretroviral treatment of HIV-infected subjects results in a decrease in IL-1 and an increase in IL-1RA, thus implicating an inhibitory role of HIV in IL-1RA production. HIV also regulates its replication by reducing IL-1RA through feedback inhibition mediated by IL-1 (Sadeghi *et al.,* 1995). Interleukin-1, in turn, stimulates HIV in the enhancer region of the LTR. Interleukin-1 also has the ability to stimulate the production of corticotropin releasing hormone, adrenocorticotropic hormone and glucocorticoid hormones. When one considers that IL-1 and glucocorticoid hormones are both able to stimulate CD8+ (T-suppressor) cell proliferation, the pathogenic implications become clear. Any combination of the above pathways of IL-1 induction by HIV apparently culminates in increased HIV infectivity and replication. The typical T-lymphocyte profile observed in AIDS patients, that is, overexpansion of CD8 lymphocytes with gradual depletion of CD4 lymphocytes, will understandably result (Heaves *et al.,* 2006).

2.6 The role of IL-1RA in HIV infection and AIDS pathogenesis

As previously mentioned, HIV inhibits the production of IL-1RA which is produced on

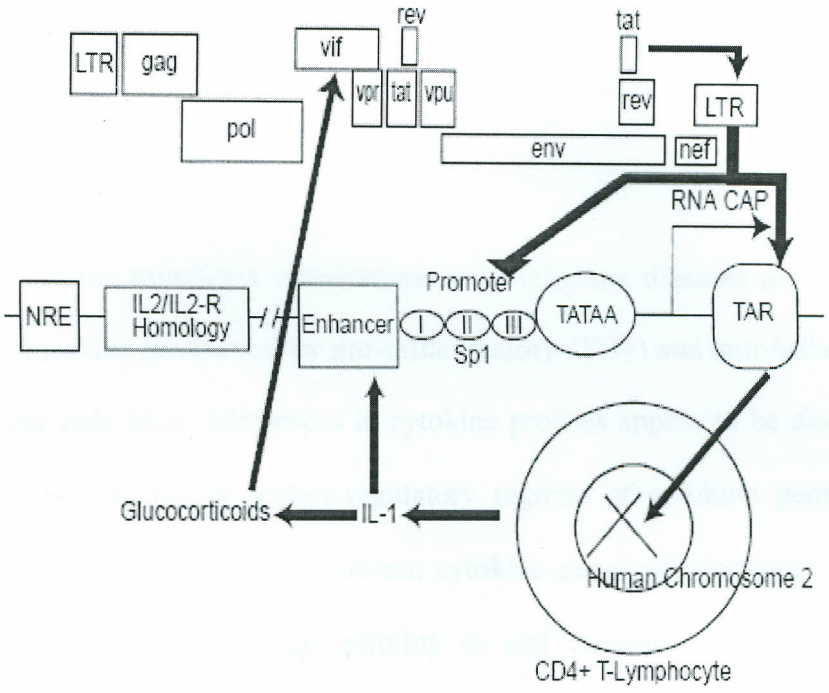


Figure 2.4 Relationship between HIV and factors produced in chromosome 2

(http://www.idealibrary.com)

HIV has an affinity for lymphoid cells and targets genetic machinery associated with IL-1 and glucocorticoid hormones to facilitate its replication (Soudeyns *et al.*, 1993). It has been observed that IL-1 regulates HCG synthesis through second messenger systems, and inhibits testosterone secretion in response to HCG. This suggests that IL-1RA also has the ability to regulate and enhance the HCG-induced testosterone secretory response which causes hypotestosteronemia frequently observed in HIV infection, IL-1RA may play several important roles as a potential treatment for AIDS, that is, as an anti-HIV factor, as a regulator of HCG and testosterone, and as a treatment for Kaposi's sarcoma (Corley, 2000). Only when new HIV infections are prevented and treatment of those

already infected addresses the HIV-induced IL-1 and glucocorticoid hormone excess, will a permanent and substantial dent be made in the defenses of this most difficult viral adversary (Strebel, 2003).

2.7 Cytokines

The pathologies of many infectious, autoimmune and malignant diseases are influenced by the profiles of cytokine production by pro-inflammatory (TH1) and anti-inflammatory (TH2) T cells. Inter-individual differences in cytokine profiles appear to be due, at least in part, to allelic polymorphism within regulatory regions of cytokine genes. Many studies have examined the relationship between cytokine gene polymorphism, cytokine gene expression *in vitro*, and the susceptibility to and clinical severity of diseases (Townsend and McKenzie, 2000). Cytokines are humoral immunomodulatory proteins or glycoproteins which control or modulate the activities of target cells, generally those within the haematopoietic system. They act on target cells by binding to specific cytokine receptor ligands, initiating signal transduction and second messenger pathways within the target cell. This can result in gene activation leading to mitotic division, growth and differentiation, migration, or apoptosis (Hideshima *et al.*, 2005).

Cytokines are produced by a wide range of cell types and have been broadly classified as monokines (produced by cells of the monocyte lineage) or lymphokines (produced by lymphocytes). Other classifications are based on functional or structural groupings (Cannon, 2000). Cytokines act in a highly complex coordinated network in which they induce or repress their own synthesis as well as that of other cytokines and cytokine

receptors. In addition, many cytokines appear to be pleiotropic, with the corollary that the cytokine network is highly flexible, since there is considerable overlap and redundancy between the function of individual cytokines. This feature continues to complicate efforts to analyze both the function of individual cytokines in addition to the influence of cytokine gene polymorphism on gene expression and disease (Frankenstein and Cohen, 2006).

Cytokine production by the cells of the immune system may occur through antigen-specific and non-antigen specific stimuli. For example, when monocytes are exposed to bacterial cell wall products, such as lipopolysaccharide, they produce cytokines which have multiple functions including influencing the expression of cytokines by other cells. Antigen-specific responses are generated by B and T cells through immunoglobulin and T cell receptors respectively (Parkin and Cohen, 2001). B cell activation may result in the production of IL-6 and other cytokines. T cells are central players in linking non-antigen specific, B cell and T cell responses together. Two classes of T cells are recognized: α and β T cells, defined by their T cell receptor (TCR) chain usage. The majority of circulating α and β T cells carry either CD4 or CD8 molecules, which bind to MHC class II or MHC class I molecules, respectively. Functionally, CD8+ T cells are typically cytotoxic T cells and can kill target cells presenting processed foreign peptide via HLA class I molecules; some CD8+ T cells secrete cytokines such as IFN γ CD4 T cells are typically helper T cells, although rare subsets have cytotoxic function (Tamaka *et al.*, 2007).

Several T helper subsets of CD4 T cells have been identified. In mouse, these subsets are well defined and include Type 1 (TH1), which promote cell-mediated effector responses; and Type 2 CD4 helper T cells (TH2) which promote B cell mediated humoral responses. Cytokines produced by TH1 cells include interleukin-2 (IL-2), Interferon gamma (IFN γ) and tumour necrosis factor beta (TNF β), and constitute a pro-inflammatory cytokine profile. Those produced by TH2 cells include IL-4, IL-5, IL-6, and IL-10, and are predominantly anti-inflammatory cytokine profile. Both TH1 and TH2 cells produce IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF). Recently a TH3 subset (characterized by TGF β) has been defined (Berger, 2000).

In humans, the distinction between TH1, TH2 and TH3 is less well defined, and a subset of TH0 cells, which produce some cytokines typical of TH1 and TH2 profiles, can be identified. The clinical outcome of many infectious, autoimmune, or malignant diseases appears to be influenced by the overall balance of production (profiles) of pro-inflammatory and anti-inflammatory cytokines (Seymour and Henderson, 2001). Hence, much interest has focused upon the regulation of genes expressing these cytokines. In particular, a significant number of studies have addressed whether genetic polymorphism within these genes might influence the levels of expression, and therefore the overall immune response (Dong and Flavell, 2001).

2.7.1 Cytokine gene polymorphism

Non-conservative mutation within the coding region of genes can result in loss, abrogation, or change of function in the expressed protein as a result of change in protein

structure. Cytokine and cytokine receptor genes are generally highly conserved in terms of exon sequences. Although conservative (silent) mutations do not affect amino acid sequence, they may influence protein expression in a variety of other ways: for example, they can alter mRNA splicing, mRNA stability, and levels of gene transcription (Ollier, 2004). Polymorphisms within the regulatory sequences or introns of genes may have a significant effect on transcription, since they may alter the structure of transcription factor binding sites within gene promoters or the structure of enhancers and silencers within introns or at more remote regulatory sites. They may also alter binding sites within the nuclear matrix for architectural transcription factors which modulate promoter geometry. Many of the reported polymorphisms within cytokine genes occur within known or putative regulatory regions (Imbonden *et al.*, 2006).

The rationale for studying cytokine gene polymorphisms in human disease can be broadly summarized as follows:-To enhance the understanding of the etiology and pathology of human disease, to identify potential markers of susceptibility, severity, and clinical outcome, to identify potential markers for responders versus non responders in therapeutic trials, to identify targets for therapeutic intervention and to identify novel strategies to prevent disease or to improve existing preventions such as vaccines. The influence of cytokine gene polymorphisms on gene expression and disease has been addressed at two levels of research. These include studies using *in vitro* gene expression, and those involving *in vivo* disease associations. Only a few studies have thus far integrated both of these approaches (Rao *et al.*, 2007).

2.7.2 *In vitro* gene expression studies

Up-regulated and/or down-regulated expression and production of cytokine mRNA and cytokines, or of their receptors, is a feature in most immune responses in human diseases. However, this response may differ significantly among individuals (Ollier, 2004). *In vitro* gene expression studies attempt to determine a genetic basis for inter-individual differences in the immune response. This is achieved by examining the relationship between individual polymorphic alleles or haplotypes of cytokine genes and the expression of the transcript or cytokine *in vitro*. The main approaches used to date include measuring the levels of cytokine or cytokine receptor mRNA, or cytokine receptor protein, expressed as a result of *in vitro* stimulation of cells in culture with a mitogen (Ananthi *et al.,* 2009). This is done by isolation of individual alleles of gene promoters by cloning adjacent to a reporter gene in an expression vector, followed by transfection of an appropriate cell line and measurement of reporter protein expression. It is becoming increasingly apparent that the results of expression studies may be critically influenced by several factors such as the cell lineage used in the assay and the therapeutic preconditioning or treatment of subjects prior to harvesting cells for the assay (Warley *et al.,* 2003).

2.7.3 *In vivo* disease association studies

These studies attempt to identify immunogenetic markers for a given disease. Association is sought between specific cytokine gene polymorphisms and clinical outcome by direct comparison of individual cytokine genotypes and the clinical features of the disease, for example, susceptibility, duration and severity (Ollier, 2004). The a priori involvement of

dysregulation of a specific cytokine or receptor in the disease is usually, though not always, the rationale for selecting a cytokine or cytokine receptor gene for analysis. Such data may be generated using population-based or family studies in humans or using animal models, and may be from analysis of secreted cell surface or intracellular protein, or of cytokine mRNA. Using these and other clues, many studies have identified statistically significant associations between cytokine alleles and disease (Nica and Dermitzakis, 2008).

2.7.4 Limitations of gene expression and disease association studies

In many of these studies, the *in vitro* expression studies have not been attempted, or are the subject of controversy, or by consensus have not indicated a convincing functional rationale for the association (Warley *et al.*, 2003). The genetic analysis of cytokines in human disease has traditionally focused on case-control association studies, in which the frequencies of marker alleles in groups of patients and healthy controls are compared, and the difference is subjected to statistical analysis. The association is often expressed as the relative risk (or odds ratio) that an individual will develop the disorder if he or she carries the particular allele or marker, compared to an individual who does not carry the allele or marker (McCarthy and Hirchorn, 2008). These studies have met with only modest success in identifying disease-causing cytokine genes, in part because of the difficulty in selecting from among the many candidate possibilities, and the likely modest effect of any single disease susceptibility gene (Janriver and Freedman, 2008).

