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Distribuição de Polimorfismos de Base Única (SNPs) em Genes Relacionados à Infecção pelo HIV-1 em uma População do Nordeste Brasileiro

Recife
2015

Ronaldo Celerino da Silva

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Tese apresentada ao Programa de Pós-Graduação em Genética da Universidade Federal de Pernambuco como parte dos requisitos exigidos para obtenção do título de Doutor em Genética.

Orientador: Prof. Dr. Sergio Crovella

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Recife

2015

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Resumo

A variabilidade genética humana tem desempenhado um papel importante para a compreensão de mecanismos envolvidos na susceptibilidade à infeção pelo HIV-1. O presente trabalho avaliou as distribuições de polimorfismos de base única (SNPs) em genes humanos relacionados à entrada (*CCL3, CCL4, CCL5, CXCL12,* CXCR6) e à replicação viral (*APOBEC3G, CUL5, TRIM5, HLA-C* e *ZNRD1*), e suas prováveis associações com a modulação da susceptibilidade à infecção pelo HIV-1 em uma população do Nordeste brasileiro (Recife-Pernambuco), a fim de estabelecer um modelo imunogenético de susceptibilidade ao HIV-1. Foi desenvolvido um estudo tipo caso-controle, utilizando pacientes infectados (HIV-1+) e controles saudáveis, os quais foram genotipados para 18 SNPs em genes reconhecidamente envolvidos na entrada e na replicação viral. Verificou-se que variantes nos genes *CCL3* (rs1719134), C*CL4* (rs1719153), *TRIM5* (rs10838525) e *CUL5* (rs11212495) foram mais frequentes em controles saudáveis; enquanto variantes em *APOBEC3G* e *ZNRD1* (rs3869068) foram mais frequentes em pacientes HIV-1+, sugerindo, respectivamente, proteção e susceptibilidade à infecção pelo HIV-1 na população pernambucana. Neste sentido, sugere–se que SNPs em genes relacionados com a entrada e a replicação viral podem modular a susceptibilidade a infecção pelo HIV-1.

Palavras-chave: HIV-1, SNPs, Susceptibilidade, Quimiocinas, Fatores de restrição, Genes HLA, Variabilidade genética.

Abstract

Human genetic variability has played an important role in understanding the mechanisms involved in susceptibility to infection by HIV-1. This study evaluated the distributions of single nucleotide polymorphisms (SNPs) in human genes related with the entry (*CCL3, CCL4, CCL5, CXCL12, CXCR6*) and viral replication (*APOBEC3G, CUL5, TRIM5, HLA-C and ZNRD1*), and their likely associations with the modulation of susceptibility to HIV-1 in a population of Northeast Brazil (Recife-Pernambuco) in order to establish a model immunogenetic susceptibility to HIV-1. A study case-control was developed using infected patients (HIV-1+) and healthy controls, which were genotyped for 18 SNPs in genes known to be involved in the entry and viral replication. It was found that variants in *CCL3* (rs1719134), *CCL4* (rs1719153), *TRIM5* (rs10838525) and *CUL5* genes (rs11212495) were more frequent in healthy controls; while variants in *APOBEC3G* and *ZNRD1* (rs3869068) were more frequent in infected patients, suggesting respectively, protection and susceptibility to HIV-1 in Pernambuco population. In this regard, it is suggested that SNPs in genes involved in viral entry and replication can modulate the susceptibility of HIV-1.

Key words: HIV-1, SNPs, Susceptibility, Chemokines, Restriction factors, HLA gene, Genetics variability.

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Lista de Abreviaturas, Siglas e Símbolos

Item	Definição
AIDS	Acquired Immunodeficiency Syndrome
APOBEC3G	Apolipoproteina B mRNA editing enzyme catalytic polypeptide-like 3G
AZT	Azidothymidine
CCL3	Chemokine (C-C motif) ligand 3
CCL4	Chemokine (C-C motif) ligand 4
CCL5	Chemokine (C-C motif) ligand 5
CCR5	Chemokine (C-C motif) receptor 5
CD4+	CD4 receptor positive
CGA	Candidate gene association
CTL	Cytotoxic T lymphocyte
CUL5	Cullin 5
CXCL12	Chemokine (C-X-C motif) ligand 12
CXCR4	Chemokine (C-X-C motif) receptor 4
CXCR6	Chemokine (C-X-C motif) receptor 6
DC-SIGN	Dendritic cell-specific Intercellular adhesion molecule-3-grabbing non-integrin
gp120	Glycoprotein 120
gp41	Glycoprotein 41
GWA	Genome-wide association study
HAART	Highly active antirretroviral therapy

HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HLA-C	Human Leucocyte Antigen, class I, C
HTLV-III	Human T-lymphotrophic virus type III
IFNG	Interferon gamma
kDa	kDalton
KIR	Killer immunoglobulin-like receptor
LAV	Lymphadenopathy-associated virus
MIP-1α	Macrophage inflammatory protein-1 alfa
MIP-1β	Macrophage inflammatory protein-1 beta
mL	Milliliter
NK	Natural Killer
PARD3B	Par-3 partitioning defective 3 homolog B
PROX1	Prospero homeobox 1
RANTES	Regulated upon activation normal T cell expressed and secreted
RT	Reverse transcriptase
SDF-1	Stromal cell derived factor
SNPs	Single-nucleotide polymorphism
TGFβ	Transforming Growth Factor Beta
TRIM5	Tripartite motif containing 5
UNAIDS	United Nations Programmer on HIV/AIDS
USA	United States of American
Vif	Viral infectivity factor
ZDV	Zidovudine
ZNRD1	Zinc ribbon domain containing 1

Sumário

1. Introdução

A Síndrome da Imunodeficiência Adquirida (AIDS) é uma doença infecciosa, ocasionada predominantemente pelo Vírus da Imunodeficiência Humana do tipo 1 (HIV-1). É caracterizada pela perda progressiva de linfócitos T $CD4^+$ auxiliares, ocasionando um quadro severo de deficiência imune, que na ausência de tratamento, pode conduzir o indivíduo a morte.

Apesar de significativos avanços na prevenção e tratamento, estimativas revelam que mais de 35 milhões de pessoas vivem com o vírus em todo o mundo, fazendo da AIDS, uma das principais causas de morbidez e mortalidade por doenças infecciosas.

A susceptibilidade à infecção pelo HIV-1 apresenta um acentuado grau de heterogeneidade individual, o que pode ser atribuída às variações genéticas do hospedeiro, aliada aos fatores virais e ambientais, sendo assim um processo complexo e de caráter multifatorial.

Alguns fatores hospedeiros como: as quimiocinas e seus receptores, os fatores de restrição viral e algumas proteínas codificadas no locus HLA, desempenham importantes papéis na susceptibilidade à infecção pelo HIV-1 e na progressão para AIDS. Tais fatores podem ser utilizados pelo vírus para entrar na célula hospedeira (receptores de quimiocinas), ou mesmo, através da competição pela ligação aos coreceptores, podem atrapalhar a entrada viral (quimiocinas). Outros podem bloquear a replicação viral pós-entrada (fatores de restrição), ou mesmo desencadear mecanismos envolvidos na resposta imune inata e adapatativa ao vírus (proteínas codificadas pelo locus do antígeno leucocitário humano - HLA).

Ao longo dos anos, diversos grupos de pesquisa têm estudado o genoma humano em busca de variações genéticas relacionadas à infecção pelo HIV-1 e a progressão para AIDS, visando compreeender o papel dos fatores genéticos no curso da infecção, e também buscando novos alvos biológicos para o desenvolvimento de novas intervenções profiláticas e terapêuticas.

Neste sentido, propusemo-nos a avaliar a distribuição e o envolvimento, na susceptibilidade a infecção pelo HIV-1, de polimorfismos de base única (SNPs) em genes relacionados à entrada (*CCL3, CCL4, CCL5, CXCL12,* CXCR6) e a replicação viral (*APOBEC3G, CUL5, TRIM5, HLA-C* e *ZNRD1*) em pacientes infectados (HIV-1^{+}) e controles saudáveis de uma população do Nordeste brasileiro, visando estabelecer um modelo imunogenético para a susceptibilidade à infecção pelo HIV-1.

2. Revisão da Literatura

2.1 Síndrome da Imunodeficiência Adquirida (AIDS)

A Síndrome da Imunodeficiência Adquirida (AIDS) é uma doença ocasionada pelo Vírus da Imunodeficiência Humana (HIV), que se caracteriza por uma perda progressiva de linfócitos T $CD4^+$ auxiliares, e consequentemente, um quadro de deficiência imune severa, doenças constitucionais (sinais e sintomas com período de duração maior que um mês: febre, diarreia e perda de peso), infecções oportunistas, agravos neurológicos e neoplasias incomuns a indivíduos imunocompetentes (Hutchinson 2001; Weiss 2008).

2.1.1 Histórico

Os primeiros seres humanos que entraram em contato com o HIV, viviam em florestas e praticavam atividades silvícolas (caça), fato que, provavelmente, intermediou os primeiros contatos com fluídos corpóreos do hospedeiro natural do vírus, a espécie de chimpanzé *Pan troglodytes troglodytes* (Gao et al. 1999), e as primeiras infecções entre os humanos. O isolamento dessas comunidades impediu a disseminação do vírus por muito tempo. Na maioria das vezes, as pessoas infectadas morriam sem nunca ter entrado em contato com outras pessoas (Sharp et al. 2001; Wain et al. 2007).

A disseminação viral para os centros urbanos teve início após a Segunda Guerra Mundial, impulsionada pelo aumento do êxodo rural, e coincidentemente, com o crescimento da prostituição e do uso de drogas injetáveis (Karpas 2004; Gallo 2006).

Os primeiros registros oficiais de indivíduos com AIDS datam de 1981, quando jovens homossexuais das cidades de Nova Iorque, São Francisco e Los Angeles, nos Estados Unidos, foram diagnosticados com pneumonia ocasionada por *Pneumocystis carinii* e sarcoma de Kaposi, apresentando uma forte depleção de células T $CD4^+$ auxiliares. No entanto, não se tinha conhecimento das causas dessa deficiência imune (Karpas 2004; Gallo 2006; Weiss 2008).

Apenas em 1982, a doença passou a ser denominados de AIDS, ficando clara a existência de um agente infeccioso na promoção da doença, que acometia em sua maioria, homossexuais, usuários de drogas e indivíduos submetidos a transfusões sanguíneas. Com a manifestação da doença em pacientes com hemofilia, especulou-se que a AIDS era ocasionada por algum tipo de vírus, sendo identificado o primeiro grupo de risco a infecção, denominado de “Os quatro Hs” (hemofílicos, viciados em heroína, homossexuais e haitianos) (Karpas 2004; Gallo 2006; Weiss 2008).

Em 23 de maio de 1983, Françoise Barré-Sinoussi e colaboradores, conduzidos por Luc Montagnier, publicaram a descrição de um tipo viral desconhecido presente em pacientes com linfadenopatia, denominado de LAV - Vírus associado à Linfadenopatia (Barré-Sinoussi et al. 1983). No mesmo ano, Robert Gallo e colaboradores descreveram outro tipo viral, o HTLV-III - Vírus Linfotrópico Humano tipo III (Gallo et al. 1983), como possível causa da AIDS

(Gallo 2006; Weiss 2008). Após clonagem e sequenciamento do material genético viral, percebeu-se que todos os vírus previamente descritos como agentes causadores da AIDS, tratavam-se de uma única espécie, e a partir de 1986 passou a ser denominado “Vírus da Imunodeficiência Humana – HIV” (Weiss 2008).

Os primeiros testes sorológicos começaram ser disponibilizados a partir de 1984, clarificando que a AIDS era ocasionada por um vírus (HIV) e que não estava restrita aos países ocidentais (Gallo 2006; Weiss 2008).

Em 1987, foi lançada a azidotimidina (AZT) ou zidovudina (ZDV), como a primeira droga antirretroviral, mas seus resultados não foram satisfatórios. Depois dela, várias outras drogas foram desenvolvidas e testadas, culminando, em 1996, com a terapia antirretroviral altamente ativa (do inglês “*highly active antirretroviral therapy”*, HAART), uma combinação de três drogas antirretrovirais que promoveu uma verdadeira revolução no tratamento, reduzindo a carga viral, com consequente recuperação do sistema imune na maioria dos pacientes (Vella et al. 2012).

2.1.2 Epidemiologia

A Pandemia HIV/AIDS é considerada uma das principais causas de morbidez e mortalidade por doenças infecciosas no mundo. Segundo o relatório Anual do Programa das Nações Unidas para HIV-AIDS (UNAIDS) divulgado em 2014, estima-se que em média 35 milhões de pessoas vivem com HIV em todo mundo. As estimativas mostraram 2,1 milhões de novas infecções (equivalente a

6 mil novas infecções por dia) e 1,5 milhões de óbitos distribuídos por todo mundo (Tabela 1) (UNAIDS 2014).

Tabela 1. Estimativa Mundial da Epidemia da AIDS em 2013, segundo o Relatório Anual da UNAIDS 2014.

Número de Pessoas vivendo com o HIV	Total	35,0 milhões (33,2 – 37,2 milhões)
	Adultos	31,8 milhões (30,1 – 33,7 milhões)
	Mulheres	16,0 milhões (15,2 – 16,2 milhões)
	Crianças < 15 anos	3,2 milhões (1,6 – 3,4 milhões)
Pessoas infectadas com HIV em 2013	Total	2,1 milhões (1,9 – 2,4 milhões)
	Adultos	1,9 milhões (1,7 – 2,1 milhões)
	Crianças < 15 anos	240 mil (210 – 280 mil)
Mortos por AIDS em 2013	Total	1,5 milhões (1,4 – 1,7 milhões)
	Adultos	1,2 milhões (1,2 – 1,5 milhões)
	Crianças < 15 anos	190 mil (170 – 220 mil)

Fonte: UNAIDS (2014).

O Brasil ocupa o topo do ranking entre os países latino americanos, com mais de 757 mil casos e mais de 270 mil óbitos em decorrência de AIDS (Ministério da Saúde/Brasil 2013; UNAIDS 2014). Considerando os dados acumulados de 1980 a julho de 2013, no Brasil já foram notificados um total de 686.478 casos de AIDS, dos quais 445.197 (64,9%) são do sexo masculino e 241.233 (35,1%) do sexo feminino (Ministério da Saúde/Brasil 2013). Dentre as regiões do país mais afetadas, a região Sudeste apresenta o maior percentual de notificações (55,2%), seguida pelas regiões Sul (20,0%) e a região Nordeste (13,9%). As regiões Centro-Oeste e Norte apresentam 5,8% e 5,1% dos casos, respectivamente (Tabela 2) (Ministério da Saúde/Brasil 2013).

Tabela 2. Indicadores Epidemiológicos da Epidemia de HIV/AIDS no Brasil.

Indicadores Epidemiológicos	Brasil	Indicadores por Região do País				
		Norte	Nordeste	Sudeste	Sul	Centro-Oeste
Casos Registrados*	686.478	35.100	95.516	379.045	137.126	39.691
Incidência**	20,2	21,0	14,8	20,1	30,9	19,5
Óbitos*	265.698	9.993	30.717	166.343	45.508	13.126
Mortalidade**	5,5	5,6	4,0	5,6	7,7	4,7

Fonte: Ministério da Saúde
** Registros de 1980 até julho 2013; ** A cada 100.000 habitantes - ano base de 2012*

O Estado de Pernambuco, entre os estados nordestinos, ocupa o primeiro lugar em números de casos acumulados (23.024 casos) e em número de óbitos (8.531 óbitos) (Ministério da Saúde/Brasil 2013). Adicionalmente, a cidade do Recife se destaca como a 7ª capital brasileira com maior taxa de incidência da doença, com 39 indivíduos infectados para cada 100 mil habitantes em 2012 (Ministério da Saúde/Brasil 2013).

2.1.3 Vírus da Imunodeficiência Humana - 1 (HIV-1)

2.1.3.1 Características gerais

O vírus da imunodeficiência humana (HIV), pertence à família Retroviridae, sub-família Lentivirinae e ao gênero dos Lentívirus. É responsável por uma infecção crônica que gradualmente danifica o sistema imunológico do hospedeiro e quando não tratada pode conduzi-lo ao óbito (Hahn et al. 2000; Hutchinson 2001; Naif 2013).

Dois tipos virais são responsáveis pela infecção: o HIV-1, com distribuição cosmopolita; e o HIV-2, restrito a região da África Ocidental e alguns poucos países como Portugal e Índia (Karpas 2004; Weiss 2008).

O HIV-1 é considerado um dos vírus mais polimórficos conhecidos, característica que pode ser diretamente atribuída à mudança de nucleotídeos promovidas pela transcriptase reversa (RT) viral e a sua complexidade na formação do cDNA. Assim, juntamente com fatores hospedeiros, a evolução do genoma viral é considerada base de todas as mudanças nas características biológicas do HIV-1, incluindo a capacidade citopática, a resistência aos antirretrovirais, o uso de correceptores e o tropismo viral (Naif 2013).

Filogeneticamente, o HIV-1 pode ser divido em quatro grupos (M, N, O e P), além dos subtipos (Grupo M: A-D, F-H, J e K) e formas recombinantes (CRFs e URFs) (Robertson et al. 2000; Geretti 2006; Plantier et al. 2009).

Considerando o tropismo viral, o HIV-1 pode ser dividido em três grupos principais: macrófagos-trópicos (M - trópico), células T trópicos (T - trópico) e com tropismo duplo (dual-trópico) (Clapham and McKnight 2001; Naif 2013).

A transmissão do HIV-1 ocorre por meio do contanto entre fluídos corporais, oriundos de indivíduos infectados (sangue e sêmen), com regiões de mucosas ou com ferimentos, através da exposição sexual (homossexual e heterossexual), da exposição intravenosa entre usuários de drogas (compartilhamentos de objetos pontiagudos e perfurantes) e durante procedimentos de transfusões sanguíneas, ou mesmo, através da exposição vertical (transmissão de mãe para filho) (Hutchinson 2001).

2.1.3.2 Estrutura Viral

Estruturalmente, o HIV-1 assume uma forma esférica (aproximadamente 100 nm de diâmetro) constituída, basicamente, por uma bicamada lipídica, de origem hospedeira, onde podemos encontrar duas importantes glicoproteínas: uma transmembranar (gp41) e outra de superfície (gp120), as quais atuam na fusão da membrana e na ligação aos receptores celulares, respectivamente (Figura 1) (Hahn et al. 2000; Hutchinson 2001).

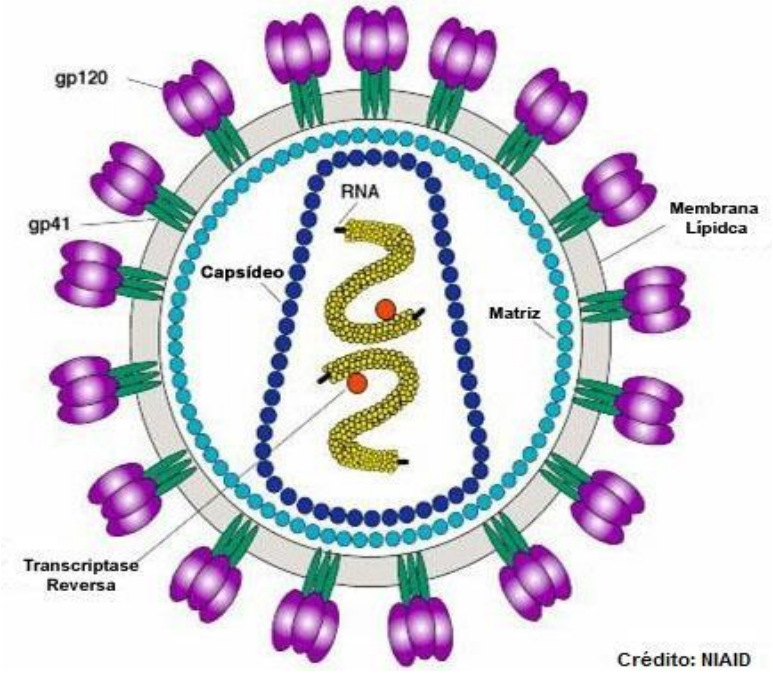


Figura 2. Vírus da Imunodeficiência Humana tipo 1. Em destaque as duas fitas de RNA, a enzima transcriptase reversa, a membrana lipídica, a matriz proteica, e as glicoproteínas do envelope viral (gp120 e gp41). *Fonte: www.stanford.edu/gruop/virus/retro/2005gongishmail/HIV.html*

Logo abaixo da membrana, encontra-se uma matriz estrutural, constituída por proteínas de matriz associadas (p17), igualmente necessárias para

incorporação do complexo gp120-gp41 na formação de novos vírus (Yu et al. 1992; Hutchinson 2001; Freed 2001).

No centro da estrutura viral, encontra-se um capsídeo com formato cônico e constituído de várias unidades da proteína p24, que abriga o genoma viral, o qual é composto por duas moléculas de RNA de fita simples, idênticas, não-complementares e associadas a transcriptase reversa (RT), com aproximadamente 10.000 nucleotídeos, organizados em nove regiões gênicas responsáveis pela codificação de 19 proteínas diferentes (Figura 2) (Hutchinson 2001; Freed 2001).

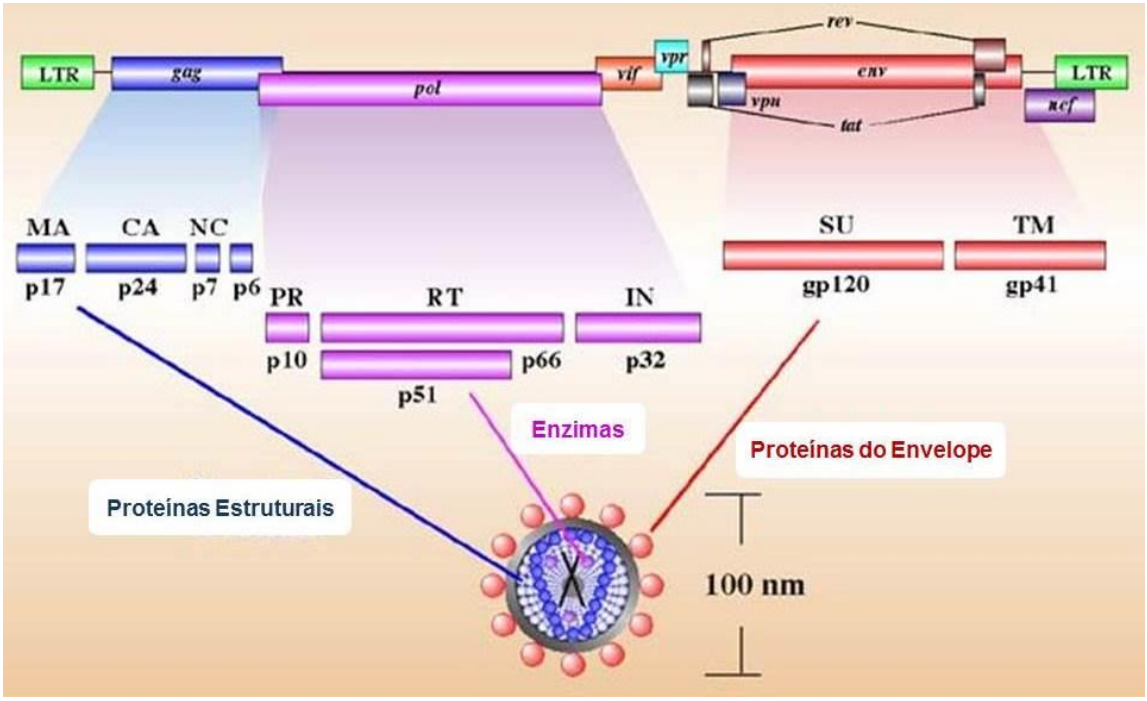


Figura 2. Genoma do Vírus da Imunodeficiência Humana tipo 1. MA = proteínas matriz, CA = capsídeo, NC = proteínas núcleo-estabilizadoras de RNA, PR = protease, RT = transcriptase reversa, IN = integrasse, SU = proteínas de superfície, TM = proteínas transmembranares. Adaptada a partir de: http://www.liquidarea.com/2009/08/aids-decifrato-genoma-virus/.

Estas proteínas podem ser, genericamente, divididas em três classes, de acordo com sua funcionalidade (Turner and Summers 1999; Hutchinson 2001; Freed 2001; Gummuluru and Emerman 2002):

- Proteínas estruturais (*Gag, Pol* e *Env*) – responsáveis pela maquinaria de replicação e estrutura dos vírions;
- Proteínas regulatórias (*Tat* e *Rev*) – responsáveis pela transcrição e tradução dos genes virais;
- Proteínas acessórias (*Nef, Vif, Vpr e Vpu/Vpx*) – responsáveis por diversas funções incluindo: estimulação da replicação, infectividade viral, processamento de proteínas virais e escape imunológico.

O capsídeo viral abriga também nucleoproteínas estabilizadoras de RNA (p7) e as enzimas virais: transcriptase reversa, protease e integrase, as quais estão diretamente relacionadas com à transcrição viral, o processamento de proteínas e integração do genoma viral no genoma hospedeiro, respectivamente (Turner and Summers 1999; Hutchinson 2001; Freed 2001; Gummuluru and Emerman 2002).

2.1.3.3 Ciclo Viral

Primariamente, o HIV-1 infecta linfócitos T $CD4^+$, macrófagos e células dendríticas, comprometendo o sistema imunológico humano. O ciclo viral é iniciado pela ligação da glicoproteína do envelope viral gp120 aos receptores de superfície celular (CD4) e correceptores presentes em macrófagos (CCR5) e

linfócitos T (CXCR4), promovendo a fusão do envelope viral à membrana da célula hospedeira (Hutchinson 2001; Freed 2001; Smith et al. 2009; Fanales-Belasio et al. 2010).

À medida que ocorre a fusão entre membranas, o capsídeo é liberado para o citoplasma, onde ocorre a desencapsulação, liberando o material genético viral. A transcriptase reversa se acopla e percorre a fita de RNA viral, produzindo uma fita de DNA complementar. Depois de formada a primeira fita de DNA complementar, a transcriptase reversa dá inicio à formação da segunda fita de DNA, usando a primeira como molde (Figura 3) (Hutchinson 2001; Freed 2001; Smith et al. 2009; Moir et al. 2011; Naif 2013).

A dupla fita de DNA viral recém-formada é conduzida para o núcleo celular, onde através da ação da integrase, é inserida no genoma hospedeiro, tornando-se um pró-vírus e estabelecendo a infecção permanente. O pró-vírus pode permanecer latente ou usar a maquinaria de transcrição da célula hospedeira para induzir a expressão de genes virais (Hutchinson 2001; Freed 2001; Smith et al. 2009).

O HIV-1 induz a transcrição em duas fases distintas. Na primeira fase, com duração aproximada de 24 horas, a maquinaria da célula hospedeira é induzida a transcrever o DNA pro-viral (integrado) em cópias complementares de RNA, as quais são processadas gerando moléculas de RNA mensageiro maduras que serão traduzidas em proteínas regulatórias. Após a tradução, essas proteínas são dirigidas para o citoplasma, onde produzirão novas partículas virais (Hutchinson 2001; Freed 2001; Smith et al. 2009).

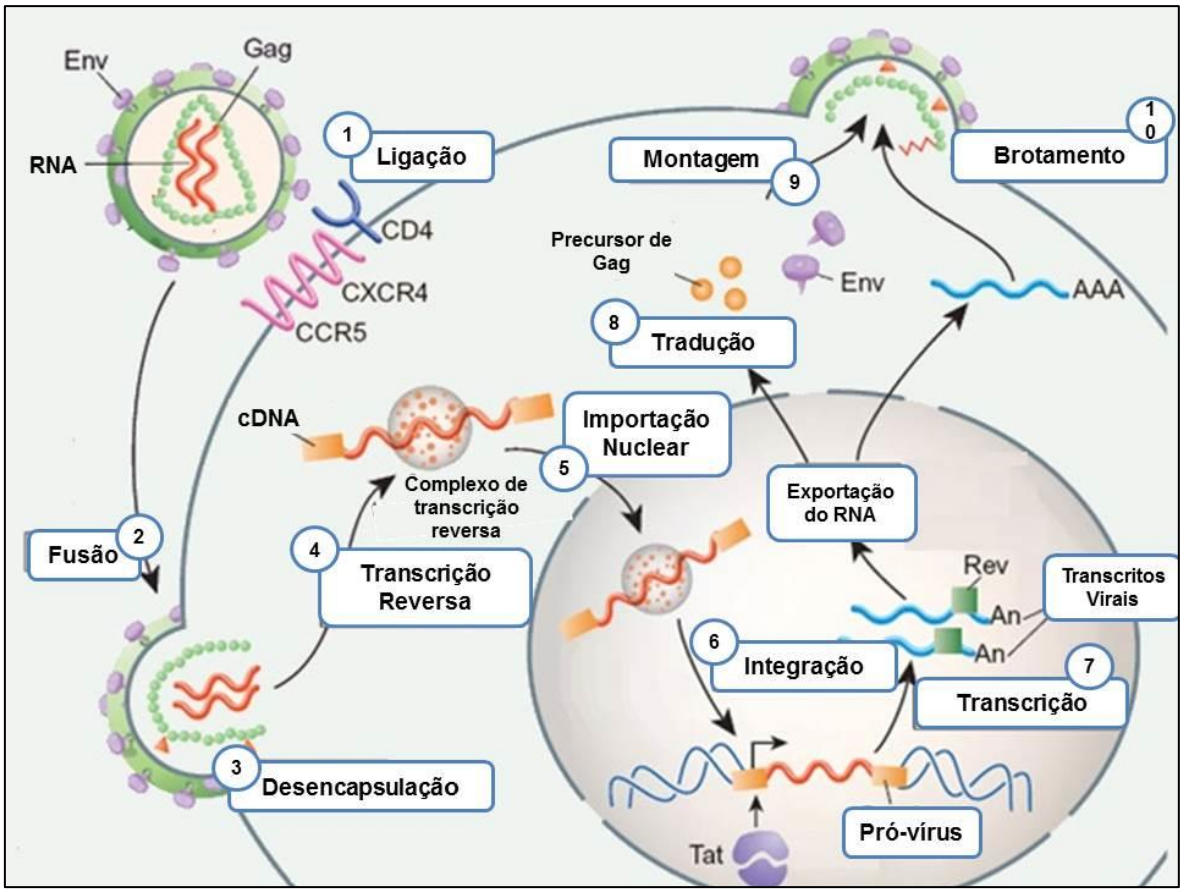


Figura 3. Ciclo Viral do HIV-1. O vírus se liga a receptores celulares (1) e se fusiona com a membrana celular entrando na célula (2). Ao entrar, é desencapsulado (3) e o RNA viral é retrotranscrito em uma dupla fita de cDNA viral (4), a qual é direcionada para o núcleo celular (5), onde será integrada ao genoma hospedeiro, tornando-se um pró-vírus (6). Posteriormente, o pró-vírus se utiliza da maquinaria da célula hospedeira e induz transcrição (7) e tradução em proteínas virais (8), as quais se unem ao RNA viral formando novos vírus (9), os quais deixam a célula hospedeira (10). Adaptada de: http://www.lookfordiagnosis.com/mesh_info.php.

Em uma segunda fase de transcrição, o transcrito de RNA não processado, torna-se uma nova fita viral, migrando para o citoplasma, onde duas novas classes de RNA com tamanhos diferenciados são produzidas. Os transcritos longos (~10.000 bases) não processados formarão o genoma viral, enquanto os transcritos menores (~4500 bases) processados (virions) codificarão as proteínas

estruturais e as enzimas virais. Por fim, o capsídeo é reestabelecido em torno desse material e direcionado para a periferia celular, gerando novos vírus e destruindo a célula hospedeira infectada (Hutchinson 2001; Freed 2001; Smith et al. 2009).

