UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE – UFCSPA
PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA

Tales Bohn Mirandolli

Associação de Polimorfismos no Gene *GATA3* com o Tempo de Progressão à AIDS

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Dissertação submetida ao Programa de Pós-Graduação em Patologia da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre

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Dedicatória

Dedico este trabalho aos pacientes por participarem deste trabalho, autorizando a coleta de material biológico, assim como por cederem tempo para a coleta de dados clínicos. Sem sua participação e compreensão, esta pesquisa não poderia ser realizada.

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Sumário

Lista de Abreviaturas

AIDS: *Acquired Immunodeficiency Syndrome*

APOBEC3G: *Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G*

CCR: Câncer de Colo Retal

CCR5: *C-C Chemokine Receptor Type 5*

CCR5del32: *CCR5 Delta 32*

CD4: *Cluster of Differentiation 4*

CD8: *Cluster of Differentiation 8*

CXCR4: *C-X-C Chemokine Receptor Type 4*

dbSNP: *Single Nucleotide Polymorphism database*

DG: Doença de Graves

DH: Doença de Hashimoto

DNA: Ácido Desoxirribonucleico

GWAS: *Genome-Wide Association Study*

HIV: *Human Immunodeficiency Virus*

HLA: *Human Leukocyte Antigen*

IFNγ: Interferon Gama

IL13: Interleucina 13

IL4: Interleucina 4

IL5: Interleucina 5

KIR: *Killer-cell Immunoglobulin-like Receptors*

LH: Linfoma de Hodgkin

LLA: Leucemia Linfoblástica Aguda

LTNP: *Long-Term NonProgressor*

mRNA: *Messenger RNA*

NK: *Natural Killer*

OMS: Organização Mundial da Saúde

RNA: Ácido Ribonucleico

PCR: *Polymerase Chain Reaction*

SIDA: Síndrome da Imunodeficiência Adquirida

SNC: Sistema Nervoso Central

SNP: Single Nucleotide Polymorphism

TF: *Transcription Factors*

UNAIDS: *Joint United Nations Programme on HIV/AIDS*

5'UTR: *Five Prime Untranslated Region*

Resumo

Introdução:

A AIDS caracteriza-se por um conjunto de danos no sistema imunológico ocasionado pelo vírus HIV. A infecção pode ser dividida em fase aguda, crônica e AIDS, e o tempo desde a infecção até a AIDS pode variar consideravelmente.

Objetivo:

Avaliar o papel de SNPs no gene *GATA3* no tempo de progressão à AIDS investigando pacientes com diferentes tempos de progressão à doença.

Material e Métodos:

Foram coletados dados clínicos de 201 pacientes para determinação do tempo de progressão e material biológico para extração de DNA. A genotipagem dos SNPs foi realizada por PCR em Tempo Real.

Resultados:

Foi observada associação entre o SNP rs1269486 e o tempo de progressão à AIDS na qual homozigotos AA progridem mais rápido que homozigotos GG em regressão univariada. Considerando modelos recessivo e dominante, foi encontrada uma associação na qual a progressão é mais rápida em homozigotos AA comparando portadores do alelo G em regressão multivariada.

Conclusões:

O SNP rs1269486 está associado com progressão mais rápida à AIDS, desconsiderando outros fatores que poderiam afetar a progressão à doença.

Palavras-chaves:

AIDS, HIV, *GATA3*, progressão, polimorfismos, SNP, genética.

1. Introdução

1.1. HIV e AIDS

A Síndrome da Imunodeficiência Adquirida (SIDA ou do inglês, AIDS) é uma doença causada pela infecção pelo Vírus da Imunodeficiência Humana (HIV) caracterizada pela imunossupressão profunda associada a infecções oportunistas e a tumores malignos, à perda de peso e à degeneração do sistema nervoso central (SNC) (Abbas e Lichtman, 2012).

Atualmente, a AIDS é uma das principais causas de mortalidade por doenças infecciosas no globo e caracteriza-se por um conjunto de sintomas e de infecções advindos do dano específico do sistema imunológico ocasionado pelo vírus (Fauci, 2003). A epidemia por HIV foi identificada inicialmente nos anos de 1980, mesmo assim o grau de morbimortalidade e os impactos nos gastos no sistema de saúde e na economia decorrentes da epidemia cada vez mais continuam a crescer (Abbas e Lichtman, 2012).

Segundo dados da Organização Mundial de Saúde, há em torno de 37 milhões de infectados pelo HIV no mundo e cerca de 16 milhões têm acesso à medicação antirretroviral. A África concentra o maior número de infectados do mundo (UNAIDS, 2015).

No Brasil, de 1980 a 2014 já foram registrados mais de 290 mil óbitos em consequência da epidemia. Há mais de 798 mil indivíduos infectados pelo HIV no País, e a distribuição dos casos de AIDS do Brasil apresenta maior concentração nas regiões Sudeste e Sul (Boletim Epidemiológico HIV, 2015).

1.2. Ciclo da Infecção pelo HIV

O vírus infecta diversos tipos celulares do sistema imunológico, principalmente as células T auxiliares $CD4^+$. A infecção por HIV inicia no momento em que a glicoproteína do envelope *Env* se liga à molécula CD4 como também a um correceptor de quimiocinas. Os receptores de quimiocinas mais importantes que atuam como correceptores para o vírus são o CXCR4 e o CCR5 (Abbas e Lichtman, 2012). O ciclo de infecção e de replicação do HIV pode ser dividido em sete fases: interação com a célula hospedeira e entrada que envolve a ligação da proteína viral gp120 às moléculas presentes na superfície da célula hospedeira, CD4 e CCR5 ou CXCR4; desligamento e liberação do RNA viral no interior da célula; transcrição reversa; integração do provírus ao genoma humano; síntese de proteínas; montagem da partícula viral; brotamento e processo de saída da partícula viral da célula (Levy, 1993; McMichael e Rowland-jones, 2001). Algumas cepas virais podem infectar e replicar nas células T $CD4^+$ (vírus T-tróficos), todavia certas cepas têm preferência por macrófagos (vírus M-tróficos) e outras os dois tipos celulares (vírus dual-tróficas). O vírus M-trófico expressa uma gp120 que se liga ao CCR5, expresso em macrófagos (e em algumas células T de memória). Por outro lado, o vírus T-trófico se liga ao CXCR4, expresso nas linhas de células T. As variantes virais são descritas como X4, quando se ligam ao CXCR4; R5 quando se ligam ao CCR5; R5X4 quando se ligam a ambos receptores (Abbas e Lichtman, 2012). O HIV tem acesso ao organismo humano através das mucosas, mais usualmente, pelo contato sexual, como também por transfusão sanguínea e pelo uso de drogas injetáveis (Fauci, 2003).

1.3. Curso Clínico da Infecção pelo HIV

Clinicamente, a infecção pelo HIV pode ser dividida em três fases: infecção aguda, infecção crônica e AIDS (Figura 1). A história natural da infecção aguda caracteriza-se por uma carga viral elevada e uma resposta imune intensa. Durante o primeiro pico de viremia, ocorre uma rápida diminuição do número de linfócitos T-CD4$^+$ que posteriormente volta a aumentar; no entanto, geralmente não retorna aos níveis prévios à infecção (Levy, 1993). Observa-se também um aumento do número absoluto de linfócitos T-CD8$^+$, o que reflete em uma resposta T citotóxica potente (O'Brien e cols., 1996). Durante esta fase, a maior causa de depleção das células T-CD4$^+$ deve-se à resposta citotóxica das células T-CD8$^+$ e à morte programada desencadeada por mecanismos endógenos (Paroli e cols., 2001).

