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CCR5 e variante CCR5Δ32: Funções, aplicações e impactos clínicos em condições inflamatórias, doenças infecciosas e câncer

Bruna Kulmann Leal

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Orientador: Dr. José Artur Bogo Chies

Coorientador: Dr. Joel Henrique Ellwanger

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SUMÁRIO

LISTA DE ABREVIATURAS, SÍMBOLOS E UNIDADES

AIDS: *Acquired Immunodeficiency Syndrome* / Síndrome de Imunodeficiência Adquirida

CCR5: *C-C Chemokine Receptor 5* / Receptor C-C de Quimiocinas 5

CCR5Δ32: Polimorfismo que consiste na deleção de 32 pares de bases no gene *CCR5*

CD34: *Cluster of Differentiation 34* / Grupamento de Diferenciação 34

CD4: *Cluster of Differentation 4* / Grupamento de Diferenciação 4

CD8: *Cluster of Differentation 8* / Grupamento de Diferenciação 8

DNA: *Deoxyribonucleic Acid* / Ácido Desoxirribonucleico

HIV: *Human Immunodeficiency Virus* / Vírus da Imunodeficiência Humana

HLA: *Human Leukocyte Antigen* / Antígeno Leucocitário Humano

MIP-1α/CCL3: *Chemokine (C-C motif) Ligand 3* / Ligante de Quimiocina 3

MIP-1β/CCL4: C*hemokine (C-C motif) Ligand 4* / Ligante de Quimiocina 4

NK: *Natural Killer Cell* / Células Matadoras Naturais

pb: Pares de bases

PCR: *Polymerase Chain Reaction* / Reação em Cadeia da Polimerase

RANTES/CCL5: *Chemokine (C-C motif) Ligand 5* / Ligante de Quimiocina 5

REDOME: Registro Nacional de Doadores Voluntários de Medula Óssea

Δ: Delta

LISTA DE FIGURAS E TABELAS

RESUMO

O CCR5 é um importante receptor celular do sistema imune, atuando principalmente no controle da migração de monócitos/macrófagos e linfócitos T. Além disso, possui um papel bem definido na infecção pelo HIV-1, sendo o principal correceptor utilizado pelo vírus para realizar sua penetração na célula hospedeira. A variante CCR5Δ32 (rs333) é um polimorfismo do gene *CCR5* que consiste em uma deleção de 32 pb no éxon 3, acarretando a expressão de uma proteína truncada não-funcional. O alelo Δ32 originou-se no continente Europeu e atualmente possui uma distribuição bastante heterogênea. No Brasil, verifica-se uma maior frequência da variante na região sul em razão da configuração predominantemente de origem Europeia da população. Diversos trabalhos avaliaram o papel da variante CCR5Δ32 em condições inflamatórias, doenças infecciosas e câncer, apresentando resultados controversos para vários dos desfechos investigados. Na presente dissertação, os estudos avaliando a frequência deste alelo de maneira ampla no Brasil são revisados (artigo apresentado no Capítulo 1). Quando tratamos especificamente de câncer, verificamos que o alelo Δ32 atua de maneira diferente em tipos distintos de neoplasias. Além dos trabalhos brasileiros, percebe-se que a expressão de CCR5 pode estar associada com malignidades, como o surgimento de metástase e controle inflamatório do microambiente tumoral. Neste contexto, hipotetizamos sobre o papel da interação entre a molécula CD34, marcadora de células-tronco hematopoiéticas, com o CCR5 no desenvolvimento de câncer (artigo apresentado no Capítulo 2). Em seguida, discutimos o papel da variante CCR5Δ32 na infecção pelo HIV, trazendo a estratégia de remissão sustentada realizada pela primeira vez há doze anos e, mais recentemente, pela segunda vez, há dois anos, com base no transplante de células tronco com genótipo Δ32/Δ32. Aqui, apresentamos um artigo original (Capítulo 3) avaliando a frequência do CCR5Δ32 em doadores voluntários de medula óssea do Rio Grande do Sul cadastrados no Registro Nacional de Doadores de Medula Óssea (REDOME), além de investigar uma possível associação da presença de alelos específicos de HLA com tal polimorfismo. Encontramos uma frequência alélica de 7,1%, e uma frequência do genótipo Δ32/Δ32 de 0,76%. Esses números provavelmente são superestimados em relação à população brasileira como um todo, visto que apenas indivíduos da região sul foram incluídos no estudo. No entanto, o estudo revela um número substancial de doadores disponíveis em um contexto nacional que podem fornecer células com o genótipo Δ32/Δ32 para novas tentativas de remissão sustentada da infecção pelo HIV. Por fim, não encontramos associações dos alelos de HLA com a variante, mas o locus *HLA-B* se mostrou um interessante objeto de estudo futuro.