2.1.3.4 Patogênese do HIV-1

Durante os dias iniciais do contato com o vírus, as células infectadas são imunologicamente ativadas (principalmente macrófagos distribuídos nas mucosas), e migram para tecidos linfoides, onde o contato direto intercelular favorece a disseminação viral. A resposta imune local não depura a replicação viral, conduzindo para a uma fase aguda, caracterizada por uma grande quantidade de vírus circulando no plasma sanguíneo, aumentando o potencial infeccioso do indivíduo. Apenas um terço dos infectados apresentam sintomas (febre, mal-estar ou até mesmo encefalite) nos primeiros seis meses após a exposição ao vírus (Sleasman and Goodenow 2003; Stevenson 2003; Moir et al. 2011; Naif 2013).

Após a fase aguda da infecção (Figura 4), o sistema imune do hospedeiro começa a gerar resposta contra o vírus, fazendo com que a viremia diminua em magnitude e seja estabilizada. No entanto, o HIV-1 apresenta vários mecanismos de escape da resposta imune, entrando em latência nas células infectadas, formando “reservatórios” virais. Transientemente, ocorrem picos de replicação virais, causando a doença crônica e assintomática nos tecidos linfoides (Fanales-Belasio et al. 2010).

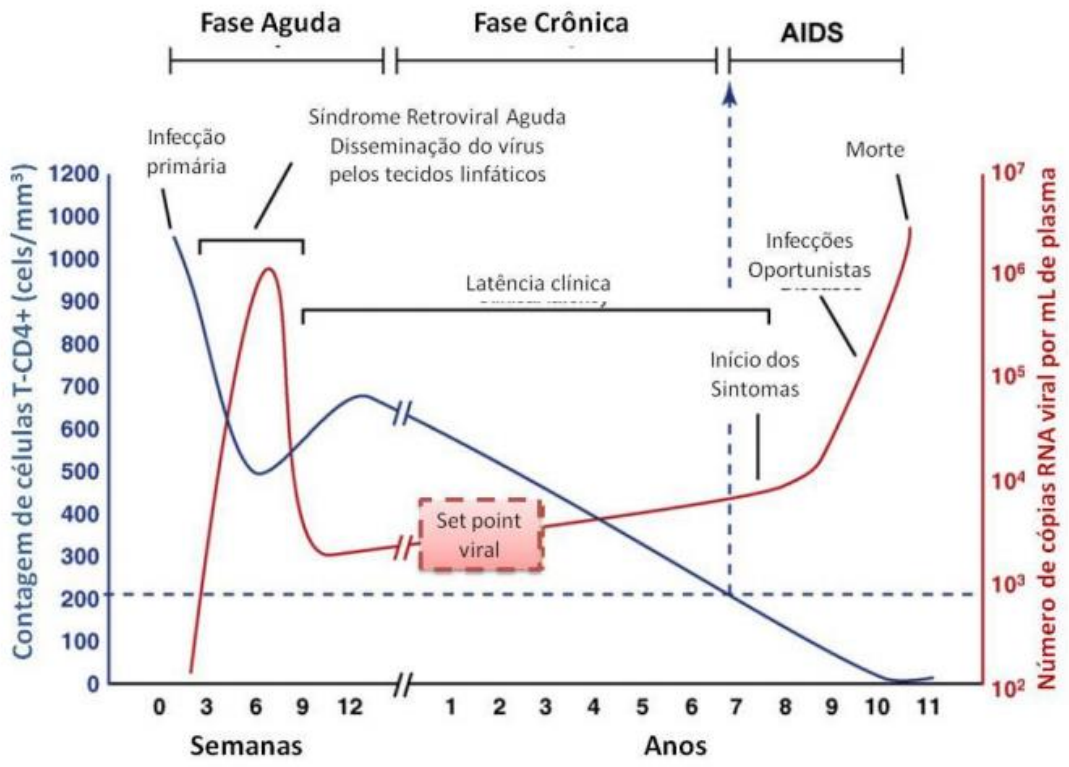


Figura 4. Curso clínico natural da infecção pelo HIV-1. Adaptado de: An e Winkler, 2010.

Dessa forma, a história natural da infecção pelo HIV-1 pode ser entendida como um mecanismo de “torneira e ralo” (*ipsis litteris* do inglês “tap and drain”). O componente “torneira” equivaleria à capacidade do timo do hospedeiro em repor células T $CD4^+$, enquanto o “ralo” equivaleria ao ritmo em que as células T $CD4^+$ são destruídas pelo vírus e pela resposta imune citotóxica. Ao longo dos anos de doença crônica pelo HIV-1, a capacidade de restauração do sistema imune vai sendo exaurida e não mais consegue equilibrar as perdas causadas pelo HIV-1, resultando num crescente déficit de células T $CD4^+$ (Sleasman and Goodenow 2003; Moir et al. 2011).

Apesar de mais de três décadas de estudo, os mecanismos que determinam precisamente a diminuição das concentrações de células T $CD4^+$ ainda não foram elucidados, consistindo em uma das principais áreas de pesquisa

do HIV/AIDS. É sabido que a perda progressiva de células T $CD4^+$ em pacientes infectados pelo HIV-1 possui grande contribuição da morte dessas células nos tecidos linfoides. O déficit na recuperação dos níveis de $CD4^+$ também podem ser atribuído à produção reduzida de células T CD4 naïve, especialmente recém-imigradas do timo, elevados níveis de morte celular (apoptose e piroptose) e excessiva ativação das células T (Doitsh et al. 2014)

Com isso, 10 anos após a exposição (em média), níveis de linfócitos T $CD4^+$ atingem valores abaixo de 200 células/µL de sangue, o que caracteriza o estágio clínico de AIDS (CDC, 1993), visto que nesse momento, o indivíduo passa a estar em grande risco de desenvolver doença constitucional (sinais e sintomas com duração maior que um mês; febre, diarreia e perda de massa corporal) e cânceres, bem com contrair infecções oportunistas, que, na ausência de tratamento antirretroviral, podem conduzir o portador à morte (Hutchinson 2001; Naif 2013).

2.2 Fatores hospedeiros envolvidos na infecção pelo HIV-1

Ao longo do processo evolutivo, o hospedeiro humano e seus patógenos seguem lado a lado em uma luta interminável pela adaptação. O hospedeiro desenvolve sofisticadas estratégias visando à eliminação do patógeno, enquanto o patógeno busca escapar do ataque hospedeiro, se estabelecer e dar continuidade ao seu ciclo infeccioso. Um exemplo clássico desta luta pela sobrevivência é a infecção pelo HIV-1, o qual se utiliza da maquinaria das células de defesa do hospedeiro para se replicar, enquanto o hospedeiro desencadeia

uma série de respostas imunológicas, visando restringir a replicação viral e debelar a infecção (Derdeyn and Silvestri 2005; Baumann 2006; Pitha 2011; Guha and Ayyavoo 2013).

A resposta hospedeira frente à infecção pelo HIV-1 utiliza uma série de fatores inatos, dentre os quais, merecem destaque: as quimiocinas e seus receptores, os fatores de restrição viral e proteínas codificadas pelo lócus HLA. Tais fatores são necessários durante o processo de entrada viral (receptores de quimiocinas e quimiocinas) e também na replicação viral (fatores de restrição e proteínas codificadas pelo lócus HLA) (Derdeyn and Silvestri 2005; Baumann 2006; Pitha 2011; Guha and Ayyavoo 2013).

2.2.1 Quimiocinas e seus receptores

As quimiocinas são proteínas quimiotáticas, solúveis e de baixo peso molecular (~8-15kDa) que mediam suas funções através do recrutamento de células portadoras de receptores acoplados à proteína G, como parte de tráfego de células imunes homeostáticas ou durante a resposta inflamatória. Na infecção pelo HIV-1, merecem destaque, visto que o vírus utiliza os receptores de quimiocinas (CCR5, CXCR4) para entrar na célula hospedeira (Murdoch and Finn 2000; Jr et al. 2003; Modi et al. 2006; Allen et al. 2007; Paximadis et al. 2013)

É sabido que a entrada do HIV-1 na célula hospedeira é mediada pela ligação entre a proteína gp120 e o receptor CD4 presente, principalmente, em células T, com a participação de outros receptores ou correceptores. Os principais correceptores do HIV-1 são os receptores de quimiocinas CCR5 (usados por

cepas R5 HIV-1) e CXCR4 (usados por cepas X4 HIV-1) (Singh et al. 2008; An and Winkler 2010). No entanto, o HIV-1 também pode utilizar outros correceptores, como DC-SIGN (Da Silva et al. 2011) e o CXCR6 (Matloubian et al. 2000).

O correceptor CXCR6 é um receptor de quimiocina, que suporta variáveis níveis de replicação do HIV-1. É codificado pelo gene *CXCR6*, localizado no cromossomo 3 (3p21) e possui como ligante natural a quimiocina CXCL16, a qual compete com o vírus pela ligação ao correceptor CXCR6 (Matloubian et al. 2000; Kim et al. 2001; Blaak et al. 2005; Passam et al. 2007; Limou et al. 2010).

As quimiocinas inflamatórias (*MIP-1α, MIP-1β* e *RANTES)* apresentam uma acentuada atividade supressora do HIV-1 atribuída à ligação ao receptor CCR5. Elas inibem a entrada viral na célula pela competição com a proteína do HIV-1 Env (gp120), bem como, através da sub-regulação da expressão CCR5 na superfície celular, ou seja, à medida que a quimiocina se liga ao CCR5 ocorre a internalização do receptor. Essas proteínas são codificadas pelos genes *CCL3* (*MIP-1α* – "Macrophage inflammatory protein-1 alfa"), *CCL4* (*MIP-1β* – "Macrophage inflammatory protein-1 beta") e *CCL5* (*RANTES* – "Regulated upon activation normal T cell expressed and secreted") que formam um cluster gênico no cromossomo 17 (17q12) (Colobran et al. 2007; Hu et al. 2012).

Além dos ligantes naturais do receptor CCR5, também temos quimiocinas que se ligam ao correceptor CXCR4, como SDF-1 (Stromal cell derived factor), codificado pelo gene *CXCL12* localizado no cromossomo 10 (Modi et al. 2005; Petersen et al. 2005; Colobran et al. 2007; Chaudhary et al. 2008; Garcia-moruja et al. 2009; Gianesin et al. 2012). Essa quimiocina está envolvida na migração de

células hematopoiéticas e na migração transendotelial de leucócitos, desempenhando também papel na infecção pelo HIV-1. SDF-1 compete com a gp120 de vírus X4 T-trópico pela ligação ao CXCR4. A interação entre a quimiocina e o receptor promove sua internalização e previne a entrada viral (Modi et al. 2005; Petersen et al. 2005; Colobran et al. 2007; Chaudhary et al. 2008; Garcia-moruja et al. 2009; Gianesin et al. 2012).

2.2.2 Fatores de restrição viral e proteínas associadas

Fatores de restrição são proteínas celulares que podem restringir ou bloquear a replicação viral de uma maneira célula-específica. Vários fatores de restrição têm sido identificados, entre eles: APOBEC3G e TRIM5α (Wolf and Goff 2008; Lever and Jeang 2011; Lever and Lever 2011; Sze et al. 2013).

A “tripartite motif-containing protein 5 alpha”, ou simplesmente, proteína TRIM5α é um importante fator de restrição que promove o bloqueio da replicação do HIV-1, no estágio de pós-entrada e pré-integração, pelo reconhecimento e prematuro desnudamento do capsídeo viral, prevenindo a transcrição reversa (Stremlau et al. 2004; Stremlau et al. 2006; Chatterji et al. 2006; Battivelli et al. 2011).

A proteína TRIM5α é codificada pelo gene *TRIM5*, localizado no cromossomo 11 (11p15) (Reymond et al. 2001), sendo constituído por 8 éxons e 7 introns. É capaz de produzir vários transcritos por processamento alternativo, incluindo a isoforma TRIM5α (Stremlau et al. 2004). A proteína formada é composta por quatro domínios distintos (“RING”, “B-box 2”, “Coiled-coil” e o

terminal C SPRY) envolvidos em importantes mecanismos para restrição viral, tais como: atividade ubiquitina E3 ligase (Javanbakht et al., 2005; Stremlau et al., 2004), formação de corpos citoplasmáticos contendo TRIM5α (Javanbakht et al. 2005; Diaz-Griffero et al. 2009), reconhecimento do capsídeo (Mische et al. 2005; Javanbakht et al. 2006b; Maillard et al. 2010) e reconhecimento específico e restrição de retrovírus (Li et al., 2007; Ohkura et al., 2006; Stremlau et al., 2006; Stremlau et al., 2005).

Outro fator de restrição viral, bastante conhecido é a apolipoproteina B catalítico-enzimática de edição de RNA mensageiro tipo 3G (APOBEC3G), uma proteína antiviral, membro de uma família de enzimas de edição de RNA, que inibe a replicação do HIV-1 através da promoção de mutações deletérias no genoma viral (Biasin et al. 2007; Desimmie et al. 2014). É codificada pelo gene *APOBEC3G*, localizado no cromossomo 22 (22q13.1-13.2), com uma extensão de ~9 kb distribuído em oito éxons e sete introns (Sheehy et al. 2002; An et al. 2004).

Em células infectadas com ausência do fator de infectividade viral (Vif), APOBEC3G é eficientemente incorporada no interior das partículas virais, sendo transferida para a próxima geração de células alvos, onde exercerá sua atividade antiviral (Wissing et al. 2010; Kitamura et al. 2011). Dentre os principais efeitos da APOBEC3G, pode-se destacar: a inibição da extensão da transcriptase reversa através de sua ligação direta ao RNA viral; deaminação de resíduos de citosina na fita de DNA negativa recém-sintetizada ocasionando a degradação do cDNA através da ação de uma uracilo-DNA-glicosidade celular ou da hipermutação G→A na cadeia positiva do DNA pro-viral; e a inibição da integração do DNA e a formação do pró-vírus (Sheehy et al. 2002; Malim 2009; Smith et al. 2009; Wissing et al. 2010; Kitamura et al. 2011; Jäger et al. 2012; Desimmie et al. 2014).

Por outro lado, em células contendo Vif, os efeitos antivirais da APOBEC3G são suprimidos. A interação de Vif com as proteínas celulares Cullina 5, Elongina B, Elongina C e Rbx1 permitem a formação do complexo ubiquitina E3 ligase, responsável pela indução da poliubiquitinação de proteínas alvos, como a APOBEC3G, e posterior degradação proteossomal (Kobayashi et al. 2005; Wissing et al. 2010; Jäger et al. 2012).

Neste processo, a proteína Cullina é tida com uma componente chave do complexo ubiquitina E3 ligase, interagindo diretamente com a proteína Vif do HIV-1(Kobayashi et al. 2005). A proteína Cullina 5 é uma membro da família ubiquitina E3 Cullina-RING, codificada pelo gene *CUL5*, o qual cobre aproximadamente 100 kb no cromossomo 11 (11q22) e consiste de 19 éxons e 18 introns (An et al. 2007a). Estudos têm mostrando que mutações ou sub-regulação por RNA de interferência no complexo Cullina pode bloquear a poliubiquinação e degradação da APOBEC3G induzida pela Vif, sugerindo que a habilidade supressora de Vif sobre APOBEC3G depende especificamente da função do complexo Cullina 5 (Yu et al. 2003; Liu et al. 2005).

2.2.3 Proteínas Codificadas pelo Lócus do Antígeno Leucocitário Humano (HLA)

O antígeno leucocitário humano (HLA) ou complexo de histocompatibilidade humana I (MHC-I), localizado no cromossomo 6, compreende moléculas clássicas de classe 1 (HLA-A, HLA-B e HLA-C), moléculas de classe 2 (DP, DQ, DR) e moléculas não clássicas (HLA-E, HLA-F e HLA-G),

que desempenham um papel central na resposta imune inata e adaptativa contra patógenos, como HIV-1 (Adams and Parham 2001; Zipeto and Beretta 2012). Dentre as várias proteínas codificadas no lócus HLA e em sua proximidade, duas, particularmente, têm sido evidenciadas por seus papéis no controle da replicação viral: HLA-C e ZNRD1 (Fan et al. 2000; Zipeto and Beretta 2012).

A proteína HLA-C é um heterodímero composto de uma cadeia pesada ancorada na membrana e uma cadeia leve, β_2-microglobulina (β_2M), naturalmente expressa na superfície celular cerca de 10 vezes menos que outros HLA de classe I (Zipeto and Beretta 2012). Essa molécula tem um duplo papel, visto que pode apresentar antígenos para linfócitos T citotóxicos (CTLs) e também pode inibir a lise de células "natural killer" (NK) via sua interação inibitória com o receptor KIR (do inglês "Killer immunoglobulin-like receptor") (Kulpa and Collins 2011; Zipeto and Beretta 2012; Celsi et al. 2013).

Muitos vírus, incluindo HIV-1, usam essa capacidade inibitória do HLA-C para facilitar a infecção no organismo hospedeiro. Eles promovem uma sub-regulação dos HLA-A e B, mas não HLA-C, visando se proteger do ataque por linfócitos T citotóxicas (CTL). Neste caso, a presença do HLA-C pode permitir a inibição de células NK expressando KIR. No entanto altos níveis de expressão de HLA-C pode aumentar a apresentação de antígenos a linfócitos T citotóxicos, interferindo na infecção viral (Kulpa and Collins 2011; Zipeto and Beretta 2012; Celsi et al. 2013). Esta proteína é codificada pelo gene *HLA-C*, mapeado no cromossomo 6 (6p21.33), e constituído por 8 éxons e 7 introns, distribuídos por uma extensão de aproximadamente 3,5 kb (Sodoyer et al. 1984).

Outra proteína relacionada codificada nas proximidades do lócus HLA, a ZNRD1 (do inglês “zinc ribbon domain-containing 1 protein”) também tem se destacado no contexto da infecção pelo HIV-1. ZNRD1 é uma RNA polimerase dependente de DNA envolvida na transcrição de DNA em RNA, requerida para replicação do HIV-1 (Brass et al. 2008). É codificada pelo gene *ZNRD1*, mapeado no cromossomo 6 (6p21.1), consiste de 4 éxons e 3 introns, ocupando aproximadamente 3.6 kb do DNA genômico (Fan et al. 2000). Estudos tem revelado que a ausência de ZNRD1 ocasiona a redução superior a 50% na replicação de cepas R5 ou X4-trópicos em células linfoides e não-linfoides, fato que pode ser relacionado com o processamento dos transcritos de HIV-1 mediado pela proteína regulatória viral Rev (Michienzi et al. 2000).

2.3 Variabilidade genética humana e a modulação da infecção pelo HIV-1

A variabilidade genética humana tem desempenhado um importante papel para a compreensão de mecanismos envolvidos na susceptibilidade a infecções humanas (Frazer et al. 2009). Grande impulso foi dado, com a publicação do genoma humano, onde milhares de variantes foram descritas, dentre os quais os polimorfismos de única base (SNPs) (Sachidanandam et al. 2001).

SNPs são variações pequenas e pontuais, ou seja em apenas uma única posição na sequência de DNA, presentes por todo genoma humano, que representam a maior fonte de variações genéticas interindividuais. De acordo com seu efeitos na cadeias de aminoácidos podem ser classificados em: SNPs sinônimos (variações que não alteram a sequência de aminoácidos) e SNPs não-sinônimos (variações que alteram a sequência de aminoácido) (Sachidanandam

et al. 2001; Frazer et al. 2009). São utilizadas na identificação de contribuições poligênicas em doenças, funcionando como uma extraordinária ferramenta na análise de marcadores genéticos, devido a sua abundância. Dependendo de sua localização podem promover alteração na expressão gênica, na ligação de fatores de transcrição e síntese proteica (Sachidanandam et al. 2001).

Neste contexto, a modulação da susceptibilidade ao HIV-1 é atribuída a uma interação complexa, entre fatores ambientais, fatores virais e fatores genéticos do hospedeiro, configurando um caráter multifatorial. Adicionalmente, a infecção pelo HIV-1 apresenta um grau substancial de heterogeneidade individual, em parte, explicada por variações genéticas (An e Winkler, 2010).

O uso combinado de análises de associação com genes candidatos (CGA) e estudos de associação de genoma completo (GWAS) permitiram a identificação e o estudo de diversas variantes genéticas, que direta ou indiretamente ajudam a entender a modulação da susceptibilidade ao vírus, bem como aspectos relacionados à patogênese e a progressão da AIDS (Cohen et al. 1997; Rowland-Jones et al. 2001; Nolan et al. 2004; Telenti and Carrington 2008; Kaur and Mehra 2009b; An and Winkler 2010; Telenti and McLaren 2010; Bol et al. 2011; Petrovski et al. 2011; Aouizerat et al. 2011; Troyer et al. 2011; Liu et al. 2011b) (Tabela 3).

Tabela 3. Fatores genéticos do hospedeiro envolvidos com HIV/AIDS.

Gene	SNPs	Mecanismos envolvidos	Efeitos na HIV/AIDS	Referências
CCR5	rs333 (Δ32)	Proteína trucada	Δ32/ Δ32: previne a aquisição; Δ32/+: retardo da progressão para AIDS	(Blanpain et al. 2002)
	rs1799987 (59029A)	Hiper-regulação da expressão de CCR5	Acelera a progressão para AIDS	(Clegg et al. 2000)

CCR2	rs1799864 (V64I)	Possível desequilíbrio de ligação com CCR6	Retardo da progressão para AIDS	(Smith 1997)
CXCR6	rs2234355		Aumenta tempo de sobrevivência após o diagnóstico de PCP	(Duggal et al. 2003)
CXCL12 (SDF1)	rs1801157 (3'A – 3'UTR)	Aumenta os níveis e a estabilidade do mRNA de CXCL12 comparado a 3'G	Retardo da progressão para AIDS	(Garcia-moruja et al. 2009)
CCL5 (RANTES)	rs2280789 (In1.1C – intron)	Hipo-regulação de CCL5	Aceleração da progressão para AIDS, risco de aquisição do HIV e infecção	(An et al. 2002; Ahlenstiel et al. 2005; Duggal et al. 2005; Koizumi et al. 2007; Rathore et al. 2008a)
	Haplótipo R3+R5		Aceleração da progressão para AIDS	(An et al. 2002)
	rs2107538 (-403)		Aumenta risco de infecção	(An et al. 2002)
	rs1800825 (-28)	Hiper-regulação	Retarda a progressão para AIDS	(Koizumi et al. 2007)
	3'222C		Aumenta o risco de infecção	(An et al. 2002)
CCL3L1	Polimorfismo no número de cópias em 17q11.2	Correlação com os níveis de CCL3L1	Aumento do número de cópias pode ser associado com resultados favoráveis	(Gonzalez et al. 2005; Mackay 2005; Huik et al. 2010; Liu et al. 2010)
CCL2-CCL17-CCL11	Hap 7 (31 kb) em 17q11.2-q12	Prováveis modificadores imunes	Prevenção na aquisição do HIV	(O'Brien and Nelson 2004)
CCL18-CCL3-CCL4	rs1719153, rs1719134, haplótipo (47kb) em 17q12		Aceleração da progressão para AIDS	(O'Brien and Nelson 2004)

DC-SIGN	rs4804803 (-336G)	Baixo nível de expressão de DC-SIGN em células dendríticas	Aumentado risco de aquisição do HIV-1	(Martin et al. 2004)
CUL5	rs7117111 (SNP5)		Aceleração progressão para AIDS	(An et al. 2007a)
	rs11212495 (SNP6)	Ligação de fator de transcrição		(An et al. 2007a)
	rs7103543 (SNP4)		Retardo na progressão para AIDS	(An et al. 2007a)
PPIA/ CypA	rs8177826 (1604G)	Ligação de fatores de transcrição	Aceleração da progressão para AIDS	(An et al. 2007b)
	rs6850 (1650G)	Aumento da infectividade do HIV-1	Aumento do risco de aquisição do HIV-1	(Bleiber et al. 2005; An et al. 2007b)
Tsg101	Haplótipo C		Retardo da progressão para AIDS	(Bashirova et al. 2006)
APOBEC3 G	rs8477832 (H186R)		Aceleração da progressão para AIDS e alta carga viral	(An et al. 2004; van Loggerenberg et al. 2008; Reddy et al. 2010)
	rs3736685 (197193C)	SNP intrônico em desequilíbrio de ligação com H186R	Aceleração da progressão para AIDS	(An et al. 2004)
	rs2294367 (199376C)	SNP intrônico	Aceleração da progressão para AIDS	(An et al. 2004)
	C400693T	SNP intrônico	Aumentado risco de aquisição do HIV-1	(Valcke et al. 2006)
APOBEC3 B	Δ3B/ Δ3B	Deleção do gene APOBEC3B	Aumentado risco de aquisição do HIV-1	(An et al. 2009)
TRIM5	rs16934386	Provável regulação da expressão de TRIM5	Aumenta o risco de aquisição do HIV-1	(Javanbakht et al. 2006a)
	rs10838525 (R136Q)	Melhor atividade anti-HIV-1	Prevenção da aquisição do HIV-1	(Javanbakht et al. 2006a)
	rs3740996 (H43Y)		Prevenção da aquisição do HIV-1	(Javanbakht et al. 2006a)

IL10	Promotor	Hiporegulação de IL10	Acelerada progressão para AIDS	(Shin et al. 2000)
IFNG	rs2069709 (-179T)	Aberrante regulação de IFNG	Acelerada progressão para AIDS	(An et al. 2003)
IRF-1	rs17848395 (619A) rs17848424 (6516G)	Hiporegulação e diminuição da resposta a IFN-r	Prevenção da aquisição de HIV-1	(Ball et al. 2007)
CXCR1 (receptor de IL8)	Haplótipo Ha	Modulação da expressão de CD4 e CXCR4	Retardo na progressão para AIDS	(Vasilescu et al. 2007)
HLA	Classe I A, B e C homozigotos	Redução do reconhecimento de epitopos		(Tang et al. 2008)
	B*35-Px	Fraca ligação de epitopos de ajuda e escape imune do HIV-1	Acelerada progressão para AIDS	(Gao et al. 2001)
	Concordância de HLA de classe I	Restrição de reconhecimento do repertório de epitopos	Aumento do risco de transmissão sexual e de mãe para filho do HIV-1	(Mackelprang et al. 2008)
	B*27	Dificulta o escape imune do HIV	Retardo na progressão para AIDS	(Carrington et al. 2008)
	B*57			(Carrington et al. 2008)
	rs9264942 (5' UTR de HLA-C)	Regula a expressão de HLA-C	Controle da carga viral no set point	(Fellay et al. 2007)
HCP5	rs2395029-G	Provável desequilíbrio de ligação com B*57	Controle da carga viral no set point	(Fellay et al. 2007)
KIR	KIR3DS1+HLA Bw4-80I	Sinalização das células NK para matar as células infectadas	Retardo na progressão para AIDS	(Carrington et al. 2008)
	KIR3DS1 na ausência de ligantes	Pobre regulação da atividade das células NK	Aceleração da progressão para AIDS	(Carrington et al. 2008)

ZNRD1	rs9261174 (5'UTR de ZNDR1)	Regula a expressão de ZNRD1	Retardo na progressão para AIDS	(Fellay et al. 2007)
Ly6	rs2572886	Alta susceptibilidade celular ao HIV-1 em células B e T primárias	Alta carga viral, rápido diminuição da contagem de células T $CD4^+$	(Loeuillet et al. 2008)
PROX1	rs17762192-C	Provável regulação da expressão de IFNG em células T	Retardo na progressão para AIDS	(Herbeck et al. 2010)

Adaptada a partir de An e Winkler (2010)

Como podemos observar na tabela 3, as variações em genes codificantes das quimiocinas e seus receptores, dos fatores de restrição e do lócus HLA estão entre os principais componentes envolvidos na modulação do curso da infecção pelo HIV-1. No entanto, inúmeros outras variantes gênicas, não listados na tabela, têm sido relacionados com a infecção pelo HIV-1 e progressão para AIDS (Nolan et al. 2004; Piacentini et al. 2009; Kaur and Mehra 2009a; Ortiz et al. 2009; Segat et al. 2010; Mogensen et al. 2010; Lever and Jeang 2011; Petrovski et al. 2011; Sobieszczyk et al. 2011; Segat and Crovella 2012; da Silva et al. 2012; Santa-Marta et al. 2013).

Adicionalmente, observa-se também que o papel das variações genéticas na infecção pelo HIV-1 pode variar em consonância com o perfil étnico da população analisada (Winkler et al. 2004). Os achados da literatura apontam consideráveis diferenças entre populações caucasianas e africanas, havendo carência de estudos em populações com grande mistura étnica, como a população do Brasil.

Considerando o perfil étnico da população do nordeste do Brasil (Recife) com diferenciado em relação as demais populações mundiais, chega-se a hipótese de que polimorfismos de base única em genes relacionados à entrada (*CCL3, CCL4, CCL5, CXCL12,* CXCR6) e a replicação viral (*APOBEC3G, CUL5, TRIM5, HLA-C* e *ZNRD1*) podem modular a infecção ocasionada pelo HIV-1, conduzindo a uma maior e/ou menor susceptibilidade à infecção.

3. Objetivos

3.1 Objetivo Geral

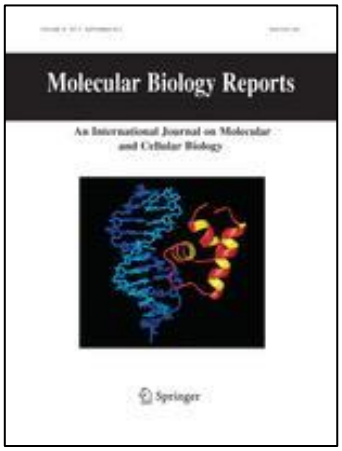
Verificar as distribuições de polimorfismos de base única (SNPs) em genes humanos relacionados à entrada e à replicação viral, e as prováveis associações com a modulação da susceptibilidade a infecção pelo HIV-1 em uma população do Nordeste brasileiro (Recife-Pernambuco), a fim de estabelecer um modelo imunogenético de susceptibilidade ao HIV-1.

3.2 Objetivos Específicos

1. Detectar e calcular as frequências alélicas, genotípicas e haplotípicas dos genes codificadores de quimiocinas e seus receptores (*CCL3, CCL4, CCL5, CXCL12, CXCR6*) em pacientes HIV-1+ e controles saudáveis.
2. Detectar e calcular as frequências alélicas, genotípicas e haplotípicas dos genes codificadores de fatores de restrição viral (*APOBEC3G, CUL5, TRIM5*) em pacientes HIV-1+ e controles saudáveis.
3. Detectar e calcular as frequências alélicas, genotípicas e haplotípicas de genes codificados pelo lócus HLA (*ZNRD1 e HLA-C*) em pacientes HIV-1+ e controles saudáveis.

4. Verificar a existência de associação entre as frequências alélicas, genotípicas e haplotípicas dos diferentes genes estudados com a modulação da susceptibilidade à infecção pelo HIV-1.

4. Capítulo I - Chemokines and co-receptor genes in HIV-1+ patients and healthy controls from Northeast Brazil: association with HIV-1 infection protection



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Chemokines and co-receptor genes SNPs in HIV-1+ patients and healthy controls from Northeast Brazil: association with HIV-1 infection protection

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Abstract

Background: HIV-1 virus entry in host cell is done mainly through $CD4^+$ T-lymphocyte cells, by interactions among the viral envelope proteins, CD4 receptor and HIV-1 coreceptors, such as chemokine receptors. Variations in the genes encoding HIV-1 coreceptors and their natural ligands have been shown to modify

HIV-1 infection susceptibility and disease progression. In this sense, we analysed the distribution of SNPs in chemokines (*CCL3, CCL4, CCL5, CXCL12*) and chemokine receptor (*CXCR6*) genes, in 268 HIV-1 infected patients (HIV-1+) and 221 healthy controls from Northeast Brazilian, and their possible connection with susceptibility to HIV-1 infection.