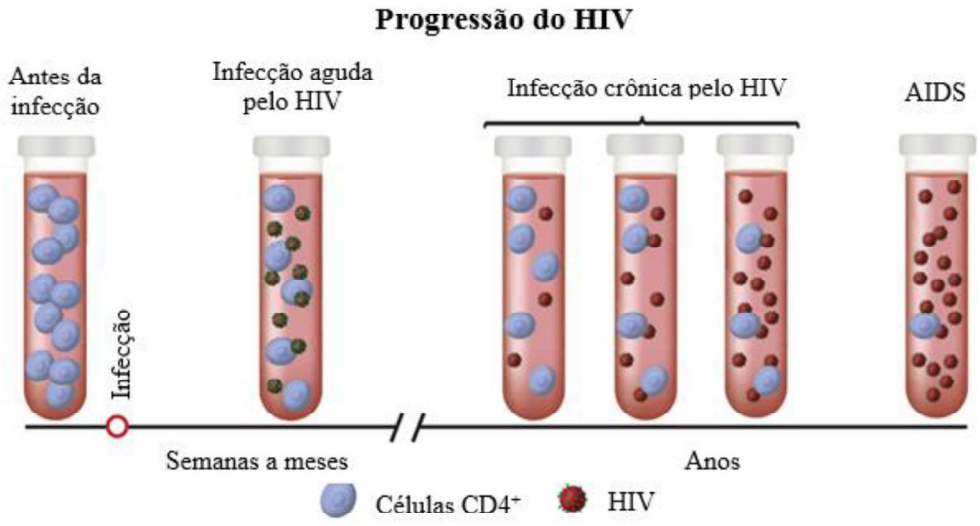

Figura 1 - Curso Clínico da Infecção pelo HIV (Fonte: www.aidsinfo.nih.gov)

Após a resolução da infecção aguda, inicia-se a fase crônica, quando ocorre a estabilização da queda do número de linfócitos T-CD4$^+$ e da viremia (os chamados *set points* virais) (Casado e cols., 2010). Esse quadro está diretamente relacionado à velocidade da replicação viral, e consequentemente, à progressão à AIDS (O'Brien e cols., 1996; Casado e cols., 2010). A infecção crônica inicia-se assintomática, porém gradativamente progride e o indivíduo começa a apresentar sinais e sintomas de intensidade variável (Levy, 1993). Nas fases mais tardias da infecção crônica, além da ativação imune generalizada, a depleção de células T-CD4$^+$ dá-se pela perda gradual da capacidade do organismo de renovar essas células (Costin, 2007).

No estágio de AIDS, as células T-CD4$^+$ geralmente estão abaixo do nível crítico de 200 células/mm^3, dosagem que remete o paciente a uma resposta imunitária ineficaz a patógenos invasores, permitindo o estabelecimento de doenças oportunistas (Costin, 2007). Neste período ou até mesmo um pouco antes, recomenda-se o início do tratamento antirretroviral, com intuito de inibir a replicação viral e, consequentemente, restaurar os níveis de células T-CD4$^+$, aumentando a sobrevida do paciente (Fauci, 2003). Apesar de todo paciente portador do vírus apresentar estas três fases clínicas, o tempo que transcorre desde a infecção até a AIDS varia consideravelmente entre os indivíduos (O'Brien e Nelson, 2004; Okulicz e cols., 2009).

1.4. Progressão à AIDS

Após a infecção pelo HIV, o tempo e o modo como os indivíduos prosseguem para AIDS são extremamente variáveis. Logo após as primeiras

descobertas acerca da síndrome, observou-se que uma pequena quantia dos infectados mantinha o controle da replicação viral e/ou não apresentava sintomas clínicos por longos períodos (Paroli e cols., 2001). Nos últimos 30 anos, uma variedade de definições para a progressão da doença foi proposta, todas considerando a estabilidade na contagem das células T-CD4$^+$, a carga viral e o tempo em que o paciente soropositivo permanecia livre de sintomas, para categorizar os indivíduos infectados dentro de uma categoria de progressão à síndrome (O'Connell e cols., 2009).

Atualmente, os pacientes infectados que seguem o curso normal da doença são conhecidos como progressores crônicos ou típicos. Esses indivíduos representam entre 80% e 90% da população soropositiva e desenvolvem uma infecção sintomática, iniciando o tratamento antirretroviral entre o 3º e o 10º ano após a soroconversão (Paroli e cols., 2001). De maneira oposta, alguns pacientes podem desenvolver uma infecção diferenciada. Enquanto alguns indivíduos progridem para a AIDS rapidamente – em menos de três anos, outros podem levar mais de dez anos para manifestar os sintomas característicos da doença (O'Connell e cols., 2009).

No grupo de indivíduos conhecidos como progressores lentos ou *long-term nonprogressors* (*LTNPs*), o organismo consegue controlar o vírus de forma natural (Okulicz e cols., 2009; Deeks e Walker, 2007). Estes pacientes têm diferentes valores de carga viral, porém exibem como características comuns a habilidade de manter níveis elevados de células T-CD4$^+$ e não desenvolvem sintomatologia de AIDS na ausência da terapia antirretroviral (O'Connell e cols., 2009; Winkler e cols., 2004; O'Brien e Nelson, 2004). Os progressores lentos correspondem de 5 a 15% da população infectada pelo

HIV. Todavia, os indivíduos que progridem de forma rápida para a AIDS apresentam duas ou mais medidas consecutivas de células T-CD4^{+} abaixo de 350 céls/mm^{3} em até três anos após a soroconversão e/ou iniciam a terapia antirretroviral neste período (Migueles e Connors, 2010). Consoante estimativas recentes, cerca de 5% dos infectados são progressores rápidos (O'Brien e cols., 1996).

Segundo o Ministério da Saúde, a grande maioria dos indivíduos infectados já se interna no hospital com sintomatologia de AIDS; consequentemente, são escassos os dados relativos à progressão da AIDS no Brasil. Na tentativa de compreender os mecanismos envolvidos nos diferentes tipos de progressão, diversos estudos investigam o quanto fatores virais podem contribuir para a progressão à AIDS. E ainda, outros trabalhos tentam correlacionar variações do sistema imunológico do hospedeiro com o tempo de progressão para a síndrome (Costin, 2007; Winkler e cols., 2004; Benkirane e cols., 1997).

1.5. Diversidade Genética do Hospedeiro e Progressão à AIDS

A descoberta do alelo CCR5del32 proporcionou a primeira evidência conclusiva à existência de resistência genética do hospedeiro à infecção pelo HIV (An e Winkler, 2010). A deleção de 32 nucleotídeos no gene *CCR5* leva à produção de uma proteína incompleta que não é transportada para a superfície da célula e, portanto, é incompetente para a entrada viral (Ekene, 2008). Esse achado levou ao desenvolvimento de fármacos que atuam como antagonistas do CCR5 (Benkirane e cols., 1997).