Palavras-chave: Brasil; câncer; CCR5; CCR5Δ32; inflamação; HLA; HIV

ABSTRACT

CCR5 is an important immune system cell receptor, acting mainly on the migration control of monocytes/macrophages and T lymphocytes. Also, it has a well-defined role in HIV-1 infection, being the main coreceptor used by the virus to penetrate the host cell. The variant CCR5Δ32 (rs333) is a polymorphism of the *CCR5* gene, which consists of a 32-bp deletion in exon 3, leading to the expression of a non-functional truncated protein. The Δ32 allele originated in the European continent and currently has a very heterogeneous distribution. In Brazil, there is a high frequency of the variant in the southern region, due to the predominantly European origin of the population. Several studies evaluated the role of the Δ32 variant in inflammatory conditions, infectious diseases and cancer, with controversial results for several of the investigated outcomes. In the present work, studies evaluating the frequency of this allele in different outcomes in Brazil are reviewed (article included in Chapter 1). When we specifically focus on cancer, we find that the Δ32 allele acts differently in different types of neoplasms. In addition, the expression of CCR5 may be associated with malignancies, such as the appearance of metastasis and in the inflammatory control of the tumor microenvironment. In this context, we hypothesized about the interaction between the CD34 molecule, a hematopoietic stem cell marker, with CCR5 in cancer development (article included in Chapter 2). Next, we discussed the role of the CCR5Δ32 variant in HIV-1 infection, bringing the strategy of sustained remission carried out for the first time twelve years ago and, more recently for the second time, two years ago, based on stem cell transplantation with Δ32/Δ32 genotype. Here, we present an original study (Chapter 3) evaluating the CCR5Δ32 frequency in voluntary bone marrow donors from Rio Grande do Sul registered in *Registro Nacional de Doadores de Medula Óssea* (REDOME), in addition to investigating a possible association of the presence of specific HLA alleles with such polymorphism. We found an allele frequency of 7.1%, and a frequency of the Δ32/Δ32 genotype of 0.76%. These numbers are probably overestimated in relation to the Brazilian population as a whole, as only the southern region was included in the study. However, it suggests a substantial number of available donors in a national context that can supply cells with the Δ32/Δ32 genotype for further attempts of sustained remission of HIV infection. Finally, we did not find associations between the HLA alleles with a variant, but the *HLA-B* locus is an interesting object for future studies.

Keywords: Brazil; cancer; CCR5; CCR5Δ32; inflammation; HLA; HIV

INTRODUÇÃO

O sistema imune e suas diversas moléculas orquestram uma miríade de processos, participando de respostas a patógenos e defesa contra danos e lesões celulares e teciduais. Por outro lado, o sistema imune pode, quando em desequilíbrio, ser o responsável pela geração de doenças. Dentre esse sistema complexo, as quimiocinas e seus receptores atuam como moléculas chave para a migração celular, função característica das células componentes do sistema imune, que precisam se locomover para os sítios de ação em que a resposta imunológica deve ocorrer em determinada situação (Zlotnik and Yoshie 2012; Sokol and Luster 2015; Ellwanger et al. 2020a).

Além disso, as moléculas quimiotáticas promovem uma comunicação entre sistema imune inato e adaptativo, sendo especialmente relevantes para o refinamento da resposta imune celular (Zlotnik and Yoshie 2012; Sokol and Luster 2015). As quimiocinas são moléculas que, ao interagirem com seus respectivos receptores, promovem a mobilidade da célula e a migração da mesma para os locais originais de suas produções, guiando o caminho por gradientes de concentração (Zlotnik and Yoshie 2012; Sokol and Luster 2015; Ellwanger et al. 2020a). Já os receptores de quimiocinas são receptores associados à proteína G com uma ampla variedade de especificidades, sendo alguns mais promíscuos enquanto outros se ligam apenas a quimiocinas específicas (Zlotnik and Yoshie 2012; Sokol and Luster 2015; Ellwanger et al. 2020a).

1. CCR5 e a variante CCR5Δ32

O Receptor CC de Quimiocinas 5 (CCR5) é um importante mediador da resposta imune adaptativa, sendo expresso principalmente por monócitos e macrófagos e células T CD4+ de perfil Th1, mas também pode ser encontrado em outros tipos celulares como células NK e T reguladoras (Zlotnik and Yoshie 2012; Sokol and Luster 2015; Ellwanger et al. 2020a). Seus principais ligantes são as quimiocinas CCL3 (MIP-1α), CCL4 (MIP-1β) e CCL5 (RANTES), podendo ocorrer interações também com CCL8, CCL13 e CCL16 (Zlotnik and Yoshie 2012; Sokol and Luster 2015; Ellwanger et al. 2020a). O CCR5 consiste em um receptor associado à proteína G com sete domínios transmembrana de 352

aminoácidos codificado pelo gene *CCR5*, localizado no braço curto do cromossomo 3 (3p21.31) humano (Samson et al. 1996; Alkhatib 2009). Além de seu papel fisiológico, o CCR5 é conhecido por ser um importante correceptor utilizado pelo HIV-1 para a penetração na célula hospedeira (Ellwanger et al. 2020a).