Methods and Results: The genotyping were performed through allele specific fluorogenic probes using real time PCR. We observed that the GA genotype of rs1719134 *CCL3* SNP were more frequent in healthy controls (33.3%) than in HIV-1+ patients (24.6%; OR=0.64; 95%CI=0.42-0.98; p-value=0.033). For rs1719153 *CCL4* SNP, the T allele and AT genotype were more frequent in healthy controls (19.8% and 35.0%, respectively) than in HIV-1+ patients (T allele: 14.1%; OR=0.67; 95%CI=0.47-0.95; p-value=0.020 and AT genotype: 24.4%; OR=0.59; 95%CI=0.39-0.90; p-value=0.012). The rs1719134 (*CCL3*) and rs1719153 (*CCL4*) SNPs presented linkage disequilibrium (D'=0.83). The AT haplotype frequency was increased in healthy controls (17.3%) in relation to HIV-1+ patients (11.0%; OR=0.60; 95%CI=0.41-0.89; p-value=0.008).

Conclusions: Since our results revealed an increased frequency of alleles and genotypes of *CCL3*/*CCL4* SNPs and haplotype (*CCL3-CCL4*) among healthy controls, we suggest a potential role these variations in modulation of susceptibility/protection to HIV-1 infection.

Key-words: *CCL3*, *CCL4*, SNPs, HIV-1, infection

1. Introduction

The HIV/AIDS epidemic is the main cause of morbidity and mortality for infectious diseases in the world. The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that more than 35 million of individuals live with the virus [1].

Virus entry is realized mainly through of $CD4^+$ T-lymphocyte cells, by interactions between the viral envelope glycoproteins (gp120), the CD4 receptor and HIV-1 coreceptors, such as chemokine receptors CCR5 (used by R5 HIV-1 strains) and CXCR4 (used by X4 HIV-1 strains) [2,3]. Others receptors also may be used in minor scale, such as DC-SIGN [4] and CXCR6 [5].

The CXCR6 receptor, a secondary HIV-1 coreceptor, is able of support variable replication levels of HIV-1, HIV-2 and SIV viruses, *in vitro*. This receptor is encoded by *CXCR6* gene, on chromosome 3 (3p21), and expressed by T-helper type 1 cells. It has as natural ligand CXCL16, which competes with the HIV-1 Env protein by binding to CXCR6 coreceptor [5–9].

The chemokines are a large superfamily of low molecular weight (~8-15 kDa), that mediate their biological functions by recruitment of cells bearing seven-transmembrane G-protein-coupled receptors, as part of homeostatic immune cell-trafficking or during inflammatory response [10–14].

The CC or inflammatory chemokines, namely *CCL3* (*MIP-1α* - Macrophage inflammatory protein-1 alfa), *CCL4* (*MIP-1β* - Macrophage inflammatory protein-1 beta) and *CCL5* (*RANTES* - Regulated upon activation normal T cell expressed and secreted) integrate a gene cluster on chromosome 17q12 and have been

demonstrated to possess HIV-1 suppressor activity thought competing with viral *Env* protein as well as by down-regulation of CCR5 surface expression [15,16].

On other hand, the *CXCL12* chemokine gene (*SDF-1* - Stromal cell derived factor), encoded on chromosome 10, is the only natural ligand of CXCR4 coreceptor and is involved in the hematopoietic cells migration and leukocytes transendothelial migration, playing an important role in blocking virus infection. *SDF-1* also competes with viral *Env* of X4 T-tropic strains by binding to CXCR4, causing receptor internalization e preventing viral entry [15,17–21]

In this sense, HIV-1 host susceptibility is the result of multiple factors, such as virulence, environmental conditions and immunogenetic factors, the latter depending upon the host genome [2,3,22]. Some studies have associated polymorphisms in *CCL3*, *CCL4*, *CCL5*, *CXCL12 and*, *CXCR6* genes with modulation of HIV-1 infection susceptibility and AIDS progression [9–11,17,23–28]. However, the results are still controversial and will vary depending on the ethnicity and studied population. So there is need for further replica studies considering all the mentioned genes in the same population.

Thus, we aimed at evaluating the distribution of single nucleotide polymorphisms (SNPs) in *CCL3*, C*CL4*, *CCL5*, *CXCL12* and *CXCR6* genes in a population of HIV-1 infected patients (HIV-1+) and healthy controls from Northeast of Brazil, in order to find their relation with modulation of susceptibility to HIV-1 infection.

2. Materials and Methods

2.1 Population Study

Variations in chemokines and coreceptor genes involved with HIV-1 entry were studied in 490 individuals (HIV-1+ patients and healthy controls) from Recife metropolitan region and/or minor towns of Pernambuco State (Brazil). The epidemiological characteristics of studied population were described in Table 1.

The HIV-1+ patients were recruited during the period from 2011 to 2013, at the Institute of Integral Medicine of Pernambuco Professor Fernando Figueira (IMIP). Their medical records were reviewed and clinical information collected. Healthy controls were blood donors, HIV-1 negative, recruited at Institute of Hematology and Hemotherapy of Pernambuco State (HEMOPE), during between 2011 and 2013.

Written informed consent was obtained from all individual enrolled in the study and the patients underwent a standardized clinical-epidemiological questionnaire. The Human Research Ethics Committees from IMIP (registration n2273-11) and HEMOPE (registration nº00880313.0.00005208) have approved the study.

Considering that Northeast Brazilian population has a strong ethnic admixture, we evaluated genetic ancestry markers (AIMs) distributions in HIV-1+ patients and healthy controls. This markers were assessed using the criteria of Kosoy et al [29] with modifications. The genotyping was performed by real time allele specific PCR, using the following 12 SNPs: rs4908343, rs7554936, rs6548616, rs7657799, rs10007810, rs6451722, rs1040045, rs10108270, rs772262, rs9530435, rs11652805 and rs4891825.

Both HIV-1+ patients and healthy controls presented the following distribution of genomic contribution: 59% European, 23% African and 18%

Amerindian. These percentages were corroborated the previous results observed by Coelho et al [30] in press.

2.2 Genomic DNA extraction, SNPs selection and genotyping

Genomic DNA was obtained from 5 mL of peripheral whole blood using the Genomic Prep DNA Isolation Kit® (Promega, Madison MD), according to the manufacturer's protocol.

We chose a panel of six SNPs in chemokines and receptor genes: *CCL3* (rs1719134 A>G), *CCL4* (rs1719153 A>T), *CCL5* (rs2280789 A>G; rs2107538 C>T), *CXCR6* (rs2234358 G/T) and *CXCL12* (rs1801157 C>T), based on literature data, on the functional role of the gene in HIV-1 infection, the minor allele frequency (MAF>0.10 in Caucasian and Yoruba) of each variation and the previous associations with HIV-1 infection and/or AIDS progression in others populations. The selected SNPs were genotyped using allele specific fluorogenic probes (TaqMan® assays of Life technologies: C_9458936-1, C_12120537_10, C_26924091-10, C_15874407-10, C_3223115_10, C_1929536-1) on a real time PCR platform (ABI 7500 SDS System).

2.3 Statistical Analysis

We estimated the allelic and genotypic frequencies of studied SNPs by direct counting and the Hardy-Weinberg Equilibrium adherence was verified through Chi-Square test (X^2), using the Genotype Transposer software [31].

Linkage disequilibrium (LD) and haplotypic frequencies were evaluated using Haploview software version 4.2 [32].

The allelic, genotypic and haplotypic frequencies among HIV-1+ patients and healthy controls were compared by Exact Fisher Test. The odds ratios (OR) and the 95% confidence intervals (CI95%) were calculated using as reference alleles, genotypes and haplotypes more frequently in healthy controls. The *p-value* less than 0.05 were considered statistically significant. Statistical analyses were performed with the R software 2.11.1 [33].

3. Results

3.1. Alleles, genotypes frequencies and association tests

The allelic and genotypic frequencies of *CCL3, CCL4*, *CCL5, CXCL12 a*nd *CXCR6* SNPs are reported in Table 2. The genotypic distributions for all SNPs studied were in accordance with Hardy-Weinberg equilibrium, except for SNP rs2107538 in HIV-1+ patients. Two SNPs, located in *CCL3* (rs1719134) and *CCL4* (rs1719153) genes, showed significant differences among HIV-1+ patients and healthy controls (Table 2).

For rs1719134 *CCL3* SNP, an A>G substitution in intron 1 region, the A allele was significantly more frequent in healthy controls (19.9%) than in HIV-1+ patients (14.9%; OR=0.71; CI95%=0.50-1.00; p-value=0.049, board line). Similarly, the GA genotype was significantly more frequent in healthy controls (33.3%) than in HIV-1+ patients (24.6%; OR=0.64; CI95%=0.42-0.98; p-value=0.033) (Table 2). Significant differences also were observed between

healthy controls and HIV-1+ patients according to dominant (GG vs GA+AA: OR=0.65; CI95%=0.43-0.97; p-value=0.031) and overdominant models (GG+AA vs GA: OR=0.65; CI95%=0.43-0.99; p-value=0.043).

For rs1719153 *CCL4* SNP, an A>T substitution in 3' UTR region, the T allele was significantly more frequent in healthy controls (19.9%) than in HIV-1+ patients (14.1%; OR=0.67; CI95%=0.47-0.95; p-value=0.020). Similarly, the AT genotype was significantly more frequent in healthy controls (35%) than in HIV-1+ patients (24.4%; OR=0.59; CI95%=0.39-0.90; p-value=0.012) (table 2). Additionally significant differences were observed between healthy controls and HIV-1+ patients according to dominant (AA vs AT+TT: OR=0.60; CI95%=0.40-0.90; p-value=0.011) and overdominant models (AA+TT vs AT: OR=0.60; CI95%=0.40-0.91; p-value=0.012).

For the others SNPs, we did not observe differences significant between healthy controls and HIV-1^{+} patients.

3.2. LD results, haplotypes frequencies and association tests

Considering that *CCL3*, *CCL4* and *CCL5* genes are localized in chromosome 17, we evaluated the linkage disequilibrium between SNPs, observing the formation of two blocks.

The rs1719134 (*CCL3*) and rs1719153 (*CCL4*) SNPs presented linkage disequilibrium (D'=0.83), forming four possible haplotypes (Table 2). The AT haplotype was significantly more frequent in healthy controls (17.3%) than in HIV-1+ patients (11.0%, OR=0.60; CI95%=0.41-0.89; p-value=0.008) (Table 3).

Additionally, the rs2280789 and rs2107538 *CCL5* SNPs showed strong linkage disequilibrium (D'=0.91), forming four haplotypes, but not showed significant difference (p-value >0.05) among healthy controls and HIV-1+ patients (Table 3).

4. Discussion

It is known that host genetic factors may influence HIV-1 infection susceptibility, and that the frequencies of these variants differs among populations [2,34–36]. Thus, we studied a panel of 6 SNPs in chemokines and a chemokine receptor genes in HIV-1+ patients and healthy controls from Northeast Brazil and verified that variants in *CCL3* (rs1719134: A allele and GA genotype) and *CCL4* genes (rs1719153: T allele and AT genotype) and the AT haplotype (rs1719134-rs1719153) were significantly more frequent among healthy controls than HIV-1+ patients, suggesting a potential protection against HIV-1 infection.

Variations in *CCL3* and *CCL4* chemokines genes have been associated to HIV-1 infection and AIDS progression in different populations [10,11,23,24,28,37,38]. However, few studies address all specifics variants analysed in this study [10,35]. Modi et al. [10] studying 21 SNPs of the gene cluster formed by *CCL3-CCL4-CCL18* in 5 cohorts HIV/AIDS in the United States, suggested that the studied SNPs (including rs1719134 and rs1719153) were not related to HIV-1 infection, but could be involved in AIDS progression, in contrast, at least in part, with our results. On other hand, Gonzalez et al. [35] found that the mutant haplotype (AA), formed by SNPs -113 and +456 (rs1719134) in *CCL3* gene, was more frequent in African American individual HIV-1 exposed and

uninfected, suggesting protection against HIV-1 infection, corroborating in part our data, since our controls were blood donors not exposed to HIV-1.

Although, we found no significant differences between HIV-1+ patients and healthy controls for the other SNPs studied in our population. Some studies showed the involvement of these variations in susceptibility to HIV-1 infection. Garcia-Moruja et al. [18] showed that 3'AA genotype (rs1801157) ensures greater mRNA stability in relation to 3'GG genotype, controlling the amount of protein produced and consequently leading to protection against HIV-1 infection; Modi et al. [17] verified that 3'A allele was associated with HIV-1 infection protection in European American individuals. On other hand, An et al [39] verified that G allele (rs2280789 in *CCL5*) was associated with decreased gene transcription levels and increased susceptibility to HIV-1 infection in European American individuals. Another study, described a higher frequency of T allele among Indian HIV-1+ subjects, suggesting susceptibility to HIV-1 infection [26]. These results may reflect differences in allelic and genotypic distributions within different populations characterized by distinct ethnic background, helping to explain our results. Northeast Brazilian individuals, as observed by our and Coelho et al. [30] studies, has a unique ethnic profile, characterized by a mixture of European (59%), African (23%) and Native Amerindian (18%), conferring a differentiated profile in modulation of HIV-1 infection susceptibility.

Our results and the evidences cited above, allow the elaboration of a theoretic HIV-1 susceptibility model. Variants in regulatory regions of *CCL3* and *CCL4* genes could increase the chemokines levels, allowing the interaction of these soluble chemokines with a large amount of CCR5 receptor, promoting their internalization and restricting entry of HIV-1 R5 strains. We suggest that the

presence of theses variations could result in advantage to host, by competition of the chemokines with virus.

Despite the limitations of our study, due to the small sample number analysed and absence of HIV-1 exposed and uninfected individuals, this was the first study to analyse this SNPs panel in *CCL3, CCL4, CCL5, CXCL12* and *CXCR6* genes in HIV-1+ patients and healthy controls from Northeast Brazil. Our results revealed that variations in *CCL3* (A allele and GA genotype), *CCL4* (T allele and AT allele) and AT haplotype (*CCL3-CCL4*) were more frequent in healthy controls suggesting a potential role for these variations in modulating susceptibility to HIV-1 infection.

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Tables

Table 1. Epidemiological characteristics of the study population

Epidemiological characteristics	Healthy Controls	HIV-1$^+$ Patients
N	221	268
Male - N(%)	61 (27.6)	74 (27.6)
Female - N(%)	160 (72.4)	194 (72.4)
Age (mean±SD)	29.8 ± 12.4	36.4 ± 8.8
Viral Load (log10 copies/mL) (mean±SD)	-	4.5 ± 0.97
CD^4 T Cells Count (cells/uL) (mean±SD)	-	255.7 ± 151.74
Transmission route		Sexual

Table 2. Allelic and genotypes frequencies of SNPs in chemokines and chemokines receptor genes among HIV-1+ patients and healthy controls of a Northeast Brazilian population.

SNPs	Healthy Controls n (%)	HIV-1 Patients n (%)	Fisher's Exact Test OR (95%CI), p-value
***CCL3* - rs1719134**			
G	351 (80.1)	456 (85.1)	Reference
A	87 (19.9)	80 (14.9)	0.71 (0.50-1.00), 0.049*
GG	139 (63.5)	195 (72.8)	Reference
GA	73 (33.3)	66(24.6)	0.64 (0.42-0.98), 0.033*
AA	7 (3.2)	7 (2.6)	0.71 (0.21-2.44), 0.587
***CCL4* - rs1719153**			
A	353 (80.2)	457(85.9)	Reference
T	87 (19.8)	75 (14.1)	0.67 (0.47-0.95), 0.020*
AA	138 (62.7)	196 (73.7)	Reference
AT	77 (35.0)	65 (24.4)	0.59 (0.39-0.90), 0.012*
TT	5 (2.3)	5 (1.9)	0.70 (0.16-3.12), 0.747
***CCL5* - rs2280789**			
A	388 (87.4)	397 (83.1)	Reference
G	56 (12.6)	81 (16.9)	1.41 (0.96-2.08), 0.078
AA	169 (76.1)	164 (68.6)	Reference
AG	50 (22.5)	69 (28.9)	1.42 (0.91-2.22), 0.110
GG	3 (1.4)	6 (2.5)	2.06 (0.43-12.92), 0.335
***CCL5* - rs2107538**			
C	302 (74.8)	330 (70.8)	Reference
T	102 (25.2)	136 (29.2)	1.22 (0.89-1.67), 0.196
CC	115 (56.9)	125 (53.6)	Reference
CT	72 (35.6)	80 (34.3)	1.02 (0.67-1.58), 0.918
TT	15 (7.4)	28 (12.0)	1.71 (0.83-3.64), 0.136
***CXCL12* - rs1801157**			
C	345 (83.3)	424 (83.8)	Reference
T	69 (16.7)	82 (16.2)	0.97 (0.67-1.39), 0.858
CC	144 (69.6)	178 (70.4)	Reference
CT	57 (27.5)	68 26.9)	0.96 (0.62-1.49), 0.916
TT	6 (2.9)	7 (2.8)	0.94 (0.26-3.48), 1.000
***CXCR6* - rs2234358**			
G	225 (50.9)	268(50.0)	Reference
T	217 (49.1)	268 (50.0)	0.83 (0.64-1.07), 0.151
GG	61 (27.6)	70 (26.1)	Reference
GT	103 (46.6)	128 (47.8)	1.08 (0.69-1.70), 0.742
TT	57 (25.8)	70 (26.1)	1.07 (0.64-1.80), 0.804

*Significant p-value ($p<0.05$)

Table 3. Haplotype frequencies of SNPs *chemokines* gene among HIV-1+ patients and healthy controls from Northeast Brazilian population.

Haplotypes		Healthy Controls n (%)	HIV-1 Patients n (%)	Fisher's Exact Test OR (95%CI), p-value
CCL3 **rs1719134**	***CCL4*** **rs1719153**			
G	**A**	337 (77.6)	433 (82.0)	Reference
A	**T**	75 (17.3)	58 (11.0)	0.60 (0.41-0.89), 0.008*
A	**A**	12 (2.8)	21 (4.0)	1.36 (0.63-3.08), 0.475
G	**T**	10 (2.3)	16(3.0)	1.24 (0.52-3.11), 0.689
CCL5 **rs2280789**	**rs2107538**			
A	**C**	302 (74.8)	321 (70.4)	Reference
A	**T**	52 (12.9)	56 (12.3)	1.01 (0.66-1.56), 1.000
G	**T**	50 (12.4)	77 (16.9)	1.45 (0.97-2.19), 0.064
G	**C**	0 (0.0)	2 (0.4)	nc

* Significant p-value ($p<0.05$), nc= not calculated

5. Capítulo II – *TRIM5* gene polymorphisms in HIV-1 infected patients and healthy controls from Northeast Brazil

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TRIM5 gene polymorphisms in HIV-1 infected patients and healthy controls from Northeastern Brazil

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Abstract

Background: Humans show heterogeneity in their vulnerability to HIV-1 infection, known to be, at least partially, under control of genes involved in host immunity and virus replication. TRIM5α protein has species-specific restriction activity against replication of many retroviruses; and *TRIM5* gene polymorphisms have been reported as possibly involved in susceptibility to HIV-1 infection. So, we evaluated the role of *TRIM5* single nucleotide polymorphisms (SNPs) in the context of HIV-1 infection in a population from Northeast of Brazil. Four hundred

and forty seven individuals (213 HIV-1+ patients and 234 healthy uninfected controls) were recruited; two non-synonymous SNPs in exon 2, rs10838525 (R136Q) and rs3740996 (H43Y), and one regulatory SNP (rs16934386) in the 5'UTR region of *TRIM5* gene were analyzed.

Results: Among the studied SNPs, only the R136Q variation presented significant differences among HIV-1+ patients and healthy controls. The T allele (136Q) and the TT genotype (136QQ) was more frequent in healthy controls (32.7% and 10.2%, respectively) than HIV-1+ subjects (T allele: 24.4%; OR=0.66; CI95%=0.49-0.90; p-value=0.008/ TT genotype: 4.2%; OR=0.33; CI95%=0.13-0.79, p=0.008). We observed that rs10838525 (R136Q) and rs3740996 (H43Y) SNPs were in moderate linkage disequilibrium (D'=0.71), forming four possible haplotypes. The AT haplotype was significantly more frequent in healthy controls (28.2%) than HIV-1+ patients (21.4%; OR=0.69; CI 95%=0.50-0.96; p=0.022).

Conclusions: Since an increased frequency of allele (136Q) and genotype (136QQ) of the non-synonymous SNP rs10838525 (R136Q) and the AT haplotype (136Q-43Y) of the *TRIM5* was observed among healthy controls individuals, we can hypothesize, being aware of the main limitation of this study related to the absence of exposed uninfected individuals, a potential role for these *TRIM5* variations in the modulation of susceptibility to HIV-1 infection.

Key-words: *TRIM5*, susceptibility, HIV-1, association studies, restriction factors.

Background

Retroviruses, as human immunodeficiency virus type 1 (HIV-1), are extremely successful pathogens. These viruses are persistent, maintained as

latent provirus DNA integrated in the genome of host cells. In parallel, host cells have evolved a number of powerful mechanisms to limit or restrict virus replication, called restriction factors [1]. In HIV-1 infection context, the restriction factors may help host cells in viral replication control.

Initially discovered in rhesus monkey cells [2], the tripartite motif-containing protein 5 alpha (TRIM5α) is a restriction factor, that can block HIV-1 replication at a post-entry, pre-integration stage of HIV-1 life cycle, by recognition and premature disassembly of incoming viral capsids (CA), thereby preventing the completion of reverse transcription [3]. Human TRIM5α restricts HIV-1, though to a lesser extent when compared to rhesus monkey [2, 4, 5].

TRIM5α is encoded by *TRIM5* gene (11p15) [6], consisting of 8 introns and 9 exons. It is able to produce several transcripts by alternative splicing, including the longest isoform originating TRIM5α [2]. The protein is composed of four distinct domains (RING, B-box 2, Coiled-coil and C-terminal SPRY) involved in important mechanism for virus restriction, such as E3 ubiquitin ligase activity [2, 7], formation of TRIM5α-containing cytoplasmic bodies [7, 8], capsid recognition [9–11] and specific recognition and restriction of retroviruses [5, 12–14].

Some studies have indicated that genetic variations may alter the activity of human TRIM5α in the context of HIV-1 infection [15–19]. The amino acid changes H43Y (rs3740996) and R136Q (rs10838525) were associated with protection to HIV-1 infection in African American individuals and the residue 136QQ is known to possess higher anti-HIV-1 activity than RR136 variant [15]. Similarly, the shift from arginine to glutamine at codon 136 in coiled-coil region of TRIM5α conferred protection against HIV-1 in a Pumwani sex workers cohort (Kenya) [17]. On the other hand, the 136Q variation was associated with increased susceptibility to

HIV-1 infection in European American individuals [19]. These contradictory results suggest a need for further investigation upon the effect of human *TRIM5* SNPs on HIV-1 infection.

All this considered, we analyzed selected *TRIM5* gene SNPs, in order to evaluate the distribution of *TRIM5* variations in HIV-1 positive (HIV-1+) patients and healthy controls from Northeast of Brazil. We hypothesized that *TRIM5* gene variations, particularly at exon 2 (R136Q and H43Y) and 5'UTR region (rs16934386), might potentiate the viral restriction, modulating the susceptibility to HIV-1 infection.

Material and Methods

Study Population

The study population consisted of 447 individuals (HIV-1+ patients and healthy controls) from Recife metropolitan region or minor towns of Pernambuco (Brazil) (Table 1).

All patients were recruited at the Institute of Integral Medicine of Pernambuco Professor Fernando Figueira (IMIP) and had their medical records reviewed. Healthy controls were blood donors, HIV-1 negative, recruited at the Institute of Hematology and Hemotherapy from Pernambuco State (HEMOPE).

Written informed consent was obtained from each individual. The Human Research Ethics Committee from IMIP (registration nº 2273-11) and HEMOPE (registration nº 00880313.0.0000.5208) approved the study. Patients underwent a standardized clinical-epidemiological questionnaire.

Since strong ethnic admixture characterizes Northeast Brazilian individuals, we evaluated HIV-1+ patients and healthy controls using genetic ancestry informative markers (AIMs). The genetic ancestry of both patients and controls was assessed using the criteria of Kosoy et al. [20] with modifications. We analyzed 12 SNPs: rs4908343, rs7554936, rs6548616, rs7657799, rs10007810, rs6451722, rs1040045, rs10108270, rs772262, rs9530435, rs11652805 and rs4891825, by real time PCR.

HIV-1+ patients and healthy controls presented the following distribution of genomic contribution: 59% European, 23% African and 18% Amerindian; these percentages are concordant with what previously reported by Coelho et al [21]. They showed that Pernambuco population is characterized by a so strong genetic admixture that, in spite of having a contribution from different ethic groups, is now a novel highly admixed population with characteristics different from the original founder genomes.

Genomic DNA extraction, SNPs selection and genotyping

Genomic DNA was extracted from peripheral whole blood using the Genomic Prep DNA Isolation Kit® (Promega, Madison MD).

The rs16934386, rs10838525 and rs3740996 SNPs have been selected based on literature data [22], according with the minor allele frequency (MAF>0.10 in Caucasian and Yoruba, being present both ethnic groups in the admixed genome of our study population), functional effects and previous association in other populations. All SNPs were genotyped using allele specific fluorogenic probes (TaqMan® probes assays: C_339444114_10, C_1452187_20,

C_25923723_20) on a real-time PCR platform (ABI 7500 SDS System, Life Technology).

Statistical Analysis

Alleles and genotypes frequencies were calculated by direct counting. Chi-square (X^2) test was used to assess if there were any departures from Hardy-Weinberg equilibrium (HWE); Exact Fisher Test was employed to assess the possible differences among HIV-1+ patients and healthy controls. The alleles, genotypes and haplotypes with the highest frequency in healthy controls were set as references. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated relative to these references. Linkage disequilibrium (LD) and estimated haplotype frequencies were calculated using Haploview software [23].

Statistical analyses were performed with the R software 2.11.1 [24]. Bonferroni method was used to correct for multiple comparisons and statistical significance threshold was thus set at 0.017 for association tests (0.05 for testing differences of haplotype frequencies).

Results

TRIM5 SNPs frequencies are reported in Table 2. Frequencies distribution for all SNPs was in Hardy-Weinberg equilibrium in the study population. The 136Q allele (rs10838525T) was more frequent in healthy individuals (32.7%) than in HIV-1+ patients (24.4%; OR=0.66; CI95%=0.49-0.90; p-value=0.008). In addition, the TT genotype (136QQ) was also significantly more frequent in healthy controls

(10.2%) than in HIV-1+ patients (4.2%; OR=0.33; CI95%=0.13-0.79; p-value=0.008) (Table 2).

We observed that rs10838525 (R136Q) and rs3740996 (H43Y) SNPs were in moderate linkage disequilibrium (D'=0.71), forming four possible haplotypes (Table 3). The AT haplotype, carrying the 43Y-136Q residue's change, was significantly more frequent in healthy controls (28.2%) than HIV-1+ patients (21.4%; OR=0.69; CI 95%=0.50-0.96; p=0.022) (Table 3).

Discussion

In this study, we observed a different frequency distribution of T allele and TT genotype of rs10838525 (R136Q) *TRIM5* SNP as well as AT haplotype (rs3740996-rs10838525) among healthy controls individuals in comparison with HIV-1+ patients from Northeast of Brazil (Pernambuco), suggesting a possible protective effect against HIV-1 infection.

Our studied population is composed by an admixture of Caucasian, African and Amerindians populations, which resulted in the unique genetic Brazilian composition. As an example, the allelic distribution of rs10838525 in the healthy individuals and HIV-1+ patients enrolled in this study is statistically different when compared to African, European and Amerindian distribution as available in the 1000 Genome Project database (www.1000genomes.org).

Despite the differences in genetic compositions and ethnic background, our results are in agreement with previously findings, *i.e.* a higher frequency of 136Q residues in healthy individuals. Javanbakht et al. [15] observed that in African-

American individuals, the frequencies of 136Q *TRIM5* variant were higher in uninfected individuals than in HIV-1+ patients, suggesting a possible protective effect. Similarly, Price et al. [17] found that the same allele was more frequent in HIV-1 infection resistant individuals from Nairobi. It is interesting to note that all these association were described in populations with African genetic ancestries. Thus, it seems that individuals carrying the 136Q residues from populations that possess an African genetic contribution could present a lower susceptibility to be infected by HIV-1.

On the other hand, Speelmon et al. [19] showed an increased frequency of haplotype containing H43Y and R136Q among HIV-1+ individuals in comparison to exposed seronegative individuals from United States. Interesting, their patients were predominantly of European-American ethnic origin and they did not find the haplotype AT (43Y-136Q) as we found in our studied population.

Additionally, we observed no differences among healthy controls and HIV-1+ patients for rs3740996 (H43Y) and rs1693438 *TRIM5* SNPs. However, the AT haplotype (containing the 43Y-136Q variation), despite moderate linkage disequilibrium (D'=0.71), was significantly more frequent in healthy controls than HIV-1+ patients, suggesting a protection against HIV-1 infection. This result is concordant with the findings reported in Japanese, Indian [25], and Chinese [26] populations. In these populations, the frequency of 43Y residue change (rs3740996) was significantly lower in the HIV-1+ patients than in controls, so the authors suggested a protective effect. Furthermore, Sawyer et al. [27] suggested that the presence of 43Y variation could negatively affect the activity of E3 ubiquitin ligase, leading to susceptibility to HIV-1 infection, thus explaining the lower frequencies of the allele/haplotype that carries this variant in HIV+ patients.

Based on evidence from previous findings cited above and our results, we can conjecture that the variations in amino acid sequence of TRIM5α, specially at position 136, located in a region required for effective recognition and binding of HIV-1 [5, 7, 10] (the coiled-coil domain), may alter the protein multimerization [7, 10, 12], which in turn would affect the viral protein binding affinity. Additionally, if the substitution is from an arginine to a glutamine at the codon 136 in TRIM5 protein, it result in a higher activity against HIV-1 [15], modulating the HIV-1 infection susceptibility.

Conclusions

To our knowledge, this is the first study analyzing *TRIM5* gene polymorphisms in Brazilian HIV-1+ patients and healthy controls. Despite the limitations of our study, consisting in small sample size and overall the absence of exposed uninfected individuals, we can suggest that polymorphisms in exon 2 of the *TRIM5* gene (R136Q), an important region for the antiretroviral activity of the protein, may enhance the retroviral restriction function and thereby modulating susceptibility to HIV-1, interfering in the establishment of the virus.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

RCS performed SNPs genotyping, data analysis and drafted the manuscript. AVCC participated in patient recruitment, DNA extraction and drafted the manuscript. LACB and RLG participated in drafting the manuscript. LCA enrolled patients and performed the clinical evaluations. SC conceived the study design and critically revised the manuscript

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Tables

Table 1. Epidemiological characteristics of the study population

Epidemiological characteristics	Healthy Controls	HIV-1+ Patients
N	234	213
Male - N(%)	69 (29.5)	62 (29.1)
Female - N(%)	165 (70.5)	151 (70.90)
Age (mean±SD)	29.3 ± 9.2	36.3 ± 9.1
Viral Load (log_{10} copies/mL) (mean±SD)	-	4.5 ± 1.0
$CD4^+$ T Cells Count (cells/uL) (mean±SD)	-	257.4 ± 151.6
Transmission route		Sexual

Table 2. Allelic and genotypes frequencies of *TRIM5* SNPs in HIV-1^+ patients and healthy controls of a Northeast Brazilian population.