Além da deleção no gene *CCR5*, entre outras variantes genéticas já estudadas, destacam-se as associações entre variações do sistema HLA, cuja principal função é codificar moléculas apresentadoras de antígenos ao sistema imune (Thorner e cols., 2016), dos receptores KIR, os quais participam da regulação das células NK por meio do reconhecimento de ligantes HLA nas células-alvo (Mosmann e Coffman, 1989), e de fatores de restrição viral como a poliproteína APOBEC3G (An e cols., 2016). Outro mecanismo já associado com o andamento da infecção pelo HIV é a expressão diferencial de citocinas; empregadas na comunicação celular, que estão estreitamente relacionadas com a resposta imune inata e adaptativa (Ouyang e cols., 2009). No entanto, a variabilidade destes genes e perfil de expressão de citocinas somente responde por parte da variabilidade de tempo de progressão à AIDS devido a fatores genéticos, e consequentemente, muitos outros genes e/ou fatores podem ser importantes para o desfecho da doença.

Recentemente, uma nova abordagem (análise de redes de coexpressão) vem tentando elucidar os mecanismos envolvidos no desenvolvimento de doenças por meio da identificação de reguladores precoces de mudanças de perfil de resposta imune. Entre estes reguladores precoces, estão citados alguns fatores de transcrição, denominados como “reguladores mestre” ou “genes mestre”, que são capazes de gerar uma diferenciação celular específica capaz de alterar o curso da doença ou da infecção viral. Através desta abordagem foi verificado que alguns fatores de transcrição (TF) apresentam alterações significativas em seu padrão de expressão nos diferentes estágios da progressão à AIDS (Ray e Bandyopadhyay, 2015) e doenças associadas à modificação de células T (Gustafsson e cols., 2015). Entre esses fatores de

transcrição, observou-se que o fator GATA3 apresentou uma forte correlação negativa de perfil de expressão entre as fases aguda e de não-progressão à AIDS (Ray e Bandyopadhyay, 2015), além de ser um gene mestre para diferenciação Th2 (Li e cols., 2015). Estes resultados tornam *GATA3* um potencial gene a ser estudado no contexto do HIV.

O fator de transcrição GATA pertence a uma família de proteínas ativadoras de transcrição e é ligado a uma região de DNA altamente conservada. O gene *GATA3*, localizado no cromossomo 10, membro dessa família multigene, é predominantemente expressado nos linfócitos T-CD4^{+} e está relacionado ao estímulo da expressão do HIV (Yang e Engel, 1993). Uma forte ligação entre a expressão de fatores de transcrição específicos, incluindo membros da família de proteínas de ligação GATA, e diferenciação de células T foi bem estabelecida. No entanto, o repertório preciso de fatores de transcrição que regulam a expressão de CCR5 em células T humanas ainda permanece pouco claro. Análises de promotor de CCR5 têm identificado regiões de DNA ligadoras para diversos fatores de transcrição, incluindo os fatores GATA.

É sabido que os fatores *GATA1* e *GATA3* suprimem de modo diferente a expressão de CCR5 em células dendríticas derivadas de células tronco e subconjuntos de células T primárias. O *GATA3* é o mais importante indutor de diferenciação de células Th0 em células Th2 efetoras e supressor em células Th1. Portanto, esse fator aumenta a produção de citocinas IL4, IL5 e IL13 (resposta Th2). Todavia, diminui a secreção de IFNγ (resposta Th1) e promove o remodelamento de cromatina no loco de IL4 durante a diferenciação de células T. Entretanto, o *GATA1* é mais potente que o *GATA3* na supressão de

Th1 – genes associados, IFNγ e CXCR3. O fator *GATA1*, mas não o *GATA3*, potencialmente suprime a transcrição de CCR5, assim tornando células T humanas resistentes à infecção por cepas virais trópicas ao CCR5 (Sundrud e cols., 2005).

De acordo com o dbSNP (http://www.ncbi.nlm.nih.gov/SNP), 2.191 variantes genéticas já foram descritas no gene *GATA3*. No entanto, poucas variantes genéticas foram investigadas no contexto de desfechos clínicos. Por meio de estudos recentes de associação genômica (do inglês, GWAS) e por abordagem gene candidato, entretanto, foi identificado que variantes do gene *GATA3* estão relacionadas à suscetibilidade à leucemia linfoblástica aguda em adolescentes e jovens adultos (Migliorini e cols., 2013), à rinite alérgica (Zhang e cols., 2009), à asma em adultos (Guthikonda e cols., 2014), ao desenvolvimento de Linfoma de Hodgkin (Enciso-Mora e cols., 2010) e ao câncer de colo retal (Figueiredo e cols., 2014) (Tabela 1).

Considerando que o gene investigado apresenta estreita ligação com a diferenciação de células T CD4+ e que nenhum trabalho desenvolvido até o momento investiga a relação de variantes genéticas de *GATA3* com HIV e a progressão à AIDS, este trabalho focar-se-á em três variantes de *GATA3* selecionadas no contexto do HIV: rs1269486, rs3824662 e rs1271899. As variantes rs1271899 (C>T) e rs1269486 (G>A) estão localizadas na região 5`UTR do gene *GATA3*, enquanto a variante rs3824662 (C>A) está localizada em uma região de íntron.

Tabela 1 - Associações de SNPs no gene *GATA3* com diferentes doenças.

SNP	Doença	Delineamento	Método	Observado	Referência
rs1269486	RA	Caso (n=109) e controle (n=112)	Gene candidato	Alelo G mais frequente no caso	Zhang 2009
rs1269486	DH e DG	Caso (DH n=90; DG n=113) e controle (n=79)	Gene candidato	N.S.	Morita 2012
rs1269486	CCR	Caso (n=9287) e controle (n=9117)	GWAS	Alelo A maior risco CCR	Figueiredo 2014
rs1269486	Asma	660 mulheres asmáticas	Gene candidato	Alelo G maior risco de asma - interfere na metilação	Guthikonda 2014
rs3824662	LLA	Caso (n=1658) e controle (n=4723)	GWAS	Alelo T associado a maior idade de diagnóstico	Migliorini 2013
rs3824662	LLA	Caso (n=297) e controle (n=454)	GWAS	Alelo T associado a LLA	Walsh 2013
rs3824662	LLA em jovens	Caso (n=309) e controle (n=6661)	GWAS	Alelo A (T) mais frequente nos casos	Perez-Andreu 2015
rs485411	CCR	Caso (n=9287) e controle (n=9117)	GWAS	Alelo C maior risco CCR	Figueiredo 2014
rs485411	LH	Caso (n=589) e controle (n=5199)	GWAS	Alelo A mais frequente no grupo caso	Enciso-Mora 2010
rs501764	LH	Caso (n=589) e controle (n=5199)	GWAS	Alelo C mais frequente no grupo caso	Enciso-Mora 2010
rs1271899	LLA	Caso (n=297) e controle (n=454)	GWAS	Associação com LLA (direção – alelo – não descrito)	Walsh 2013
rs4143094	CCR	Caso (n=9287) e controle (n=9117)	GWAS	Alelo T maior risco CCR	Figueiredo 2014
rs3781093	LLA em jovens	Caso (n=309) e controle (n=6661)	GWAS	Alelo C mais frequente nos casos	Perez-Andreu 2015

RA: Rinite Alérgica; DH: Doença de Hashimoto; DG: Doença de Graves; CCR: Câncer do Colo Retal; LLA: Leucemia Linfoblástica Aguda; LH: Linfoma de Hodgkin; GWAS: Genome-Wide Association Study.

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2. Objetivos

Este trabalho teve como objetivo avaliar o papel de três polimorfismos no gene *GATA3* (rs1269486, rs1271899 e rs3224662) no tempo de progressão à AIDS investigando um grupo soropositivo de forma retrospectiva, a partir de dados clínicos e de amostras biológicas obtidos em um grande centro de atendimento a pacientes HIV+ em Porto Alegre.