Dada a relevância desse receptor no processo infeccioso do HIV-1, fármacos antagonistas de CCR5 foram desenvolvidos para inibir a ligação da partícula viral à molécula, assim como foram desenvolvidas tentativas de edição gênica para excisar o *CCR5*, com o objetivo de ampliar os protocolos terapêuticos contra a infecção (Zlotnik and Yoshie 2012; Ellwanger et al. 2019). Além dessas abordagens, um polimorfismo genético confere resistência ao HIV de maneira natural. A variante CCR5Δ32 (rs333) consiste em uma deleção de 32 pares de bases na região codificante (exon 3) do gene *CCR5*, ocasionando a geração de um códon de parada prematuro. A deleção ocorre na região correspondente à formação do segundo *loop* extracelular da proteína, e com o códon de parada gerado pela variante, o terceiro *loop* extracelular e a região C-terminal do receptor não são formados. Dessa forma, há a produção de uma proteína truncada que não é levada a superfície celular, impedindo sua função como receptor de quimicionas (Dean et al. 1996; Liu et al. 1996; Samson et al. 1996; Silva-Carvalho et al. 2016; Ellwanger et al. 2020a). A **Figura 1** demonstra a estrutura molecular simplificada do *CCR5* e da variante CCR5Δ32.

O CCRΔ32 possui frequências bastante diversas em diferentes populações humanas, sendo mais comum em países do norte da Europa, com frequências de 10-20%, e praticamente ausente em países africanos e asiáticos (Libert et al. 1998; Silva-Carvalho et al. 2016). Isso se deve a origem da variante, que parece ter ocorrido relativamente recentemente, em um único evento mutagênico em alguma população Europeia (Libert et al. 1998).

Devido a processos de colonização e a atual globalização, povos com grandes taxas de miscigenação também podem apresentar a variante em uma frequência considerável. No Brasil, a frequência média do alelo Δ32 é de 4%, porém é bastante variável ao longo do território, podendo variar entre frequências tão baixas quanto menos de 1% a tão altas quanto 9%. Em geral, a frequência da variante é mais alta em populações com maiores

contribuições europeias na composição genética (Boldt et al. 2009; Farias et al. 2012; Silva-Carvalho et al. 2016; Solloch et al. 2017).

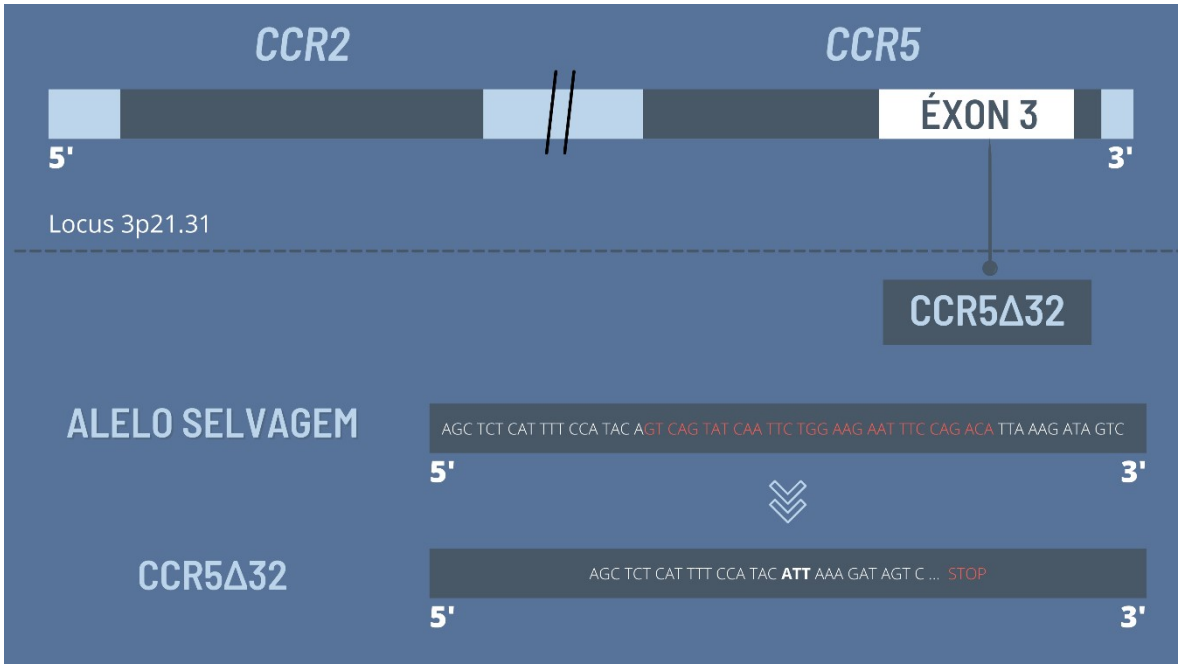

Figura 1. Representação esquemática do gene *CCR5* e a variante CCR5Δ32. No alelo selvagem, a região em vermelho destaca os nucleotídeos deletados, inexistentes no alelo Δ32.