SNPs	Healthy Controls n (%)	HIV-1 Patients n (%)	Fisher's Exact Test OR (95%CI), p-value
rs16934386 (5'UTR)			
A	456 (97.4)	418 (98.1)	Reference
G	12 (2.6)	8 (1.9)	0.73 (0.25-1.96), 0.508
AA	222 (94.9)	205 (96.2)	Reference
AG	12 (5.1)	8 (3.8)	0.72 (0.25-1.97), 0.503
rs3740996 (H43Y)			
A	424 (90.6)	381 (89.4)	Reference
G	44 (9.4)	45 (10.6)	1.14 (0.72-1.81), 0.578
AA	191 (81.7)	170 (79.8)	Reference
AG	42 (17.9)	41 (19.3)	1.10 (0.66-1.82), 0.716
GG	1 (0.4)	2 (0.9)	2.24 (0.12-133.1), 0.604
rs10838525 (R136Q)			
C	315 (67.3)	322 (75.6)	Reference
T	153 (32.7)	104 (24.4)	0.66 (0.49-0.90), 0.008*
CC	105 (44.9)	118 (55.4)	Reference
CT	105 (44.9)	86 (40.4)	0.73 (0.48-1.09), 0.116
TT	24 (10.2)	9 (4.2)	0.33 (0.13-0.79), 0.008*

*Significant p-value with Bonferroni correction (p<0.017)

Table 3. Haplotype frequencies of *TRIM5* non-synonymous SNPs in in HIV-1+ patients and healthy controls of a Northeast Brazilian population

Haplotypes		Healthy Controls n (%)	HIV-1 Patients n (%)	Fisher's Exact Test OR (95%CI), p-value
rs3740996	**rs10838525**			
A	**C**	292 (62.4)	290 (68.1)	Reference
A	**T**	132 (28.2)	91 (21.4)	0.69 (0.50-0.96), 0.022*
G	**C**	23 (4.9)	32 (7.5)	1.40 (0.77-2.57), 0.261
G	**T**	21 (4.5)	13 (3.1)	0.62 (0.28-1.33), 0.218

*Significant p-value (p<0.05)

6. Capítulo III - *APOBEC3G* and *CUL5* polymorphisms in HIV-1+ patients and healthy controls from Northeast Brazil: implications in HIV-1 susceptibility

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APOBEC3G and *CUL5* Polymorphisms in HIV-1+ patients and healthy controls from Northeast Brazil: implications in HIV-1 susceptibility

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Abstract

APOBEC3G cause hypermutation of proviral DNA leading to degradation or replication-incompetent HIV-1. The HIV-1 viral infectivity factor (Vif) suppresses

APOBEC3G activity through the Cullin 5 complex. *APOBEC3G* and *CUL5* polymorphisms have been involved with modulation of susceptibility to HIV-1 infection. In this sense, we evaluated the distribution of single nucleotide polymorphisms (SNPs) in *APOBEC3G* (rs3736685, rs2294367) and *CUL5* (rs7103534, rs7117111, rs11212495) genes, in 264 HIV-1+ patients and 259 healthy controls from Northeast Brazil, and their relation with HIV-1 infection susceptibility. All genotyping were performed through allele specific probes by real time PCR. We verified that all that frequencies distributions were in Hardy-Weinberg equilibrium. The G allele of rs11212495 *CUL5* SNP was more frequent in healthy controls than in HIV-1+ patients (p=0.029). Significant difference among healthy controls and HIV-1+ patients were observed in the dominant model (p-value=0.035). The linkage disequilibrium analysis revealed that *APOBEC3G* SNPs were not linked (D'=0.16), but the GC allelic combination frequency was increased in HIV-1+ patients in relation to healthy controls (p=0.0002). The rs7103534 and rs1717111 *CUL5* SNPs were in moderate linkage disequilibrium (D'=0.72) and the CG haplotype was more frequent in healthy controls than in HIV-1+ patients (p=0.032). The *CUL5* SNPs CGA and TAG allelic combination were more frequent in healthy controls than HIV-1+ patients (p=0.031 and p=0.015, respectively). Considering the main limitation of study (absence of HIV-1 exposed and uninfected individuals), we can suggest that variations in *APOBEC3G* and *CUL5* may potentially modulate HIV-1 infection susceptibility.

Key-words: HIV-1, *CUL5*, *APOBEC3G*, SNPs, Susceptibility.

1. Introduction

Over the evolutionary process, hosts and pathogens are involved in an endless adaptive battle, in which the host develops sophisticated immunological strategies for elimination of pathogens, while the pathogens develop mechanisms to escape host response. One well-studied example of this fight is during human immunodeficiency virus 1 (HIV-1) infection. Since HIV-1 utilizes immune host cells for its replication, while the host has a series of restriction factors that interfere with the virus' ability to replicate [1–3].

Host restriction factors are cellular proteins that can restrict or block viral replication in a cell-specific way. Several host restriction factors have been identified so far, such as TRIM5α, BST2, SAMHD1 and APOBEC3G [4–6].

Human Apolipoprotein B mRNA-editing enzyme catalytic polypeptide like 3G (APOBEC3G) is an antiviral protein, member of a family of RNA editing enzymes able to inhibit HIV-1 replication by causing deleterious mutations in the HIV-1 genome [7,8]. It is encoded by *APOBEC3G* gene, which spans ~9 kb on chromosome 22q13.1-13-2 and has 8 exons and 7 introns [9,10].

In cells infected with viral infectivity factor-deleted (ΔVif), APOBEC3G is not degraded and is effectively incorporated into the budding virus, being transferred to the next target cell, exerting antiviral effects [11,12]. It has been associated with the inhibition of reverse transcriptase elongation through direct binding to the viral RNA, and with the deamination of cytidine residues in newly synthesized DNA negative strands, causing the degradation of the complementary DNA (cDNA) through a cellular uracil-DNA-glycosidase pathway or by a pervasive

G to A hypermutation in the plus-strand provirus DNA. Its antiviral action thus can lead to inhibition of viral DNA integration and provirus formation [8,11–14].

APOBEC3G antiretroviral activities can be suppressed by HIV-1 Vif, which effectively reduces the amount of APOBEC3G encapsulated in HIV-1 virions, primarily by induction of proteasomal degradation [11,13]. In this process, the interaction among HIV-1 Vif and the cellular proteins Cullin 5, Elongin B, Elongin C and Rbx1 allows the formation of E3 ubiquitin ligase complex, responsible by induction of polyubiquitination and, consequently, proteasomal degradation of APOBEC3G [15].

Cullin 5 protein is a member of the Cullin-RING E3 ubiquitin family, encoded by human *CUL5* gene, which covers approximately 100 kb in chromosome 11q22 and consists of 19 exons and 18 introns [16]. It is a core component of E3 ubiquitin protein ligase complexes and interacting directly with HIV-1 Vif protein [15]. Vif-Cullin 5 binding domain has been mapped to a highly conserved HCCH motif within the HIV-1 zinc-binding domain [17], and within the loop region between helices 6 and 7 (amino acids 120-138) of Cullin 5 protein [18]. Mutations or down-regulation by RNA interference in Cullin 5 complex can block polyubiquitination and degradation of APOBEC3G induced by HIV-1 Vif, suggesting that the suppressor activity of HIV-1 Vif depends on Cullin 5 complex function [19,20].

Some studies suggested that genetic variations in *APOBEC3G* and *CUL5* genes could be involved in modulation of susceptibility to HIV-1 infection and AIDS progression in some populations [7,16,21–26]. Therefore, new studies in others populations are necessary to replicate these findings.

Thus, considering the importance of APOBEC3G and Cullin 5 protein in HIV-1 infection context, we evaluated the distribution of SNPs in *APOBEC3G*

(rs3736685 and rs2294367) and *CUL5* (rs7117111, rs7103534, rs11212495) genes, among HIV-1+ patients and healthy controls from Northeast of Brazil, and their relation with modulation of susceptibility to HIV-1 infection.

2. Material and Methods

2.1 Population Study

This study enrolled 523 individuals (264 HIV-1+ patients and 259 healthy controls) (Table 1) from Recife metropolitan region and/or minor towns of Pernambuco State (Brazil).

HIV-1+ patients and healthy controls (HIV-1 negative blood donors) individuals were recruited at Institute of Integral Medicine of Pernambuco Professor Fernando Figueira (IMIP) and Institute of Hematology and Hemotherapy of Pernambuco State (HEMOPE), among 2011 and 2013.

Written informed consent was obtained from all individuals enrolled in the study; the patients and controls underwent a standardized clinical-epidemiological questionnaire. The Human Research Ethics Committees from IMIP (registration nº 2273-11) and HEMOPE (registration nº 00880313.0.00005208) approved the study.

Since the Northeast Brazilian population has a strong ethnic admixture, we evaluated a panel of genetic ancestry markers (AIMs) in our healthy controls and HIV-1+ patients individuals, using the criteria of Kosoy et al. [27] with modification. We analyzed 12 AIMs SNPs (rs4908343, rs7554936, rs6548616, rs7657799, rs10007810, rs6451722, rs1040045, rs10108270, rs772262, rs9530435,

rs11652805 and rs4891825) using allele specific probes by real time PCR. We observed that both HIV-1+ patients and healthy controls presented the following distribution of genomic contribution: 59% European, 23% African and 18% Amerindian, corroborating the previous results of Coelho et al. [28] in press.

2.2 Genomic DNA extraction, SNPs selection and genotyping

Genomic DNA was obtained from 5 mL of peripheral whole blood using the Genomic Prep DNA Isolation Kit® (Promega, Madison MD), according to the manufacturer's protocol.

Single nucleotide polymorphisms (SNPs) in A*POBEC3G* (rs3736685, rs2294367) and *CUL5* (rs7117111, rs11212495, rs7103534) genes were chosen, according to the following criteria: functional role in HIV-1 infection, minor allele frequency (MAF>0.10 in Caucasian and Yoruba representative populations) and previous associations with HIV-1 infection in others populations. The SNPs selected were genotyped using allele specific fluorogenic probes (TaqMan® assays APOBEC3G: C_27489853_20, C_16186714; CUL5: C_1345246_10, C_1345248_10, C_29674421_10) on a real time PCR platform (ABI 7500 SDS System).

2.3 Statistical Analysis

Allelic and genotypic frequencies of studied SNPs were obtained by direct counting, and the Hardy-Weinberg equilibrium was measured through Chi-square test (X^2) using the Genotype Transposer software [29]. Linkage disequilibrium (LD)

and haplotypic frequencies were estimated through Haploview software version 4.2 [30].

The allelic, genotypic, haplotypic and allelic combination frequencies among HIV-1+ patients and healthy controls were compared using Exact Fisher Test. The odds ratios (OR) and corresponding 95% confidence intervals (95%CI) were calculated using the most frequent alleles, genotypes and haplotypes in the healthy controls group as a reference categorical variable during statistical analysis. *P-values*<0.05 were considered statistically significant. Statistical analyses were performed with the R software 2.11.1 [31].

3. Results

APOBEC3G and *CUL5* SNPs frequencies distribution in HIV-1+ patients and healthy controls from Northeast Brazil were in Hardy-Weinberg equilibrium, and significant difference was observed for rs11212495 (*CUL5*) (Table 2).

The G allele (rs11212495 *CUL5*) was significantly more frequent in healthy controls (10.5%) than in HIV-1+ patients (6.4%, OR=0.58, CI95%=0.35-0.96, p-value=0.029). Significant difference was observed among healthy controls and HIV-1+ patients according to dominant model (OR=0.56, CI95%=0.32-0.97, p-value=0.035).

Linkage disequilibrium analyzed revealed that *APOBEC3G* SNPs were not linked (D'=0.16), but the GC allelic combination was significantly more frequent in HIV-1+ patients (4.7%) than in healthy controls (0.8%, OR=6.87, CI95%=2.05-36.1, p-value=0.0002) (Table 3).

In the other hand, the rs7103534 and rs1717111 *CUL5* SNPs were in moderate linkage disequilibrium (D'=0.72), constituting four possible haplotypes (Table 3). Significant differences were verified among healthy controls (2.7%) and overall HIV-1+ patients (0.9%) for the CG haplotype (OR=0.33, CI95%=0.09-0.99, p-value=0.032). Additionally, the *CUL5* allelic combination analysis revealed that CGA and TAG combinations were significantly more frequent in healthy controls (2.7% and 1.6%, respectively) than HIV-1+ patients (CGA: 0.9%, OR=0.31, CI95%=0.08-1.00, p-value= 0.031/ TAG: 0.2%, OR=0.10, CI95%=0.002-0.85, p-value=0.015) (Table 4).

4. Discussion

In this study, we evaluated the distribution of *APOBEC3G* and *CUL5* SNPs in individuals from a Northeast Brazilian population and we found that GC allele combination (*APOBEC3G*: rs2294367-rs3736685) frequency was increased among HIV-1+ patients, while the G allele (rs11212495 - *CUL5*) CG haplotype (*CUL5:* rs7103534-rs7117111) and CGA and TAG allele combinations (*CUL5:* rs7103534-rs7117111-rs11212495) were more frequent among healthy controls.

Some studies conducted in United States [10], Indian [23] and Argentinian [21] populations suggested that SNPs in the *APOBEC3G* gene have no effect on HIV-1 infection susceptibility. Despite the absence of individuals exposed to HIV-1 but uninfected, our study showed a predominance of GC allele combined (*APOBEC3G*: rs2294367-rs3736685) among HIV-1+ patients in relation to healthy controls individuals, suggesting a possible higher susceptibility to HIV-1 infection and corroborating, at least, in part the findings of Valcke et al. [25], that found a

intron 4 *APOBEC3G* variant (40693-C/T) strongly associated with increased infection risk in a Caucasian cohort.

The rs3736685 SNP, localized in *APOBEC3G* intron 3, has been showed that is in strong linkage disequilibrium with a nonsynonymous rs8477832 (H186R) SNP [10], so most of the studies only address the H186R variants. The rs8477832 variants (186R and 186RR) have been associated with accelerated AIDS progression in African American subjects [10,24], but not in Caucasian individuals [21,26]. However, no studies have linked this variation with susceptibility to HIV-1.

As discussed by An et al. [10], the change of histidine to arginine amino acid at the 186 position (H186R), can promoted a potential alteration in APOBEC3G protein expression or function, suggesting that the amount of protein can be important against HIV-1 infection. Biasin et al. [7] found that *APOBEC3G* expression was significantly increased in peripheral blood mononuclear cells (PBMCs) and in cervical tissues of individuals exposed to HIV-1 but uninfected. In addition, a higher APOBEC3G expression was also correlated with a reduced HIV-1 R5 strain infection susceptibility *in vitro* experiments with PBMCs [7].

In our study, we also observed an increased frequency of rs11212495 *CUL5* SNP variants (G allele), CG haplotype (rs7103534-rs7117111) and the CGA and TAG allelic combinations (rs7103534-rs7117111-rs11212495) among healthy controls in relation to HIV-1+ patients, indicating a possible protection against HIV-1 infection.

Despite the role of *CUL5* gene in HIV-1 infection, only one study has examined the role of *CUL5* SNPs in modulation of susceptibility to HIV-1 infection. An et al. [16] studying five United States-based natural history HIV/AIDS cohorts, suggested the C*UL5* SNPs variations (including rs7103534, rs7117111,

rs11212495) have no direct effect on susceptibility to HIV-1 infection. However, the rs11212495 SNP variation has been associated with more rapid CD4+ T cell loss [16] and others have found a lower HIV-1 editing promoted by *APOBEC3G* in perinatal HIV-1+ patients from Argentine paediatric cohort [22].

Functionally, rs11212495 SNP, an A to G change in intron 3 of *CUL5*, has been suggested as a modifier of T lymphocytes nuclear proteins DNA binding affinity. Thus, it possibly affects post-transcriptional gene regulation by interfering with transcriptional protein complex interaction with the primary RNA transcript. As discussed by An et al. [10], higher levels of Cullin 5, due to a potential upregulation of *CUL5* caused by the SNPs, would enhance the Cullin 5-Vif interaction, leading to APOBEC3G inhibition, reducing HIV-1 editing [22] and thus increasing HIV-1 infectivity. On the other hand, Liu et al. [20] observed that Cullin 5 depletion (through RNA interference, or overexpression of Cullin 5 mutants experiments) blocks the HIV-1 Vif suppressive ability over both APOBEC3G and APOBEC3F. Therefore, it is suggested that lower levels of Cullin 5 can be a protective factor against HIV-1 infection, corroborating, at least in part, our results, since we found an increased frequency of rs11212495 variants in healthy controls.

Thus, the combination of our results with the literature evidence, allows us to propose the following hypothesis for involvement of *APOBEC3G* and *CUL5* SNPs in HIV-1 infection. Since the rs3736685 SNP is in strong linkage disequilibrium with rs8477832 SNP, which is potentially involved in changes in the expression and function of APOBEC3G protein, we hypothesize that the presence of *APOBEC3G* variants (GC allele combined), containing the SNP rs3736685, leads to a reduction in the amount of protein or changes in its antiviral functions, enabling the suppressive action of Vif protein on APOBEC3G, conferring

susceptibility to HIV-1 infection. Moreover, the presence of rs11212495 *CUL5* SNP variants (involved in gene regulation) may potentially have an effect in decreasing the expression of Cullin 5, impairing its interaction with Vif and resulting in the interruption of the suppressor activity of Vif on APOBEC3G, giving protection against HIV-1 infection. However, we recognize the need of functional studies, aiming at clarifying the true role of these variations in HIV-1 infection.

To our knowledge, this is the first study analysing *APOBEC3G* and *CUL5* polymorphisms in Brazilian HIV-1+ patients and healthy controls. We can suggest, despite the limitations of our study (small sample size and absence of individuals exposed to HIV-1 but uninfected), that variations in regions involved with regulation of *APOBEC3G* and *CUL5* expression, may potentially modulate the susceptibility to HIV-1 infection, modifying infection risk .

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Tables

Table 1. Epidemiological characteristics of the study population

Epidemiological Characteristics	Healthy Controls	HIV-1 Patients (HIV-1+)
N	259	264
Male - N(%)	86 (33.2)	72 (27.3)
Female - N(%)	173 (66.8)	192 (72.7)
Age (mean±SD)	33.2 ± 11.8	36.3 ± 8.9
Viral Load (log10 copies/mL) (mean±SD)	-	4.5 ± 1.0
CD^4 T Cells Count (cells/uL) (mean±SD)	-	253.6 ± 151.8
Transmission route	-	Sexual

Table 2. Allelic and genotypes frequencies of *APOBEC3G* and *CUL5* SNPs in HIV-1+ patients and healthy controls of a Northeast Brazilian population

Gene/SNPs	Healthy Controls n (%)	HIV-1 Patients n (%)	Fisher's Exact Test OR (95%CI), p-value
APOBEC3G – rs2294367			
G	234 (55.4)	280 (53.0)	Reference
C	188 (44.6)	248 (47.0)	1.10 (0.85-1.44), 0.471
GG	58 (27.5)	75 (28.4)	Reference
GC	118 (55.9)	130 (49.2)	0.85 (0.54-1.33), 0.518
CC	35(16.6)	59 (22.4)	1.30 (0.73-2.32), 0.342
APOBEC3G – rs3736685			
T	369 (89.1)	453 (85.8)	Reference
C	45 (10.9)	75 (14.2)	1.36 (0.90-2.06), 0.140
TT	167 (80.7)	196 (74.2)	Reference
CT	35 (16.9)	61 (23.1)	1.48 (0.91-2.44), 0.106
CC	5 (2.4)	7 (2.7)	1.19 (0.32-4.86), 1.000
CUL5 – rs7103534			
T	438 (86.6)	457 (86.5)	Reference
C	68 (13.4)	71 (13.5)	1.00 (0.69-1.45), 1.000
TT	190 (75.1)	201 (76.1)	Reference
CT	58 (22.9)	55 (20.8)	0.90 (0.58-1.39), 0.669
CC	5 (2.0)	8 (3.1)	1.51 (0.43-5.98), 0.578
CUL5 – rs7117111			
G	302(61.9)	336 (63.6)	Reference
A	186 (38.1)	192 (36.4)	0.93 (0.71-1.21), 0.603
GG	100 (41.0)	109 (41.3)	Reference
GA	102 (41.8)	118 (44.7)	1.06 (0.71-1.58), 0.772
AA	42 (17.2)	37 (14.0)	0.81 (0.46-1.40), 0.431
CUL5 – rs11212495			
A	356 (89.5)	494 (93.6)	Reference
G	42 (10.5)	34 (6.4)	0.58 (0.35-0.96), 0.029*
AA	161 (80.9)	233 (88.3)	Reference
AG	34 (17.1)	28 (10.6)	0.57 (0.32-1.01), 0.052
GG	4 (2.0)	3 (1.1)	0.52 (0.07-3.11), 0.453

*Significant p-value ($p<0.05$); nc = no calculated.

Table 3. *APOBEC3G* allele combination and *CUL5* haplotypes frequencies in HIV-1^{+} patients and healthy controls of a Northeast Brazilian population.

Haplotypes		Healthy Controls n (%)	HIV-1 Patients n (%)	Fisher's Exact Test OR (95%CI), p-value
APOBEC3G				
rs2294367	**rs3736685**			
G	**T**	211 (53.8)	255 (48.3)	Reference
C	**T**	141 (36.0)	198 (37.5)	1.16 (0.87-1.56), 0.314
C	**C**	37 (9.4)	50 (9.5)	1.12 (0.69-1.83), 0.725
G	**C**	3 (0.8)	25 (4.7)	6.87 (2.05-36.1), 0.0002*
CUL5				
rs7103534	**rs7117111**			
T	**G**	281 (59.0)	331 (62.7)	Reference
T	**A**	132 (27.7)	126 (23.9)	0.81 (0.60-1.10), 0.159
C	**A**	50 (10.5)	66 (12.5)	1.12 (0.74-1.71), 0.612
C	**G**	13 (2.7)	5 (0.9)	0.33 (0.09-0.99), 0.032*

*Significant p-value (p<0.05); nc = no calculated.

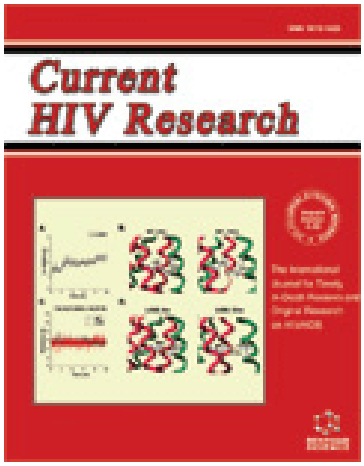
Figure 4. *CUL5* alleles combined frequencies in HIV-1+ patients and healthy controls of a Northeast Brazilian population

Combination			Healthy Controls N (%)	HIV-1 Patients N (%)	Fisher's Exact Test OR (95%CI), p-value
SNP1	**SNP2**	**SNP3**			
T	**G**	**A**	182 (48.9)	298 (56.4)	Reference
T	**A**	**A**	99 (26.6)	125 (23.7)	0.77 (0.55-1.08), 0.117
C	**A**	**A**	43 (11.6)	66 (12.5)	0.94 (0.60-1.47), 0.827
T	**G**	**G**	31 (8.3)	33 (6.3)	0.65 (0.37-1.14), 0.133
C	**G**	**A**	10 (2.7)	5 (0.9)	0.31 (0.08-1.00), 0.031*
T	**A**	**G**	6 (1.6)	1 (0.2)	0.10 (0.002-0.85), 0.015*

*Significant p-value (p<0.05); nc = no calculated.

SNP1=rs7103534; SNP2= rs7117111; SNP3= rs11212495

7. Capítulo IV - Single nucleotide polymorphisms in *ZNRD1* gene: implication in susceptibility to HIV-1 infection in a Northeast Brazilian population



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Single nucleotide polymorphisms in *ZNRD1* gene: implication in susceptibility to HIV-1 infection in a Northeast Brazilian population

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ABSTRACT

Human immunodeficiency virus (HIV) takes advantage of multiple host proteins to support its own replication. The gene HLA-C and ZNRD1 has been identified as encoding a potential host factor that influenced the HIV-1 infection. In this sense, we evaluated the distribution of *HLA-C* (rs10484554, rs9264942) and

ZNRD1 (rs3869068, rs8321) single nucleotide polymorphisms (SNPs), in 266 HIV-1 positive patients (HIV-1+) and 223 healthy controls from Northeast Brazilian, and their relation with susceptibility to HIV-1 infection. All SNPs were genotyping though allele specific probes by real time PCR. We observed that CT genotype of rs3869068 *ZNRD1* SNP genotype was more frequent in HIV-1+ patients (40.1%) than healthy controls (29.0%; OR=1.65; 95%CI=1.08-2.55; p-value=0.019). HLA-C SNPs were in linkage disequilibrium (D'=0.84), constituting four possible haplotypes (CT; CC; TC and TT). Haplotype distribution showed no significant differences among healthy controls and HIV-1+ patients. On the other hand, *ZNRD1* SNPs presented weak linkage disequilibrium (D'=0.24), not forming haplotypes blocks nor showed allelic combinations with significant differences among healthy controls and HIV-1+ patients. Being aware of the relative small number of individuals analysed and the absence of exposed uninfected individuals, our results suggest that variations in *ZNRD1* (rs3869068 - CT) could be potentially involved in susceptibility to HIV-1 infection.

Key-words: SNPs, HLA-C, *ZNRD1*, infection, pathogenesis

1. Introduction

Susceptibility to human immunodeficiency virus type 1 (HIV-1) infection can be seen as the final result of a dynamic interaction between host genome and the pathogen, together with environmental influences [1,2]. In the midst of this complicated puzzle, several genetic polymorphisms have been described as able

to influence susceptibility to HIV-1 infection or AIDS progression, especially in the HLA locus [3–10].

The human leukocyte antigen (HLA) or major histocompatibility complex-1 (MHC-1), mapping at chromosome 6p21, comprises a classical class I (HLA-A, HLA-B and HLA-C) and II (DP, DQ, DR) molecules and a non-classical molecules (HLA-E, HLA-F and HLA-G). It plays a central role in adaptive immunity as well as in innate defences [11,12].

The *HLA-C* gene (6p21.33), is composed 8 exons and 7 introns spanning approximately 3.5 kb [13]. This gene encodes the HLA-C protein, a heterodimer composed of a membrane-bound mature heavy chain and a light chain, β_2-microglobulin (β_2M), naturally expressed on the cell surface about 10-fold less than others class I HLA [12]. It plays a dual role, being able to present antigen to cytotoxic T lymphocytes (CTLs) and inhibiting natural killer (NK) cells lysis via its interaction with inhibitory killer cell immunoglobulin-like receptor (KIR) [12,14,15].

Many viruses, including HIV-1, use this inhibitory capacity of HLA-C to facilitate their infections in host organism. HIV-1 promotes the down-regulation of HLA-A and B, but not HLA-C, with the aim of protecting himself from attack by cytotoxic T lymphocytes (CTL). In this case, the presence of HLA-C may allow inhibition of NK cells expressing KIR. However, high HLA-C expression levels could increase the antigen presentation to CTL interfering in viral infections [12,14,15].

ZNRD1 is one of several genes in the HLA region, mapping at chromosome 6p21.3, consists of 4 exons and 3 introns, occupying approximately 3.6 kb of genomic DNA [16]. This gene encodes the ZNRD1 protein (a zinc ribbon domain-containing 1 protein), a DNA-dependent RNA polymerase involved in transcription

of DNA into RNA. It was first identified throught genome-wide small interfering RNA (siRNA) knock-down, being described as one of 250 HIV-1 dependency factors required for HIV-1 replication [17]. The ZNRD1 absence was responsible for HIV-1 restriction, reducing the R5 or X4-tropic HIV-1 replication (>50%) in HeLa derived cells or lymphoid cells [3]. This restriction could involve interference with the processing of HIV-1 transcripts by regulatory protein Rev [18].

Some genome-wide and case-controls studies suggested that single nucleotide polymorphisms (SNPs) in *HLA-C* and *ZNRD1* genes could to influence susceptibility to HIV-1 infection and AIDS progression [3–8,19,20]. Despite their enormous power and interest, large-scale genome-wide screens should be taken only as starting points. The identified genes and genetic associations should be validated in independent populations with different ethnic background. In this sense, we aimed at evaluating the distribution of *HLA-C* (rs10484554 and rs9264942) and *ZNRD1* (rs3869068 and rs8321) SNPs among HIV-1 positive patients (HIV-1+) and healthy controls from Northeast Brazil and their possible relation with susceptibility to HIV-1 infection.

2. Materials and Methods

2.1 Population Study

Four hundred ninety-nine individuals (HIV-1+ patients and healthy controls) from Recife metropolitan region and/or minor towns of Pernambuco State (Brazil) were enrolled in this study; see Table 1 for epidemiological features of the population.

All HIV-1+ patients and healthy controls (blood donors HIV-1 negative) individuals were recruited at Institute of Integral Medicine of Pernambuco Professor Fernando Figueira (IMIP) and Institute of Hematology and Hemotherapy of Pernambuco State (HEMOPE), respectively, during the period 2011 to 2013.

Written informed consent was obtained from all individual enrolled in the study; HIV-1+ patients and healthy controls were underwent a standardized clinical-epidemiological questionnaire. In addition, clinical information's were obtained from medical records. The Human Research Ethics Committee from IMIP (registration n2273-11) and HEMOPE Foundation (registration nº00880313.0.00005208) approved the study.

Additionally, we evaluated the ethic admixture of HIV-1+ patients and healthy controls from Northeast Brazilian. The genetic ancestry of all subjects enrolled in study was assessed through ancestry informative markers (AIMs) using the criteria of Kosoy et al. (2011) with modification. We genotyped a panel of 12 AIMs SNPs (rs4908343, rs7554936, rs6548616, rs7657799, rs10007810, rs6451722, rs1040045, rs10108270, rs772262, rs9530435, rs11652805 and rs4891825), using allele specific probes by real time PCR platform.

We observed that both HIV-1+ patients and healthy controls presented the following distribution of genomic contribution: 59% European, 23% African and 18% Amerindian; corroborating the previous report of Coelho et al. [21] (in press).

2.2 Genomic DNA extraction, SNPs selection and genotyping

Genomic DNA was obtained from 5 mL of peripheral whole blood using the Genomic Prep DNA Isolation Kit® (Promega, Madison MD), according to the manufacturer's protocol.

Single nucleotide polymorphisms (SNPs) in *HLA-C* and *ZNRD1* gene were chosen, based on literature data, also considering their functional role in HIV-1 infection and pathogenesis, the minor allele frequency (MAF>0.10 in Caucasian and Yoruba) and the previous associations with HIV-1 in others populations. SNPs selected were: rs10484554 C>T (C_29612773_20) and rs9264942 C>T (C_29901957_10) in *HLA-C*; rs3869068 C>T (C_26544924_10) and rs8321 A>C (C_2437466_10) in *ZNRD1*. SNPs genotyping was performed using allele specific fluorogenic probes (TaqMan® assays - Life technologies) on a real-time PCR platform (ABI 7500 SDS System).

2.3 Statistical Analysis

The allelic and genotypic frequencies of SNPs studied were obtained by direct counting, and the Hardy-Weinberg Equilibrium was evaluated through Chi-square test (X^2), using the Genotype Transposer software [22]. Linkage disequilibrium (LD) and haplotypic frequencies were estimated through Haploview software version 4.2 (Barrett et al., 2005). The allelic combinations were analyzed in order to verify their influence on HIV-1 infection susceptibility.

The allelic, genotypic, haplotypic and allelic combined frequencies among HIV-1^+ patients and healthy controls were compared by Exact Fisher Test. The odds rations (OR) and 95% confidence intervals (CI95%) were calculated using as reference alleles, the more frequent genotypes and haplotypes in healthy controls.

The *p-value* less than 0.05 were considered as statistically significant. Statistical analyses were performed with the R software 2.11.1 [24].

3. Results

HLA-C and *ZNRD1* SNPs frequencies distribution in HIV-1+ patients and healthy controls were in Hardy-Weinberg equilibrium (Table 2).

We verified that the *ZNRD1* CT genotype (rs3869068) was significantly more frequent in HIV-1+ patients (40.1%) than in healthy controls (29.0%) by codominant (OR=1.65, CI95%=1.08-2.55, p-value=0.019) and overdominant models (CT vs CC+TT: OR=1.64, CI95%=1.08-2.51, p-value=0.020). Significant differences were also verified among HIV-1+ patients and healthy controls by dominant model (CT+TT vs CC: OR=1.57, CI95%=1.05-2.38, p-value=0.024).

Linkage disequilibrium analysis showed that HLA-C SNPs were strongly linked (D'=0.84), constituting four possible haplotypes (CT; CC; TC and TT). The haplotype distribution showed no significant differences between healthy controls and HIV-1+ patients (p-value>0.05) (data not shown). On the other hand, *ZNRD1* SNPs presented weak linkage disequilibrium (D'=0.24), not forming haplotypes blocks. The *ZNRD1* combined alleles were analyzed, but no significant differences were achieved (data not shown).