The difficulty in identifying a perfectly matched control group creates an additional limitation, increasing the possibility that a potentially positive association is biologically irrelevant because of population admixture. Furthermore, even when cases and controls are adequately matched, most study designs involve relatively small sample sizes which lack the statistical power to detect small or moderate gene effects (Bidwell *et al.,* 1999). While combined analysis of data from several studies can be pooled to increase confidence in the strength of observed associations, biases in reporting positive or weak associations as opposed to lack of reporting negative associations also influences interpretation of published observations (Janriver and Freedman, 2008). One of the sometimes overlooked aspects of such disease association studies is that the cytokine network is highly complex, containing interactive cascades of gene activation and suppression (Shannon *et al.,* 2001).

One consequence of mutual TH1–TH2 antagonism may be the predominance of one or the other subset, which might directly influence the clinical outcome of disease. Therefore, genetic polymorphisms in cytokine genes and their receptors which regulate expression should not in all cases be studied strictly in isolation. This is because individual associations may be non-informative, whereas specific combinations of cytokine genotypes might predispose to disease susceptibility or outcome (Seymour and Henderson, 2001). Only very few studies to date have attempted to analyze the combined contribution of more than one cytokine gene polymorphism to disease. For certain combinations of polymorphisms and diseases, contradictory results have been published. In these cases, the discordance may be attributable to differences in ethnicity of

populations, patient and/or control cohort selection *or* size, disease classification or status, or methods of statistical analysis (Cookson *et al.*, 2009).

2.8 Introduction to the interleukin-1 system

The term Interleukin 1(IL-1) usually designates a group of three molecules; IL-1α, IL-1β and IL-1 receptor antagonist. Interleukin-1 production by mononuclear phagocytes can be triggered by bacterial products such as lipopolysaccharide (LPS), macrophage derived cytokines such as TNF and by contact with CD4+ T cells. The biologic effects of IL-1 depend on the quantity of cytokine released (Weber *et al.*, 2010). At low IL-1 concentrations its principal function is as a mediator of local inflammation. For instance, IL-1 acts on endothelial cells to promote coagulation and to increase expression of surface molecules that mediate leukocyte adhesion. When secreted in large quantities, IL-1 enters the bloodstream and exerts endocrine effects. Systemic IL-1 has the ability to cause fever, to induce synthesis of acute phase plasma proteins by the liver and to initiate metabolic wasting (cachexia) (Dinarello, 2011).

Both IL-1α and IL-1β are synthesized as 33 kDa precursors that are proteolytically cleaved to generate the mature 17 kDa proteins. IL-1 receptor antagonist gene is located on chromosome two in close proximity to the genes coding for IL-1α and IL-1β. Interleukin-1 alpha (IL-1α) and IL-1β are major inducers of pro-inflammatory immune responses (Cannon, 2000). They both bind to the same IL-1 receptor on the surface of a variety of cells and initiate a cascade of events leading to recruitment and activation of macrophages and neutrophils, vascular dilation and fever, and a potent pro-inflammatory

immune response. Interleukin -1 receptor antagonist binds to the same IL-1 receptor but does not initiate signal transduction (Berger, 2000). Interleukin -1 receptor antagonist is thus a competitive inhibitor of IL-1 bioactivity. There are two types of IL-1 receptors, type I IL-1 receptor designated as IL1RI and type II receptor designated as IL-1RII and are both members of immunoglobulin super gene family. The type 1 receptor has a higher affinity for IL-1β and is the major receptor for IL-1 mediated responses. It is expressed on all cell types. The type II receptor has greater affinity for IL-1α and its major function is to act as a 'decoy' that competitively inhibit IL-1 binding to the type I signaling receptor. The type II receptor is expressed on B cells but may be induced on other cell types (Warley *et al.,* 2003). The relative levels of IL-1RA and IL-1 at an inflammatory site will thus determine whether a pro-inflammatory response will be initiated and persist or will be terminated. Typically, the concentration of IL-1RA increases late during the course of an inflammatory event so that an induced acute inflammation can terminate. If not, the inflammation can become chronic and damage healthy cells (Arend, 2003).

2.9 Interleukin-1 receptor antagonist structure and function

Interleukin-1 receptor antagonist (IL-1RA) is the first described naturally occurring specific receptor antagonist of any cytokine or hormone-like molecule. Its inhibitory activity was originally demonstrated in the urine of patients with fever and in the supernatants of monocytes cultured on adherent IgG (Arend, 2003). Several structural variants of the molecule have been described. Its two main variants are a 17kDa (sIL-1RA) secreted form produced by monocytes, macrophages, neutrophils and other cells and an 18kDa (icIL-1RA) form that remains in the cytoplasm. This form is found mainly

in keratinocytes and other epithelial cells and also in monocytes and fibroblasts. The intracellular form has 16kDa isoform which has been found in neutrophils, monocytes and hepatic cells. The main isoform of IL-1RA are translated from the same gene by alternative splicing of the first exons. Production of the cytokine antagonist is stimulated by the adherent IgG, other cytokines such as GM-CSF and IL-4, bacterial or viral components and many other substances (Arend, 2003).

2.10 IL-1RA and its clinical use and significance

The IL-1 receptor antagonist gene has been shown to contain eighty six base pairs tandem repeats. Several studies suggest an association of allele 2 (IL1RN*2) of this polymorphism with disease severity in several chronic inflammatory diseases, including systemic lupus erythematosus (Huang *et al.,* 2002), ulcerative colitis (Carter and Giovine, 2001), and alopecia areata (Tarlow *et al.,* 1994). A significant association between this allele and diabetic nephropathy has been reported by Blakemore *et al.,* (1996). A recombinant form of IL-1RA (Kineret) has been approved for use in the treatment of rheumatoid arthritis (Lee *et al.,* 2004). IL-1RA acts as a non-steroidal anti-inflammatory substance and may be useful also in the treatment of chronic inflammatory diseases such as autoimmune diabetes. It can prevent the systemic inflammatory response syndrome usually observed during Gram-negative sepsis in animal experiments (Wakabayashi *et al.,* 1991).

In an experimental study with human volunteers receiving intravenous injections of gram-negative endotoxins, systemic administration of IL-1RA has been shown to lessen

the severity of symptoms (Van Zee *et al.,* 1995). It has been suggested that IL-1RA may improve hemorrhagic shock survival by preventing adenosine triphosphate depletion in vital organs (Pellicane *et al.,* 1993). In experimental animals, pretreatment with IL-1RA has been shown to prevent death caused by injections of TNF-alpha and IL1 (Everaerdt, 1994). Pretreatment with IL-1RA also prevents the development of immune-complex induced colitis (Ferretti *et al.,* 1994). It has been observed that an imbalance between IL-1RA and IL-1 has been suggested to play a role in human inflammatory bowel disease (Casini *et al.,* 1995). Graft-versus-host reactions have been shown also to be inhibited by administration of IL1RA (Cullup *et al,* 2001).

IL-1RA may be of clinical relevance also since it is known that IL-1 synthesized by myeloma cells, Hodgkin lymphoma cells, B-cells, T-cells, and a variety of leukemic cell types in turn promotes the synthesis of colony stimulating factors. It has been shown that spontaneous as well as IL-1 stimulated proliferation of acute myelogenous leukemia cells in vitro can be inhibited significantly by recombinant IL-1RA in a dose dependent manner (Estrov *et al.,* 1991). This effect correlates with a reduction or disappearance in culture conditioned medium of granulocyte-monocyte colony stimulating factor, which is normally secreted by these cells. IL-1RA has been shown to inhibit chronic myelogenous leukemia (CML) colony growth in a dose-dependent fashion (Estrov *et al.,* 1991). IL1RA has been shown also to inhibit augmentation of metastasis induced by IL-1 or bacterial lipopolysaccharide in a human melanoma/nude mouse system (Chirivi *et al.,* 1993). IL-1RA also has been shown to block IL-1 mediated induction of expression of Human Immunodeficiency Virus in infected cells (Price *et al.,* 2004). HIV predominantly

induces IL-1RA over IL-1 synthesis in human primary monocytes (Heaves *et al.,* 2006). These effects may be critical in the control of inflammation associated with HIV infection. It has also been shown to suppress allergic eye disease in a mouse model, probably by a down-modulation of the recruitment of eosinophils and other inflammatory cells essential for the immunopathogenesis of ocular atopy (Keane *et al.,* 1999).

The functions of this molecule have further been established through experiments involving Interleukin-1 receptor antagonist gene knockout mice or those having extra copies of the gene. It was observed that IL-1RA appeared to trigger IL-1 production in the presence of endotoxins. The highest IL-1 serum levels were observed in IL-1RA overproducers and lowest in the knockout mice (Witkin *et al.,* 2002). The nature of interactions among cytokines is complex. This may explain in part the influence of IL-1RA on IL-1. Depending on the prevailing conditions, IL-1RA may inhibit IL-1 activity or up regulate IL-1 gene expression. Several published findings have added to an understanding of the complexity of this cytokine. Its elevated levels during chronic rheumatic diseases and in patients following surgical traumas indicate that the cytokine antagonist is also an acute phase protein. Like other acute phase proteins, its levels correlate with those of IL-1 and IL-6 (Chirivi *et al.*, 1993). Studies have also demonstrated production of sIL-1RA by both cultured human hepatocytes and the human hepatoma cell line in response to stimulation with IL-1 and IL-6 (Lee *et al.*, 2004).

2.11 IL-1RA and sex

The regulation of cytokine production is influenced by various factors, including

genetics, sex, environment, age and diurnal rhythm. It has been reported that genes encoding proinflammatory and anti-inflammatory cytokines are polymorphic, a phenomenon important for regulation of their level. The presence of distinct alleles of cytokine polymorphic genes could explain the individual differences in rate and extent of production of various cytokines. The polymorphism of the interleukin-1 (IL-1) gene cluster is implicated in the regulation of IL-1 and IL-1 receptor antagonist (IL-1RA) production and is associated with several diseases linked to inflammation (Witkin *et al.*, 2002).

The production of IL-1RA and IL-1 differs between males and females. This difference has been demonstrated by *in vitro* studies indicating that IL-1RA secretion by monocytes isolated from healthy females was significantly higher than that in males, whereas whole blood from males produced significantly more IL-1 than that from females. Moreover, the IL-1RA level was found to be significantly higher in the amniotic fluid and neonatal urine from female newborns than in male ones, asexual difference that has been attributed mainly to variations in the level of sex hormones (Vardhana *et al.*, 2003). Genetic polymorphism has been shown to affect IL-1 production by peripheral blood mononuclear cells (PBMCs) activated *in vitro* (Price *et al.*, 2004).

The human IL-1RA gene has been mapped on the long arm of chromosome 2. A polymorphism present in intron 2 of the IL-1Ra gene is characterized by variations in the number of 86-base pair (bp) tandem repeat sequence. The number of times this sequence is repeated varies from two to six. Allele 1 (IL-1RN*1), containing four repeats, is more

common in the general population than is allele 2 (IL-1RN*2), containing two repeats. The remaining alleles, representing three, five, and six repeats, occur in 1% of most ethnic groups (Dinarello *et al.*, 2003). It has been demonstrated that the IL-1RA genotype is involved in IL-1 bioactivity within the IL-1 gene cluster, regulating both constitutive and stimulated IL-1RA and IL-1 release. Allele 2 of the IL-1RA gene (IL-1RN*2) has been consistently associated with higher IL-1RA release, whereas individuals homozygous for allele 1 (IL-1RN*1) released more IL-1 compared with carriers of at least one IL-1RN*2 allele. The IL-1β gene, encoding IL-1 cytokine, contains several single nucleotide polymorphisms (SNP). One of them, which is in position 511 (C-T) related to the promoter region, has been associated with increased IL-1 production (Vamvakopoulos *et al.*, 2002).

Based on sexual differences in IL-1 and IL-1RA levels and on the observation that the production of these cytokines is dependent on gene polymorphism, the question was raised whether a sexual difference in the genetic polymorphism of these cytokines exists. A positive finding could contribute to the understanding of the difference in cytokine production and immune response observed in males and females.

2.12 Association of IL-1RA polymorphisms and disease

There have been numerous studies linking IL-1, IL-1RA alleles, genotypes and sexual differences in other diseases and conditions. Several studies have shown the existence of sexual differences in pain perception during the postoperative period (Uchiyama *et al.*, 2006). Accordingly, it has been found that females show lower pain thresholds and less

pain tolerance than males. It is notable that although males reported higher tolerance to postoperative pain, they required higher doses of opioid analgesics. It is suggested that the higher incidence of the allele 2 found in females may be associated with an elevated IL-1RA level, whereas the increased frequency of allele 1 homozygotes in males may explain their higher IL-1 level. These findings require an explanation for the existence of a sexual difference in pain perception and in the analgesic response to opioid.