Há uma quantidade considerável de trabalhos avaliando a frequência da variante CCR5Δ32 no Brasil. No entanto, é importante ressaltar que boa parte desses estudos são realizados com o objetivo de avaliar a frequência do alelo em contextos clínicos específicos, e mesmo em alguns desses contextos, o papel do CCR5Δ32 ainda não é bem estabelecido. Assim, o **Capítulo 1** dessa dissertação retoma conceitos importantes sobre a variante e faz uma ampla revisão nos trabalhos investigando a frequência do alelo Δ32 no Brasil.

2. CCR5 e câncer

Conforme discutido anteriormente, as quimiocinas são moléculas-chave para a migração celular, inclusive em situações de câncer: essas proteínas e seus receptores atuam

controlando o tráfego de células do sistema imune no microambiente tumoral. O papel atribuído às quimiocinas e seus receptores no câncer se torna uma 'faca de dois gumes', podendo contribuir para respostas antitumorais e, quando produzidas por células malignas, para a manutenção do tumor (Aldinucci et al. 2008; Balkwill 2012; Che et al. 2015; Halama et al. 2016). O CCR5, sendo um receptor de quimiocinas fundamental para a migração leucocitária, é um fator notável para o estabelecimento de processos inflamatórios. Dentre as atuações já levantadas do CCR5 na tumorigênese estão a migração de células malignas e geração de metástase, assim como o controle da inflamação no microambiente tumoral a partir de células regulatórias CCR5+ (Kulmann-Leal et al. 2020).

Interessantemente, Enrich et al. (2017) observaram um repertório menor de células CD34+ em indivíduos homozigotos para a variante CCR5Δ32. A molécula CD34 é um marcador biológico de células-tronco hematopoiéticas e está associada a processos angiogênicos, podendo ser encontrada em células tumorais. Visto que tanto CCR5 como CD34 podem ser fatores pró-tumorais, e que pode potencialmente haver uma conexão funcional entre ambas as moléculas, a presente dissertação aborda no **Capítulo 2** a hipótese de que as moléculas CCR5 e CD34, juntas, podem atuar na proliferação de células tumorais e no estabelecimento de câncer (Kulmann-Leal et al. 2020).

3. CCR5Δ32 e supressão viral sustentada em pacientes infectados pelo HIV

A respeito do papel da variante CCR5Δ32 na infecção pelo HIV-1, observa-se que indivíduos que possuem o alelo em homozigose apresentam uma alta resistência a infecção, pois, apesar de o CCR5 não ser o único correceptor possível para a ligação da proteína viral gp41, a maioria das cepas de HIV-1 o tem como preferência. Indivíduos heterozigotos para a variante, no entanto, não possuem a mesma resistência. Porém, verifica-se nesses últimos uma progressão mais lenta a AIDS quando comparados a indivíduos homozigotos para o alelo selvagem (sem deleção) (Huang et al. 1996; Hutchinson 2001; Ellwanger et al. 2020b). Essas consequências se dão pelos perfis de expressão ocasionados pelos genótipos, conforme demonstrados na **Figura 2**.

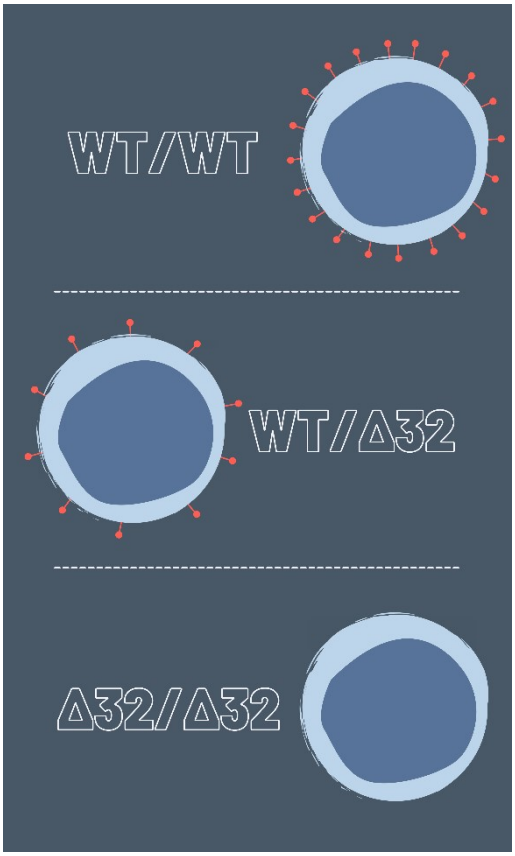

Figura 2. Perfil de expressão do receptor CCR5 (em vermelho) de acordo com os genótipos da variante CCR5Δ32. Indivíduos homozigotos para o alelo selvagem (WT/WT, sem deleção) expressam uma quantidade normal do receptor em suas superfícies celulares. Indivíduos heterozigotos (WT/Δ32, portando uma cópia do alelo com deleção) expressam aproximadamente metade da quantidade que indivíduos WT/WT produzem. Indivíduos homozigotos para a variante (Δ32/Δ32, portando duas cópias do alelo com deleção) não expressam CCR5 nas membranas celulares.