4. Discussion

In this study, we evaluated the distribution of *HLA-C* and *ZNRD1* SNPs in HIV-1+ patients and healthy controls in a Northeast Brazilian population and

founding significant differences among HIV-1+ patients and healthy controls, for the rs3869068 *ZNRD1* SNPs.

A *ZNRD1* gene variant of rs3869068 SNP in (CT genotype) presented significantly increased frequency in HIV-1+ patients in comparison to healthy controls in our population, suggesting an increased in the susceptibility to HIV-1 infection. Our results were not in agreement with An et al. [6] study, which verified a 35% decreased risk of HIV-1 infection among high risk uninfected from 5 US-based treatment-naïve natural history HIV/AIDS cohorts, in consequence of *ZNRD1* haplotype presence (including rs3869068 and rs8321). Others studies, reported the involvement of *ZNRD1* gene variants such as rs3869068 SNP, rs1048412, rs16896970 with viral load control [3,8], accelerated $CD4^+$ T-lymphocyte cells depletion [6] and AIDS progression [19,20].

As we can see, the *ZNRD1* SNPs variations (such as rs3869068) can have different implications in HIV-1 infection and AIDS progression, which can be explained by *ZNRD1* expression levels. Ballana et al. [3] demonstrated that the *ZNRD1* gene silencing impaired HIV-1 replication at transcriptional level in lymphoid and non-lymphoid cells. *ZNRD1* rs3869068 SNP, localized in regulatory region, was correlated with regulation of *ZNRD1* expression, and lower levels of *ZNRD1* expression have been considered a protective factor to HIV-1 infection [8]. Our results showed an increased frequency the CT genotype in HIV-1+ patients, leading us to hypothesize that such variation might interfere in the *ZNRD1* expression levels, but not enough to prevent the infection success. However, this is only a speculation, requiring additional studies.

In our study has demonstrated no association of HLA-C variants with modulation of susceptibility to HIV-1 infection. However, some studies have

reported that variations in HLA-C strongly associated with HIV-1 control in different populations [4,7,8,10,12,25–28].

Our findings, together with literature evidences, lead us to suggest a potential involvement of Z*NRD1* SNPs in susceptibility to HIV-1 infection. The rs3869068 SNPs *ZNRD1* variants can decrease ZNRD1 expression [8] and, consequently, the ZNRD1 expression levels could interfere in the viral transcripts processing, thus interfering in viral replication [3,18].

Even with the limitations of our study (small sample size, absence of HIV-1 exposed and uninfected individuals) and necessity of additional functional studies, our results suggest that variations of ZNRD1 (rs3869068) SNPs could be involved in modulation of susceptibility to HIV-1 infection in Northeast Brazilian individuals.

Acknowledgments

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Conflict of interest

None of the authors has any potential financial conflict of interest related to this manuscript.

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Tables

Table 1. Epidemiological characteristics of the study population.

Epidemiological Characteristics	Healthy Controls	HIV-1$^+$ Patients
N	223	266
Male - N(%)	60 (26.9)	74 (27.8)
Female - N(%)	163 (73.1)	192 (72.2)
Age (mean±SD)	29.8 ± 12.3	36.4 ± 8.8
Viral Load (log10 copies/mL) (mean±SD)	-	4.5 ± 1.0
CD^4 T Cells Count (cells/uL) (mean±SD)	-	256.5 ± 151.8
Transmission route		Sexual

Table 2. Allelic and genotypes frequencies of SNPs in *HLA-C* and *ZNDR1* genes in HIV-1^+ patients and healthy controls of a Northeast Brazilian population.

Genes/ SNPs	Healthy Controls n (%)	HIV-1 Patients n(%)	Fisher's Exact Test OR (95%CI), p-value
HLA-C – rs10484554			
C	364 (83.9)	447 (88.3)	Reference
T	70 (16.1)	59 (11.7)	0.69 (0.46-1.01), 0.057
CC	155 (71.4)	200 (79.0)	Reference
CT	54 (24.9)	47 (18.6)	0.67 (0.42-1.08), 0.090
TT	8 (3.7)	6 (2.4)	0.58 (0.16-1.96), 0.413
HLA-C – rs9264942			
T	262 (62.4)	328 (65.0)	Reference
C	158 (37.6)	177 (35.0)	0.89 (0.68-1.18), 0.450
TT	77 (36.7)	111 (44.0)	Reference
CT	108 (51.4)	106 (42.0)	0.68 (0.45-1.03), 0.058
CC	25 (11.9)	35 (14.0)	0.97 (0.52-1.84), 1.000
ZNRD1 – rs3869068			
C	295 (80.6)	395 (75.4)	Reference
T	71 (19.4)	129 (24.6)	1.36 (0.97-1.91), 0.073
CC	121 (66.1)	145 (55.3)	Reference
CT	53 (29.0)	105 (40.1)	1.65 (1.08-2.55), 0.019*
TT	9 (4.9)	12 (4.6)	1.11 (0.41-3.10), 1.000
ZNRD1 – rs8321			
A	416 (97.6)	489 (98.2)	Reference
C	10 (2.4)	9 (1,8)	0.76 (0.27-2.12), 0.645
AA	203 (95.3)	240 (96.4)	Reference
AC	10 (4.7)	9 (3.6)	0.76 (0.27-2.13), 0.641

*Significant p-value ($p<0.05$); nc = no calculated.

Table 3. *HLA-C* haplotypes frequencies in HIV-1^+ patients and healthy controls of a Northeast Brazilian population.

Haplotypes *HLA-C*		Healthy Controls n (%)	HIV-1 Patients n (%)	Fisher's Exact Test OR (95%CI), p-value
rs10484554	**rs9264942**			
C	**T**	256 (61.2)	310 (63.8)	Reference
C	**C**	94 (22.5)	118 (24.3)	1.04 (0.74-1.44), 0.871
T	**C**	62 (14.8)	55 (11.3)	0.73 (0.48-1.11), 0.128
T	**T**	6 (1.4)	3 (0.6)	0.41 (0.07-1.96), 0.312

*Significant p-value ($p<0.05$); nc = no calculated.

8. Discussão Geral

A variabilidade genética humana tem sido apontada como fator importante na modulação da susceptibilidade à infecção pelo HIV-1 e progressão para AIDS (Gonzalez et al. 2001; Anastassopoulou and Kostrikis 2003; Winkler et al. 2004; An and Winkler 2010). Neste sentido, avaliamos a distribuição de SNPs em genes relacionados à entrada (*CCL3, CCL4, CCL5, CXCR6, CXCL12)* e à replicação viral (*APOBEC3G, CUL5, TRIM5, HLA-C* e *ZNRD1*) e sua relação com a modulação da susceptibilidade à infecção pelo HIV-1, em uma população do Nordeste brasileiro (Recife-PE) e verificamos diferenças significativas entre controles saudáveis e pacientes HIV-1+ para os SNPs: rs1719134 (*CCL3*), rs1719153 (*CCL4*), rs11212495 (*CUL5*), rs10838525 (*TRIM5*) e rs3869068 (*ZNRD1*).

No primeiro capítulo, observamos que variantes nos SNPs rs1719134 no gene *CCL3* (genótipo GA) e rs1719153 no gene *CCL4* (alelo T e genótipo AT), bem como o haplótipo AT (rs1719134-rs1719153) apresentaram uma aumentada frequência entre controles saudáveis, sugerindo uma menor susceptibilidade à infecção pelo HIV-1. É sabido que CCL3 e CCL4 são ligantes naturais do correceptor CCR5 e competem com o vírus pela ligação a CCR5 (Singh et al. 2008). Variantes nos genes das quimiocinas CCL3 e CCL4 vêm sendo associados à infecção pelo HIV-1 e progressão para AIDS em diferentes populações (Modi et al. 2006; Meddows-Taylor et al. 2006; Colobran et al. 2008; Levine et al. 2009; Shrestha et al. 2009; Paximadis et al. 2009; Paximadis et al. 2013). Modi et al. (2006) observaram, em 5 coortes de pacientes HIV/AIDS dos Estados Unidos, que as diferentes variantes dos SNPs estudados para o cluster

gênico *CCL3-CCL4-CCL18* (incluindo rs1719134 e rs1719153), não possuíam efeitos na susceptibilidade à infecção e progressão para AIDS. Por outo lado, Gonzalez et al. (2001) constataram que o haplótipo mutante (AA), formado pelos SNPs -113 e 456 (rs1719134) no gene *CCL3*, predominou entre os indivíduos expostos e não infectados pelo HIV-1, corroborando, em parte, com os nossos resultados.

No segundo capítulo, observamos que variantes do SNP rs10838525 (alelo T e o genótipo TT) no gene *TRIM5*, bem como o haplótipo AT (rs3740996-rs10838525) apresentaram uma aumentada frequência entre os controles saudáveis, sugerindo uma menor susceptibilidade à infecção pelo HIV-1. Alguns estudos verificaram também o aumento da frequência do alelo 136Q (T) entre indivíduos expostos e não infectados de origem Afro-americana (Javanbakht et al. 2006a) e da coorte Pumwani de Nairóbi (Price et al. 2010), sugerindo uma menor susceptibilidade à infecção pelo HIV-1. Por outro lado, Speelmon et al. (2006) mostraram uma aumentada frequência de haplótipos contendo essa variante (136Q) em indivíduos HIV-1^{+} em comparação com indivíduos expostos e não infectados ao HIV-1 dos Estados Unidos.

Apesar de não observarmos diferenças significativas entre os grupos para os SNPs rs3740996 (H43Y) e rs1693438, o haplótipo AT (rs3740996-rs10838525), contendo a variação 43Y e 136Q, foi significativamente mais frequente em controles saudáveis, corroborando os resultados observados nas populações japonesa e indiana (Nakajima et al. 2009), e chinesa (Liu et al. 2011), onde a frequência da variante 43Y foi significativamente baixa entre pacientes HIV-1+. Por outro lado, Sawyer et al. (2006) sugeriram que a presença do variante

43Y pode negativamente afetar a atividade da ubiquitina E3 ligase, conduzindo a susceptibilidade a infecção pelo HIV-1.

No terceiro capítulo, observamos que um variante do gene *APOBEC3G* (Combinação alélica GC) foi mais frequentes entre pacientes HIV-1+, sugerindo uma susceptibilidade aumentada. Enquanto que variantes do SNP rs11212495 no gene *CUL5* (alelo G), bem como o haplótipo CG (rs7103534-rs7117111) e as combinações alélicas CGA e TAG (rs7103534-rs7117111-rs11212495) foram mais frequentes entre os controles saudáveis, sugerindo que tais variações podem modular a susceptibilidade a infecção pelo HIV-1, conferindo uma menor susceptibilidade.

Estudos conduzidos com indivíduos dos Estados Unidos (An et al. 2004), Índia (Rathore et al. 2008b) e Argentina (De Maio et al. 2011) têm descartado o envolvimento de SNPs no gene *APOBEC3G* na susceptibilidade à infecção pelo HIV-1. No entanto, Valcke et al. (2006) observaram que uma variante no intron 4 de *APOBEC3G* (40693-C/T) estava fortemente associada com risco de infecção em uma coorte de Caucasianos. Em nosso estudo, observamos a predominância da combinação alélica CT entre pacientes HIV-1+, sugerindo um provável efeito na susceptibilidade ao vírus.

O SNP rs3736685, localizado no intron 3 do gene *APOBEC3G*, tem sido mostrado estar em forte desequilíbrio de ligação com o SNP não-sinônimo rs8477832 (H186R) (An et al. 2004), o que talvez explique a predominância de estudos inerentes a esse SNP. Alguns estudos têm relacionado variantes do SNP rs8477832 com o declínio de células T $CD4^+$, acelerada progressão para AIDS e complicações do sistema nervoso central em indivíduos HIV-1+ afro-americanos (An et al. 2004; Singh et al. 2013), enquanto outros não encontraram nenhum

efeito em populações de origem caucasoides, como Argentina e França (Do et al. 2005; De Maio et al. 2011).

Como discutido por An et al (2004), a troca do aminoácido histidina para arginina na posição 186 (H186R), potencialmente pode promover alterações na expressão e funcionalidade da APOBEC3G. Ao que parece, a quantidade desta proteína é chave no controle do vírus. Aumento da expressão de *APOBEC3G* em células mononucleares do sangue periférico e tecidos cervicais de indivíduos expostos e não infectados foi relacionada a uma reduzida susceptibilidade de PBMCs a infeção por cepas R5 HIV-1 *in vitro* (Biasin et al. 2007), corroborando em parte nossos resultados.

Quanto as variantes do gene *CUL5,* An et al. (2007) estudaram 5 coortes de pacientes HIV/AIDS dos Estados Unidos e não encontraram nenhum efeito de SNPs neste gene (incluindo rs7103534, rs7117111, rs11212495) com a susceptibilidade à infecção pelo HIV-1. No entanto, variantes do SNP rs11212495 tem sido associados com rápida depleção de células T $CD4^+$ (An et al. 2007a) e com baixa atividade de edição do HIV-1, promovida pela APOBEC3G (De Maio et al. 2012).

Funcionalmente, o SNP rs11212495, uma variação no intron 3 do gene *CUL5*, tem sido sugerido por modificar a afinidade de ligação ao DNA de proteínas nucleares de linfócitos T, podendo afetar a regulação gênica ou a interação com proteínas. Com discutido por An et al. (2004), altos níveis de Culina 5 podem aumentar a interação Culina 5–Vif, inibindo a atividade da APOBEC3G, como já foi observado pela redução da edição do HIV-1 (De Maio et al. 2012), e aumentando a infectividade. Por outro lado, Liu et al. (2005) observaram que a depleção ou uma super-expressão de mutantes de Culina 5 pode bloquear a

atividade supressora de Vif sobre APOBEC3G e 3F, sugerindo que baixos níveis de Culina 5 pode fornecer uma vantagem ao hospedeiro.

No quarto e último capítulo, observamos que um variante SNP rs3869068 no gene *ZNRD1* (genótipo CT) predominou entre os pacientes HIV-1+, sugerindo um provável envolvimento na modulação da susceptibilidade ao vírus, discordando em parte, com o estudo de An et al. (2014), que verificaram um risco, aproximadamente 35% menor para a infecção pelo HIV-1 entre indivíduos de expostos e não-infectados, em consequência de um haplótipo em *ZNRD1* (incluindo rs3869068 e rs8321). Adicionalmente, variantes do SNP rs3869068 têm sido correlacionados com o controle da carga viral, acelerada depleção de células T $CD4^+$ e progressão para AIDS (Fellay et al. 2007; Limou et al. 2009; Ballana et al. 2010; Lin et al. 2013; An et al. 2014).

Os resultados observados podem ser relacionados com o nível de expressão de *ZNRD1,* visto que a replicação viral pode ser seriamente prejudicada na ausência de ZNRD1, conforme observado por Ballana et al. (2010). Baixos níveis de ZNRD1 também têm sido correlacionados com uma menor susceptibilidade à infecção pelo HIV-1 (Fellay et al. 2007). Alguns SNPs, como rs3869068, têm sido relacionado com a regulação da expressão de *ZNRD1*. Em nossa população verificamos uma elevada frequência para o genótipo CT em pacientes, levando-nos a especular que essa variação em *ZNRD1* pode ter interferido nos níveis de ZNRD1, mas não o suficiente para impedir a infecção.

Em nosso estudo não demostramos nenhuma associação entre variantes no gene *HLA-C* e a modulação da susceptibilidade à infecção pelo HIV-1. No entanto, alguns estudos têm sugerido que variantes no gene *HLA-C* estão fortemente associadas com o controle viral em diferentes populações (Stranger et

al. 2005; Fellay et al. 2007; Shrestha et al. 2009; van Manen et al. 2009; Fellay et al. 2009; Thomas et al. 2009; Trachtenberg et al. 2009; Zipeto and Beretta 2012; Apps et al. 2013).

Considerando as evidências disponíveis na literatura quanto à funcionalidade de cada proteína, juntamente com nossos resultados obtidos para os vários genes estudados em nossa população, e reconhecendo as limitações metodológicas do nosso estudo (ausência de indivíduos expostos e não-infectados e ensaios funcionais), propomos um potencial modelo para o papel de SNPs nos genes associados na infecção pelo HIV-1 (Figura 5).

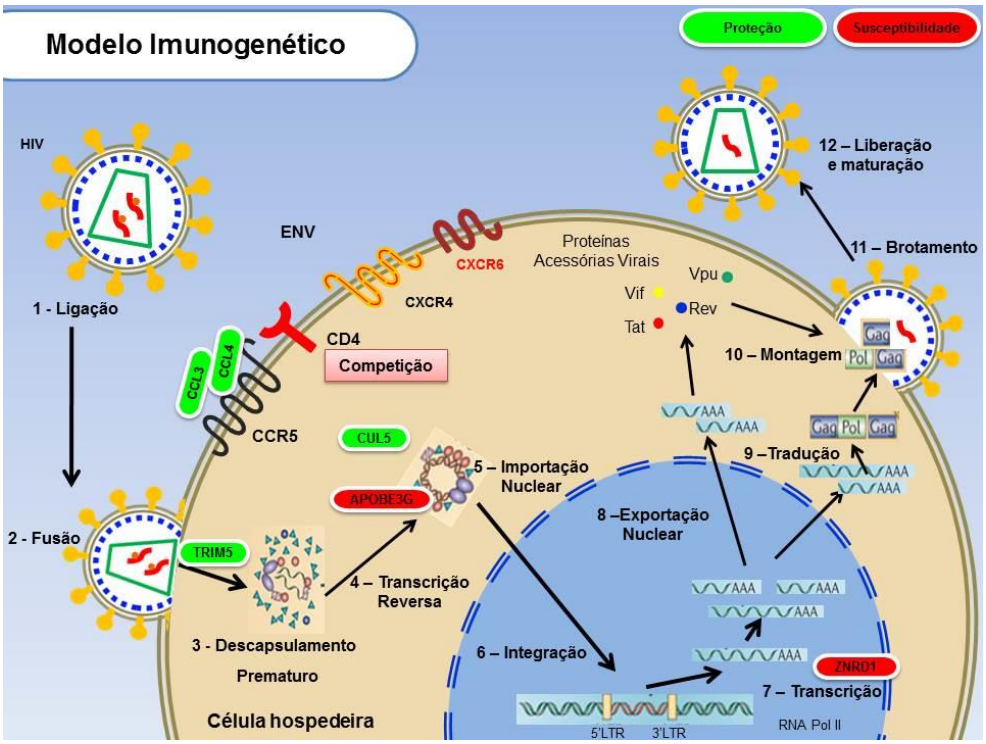

Figura 5. Modelo imunogenético da susceptibilidade/proteção à infecção pelo HIV-1.

Variações em regiões reguladoras de genes codificadores de quimiocinas (rs1719134 – *CCL3*; rs1719153 – *CCL4*), podem potencialmente aumentar os níveis de expressão destas proteínas, permitindo uma vantagem competitiva frente os vírus pela ligação ao correceptor CCR5, dificultando a entrada do vírus.

No entanto, o HIV-1 pode se utilizar outros correceptores e entrar na célula, onde, prontamente, pode ser contido por fatores de restrição como: TRIM5α e APOBEC3G. Variantes do SNP rs10838525 do gene *TRIM5*, reconhecidamente alteram a sequência de aminoácidos (posição 136) em uma região requerida para efetivo reconhecimento e ligação ao HIV-1 (Javanbakht et al. 2005; Mische et al. 2005; Stremlau et al. 2006) - o domínio coiled-coil, que potencialmente pode alterar a multimerização proteica (Javanbakht et al. 2005; Mische et al. 2005; Li et al. 2007), afetando a afinidade de ligação ao vírus, e, possivelmente, tornando a proteína mais ativa na supressão viral. Por outro lado, variações no gene *APOBEC3G* podem promover a redução dos níveis da proteína ou alterações em sua função antiviral, permitindo a ação supressora do fator viral Vif, o qual destruirá a APOBEC3G, conferindo susceptibilidade à infecção. Mas, a presença de variantes do SNP rs11212495 no gene *CUL5,* variante potencialmente envolvido na regulação gênica, pode conduzir a um decréscimo na expressão de Culina 5, impedindo sua interação com Vif e consequentemente impedindo a atividade supressora de Vif sobre APOBEC3G, conferindo assim, uma menor susceptibilidade à infecção pelo HIV-1. Por fim, variações no gene *ZNRD1* (rs3869068) potencialmente podem promover a diminuição da expressão (Fellay et al., 2007). Baixos níveis de expressão de ZNRD1 podem interferir no processamento de transcritos virais, e assim na replicação viral (Ballana et al, 2010;Michienzi et ai, 2000).

Enfim, o somatório de todos os fatores imunogenéticos, juntamente com os inúmeros fatores viras e ambientais, fazem a infecção pelo HIV-1 um complicado quebra-cabeças, onde cada peça é essencial para o entendimento do todo.

9. Conclusões Gerais

O estudo da distribuição de polimorfismos em genes envolvidos na resposta hospedeira frente à infecção pelo HIV-1 permitiu, de uma forma geral, uma melhor compreensão de como a variabilidade genética humana pode atuar na modulação da susceptibilidade à infecção pelo HIV-1. Permitindo-nos chegar as seguintes inferências:

- ✓ As distribuições de SNPs em genes envolvidos com a entrada viral revelaram que variações em regiões reguladoras dos genes *CCL3* (rs1719134: GA) e *CCL4* (rs1719153: T e AT; haplótipo AT- rs1719134-rs1719153) foram mais frequente em controles saudáveis, sugerindo uma menor susceptibilidade à infecção pelo HIV-1, possivelmente por alterações na expressão gênica.
- ✓ As distribuições de SNPs em genes envolvidos com a restrição da replicação viral revelaram que variantes nos genes *TRIM5* (rs10838525: alelo T e genótipo TT; rs370996-rs10838525: haplótipo AT) e *CUL5* (rs11212495: alelo G, rs1703534-rs7117111: haplótipo CG; combinações alélicas CGA e TAG) foram mais frequentes em controles saudáveis, enquanto que variantes em *APOBEC3G* (rs3736685-rs2294367: alelos combinados CG) em pacientes HIV-1+, sugerindo papéis diferenciados na modulação da infecção, seja protegendo (*TRIM5-CUL5*) e/ou conferindo susceptibilidade (*APOBEC3G*).
- ✓ As distribuições de SNPs em genes do lócus HLA, evidenciaram que um variante no gene *ZNRD1* (rs3869068: genótipo CT) foi mais frequente em pacientes HIV-1+, possivelmente conferindo uma maior susceptibilidade à

infecção, em decorrência da modulação da expressão gênica por essa variante.

Apesar das limitações, o presente estudo apresenta um caráter inovador, visto que foi o primeiro, de sua natureza, realizado com indivíduos brasileiros, permitindo a visualização de variações genéticas importantes na modulação da infecção pelo HIV-1.

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11. Anexos

Anexo 1

Instituto de Medicina Integral
Prof. Fernando Figueira
Escola de Pós-graduação em Saúde Materno Infantil
Instituição Civil Filantrópica

IMIP

DECLARAÇÃO

Declaro que o projeto de pesquisa **nº 3629 - 13** intitulado **"Fatores Genéticos humanos envolvidos no curso da Infecção pelo HIV: Transmissão vertical, imunidade e resposta à terapia antirretroviral."** apresentado pelo (a) pesquisador (a) **Antonio Victor Campos Coelhos** foi APROVADO pelo Comitê de Ética em Pesquisa em Seres Humanos do Instituto de Medicina Integral Prof. Fernando Figueira – IMIP, em reunião ordinária de 13 de novembro de 2013

Recife, 18 de novembro de 2013

Dr. José Eulálio Cabral Filho
Coordenador do Comitê de Ética
em Pesquisa em Seres Humanos do
Instituto de Medicina Integral Prof. Fernando Figueira

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Anexo 2

Molecular Biology Reports - Instructions for Authors

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

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Please provide 4 to 6 keywords which can be used for indexing purposes.

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Abbreviations should be defined at first mention and used consistently thereafter.

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Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

REFERENCES

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

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The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list. The entries in the list should be numbered consecutively.

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

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All tables are to be numbered using Arabic numerals. Tables should always be cited in text in consecutive numerical order. For each table, please supply a table caption (title) explaining the components of the table. Identify any previously published material by giving the original source in the form of a reference at the end of the table caption. Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

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Anexo 3

Journal of Biomedical Sciense – Instructions for Authors

Submission process

Manuscripts must be submitted by one of the authors of the manuscript, and should not be submitted by anyone on their behalf. The submitting author takes responsibility for the article during submission and peer review.

The publication costs for *Journal of Biomedical Science* are covered by the Ministry of Science and Technology (MOST), Taiwan, so authors do not need to pay an article-processing charge.

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Preparing main manuscript text

General guidelines of the journal's style and language are given below.

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Preparing additional files

The **Accession Numbers** of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example, [EMBL:AB026295, EMBL:AC137000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116]. The databases for which we can provide direct links are: EMBL Nucleotide Sequence Database (EMBL), DNA Data Bank of Japan (DDBJ), GenBank at the NCBI (GenBank), Protein Data Bank (PDB), Protein Information Resource (PIR) and the Swiss-Prot Protein Database (Swiss-Prot). You can download a template (Mac and Windows compatible; Microsoft Word 98/2000) for your article. For reporting standards please see the information in the About section.

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The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: **Background,** the context and purpose of the study; **Results**, the main findings; **Conclusions**, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract.

Keywords

Three to ten keywords representing the main content of the article.

Background

The Background section should be written in a way that is accessible to researchers without specialist knowledge in that area and must clearly state - and, if helpful, illustrate - the background to the research and its aims. The section should end with a brief statement of what is being reported in the article.

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The methods section should include the design of the study, the type of materials involved, a clear description of all comparisons, and the type of analysis used, to enable replication. For further details of the journal's data-release policy, see the policy section in 'About this journal'.

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The Results and discussion may be combined into a single section or presented separately. The Results and discussion sections may also be broken into subsections with short, informative headings.

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This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided, which should precede the competing interests and authors' contributions.

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A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organizations. Authors must disclose any financial competing interests; they should also reveal any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript. Authors are required to complete a declaration of competing interests. All competing interests that are declared will be listed at the end of published articles. Where an author gives no competing interests, the listing will read 'The author(s) declare that they have no competing interests'. When completing your declaration, please consider the following questions:

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research group, alone, does not justify authorship. We suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript. All contributors who do not meet the criteria for authorship should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support.

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Endnotes should be designated within the text using a superscript lowercase letter and all notes (along with their corresponding letter) should be included in the Endnotes section. Please format this section in a paragraph rather than a list.

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All references, including URLs, must be numbered consecutively, in square brackets, in the order in which they are cited in the text, followed by any in tables or legends. Each reference must have an individual reference number. Please avoid excessive referencing. If automatic numbering systems are used, the reference numbers must be finalized and the bibliography must be fully formatted before submission.

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Each table should be numbered and cited in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.). Tables should also have a title (above the table) that summarizes the whole table; it should be no longer than 15 words. Detailed legends may then follow, but they should be concise. Tables should always be cited in text in consecutive numerical order. Smaller tables considered to be integral to the manuscript can be pasted into the end of the document text file, in A4 portrait or landscape format. These will be typeset and displayed in the final published form of the article. Such tables should be formatted using the 'Table object' in a word processing program to ensure that columns of data are kept aligned when the file is sent electronically for review; this will not always be the

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SI units should be used throughout (liter and molar are permitted, however).

Anexo 4

Journal of Medical Virology - Instructions for Authors

AIMS AND SCOPE. Journal of Medical Virology provides a means of rapid publication of original scientific papers on fundamental as well as applied research concerning viruses affecting humans. These include reports describing the characterisation, diagnosis, epidemiology, immunology and pathogenesis of human virus infection, as well as basic studies on virus morphology, genetics, replication and host-cell interactions.
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ABSTRACT. This should be a factual condensation of the entire work and include statements of the problem, method of study, results, and conclusions. The abstract may not exceed 250 words.
KEY WORDS. Supply a list of three to six key words (without repeating words in the title), pertinent to the article, which will appear below the abstract and will be included in the index at the end of the volume.
SEARCH ENGINE OPTIMIZATION. Driving usage and readership is critically important to raising the visibility of your published research. One of the key factors in sustaining long-term usage is through search engine optimization (SEO). Below is a list of suggested ways of maximizing your SEO.
1. Make sure your article title is SEO-friendly. It should be descriptive, and it must include a key phrase from your topic. Key words should appear within the title's first 65 characters.
2. Provide up to five topic-specific key words or phrases in the key word field.
3. Be sure your key words and phrases appear in your abstract several times, but don't go overboard or the search engine may kick you out.
4. When referencing authors, be consistent. Use their names as they generally appear in past online publications.
5. When appropriate, use your key words in article section headings. Remember: They can't read it if they can't find it! For more detailed information on SEO, including helpful examples, go to http://authorservices.wiley.com/bauthor/seo.asp.
VIRUS NOMENCLATURE. Each virus should be identified at least once, preferably in the*Introduction* or *Materials and Methods* section, using formal family, genus, and species terms, and where possible by using a precise strain designation term as developed by an internationally recognized specialty group or

culture collection. Please note that the word type is not used before species designations that include a number. Formal terms used for virus families, genera, and species, should be those approved by the International Committee on Taxonomy of Viruses (ICTV): Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., McGeoch, D.J., Maniloff, J., Mayo, M.A., Pringle, C.R., and Wickner, R.B. Virus Texonomy, Classification and Nomenclature of Viruses, Seventh ICTV Report, Academic Press. This volume also includes standard abbreviations for species. Once formal taxonomic names have been given in a paper, vernacular terms may be used.

Formal taxonomic nomenclature

In formal taxonomic usage, the first letters of virus order, family,subfamily, genus and species names are capitalized and the terms are printedin italics. Other words in the species name are not capitalized unless theyare proper nouns or parts of nouns, for example *West Nile virus* . Informal usage, the name of the taxon should precede the term for the taxonomicunit; for example: "the family *Paramyxoviridae*," "the genus *Morbillivirus*." The following represent examples of full formal taxonomic terminology:

1 Order *Mononegavirales*, Family *Rhabdoviridae*, genus *Lyssavirus*, Species *Rabies virus*.

2 Family *Poxviridae*, subfamily *Chordopoxvirinae*, genus *Orthopoxvirus*, species *Vaccinia virus*.

3 Family *Picornaviridae*, genus *Enterovirus*, species *Poliovirus*.

4 Family *Bunyaviridae*, genus *Tospovirus*, species *Tomato spotted wilt virus*.

Vernacular taxonomic nomenclature

In formal vernacular usage, virus order, family, subfamily, genus and species names are written in lower case Roman script; they are not capitalized, nor are they printed in italics or underlined. In informal usage,the name of the taxon should not include the formal suffix, and the name of the taxon should follow the term for the taxonomic unit; for example "the picornavirus family," "the enterovirus genus." One particular source of ambiguity in vernacular nomenclature lies in the common use of the same root terms in formal family, genus or species names. Imprecision stems from not being able to easily identify in vernacular usage which hierarchical level isbeing cited. For example, the vernacular name "paramyxovirus" might refer to the family *Paramyxoviridae*, the subfamily *Paramyxovirinae*, or one species in the genus *Respirovirus*, such as *Human parainfluenza virus 1* .The solution in vernacular usage is to avoid ‘‘jumping’’ hierarchical levels and to add taxon identification wherever needed. For example, when citing thetaxonomic placement of *Human parainfluenza virus 1*, taxon identification should always be added: " *Human parainfluenza virus 1* is a species in the genus *Respirovirus*, family *Paramyxoviridae*." In this example, as is usually the case, adding the information that this virus is also a member of the subfamily *Paramyxovirinae* and the order *Mononegavirales* isunnecessary. It should be stressed that italics and capitals initial letters need to be used only if the species name refers to the taxonomic category. When the name refers to viral objects such as virions present in a preparation or seen in an electron micrograph, italics and capitals initial letters are not needed and the names are written in lower case Roman script. This also applies when the names are used in adjectival form, for instance tobacco mosaic virus polymerase. The use of italics when referring to the name of a species as a taxonomic entity signals that it has the status of an officially recognized species. The 7th ICTV Report (Van Regenmortel, M.H.V. et al., 1999, Academic Press)

should be consulted to ascertain which names have been approved as official species names. When the taxonomic status of a new putative species is uncertain or its position within an established genus has not been clarified, it is considered a tentative species and its name is not written in italics although its initial letter is capitalized.