3. Artigo Científico Redigido em Inglês

Association of *GATA3* Polymorphisms in HIV-1 Susceptibility and in the Time of Progression to AIDS

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Running title: *GATA3* SNPs and progression to AIDS

Abstract

The infection of HIV-1 can be divided into acute, chronic and AIDS, and the time elapsed since the infection to AIDS varies greatly, it may be related to virus and host factors. The objective of this study is to evaluate the role of polymorphisms in *GATA3* gene in the time of progression to AIDS. Data was collected from 201 HIV+ patients to determine the time of progression and biological material for DNA extraction. Genotyping of the SNPs rs1269486, rs1271899 and rs3824662 was performed by RT-PCR using commercial probes. Comparing the groups of fast and slow progressors, there was no statistical difference between genotypic frequencies. It was observed association between SNP rs1269486 and AIDS progression of time in which AA homozygotes progress faster than GG homozygotes in univariate regression. Whereas dominant and recessive models, an association in which the progression is faster in comparing AA homozygotes carrying the G allele regression multivariate. Data shows that the rs1269486 polymorphism in *GATA3* gene is associated with more rapid progression to AIDS has been found, disregarding other factors and variations that could affect the progression to disease.

Keywords: genetics, AIDS, progression, polymorphisms, *GATA3.*

Introduction

The natural progression of human immunodeficiency virus (HIV) infection starts with a short acute retroviral condition which usually transit to chronic and clinical latency phases and then progresses to a symptomatic and severe disease known as Acquired Immunodeficiency Syndrome (AIDS) (Ray and Bandyopadhayay, 2015). The majority of infected individuals eventually progress to AIDS in different times of progression: rapid progression, when AIDS develops within three years of infection; intermediate progression, when AIDS develops slowly between three and ten years after seroconversion; and slow progressors when infected people preserve high CD4+ and CD8+ T lymphocytes counts and eventually remain therapy naïve. Slow progressors can subsequently be divided into two groups, one showing low detectable plasma viremia, termed long-term non-progression (LTNP); and other showing plasma viremia undetectable, named elite controllers. Many studies have intended to discover genetic host and viral factors which could be useful to relate to progression (Kumar, 2013).

The GATA factor belongs to a family of transcriptional activator proteins and is connected to a highly conserved region of DNA. In addition to its normal role in T lymphocyte gene regulation, h*GATA3* may also be significant in HIV-1 transcriptional activation (Yang and Engel, 1993). The *GATA3* gene is predominantly expressed in CD4+ T lymphocytes and shows significant change in expression pattern over HIV progression stages (Ray and Bandyopadhayay, 2015). Additionally, it was demonstrated that *GATA1*, but not *GATA3*, potently suppressed CCR5 (the major HIV-1 correceptor) transcription, thereby rendering human T cells resistant to CCR5-tropic HIV-1 infection. However,

GATA1 could also serve as a surrogate for *GATA3* in its canonic role of programming Th2 gene expression. These findings provide insight into *GATA3* mediated gene regulation during T-cell differentiation. *GATA3* is a master regulator of T naïve cell differentiation into Th2 effector cells. Indeed, expression of *GATA3* enhances Th2 cytokine production, decreases IFNγ secretion and promotes chromatin remodeling of the IL-4 locus during T-cell differentiation (Sundrud *et al.*, 2005). It is possible to think that *GATA3* mutations could be related to progression to AIDS as *GATA3* influence T-cell differentiation in Th2 effector cells.

Recently, through genomic wide association studies, it was identified that the variants in *GATA3* (rs3824662 and rs1271899) are related to the susceptibility to acute lymphoblastic leukemia (Perez-Andreu *et al.*, 2015; Walsh *et al.,* 2013). Moreover, other studies have correlated another variant (rs1269486) with allergic rhinitis and asthma (Guthikonda *et al.*, 2014). Therefore, our study aims to investigate the association between these SNPs and time of progression to AIDS in a sample of HIV+ patients.

Material and Method

Study population

The 201 HIV+ blood samples were collected after screening more than 3.500 medical records in Conceição Hospital Group Infectious Center in Porto Alegre City, Brazil. Through their medical history, covering the date of negative and positive serology and the beginning of antiretroviral therapy it was possible to define the time of progression to AIDS. The 201 patients were classified in subgroups: 40 as rapid progressors, 82 as slow progressors and three as

intermediate progressors, which were not included in the analysis. The progression for the others 76 subjects could not be determined. Antiretroviral Treatment in Brazil, according to the Ministry of Health Guidelines, specifies that the initiation of Highly Active Antiretroviral Therapy should start for patients with CD4+ T lymphocytes counts under 350 cells/mm^3 and/or a insistently high viral load (National Program for STD and AIDS, 2012). In Porto Alegre city, our population is composed mainly by Eurodescendant and Afrodescendant. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected (Pena *et al.,* 2011).

Clinical definitions of the patients

Data from medical records were used to group patients in rapid and slow progressors whose characteristics were acquired by a socio-demographic form. Subjects with time between seroconversion and AIDS diagnosis below three years, with no less than two consecutive counts of CD4+ T-cell under 350 cells/mm^3, were considered rapid progressors. Individuals that were not diagnosed with AIDS even nine years after seroconversion and showed more than two consecutive counts of CD4+ T-cell 500 cells/mm^3 were classified as slow progressors. Moreover, according to the dates of the antiretroviral treatment starting and seroconversion, it was possible to estimate the time in years to progression to AIDS. Intermediate progressors subjects were not included in the analysis. This protocol study was approved by Ethics Committees of Conceição Hospital Group and written informed consent form was obtained from all patients.

DNA extraction and genotyping of GATA3 SNPs

Total DNA extraction from blood samples was performed using the salting-out method, as described by Lahiri and Nurnberg (1991). From the extracted DNA, genotypes were determined using Real-Time PCR with validated TaqMan assays to assess the following three SNPs located in *GATA3*: rs1269486 (G>A), rs1271899 (C>T) and rs3824662 (C>A) (Applied Biosystems, CA, USA). The *CCR5del32* (rs333) variant was evaluated by PCR and visualized on 3% agarose gel, and used to control for known genetic factors involved in the progression to AIDS.

Statistical analysis

Categorical variables are described in proportions and continuous variables are expressed as the mean (standard deviation [SD]) or median (interquartile range [IQR]). Allelic frequencies were calculated using the gene counting method. The agreement of the genotypic frequencies with the Hardy–Weinberg equilibrium expectations was tested using chi-square Test.

After the subjects were classified as rapid progressors or slow progressors according to the initiation of HAART and the seroconversion date. The CD4+ T lymphocytes slope values before HAART for each group were estimated using linear regression with the square root of the CD4+ T lymphocytes count (Wolbers *et al*., 2012). The non-parametric Mann-Whitney test and Fisher's exact test were used to characterize samples according to the AIDS progression classification (rapid progressors and slow progressors).

In addition, a Cox regression analysis was performed using the time of progression to AIDS in years as the dependent variable. To estimate the hazard

ratio (HR), variables that could influence the course of the disease were tested using Cox regression in a univariate model. The following variables were included: baseline CD4 levels, sex, ethnicity and the genotypes for *CCR5del32*. *CCR5del32* and variables with a p<0.1 were maintained in the multivariate model to address the effect of *GATA3* polymorphisms. Statistical test differences were considered significant if the *P* values were less than 0.05. Analyses were performed with IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NYC, USA).