Interleukin-1 (IL-1) is a major factor in initiation of infections related to preterm delivery, and polymorphism in cytokine genes may influence the pregnancy outcome (Murtha *et al.,* 2006). On the other hand, IL-1RA present in the amniotic fluid and of fetal origin may reduce the proinflammatory immune activation and prevent premature delivery. It is suggested that the genotype of the fetus and not only that of the mother might affect the outcome of pregnancy. Studies have reported an association between IL-1RN*1 homozygosity and a history of increased occurrence of spontaneous abortions in previous pregnancies, leading to the suggestion that fetal carriage of only the IL-1RN*1 allele (i.e., the absence of IL-1RN*2) is a genetic factor that increases susceptibility to spontaneous abortion (Perni *et al.,* 2004). Based on this observation, it is presumed that during pregnancy, a selection associated with the fetal genome occurs, causing an increased number of abortions of male fetuses carrying certain alleles of the IL-1RA gene.

It is known that male gender is associated with higher neonatal mortality and long-term morbidity in preterm infants. In addition, it has been reported that among twins, male-male pairs have a worse outcome than female-female pairs. This gender difference in the

polymorphism of the IL-1RA gene, with a possible effect on IL-1 and IL-1RA levels, provides an explanation for the fetal and newborn gender differences in inflammatory response and could explain the male disadvantage in neonatal mortality that has been recognized for over two decades (Murtha *et al.,* 2006). It is conceivable that this difference is associated with the higher rate of postnatal complications in very low birth weight boys as compared with girls. In summary, the present results point to the existence of a gender difference in IL-1RA gene polymorphism. This genetic diversity may affect IL-1 and IL-1RA production, with subsequent sexual differences in various physiologic and pathologic conditions, such as autoimmune diseases, pain perception, and premature delivery and outcome. As the gene for IL-1RA is located on the long arm of chromosome 2, the study offers an insight into gender differences that are neither linked to sex chromosome nor associated with sex hormones. Based on sexual differences in IL-1 and IL-1RA levels and on the observation that the production of these cytokines is dependent on gene polymorphism, the question was raised whether a sexual difference in the genetic polymorphism of these cytokines exists. A positive finding could contribute to the understanding of the difference in cytokine production and immune response observed in males and females.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study sites

Clinical samples were obtained from Kenyatta National Hospital comprehensive care clinic (CCC) and the HIV Immunology Laboratory at Kenya Medical Research Institute (KEMRI), being representative of Nairobi County (appendix I). The sample population consisted of Kenyans of African descent aged 18 yrs to 64 years diagnosed as HIV positive and HIV negative, being the age bracket hard hit by HIV/AIDS (Kenya Aids Indicator Survey, 2007). This study was part of an ongoing study and no consent was obtained for the said program.

3.2 Study population

This was a cross-sectional study which included 170 clinical samples from HIV infected adults and 70 clinical samples from HIV negative adults in Nairobi province.

3.3 Study design

3.3.1 Sample size determination

The sample size was determined using the formula as described by Fisher (1992).

$$n=\frac{Z^2P(1-P)}{d^2}$$

Where:

n= minimum sample size required.

Z= 1.96 standard error.

P= 0.16 i.e. 16% expected prevalence of HIV in Nairobi as by the year 2003: (National Aids Control Council, 2003).

d= 0.05 the inverse of 95% confidence limit (the allowable error).

Therefore:

$$n = \frac{1.96^2 (0.16) (0.84)}{(0.05)^2} = 240 \text{ Samples}$$

Thus, the calculated sample size was 240.

3.3.2 Sampling procedure

Qualified technical staff from KEMRI assisted in collecting about 5 ml of blood samples through venipuncture using disodium EDTA containers. The collected samples had no names of the source individuals but were given codes for their identification (Appendix V). The samples were then transported to KEMRI HIV laboratory for analysis in a cooler packed with ice to prevent degradation of cells. The collected samples were stored in the freezer at a temperature of – 20 °C until the DNA extraction was ready to be done.

3.4 Extraction of genomic DNA

PBMC's extraction was carried out as previously described (Khamadi *et al.*, 2005). Briefly, 5 ml of whole blood collected was added to a falcon tube containing 10 ml of 0.84% ammonium chloride and vortexed to mix completely, and then incubated at 37°C for 10 minutes. The mixture was then spun at 1500 x g in a centrifuge for 10 minutes at room temperature and the supernatant discarded. A 10 ml aliquot of 0.84% ammonium chloride was added to the pellet and the procedure was repeated three times until the PBMC pellet appeared white in color. The supernatant was discarded and 1 ml of 0.84% ammonium chloride was added to the pellet and mixed. The suspension was span at 1200 x g in a micro centrifuge at room temperature and the supernatant was discarded. To the pellet 500 µl of DNAzol genomic DNA extraction reagent (Gilco BRL® USA) was added. The pellet was dissolved completely by pipetting the reagent–pellet mixture up and

down. Two volumes (1000 µl) of chilled (cooled at 4°c) absolute ethanol was added to the dissolved pellet and mixed gently without vortexing. The mixture was then spun at 12000 rpm in a centrifuge at 4°C for 15 minutes. The supernatant was discarded and 1000 µl of 70% ethanol was added to the pellet and vortexed thoroughly. The 70% ethanol acted as a wash solution. The mixture was then spun again at 12000 rpm in a centrifuge at 4°C for 15 minutes and supernatant discarded. The pellet was air dried in a safety cabinet at room temperature and then dissolved in 100 µl of DNAse free water and stored at -20°C till further use.

3.5 IL-1RA genotyping by Polymerase Chain Reaction

Polymerase Chain Reaction was carried out as described by Khamadi *et al.* (2005). Briefly, a master mix was constituted as per the number of samples for the PCR reaction. The proviral DNA extracted as above served as the starting template. PCR was carried out using primers specific for the IL-1RA gene. The conditions of the PCR were 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 70°C for 2 min and a final extension for 7 min at 72°C for PCR amplification of variable number of tandem repeats (VNTR) region of the intron two (Tarlow *et al.*, 1994). The following sets of primers were used: Forward primer (F1) 5-CTCAGCAACACTCCTAT-3 Reverse primer (R1) 5-TCCTGGTCTGCAGGTAA-3(Perkin-Elmer Corporation, Norwalk, CT-USA). Each PCR was conducted in a final volume of 25 µl containing 0.2-0.5 µl template DNA, 1x PCR buffer, 200 µl each dNTPs, 1 mM MgCl, 1.25 µl Thermus aquaticus DNA polymerase, 50 mM each primer and distilled water.

3.6 Gel electrophoresis

Once the PCR was carried out, all the products were analyzed by conventional agarose electrophoresis. This was used to resolve the PCR products. The gels prepared for loading the sample was placed in the electrophoretic tank. The first well was loaded with 3 µl molecular marker while the consecutive wells were loaded with the samples. The samples were mixed with Tris Borate EDTA buffer before loading into the wells, that is, 3 µl of the loading dye (Bromophenol Blue) was first placed on a parafilm and 5 µl of the samples added. The gel tank was then closed and plugged in the electrodes to the power supply and turned on. Electrophoresis was done at a constant voltage of 80-120 volts/cm using a Bio-Rad model 200/2- power supply source for 30-45 minutes. Once the electrophoresis was completed, the gel was stained with 0.5 µl/ml ethidium bromides for visualization using a ultra violet (UV) spectrophotometer. The location of the various alleles was determined by direct examination of the gel and the size estimated by comparing with molecular weight size markers loaded alongside them under UV light.

3.7 Data management and statistical analysis

The distribution of the genotypes and alleles was calculated by direct counting. Chi square test or Fisher's exact test where applicable, were then used to determine the differences in allele (alternative forms of the gene) and genotype (combination of two alleles) frequencies of IL-1RA genetic variants. The genotype and allele frequency were tested to check deviation from Hardy-Weinberg equilibrium theory.

CHAPTER FOUR: RESULTS

4.1 The interleukin-1 receptor antagonist DNA products

All the samples that amplified had fragments of varying molecular sizes of 240bp, 325bp, 410bp and 500 representing allele 2, 4, 1 and allele 3, respectively. A total of two hundred and forty samples were collected and genotyped of which one hundred and seventy samples were from HIV-1 infected individuals and seventy samples were negative samples representing the control group. Of the HIV positive samples, eighty five were obtained from men and eighty five were women. Of the HIV negative samples, thirty five were men and thirty five were women.

4.2 Allele frequencies based on HIV status

In this study, the presence of IL-1RA allele polymorphism among HIV-1 infected and negative men and women was determined in Nairobi. In the study four alleles out of the possible five were identified. This included allele 1 with a fragment size of 410bp, allele 2 with 240bp, allele 3 with 500bp and allele 4 with 325bp. Allele 5 with 595bp was not observed. In both groups, allele 1 had the highest occurrence followed by allele 4, then allele 3 while allele 2 had the least occurrence (Table 4.1)

Table 4.1 Comparison of IL-1RA allele frequencies between HIV positive and HIV negative individuals

ALLELES	HIV POSITIVE		HIV NEGATIVE	
	Frequency(n)	%	Frequency(n)	%
Allele 1 (410bp)	137	80.59	52	74.3
Allele 2 (240bp)	8	4.71	0	0.0
Allele 3 (500bp)	10	5.88	0	0.0
Allele 4 (325bp)	15	8.82	18	25.7
Total	**170**	**100.0%**	**70**	**100.0%**

In the study (HIV positive) group (n = 170), four alleles out of the possible five were observed (Table 4.1). Allele 1 (A1) had the highest frequency of 80.59% followed by allele 4 (A4) with 8.82%, then by allele 3 (A3) with 5.88% and the lowest was Allele 2 (A2) with 4.71%. In the control (HIV negative) group, only two of the alleles were observed. These were allele 1 (74.3%) and A4 (25.7%) (Table 4.1). After statistical analysis, the overall distribution of the alleles between the HIV positive and HIV negative groups differed significantly ($\chi 2 = 17.94$, $df\,3$, $p = 0.00045$) with allele 1, allele 2 and allele 3 being high in the HIV positive group while allele 4 was high in HIV negative group.

4.3 Genotype frequencies based on HIV status

The genotype frequencies were identified based on the size and number of bands observed after gel electrophoresis. Based on the number of bands observed after gel electrophoresis in each sample, the individual was classified as either being homozygous or heterozygous for a certain allele. Homozygous individuals had one band while heterozygous individuals had two bands of different sizes. If an individual was homozygous, he/she had a combination of two alleles that were similar forming a particular genotype. For example, an individual homozygous for allele one had two alleles of 410bp (A1) combining and this means they had genotype 1/1. On the other hand, if an individual is heterozygous, they had two different alleles of different molecular sizes combining to form a particular genotype. For example, when allele 1 and 2 combine together they form genotype 1/2 (Figure 4.1).