TEXT:

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REFERENCES:

In the text, references with one or two authors should be cited with the author's or authors' surname(s) and year of publication in brackets; references with three or more authors should be cited with the first author's surname followed by "et al." and the year of publication in brackets. In the final list, they should be in alphabetical order, and chronologically for more than one reference with the same authorship. Each reference begins with the names of *all* authors and the year of publication. For references to journals give titles of articles in full, inclusive pagination, and journal titles. For references to books, include all author's names, chapter titles (if any), editor (if any), book title, city of publication, publisher's name, and year of publication. Note the following examples:

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Gordon MT, Bell SC, Mee AP, Mercer S, Carter SD.1993. Prevalence of Canine Distemper Antibodies in the Pagetic Population. J Med Virol 40:313–317.

Books:

Zuckerman AJ, Banatvala JE, Patison JR, editors. 2000. Principles and practice of clinical virology, 4th ed. Chichester and New York: John Wiley & Sons, Inc. 776 p.

Chapters in Books:

Lazinski DW, Taylor JM. 1993. Structure and function of the delta virus antigens. In: Hadziyannis SJ, Taylor JM, Bonino F, editors. Hepatitis delta virus—molecular biology, pathogenesis, and clinical aspects. New York: Wiley-Liss, Inc. p 35–44.

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14. Apêndices

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HIV mother-to-child transmission: A complex genetic puzzle tackled by Brazil and Argentina research teams

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ABSTRACT

Human immunodeficiency virus (HIV) mother-to-child transmission is a complex event, depending upon environmental factors and is affected by host genetic factors from mother and child, as well as viral genetic elements. The integration of multiple parameters (CD4 cell count, virus load, HIV subtype, and host genetic markers) could account for the susceptibility to HIV infection, a multifactorial trait. The goal of this manuscript is to analyze the immunogenetic factors associated to HIV mother-to-child transmission, trying to unravel the genetic puzzle of HIV mother-to-child transmission and considering the experience in this topic of two research groups from Brazil and Argentina.

1. Introduction

The mother-to-child transmission (MTCT) or vertical transmission of human immunodeficiency virus type 1 (HIV-1) occurs at an estimated rate of more than 30% and is the major cause of AIDS in children. The transmission can occur at three different times (Newell et al., 1996):

- Prepartum (*in uterus*), due to feto-maternal blood shunts within the placenta;
- Intrapartum (delivery), when infant's oral mucosa is contaminated with infected vaginal secretions;
- Through breast feeding.

Numerous maternal parameters, including mother's advanced clinical stages, low CD4+lymphocyte counts, high viral load, immune response, and disease progression have been implicated in the increased risk of vertical transmission. While the use of antiretroviral therapy (ART) during pregnancy has been shown to reduce the risk of vertical transmission, selective transmission of ART-resistant mutants has also been documented. Elucidation of the molecular mechanisms of vertical transmission might provide relevant information for the development of effective strategies for prevention and treatment (Ahmad, 2011).

The time of delivery and breastfeeding are the principal routes of viral transmission and account for about 70% of pediatric infections in resource-poor countries. The effect of innate immunity (i.e.: anti-microbial peptides, pattern recognition receptors/PRRs) may be of particular relevance because infants are exposed to HIV-1 and acquire infection when the adaptive immune system is still under development.

On the other hand, the risk of in utero transmission is less than 7%; so that even in the absence of virologic suppression with maternal antiretroviral therapy, over 90% of HIV-1-exposed newborns are "naturally" protected from infection in utero. These observations suggest the placenta has evolved mechanisms that restrict establishment of viral infection at the feto-maternal interface. Elucidating these mechanisms may help to determine biologic correlates of protection against HIV-1 transmission in humans (Ahmad, 2011; Johnson and Chakraborty, 2012).

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The feto-maternal interface is characterized by intimate contact between uterine decidual cells and invading chorionic villi. For HIV-1 transmission occurring in utero, the virus must cross the selective placental barrier. An individual villus is lined by trophoblasts, which enclose connective tissue stroma containing fetal blood vessels and numerous fetalmacrophages or Hofbauer cells (HCs). The chorionic villi are directly bathed in maternal blood. Moreover maternal cells have been identified in fetal lymph nodes and are involved in fetal T cells development. So the fetus of an HIV-1-infected mother may be exposed to free and cell-associated virus during gestation (Johnson and Chakraborty, 2012).

HIV-1 has been shown to productively infect trophoblasts, however, they exhibit a lower susceptibility to productive HIV-1 infection than CD4+ T cells do. The trophoblasts express no or few receptors/coreceptors required for virus internalization and its entry in these cells is associated with unusual endocytosis (Vidricaire et al., 2007). Moreover HIV-1 may infect trophoblast by T cell adherence (Arias et al., 2003). Fetal trophoblasts are known to express HLA-G, a non-classical class I HLA, involved in immune tolerance during pregnancy. HLA-G is a ligand for NK cell inhibitory receptor KIR2DL4. Both HLA-G and KIR2DL4 have been described to be involved in HIV infection (Huang et al., 2010; Chaichompoo et al., 2010), emphasizing the possible role of trophoblast in HIV transplacental infection.

An HIV-1 virion can potentially encounter HCs after breaching the trophoblast cell layer. HCs express the HIV-1 (co)-receptors CD4, CCR5 and CXCR4, and also DC-SIGN on their cell surface. HCs express very high levels of DC-SIGN. During pregnancy, there is increased expression of DC-SIGN on HCs; this expression has been correlated with increased rates of HIV-1 vertical transmission. Intuitively, the presence of DC-SIGN and HIV-1 (co)-receptors on HCs, should promote viral entry by free or cell-mediated transmission of HIV-1 facilitating infection in the unborn fetus (Johnson and Chakraborty, 2012).

Another aspect to be considered is that cytokines may influence HIV-1 replication in placenta. Placentas from non-transmitting mothers appear to sustain an immunoregulatory (i.e., IL-10, TGF-β) predominance while placentas from transmitting mothers exhibit a pro-inflammatory pattern (i.e., IL-1β, TNF) of cytokine release (Johnson and Chakraborty, 2012).

2. Aim of the review

MTCT is a complex event, which depends on environmental factors and is affected by host genetic factors from mother and child, as well as viral genetic elements. The aim of this work is to review the current knowledge about genetics factors associated to HIV MTCT, using as starting point the previous experience of two research groups from Brazil and Argentina, widely working on the host immunogenetic restriction factors responsible for susceptibility/protection to HIV-1 infection in children.

We categorize the host immunogenetic restriction factors in: soluble innate immune HIV restriction, HIV (co)-receptors, chemokines and cytokines, human leukocyte antigen, natural killer cells receptors and products. Several studies address the role of host immunogenetic variations in MTCT. Table 1 reports selected association studies conducted on MTCT and the polymorphisms analyzed.

3. Soluble innate immune HIV restriction factors

3.1. Human beta defensin (DEFB1)

Defensins are small cationic amphipathic peptides (30–48 amino acids), produced by leukocytes and epithelial cells, especially in mucosa, with direct and indirect antiviral activity. They inactivate viruses interacting with envelope proteins, or acting as chemoattractive on immune cells.

Within the different human defensins, the beta defensin 1 (hBD1), encoded by *DEFB1* gene, has been reported to be involved in the protection against HIV-1 infection (Ricci et al., 2009). Moreover hBD1has been found in oral and vaginal mucosa as well as in breast milk (Armogida et al., 2004; Jia et al., 2001) and for this reason has been deeply investigated as natural factor involved in susceptibility to MTCT.

When considering the impact of genetic variations in *DEFB1* gene expression, we can observe that single nucleotide polymorphisms (SNPs) localized at regulatory region affect DEFB1 expression that varies within individuals depending upon these SNPs.

In this context, Braida et al. (2004) and Milanese et al. (2006) studied the frequencies of three single nucleotide polymorphisms (SNPs) at the 5′-untranslated region of *DEFB1* gene: −52 (G/A); −44 (C/G); and −20 (G/A) (rs1799946, rs1800972, and rs11362, respectively) in MTCT cohorts from Brazil and Italy.

In Braida et al. (2004) study, allele frequencies of the −44 C/G SNP were significantly different in HIV positive Italian children compared to the healthy controls, because of the difference in the frequency of −44 C/C homozygous individuals. The odds ratio for the −44 C/C genotype in HIV-infected children was 3.6 (95% CI = 1.89–6.90). Genotype and allele frequencies of the −20 G/A SNP in HIV positive children were similar to the controls.

In a similar study, Milanese et al. (2006) analyzed a group of Brazilian children and obtained different results reporting a significant increase of the −52 A/A and −20 G/G genotypes in HIV infected children, when compared with healthy controls. These data suggest a role for −52 A/A and −20 G/G genotypes in increasing the susceptibility to infection. They also found a sensible, even if not significant, reduction of the frequency of the −44 G allele. The frequency of this polymorphism was very low in the Brazilian population when compared with other populations, and this fact could account for the lack of statistical significance.

Conversely the association mentioned above was not confirmed in another replica study by Segat et al. (2009a): the authors showed non-significant results comparing the frequencies of *DEFB1* polymorphisms between HIV positive and healthy control groups. Moreover, when the Brazilian HIV positive populations from Milanese's and Segat's studies were compared, a significant difference between the −20 G/A SNP genotype distribution ($p < 10^{-5}$) was found, evidencing that *DEFB1* 5′UTR polymorphisms frequencies could vary among different populations, and even within groups from the same population.

Other research groups performed analogous studies on DEFB1 5′UTR SNPs associating with risk of MTCT in different populations.

Ricci et al. (2009) studied the distribution of −44 C/G (rs1800972) and −52 G/A (rs1799946) polymorphisms in 118 HIV infected and 182 HIV uninfected children, born of HIV infected mothers. The −52 G/G genotype and the −44G/−52G haplotype were associated with protection against HIV infection ($p = 0.03$, OR = 0.52, 95% CI = 0.31–0.86 and $p = 0.014$, OR = 0.50, 95% CI = 0.31–0.83; respectively). They also studied 84 HIV-infected mothers and showed that the −52G/G genotype and the −44G/−52G haplotype were associated with low levels of HIV plasma viremia (<1000 copies/mL) and a consequent lower risk of HIV MTCT ($p = 0.009$, OR = 0.14, 95% CI = 0.03–0.67 and $p = 0.012$, OR = 0.23, 95% CI = 0.08–0.66, respectively).

Segat et al. (2006a) evaluated the frequency of the same three SNPs at the 5′UTR region of *DEFB-1* gene, in a cohort of 130 HIV infected Italian mothers and their children: the frequency of −44 C allele was significantly different in both HIV positive mothers and their children, in comparison with healthy controls. The odds ratio for −44 C allele in children born to HIV infected mothers

Table 1
Association studies conducted in different populations involving HIV mother to children transmission.

Study	Population	N	Variation	MAFs in studied population				MAFs – HapMap			
				HIV+	HIV−	HC	GERAL	Allele referency	CEU	YRB	CHB
HLA											
Kilpatrick et al. (1991)	UK	53	HLA-DR3	0.43	0.15	NA	0.19				
			HLA–A3	0.13	0.42	NA	0.14				
Greggio et al. (1993)	Italy	172	HLA - DRB1-14a	0.00	0.10	0.05	0.06				
			HLA - DRB1 - 13a.4	0.00	0.06	0.04	0.03				
Winchester et al. (1995)	USA	109	HLA - DR2	0.38	0.44	NA	0.42				
			HLA - DRB1∗1501	0.15	0.67	NA	0.20				
			HLA - DRB1∗11011	0.03	0.12	NA	0.07				
			HLA - DRB1∗1102	0.15	0.12	NA	0.13				
			HLA - DRB1∗03011	0.18	0.19	NA	0.18				
Segat et al. (2009)	Brazil	397	HLA-G - rs1707	0.39	0.40	0.41	0.40	C	0.115(C)	0.123(C)	0.047(C)
Fabris et al. (2009)	Brazil	421	HLA-G - rs1704	0.42	0.21	0.40	0.40	–	0.320 (–)	0.430 (–)	0.309 (–)
CCR5-CXCR4											
Mandl et al (1998)	Austria	79	rs333 (CCR5Δ32)	0.11	0.03	NA	0.08	+	0.048 (–)	0.000 (–)	NA
Philpott et al. (1999)	USA	1104	rs333 (CCR5Δ32)	0.02	0.03	NA	0.03				
Mangano et al. (2000)	Argentina	983	rs333 (CCR5Δ32)	0.04	0.04	0.05	0.04				
DEFB1											
Braida et al. (2004)	Italy	217	rs11362 -A	0.38	NA	0.38	0.38	C	0.363 (T)	0.403 (C)	0.435 (T)
			rs1800972 - G	0.10	NA	0.22	0.16	C	0.258 (G)	0.042 (G)	0.125 (G)
			rs1799946 – A	0.52	NA	0.42	0.47	C	0.394 (T)	0.292 (T)	0.405 (T)
Milanese et al. (2006)	Brazil	303	rs11362 -A	0.52	0.42	0.37	0.44				
			rs1800972 – G	0.07	0.13	0.14	0.11				
			rs1799946 – A	0.33	0.46	0.46	0.40				
Segat et al. (2006)	Italy	250	rs11362 –A	NA	0.37	0.38	0.38				
			rs1800972 – G	NA	0.04	0.22	0.10				
			rs1799946 – A	NA	0.55	0.42	0.50				
Ricci et al. (2009)	Italy	384	rs1800972 – G	0.12	0.16	NA	0.15				
			rs1799946 – A	0.20	0.38	NA	0.40				
MBL2											
Boniotto et al. (2000)	Italy	101	Position –550 - H	NA	0.48	0.36	0.39				
			Position – 328 - del	NA	0.14	0.19	0.18				
Boniotto et al. (2003)	Brazil	306	Allele O	0.29	0.19	0.20	0.23				
Mangano et al. (2008)	Argentina	492	Allele X	0.16	0.11	0.15	0.14				
			Allele O	0.25	0.26	0.21	0.24				
			rs1800450 (B)	0.20	0.20	0.18	0.19	C	0.150 (T)	0.009 (T)	0.155 (T)
			rs5030737 (D)	0.05	0.05	0.03	0.04	G	0.071 (T)	0.021 (T)	0.012 (T)
			rs1800451 (C)	0.00	0.00	0.00	0.00	C	0.018 (A)	0.167 (A)	0.012 (A)
PRF1											
Padovan et al. (2011)	Brazil	395	rs885822 – C	0.32	0.49	NA	0.35	G	0.425 (G)	0.133 (G)	0.321 (G)
SDF1											
Mangano et al. (2000)	Argentina	983	SDF1 3'A (rs1801157)	0.18	0.21	0.24	0.20	C	0.208 (T)	0.022 (T)	0.298 (T)
Sei et al. (2001)	USA	127		0.05	NA	NA	NA				
Tresoldi et al. (2002)	Italy	544		0.24	0.26	0.27	0.25				
DC/L-SIGN											
Da Silva et al. (2012)	Brazil	346	rs735240 – A	0.42	0.36	0.40	0.41	G	0.451 (A)	0.333 (A)	0.270 (A)
			rs735239 – G	0.37	0.28	0.29	0.33	A	0.380 (G)	0.003 (G)	0.180 (G)
			rs4804803 - G	0.32	0.41	0.31	0.33	G	0.258 (G)	0.432 (G)	0.042 (G)
			rs11465366 – T	0.02	0.12	0.03	0.03	C	NA	0.085 (T)	NA
			rs2287886 - A	0.27	0.16	0.28	0.26	A	0.305 (A)	0.184(A)	0.303 (G)
INFAMMASOME											
Pontillo et al. (2010)	Brazil	1038	rs1143634 - G	0.40			0.40	G	0.208(A)	0.099(A)	0.015 (A)
Segat et al. (2006)	Brazil		*rs*1946518	0.35	0.44	0.46	0.41	T	0.392 (T)	0.345 (T)	0.390 (G)
			*rs*187238	0.22	0.25	0.26	0.24	G	0.233 (C)	0.142 (C)	0.153 (C)
TRL9											
Ricci et al. (2010)	Italian	300	rs352139 – A	0.49	0.42	NA	0.48	T	0.482 (C)	0.425 (T)	0.405 (C)
			rs352140 - G	0.45	0.42	NA	0.44	C	0.478 (T)	0.305 (T)	0.399 (T)

EIC = Exposed Infected Children.
EUC = Exposed uninfected children.
UUC = Unexposed uninfected children.
IC = Infected children.
UC = Uninfected children.
MC*p* = Mother–child pairs.
IC-IM*p* = infected child-infected mother pairs.
UC-IM*p* = Uninfected child-infected mother pairs.
UC-IM*p* = Uninfected child-infected mother pairs.
IM–Infected mothers.

was 7.09 (95% CI = 3.38–15.3), whereas for HIV infected mothers was 6.42 (confidence interval 3.14–13.4). This results evidenced an elevated frequency of the −44 C allele in HIV infected mothers. Thus, we must consider that antiretroviral drug treatment and

cesarian section of HIV positive mothers successfully prevented the potential risk of vertical transmission.

Several studies tried to unravel the function meaning of DEFB1 5′UTR SNPs associated with MTCT.

Braida et al. (2004) hypothesized that hBD-1 could be very important in protecting the skin and mucosa of newborns by interacting with the viral particles or with cells of the immune response.

Baroncelli et al. (2008) analyzed *DEFB1*–44C/G and −52 G/A polymorphisms in 78 Mozambican HIV infected mothers. They observed significantly lower levels of HIV RNA in breast milk but not in plasma, in women with the −52 G/G genotype versus women with the −52 G/A and −52 A/A genotypes, supporting the hypothesis that different expression of beta-defensins could have an impact on viral replication in breast milk.

Aguilar-Jiménez et al. (2011) performed a study in a group of 74 mothers and their infants, 36 HIV positive pregnant women and 38 pregnant women HIV negative from Colombia. They observed that hBD-1 transcript levels were significantly higher in placenta from seropositive mothers compared with controls. Additionally, the simultaneous presence of A692G A/G and A1836 G/G genotypes, was associated with high expression of hBD-1 in all groups. Contrasting results in levels of hBDs were probably due to viral stimuli, suggesting that HIV could induce an hBD differential expression in placenta, and this peptide could be involved in protection against HIV, at least early in pregnancy.

Considering that polymorphisms in *DEFB1* affect its expression and that MTCT could involve infant oral mucosa, these findings emphasize that human defensin 1 plays a prominent role in mucosal innate immune defense against HIV-1.

Finally, when considering other human beta defensins, it has been reported that beta defensin 2 and 3 (hBD-2 and hBD-3) could contrast the infection from HIV by protecting GHOST X4/R5 cells from virus infection, by directly binding to the viral envelope (Quinones-Mateu et al., 2003). Moreover, Sun et al. (2005) hypothesized an involvement of beta-defensins in HIV oral transmission, emphasizing their protective role in the oral mucosa.

3.2. Mannose binding lectin (MBL2)

Mannose-binding lectin (MBL), a protein secreted by the liver, is an important component of the innate immunity. It is an acute-phase protein that binds specific carbohydrate residues present on some virus, bacteria and yeast, and may mediate phagocytosis or activate the classical pathway of the complement (Garred et al., 2003).

Three different polymorphisms have been described at exon 1 of the *MBL2* gene, which result in single amino acid changes, affecting MBL oligomerization and funcionality. They are localized at codons 52, 54, and 57 at nucleotide positions 223-C/T (Arg52Cys), 230-G/A (Gly54Asp), and 239-G/A (Gly57Glu), respectively. These mutations generate the allelic variants named "B" (codon 54), "C" (codon 57), and "D" (codon 52), collectively designated as "O"; the wild type allele was called "A" (Garred et al., 2003).

MBL is able to bind the HIV glycoprotein complex gp120–gp41 *in vitro* (Garred et al., 2003). *MBL2* polymorphisms have been associated with susceptibility to HIV infection in Brazilian perinatally infected children (Boniotto et al., 2003) and with accelerated disease progression in HIV-infected Italian children born to seropositive mothers (Amoroso et al., 1999).

The distribution of *MBL2* alleles varies among different populations. The *B* allele is present in White, Asian and American indigenous populations. The *C* allele is found almost exclusively in African populations, while the *D* allele is found in White, East Africans and almost absent in Asians (Garred et al., 2003). Three polymorphisms also have been found in the promoter region of *MBL2*, at positions −550 *(H/L)* and −221 (*X*/*Y*) and in the 5′-untranslated region of exon 1 at position −4 (P/Q) (Mangano et al., 2008).

In Mangano et al. (2008) study, the combined genotype *XA*/*XA* associated with a 8-fold risk of HIV MTCT (OR = 8.11; 95% CI = 0.96–67.86). The polymorphism at codon 54 of exon 1, results in the replacement of a glycine with an aspartic acid, reducing the level of MBL in the serum of five to ten times in heterozygous individuals. In HIV infected children, the presence of the Gly54Asp mutation conferred a relative risk of 3.68 (95% CI = 1.1–13.1) for a rapid progression to AIDS.

Boniotto et al. (2000, 2003)) described an association between the mutated *MBL2* O allele and susceptibility to HIV infection in infants. The presence of the allele O confers a relative risk of 1.37 (95% CI = 1.02–1.84) for HIV infection through MTCT. This allele has a dominant negative effect on MBL serum levels, because it determines an incorrect assembly of MBL subunits in the collagen-like domain, producing a more vulnerable protein to degradation by metalloproteinases. In heterozygous individuals, the serum level of the protein was reduced five to ten times, whereas in O/O homozygote, the level of the protein was undetectable (Boniotto et al., 2000, 2003).

In Singh and Spector (2009) study, *MBL2* O/O genotype was associated with more rapid HIV-related disease progression, predominantly in children younger than 2 years, suggestingthat*MBL2* variants are associated with altered HIV disease progression, particularly in young children.

Crovella et al. (2005) investigated *MBL2* polymorphisms in a cohort of 90 Italian HIV pregnant seropositive women and their children, confirming the association of *MBL2* O/O genotype with an increased risk of infection by HIV MTCT. The frequency of the *MBL2* O/O homozygote was higher in HIV infected mothers than in healthy controls. Similarly, the *MBL2* O/O genotype was more frequent in infected children born from HIV positive mothers than in healthy controls. These polymorphisms were also evidenced in children born from HIV positive mothers, but the risk of infection was strongly reduced by cesarean delivery and by antiretroviral treatment.

Assuming that *MBL2* activates the complement system, promoting viral killing, and that variations at exon 1 (polymorphism A/O) lead to deficient levels of circulating protein, studies show that individuals with polymorphism A/O (codons 52, 54 and 57) are more susceptible to HIV MTCT.

4. HIV (Co-)receptors

4.1. C–C chemokine receptor type 5 (CCR5)-C–X–C chemokine receptor type 4 (CXCR4)

CCR5 and CXCR4 are recognized as the most important co-receptors used for HIV to enter the cell.

CCR5 genetic polymorphisms have been associated to MTCT (John et al., 2001). The *CCR5Δ32* mutation occurs in 10% of Caucasian and consists in a deletion of 32bpresulting in a non-functional receptor (Taborda-Vanegas et al., 2011). This mutation was associated with AIDS progression, but evidences suggest that it has no effect on the risk of HIV perinatal transmission (Contopoulos-Ioannidis et al., 2003). This could be explained by the fact that *CCR5* expression is influenced by other factors than *CCR5Δ32*; in fact, *CCR5* expression levels differ considerably among individuals with the same genotype. MTCT could occur via R5X4 or X4 strains able to initiate infection via CXCR4, the alternative co-receptor for HIV (De Souza et al., 2006).

A meta-analysis study including 10 cohorts with 1317 HIV-infected children the *CCR5Δ32* and *CCR64I* alleles were associated

with a decreased risk of death among perinatally infected children, but only for the first years of life (Ioannidis et al., 2003).

Philpott et al. (1999) studied a cohort of 552 children (13% White, 30% Latino and 56% African American) born of Americans infected mothers in relation to the *CCR5Δ32* mutation and they observed variation in allele frequency among the groups, ranging from 0.08 in Whites to 0.02 in both Latinos and African Americans. Approximately, 27% of the children in each ethnic group were infected. Four children were identified as *CCR5Δ32* homozygotes, two uninfected Whites (3.77%) and two uninfected Latinos (1.68%). None of the infected children displayed the *CCR5Δ32* homozygous genotype, suggesting that this mutant genotype may confer protection from HIV mother-to-child transmission.

Similarly, in an Argentinean cohort of 886 children born to HIV seropositive mothers (449 HIV+, 433 HIV−) of Hispanic-Caucasian descendants, only one *CCR5Δ32* homozygous was found among exposed uninfected children (Mangano et al., 2000).

Mandl et al. (1998) studied a group of 79 children born to HIV positive mothers from Austria (45 uninfected and 34 infected by MTCT) and showed that the presence of the defective HIV co-receptor gene *CCR5Δ32* was also associated with MTCT. The mutant allele frequency was 11.1% in uninfected children (17.8% heterozygous, 2.2% homozygous). In the group of infected children, there were only two heterozygous and no *CCR5Δ32* homozygous, corresponding to a significantly reduced mutant allele frequency of 2.9% ($p = 0.05$ compared to HIV negative children). These results suggest that *CCR5/CCR5Δ32* heterozygous children were less susceptible to vertical transmission of HIV.

Some genetic polymorphisms have been described in *CCR5* regulatory region, which, together with the *CCR5Δ32* mutation, define 9 human haplogroups (HHA to HHG2) (Gonzalez et al., 1999 Kostrikis at al. (1999)).

Gonzalez et al. (1999) showed that *CCR5* haplotypes pairs have been associated with different risk of transmission and AIDS progression in a large well-characterized racially mixed cohort of HIV seropositive children. The HHE/HHE haplotype was associated with increased of HIV MTCT susceptibility, disease accelerating and faster progression in Argentinean children. On the other hand, the HHC/HHG2 haplotype was associated with reduced risk of HIV MTCT and disease retarding effects. Additionally, the spectrum of *CCR5* haplotypes associated with disease acceleration or retardation differs between African Americans and Caucasians. Other studies conducted by Mangano et al. (2000, 2001) showed that other haplotypes, such as HHD/HHD (in African American children) and HHC/HHF2 (in Argentinean children) were associated with increased HIV MTCT susceptibility and disease retarding effect.

As expected, considering its role as HIV-1 co-receptor, the CCR5 Δ32 variation is associated with a protection against MTCT. However polymorphisms at*CCR5* gene regulatory region confer increased susceptibility to HIV MTCT.

4.2. C-type lectins (DC-SIGN and L-SIGN)

Some pattern recognition receptors (PRRs) located on the surface of dendritic cells (and other cells) play an important role in HIV transmission. Of particular interest are the DC-SIGN (Dendritic cell-specific ICAM-3-grabbing non-integrin) and L-SIGN (liver/lymph node–specific ICAM-3–grabbing non-integrin) receptors, two C-type lectins, long type 2 integral membrane proteins, involved in both innate and adaptive immunity. They work as pathogen-recognition receptors and are able to detect a wide range of microorganisms, including HIV (Baribaud et al., 2001; da Silva et al., 2011; Sobieszczyk et al., 2011).

The *CD209* gene family encodes both receptors. DC/L-SIGN receptors captures the HIV virus by binding to the gp120, promoting the enhancement of T cell infection *in trans*. Additionally, they can internalize the virus and promote virus degradation in a proteasoma dependent manner (da Silva et al., 2011; Sobieszczyk et al., 2011).

Only a few studies have investigated the possible involvement of DC/L-SIGN receptors in the genetic mechanisms correlated with HIV MTCT (Boily-Larouche et al., 2009; Da Silva et al., 2012).

Da Silva et al. (2012) studied polymorphisms in *DC-SIGN* and *L-SIGN* genes in children (192 HIV+ and 58 HIV−) born to HIV+ mothers, as well as in 96 healthy uninfected children not exposed to HIV, all from Northeast Brazil, and found associations of three SNPs in *DC-SIGN* promoter, being two associated with protection (rs11465366: allele T and G/T genotype; rs4804803: G/G genotype) and one with susceptibility (rs2287886: G/A genotype) to HIV MTCT. It was also observed that variations number tandem repeat (VNTR) in *L-SIGN* exon 4 were associated with susceptibility (5/5 and 6/6 homozygous genotypes) to HIV MTCT.

Another association study (Boily-Larouche et al., 2009) performed in a group of 197 HIV infected mothers and their children from Zimbabwe found that children with two copies of H1 and/or H3 haplotype of L-SIGN were about 3.6 times more at risk for intrauterine HIV MTCT and 5.7 times at risk for intrapartum transmission. The H1 and H3 haplotypes were characterized by two SNP at the promoter region (p-198A) and the intron 2 (int2–180A) that associated with a reduction of the transcriptional activity.

The role of DC-SIGN genetics in MTCT is still confusing: some polymorphisms in DC-SIGN promoter region (rs11465366, rs4804803) are found to be protective, whereas other (rs2287886) appeared to augment the risk of MTCT. L-SIGN variations were invariably associated with susceptibility to HIV MTCT.

4.3. Toll like receptor 9 (TLR9)

Another gene related with MTCT is the Toll-like receptor 9 (*TLR9*). Ricci et al. (2010) studied SNPs (rs352139: c.4–44G > A and rs352140: c.1635A > G) in *TLR9* gene associated to the risk of HIV MTCT in 300 children (118 HIV-infected and 182 HIV-uninfected) born to HIV-infected mothers. TLR9 recognizes pathogen-associated molecular patterns and play a crucial role in the host's innate immune response. The AA and GG haplotypes were associated with a higher risk of HIV infection compared to the prevalent GA haplotype ($p = 0.016$, OR = 3.16, 95% CI = 1.24–8.03 and $p = 0.004$, OR = 5.54, 95% CI = 1.76–17.50, respectively) (Ricci et al., 2010) suggesting a role for TLR9 in the modulation of susceptibility to HIV MTCT.

5. Chemokines and cytokines

5.1. Human beta chemokine ligand 3-like1 (CCL3L1)

Human beta-chemokine (CCL3L1), the most potent ligand for CCR5, may be a dominant HIV-suppressive chemokine (Nibbs et al., 1999; Menten et al., 2002; Townson et al., 2002). *CCL3L1*, a duplicated isoform of the gene encoding *CCL3*, is 30-fold more potent in inhibiting R5 HIV infection when compared with CCL3 (Menten et al., 2002). As a consequence of these duplications, the copy number of *CCL3L1* (gene dose) varies among individuals and can affect chemokine concentrations (Townson et al., 2002; Gonzalez et al., 2005; Meddows-Taylor et al., 2006).

Townson et al. (2002) found that lipopolysaccharide stimulation of peripheral blood mononuclear cells from 35 individuals increased expression of *CCL3L1* mRNA. Samples with higher *CCL3L1* copy number had a significant increase in the ratio *CCL3L1/CCL3* mRNA. A high *CCL3L1* copy number also correlated with increased functional chemokine production. Genetic variation in CCL3L1 gene copy number may affect the susceptibility to progression or

severity of diseases in which this chemokine plays a role, as for HIV infection.

Some studies have shown that genetic variation in *CCL3L1* can affect susceptibility to HIV transmission. Kuhn et al. (2007) study, conducted in 849 HIV infected mothers and their infants of Johannesburg (South Africa), observed a strong association between higher infant CCL3L1 gene copies and reduced susceptibility to HIV in the absence of maternal treatment with nevirapine.

Meddows-Taylor et al. (2006) showed that *CCL3L1* gene copy number was associated with *CCL3* production and with HIV vertical transmission. However, at equivalent *CCL3L1* gene copy numbers, infants who acquired HIV infection relative to their exposed but uninfected counterparts had lower production of *CCL3*, suggesting that they may harbor some non-functional copies of this gene.