Results

Clinical and laboratory characteristics of the study population

The characteristics of the HIV+ patients are shown in Table 1. If taken in consideration the whole HIV+ patients, the males represent only 26.9% (54 individuals), European ancestry is predominant (134 – 66.7%) and the average age is 41.05 (±10.3 years). The median of time to progression to AIDS in the rapid progressors group was 2 years (IQR = 1 to 3 years), whereas in the slow progressors group was 11 years (IQR = 9 to 13 years). Altogether, 27 patients in the non-progressor group did not start antiretroviral therapy at the time of study (follow-up median = 8.25 years; IQR = 4.5 to 12.25 years). The characterization of the disease progression included significant differences in the values of baseline CD4+ T-cell counts, baseline HIV-RNA copies and CD4+ T-cell slopes (p<0.001 for all variables). The number of CD4+ T-cells decreased more abruptly in the rapid progressors (median slope = -0.65; IQR = -1.28 to -0.27) than they did in slow progressors (median slope = 0.40; IQR = 0.25 to 0.50). Other variables did not differ between rapid and slow progressors. The

presence of coinfection in slow progressor group (29.6%) was greater than in rapid progressors group (15.8%). Heterosexual's exposure category was around 80%, similar in both groups.

GATA3 polymorphisms, linkage disequilibrium and haplotype frequencies

GATA3 is about 30kb in length, generating four splice variants. Three SNPs, including rs1271899 and rs1269486 (both located in 5'UTR of *GATA3*) and rs3824662 located in intron region of *GATA3* were genotyped. The minor allele frequencies for the investigated polymorphisms were 0.24 (rs1271899), 0.26 (rs1269486) and 0.22 (rs3824662) (Table 2). The current study showed similar Minor Allele Frequency (MAF) to Europeans and Africans according to the 1000 genomes data. The genotype frequencies did not show statistically significant differences compared with those expected under the Hardy–Weinberg equilibrium (P>0.05 for all SNPs). The three SNPs were in strong linkage disequilibrium (D'=0.96 to 1.00), and rs1271899-rs1269486 showed a high correlation (r^2=0.87). For the pairs rs1271899-rs3824662 and rs1269486-rs3824662 the correlation was very low (r^2=0.09). Four haplotypes were derived according the linkage disequilibrium structure between rs1271899-rs1269486-rs3824662: CGC (51.3%), TAC (23.9%), CGA (22.2%) and TGC (2.0%), respectively.

The possible functional consequence of the three SNPs were evaluated by SNPinfo web server (SNP Function Prediction - FuncPred), as listed on Table 2. Both rs1271899 and rs1269486, located in the 5'UTR were predicted as potential transcription factors binding sites (TFBS).

Association of GATA3 polymorphisms and the time of progression to AIDS

The genotype frequencies did not show statistically significant differences compared rapid and slow progressors groups (Table 3). Through univariate Cox regression we observed an association between the rs1269486 polymorphism and the time of progression to AIDS in which the progression was faster for AA homozygotes than for GG homozygotes (HR=1.91, CI95%=1.02-3.58, *P*=0.042). However, when multivariate Cox regression was carried out, including others well-known factors that affect the time of progression to AIDS, the association did not remain statistically significant (HR=1.90, CI95%=0.96-3.73, *P*=0.065, Table 4 and Figure 1). We also tested the dominant and recessive models and considering two genotype groups (AA x AG+GG in recessive model), we found an association in which the progression was faster for AA homozygotes than for G allele carriers in multivariate Cox regression (HR=1.98, CI95%=1.02-3.85 and *P*=0.043). No association was found for the other analyzed polymorphisms.

Discussion

Many studies are associating genetic variants with more rapid progression to AIDS (Zhang *et al.*, 2015). Until now there were no studies that we know of, that relates *GATA3* polymorphisms and HIV-1 in humans, only relating to other diseases (Riou *et al.*, 2016). However, it is known that *GATA3* is a very important transcription factor related to effective immune response and shows significant change in expression patter over HIV progression stage (Ray and Bandyopadhayay, 2015). This study focus in determine whether polymorphisms on the *GATA3* gene, which is a master regulator of T naïve cell

differentiation, are associated with the time of progression to AIDS in HIV-1 infected patients. In the current study, the analysis shows statistically significant differences in the time of progression to AIDS for rs1269486. According to the univariate Cox regression we observed an association between the rs1269486 polymorphism and the time of progression to AIDS in which the progression was faster for AA homozygotes than for GG homozygotes, however the association did not remain statistically significant in multivariate Cox regression. On the other hand, if considered two genotype groups (AA x AG+GG in recessive model), the AA homozygotes genotype showed faster progression to AIDS compared to G allele carries in multivariate Cox regression.

Although *GATA3* polymorphisms have not yet been investigated in the context of HIV, some genetic variations, in particular the variant rs1269486 has been associated with outcomes related to immune response, such as allergic rhinitis (Zhang *et al.*, 2009), Graves and Hashimoto disease (Morita *et al.*, 2012) and asthma (Guthikonda *et al.*, 2014). Furthermore, there is evidence that this variant is associated with the development of certain cancers, such as colorectal cancer (Figueiredo *et al.*, 2014).

The prediction functionality of rs1269486 was also tested in our study. According to our results, this SNP is predicted as potential transcription factors binding sites (TFBS) and could be related to changing transcriptional status. This results corroborate the analysis performed by Figueiredo *et al.* (2014), who shows that the rs1269486 variant is located 1.420 bases upstream of GATA3 in a region of open chromatin (DNase I hypersensitivity) with histone methylation patterns consistent with promoter activity in a colorectal cancer cell line.

Our study has some limitations, despite reviewing more than 3.500 medical records and selecting 201 potential patients to determine progression to AIDS, this study includes a small final sample size, especially as the progression groups and a limited investigation of number of *GATA3* SNPs. Nonetheless, more studies are required to answer association between *GATA3* genetic variants with the time to progression to AIDS. Our results necessitate further replication in other bigger samples with well-defined clinical AIDS outcomes data.

In conclusion, our data showed that the rs1269486 in *GATA3* is associated with a more rapid progression to AIDS, regardless of another factors and variant alleles that could potentially affect the disease progression.

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Conflicts of Interest

No competing financial interests exist.

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Table 1. Socio-demographic and clinical of the study sample.

Variable	Type of Progression		*P*
	Rapid (40)	Slow (82)	
Sex (female)	65%	80.5%	0.0062
Age (years)[a]	39.4 (11.6)	41.1 (8.6)	0.389
Time to AIDS (years)[b]	2 (1-3)	11 (9-13)[c]	<0.001
Baseline CD4+ T-cell (cells/μL)[b]	321 (206-480)	632 (476-841)	<0.001
Baseline HIV-RNA log10 (copies/mL)[b]	4.22 (3.91;4.77)	3.76 (3.31;4.16)	<0.001
CD4 Slope[b]	-0.65 (-1.28;-0.27)	0.40 (0.25;0.50)	<0.001
Co-infection (yes)	15.8%	29.6%	0.125
Transmission			0.110
Het	80%	78%	
MSM	15%	8.3%	
IDU	2.5%	9.8%	
Others	2.5%	4.9%	
Ethnicity			0.407
European ancestry	67.5%	59.8%	
African ancestry	32.5%	40.2%	

[a]Mean (standard deviation); [b]Median (interquartile range);[c]Data for patients who progressed to AIDS. Exposure category: Het = Heterosexual; MSM = Men who have sex with men; IDU = Injection drug user; others include vertical transmission and occupational accident. Co-infections include hepatitis B/C and HTLV.