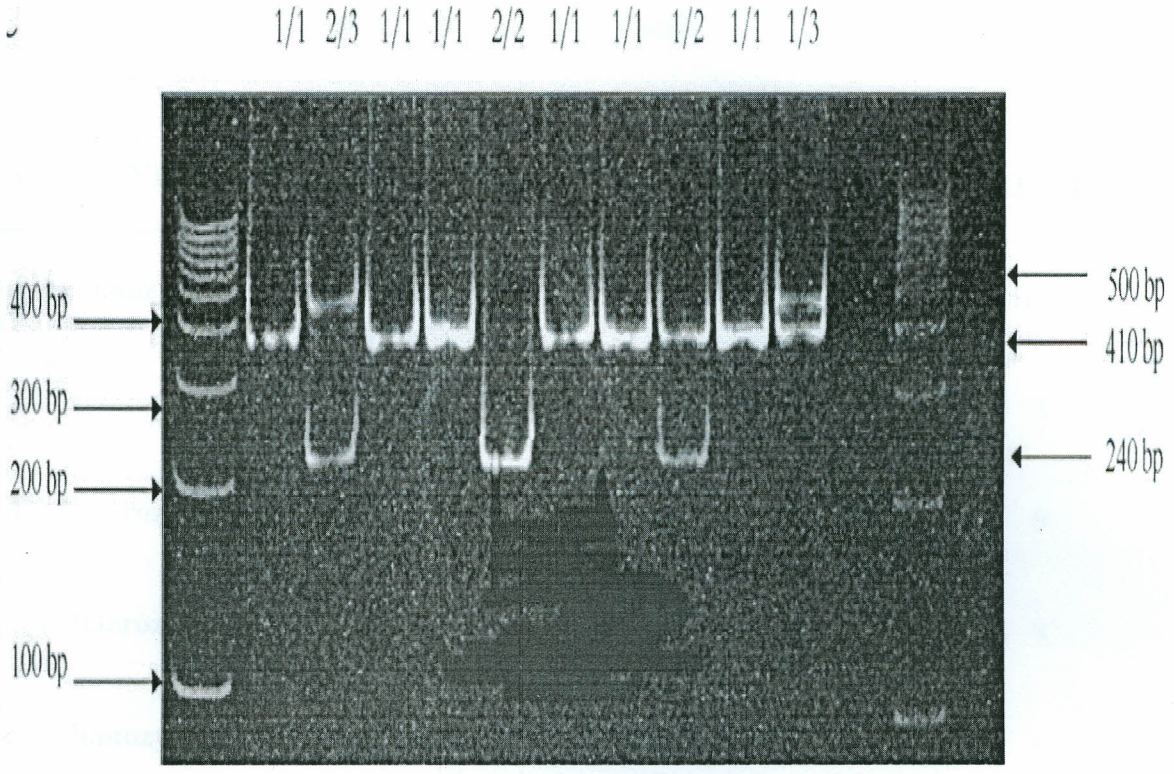


Figure 4.1 Representative electrophoresis ethidium bromide stained gels for IL-1RA alleles and genotypes

In the HIV infected (study) group, genotype 1/1 was the highest with 68.8%, followed by 4/4 with 8.8%, genotype 3/3 (5.9%), and genotype 2/2 (3.5%). The other individuals possessed other rare allelic combinations: 3.0% of them had both allele 1 and 2 (1/2) genotype, 4.7% had genotype 1/3, 4.1% had genotype 1/4 and 1.2% had genotype 2/3 (Table 4.2). In the HIV negative (control) group, only four genotypes were identified. As expected, genotype 1/1 was the highest with 70% followed by genotype 3/3 with 25.7%, then genotype 1/4 with 2.9% and the lowest was genotype 1/3 with 1.4% (Table 4.2).

Table 4.2 Comparison of the genotype frequencies

GENOTYPES	HIV POSITIVE	HIV NEGATIVE	TOTAL
1/1 Homozygous	117 (68.8%)	49 (70%)	166
1/2 Heterozygous	5 (3.0%	-	5
1/3 Heterozygous	8 (4.7%)	1 (1.4%)	9
1/4 Heterozygous	7 (4.1%)	2 (2.9%)	9
2/2 Homozygous	6 (3.5%)	-	6
2/3 Heterozygous	2 (1.2%)	-	2
3/3 Homozygous	10 (5.9%)	-	
4/4 Homozygous	15 (8.8%)	18 (25.7%)	33
TOTAL	**170**	**70**	**240**

In the study group, one hundred and seventeen (117) individuals were homozygous for allele 1 abbreviated as (IL-1RN*1), six were homozygous for allele 2 (IL-1RN*2), ten were homozygous for allele 3 (IL-1RN*3), and fifteen were homozygous for allele 4 (IL-1RN*4). The other individuals possessed other rare allelic combinations making them heterozygous. Five individuals were heterozygous for allele 1 & 2 (IL-1RN*1/IL-1RN*2), eight were heterozygous for allele 1 & 3 (IL-1RN*1/IL-1RN*3), seven

individuals were heterozygous for allele 1 & 4 (IL-1RN*1/IL-1RN*4) while two individuals were heterozygous for allele 2 & 3 (IL-1RN*2/IL-1RN*3). In the control group, forty nine individuals were IL-1RN*1 homozygous, eighteen individuals were IL-1RN*4 homozygous, one person was IL-1RN*1/IL-1RN*3 heterozygous and two were IL-1RN*1/IL-1RN*4 heterozygous. In both groups, individuals homozygous for allele 1 were the majority. The genotypes that appeared in HIV positive and negative groups were analyzed statistically using chi test. The overall distribution of the genotypes differed significantly between the HIV positive and negative individuals ($\chi 2 = 23.78$, *df* 3, $p = 0.0012$. Genotype 1/1 homozygous was almost similar in both groups while genotypes 1/2, 1/3, 1/4, 2/2, 2/3 and 3/3 were high in the HIV positive group. Genotype 4/4 was high in the HIV negative group.

4.4 Allele frequencies based on sex

In the study group, there was a sex difference in the occurrence of the alleles. Allele 1 and allele 4 were high in males with 87.05% and 10.59% as compared to females with 74.12% and 7.06% respectively. Allele 2 and allele 3 were high in females with 8.24% and 10.58% respectively as compared to males with 1.18% in both allele 2 and 3. In the control group, A1 was high in males with 85.71% as compared to females with 62.86%. A4 was high in females with 37.14% as compared to males with 14.29%. Alleles 2 and 3 were not observed in both males and females (Table 4.3). After statistical analysis, the frequency of the alleles differed significantly between males and females ($\chi 2 = 12.68$, *df* 3, $p = 0.0054$) with allele 1 being high in males who were HIV positive. Allele 2 and

allele 3 were high in females who were HIV positive while allele 4 was high in males who were HIV negative.

Table 4.3 Comparison of IL-1RA allele in HIV positive and HIV negative males and females

	HIV POSITIVE (n=170)		HIV NEGATIVE (n=70)	
	Males (n=85) n (%)	Females (n=85) n (%)	Males (n=35) n (%)	Females (n=35) n (%)
Allele 1	74 (87.1%)	63 (74.1%)	30 (85.7%)	22 (62.9%)
Allele 2	1 (1.2 %)	7 (8.2%)	0	0
Allele 3	1 (1.2%)	9(10.6%)	0	0
Allele 4	9 (10.5%)	6(7.06%)	5 (14.3%)	13 (37.1%)

4.5 Genotype frequencies based on sex

The IL-1RA genotypes in females were different from that of males (Table 4.4). In the study group (HIV positive), the incidence of genotype IL-1RN*1/ IL-1RN*1 homozygotes was higher in males (71.76%) compared with females (61.88%). The occurrence of IL-1RN*1/ IL-1RN*2 heterozygotes was high in females with 4.71% and lower in males with 1.18%. The occurrence of IL-1RN*1/ IL-1RN*3 and IL-1RN*1/ IL-1RN*4 heterozygotes were higher in males with 5.88% and 4.71% as compared to

females with 3.53% respectively. The occurrence of genotype IL-1RN*2/ IL-1RN*2 homozygote was higher in females with 5.88% as compared to males with 1.18%. Genotype IL-1RN*2/ IL-1RN*3 heterozygote was the same in both males and females with 1.18%. The incidence of genotype IL-1RN*3/ IL-1RN*3 homozygote was higher in females with 8.24% as compared to males with 3.53% while genotype IL-1RN*4/ IL-1RN* 4 homozygote was higher in males with 10.59% as compared to females with 7.06% (Table 4.4).

Table 4.4 Genotype frequencies in males and females

GENOTYPES	HIV POSITIVE		HIV NEGATIVE		TOTAL
	Females	Males	Females	Males	
1/1	56 (61.8%)	61 (71.76%)	23 (65.71%)	26 (74.29%)	166
1/2	4 (4.7%)	1 (1.18%)	-	-	5
1/3	3 (3.5%)	5 (5.9%)	1 (2.9%)	-	9
1/4	3 (3.5%)	4 (4.7%)	2 (5.7%)	-	9
2/2	5 (5.9%)	1 (1.2%)	-	-	6
2/3	1 (1.2%)	1 (1.2%)	-	-	2
3/3	7 (8.2%)	3 (3.5%)	-	-	33
4/4	6 (7.1%)	9 (10.6%)	9 (25.7%)	9 (25.7%)	10
TOTAL	85	85	35	35	240

In the control group, the genotypes observed were fewer and different in males and females. The incidence of genotype IL-1RN*1/ IL-1RN*1 homozygotes was higher in males with 74.29% compared with females with 65.71% while the incidence of genotype IL-1RN*4/ IL-1RN*4 homozygotes was the same in males and females with 25.71%. The occurrence of genotypes IL-1RN*1/ IL-1RN*3 and IL-1RN*1/ IL-1RN*4 heterozygotes were only observed in females with 2.86% and 5.71% respectively (Table 4.4). After statistical analysis, the genotypes were significantly different between males and females ($\chi 2 = 20.876$, $df 7$, $p = 0.0075$) with genotype 1/1 homozygous being high in HIV negative males. Genotypes 1/3, 1/4 heterozygous were high in HIV positive males. Genotype 1/2 heterozygous was high in females while genotypes 2/2, 3/3 homozygous were high in HIV positive females. On the other hand, genotype 4/4 homozygous was high in HIV negative males.

CHAPTER FIVE: DISCUSSION

In this study, IL-1RA alleles and genotypes were characterized to determine their distribution among HIV-positive and HIV negative individuals. In addition, the study sought to highlight the presence or absence of IL-1RA polymorphisms among adult males and females in Nairobi province.

As there were no previous reports on IL-1RA allele and genotype frequencies in the Kenyan population, we defined them as a first part of our study and compared them with the frequencies determined in other population groups. Allele 1 had an average frequency of 0.805 in our Kenyan study and control population, which is almost similar to frequencies previously reported in smaller study groups from other countries: 0.756 in the Netherlands, 0.736 in the UK and 0.710 in a white South-African population (Wang *et al.,* 2004). The frequency of allele 2 in our Kenyan group is 0.047, which was lower than a frequency of 0.238 in the Netherlands, 0.214 in the UK and 0.250 in white South Africa (Lee *et al.,* 2004). Allele 3 showed a frequency of 0.059 in the Kenyan population higher than that in the Dutch population (0.006), in the British population (0.036) and in the white South-African population (0.030). The frequency of Allele 4 in our population was 0.08 which was higher than that in the white South-African group (0.010). Allele 5 was not observed in our population or in the studied South-African group (Mwantembe *et al.,* 2001).

5.1 Association of IL-1RA alleles, genotypes and HIV status

The frequency of the alleles in the Kenyan population was determined. Four alleles were observed in the HIV positive group with allele 1 having the highest frequency. The

individuals who were homozygous for allele 1 were the majority in both groups. This results were similar to the studies done elsewhere indicating that the frequency of the individual alleles varies among different ethnic or geographic populations, where allele 1 (IL1RN*1), containing 4 repeats, is always more common than allele 2 (IL1RN*2), containing 2 repeats (Rider *et al.*, 2000). The remaining alleles, representing 3, 4, and 5 are rare in most populations. In every population studied to date, most persons are either homozygous for allele 1(IL1RN*1) or heterozygous for allele 1 and allele 2 (IL1RN*1/IL1RN*2). The prevalence of individuals homozygous for allele 2 (IL1RN*2) is typically less than 10%. In the Kenyan study, the occurrence of allele 2 was found to be 4.7% in the HIV positive group. In black Africans and African American persons, the frequency of IL1RN*2 homozygotes is considerably lower than in the white population (Mwantembe *et al.*, 2001).

In the HIV negative group, only two alleles were observed and the difference could have been due to a small sample size. In the Kenyan study the occurrence of allele 3 and allele 4 were found to be higher with a percentage of 5.9% and 8.8% respectively. Their occurrence as observed elsewhere is always less than 1% (Mwantembe *et al.*, 2001) and this could have been due to the fact that this alleles and genotypes differ geographically and also with the race. There were significant differences in the occurrence of the alleles and between the HIV positive and HIV negative groups as shown by the *p*-value confirming the importance of IL-1RA polymorphisms in HIV. Carriage of allele 1, allele 2 and allele 3 will have an effect on disease progression and severity while carriage of allele 4 has no influence on HIV since it was higher in the negative group. The genotypes also differed significantly between the two groups.