O conhecimento a respeito da resistência gerada pela variante CCR5Δ32 serviu como base para uma estratégia inovadora que resultou na remissão sustentada da infecção pelo HIV, sem a necessidade de uso contínuo de medicamentos antirretrovirais. Em 2009, o grupo de Hütter et al. (2009) publicou o trabalho que trouxe à tona a discussão sobre cura esterilizante da infecção pelo HIV, divulgando o caso do conhecido 'Paciente de Berlim'. Timothy Ray Brown, um indivíduo com sorologia positiva para HIV, havia desenvolvido uma leucemia mieloide aguda e, como tratamento para tal, passaria por um transplante de medula óssea. A equipe médica, então, buscou como doador um indivíduo com genótipo

Δ32/Δ32. Após o transplante, Timothy teve sua terapia antirretroviral interrompida e foi acompanhado por um extenso período. Após alguns meses, o Paciente de Berlim passou a apresentar carga viral indetectável, o que foi mantido até a data de seu falecimento, em 2020 (Hütter et al. 2009; Hütter and Thiel 2011; Brown 2015; Hope et al. 2020).

Em 2019, o segundo caso de remissão sustentada da infecção pelo HIV pós-transplante com células-tronco hematopoiéticas Δ32/Δ32 foi publicado. Seguindo um protocolo muito similar ao de Hütter et al. (2009), Adam Castillejo, um indivíduo HIV+ que havia desenvolvido linfoma de Hodgkin, ficou conhecido como o 'Paciente de Londres', em referência ao primeiro caso de cura esterilizante. Até então, os relatos acerca da carga viral de Adam demonstram a mesma remissão sustentada observada em Timothy (Gupta et al. 2019; Gupta et al. 2020).

Dada a relevância da revolucionária terapia demonstrada por Hütter et al. (2009), Enrich et al. (2017) e Solloch et al. (2017) realizaram triagens em bancos europeus de células-tronco hematopoiéticas para verificar a frequência do alelo Δ32 em potenciais doadores de medula óssea. A partir da observação de que é possível encontrar um número razoável de indivíduos dispostos a doar medula óssea apresentando homozigose para a variante CCR5Δ32, o **Capítulo 3** desta dissertação apresenta um artigo original avaliando a frequência do alelo Δ32 em Brasileiros cadastrados no Registro Nacional de Doadores Voluntários de Medula Óssea (REDOME), além de investigar possíveis associações CCR5Δ32 com alelos de Antígeno Leucocitário Humano (HLA). Saber se o alelo Δ32 está associado (em termos de maior frequência) a alelos HLA específicos ajudaria a prever quais indivíduos poderiam mais facilmente encontrar um doador compatível tanto para CCR5Δ32 quanto para HLA.

4. Uma breve revisão sobre HLA e transplante de medula óssea

O transplante de medula óssea é um tratamento sugerido para distúrbios envolvendo os chamados elementos figurados do sangue (eritrócitos, leucócitos e plaquetas), que são formados a partir de células-tronco hematopoiéticas, geradas na medula óssea. O procedimento consiste na substituição desse tecido do paciente por uma medula saudável, obtida de um doador compatível. Essa compatibilidade, por sua vez, é dada principalmente

pelo chamado Sistema de Antígeno Leucocitário Humano, ou HLA. As diversas moléculas de HLA constituem o Complexo Principal de Histocompatibilidade (MHC) em humanos, sendo divididas em genes de classe I e genes de classe II (Bjorkman et al. 1987; Thomas 1999; Fernandes et al. 2003).

Os genes clássicos de classe I, *HLA-A*, *HLA-B* e *HLA-C*, são expressos por praticamente todas as células do corpo e possuem uma quantidade exorbitante de polimorfismos, especialmente o *HLA-B*. Já os genes de classe II, *HLA-DR*, *HLA-DQ* e *HLA-DP*, são expressos predominantemente por células apresentadoras de antígeno, e atuam diretamente nas respostas imunes celulares (Fernandes et al. 2003). Para que um transplante tenha sucesso e não desencadeie a chamada Doença do Enxerto Contra o Hospedeiro, o tecido doado precisa possuir um elevado grau de similaridade de moléculas HLA em relação ao paciente a receber o transplante. Em geral, três loci principais de HLA são analisados nesse contexto: *HLA-A*, *HLA-B* e *HLA-DR* (Morishima et al. 2002).