Table 2. Characteristics of *GATA3 genetic variants.*

SNP rs#	Position on Chr. 10	Location *GATA3*	Allele Change	Minor Allele Frequency			Predicted SNP Function[2]
				Present study	EUR[1]	AFR[1]	
rs1271899	8050223	5'UTR	C>T	0.24	0.23	0.22	TFBS
rs1269486	8054236	5'UTR	G>A	0.26	0.23	0.23	TFBS
rs3824662	8062245	Intron	C>A	0.22	0.19	0.08	-

[1]Source: 1000genomes.org; EUR: European; AFR: African. [2] Predicted by SNPinfo web Server (http://snpinfo.niehs.nih.gov/); TFBS: transcription factor binding site

Table 3. Genotype frequencies of *GATA3* SNPs in HIV-1 positive patients according to the type of progression.

N	Type of Progression	
	Rapid (40)	Slow (82)
rs1269486		
GG	25 (62.5%)	46 (56.1%)
GA	10 (25%)	31 (37.8%)
AA	5 (12.5%)	5 (6.1%)
P	0.241	
rs1271899		
CC	26 (66.7%)	51 (63%)
CT	9 (23.1%)	26 (32.1%)
TT	4 (10.3%)	4 (4.9%)
P	0.385	
rs3824662		
CC	21 (53.8%)	46 (56.1%)
CA	17 (43.6%)	32 (39%)
AA	1 (2.6%)	4 (4.9%)
P	0.778	

Table 4. Multivariate Cox proportional hazards regression analysis to assess the independent effects of the *GATA3* polymorphisms in the time to progression to AIDS.

	HR[a]	CI95%	*P*	Adjusted HR[b]	CI95%	*P*
rs1269486						
Ethnics (white)	1.37	0.94-1.99	0.100	1.48	0.99-2.21	0.051
Sex (male)	1.59	1.06-2.38	0.026	1.21	0.77-1.90	0.401
CCR5del32 carriers	1.54	0.84-2.81	0.135	1.40	0.75-2.62	0.293
Baseline CD4+ T-cell (cells/μL)	0.99	0.98-0.99	<0.001	0.99	0.98-0.99	<0.001
GA genotype	0.87	0.58-1.29	0.500	0.87	0.58-1.32	0.514
AA genotype	1.91	1.02-3.58	0.042	1.90	0.96-3.73	0.065
rs1271899						
Ethnics (white)	1.37	0.94-1.99	0.100	1.45	0.97-2.16	0.067
Sex (male)	1.59	1.06-2.38	0.026	1.28	0.82-2.00	0.287
CCR5del32 carriers	1.54	0.84-2.81	0.135	1.32	0.70-2.46	0.385
Baseline CD4+ T-cell (cells/μL)	0.99	0.98-0.99	<0.001	0.99	0.98-0.99	<0.001
TC genotype	0.91	0.61-1.36	0.647	0.99	0.65-1.52	0.977
TT genotype	1.71	0.87-3.36	0.119	1.66	0.80-3.46	0.173
rs3824662						
Ethnics (white)	1.37	0.94-1.99	0.100	1.43	0.96-2.12	0.081

Sex (male)	1.59	1.06-2.38	0.026	1.32	0.85-2.05	0.219
CCR5del32 carriers	1.54	0.84-2.81	0.135	1.35	0.72-2.52	0.352
Baseline CD4+ T-cell (cells/μL)	0.99	0.98-0.99	<0.001	0.99	0.98-0.99	<0.001
AC genotype	1.21	0.83-1.77	0.326	1.18	0.80-1.80	0.383
AA genotype	0.52	0.16-1.66	0.269	0.53	0.17-1.72	0.291

HR: Hazard Ratio; CI: Confidence Interval. [a]Univariate model; [b]Multivariate model.

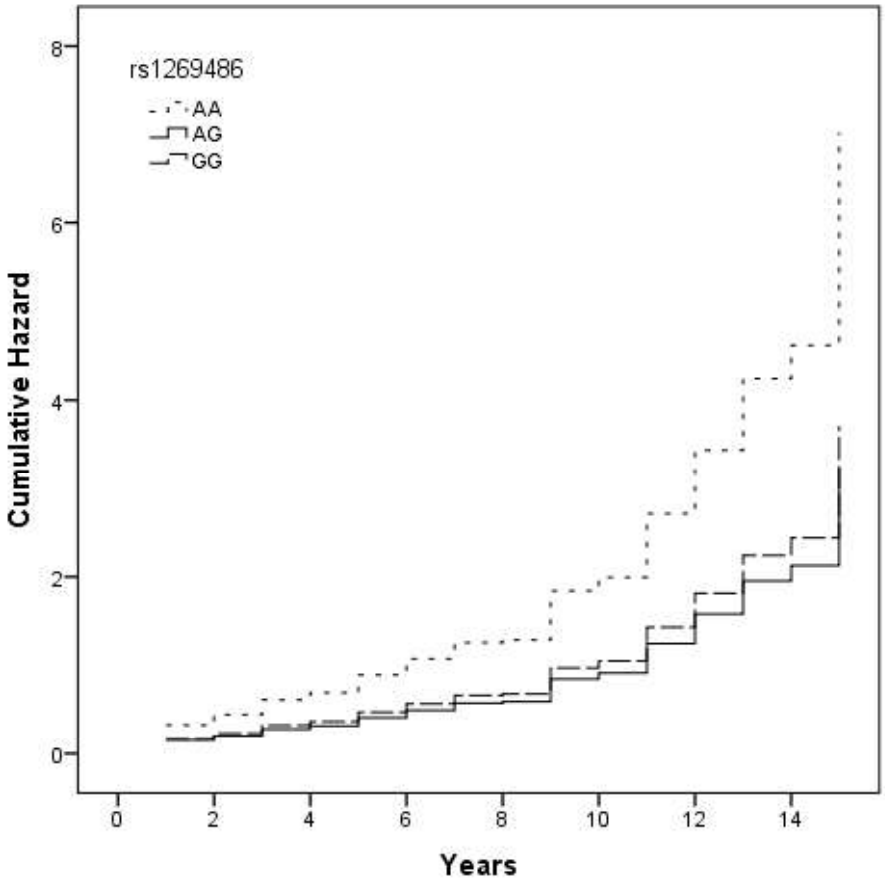

Figure 1 Univariate Cox regression analysis for rs1269486 showing the time of progression to AIDS in individuals with the AA genotype (Hazard Ratio=1.91, CI95%=1.02-3.58, *P*=0.042) and GA genotype (Hazard Ratio=0.87, CI95%=0.28-1.59, p=0.500) compared to GG genotype.

4. Considerações Finais

Muitos estudos têm associado variantes genéticas com progressão mais rápida para AIDS. Até agora não existem estudos, dos quais se tem conhecimento, que relacionam polimorfismos no gene *GATA3* e HIV-1 em humanos, somente relacionando com outras doenças (Riou e cols., 2016).

No entanto, sabe-se que o GATA3 é um fator de transcrição muito importante relacionado com a resposta imune eficaz e que ele está associado a uma alteração significativa no padrão de expressão do HIV (Ray e Bandyopadhyay, 2015). Isso nos fez escolher o gene *GATA3* como candidato para realização deste estudo, o qual focou em determinar se três polimorfismos estão associados com o tempo de progressão para AIDS.