This study showed that there was a positive relationship between HIV and IL-1RA gene polymorphisms suggesting that there will be influence on disease outcome. There are several reported associations between HIV and AIDS and IL-1RA serum levels and also with IL-1RA polymorphisms. A study involving HIV infected African women reported that plasma IL-1RA levels were elevated in asymptomatic HIV positive women whereas levels in AIDS patients were not different from those in the controls (Witkin *et al.*, 2001)). Findings from a study that analysed human promonocytic cell line indicated that both TNF and IL-1 were up regulated by HIV infection *in vitro* and *in vivo*. Interleukin-1 (IL-1) induced expression of HIV in the cell line was inhibited by IL-1RA (Pillay *et al.*, 2000). These findings imply that IL-1/IL-1RA balance is an important factor in host resistance to HIV infection. Most of the studies on the association of IL-1RA gene polymorphisms and diseases have focused mainly on allele 1 and allele 2 since they occur more often. The other alleles (alleles 3, 4 and 5) are rare in most individuals and are therefore not studied extensively.

5.2 Frequency of allele 2

One of the objectives of this study was to investigate the presence of allele 2 in the Kenyan population. Only 8 individuals (4.7%) of the study population were found to have allele 2 (IL-1RN*2). It was found only in the HIV positive group suggesting that carriage of this allele will have an influence on disease progression. The occurrence of this allele was in accordance with studies done elsewhere showing that the prevalence of IL1RN*2 homozygotes is typically less than 10%. The occurrence of allele 2 (IL1RN*2) has a lower frequency in the black as compared with the white population (Gerber *et al.*,

2005), thus it is a potential candidate to account for differences in innate immunity responses between white and black ethnic groups. The presence of allele 2 (IL1RN*2) has been implicated in increased inflammatory responses and several pathological conditions (Gerber *et al.*, 2005). As a consequence of the association between allele 2 (IL-1RN*2) and chronic inflammatory disorders, individuals with this allele show more protracted and severe proinflammatory immune response than do persons with other IL-1RA genotypes. It has been reported that allele 2 carriage is associated with inflammatory conditions such as inflammatory bowels disease, alopecia areata, psoriasis, lichen sclerosis, lupus erythematosus, multiple sclerosis, Graves' disease, and diabetic nephropathy (Heaves *et al.,* 2006). The rarity of the allele 2 in black individuals correlates well with their lower predisposition to inflammatory bowel diseases as compared with the white population (Mwantembe *et al.,* 2001).

In relation to HIV and AIDS women have been found to be more susceptible to most of the opportunistic infections. Women are more likely than men to develop bacterial pneumonia, herpes infections, thrush (a yeast infection) in their throats more often than men and many other infections (CDC, 2005). This could also be due to the presence of allele 2 which has been implicated in increased inflammatory responses. On the other hand, men are eight times more likely than women to develop Kaposi's sarcoma-a cancer-like disease caused by a herpes virus. Recent studies have proposed that IL-1RA may play an important role as a treatment for Kaposi's sarcoma (Corley, 2000). The results of this study indicated that there is an association between IL-1RA gene polymorphism and HIV Status in Kenya showing that it will have an influence on disease outcome. It is interesting to note that the IL-1RA genotypes were associated with HIV in

our study. This finding confirms a previous investigation performed in eighty six HIV sero-positive women in Sao Paulo, Brazil (Witkin *et al.,* 2001). The distribution of IL-1RA genotypes in the HIV sero-positive women studied was similar to that seen previously in HIV-seronegative women from the state of Sao Paulo, Brazil and from European and American populations implying that IL-1RA genotypes are important in the pathogenesis of HIV.

A lowered prevalence of IL-1RN*2 in a black African population and in an African-American population has also been previously reported (Rider *et al.,* 2000). Although the numbers of subjects were small, the women in these investigation who were homozygous for IL-1RN*2 had a strikingly lower concentration of circulating HIV-1 than did the other women with the other genotypes. This occurred in the absence of any relation between IL-1RA genotype and CD4 lymphocyte population or the stage of disease. This IL-1RA genotype might improve immunological defenses against microbial infections by virtue of elevated and/or prolonged proinflammatory immune responses.

Whether the lower levels of circulating HIV associated with the IL-1RN*2 genotype will result in a reduced rate of sexual and/or neonatal transmission of HIV and the progression of HIV infection to AIDS, remains an interesting unexplored possibility. Previous investigations elsewhere have identified a relationship between polymorphism in the β-chemokine receptor 5 genes and HIV transmission and progression to AIDS (Wang *et al.,* 2004). Recently, a polymorphism in the gene coding for tumor necrosis factor alpha has also been shown to possibly influence HIV progression (Knuchel *et al.,* 1998). It remains to be determined whether the IL-1RA polymorphisms identified here may further

influence disease outcome in HIV-infected individuals with these other polymorphisms. The mechanism whereby IL-1RA genotype influences levels of HIV-1 RNA remains to be determined. Individuals who are IL-1RN*2 homozygotes have been shown to produce higher levels of IL-1RA *in vitro* than do individuals with the other IL-1RA genotypes. However, IL-1β production is also increased, leading to a net decrease in the IL-1RA/IL-1β ratio (Matisuki *et al.,* 2006). This would result in a relative deficiency in the capacity to terminate a pro-inflammatory reaction, a prolongation of a Th1 cell-mediated immune response, and an increased capability of defending the body against microbial infections.

5.3 Association of IL-1RA gene polymorphisms, sex and HIV

The results of the present study favor the existence of sex differences in IL-1RA gene polymorphism expressed by higher incidence of genotype 1/1 (IL-1RN*1/IL-1RN*1) homozygotes in males and lower occurrence of genotype 1/2 (IL-1RN*1/IL-1RN*2) heterozygotes in males. Furthermore, the presence of allele 1 (IL-1RN*1) was more frequent in males, whereas allele 2 (IL-1RN*2) was more prevalent in females. Evidence has been provided that secretion of IL-1 and IL-1RA differs in males and females (Bessler *et al.,* 2007). Secreted IL-1RA is normally present in circulation of healthy persons and is normally different in males and females. These differences are regulated by genotypic and non-genotypic factors. According to Lynch *et al.* (1994), the *in vitro* production of IL-1RA by isolated monocytes was significantly higher in females than in males, whereas whole blood from males produced significantly more IL-1 than that from females. In addition, IL-1RA levels have been shown to be significantly higher in amniotic fluid and urine of female than in male newborns. These findings have been attributed mainly to the levels of sex hormones (Vardhana *et al.,* 2003).

Over the past several years, there have been discussions about how HIV disease develops in adult males and females. Research has shown sex differences in viral load. During acute/early infection, women tend to have lower viral loads than men with the same or similar CD4+ cell counts. However, this difference appears to remain only in the first three to five years of infection. No impact has been seen on disease progression overall. This lower viral load does not put females at either lesser or greater risk for disease progression (Witkin et *al.*, 2001). To the contrary, most studies suggest that males and females progress from HIV infection to symptoms of AIDS at similar rates over time. Some studies even suggest that females may actually live a bit longer, and thrive better, with HIV disease. The cause and significance of viral load differences remain unclear, although one explanation is the role of the female hormones, estrogen and progesterone.

Sex hormones in females can interact with HIV (Juan and Vicente, 2007). Currently, differences in viral load have not warranted different approaches to treating males and females with HIV. Although these differences are highlighted in the Federal Guidelines, the Guidelines Committee did not conclude that females should consider starting anti-HIV therapy at lower viral loads. Sex hormones may also affect parts of the immune system, such as the presence of proteins on cells called chemokine receptors. These proteins are used by HIV to infect a cell. One example is the CCR5 receptor. The greater the number of CCR5 receptors on the cell, the more proteins HIV can use to infect the cell. This makes it easier for HIV to enter the cell, begin to reproduce and move on to infect and destroy more cells. In general, the amount of CCR5 on a given cell is less in women than in men. Research shows that progesterone can affect the amount of CCR5 receptors. The lower the levels of progesterone, the fewer CCR5 proteins are on the cell;

the higher the level, the more CCR5 proteins are found (Easterbrook, 1999). Interpreting these differences between men and women is difficult. There are many possible conclusions but not many hard and fast answers. Based on what we know, we could conclude that lower viral load in early infection would lessen a woman's risk for HIV disease progression. Also, having lower levels of CCR5 on immune cells, in theory, should also lessen her risk. Yet studies show that women and men have similar courses of HIV disease (Schemechel *et al.,* 2001).

It's possible, as with other diseases, that female's bodies are more capable of fighting HIV infection over time. Perhaps the best news from the research to date is that females live as long as and maybe even longer than males with HIV. Females have biological factors that may enable their immune systems to better resist HIV infection. A few studies suggest that females may actually respond better and are less likely to experience disease progression. It is unclear why females have lower HIV levels than males. It has been suggested that females may be capable of clearing the virus faster than males, but that the damage to the immune system has already been done (Brouwer *et al.,* 2005). The occurrence of the lower viral load in females could be due to the effects of allele 2 although more studies are required to support this hypothesis since other mechanisms could be in play. However, studies have shown that, IL-1RA is capable of inhibiting HIV gene expression and replication which are induced by IL-1 (Corley, 2000). In a study done among the Brazilian women, individuals homozygous for allele 2 were found to have a lower concentration of HIV RNA levels as compared to those women who were homozygous for allele 1 or heterozygous for allele 1 and allele 2 (Witkin *et al.*, 2001).

In this study we observed that, IL-1RA polymorphisms belong to the large group of genetic factors that are of importance in the pathogenesis of HIV. Since the functional significance of this polymorphism remains unclear, further investigation into a possible correlation between a certain IL-1RA genotype and IL-1RA production quantity is necessary, with a view to a possible new therapeutical approach in inflammatory diseases. The knowledge that IL-1RA gene can be used to inhibit IL-1 induced HIV replication may play an important role as a potential treatment for AIDS, that is, as an anti-HIV factor, as a regulator of HCG and testosterone, and as a treatment for Kaposi's sarcoma.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study focused on a relatively small numbers of individual, that is, two hundred and forty samples obtained from individuals who were HIV positive and negative. In spite of that, the intended specific objectives of the study were met.

- The study sought to determine the presence of allele 2 in the population which has been found to be beneficial in immune defense of HIV. Only eight individuals (4.7%) were found to be homozygous for this allele in the HIV positive group suggesting that it is important to HIV progression.

- There was a difference in the occurrence of alleles between the study and the control group. Four alleles were observed in the HIV positive group namely allele 1, allele 2, allele 3 and allele 4 while only two alleles were observed in the control group namely allele 1 and allele 4.

- There was a sex difference in the occurrence of the alleles with allele 1 and 4 being more common in males while allele 2 and allele 3 were more prevalent in females. There was also a sex difference in the genotypes with genotype 1/1 homozygous being common in males while genotype 2/2 being more common in females.

6.2 Recommendations

- Only eight individuals in the studied population had allele 2. There is need for further studies to determine its association with HIV/AIDS in Kenya since it has been found to be beneficial in immune defense against HIV.

- In relation to sex, the influences of the alleles and genotypes have not been well studied in HIV in both males and females. There is need for further studies involving the polymorphisms, sex and other HIV/AIDS parameters such as CD4 counts, viral load and disease progression to determine whether they influence the outcome of the disease.

- The mechanisms by which IL-1RA gene polymorphisms influence HIV have not been studied and there is need for more studies on HIV after chromosomal integration has taken place. Understanding the mechanisms that account for the lower viral loads in some individuals is important for designing of new antiviral drugs or vaccines which may be achieved by blocking the effects of IL-1. Since the functional significance of this polymorphisms remains unclear, further investigations into a possible correlation between specific IL-1RA genotypes and alleles and HIV is necessary, with a view to a possible new therapeutical approach in the treatment of HIV especially the use of protective IL-1RA alleles.

REFERENCES

Ananthi, J. A., Magner, J. W. and Tomasi, T. B. (2009). Messenger RNA regulation of cytokine genes. *Cytokine* **45(2):** 58-69.

Arend, W. P. (2003). The balance between IL-1 and IL-1RA in disease. *Cytokine Growth Factor* **13**: 323-340.

Bank, W. (2005). Evaluating the World Bank's Assistance for fighting the HIV/AIDS Epidemic. *Nature* **12**: 24-30.

Blakemore, A. F., Cox, A., Gonzalez, A. M., Maskili, J. K., Hughes, M. E., Wilson, R. M., Ward, J. D. and Duff, G. W. (1996). IL-1RA allele 2 (IL-1RN*2) associated with nephropathy in diabetes mellitus. *Human Genetics* **97**: 369-374.