Paximadis et al. (2011) study analyzed the influence of intragenic *CCL3* haplotypes and *CCL3L* copy number (CN) in a cohort HIV MTCT from sub-Saharan Africa. The authors observed that *CCL3* Hap-A1 haplotype was associated with high *CCL3L* CN in total ($p = 0.001$) and exposed uninfected infants ($p = 0.006$), the effect was not additive, however, having either Hap-A1 or high *CCL3L* CN was more significantly ($p = 0.0008$) associated with protection from in utero infection than Hap-A1 ($p = 0.028$) or high *CCL3L* CN ($p = 0.002$) alone.

Gonzalez et al. (2005) showed that there are significant inter-individual and inter-population differences in the copy number of a segmental duplication encompassing the gene encoding *CCL3L1*. Mean *CCL3L1* copy number varied in different population groups, being generally highest in Africans, followed by East Asians, Amerindians, Central/South Asians, Middle Easterners and Europeans. Additionally, possession of a *CCL3L1* copy number lower than the population average is associated with markedly enhanced susceptibility to HIV MTCT.

As expected studies showed that individuals with high copy number of *CCL3L1* are protected from HIV MTCT.

5.2. Human alpha chemokine ligand 12 (CXCL12)/Stromal derived factor 1 (SDF1)

SDF1 gene encodes a stromal cell-derived alpha chemokine, member of the intercrine family. The gene product and its receptor CXCR4 can activate lymphocytes (Winkler et al., 1998). Mutations in this gene were associated with resistance to HIV infection (Winkler et al., 1998) and rapid disease progression in children (Tresoldi et al., 2002).

Tresoldi et al. (2002) study suggested that the presence of the *SDF-1 3′A* polymorphism was associated to a rapid disease progression in Italian HIV infected children born to seropositive mothers, but did not protect against MTCT, proposing *SDF-1 3′A* mutation as a marker of disease progression. In contrast, Mangano et al. (2000) did not find any association between the rates of HIV transmission or disease progression with *SDF-1 3′A* genotype in a group of 430 HIV infected children.

Tresoldi's findings are in agreement with other studies (Winkler et al., 1998; Sei et al., 2001) associating the homozygous *SDF-1 3′A* mutation with accelerated onset of AIDS in HIV infected adults (Winkler et al., 1998). These studies showed evidences that a large number of children were infected with MT-2–negative viruses, which are capable of using only the CCR5 receptor. Therefore, it is not surprising that SDF-1, the ligand of CXCR4, may not affect vertical transmission of R5 viruses. However it is possible that SDF-1 has an inhibitory effect on the transmission of X4 viruses harboured by the mother (Winkler et al., 1998).

Furthermore, Sei et al. (2001) did not show any significant difference in the frequency of AIDS development in children during the first 3 years of life in relation to *SDF-1 3′A* genotype. This group of children included 127 subjects (58 Caucasians, 60 African-Americans and 9 Hispanics). The overall frequency of the *SDF-1 3′A* mutation was different in the Italian children of Tresoldi's study with respect to the Caucasian children (41.4% vs. 34.5%, respectively) enrolled in the United States by Sei et al. (2001).

In pediatric AIDS, the protective effect of *CCR5wt/Δ32* appears to be abrogated by the *SDF1–3′A* genotype. Singh and Spector (2009) studied SDF1–3′-G/A polymorphism in a cohort of 1049 children with symptomatic HIV infection and observed that the *SDF1–3′*A/A variant was associated with more-rapid disease progression, occurring in <2% of the children.

John et al. (2000) showed that the maternal heterozygous *SDF1* genotype (*SDF1 3′A*/wt) was associated with perinatal transmission of HIV (risk ratio [RR], 1.8; 95% CI = 1.0–3.3) and particularly postnatal breast-milk transmission (RR = 3.1; 95% CI = 1.1–8.6). In contrast, the infant *SDF1* genotype had no effect on mother-to-infant transmission. These data suggest that *SDF1* may affect the ability of the mother to transmit the virus to her infant.

So we can conclude that SDF-1 3′A polymorphism is associated with increased susceptibility to HIV MTCT.

5.3. Interleukin-18 (IL-18)

Segat et al. (2006b) reported that the −607 C variation is associated with an increased susceptibility to MTCT in North East of Brazil, suggesting a role of IL-18 in MTCT, as proposed by Ahmad et al. (2002).

6. Human leukocyte antigen (HLA)

6.1. Human leukocyte antigen (HLA) class 1

Human leukocyte antigen (HLA) class 1 genes, located at *the HLA-A, -B*, and *-C loci*, encode molecules that differentially present endogenous viral peptides to $CD8^+$ T lymphocytes. This class of genes has been so far investigated for its role in the infection of HIV and MTCT. Several polymorphisms in *HLA* genes have been widely studied as candidates for susceptibility to MTCT (Kilpatrick et al., 1991; Greggio et al., 1993; Winchester et al., 1995; Aikhionbare et al., 2001; Fabris et al., 2009; Segat et al., 2009b; Pérez-Núñez et al., 2011).

A serologic HLA typing study found that *HLA-A3-B7-DR2* haplotype was associated with protection against HIV MTCT infection, whereas the *HLA-A1-B8-DR3* haplotype was associated with the predisposition to infection in children (Kilpatrick et al., 1991). Another study showed that *DRB1–13* allele subtypes were associated with protection against MTCT (Greggio et al., 1993).

On other hand, the *HLA-DR3* (DRB1*03011) allele was associated with the occurrence of HIV infection among American Caucasian children and the *HLA-DR13* alleles associated with protection against HIV transmission in African-American but not in Caucasian infants (Winchester et al., 1995). These studies suggest that the variability of viral peptides presentation by HLA molecules, may significantly influence the susceptibility to MTCT.

Another study showed that the concordance or discordance of *HLA* alleles between mother and child could to be a key factor for MTCT, and that *HLA* genotype could influence disease susceptibility in utero by affecting immune responses (Pérez-Núñez et al., 2011).

6.2. HLA-G

Within HLA molecules, HLA-G plays an important role at the maternal-fetal interface. HLA-G is a non-classical HLA molecule from class I involved in immune tolerance by acting as ligand for inhibitory receptors present on natural killer (NK) cells and macrophages. This molecule has a limited distribution in tissues and is

selectively expressed in placental trophoblast cells, at the maternal-fetal interface (Hunt et al., 2000; Moodley and Bobat, 2011).

HLA-G molecules appear to protect the fetus from maternal cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, playing an important role in pregnancy maintenance (Kovats et al., 1990). Therefore, HLA-G influences HIV MTCT, increasing or decreasing protection of infants against virus transmission.

Aikhionbare et al. (2001) study suggests that mother–child pairs both carrying the identical mutation in *HLA-G* exon 2 may be at higher risk for HIV MTCT: the discordance of *HLA-G* exon 2 was significantly more common among non-transmitting (93%) than transmitting mother–child pairs (40%).

Another studies in a Brazilian population, conducted by Fabris et al. (2009) and Segat et al. (2009b) showed that polymorphisms in *HLA-G* are involved in MTCT.

Fabris et al. (2009) studied 175 perinatally infected and 71 exposed uninfected children born to HIV infected mothers and 175 uninfected children, founding significant differences in allele and genotype frequencies of *HLA-G* 3′ UTR 14-bp polymorphism (rs16375). The 14-bp-deleted allele was significantly more frequent in exposed uninfected children than in HIVpositivechildren, being associated with a reduced risk of HIV MTCT ($p < 0.0001$, OR = 0.37, 95% CI = 0.23–0.58).

Segat et al. (2009a) evaluated the possible association of *HLA-G* 3777G > C and 14-bp deletion/insertion (D/I) polymorphisms with perinatal transmission, and observed that the 3777G > C polymorphism alone has no effect on HIV MTCT, but when linked with the D allele, exerts a positive role in the protection to infection.

HLA-G 14-bp insertion has been associated with a lower mRNA production for most membrane-bound and soluble isoforms in trophoblast samples. Different subjects carrying this polymorphism have been shown to undergo alternative splicing events (Kovats et al., 1990).

Moodley and Bobat (2011) showed that placental *HLA-G1* expression could contribute for MTCT. The authors observed that, in children, the risk for HIV infection increases by 1.3 with every 1 unit increase in *HLA-G1* expression. Females were 3.7 times more likely to become infected than males. A positive correlation was observed between mother's log viral load and HIV vertical transmission ($p = 0.047$; 95% CI = 1.029–11.499). Furthermore, the authors described that *HLA-G1* expression was 3.95 times higher in placentas of HIV-1 infected mothers who transmitted the virus to their children, when compared to mothers with uninfected babies.

These studies indicated that *HLA-G* polymorphism rs16375 alone or combined with 3777G/C as well as a mutation in exon 2 confer protection to HIV MTCT.

7. Natural killer cells receptors and products

7.1. Killer immunoglobulin-like receptors (KIR)

Natural killer (NK) cells perform a vital role in response to pathogen infection, with the ability to directly kill infected cells, produce cytokines and crosstalk with the adaptive immune system. These functions are dependent on activation of NK cells, which is determined by surface receptor interactions with ligands on target cells, as the killer immunoglobulin-like (KIRs) receptors that interact with MCH class 1 (Jamil and Khakoo, 2011).

When considering the susceptibility to HIV infection, is evident the role of HLA and KIR receptors. HIV can down regulate HLA class I expression to block the presentation of viral epitopes and prevent cytotoxic T lymphpcytes (CTL) killing of the infected cells. NK cells eliminate cells that fail to display correct levels of HLA receptors, and one function of KIR in NK cells is to define whether the potential target cells carry the proper set of HLA receptors (Jamil and Khakoo, 2011; Paximadis et al., 2011).

The interaction of KIR and their HLA ligands is complex (Paximadis et al., 2011): some studies showed that polymorphism in these genes may influence HIV MTCT (Winchester et al., 1995; Mackelprang et al., 2008; Paximadis et al., 2011).

During pregnancy, the child shares *MHC* genes with the mother, while the mother is induced to tolerate the paternally derived fetal

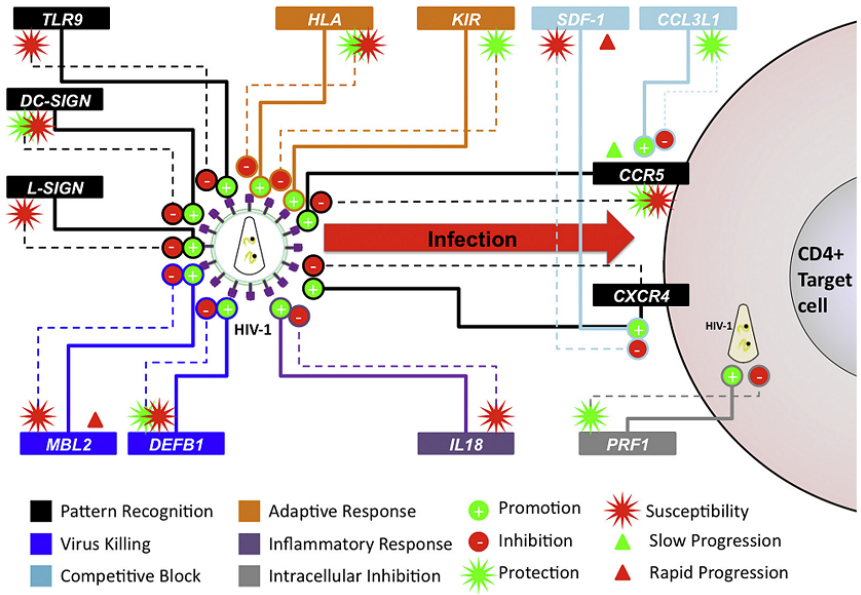

Fig. 1. How genetics can affect innate and adaptive immunity components involved in HIV mother-to-children transmission and progression to AIDS.

Table 2
Minor allele frequencies of SNPs involved in MTCT in different populations.

Study	Population	*N*	Variation	MAFs in studied population				MAFs – HapMap			
				HIV+	HIV−	HC	GERAL	Allele referency	CEU	YRB	CHB
HLA Kilpatrick et al. (1991)	UK	53									
			HLA–DR3	0.43	0.15	NA	0.19				
			HLA–A3	0.13	0.42	NA	0.14				
Greggio et al. (1993)	Italy	172	HLA–DRB1–14a	0.00	0.10	0.05	0.06				
			HLA–DRB1–13a.4	0.00	0.06	0.04	0.03				
Winchester et al. (1995)	USA	109	HLA–DR2	0.38	0.44	NA	0.42				
			HLA–DRB1∗1501	0.15	0.67	NA	0.20				
			HLA–DRB1∗11011	0.03	0.12	NA	0.07				
			HLA–DRB1∗1102	0.15	0.12	NA	0.13				
			HLA–DRB1∗03011	0.18	0.19	NA	0.18				
Segat et al. (2009a)	Brazil	397	HLA-G–rs1707	0.39	0.40	0.41	0.40	C	0.115(C)	0.123(C)	0.047(C)
Fabris et al. (2009)	Brazil	421	HLA-G–rs1704	0.42	0.21	0.40	0.40	–	0.320(–)	0.430(–)	0.309(–)
CCR5-CXCR4											
Mandl et al. (1998)	Austria	79	rs333 (CCR5Δ32)	0.11	0.03	NA	0.08	+	0.048 (–)	0.000 (–)	NA
Philpott et al. (1999)	USA	1104	rs333 (CCR5Δ32)	0.02	0.03	NA	0.03				
Mangano et al. (2000)	Argentina	983	rs333 (CCR5Δ32)	0.04	0.04	0.05	0.04				
DEFB1											
Braida et al. (2004)	Italy	217	rs11362–A	0.38	NA	0.38	0.38	C	0.363 (T)	0.403 (C)	0.435 (T)
			rs1800972–G	0.10	NA	0.22	0.16	C	0.258 (G)	0.042 (G)	0.125 (G)
			rs1799946–A	0.52	NA	0.42	0.47	C	0.394 (T)	0.292 (T)	0.405 (T)
Milanese et al. (2006)	Brazil	303	rs11362–A	0.52	0.42	0.37	0.44				
			rs1800972–G	0.07	0.13	0.14	0.11				
			rs1799946–A	0.33	0.46	0.46	0.40				
Segat et al. (2006)	Italy	250	rs11362–A	NA	0.37	0.38	0.38				
			rs1800972–G	NA	0.04	0.22	0.10				
			rs1799946–A	NA	0.55	0.42	0.50				
Ricci et al. (2009)	Italy	384	rs1800972–G	0.12	0.16	NA	0.15				
			rs1799946–A	0.20	0.38	NA	0.40				
MBL2											
Boniotto et al. (2000)	Italy	101	Position-550–H	NA	0.48	0.36	0.39				
			Position-328–del	NA	0.14	0.19	0.18				
Boniotto et al. (2003)	Brazil	306	Allele O	0.29	0.19	0.20	0.23				
Mangano et al. (2008)	Argentina	492	Allele X	0.16	0.11	0.15	0.14				
			Allele O	0.25	0.26	0.21	0.24				
			rs1800450 (B)	0.20	0.20	0.18	0.19	C	0.150 (T)	0.009 (T)	0.155 (T)
			rs5030737 (D)	0.05	0.05	0.03	0.04	G	0.071 (T)	0.021 (T)	0.012 (T)
			rs1800451 (C)	0.00	0.00	0.00	0.00	C	0.018 (A)	0.167 (A)	0.012 (A)
PRF1											
Padovan et al. (2011)	Brazil	395	rs885822–C	0.32	0.49	NA	0.35	G	0.425 (G)	0.133 (G)	0.321 (G)
SDF1											
Mangano et al. (2000)	Argentina	983	SDF1 3′A (rs1801157)	0.18	0.21	0.24	0.20	C	0.208 (T)	0.022 (T)	0.298 (T)
Sei et al. (2001)	USA	127		0.05	NA	NA	NA				
Tresoldi et al. (2002)	Italy	544		0.24	0.26	0.27	0.25				
DC/L–SIGN											
Da Silva et al. (2012)	Brazil	346	rs735240–A	0.42	0.36	0.40	0.41	G	0.451 (A)	0.333 (A)	0.270 (A)
			rs735239–G	0.37	0.28	0.29	0.33	A	0.380 (G)	0.003 (G)	0.180 (G)
			rs4804803–G	0.32	0.41	0.31	0.33	G	0.258 (G)	0.432 (G)	0.042 (G)
			rs11465366–T	0.02	0.12	0.03	0.03	C	NA	0.085 (T)	NA
			rs2287886–A	0.27	0.16	0.28	0.26	A	0.305 (A)	0.184 (A)	0.303 (G)
INFAMMASOME											
Pontillo et al. (2010)	Brazil	1038	rs1143634–G	0.40			0.40	G	0.208 (A)	0.099 (A)	0.015 (A)
Segat et al. (2006)	Brazil		*rs*1946518	0.35	0.44	0.46	0.41	T	0.392 (T)	0.345 (T)	0.390 (G)
			*rs*187238	0.22	0.25	0.26	0.24	G	0.233 (C)	0.142 (C)	0.153 (C)
TRL9											
Ricci et al. (2010)	Italian	300	rs352139–A	0.49	0.42	NA	0.48	T	0.482 (C)	0.425 (T)	0.405 (C)
			rs352140–G	0.45	0.42	NA	0.44	C	0.478 (T)	0.305 (T)	0.399 (T)

NA = not analyzed.

MHC molecules, in part through natural killer (NK) recognition of MHC polymorphisms (Winchester et al., 1995).

In the context of MTCT Winchester et al. (1995) determined the *HLA-B* alleles of mother and infants. The results revealed that almost half (48%) of mothers who transmitted with low viral loads had *HLA-B*1302, B*3501, B*3503, B*4402 or B*5001* alleles, compared with 8% of non-transmitting mothers ($p = 0.001$). Conversely, 25% of mothers who did not transmit despite high viral loads had B*4901 and B*5301, vs. 5% of transmitting mothers ($p = 0.003$), showing a distinct pattern of allelic involvement able to influence susceptibility to HIV infection. In children *HLA-B* alleles were not associated with virus transmission risk. The *HLA-B*4901* and B*5301 alleles, protective in the mother, both differed respectively from the otherwise identical susceptibility alleles, B*5001 and B*3501, by 5 amino acids encoding the ligand for the KIR3DL1 NK receptor. Results suggest that the probable molecular basis of the observed association involves definition of maternal NK recognition repertoire by engagement of NK receptors with polymorphic ligands encoded by maternal *HLA-B* alleles.

Paximadis et al. (2011) studied the *KIR*, *HLA-B* and *HLA-C* genes of 224 HIV infected mothers and 222 infants (72 infected and 150 uninfected) from South Africa. *KIR2DL2/KIR2DL3* was underrepresented in intrapartum (IP) transmitting mothers ($p = 0.036$). The frequency of homozygous for *KIR2DL3* alone, and in combination

with *HLA-C* haplotype heterozygous (C1C2), was significantly elevated in IP transmitting mothers ($p = 0.034$ and $p = 0.01$ respectively). In infants, *KIR2DL3* in combination with its *HLA-C1* ligand as well as homozygous *KIR2DL3* with *C1C2*, were underrepresented in infected infants compared to exposed uninfected subjects ($p = 0.007$ and $p = 0.03$).

Mackelprang et al. (2008) study analyzed mother–child *HLA* concordance and maternal *HLA* homozygosity in a Kenyan perinatal cohort receiving antenatal zidovudine and found that the risks of overall, in utero and breast milk HIV transmission increased with *HLA* concordance and homozygosity. The increased risk may be due to reduced alloimmunityy or less diverse protective immune responses.

These findings suggest that KIR variants in combination with others components such as HLA-C confer protection to HIV MTCT.

7.2. Perforin(PRF1)

Perforin is an important component of the secretory granule-mediated cell death pathway. It is a protein present in the granules of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells and plays an important role in the elimination of virus-infected cells (Heintel et al., 2002).

Once released, perforin polymerizes to form transmembrane pores in the phospholipid bilayer of target cells' membranes. Through these pores other components of the lytic granules, such as granzyme A, granzyme B, and granulysin (Heintel et al., 2002) can entry into the cells, leading to the activation of various apoptotic death pathways (Lichtenheld et al., 1988).

Padovan et al. (2011) analyzed *PRF1* gene polymorphisms, localized at coding and untranslated regions (UTRs), in three groups of children from Recife (Brazil): 173 perinatally infected children, 51 HIV exposed-uninfected and 170 children with no exposure to the virus. The rs885822 C allele and C/C genotype were significantly more frequent in HIV exposed-uninfected than in HIV exposed-infected children. The authors suggested that C allele and C/C genotype were associated with a protective effect toward HIV MTCT.

8. Comments

The HIV MTCT is a complex puzzle event of multiple factors that remain incomplete until now. In this article we try to share with the reader the experience of two research groups working on genetic variations of innate and acquired immunity involved in the in the susceptibility to HIV MTCT, by systematically and critically revising our findings in comparison with the literature. The innate immunity is essential in the initial detection of HIV, mounting an efficient response against the virus in newborns, since its adaptive immunity is not well developed yet. Even thus, the children adaptive immune response plays a key role on the MTCT mechanism too. In fact, the interaction of innate and adaptive immune genetic components seems to be essential in the HIVMTCT outcome (see Fig. 1).

Another interesting component in HIV MTCT, is the mother–child genetic interdependency. For example, HLA concordance between the mother and its child was associated with increased risk of HIV MTCT in utero and through breast milk. This increased susceptibility has several possible biological mechanisms. Children with the same HLA of their mothers could be less able to identify HIV that has evaded maternal immune responses via HLA-mediated selection. HLA concordance might also reduce the likelihood of babies' immune response against maternally derived lymphocytes (Mackelprang et al., 2008).

The major limitation of the genetic associations studies described in this review is the small numbers of individuals enrolled in each study. In part, this limitation is due to, fortunately, the highly effective prevention strategies for MTCT that have been successfully introduced in the clinical management of pregnant women. A definitive solution for this problem is the creation of a MTCT consortium, at least at Continental level, with the possibility of analyzing larger groups of children from different ethnic groups worldwide. Ethnicity is the other source of discordant findings, since the uneven distribution of several genetic polymorphisms in distinct ethnic group's accounts for the biases presented in the paragraphs above.

Table 2 describes the minor allele frequencies (MAFs) of all associated genetic variations with HIVMTCT in HIV patients and in different ethnic healthy individuals: data were collected in International HapMap project (http://hapmap.ncbi.nlm.nih.gov/) and NCBI Variation Database (dbSNP) (http://www.ncbi.nlm.nih.gov/snp), International HapMap Project (2013), NCBI Variation Database (2013). The distribution of some genetic variations is clearly associated with the ethnic component and could influence the rate of the HIV infection in such ethnic group.

As a multifactorial event, the MTCT does not depend on the genetic contribution of each individual factor, conferring higher/lower MTCT susceptibility in statistical odd rates. Since the role of various factors have been elucidated in the same population, it would be possible infer the overall risk factors. Until now, it does not exist a genetic association study of MTCT that includes the whole human genome and the available data, unfortunately, do not allow predicting the genetic interaction with statistical power.

As stated before, it would be very useful to create a consortium to increase the number of patients and join forces to better understand the role of each genetic factor in the susceptibility of MTCT, including children with different ethnic backgrounds.

In this article we looked at HIV-MTCT with a "geneticist eye" but the role of environment in MTCT, as described, is also very important. Moreover, studies focusing in the viral variants and subtypes could increase the knowledge and should be considered as an important variable in the future of genetic association studies.

9. Conclusions

The year of 2013 will mark the 32th anniversary of the beginning of AIDS epidemic, and the better understanding of the innate and adaptive immunity factors involved in MTCT susceptibility will be essential for unravelling the mechanisms involved in HIV infection, possibly contributing to the identification of new targets for immunological drugs. Safeguarding the health of mothers and infants provides a strong basis for the growth of new AIDS free generations. For this reason we are aware that in spite of being of scientific interest genetics just provides a little contribution in the fight against MTCT; prevention and the successful introduction in the gynaecological practice of the rapid HIV test as well as the strategies to limit MTCT including cesarian delivery, maternal milk bank to replace breastfeeding do represent the better approach that succeeded to strongly reduce MTCT in most of the world, including Latin America where we do operate.

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SHORT REPORT

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Host genomic HIV restriction factors modulate the response to dendritic cell-based treatment against HIV-1

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Keywords: DC-based immune-treatment, HIV vaccine, PARD3B, host genome and response to vaccine

Host genome is still poorly investigated in the context of vaccine or immunotherapy, however recently findings emphasized that it may affect the response to those treatments. In our retrospective study we evaluated the effect of HIV-1 genetic restriction factors on the response to dendritic cell (DC)-based immunotherapy in a Brazilian cohort of HIV positive (HIV+) patients that underwent a phase I clinical trial in 2004.

Genomic DNA from 18 HIV+ individuals that underwent DC-based immunotherapy was analyzed for selected polymorphisms known to be associated with susceptibility to HIV-1 infection and/or AIDS progression. Allelic and genotypic distribution of the 22 polymorphisms was evaluated considering the response to the treatment.

The rs11884476 SNP in *PARD3B* resulted associated with good response to ~~the~~ immune treatment according to an over-dominant model. Even if functional effect of this variation is still unknown, our data suggested that it could play a role in the control of viral replication.

Our findings, being aware of the limitation represented by the small number of subjects analyzed, suggest that genetic factors involved in AIDS progression could affect the response to immunotherapy, reinforcing the idea that deeper investigation on host genetic variations will be fundamental for a rational vaccine development.

Dendritic cell (DC)-based immune treatments have gained great interest as alternative therapy for HIV-1 infected (HIV+) individuals as they are conceived to induce durable cellular responses to control viral replication through an autologous, safe and well-tolerated protocol. However, at present, only few clinical trials reported a significant control of plasma viral load (PVL) in HIV+ patients.[1,2]

In 2004 Lu et al.[3] performed a phase I clinical trial of DC-based treatment on 18 Brazilian HIV+ patients. Three doses of autologous monocyte-derived DC pulsed with autologous chemically inactivated HIV-1 were administered every 15 d and PVL were monitored up to 1 y. Eight out of 18 individuals showed prolonged suppression of PVL (Good Responders, GR; >90% PVL decrease 1 y after immunization), whereas 10 did not (Weak or Transient Responders, WTR; <90% PVL decrease 1 y after immunization), opening discussion about factors generating this differential response.

Host genetic background has been reported to affect individual response to prophylactic anti-HIV vaccines[4,5] emphasizing that vaccine-induced cellular immunity and natural immune control against the virus share common genetic contributors. Similarly, our group showed that single nucleotide polymorphisms (SNPs) in innate immune genes, specifically *MBL2* and *NOS1*, were associated with individual response to DC-based immune treatment of Lu et al.[6]

Another point of interest concerns the genotyping of known host anti-HIV restriction factors in patients enrolled in DC-based clinical trials because of the natural impact on viral replication in these individuals. We hypothesized that individuals with a less permissive genomic profile for HIV-1 infection and/or replication may respond better to DC-based treatment.

To evaluate the impact of host anti-HIV restriction factors on the efficacy of DC-based treatment, we analyzed the 18 HIV+ patients that participate in ~~of~~ Lu clinical trial[3] for selected polymorphisms in genes known to be involved in HIV-1 infection and/or progression to AIDS.

Genomic DNA of those patients was already available and its quality/quantity checked at our laboratory using both spectrophotometer and Nanodrop. Full clinical data of the patients are available in Lu et al.,[3] whereas characteristic considered relevant for this study were summarized in **Table S1**.

Twenty two polymorphisms in 13 genes involved in HIV-1 host restriction (*APOBEC3G, CCL4, CCL5, CCR5, CUL5, CXCR6, HLA-C, IFNG, PARD3B, Prox1, SDF-1, TRIM5, ZNRD1*) were

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Table 1. Results of polymorphisms association analysis in 18 HIV+ patients underwent DC-based immune treatment against HIV-1 classified in good responder (GR) and weak or transient responder (WTR) according to Lu et al.[3]

Gene	Polymorphism	GR (n = 8)	WTR (n = 10)	P value	OR (CI 95%)
APOBEC3G	rs3736685				
	C	2	1	0.574	2.64 (0.13–168.21)
	T	14	11		
	C/C	0	0	0.396	
	C/T	2	1		
	T/T	6	9		
	rs2294367				
	G	7	9	1.0	0.95 (0.21–4.32)
	C	9	11		
	C/C	3	2	0.375	
	C/G	3	7		
	G/G	2	1		
CCL4	rs1719153				
	T	6	8	1.0	0.90 (0.19–4.21)
	A	10	12		
	T/T	0	0	0.800	
	A/T	6	8		
	A/A	2	2		
	rs1719134				
	G	4	4	1.0	1.32 (0.20–8.72)
	A	12	16		
	A/A	5	6	0.789	
	A/G	2	4		
	G/G	1	0		
CCL5	rs2280789				
	G	0	4	0.113	0 (0–1.79)
	A	16	16		
	G/G	0	0	0.092	
	A/G	0	4		
	A/A	8	6		
	rs2107538				
	T	2	6	0.257	0.34 (0.03–2.37)
	C	14	14		
	T/T	0	0	0.132	
	C/T	2	6		
	C/C	6	4		
CCR5	Δ32				
	wt	16	15	1	0 (0–48.71)
	Δ32	0	1		

selected based on previously published data (briefly revised in ref. 7; reported in **Table S2**). Genotyping was performed using commercially available TaqMan assays (Applied Biosystems/AB) and ABI7500 Real-Time platform (AB). Allelic discrimination was performed using the SDS v1.4 Software (AB). *CCR5* Δ32 deletion was evaluated by PCR-RFLP. *PARD3B* genotyping results have been double checked and confirmed by direct sequencing of the amplicon containing the rs11884476 SNP.

The frequency of *PARD3B* rs11884476 SNP was then evaluated in a population coming from Recife (same metropolitan area of the Brazilian clinical trial[3]). 119 HIV+ patients (34 males, average age = 40.4; 85 females, average age = 34.95) and 212 healthy controls (HC; 59 males, average age = 24.91; 153 females, average age = 32.04) were recruited respectively at the Immunologic Day Hospital of the "Instituto de Medicina Integral Prof. Fernando Figueira" (IMIP), and at the Transfusion Center HEMOPE of Recife.

R-project software was used to calculate allelic, genotypic and haplotypic frequencies, *P* values and Odds Ratio (OR) as well as for genotypes modeling (package "SNP assoc" version 1.5–2). Haploview software was employed to derivate haplotypes. Polymorphisms frequencies were compared with Chi-square test with Yate's continuity correction, which accounts for adjusting the p values of comparisons between data sets with a small number of observations in a genotype class (even less than five). Comparison between *PARD3B* rs11884476 genotypes and immunologic characteristic of the 18 patients submitted to immune treatment (found in[3]), such as PVL, CD4+ and CD8+ cell counts as well as IFN-γ positive cells, was done by *t* test using GraphPad Prism software. Genevar software was used to evaluate in silico the functional impact of *PARD3B* rs11884476 on mRNA biology.

The selected 22 polymorphisms in 13 HIV-1 restriction factor genes were genotyped in the 18 HIV+ individuals submitted to the Brazilian phase I clinical trial of DC-based treatment.[3] Allelic and genotypic frequencies were in Hardy–Weinberg equilibrium. Polymorphisms distribution was compared in good responder and weak or transient responder according to the classification applied by Lu et al.[3] (**Table 1**).

The rs11884476 polymorphism in *PARD3B* resulted associated with good response to the immune treatment according to an overdominant model (C/G vs. C/C+G/G;

P = 6.5exp-3), being C/G more frequent in GR than in WTR (5/8 vs. 0/10).