Como já comentado, a estratégia utilizada por Hütter et al. (2009) e Gupta et al. (2019) requer uma significativa compatibilidade tecidual entre doador e receptor. No **Capítulo 3** desta dissertação, é avaliada uma possível associação entre a presença de alelos específicos dos loci *HLA-A*, *HLA-B* e *HLA-DR* e a variante CCR5Δ32, para ampliar o conhecimento acerca da seleção de doadores com genótipo Δ32/Δ32 para a realização de transplante de medula óssea em indivíduos HIV+.

OBJETIVOS

1. *Objetivo geral*

Investigar a molécula CCR5 de forma múltipla, focando no papel da proteína e da variante genética CCR5Δ32 em doenças inflamatórias e câncer, na distribuição do alelo Δ32 no território brasileiro e na identificação de possíveis doadores de células-tronco hematopoiéticas para novas tentativas de remissão sustentada da infecção pelo HIV.

2. *Objetivos específicos*

- Revisar os trabalhos que investigaram a variante CCR5Δ32 no Brasil;
- Investigar o impacto da miscigenação sobre a frequência da variante CCR5Δ32 no Brasil;
- Construir uma hipótese sobre a correlação funcional entre CCR5 e CD34, discutindo seu impacto no desenvolvimento de câncer;
- Avaliar a frequência da variante CCR5Δ32 em indivíduos do Rio Grande do Sul registrados no REDOME;
- Identificar a frequência de indivíduos homozigotos para a variante CCR5Δ32 que estão registrados como potenciais doadores de medula óssea;
- Investigar a possível associação entre alelos específicos de HLA e a variante CCR5Δ32.

CAPÍTULO 1

O presente capítulo apresenta um artigo de revisão que contempla os dois primeiros objetivos específicos desta dissertação, a ser submetido ao periódico *Gene*:

KULMANN-LEAL, Bruna; ELLWANGER, Joel Henrique; CHIES, José Artur Bogo. **CCR5Δ32 in Brazil: Impacts of a European genetic variant on a highly admixed population**. 2021. *Artigo em preparação para submissão.*

CCR5Δ32 in Brazil: Impacts of a European genetic variant on a highly admixed population

Bruna Kulmann-Leal [a,b], Joel Henrique Ellwanger [a,b], José Artur Bogo Chies [a]

[a] Laboratório de Imunobiologia e Imunogenética, Programa de Pós-Graduação em Genética e Biologia Molecular (PPGBM), Departamento de Genética, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil.

[b] These authors contributed equally to this article.

Corresponding author: Dr. José Artur Bogo Chies. Laboratório de Imunobiologia e Imunogenética (Prédio 43323, Laboratório 212), Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul – UFRGS. Av. Bento Gonçalves, 9500, Campus do Vale, Porto Alegre, Rio Grande do Sul, Brazil. Phone: +5551 33086737. E-mail: jabchies@terra.com.br

Abstract

The genetic background of Brazilians encompasses Amerindian, African, and European components as a result of the colonization of an already Amerindian inhabited region by Europeans, associated to a massive trade of slaves from Africa. Other migratory flows introduced into the Brazilian population genetic components from Asia and the Middle East. Currently, Brazil has a highly admixed population and, therefore, the study of genetic factors in the context of health or disease in Brazil is a challenging and very interesting subject. This phenomenon is exemplified by the genetic variant CCR5Δ32, a 32 base-pair deletion in the *CCR5* gene. CCR5Δ32 originated in Europe, but the time of origin as well as the selective pressures that allowed the maintenance of this variant and the establishment of its current frequencies in the different human populations is still a field of debates. Due to its origin, the CCR5Δ32 allele frequency is high in European-derived populations (~10%) and low in Asian and African native human populations. In Brazil, the CCR5Δ32 allele frequency is intermediate (4-6%) and varies on the Brazilian States, depending on the migratory history of each region. CCR5 is a protein that regulates the activity of several immune cells, also acting as the main HIV-1 co-receptor. The CCR5 expression is influenced by CCR5Δ32 genotypes. No CCR5 expression is observed in CCR5Δ32 homozygous individuals. Thus, the CCR5Δ32 has particular effects on different diseases. At the population level, the effect that CCR5Δ32 has on European populations may be different than that observed in highly admixed populations. Besides less evident due to its low frequency in admixed groups, the effect of the CCR5Δ32 variant may be affected by other genetic traits. Understanding the effects of CCR5Δ32 on Brazilians is essential to predict the potential use of pharmacological CCR5 modulators in Brazil. Therefore, this study reviews the impacts of the CCR5Δ32 on the Brazilian population, considering infectious diseases, inflammatory conditions, and cancer. Finally, this article provides a general discussion concerning the impacts of a European-derived variant, the CCR5Δ32, on a highly admixed population.