As principais contribuições e resultados do presente estudo são:

1) Este é o primeiro estudo que avalia as variantes rs1271899, rs1269486 e rs3824662 em amostra de uma população brasileira e também o primeiro estudo no contexto HIV+.

2) As frequências gênicas das variantes rs1271899, rs1269486 e rs3824662 na população em estudo foram 0,24, 0,26 e 0,22, respectivamente. Das três variantes analisadas a única que difere em frequência entre Europeus e Africanos é a rs3824662, sendo a frequência observada no nosso estudo, mais próxima a de europeus.

3) Não houve diferença entre as frequências genotípicas das variantes analisadas entre os pacientes que apresentaram progressão rápida ou lenta.

4) Por meio da análise de regressão de Cox univariada, no entanto, se observou que homozigotos AA da variante rs1269486 progrediram mais rapidamente à AIDS.

5) A associação descrita no item 3 não se manteve significativa após correção para co-variáveis na regressão de Cox multivariada. No entanto, quando dois grupos de genótipos (AA x AG+GG) foram testados no modelo recessivo, o genótipo homozigoto AA mostrou uma progressão mais rápida para AIDS em relação aos portadores do alelo G.

6) As variantes analisadas nesta dissertação foram alvo de vários estudos com outras doenças, em abordagem gene candidato e GWAS. Apesar dos diversos resultados significativos, especialmente com a variante rs1269486, nenhum trabalho abordou a funcionalidade das variantes descritas.

7) Em nosso estudo, a predição da função do rs1269486 também foi testada. De acordo com os resultados, este SNP está potencialmente relacionado a sítios de ligação a fatores de transcrição (TFBS) e pode estar relacionado à alteração de transcrição.

Apesar de inovador, este trabalho apresenta algumas limitações: 1) embora inicialmente analisando mais de 3.500 registros médicos, este estudo inclui um grupo amostral final pequeno, especialmente os subgrupos de progressão definida. Isto ocorre devido a difícil definição do início da progressão para a maioria dos pacientes HIV+. 2) Além disso, este trabalho apresenta uma investigação limitada de polimorfismos e mais estudos serão necessários para identificar associações entre variantes genéticas do gene *GATA3* com o tempo de progressão para a AIDS. Desta maneira, acreditamos

que nossos resultados necessitam ser replicados em grupos amostrais maiores com dados de resultados clínicos de AIDS bem definidas.

No contexto atual da infecção pelo HIV, fazem-se necessários novos estudos para compreender o curso da doença com intuito de desenvolver um prognóstico mais preciso da doença e medicamentos mais eficazes no combate e manutenção da doença. Assim, os resultados encontrados neste estudo contribuirão certamente para a identificação de fatores genéticos humanos envolvidos na progressão à AIDS. Uma vez que seja possível identificar variações em genes que influenciam o curso clínico da infecção, novas estratégias no combate à doença podem ser desenvolvidas.

5. Anexos

5.1. Parecer do Comitê de Ética do Grupo Hospitalar Conceição

HOSPITAL N. S. DA CONCEIÇÃO S.A.
Av. Francisco Trein, 596
CEP 91350-200 - Porto Alegre - RS
Fone: 3357.2000
CNPJ: 92.787.118/0001-20

HOSPITAL DA CRIANÇA CONCEIÇÃO
(Unidade Pediátrica do Hospital Nossa Senhora da Conceição S.A.)

HOSPITAL CRISTO REDENTOR S.A.
Rua Domingos Rubbo, 20
CEP 91040-000 - Porto Alegre - RS
Fone: 3357.4100
CNPJ: 92.787.126/0001-76

HOSPITAL FÊMINA S.A.
Rua Mostardeiro, 17
CEP 91430-001 - Porto Alegre - RS
Fone: 3314.5200
CNPJ: 92.693.134/0001-53

Vinculados ao Ministério da Saúde - Decreto nº 99.244/90

COMITÊ DE ÉTICA EM PESQUISA - CEP/GHC

O Comitê de Ética em Pesquisa do Grupo Hospitalar Conceição (CEP/GHC), que é reconhecido pela Comissão Nacional de Ética em Pesquisa (CONEP)/MS desde 31/10/1997, pelo Office For Human Research Protections (OHRP)/USDHHS, como Institutional Review Board (IRB0001105) e pelo FWA - Federalwide Assurance (FWA 00000378), em 30 de novembro de 2010, reavaliou o seguinte projeto de pesquisa:

Projeto: 10-213 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
JOSÉ ARTUR BOGO CHIES
LUIZ FERNANDO JOBIM
MARIA CRISTINA COTTA MATTE
RÚBIA MARÍLIA MEDEIROS
DENNIS MALETICH JUNQUEIRA
LEONARDO AUGUSTO LUVISON ARAÚJO
CYNARA CARVALHO NUNES
MARINEIDE GONÇALVES DE MELO
BRENO RIEGEL SANTOS
MARIA LÚCIA ROSA ROSSETTI
SABRINA ESTEVES DE MATOS ALMEIDA

Título: Avaliação de polimorfismos em genes envolvidos na resposta imunológica de pacientes infectados com HIV-1.

Documentação: Aprovados
Aspectos Metodológicos: Aprovados
Aspectos Éticos: Aprovados

Parecer final: Este projeto, por estar de acordo com as Diretrizes e Normas Internacionais e Nacionais especialmente as Resoluções 196/96 e complementares do Conselho Nacional de Saúde, obteve o parecer de APROVADO.

Considerações Finais: Toda e qualquer alteração do projeto, deverá ser comunicada imediatamente ao CEP/GHC. Lembramos do compromisso de encaminhar dentro dos prazos estipulados, o(s) relatório(s) parcial(ais) e/ou final ao Comitê de Ética em Pesquisa do Grupo Hospitalar Conceição e ao Centro de Resultado onde a pesquisa for desenvolvida.

Porto Alegre, 30 de novembro de 2010.

Daniel Demétrio Faustino da Silva
Coordenador-geral do CEP/GHC

5.2. Parecer do Comitê de Ética da Fundação Estadual de Produção e Pesquisa em Saúde

FEPPS
Fundação Estadual de Produção e Pesquisa em Saúde

COMITÊ DE ÉTICA EM PESQUISA DA FEPPS
Av Ipiranga 5400, Prédio Administrativo.
CEP 90.610-000 – PORTO ALEGRE/RS
e-mail: cep_fepps@fepps.rs.gov.br

CEP - FEPPS

PARECER DO COMITÊ DE ÉTICA EM PESQUISA

CEP/FEPPS-RS Nº: 13/2010
PROCESSO Nº: 002964-20.69/ 10-5
PROJETO PADCT Nº: 12/2010
Deliberação conforme reunião realizada em: (27/09/2009)

Título do Projeto:

"AVALIAÇÃO DE POLIMORFISMOS EM GENES ENVOLVIDOS NA RESPOSTA IMUNOLÓGICA DE PACIENTES INFECTADOS COM HIV-1".

Nome do pesquisador principal:

Sabrina Esteves de Matos Almeida

	PARECER
x	APROVADO
	APROVADO COM RECOMENDAÇÕES
	NECESSITA DE ADEQUAÇÕES
	NÃO APROVADO

PARECER DO COMITÊ:

O Comitê de Ética em Pesquisa da FEPPS/RS em reunião do dia 27/09/2010, Ata nº 13/2010, que o presente projeto está adequado ética e metodologicamente de acordo com as Diretrizes e Normas Regulamentadoras de Pesquisa envolvendo Seres Humanos (Res.196/96/CNS e suas complementares) e portanto, **aprovado** por este Comitê.