When changes in PVL and cellular response were stratified according to patients' *PARD3B* genotypes (**Table S1**), rs11884476 C/G genotype was associated with greater PVL reduction compared with C/C or C/C+G/G (P = 4.3exp-3 or P = 2.6exp-3). Similarly, the rs11884476 C/G was associated with higher increase in IFN-γ producing CD4+ cells compared with C/C (P = 0.021) or C/C+G/G (P = 0.015) (**Fig. 1**). These findings are in agreement with previously reported data about the association of rs11884476 with better prognostic and delayed AIDS.[8]

The rs11884476 SNP is an intronic variant, with still unknown functional effect, even if it could be a tag for other polymorphisms such as for the rs10185378 reported to be associated to an alternative mRNA splicing of *PARD3B* gene.[8] As biologic samples, other than genomic DNA, of from those18 patients were no longer available and functional studies were not possible, we evaluated the possible impact of the SNP on *PARD3B* mRNA levels in silico using the GENEVAR database (www.sanger.ac.uk/humgen/genevar/), able to display mRNA expression profiles of B lymphoblastoid cell lines established from HapMap donors. The unique data available for rs11884476 genotypes are referring to YRI population and they did not significantly correlate with *PARD3B* mRNA level variation (P = 0.181) (**Fig. S3**), suggesting that, in this population, this SNP could not affect mRNA production.

To investigate the prevalence of this polymorphism in HIV+ population from the same geographical region of the 18 patients submitted to immune treatment,[3] *PARD3B* rs11884476 was then genotyped in a case/control study (n = 119/212) with patients and controls coming from Recife. We did not find any significant association between *PARD3B* rs11884476 SNP and susceptibility to HIV-1 infection (**Table S4**), however it is interesting to emphasize that the HIV+ cohort enrolled for the study were not efficient controllers of virus replication, being PVL always greater than 2 log copy/ml before starting the HAART treatment, whereas *PARD3B* rs11884476 was previously associated with delayed AIDS.[8]

Allelic and genotypic frequencies of the 18 patients submitted to DC immune-treatment (classified as GR and WTR) were then compared with those of HIV+ patients form Recife. No significant difference was found between

Table 1. Results of polymorphisms association analysis in 18 HIV+ patients underwent DC-based immune treatment against HIV-1 classified in good responder (GR) and weak or transient responder (WTR) according to Lu et al.[3] (continued)

Gene	Polymorphism	GR (n = 8)	WTR (n = 10)	P value	OR (CI 95%)
	wt/wt	8	9	1	
	wt/Δ32	0	1		
	Δ32/Δ32	0	0		
CUL5	rs7117111				
	A	6	9	0.741	0,74 (0.15–3.38)
	G	10	11		
	A/A	0	3	0.142	
	A/G	6	3		
	G/G	2	4		
	rs11212495				
	G	1	5	0.196	0.21 (0.00–2.19)
	A	15	15		
	G/G	0	1	0.588	
	A/G	1	3		
	A/A	7	6		
	rs7103534				
	C	0	2	0.492	0 (0–6.64)
	T	16	18		
	C/C	0	0	0.477	
	C/T	0	2		
	T/T	8	8		
SDF1	rs2234358				
	T	6	7	1	1.11 (0.23–5.35)
	G	10	13		
	T/T	0	2	0.214	
	G/T	6	3		
	G/G	2	5		
HLA-C	rs10484554				
	T	4	2	0.374	2.91 (0.35–36.98)
	C	12	18		
	T/T	2	0	0.2288	
	C/T	0	2		
	C/C	6	8		
	rs9264942				
	C	6	6	0.730	1.39 (0.28–7.0)
	T	10	14		
	C/C	1	1	0.868	
	C/T	4	4		
	T/T	3	5		
IFNG	rs2069709				

Table 1. Results of polymorphisms association analysis in 18 HIV+ patients underwent DC-based immune treatment against HIV-1 classified in good responder (GR) and weak or transient responder (WTR) according to Lu et al.[3] (continued)

Gene	Polymorphism	GR (n = 8)	WTR (n = 10)	P value	OR (CI 95%)
	A	0	2	0.492	0 (0–6.64)
	C	16	18		
	A/A	0	1	1	
	A/C	0	0		
	C/C	8	9		
PARD3B	**rs11884476**				
	G	5	2	0.204	3.93 (0.53–48.04)
	C	11	18		
	G/G	0	1	6.5 exp-3*	* co-dominant and over-dominant
	C/G	5	0		
	C/C	**3**	**9**		
Prox1	rs17762192				
	C	7	10	0.749	0.78 (0.17–3.51)
	G	9	10		
	C/C	2	2	0.607	
	C/G	3	6		
	G/G	3	2		
CXCL12	rs1801157				
	T	4	3	0.675	1.86 (0.26–15.08)
	C	12	17		
	T/T	1	1	0.665	
	C/T	2	1		
	C/C	5	8		
TRIM5	rs16934386				
	G	1	0	0.444	inf (0.03–inf)
	A	15	20		
	G/G	0	0	0.444	
	A/G	1	0		
	A/A	7	10		
	rs10838525				
	C	0	0	monomorphic	monomorphic
	T	16	20		
	C/C	0	0	monomorphic	monomorphic
	C/T	0	0		
	T/T	8	10		
	rs3740996				
	G	5	4	0.470	1.79 (0.31–11.26)
	A	11	16		
	GG	0	1	0.145	
	AG	5	2		
	AA	3	7		

WTR and HIV+ patients, while a significant difference has been observed between GR and HIV+ patients considering the over-dominant model (P = 0.025) (**Table S4**), reinforcing the idea that individuals with rs11884476 in heterozygosis were able to control viral replication more efficiently than individuals homozygotes for the SNP, and that these "controllers" of virus replication may have a greater chance to better respond to immune treatment.

PARD3B protein interacts with TGFß signaling proteins SMAD, which directly binds HIV-1 proteins Tat and gp120.[8] As increasing levels of TGFß are typically detected during HIV-1 replication and progression to AIDS, rs11884476 variant could affect PARD3B-SMAD interaction resulting in TGFß signaling downregulation, leading to better control of AIDS progression. However, reducing production of TGFß is recommended in HIV vaccine design due to its immunomodulatory function on DC activation,[9] suggesting that polymorphisms in *PARD3B* could affect both AIDS progression as well as DC-mediated lymphocytes activation.

The possible dual role of PARD3B in terms of viral replication and AIDS progression, or DC-mediated lymphocytes activation is depicted in the cartoon reported in **Figure 2**. According to the over-dominant model, we may hypothesize that individual heterozygotes for rs11884476 SNP could have a TGFß signaling leading to a balance between regulatory and stimulatory DC profile.

Despite some limitations of this study, such as the very low number of individuals analyzed for few genetic variants and the lack of biologic samples to deeper corroborate our data, our findings lead us to hypothesize that genetic factors involved in AIDS progression could affect the response to therapeutic DC vaccine, reinforcing the idea that deeper investigation on host genetic variations will be fundamental for a rational vaccine development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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Supplemental Materials

Supplemental materials may be found here: www.landesbioscience.com/journals/vaccines/article/27125/

Table 1. Results of polymorphisms association analysis in 18 HIV+ patients underwent DC-based immune treatment against HIV-1 classified in good responder (GR) and weak or transient responder (WTR) according to Lu et al.[3] (continued)

Gene	Polymorphism	GR (n = 8)	WTR (n = 10)	P value	OR (CI 95%)
ZNRD1	rs3869068				
	T	5	3	0.422	2.51 (0.39–19.52)
	C	11	17		
	TT	1	0	0.472	
	CT	3	3		
	CC	4	7		
	rs8321				
	C	0	0	monomorphic	monomorphic
	A	16	20		
	CC	0	0	monomorphic	monomorphic
	AC	0	0		
	AA	8	10		

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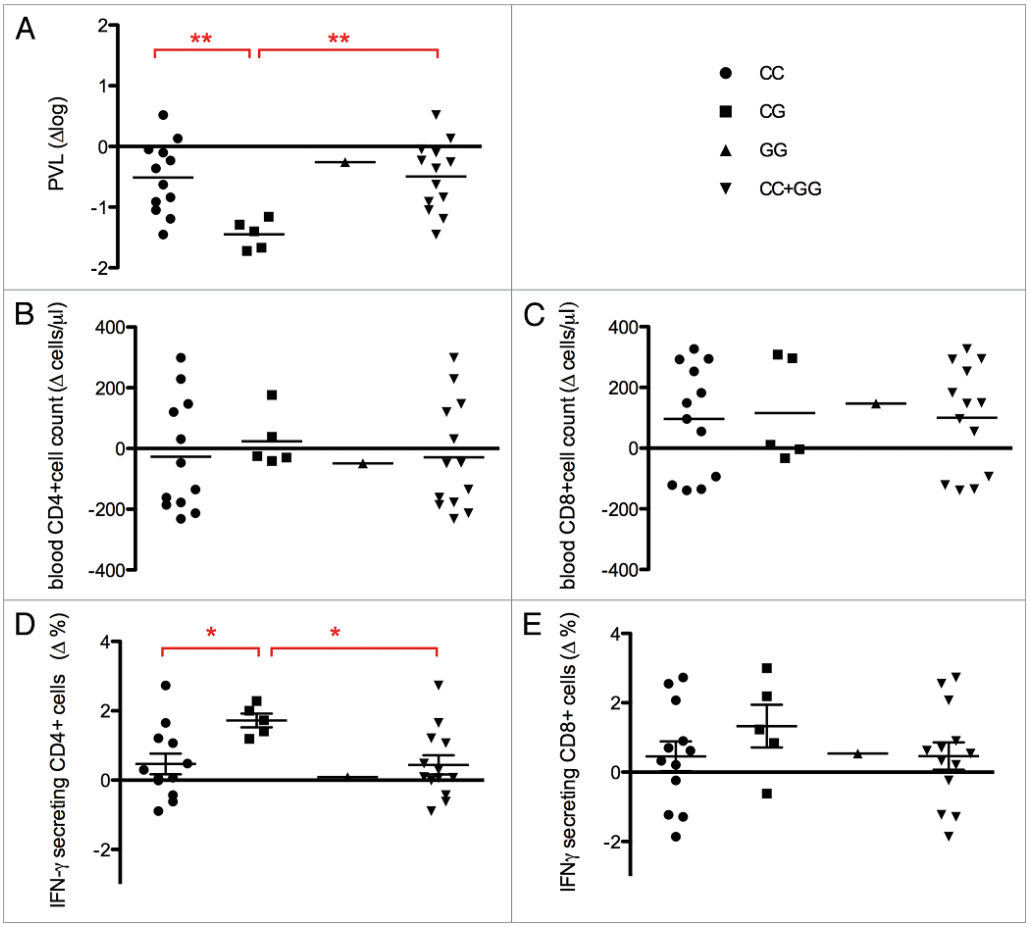

Figure 1. Plasma viral load (PVL) reduction and cellular response in 18 HIV+ patients who's underwent DC-based immune treatment against HIV-1 according to *PARD3B* rs11884476 genotypes. Change in PVL expressed as log change (Δlog), change in CD4+ and CD8+ cells counts (Δcells/μl) and change in percentage of CD4+ and CD8+ cells producing IFN-γ (Δ%) are reported for the 18 HIV+ patients included in the phase I clinical trial of DC-based immune-therapy[3] classified according to *PARD3B* rs11884476 genotypes. The data, obtained from Lu et al.,[3] represent difference (Δ) between values presented 1 y after immunization and before the starting of the trial. Individual data and media were reported. (**A**) Plasma viral load (PVL). Individual blood CD4+ (**B**) and CD8+ (**C**) cell counts. (**D**-**E**) Intracellular IFN-γ detection of T cells following stimulation with HIV-1-pulsed DC. Percentage of total CD4+ (**D**) or CD8+ (**E**) cell secreting IFN-γ is reported. T test analysis was performed between C/C and C/G groups and between C/C+G/G and C/G groups according to an over-dominant model. Being unique value the G/G has been excluded from the analysis. *$P < 0.05$; ** $P < 0.01$.

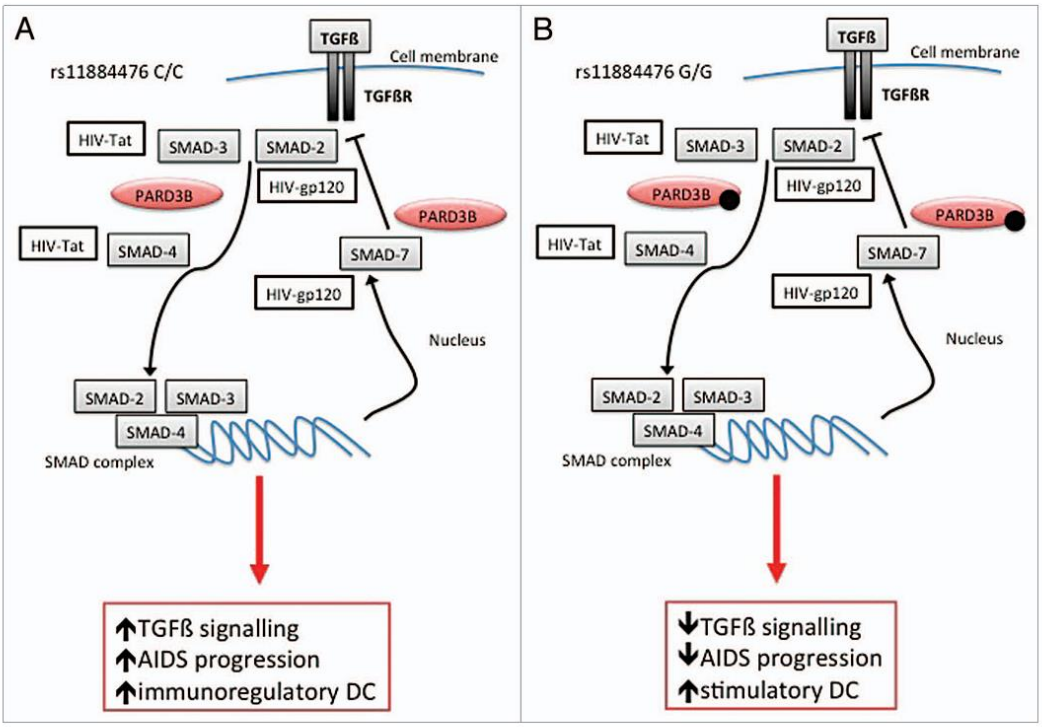

Figure 2. Possible interactions between PARD3B and SMAD proteins influencing TGFß signaling. The two hypotheses concerning the dual role of PARD3 in terms of viral replication control or DC-mediated lymphocytes activation are reported according to rs11884476 genotypes (2A: wild type C/C genotype; 2B: G/G genotype). The up or downregulation of TGFß signaling and the consequences in terms of AIDS progression or DC immune-regulation are evidenced in red rectangles.

15. Currículo Lattes

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Citações: WEB OF SCIENCE ™ 7 | SCOPUS 9

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1. Filho, E. F. A. ; SILVA, L. R. S. ; LIMA, P. A. S. ; AMANCIO, F. F. ; **SILVA, R. C.** ; MELO, A. M. M. A. . Use of Micronucleus Test in the Assessment of Radiation Effects in Aquatic Environments. In: 2011

International Nuclear Atlantic Conference - INAC 2011, 2011, Belo Horizonte - MG. 2011 International Nuclear Atlantic Conference - INAC 2011, 2011.

2. SILVA, L. R. S. ; SILVA, E. B. ; **SILVA, R. C.** ; AMANCIO, F. F. ; MELO, A. M. M. A. . Micronuclei as Biomarkers of Genotoxicity of Gamma Radiation in Aquatic Environments. In: 2011 International Nuclear Atlantic Conference - INAC 2011, 2011, Belo Horizonte - MG. 2011 International Nuclear Atlantic Conference - INAC 2011, 2011.

3. **3.**SIQUEIRA, W. N. ; SILVA, L. R. S. ; **SILVA, R. C.** ; LACERDA, L. B. N. ; SILVA, H. A. M. F. ; Santos, M. L. O. ; SILVA, E. B. ; MELO, A. M. M. A. . Radioprotective Effect of the Extract of Ziziphus Joazeiro and Anacardium Occidentale on Embryos of Biomphalaria Glabrata Submitted to Ionizing Radiation. In: 2011 International Nuclear Atlantic Conference - INAC 2011, 2011, Belo Horizonte - MG. 2011 International Nuclear Atlantic Conference - INAC 2011, 2011.

Resumos publicados em anais de congressos

1. **CELERINO DA SILVA, R.** ; COELHO, A. V. C. ; Crovella, Sergio . The role of single nucleotide polymorphisms (SNPs) in ZNRD1 gene in HIV-1 infection in a Pernambuco Population. In: 60º Congresso Brasileiro de Genética, 2014, Guarujá - SP.

2. **CELERINO DA SILVA, R.** ; PONTILLO, A. ; MOURA, R. R. ; Crovella, Sergio. Host genetic HIV restriction factors may modulate the response to HIV therapeutic dendritic cell based vaccine. In: 59º Congresso Brasileiro de Genética, 2013, Águas de Lindóia - SP.

3. **SILVA, R. C.** ; Crovella, Sergio ; CRUZ, H. L. A. ; SCHINDLER, H. C. ; MONTENEGRO, L. M. L. . CD209 and CD209L polymorphisms associated with tuberculosis in Pernambuco population. In: XIX Encontro de Genética do Nordeste/ I Simpósio de Genética Humana e Médica do Nordeste, 2012, Petrolina-PE/Juazeiro-BA.

4. **SILVA, R. C.** ; COELHO, A. V. C. ; SERAFIN-SILVA, S. P. ; BRANDAO, L. A. C. ; CROVELLA, S. . HLA-G 14-pb del/ins polymorphism is not associated with heterosexual HIV-1 infection susceptibility in Recife population. In: XIX Encontro de Genética do Nordeste/ I Simpósio de Genética Humana e Médica do Nordeste, 2012, Petrolina-PE/Juazeiro-BA.

5. **SILVA, R. C.** ; COELHO, A. V. C. ; Crovella, Sergio . Polimorfismos de Base Única nos Genes Cul5 e TRIM5 Associados a Infecção pelo Vírus HIV-1 em Pernambucanos. In: II Jornada de Pós-Graduação em Genética, 2012, Recife - PE.

6. **SILVA, R. C.** ; COELHO, A. V. C. ; LOUREIRO, P. ; BRANDAO, L. A. C. ; CROVELLA, S. . SNP (rs735240) in DC-SIGN gene is not involved with HTLV-1 infection susceptibility in Recife population, Brazil. In: XI Simpósio Internacional sobre HTLV no Brasil, 2011, Recife. Revista das Ciências Médicas de Pernambuco. Recife: Faculdade de Ciências Médicas da Universidade de Pernambuco, 2011. v. 7. p. 0-0.

7. **SILVA, R. C.** ; SEGAT, L. ; ROCCA, G. ; ZANIN, V. ; CROVELLA, S. . Polymorphisms in DC-SIGN and L-SIGN Genes Are Associated with HIV-1 Vertical Transmission in a Northeastern Brazilian Population. In: 57º Congresso Brasileiro de Genética, 2011, Águas de Lindóia – SP.

8. CARVALHO, M. S. Z. M. G. ; CRUZ, H. L. A. ; **SILVA, R. C.** ; SANDRIN-GARCIA, P. ; MONTENEGRO, L. M. L. ; SCHINDLER, H. C. ; CROVELLA, S. . Distribution of Single Nucleotide Polymorphisms (SNPs) in MBL2 Gene among Patients with HIV and Tuberculosis. In: 57º Congresso Brasileiro de Genética, 2011, Águas de Lindóia - SP.

9. **SILVA, R. C.** ; Crovella, Sergio . FATORES IMUNOGENÉTICOS ENVOLVIDOS NA TRANSMISSÃO VERTICAL DO VÍRUS DO HIV-1 EM CRIANÇAS PERNAMBUCANAS. In: I Jornada de Pós-graduação em Genética da UFPE, 2011, Recife.

10. **SILVA, R. C.** ; TAVARES, N. A. C. ; SANDRIN-GARCIA, P. ; SCHINDLER, H. C. ; MONTENEGRO, L. M. L. ; Crovella, Sergio . Single Nucleotide Polymorphisms in DC-SIGN Promoter Are Associated With Protection to Tuberculosis Development in a Northeastern Brazilian Population. In: III Simpósio Internacional em Diagnóstico e Terapêutica/ VI Jornada Científica do LIKA, 2011, Recife.

Apresentações de Trabalho

1. **DA SILVA, R. C.** . Polimorfismos nos Genes DC-SIGN e L-SIGN e suas Implicações com a Transmissão Vertical do HIV-1. 2012. (Apresentação de Trabalho/Conferência ou palestra).

2. **SILVA, R. C.** . Associação de Polimorfismos nos Genes DC-SIGN e L-SIGN com a Transmissão Vertical do HIV-1 em uma População do Nordeste do Brasil. 2011. (Apresentação de Trabalho/Conferência ou palestra).

Demais tipos de produção técnica

1. MELO, A. M. M. A. ; SILVA, L. R. S. ; SIQUEIRA, W. N. ; AMANCIO, F. F. ; **SILVA, R. C.** ; Filho, E. F. A. . Radiobiologia em Biomedicina. 2011. (Curso de curta duração ministrado/Outra).

Bancas

Participação em bancas de trabalhos de conclusão

Monografias de cursos de aperfeiçoamento/especialização

1. Cornélio, M.T. M. N.; **DA SILVA, R. C.**; SANDRIN-GARCIA, P.. Participação em banca de Catarina Addobbati Jordão Cavalcanti. Estudo de Associação do Polimorfismo Indel 14pb no Gene HLA-G Com a Susceptibilidade ao Lúpus Eritomatoso Sistêmico. 2012. Monografia (Aperfeiçoamento/Especialização em Patologia Clínica) - Universidade de Pernambuco.

Trabalhos de conclusão de curso de graduação

1. **CELERINO DA SILVA, R.**. Participação em banca de Paloma da Silva.Variabilidade genética de Drosophila equinoxialis (Insecta: Diptera) baseada em genes do elemento F/E de Müller. 2014. Trabalho de Conclusão de Curso (Graduação em Licenciatura em Ciências Biológicas) - Universidade Federal de Pernambuco.

2. **DA SILVA, RONALDO CELERINO**; AMANCIO, F. F.; MELO, A. M. M. A.. Participação em banca de Michelle Cardinalli Araújo Costa.Avaliação da Toxicidade do Extrato Metanólico de Mimosa tenuiflora e de Rhamnidium molle Reissek. 2013. Trabalho de Conclusão de Curso (Graduação em Biomedicina) - Universidade Federal de Pernambuco.

3. **DA SILVA, RONALDO CELERINO**; AMANCIO, F. F.; MELO, A. M. M. A.. Participação em banca de Laila Bezerra Nascimento de Lacerda.Análise do Efeito Radioprotetor do Extrato Metanólico de Caesalpinia pyramidalis. 2013. Trabalho de Conclusão de Curso (Graduação em Biomedicina) - Universidade Federal de Pernambuco.

4. AMANCIO, F. F.; MELO, A. M. M. A.; **DA SILVA, RONALDO CELERINO**. Participação em banca de Taciane Leal Botelho.Cancer de Prostata Uma Revisão. 2013. Trabalho de Conclusão de Curso (Graduação em Biomedicina) - Universidade Federal de Pernambuco.

5. AMANCIO, F. F.; MELO, A. M. M. A.; **DA SILVA, R. C.**. Participação em banca de José Luís Ferreira Sá.Avaliação da Atividade Moluscicida do Extrato Bruto de Casca de Anadenanthera colubrina Sobre Biomphalaria glabrata. 2013. Trabalho de Conclusão de Curso (Graduação em Biomedicina) - Universidade Federal de Pernambuco.

6. **DA SILVA, RONALDO CELERINO**. Participação em banca de Jalva Pereira da Silva.Uma Proposta de Orientações sobre Reeducação Alimentar na Comunidade Escolar do Sítio Tamanduá, Passira-PE. 2013. Trabalho de Conclusão de Curso (Graduação em Lic. Plena em Ciências com Habilitação em Biologia) - Faculdades Integradas da Vitória de Santo Antão.

7. **DA SILVA, RONALDO CELERINO**. Participação em banca de Felipe Salviano Cabral.Análise dos Perfis de Jovens e Adultos que Fazem Uso de Esteróides Anabólicos Androgênicos (EAA) nas Academias do

Município de Passira. 2013. Trabalho de Conclusão de Curso (Graduação em Lic. Plena em Ciências com Habilitação em Biologia) - Faculdades Integradas da Vitória de Santo Antão.

8. **DA SILVA, RONALDO CELERINO**. Participação em banca de Wanessa Kassia Amancio da Silva.Diabetes Mellitus: Avaliando o Nível de Conhecimento entre Alunos do Ensino Médio de Escolas Públicas e Privadas no Município de Moreno-PE. 2013. Trabalho de Conclusão de Curso (Graduação em Lic. Plena em Ciências com Habilitação em Biologia) - Faculdades Integradas da Vitória de Santo Antão.

9. SANDRIN-GARCIA, P.; TAVARES, N. A. C.; SILVA, J. A.; **SILVA, R. C.**. Participação em banca de Karina Monteiro Fernandes.Estudo de Associação de Polimorfismos de Base Única (SNPs) do Gene VDR (Receptor de Vitamina D) com a Susceptibilidade ao Lúpus Eritematoso Sistêmico. 2012. Trabalho de Conclusão de Curso (Graduação em Ciências Biológicas Bacharelado) - Universidade Federal de Pernambuco.

10. SILVA, E. B.; MELO, A. M. M. A.; SILVA, L. R. S.; **SILVA, R. C.**. Participação em banca de Pedro André de Souza Lima.Detecção de Micronúcleo em Hemócitos de Biomphalaria glabrata Exposto ao Oxifluorfen (Goal BR). 2011. Trabalho de Conclusão de Curso (Graduação em Licenciatura em Ciências Biológicas) - Universidade Federal de Pernambuco.

11. Souza, P. R. E.; Guimarães, R. L.; **SILVA, R. C.**. Participação em banca de Manuella Maria Silva Santos.O Papel do Gene da Citocina Pró-Inflamatória IL-18 no Desenvolvimento do Diabetes Mellitus do Tipo I na População do Estado de Pernambuco. 2011. Trabalho de Conclusão de Curso (Graduação em Bacharelado em Ciências Biológicas) - Universidade Federal Rural de Pernambuco.

Eventos

Participação em eventos, congressos, exposições e feiras

1. 60º Congresso Brasileiro de Genética. The role of single nucleotide polymorphisms (SNPs) in ZNRD1 gene in HIV-1 infection in a Pernambuco Population. 2014. (Congresso).

2. 59º Congresso Brasileiro de Genética. Host genetic HIV restriction factors may modulate the response to HIV therapeutic dendritic cell based vaccine. 2013. (Congresso).

3. XIX Encontro de Genética do Nordeste/ I Simpósio de Genética Humana e Médica do Nordeste.HLA-G 14-pb del/ins polymorphism is not associated with heterosexual HIV-1 infection susceptibility in Recife population. 2012. (Encontro).

4. II Jornada de Pós-Graduação em Genética.Polimorfismos de Base Única nos Genes Cul5 e TRIM5 Associados a Infecção pelo Vírus HIV-1 em Pernambucanos. 2012. (Outra).

5. 57º Congresso Brasileiro de Genética. Polymorphisms in DC-SIGN and L-SIGN Genes Are Associated with HIV-1 Vertical Transmission in a Northeastern Brazilian Population. 2011. (Congresso).

6. XI Simpósio Internacional sobre HTLV no Brasil.SNP (rs735240) in DC-SIGN gene is not involved with HTLV-1 infection susceptibility in Recife population, Brazil. 2011. (Simpósio).

7. IV Simpósio Paraibano de Biomedicina.Polimorfismos nos Genes DC-SIGN e L-SIGN: Implicações na Transmissão Vertical do HIV-1 em uma População do Nordeste do Brasil. 2011. (Simpósio).

8. III Simpósio Internacional em Diagnóstico e Terapêutica/ VI Jornada Científica do LIKA.Single Nucleotide Polymorphisms in DC-SIGN Promoter Are Associated With Protection to Tuberculosis Development in a Northeastern Brazilian Population. 2011. (Simpósio).

9. I Jornada de Pós-graduação em Genética da UFPE.FATORES IMUNOGENÉTICOS ENVOLVIDOS NA TRANSMISSÃO VERTICAL DO VÍRUS DO HIV-1 EM CRIANÇAS PERNAMBUCANAS. 2011. (Outra).

Orientações

Orientações e supervisões concluídas

Orientações de outra natureza

1. Carlos Eduardo Gomes Barros. Monitoria da Disciplina de Genética de Populações (BIOL0026) - Semestre 2013.1. 2013. Orientação de outra natureza. (Ciências Biológicas/ Licenciatura) - Centro Acadêmico de Vitória - Universidade Federal de Pernambuco, Pró-Reitoria para Assuntos Acadêmicos. Orientador: Ronaldo Celerino da Silva.

2. Alyson Mykael Albuquerque Florenço. Monitoria da Disciplina Evolução (BIOL0024) - Semestre 2013.1. 2013. Orientação de outra natureza. (Ciências Biológicas/ Licenciatura) - Centro Acadêmico de Vitória - Universidade Federal de Pernambuco, Pró-Reitoria para Assuntos Acadêmicos - Universidade Federal de Pernambuco. Orientador: Ronaldo Celerino da Silva.

3. Carlos Eduardo Gomes Barros. Monitoria da Disciplina de Genética de Populações (BIOL0026) - Semestre 2013.2. 2013. Orientação de outra natureza. (Licenciatura em Ciências Biológicas) - Universidade Federal de Pernambuco, Pró-Reitoria para Assuntos Acadêmicos - Universidade Federal de Pernambuco. Orientador: Ronaldo Celerino da Silva.

4. Alyson Mykael Albuquerque Florenço. Monitoria da Disciplina de Evolução (BIOL0024) - Semestre 2013.2. 2013. Orientação de outra natureza. (Licenciatura em Ciências Biológicas) - Universidade Federal de Pernambuco, Pró-Reitoria para Assuntos Acadêmicos - Universidade Federal de Pernambuco. Orientador: Ronaldo Celerino da Silva.

5. Mercia Maria Bezerra Barbosa. Monitoria da Disciplina de Genética de Populações (BIOL0026) - Semestre 2013.2. 2013. Orientação de outra natureza. (Licenciatura em Ciências Biológicas) - Universidade Federal de Pernambuco. Orientador: Ronaldo Celerino da Silva.

6. Paloma da Silva. Monitoria da Disciplina de Genética de Populações (BIOL0026) - Semestre 2012.1. 2012. Orientação de outra natureza. (Ciências Biológicas/ Licenciatura) - Centro Acadênico de Vitória - Universidade Federal de Pernambuco. Orientador: Ronaldo Celerino da Silva.

7. Adriano Oliveira. Monitoria da Disciplina de Evolução (BIOL0024) - Semestre 2012.1. 2012. Orientação de outra natureza. (Ciências Biológicas/ Licenciatura) - Centro Acadêmico de Vitória - Universidade Federal de Pernambuco. Orientador: Ronaldo Celerino da Silva.

8. Cleciana Maristela de Souza. Monitoria da Disciplina de Genética de Populações (BIOL0026) - Semestre 2012.2. 2012. Orientação de outra natureza. (Ciências Biológicas/ Licenciatura) - Centro Acadêmico de Vitória - Universidade Federal de Pernambuco. Orientador: Ronaldo Celerino da Silva.

9. Alyson Mykael Albuquerque Florenço. Monitoria da Disciplina Evolução (BIOL0024) - Semestre 2012.2. 2012. Orientação de outra natureza. (Ciências Biológicas/ Licenciatura) - Centro Acadêmico de Vitória - Universidade Federal de Pernambuco. Orientador: Ronaldo Celerino da Silva.

10. Adriano Oliveira Lima. Monitoria da Disciplina de Genética de Populações (BIOL0026) - Semestre 2012.2. 2012. Orientação de outra natureza. (Licenciatura em Ciências Biológicas) - Universidade Federal de Pernambuco. Orientador: Ronaldo Celerino da Silva.

Outras informações relevantes

Aprovado em 5° Lugar no Concurso para Professor Temporário – Área: Genética – Universidade Federal Rural de Pernambuco – 2011 Aprovado em 1° Lugar no Concurso para Professor Substituto – Área: Genética – Centro Acadêmico de Vitória – Universidade Federal de Pernambuco – 2012