Keywords: Brazil; cancer; CCR5; infectious disease; inflammation; population genetics

1. Introduction

1.1. Genetic aspects of the Brazilian population

Until the year 1500 CE, Brazil was inhabited only by Native Americans belonging to different linguistic groups, distributed along the coast and hinterland of the country. This scenario changed dramatically after the arrival of the Portuguese explorers in the Brazilian territory that year, affecting many cultural and biological aspects of the native populations. The European colonization of Brazil and the associated trade of African slaves had a strong influence on the genetic makeup of the Brazilian population. In Brazil, as well as in other countries colonized by the Europeans, the Native American population deeply declined after colonization (contracted around 90% in the Americas) (Pena et al., 2011; Adhikari et al., 2017; Mas-Sandoval et al.,2019). The remaining native population underwent a strong process of genetic miscegenation. However, the processes of population change continued throughout Brazilian history, even in more recent times. Over the past 200 years, Brazil has received a large influx of European immigrants from various countries (also described as the last migration pulse), who sought better living and working conditions in the American continent, and which added another layer to the genetic makeup of the Brazilian population. Specifically, after the prohibition of the slave trade in Brazil in 1850, the country received many Europeans as a governmental strategy for "whitening" the population (Pena et al., 2011; Adhikari et al., 2017; Mas-Sandoval et al., 2019; Castro e Silva et al., 2020). It is critical to stress that this racist strategy had many undesirable social and economic impacts on Brazilian society (Pena et al., 2011).

In general terms, the genetic background of current Brazilians has Amerindian, African, and European components in different proportions (Lins et al., 2010; Giolo et al., 2012; Suarez-Kurtz et al., 2014; Adhikari et al., 2017; Mas-Sandoval et al., 2019), depending on the Brazilian region under investigation (North, Northeast, Center-West, Southeast, or South). For example, the genetic makeup of Brazilians in the southern region of Brazil was strongly influenced by migratory flows from Europe in the 19th and 20th centuries. In the Northeast of the country, the African genetic component is high due to the region having received a large number of African slaves (Callegari-Jacques et al., 2003; Pena et al., 2011; Adhikari et al., 2017). Of note, the European component is preponderant

in different Brazilian regions when the Amerindian, African, and European components are compared, but even observing some regional peculiarities as those mentioned above, the genetic composition of the Brazilian population is rather uniform in its miscegenation in different regions of the country (Pena et al., 2011).

Throughout history, Brazil also received migrants from other countries beyond those from Europe and Africa, including countries from Asia and Middle East (Suarez-Kurtz et al., 2014; Halagan et al., 2018). The intense migration within the national territory (Batista et al., 2018) allowed the exchange of genetic information between Brazilians from different regions, ethnic and genetic groups. As a result of the interactions of these different groups, the Brazilian population is currently highly miscegenated, a characteristic evident in the rich genetic and phenotypic diversity observed among the Brazilian population (Giolo et al., 2012; Ruiz-Linares et al., 2014; Adhikari et al., 2017; Chacón-Duque et al., 2018). Considering the scenario mentioned above, the Brazilian population can be considered genetically heterogeneous and admixed, in addition to being relatively uniform throughout the country (Pena et al., 2011). Interestingly, admixed Brazilian populations are probable "reservoirs" of the diverse Native American genetic component (Mas-Sandoval et al., 2019), currently the least prevalent genetic component in the population (Callegari-Jacques et al., 2003; Pena et al., 2011).

1.2. Pivotal information regarding the CCR5Δ32 variant

The CCR5Δ32 polymorphism (reference SNP ID number: rs333) is a genetic variant that originated in the European population (Ellwanger et al., 2020a), and therefore can be used as an ancestry-informative marker in studies involving population genetics and genome ancestry (Carvalho et al., 2004; Vargas et al., 2006). This variant represents a 32-base pair deletion in the *CCR5* gene (chromosome 3; 3p.21.31), a fundamental component of the immune system responsible for encoding the CCR5 protein, which acts mainly in the regulation of inflammatory cell migration. It is unclear what selective pressures (considering positive selection) were responsible for fixing CCR5Δ32 in the human genome. Smallpox, bubonic plague, and other infectious diseases have already been suggested, but there is no consensus on this aspect (Ellwanger et al., 2020a). Neutral evolution is also a possibility (Sabeti et al., 2005). What is somehow certain is that the

variant probably originated in the European population at 700-5,000 years ago (Stephens et al., 1998; Sabeti et al., 2005), potentially even earlier than 5,000 years (Lidén et al., 2016; Faure and Royer-Carenzi, 2018), and later spread heterogeneously across the world.

The frequency of the CCR5Δ32 allele is higher in northern Europe (greater than 15% in Norway, Latvia, and Estonia), being observed less frequently in countries located in the south of the European continent. For example, the frequency of the CCR5Δ32 allele is 8.1% in Spain, 6.9% in Portugal, 6.2% in Italy, and 5.1% in Greece. The allele frequency is low or even absent in most Asian and African countries: for example, 0.4% in China, 2.2% in Korea, 0.7% in Cameroon, 0.26% in Eritrea, and 2.9% in Egypt (Soloch et al., 2017). A recent study reports the absence of the CCR5Δ32 allele in the Nepalese population (Shrestha et al., 2020). Similarly, CCR5Δ32 is rare in Native American groups, showing an overall CCR5Δ32 allele frequency of 0.2%, mostly probably due to miscegenation (Vargas et al., 2006). In the contemporary Brazilian population, the overall frequency of the CCR5Δ32 allele usually ranges from 4 to 6% but showing significant variations between different Brazilian regions and ethnic groups (Vargas et al., 2006; Silva-Carvalho et al., 2016), as will be discussed in the next sections of this article.