Reiteramos que relatórios semestrais do projeto em andamento, relatório final e cópia do trabalho de conclusão e/ou publicação deverão ser entregues ao Comitê de Ética em Pesquisa da FEPPS.

Porto Alegre, 27 de setembro de 2010.

Maria da Graça Boucinha Marques
Coordenadora CEP-FEPPS/RS

5.3. Normas da Revista Journal of Infectious Disease

Manuscript Format

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Brief Report: 2000 words, 15 references, 2 inserts (with no more than 4 panels per figure) in print, and no more than 25 MB of online-only supplementary data.
2. Include a cover letter with the following information:
A statement that the manuscript has not been submitted or accepted elsewhere
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5. Ensure that all text, including tables and references, is double spaced
6. Ensure that the manuscript has page numbers
7. Use a title of no more than 160 characters and spaces and a running title of no more than 40 characters and spaces
8. Include the word count of the abstract and of the text
We require that all submissions use line-numbering within the text.
9. Include a footnote page with the following items:
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A funding statement
Mention of any meeting(s) where the information has previously been presented
Corresponding author contact information
10. Include 3–10 key words at the end of the abstract
11. Include, in the Methods section, a statement regarding informed consent and human and/or animal experimentation guidelines, when indicated
12. Include the registry number for a report of a clinical trial
13. Provide written permission for all personal communications
14. Provide accession numbers for nucleotide sequences
15. Use only approved human genetic nomenclature and notation (see the relevant subsections of the "Manuscript Preparation" section, below)

16. Submit newly identified single-nucleotide polymorphisms (SNPs) to the appropriate database; include previously recognized or recently submitted SNP numbers

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Major Articles describe original investigations that are an important advance in the field and that have been brought to an acceptable degree of completion. Major Articles must be no longer than 3500 words of text (from the beginning of the introduction to the end of the discussion; do not count the abstract or the references), and illustrations must be limited to the minimum necessary for clear and concise presentation. For Major Articles describing results of clinical trials (see "Clinical trials registration," below), the abstract must be structured with the headings Background, Methods, Results, and Conclusions and must be no more than 200 words; for other Major Article manuscripts, the abstract may be structured (200-word limit) or unstructured (150-word limit). The order of the text should be Background, Methods, Results, and Discussion. Major Articles are limited to a maximum of 7 inserts (tables and figures combined, with no more than 4 panels allowed per figure) in print, 50 references, and no more than 25 MB of online-only supplementary data.

Brief Reports present complete studies that are narrower in scope than those described in Major Articles or that represent new developments. Manuscripts that are descriptive or primarily methodologic in nature, that report results of phase I and II vaccine trials (see "Clinical trials registration," below), or that describe in vitro chemotherapeutic studies should, in general, be submitted as Brief Reports. Brief Reports include an abstract (no more than 100 words) and are limited to no more than 2000 words of text, a total of 2 inserts (tables or figures) in print, 15 references, and no more than 25 MB of online-only supplementary data. The order of the text should be Background, Methods, Results, and Discussion.

Correspondence (letters) must be submitted in reference to a previous publication in JID (within the preceding 12 months); otherwise they will not be considered. Please prepare the letter in manuscript format, including a title page. The letter cannot exceed 750 words of text, 1 insert (table or figure), and 10 references.

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Reviews that are research oriented will be considered. They should be no longer than 3500 words (from the beginning of the introduction to the end of the discussion) and have no more than 7 inserts (tables and figures combined, with no more than 4 panels per figure) in print, 50 references, and no more than 25 MB of online-only supplementary data. Authors should contact the Editor in advance of submission to determine whether a specific topic is deemed appropriate and timely.

Tables and Figures

Major Articles are limited to a maximum of 7 inserts (tables and figures combined, with no more than 4 panels allowed per figure) in print and no more than 25 MB of online-only supplementary data. Brief Reports are limited to a maximum of 2 inserts in print and no more than 25 MB of online-only supplementary data. A single insert should not contain both tables and figures.

Data should not be repeated in both a table and a figure. Abbreviations and acronyms used in tables and figures must be explained in the table footnotes and figure legends, respectively, even if they were already defined in the text.

Tables

Tables should be numbered in the order of mention in the text. Tables should be typed double spaced throughout, with no vertical or internal rules. Footnotes and accompanying explanatory

material should be kept to a minimum. Footnotes should be placed below the table and designated by superscript lowercase letters (listed in order of location when the table is read horizontally). Each column must have an appropriate heading describing the data in the column below, and units of measure must be clearly indicated. For further instructions on the preparation of tables in Word or WordPerfect, consult the Guidelines for Tables.

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Figures should be clear and comprehensible, and should generally contain one panel. No more than four panels are allowed per figure. Each figure should have a resolution of at least 300 DPI, and should be provided, individually, in its native file format, i.e., the file type in which the image was created.

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All manuscripts submitted to JID must be accompanied by a letter declaring that the manuscript has not been submitted or accepted for publication elsewhere. This letter must warrant that all authors have seen and approved the content and have contributed significantly to the work. Authors should suggest five potential unbiased reviewers who are qualified to review their manuscript. A cover letter must also accompany a revised submission and must address, point by point, issues raised in the review process.

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Footnotes must include (1) a statement that the authors either have or do not have a commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding); (2) a statement naming sources of financial support (including grant numbers); (3) the name, date (month and year), and location (city, state, and, if not USA, country) of a meeting at which all or part of the information has been presented (include an abstract number if possible); (4) the name, address, telephone and fax numbers, and e-mail address of the person to whom correspondence and requests for reprints should be addressed; and (5) current affiliations and addresses for authors whose affiliations have changed since completion of the study.

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The statistical analyses used should be identified both in the text and in all tables and figures where the results of statistical comparison are shown.

Units of Measure

All data should be expressed in metric units; use of SI units is encouraged. Use °C for temperature.

Funding

Details of all funding sources for the work presented should be given in a separate section entitled “Funding.” This information should appear before the “Acknowledgment” section.

The following rules should be followed:

The sentence should begin: “This work was supported by …”

The full official funding agency name should be given, i.e., “the National Cancer Institute at the National Institutes of Health” or simply “National Institutes of Health” not “NCI” (one of the 27 subinstitutions) or “NCI at NIH.”Please go to this Web site for a full RIN-approved list of UK funding agencies.

Grant numbers should be complete and accurate and provided in brackets as follows: “[grant number ABX CDXXXXXX]”

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Agencies should be separated by a semicolon (plus “and” before the last funding agency)

Where individuals need to be specified for certain sources of funding the following text should be added after the relevant agency or grant number “to [author initials].”

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Acknowledgments

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References

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Book chapter

McIntosh K. Diagnostic virology. In: Fields BN, Knipe DM, Chanock RM, et al., eds. Fields virology. 2nd ed. Vol 1. New York: Raven Press, 1990:411-40.
Conference program

Lyon DJ, Cheng AFB, Norrby SR. Mechanisms of cefotaxime resistance in blood culture isolates of Enterobacter high prevalence of extended-spectrum β-lactamases [abstract C43]. In: Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco). Washington, DC: American Society for Microbiology, 1995:47.
Internet site

Public Health Service Task Force. Recommendations for the use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States. Available at: http://www.hivatis.org. Accessed 24 April 2002.

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