Berger, A. (2000). Th1 and Th2 responses. What are they? *British Medical Journal* **321**: 7258-7424.

Bessler, H., Osovsky, M., Benzion, B., Alcalay, Y. and Sirota, L. (2007). The Existence of Gender Difference in IL-1RA Gene Polymorphism. *Journal of Interferon & Cytokine Research* **27**: 931–935

Bidwell, J., Keen, L. and Gallagher, G. (1999). Cytokine gene polymorphism in human Disease: online databases. *Gene Immunology* **1**: 3-19.

Brouwer, K. C., Yang, C., Parekh, S., Mirel, L. B., Shi, Y. P., Otieno, J., Lal, A. A. and Lal, R. B. (2005). Effect of CCR2 chemokine receptor polymorphism on HIV type 1 mother to child transmission and child survival in western Kenya. *AIDS* **21**: 358-362.

Campbell, G. R., Pasquier, E., Watkins, J., Rey, V., Peyrot, V., Esquieu, D., Barbier, P. and Loret, E. P. (2004). The glutamine rich region of the HIV-1 Tat protein is involved in T-cell apoptosis. *Journal of Biology and Chemistry* **279**: 48197-48204.

Cannon, J. G. (2000). Inflammatory cytokines in non-pathological state. *News Physiological Sciences Journal* **15**: 298-303.

Carter, M. J. and Giovine, F. S., (2001). Association of IL-1RA gene with ulcerative colitis in North European Caucasians. *Gut Journal* **48**: 461-467.

Casini, R. V., Barr, J. A., Driscoll, G. L., Gold, J. and Lamont, B. J. (1995). Mucosal imbalance of IL-1 and IL-1RA in inflammatory bowel disease: A novel mechanism of chronic intestinal inflammation. *Journal of Immunology* **154**: 2434-2440.

Center for Disease Control (1981). Pneumocystis pneumonia- Los Angeles. *Mortal Weekly Report* **30**: 250-252.

Centre for Disease Control (2005). U.S. HIV and AIDS cases reported through December 1996.

Centers for Disease Control and Prevention (2005). Women are more vulnerable to HIV/AIDS infection than men.

Chirivi, R. G., Hlandik, F., Markee, J., Alef, C., Kelley, H., Collier, A. and McElrath, M. J. (1993). IL-1RA inhibits augmentation of metastasis induced by IL-1 or LPS in a human melanoma or nude mouse system. *Cancer Research* **53**: 5051-5054.

Coffin, J., Haase, A., Levy, J. A., Montagnier, L., Oroszlan, S., Teich, N., Temin, H., Toyoshima, K., Varmus, H. and Vogt, P. (1986). What to call the AIDS virus? *Nature* **321**: 10-11.

Cohen, J. (1994). The Dues berg phenomenon. *Science* **266**:1642-1644.

Cookson, W., Liang, L., Abecasis, G., Moffat, M. and Lathrop, M. (2009). Mapping complex disease traits with global gene expression. *Nature Review Genetics* **10**: 184-194.

Corley P.A. (2000). Interleukin-1 receptor antagonist as a treatment of HIV infection. *Medical Hypotheses* **54:** 513–518.

Courgnaud, V., Pourrut, X., Bibollet, F., Mpoundi, E., Bourgeois, A., Delaporte, E. and Peeters, M. (2001). Characterization of a novel simian immunodeficiency virus from guereza colobus monkeys in Cameroon. *Journal of Virology* **75**: 857-866.

Cullup, H., Dickson, A. M., Jackson, G. H., Taylor, P. R., Cavet, J. I. and Middleton, P. G. (2001). Interleukin-1 receptor antagonist genotype associated with acute graft-versus- host disease in human leukocyte antigen. *British Journal of Hematology* **113(3)**: 807-813.

Dinarello, C.A. (2009). Immunological and inflammation functions of the interleukin-1 family. *Annual Reviews in Immunology* **27**: 519-550.

Dinarello, C. A. (2011). Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* **117:** 3720-3732.

Dinarello, C. A., Thompson, A. and Lotze, M. (2003). Interleukin-1 family: The cytokine handbook. *Elsevier Science Publishers* **57**: 2095-147.

Dong, C. and Flavell, R. A. (2001). Th1 and Th2 cells. *Current Opinions in Hematology* **8(1)**: 47-51.

Easterbrook, P. J. (1999). Chemokines receptor polymorphisms and human immunodeficiency virus disease progression. *Journal of Infectious Diseases* **180**: 1096-1105.

Estrov, Z., Chanadec, J., Chambri, V., Allemon, M. C., Vilmer, E. and Tricoire, J. (1991). Suppression of chronic myelogenous leukemia colony growth by IL-1RA and soluble IL-1 receptors: a novel application for inhibitors of IL-1 activity. *Blood* **78**: 1476-

1484.

Evaraerdt, B. (1994). Recombinant Interleukin-1 receptor antagonist protects against TNF induced lethally in mice. *Journal of Immunology* **152**: 5041-5049.

Fauci, A. S. (1988). The Human Immunodeficiency Virus: infectivity and mechanisms of pathogenesis. *Science* **239**: 617-622.

Ferretti, M., Jackson, J. B., Kroner, B. L., White, G. C., Aledort, L. M., Kessler, C. M. and Kiger, K. P. (1994). Neutralization of endogenous IL-1RA exacerbates and prolongs inflammation in rabbit immune colitis. *Journal of Clinical Investigation* **94**: 449-453.

Fisher, R. A. (1992). On the interpretation of $\chi 2$ from contingence tables and calculation of *p. Journal of the Royal Statistical Society*, Series A, Vol.85, pp.87-94

Frankenstein, Z., Alon, U. and Cohen, I. R. (2006). The immune- body cytokine network defines a social architecture of cell interactions. *Biology Direct* **1**: 32-54.

Fujiwara H., Fukuoka, M. and Yasuda, K. (1994). Cytokines stimulate dipeptidyl peptidase-IV expression on human luteinizing granuloma cells. *Journal of Clinical Endocrinology Metabolism* **79**: 1007–1011.

Gao, F., Yue, L., Robertson, D. L., Chen, Y., Rodenburg, C. M., Michael, S. F., Huang, Y., Paxton, W. A., Wolinsky, S. M., Neumann, A. U., Zhang, L., He, T., Kang,

S., Ceradini, D., Jin, Z., Yazdanbakhsh, K., Kunstman, K., Erickson, D., Dragon, E., Landa, N. R., Phair, J. K., Ho, D. and Koup, R. A. (1996). The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nature Medicine* **2**: 1240–1243.

Goletti, D., Kinter, A. L., Hardy, E. C., Poli, G. and Fauci, A. S. (1996). Modulation of endogenous IL-1beta and IL-1 receptor antagonist results in opposing effects on HIV expression in chronically infected monocytic cells. *Journal of Immunology* **156**: 3501-3508.

Gerber, S., Vardhana, S., Meagher, K., Vial, Y., Hohlfeld, P. and Witkin, S. S. (2005). Association of fetal interleukin-1 receptor antagonist gene polymorphism and unexplained fetal death. American Journal of Obstetric Gynecology **193**: 1472-1477.

Heaves, D., Alexandre, V., Wassila, C., Laurence, M., Diop, G., Fumihiko, M. and Jean, F. Z. (2006). Exhaustive genotyping of IL-1 family genes and associations with AIDS progression in a French cohort. *Journal of Infectious diseases* **194 (11)**: 1492:1504.

Hideshima, T., Podar, K., Chauhan, D. and Anderson, K. C. (2005). Cytokines and signal transduction. *Best Practice Resources on Clinical Hematology* **18(4)**: 509-524.

Http://www.blissites.com/kenya/map.html Map of Nairobi province

Http://www.idealibrary.com HIV structure

Huang, C. M., Wu, M. C. and Tsai, F. J. (2002). IL-1RA gene polymorphism as a disease severity in systemic lupus erythematosus. *Arthritis and Rheumatism* **37**: 1380-1385.

Hull, R. (2001). Classifying reverse transcribing elements: a proposal and a challenge to the ICTV. International Committee on Taxonomy of Viruses. *Archive Virology* **14**: 2255-2261.

Imbonden, M., Nieters, A., Bircher, A. J., Brutsce, M., Liebrich, A. U., Berger, W. and Probst, N. M. (2006). Cytokine gene polymorphism and atopic disease in two European cohorts. *Clinical and Molecular Allergy* **4**: 9-10.

Janriver, K. T. and Freedman, J. E. (2008). The promise and limitations of gene expression analysis. *Circulation and Cardiovascular Genetics* **1**: 7-9.

Juan, L. and Vicente, P. (2007). Host factors influencing susceptibility to HIV infection and AIDS progression. *Journal of Retrovirology* ***4***: 52-53.

Kawa, S., Cloyd, M. W. and Thompson, E. B. (1993). Resistance to HIV-1 infection by CD4 positive lymphoid cells that vary in their glucocorticoid receptors and responses. *Cell Developmental Biology Animal Journal* **29**: 255-257.

Keane, A. M., Todd, J., Corrah, T., Wilkins, A., Demba, E., Morgan, G., Rolfe, M., Berry, N. and Tedder, R. (1999). Prevention of allergic eye disease by treatment with IL-

1RA investigative ophthalmology and visual sciences. *Lancet* **40**: 3041-3046.

Kedzierska, K. and Crowe, S. M. (2001). Cytokines and HIV-1: interactions and clinical implications. *Antiviral Chemotherapy* **12(3)**: 133-50.

Kenya AIDS Indicator Survey (2007). Final report on the status of HIV/AIDS in Kenya.

Kenya AIDS Indicator Survey (2008). Report on the status of HIV/AIDS in Kenya.

Khamadi, S., Ochieng, W., Lihana, R. W., Kinyua, J., Muriuki, J., Mwangi, M., Lwembe, R., Kiptoo, M., Osman, S., Lagat, N., Pelle, R., Muigai, A., Carter, J. Y., Oishi, I., Ichimura, H., Mwaniki, D. L. and Okoth, F. A. (2005). HIV Type 1 Subtypes in Circulation in Northern Kenya. *AIDS Research and Human Retroviruses* **9**: 810 -814.

Knuchel, M. C., Spira, T. J., Neumann, A. U., Xiao, L., Rudolph, D. L., Phair, J., Wolinsky, S. M., Koup, R. A., Cohen, O. J., Folks, T. M. and Lal, R. B. (1998). Analysis of a biallelic polymorphism in the tumor necrosis factor promoter and HIV type 1 disease progression. *AIDS* **14**:305-309.

Lee, Y. H., Kim, H. J., Rho, Y. H., Choi, S. J. and Song, G. G. (2004). Interleukin-1 receptor antagonist gene polymorphism and rheumatoid arthritis. *Rheumatology International* **24(3)**: 13-16.

Levy, J. A. (1993). HIV pathogenesis and long-term survival. *Aids* **7**: 1401-1410.

Lynch, E. A., Dinarello, C. A. and Cannon, J. G. (1994). Gender differences in IL-1, and IL-1 receptor antagonist secretion from mononuclear cells and urinary excretion. *Journal of Immunology* **153**: 300–306.

Matisuki, T., Nakae, S., Sudo, K., Horai, R. And Iwakura, Y. (2006). Abnormal T cell activation caused by the imbalance of the IL-1/IL-1RA system is responsible for development of experimental autoimmune encephalopathy. *International Immunology* **18(2)**: 399-407.

Mayo, M. A. and Haenni, A. L. (2006). Report from the 36th and the 37th meetings of the Executive Committee of the International Committee on Taxonomy of Viruses. *Archives Virol*ogy **151**: 1031-1037.

McCarthy, M. and Hirchorn, J. N. (2008). Genome wide association studies: past, present and future. *Human Molecular Genetics* **17**: 100-101.

Ministry of Health (2005). AIDS in Kenya: Trends, interventions and impact, 7th Edition.

Mwantembe, O., Gaillard, M. C. and Barkhuizen, M. (2001). Ethnic differences in allelic associations of the interleukin-1 gene cluster in South African patients with inflammatory bowel disease and in control individuals. *Immunogenetics* **52**: 249-54.

Murtha, A. P., Nieves, A., Hauser, E., Swamy, G. K., Yonish, B. A., Sinclair, T. R. and Heine, R. P. (2006). Association of maternal IL-1 receptor antagonist intron 2 gene

polymorphism and preterm birth. *Journal of Obstetrics and Gynecology* **195**: 1249–1253.

National AIDS Control Council (2003). Annual report on HIV prevalence in Kenya.

Nica, A. C. and Dermitzakis, E. T. (2008). Using gene expression to investigate basis of complex disorders. *Human Molecular Genetics* **17**: 128-133.

Ollier, W. E. (2004). Cytokine genes and disease susceptibility. *Cytokine* **28**: 174-8

Papadopoulos, E. E., Turner, V. F., Page, B., Causer, D., Alfonso, H., Miller, T., Maniotis, A. And Fiala, C. (2004). A critique of Montagnier evidence for the HIV/AIDS hypothesis. *Medical Hypothesis* **63**: 597-601.

Parkin, J. and Cohen, B. (2001). An overview of the immune system. *Lancet* **357**: 1777-1789.

Pellicane, J. V., Levy, J. A., Montagnier, L., Oroszlan, S., Teich, N., Temin, H., Toyoshima, K., Varmus, H. and Vogt, P. (1993). IL-1RA improves survival and preserves organ adenosine-5'-triphosphate after hemorrhagic shock. *Surgery* **114**: 278-283.

Perni, S. C., Vardhana, S., Tuttle, S. L., Kalish, R. B., Chasen, S. T. and Witkin, S. S. (2004). Fetal interleukin-1 receptor antagonist gene polymorphism, intra-amniotic interleukin-1 levels, and history of spontaneous abortion. *American Journal of Obstetric Gynecology* **191**: 1318–1323.

Pillay, V., Gaillard, M. C., Halkas, A., Song, E. and Dewar, J. B. (2000). Differences in the genotypes and plasma concentrations of the interleukin-1 receptor antagonist in black and white South African asthmatics and control subjects. *Cytokine* **12**: 819-821.

Price, P., James, I., Fernandez, S. and French, M. A. (2004). Alleles of genes encoding IL-1 alpha may predict control of the plasma viraemia in HIV-1 patients on HAART. *AIDS* **18**: 1495-1501.

Rao, M., Wong, C., Kanetsky, P., Girndt, M., Stenvinkel, P., Reilly, M. and Raj, D. (2007). Cytokine gene polymorphism and progression of renal and cardiovascular diseases. *Kidney International* **72**: 549-556.

Reeves, J. D. and Doms, R. W. (2002). Human Immunodeficiency virus type 2. *Journal of Genetics and Virology* **83**: 1253-1265.

Richter, S. and Palu, G. (2006). Inhibitors of HIV-1 Tat mediated transactivation. *Current views in medicine* **13(11)**: 1305-1315.

Rider, L. G., Artlett, C. M. and Foster, C. B. (2000). Polymorphisms of the IL-1RA gene VNTR are possible risk factors for juvenile idiopathic inflammatory myopathies. *Clinical Experiments in Immunology* **121**: 47-52.

Roberts, L., Carolyna, C., Lisa, M. C., Wendy, A., Joel, F. F. and Salim, S. C. (2010). Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. *AIDS* **24(6)**: 819-831.

Sadeghi, H. M., Weiss, L., Kazatchkine, M. D. and Haeffner, N. C. (1995). Antiretroviral therapy suppresses the constitutive production of interleukin-1 associated with human immunodeficiency virus infection. *Journal of Infectious Diseases* **172**: 547-550.

Schemechel, S. C., Russell, N., Hladik, F., Lang, J., Wilson, A., Ha, R., Desbien, A. and McElrath, M. J. (2001). Immune defense against HIV-1 infection in HIV-1 exposed seronegative persons. *Immunology Letters* **79**: 21-27.

Seymour, R. M. and Henderson, B. (2001). Pro-inflammatory anti-inflammatory cytokine dynamics mediated by cytokine receptor dynamics in monocytes. *Mathematics, Medicine and Biology Journal* **18(2)**: 159-192.

Soudeyns, H., Geleziunas, R., Shymala, G., Hiscott, J., Wainberg, M. A. (1993). Identification of a novel glucocorticoid response element within the genome of the human immunodeficiency virus type 1. *Virology* **194**: 758–768.

Shannon, M. F., Coles, L. S., Attema, J., and Diamond, P. (2001). The role of architectural transcription factors in cytokine gene transcription. *Journal of Leukocyte*

Biology **69**: 21-32.

Strebel, K. (2003). Virus host interactions. Role of HIV proteins, Vif, Tat and Rev. *AIDS* **17(1)**: 45-52.

Stewart, G. J., Tyler, J. P., Cunningham, A. L., Barr, J. A., Driscoll, G. L., Gold, J. and Lamont, B. J. (1985). Transmission of human T cell lymphotropic virus type III by artificial insemination by donor. *Lancet* **2**: 581-585

Tamaka, S., Aso, H., Kohtaro, M., Nagai, Y., Kobayashi, Y. and Takahiro, Y. (2007). Differential cytokine gene expression in CD4+ and CD8+ T cell subsets. *Veterinary Immunology* **1(2)**: 84-91.

Tarlow, J. K., Clay. F. E., Cork, M. J., Blakemore, A. I., McDonagh, A. J., Messenger, A. G. and Duff, G. W. (1994). Severity of alopecia areata is associated with a polymorphism in the interleukin-1 receptor antagonist gene. *Journal of Investigations in Dermatology* **103**: 387-390.

Terwilliger, E., Sodroski, J. G., Rosen, C. A. and Haseltine, W. A. (1986). Effects of mutations within the open reading frame region of human T-cell lymphotropic virus type III (HTLV-III/LAV) on replication and cytopathogenicity. *Journal of Virology* **60**: 754–760.

Townsend, M. J. and McKenzie, A. N. (2000). Cytokines and diseases. *Journal of Cellular Sciences* **113**: 3549-3550.

Uchiyama, K., Kawai, M., Tani, M., Ueno, M., Hama, T. and Yamaue, H. (2006). Gender differences in postoperative pain after laparoscopic cholecystectomy. *Surgical Endoscopy* **20**: 448–451.

UNAIDS/WHO (2005a). Special session of general assembly on HIV/AIDS round table 3 Socio-economic impact of the epidemic and the strengthening of national capacities to combat HIV/AIDS.

UNAIDS/WHO (2005b). AIDS epidemic update.

UNAIDS (2006a). HIV/AIDS estimates and data.

UNAIDS (2006b). Overview of the global AIDS epidemic.

Vamvakopoulos, J., Green, C. and Metcalfe, S. (2002). Genetic control of IL-1 bioactivity through differential regulation of IL-1 receptor antagonist. *European Journal of Immunology* **32**: 2988–2996.

Van Zee, K. J. (1995). Influence of interleukin-1 receptor blockade on the human response to endotaxaemia. *Journal of Immunology* **154**: 1499-1507.

Vardhana, S., Witkin, S. S., Yih, M. and Gerber, S. (2003). Polymorphism in intron 2 of the fetal IL-1RA genotype influences mid trimester amniotic fluid concentration of IL-1beta and IL-1RA and pregnancy outcome. *Annual Journal of Obstetrics Gynecology* **189(5)**: 1413-1417.

Walter, J. (2010). Clinical features and serum biomarker in HIV immune reconstitution inflammatory syndrome after cryptococcal meningitis. *AIDS* **179(3)**: 123-130.

Warley, M. C., Farhan, A., Metselaar, H. J., Hop, W. C. and Bouma, G. J. (2003). Are cytokine gene polymorphisms related to in vitro cytokine production profiles? *Liver Transplantation* **9(2)**: 170-81.

Wasik, T. J., Smolen, J., Kruszynski, P., Wasik, J. B. and Beniowski, M. (2005). Effects of CCR5 delta 32, CCR2-641 and SDF-1-3'A polymorphic alleles on HIV-1 infection in the Polish population. *Nature* **58**: 500-507.

Wakabayashi, G., Markee, J., Akridge, R. E., Meldorf, M., Musey, L. and McElrath, M. J. (1991). A specific receptor antagonist for IL-1 prevents Escherichia coli induced shock in rabbits. *FASEB* **5**: 338-343.

Wang, C., Song, W. and Lobashevsky, E. (2004). Cytokine and chemokine gene polymorphism among ethnically diverse North Americans with HIV infection. *AIDS* **35**: 446-450.

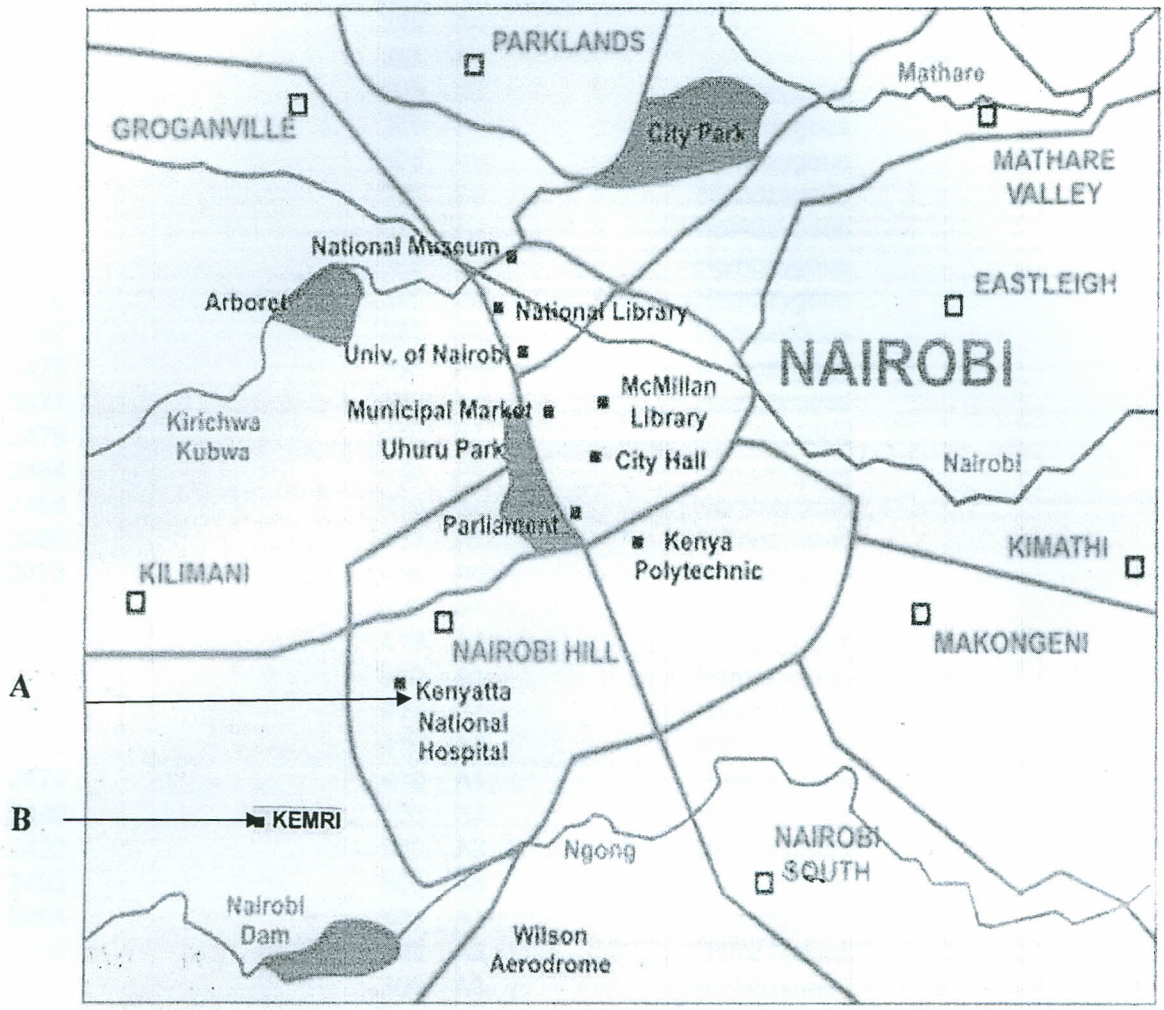
Weber, A., Wasiliew, P. and Kracht, M. (2010). Interleukin-1 (IL-1) pathway. *Science Signaling* **3(105)**: 1126-3105.

Witkin, S., Iara, M., Gerber, S., Caetano, E. M., and Alusio, C. (2001). Interleukin-1 Receptor Antagonist Gene Polymorphism and Circulating Levels of Human Immunodeficiency Virus Type 1 RNA in Brazilian Women. *Journal of Virol*ogy **75**: 6242–6244.

Witkin, S., Gerber, S. and Ledger, W. J. (2002). Influence of interleukin-1 receptor antagonist gene polymorphism on disease. *Clinical Infectious Journal* **34**: 204-209.

APPENDICES

APPENDIX I: Map of Nairobi Province showing location of KNH & KEMRI (http://www.blissites.com/kenya/map.html)



Key: **A**: Kenyatta National Hospital (KNH)
B: Kenya Medical Research Institute (KEMRI)

APPENDIX II

RAW DATA FROM THE TWO STUDY GROUPS

I. Raw data from the HIV positive group

Sample code	DNA size (bp)	Allele type		
STC 2469	325	A4		Heterozygous
STC 2460	325	A4		Heterozygous
STC 2459	325	A4	Homozygous	
STC 2452	325	A4	Homozygous	
STC 2456	325	A4	Homozygous	
STC 2457	325	A4	Homozygous	
STC 2453	325	A4	Homozygous	
STC 2451	325	A4	Homozygous	
STC 2466	325	A4	Homozygous	
STC 2467	325	A4	Homozygous	
STC 2475	325	A4	Homozygous	
STC 2471	325	A4	Homozygous	
STC 2479	410	A1	Homozygous	
STC 2464	410	A1	Homozygous	
STC 2465	410	A1	Homozygous	
STC 2486	410	A1	Homozygous	
STC 2473	410	A1	Homozygous	
STC 2483	410	A1	Homozygous	
STC 2481	410	A1	Homozygous	
STC 2491	410	A1	Homozygous	
STC 2462	410	A1	Homozygous	
STC 2472	410	A1	Homozygous	
STC 2474	410	A1	Homozygous	
STC 2482	500	A3	Homozygous	
STC 2492	500	A3	Homozygous	
STC 2493	500	A3	Homozygous	
STC 2495	500	A3	Homozygous	
STC 2498	500	A3	Homozygous	
STC 2468	500	A3	Homozygous	
STC 2496	500	A3	Homozygous	
STC 2494	500	A3	Homozygous	
STC 2487	500	A3	Homozygous	
STC 2490	500	A3	Homozygous	
STC 2485	500	A3		Heterozygous
STC 2489	325	A4	Homozygous	
STC 2469 E.P	325	A4	Homozygous	
STC 2265	325	A4	Homozygous	
STC 2266	325	A4		Heterozygous
STC 2291	325	A4	Homozygous	
STC 2292	325	A4		Heterozygous
STC 2267	325	A4	Homozygous	
STC 2290	240	A2		Heterozygous
STC 2268	240	A2	Homozygous	

STC 2157	410	A1	Homozygous	
STC 2156	410	A1	Homozygous	
STC 2155	410	A1	Homozygous	
STC 2154	410	A1		Heterozygous
STC 2153	410	A1	Homozygous	
STC 2152	410	A1		
STC 2151	410	A1	Homozygous	
STC 2150	410	A1	Homozygous	
STC 2110	410	A1	Homozygous	
STC 2113	410	A1		Heterozygous
STC 2114	410	A1		Heterozygous
STC 2112	410	A1	Homozygous	
STC 2116	410	A1	Homozygous	
STC 2117	410	A1	Homozygous	
STC 2111	410	A1	Homozygous	
STC 2115	410	A1	Homozygous	
STC 2206	410	A1	Homozygous	
STC 2214	410	A1	Homozygous	
STC 2207	410	A1	Homozygous	
STC 2208	410	A1	Homozygous	
STC 2209	410	A1	Homozygous	
STC 2210	410	A1	Homozygous	
STC 2211	410	A1	Homozygous	
STC 2211B	410	A1		Heterozygous
STC 2284	410	A1	Homozygous	
STC 2277	410	A1	Homozygous	
STC 2290	410	A1	Homozygous	
STC 2283	410	A1	Homozygous	
STC 2285	410	A1		Heterozygous
STC 2286	410	A1	Homozygous	
STC 2287	410	A1		Heterozygous
STC 2298	410	A1	Homozygous	
STC 2288	410	A1	Homozygous	
STC 2281	410	A1	Homozygous	
STC 2278	410	A1		Heterozygous
STC 2282	410	A1	Homozygous	
STC 2293	410	A1	Homozygous	
STC 2294	410	A1	Homozygous	
STC 2296	410	A1	Homozygous	
STC 2295	410	A1	Homozygous	
STC 2305	410	A1	Homozygous	
STC 2307	410	A1	Homozygous	
STC 2308	410	A1	Homozygous	
STC 2309	410	A1	Homozygous	
STC 2312	410	A1	Homozygous	
STC 2313	410	A1	Homozygous	
STC 2314	410	A1	Homozygous	
STC 2315	410	A1	Homozygous	
STC 2317	410	A1	Homozygous	
STC 2318	240	A2	Homozygous	

STC 2319	410	A1	Homozygous	
STC 2320	410	A1	Homozygous	
STC 2323	410	A1	Homozygous	
STC 2324	410	A1	Homozygous	
STC 2325	410	A1	Homozygous	
STC 2326	410	A1	Homozygous	
STC 2327	410	A1		Heterozygous
STC 2328	410	A1	Homozygous	
STC 2329	410	A1	Homozygous	
STC 2330	410	A1		Heterozygous
STC 2331	410	A1	Homozygous	
STC 2398	410	A1	Homozygous	
STC 2395	410	A1	Homozygous	
STC 2394	410	A1	Homozygous	
STC 2397	410	A1		Heterozygous
STC 2394	410	A1	Homozygous	
STC 2393	410	A1	Homozygous	
STC 2392	410	A1	Homozygous	
STC 2391	410	A1	Homozygous	
STC 2450	410	A1	Homozygous	
STC 2449	410	A1		Heterozygous
STC 2448	410	A1	Homozygous	
STC 2447	410	A1	Homozygous	
STC 2446	410	A1	Homozygous	
STC 2445	410	A1	Homozygous	
STC 2444	410	A1	Homozygous	
STC 2443	410	A1	Homozygous	
STC 2109	410	A1	Homozygous	
STC 2108	410	A1	Homozygous	
STC 2107	410	A1	Homozygous	
STC 2106	410	A1	Homozygous	
STC 2105	410	A1	Homozygous	
STC 2104	410	A1	Homozygous	
STC 2103	410	A1	Homozygous	
STC 2102	410	A1	Homozygous	
STC 2085	410	A1	Homozygous	
STC 2084	410	A1	Homozygous	
STC 2092	410	A1	Homozygous	
STC 2093	410	A1	Homozygous	
STC 2082	410	A1	Homozygous	
STC 2083	410	A1	Homozygous	
STC 2091	410	A1	Homozygous	
STC 2090	410	A1	Homozygous	
STC 2100	410	A1	Homozygous	
STC 2081	410	A1	Homozygous	
STC 2089	410	A1	Homozygous	
STC 2078	410	A1	Homozygous	
STC 2079	410	A1	Homozygous	
STC 2088	410	A1		Heterozygous
STC 2080	410	A1		Heterozygous

STC 2149	410	A1	Homozygous	
STC 2148	410	A1	Homozygous	
STC 2147	410	A1		Heterozygous
STC 2146	410	A1		Heterozygous
STC 2145	410	A1	Homozygous	
STC 2144	410	A1	Homozygous	
STC 2143	410	A1		Heterozygous
STC 2142	410	A1	Homozygous	
STC 2141	410	A1	Homozygous	
STC 2140	410	A1	Homozygous	
STC 2139	410	A1	Homozygous	
STC 2138	410	A1	Homozygous	
STC 2137	410	A1	Homozygous	
STC 2136	410	A1	Homozygous	
STC 2135	410	A1	Homozygous	
STC 2134	410	A1		Heterozygous
STC 2133	410	A1	Homozygous	
STC 2132	410	A1	Homozygous	
STC 2131	410	A1		Heterozygous
STC 2130	410	A1	Homozygous	
STC 2129	410	A1	Homozygous	
STC 2128	410	A1	Homozygous	
STC 2127	410	A1	Homozygous	
STC 2126	410	A1	Homozygous	
STC 2193	410	A1	Homozygous	
STC 2192	240	A2	Homozygous	
STC 2190	325	A4	Homozygous	
STC 2470	240	A2	Homozygous	
STC 2483	240	A2	Homozygous	
STC 2481	325	A4	Homozygous	
STC 2101	325	A4	Homozygous	
STC 2443	325	A4	Homozygous	
STC 2279	325	A4	Homozygous	
STC 2197	325	A4	Homozygous	
STC 2196	325	A4	Homozygous	
STC 2455	325	A4	Homozygous	
STC 2477	325	A4	Homozygous	

II. Raw data from the HIV negative group

Sample code	DNA size (Bp)	Allele type	
NRB 0001	410	A1	Homozygous
NRB 0002	410	A1	Homozygous
NRB 0003	325	A4	Homozygous
NRB 0004	410	A1	Homozygous
NRB 0005	410	A1	Homozygous
NRB 0006	325	A4	Homozygous
NRB 0007	410	A1	Homozygous
NRB 0008	325	A4	Heterozygous
NRB 0009	410	A1	Homozygous
NRB 0010	325	A4	Homozygous
NRB 0011	410	A1	Homozygous
NRB 0012	410	A1	Homozygous
NRB 0013	410	A1	Homozygous
NRB 0014	410	A1	Homozygous
NRB 0015	325	A4	Homozygous
NRB 0016	410	A1	Homozygous
NRB 0017	410	A1	Homozygous
NRB 0018	410	A1	Homozygous
NRB 0019	410	A1	Homozygous
NRB 0020	325	A4	Homozygous
NRB 0021	410	A1	Homozygous
NRB 0022	410	A1	Homozygous
NRB 0023	325	A4	Homozygous
NRB 0024	325	A4	Homozygous
NRB 0025	410	A1	Homozygous
NRB 0026	410	A1	Homozygous
NRB 0027	410	A1	Homozygous
NRB 0028	410	A1	Homozygous
NRB 0029	410	A1	Homozygous
NRB 0030	410	A1	Homozygous
NRB 0031	325	A4	Homozygous
NRB 0032	410	A1	Homozygous
NRB 0033	410	A1	Homozygous
NRB 0034	325	A4	Homozygous
NRB 0035	410	A1	Homozygous
NRB 0036	410	A1	Homozygous
NRB 0037	325	A4	Homozygous
NRB 0038	410	A1	Heterozygous
NRB 0039	410	A1	Homozygous
NRB 0040	410	A1	Homozygous
NRB 0041	325	A4	Homozygous
NRB 0042	410	A1	Homozygous
NRB 0043	410	A1	Homozygous
NRB 0044	410	A1	Homozygous
NRB 0045	410	A1	Homozygous
NRB 0046	410	A1	Homozygous
NRB 0047	410	A1	Homozygous
NRB 0048	410	A1	Homozygous

NRB 0049	325	A4	Homozygous	
NRB 0050	325	A4	Homozygous	
NRB 0051	410	A1	Homozygous	
NRB 0052	325	A4	Homozygous	
NRB 0053	325	A4	Homozygous	
NRB 0054	410	A1	Homozygous	
NRB 0055	410	A1	Homozygous	
NRB 0056	410	A1		Heterozygous
NRB 0057	325	A4	Homozygous	
NRB 0058	410	A1	Homozygous	
NRB 0059	410	A1	Homozygous	
NRB 0060	325	A4		Heterozygous
NRB 0061	410	A1	Homozygous	
NRB 0062	410	A1		Heterozygous
NRB 0063	410	A1	Homozygous	
NRB 0064	410	A1	Homozygous	
NRB 0065	410	A1	Homozygous	
NRB 0066	410	A1	Homozygous	
NRB 0067	410	A1	Homozygous	
NRB 0068	410	A1	Homozygous	
NRB 0069	410	A1	Homozygous	
NRB 0070	410	A1	Homozygous	