The main function of the CCR5 is coordinating leukocyte migration during inflammatory reactions through interaction with different chemokines, especially CCL3, CCL4, and CCL5 (Ellwanger et al., 2020a). Of note, these chemokines were historically called “MIP-1α”, “MIP-1β” and “RANTES”, respectively, but that denomination has fallen into disuse (Zlotnik and Yoshie, 2000; IUIS/WHO Nomenclature Committee, 2003). The CCR5 protein is expressed on the cell surface and has seven transmembrane domains connected by three extracellular loops and three intracellular loops. Leukocytes are the main cells that express the CCR5 (Ellwanger et al., 2020a), although the protein is also detected in other cell types, such as human embryonic neurons (Boutet et al., 2001), adipocytes (Hazan et al., 2002), and several types of cancer cells and tissues (Vaday et al., 2006; Sales et al., 2014; Kranjc et al., 2019; Liu et al., 2019; Suarez-Carmona et al., 2019), indicating that CCR5 performs immune functions that go beyond coordinating the migration of inflammatory cells.

Carriers of the wild-type *CCR5* gene have CCR5 expression constitutively, with some variation between individuals. CCR5Δ32 causes important phenotypic effects, affecting the interaction of the CCR5 with chemokines. Due to the induction of a change in

the *CCR5* gene reading frame, the CCR5Δ32 produces a truncated protein that is not expressed on the cell surface, presenting a gene-dosage effect. In brief, the presence of the CCR5Δ32 allele in heterozygous causes a reduction in the expression of CCR5 at the membrane. The presence of the CCR5Δ32 allele in homozygosis culminate in virtually no expression of CCR5 molecules on the cell surface (Liu et al., 1996; Wu et al., 1997; Husman et al., 1999; Segerer et al., 1999; Venkatesan et al., 2002). The CCR5Δ32-derived molecules are not phosphorylated and remain retained in the endoplasmic reticulum (Benkirane et al., 1997). Interestingly, it was suggested that in addition to the gene-dosage effect associated to CCR5Δ32, the CCR5Δ32-derived truncated protein could promote the sequestration of the CCR5 and CXCR4 proteins, both HIV-1 co-receptors, from the cell surface (Agrawal et al., 2004; Agrawal et al., 2007).

These changes in the expression of CCR5 associated to CCR5Δ32 culminate in a disrupted CCR5-mediated immune response, which can be beneficial in some situations or harmful in others (Ellwanger et al., 2019) since the 'chemokine system' is not completely redundant. The absence of CCR5 can impact the cell signaling coordinated by CCL3, CCL4 and CCL5, thus perturbing the proper CCR5-mediated immune responses (Ellwanger et al., 2020b). Disruptions in the chemokine system can significantly alter the susceptibility and progression of different diseases. For instance, COVID-19 severe cases are associated with uncontrolled receptor-ligand interactions and consequent inflammatory dysregulation, which characterizes the cytokine storm frequently observed in such severe disease cases (Coperchin et al., 2020; Mehlotra, 2020). Recently, CCR5Δ32 deletion was identified as a protective factor in Czech First-Wave COVID-19 subjects (Hubacek et al., 2021). Different *CCR5*-editing techniques are currently available and can be used to test *in vitro* the impacts of the CCR5 absence in different conditions, simulating the consequences of CCR5Δ32 on the immune system and disease conditions (Badia et al., 2014; Liu et al., 2020). However, it is essential to emphasize that the *CCR5*-editing in human embryos raises many ethical concerns and may have deleterious consequences (Ellwanger et al., 2019; Niemiec and Howard, 2020).

Looking at the desirable effects, CCR5Δ32 protects against HIV infection, since the homozygous state of the variant impairs the proper expression of CCR5, preventing the interaction of CCR5 (the main HIV co-receptor) with the virus on the cell surface, thus avoiding infection of the host (Huang et al., 1996; Samson et al., 1999). As mentioned

above, CCR5Δ32-derived molecules (CCR5 truncated proteins) can also have an important protective effect against HIV by sequestrating CCR5 and CXCR4 from cell surface (Agrawal et al., 2004; Agrawal et al., 2007). The discovery of this effect was very relevant because it gives support to the use of CCR5 blockers for the clinical control of HIV infection. The best example of this case is maraviroc, a noncompetitive CCR5 antagonist that prevents the proper interaction between the HIV envelope glycoprotein and the CCR5. Currently, other CCR5 blockers (e.g. cenicriviroc, leronlimab) are being tested to treat HIV infection and other inflammatory conditions, and maraviroc emerges as a potential drug to treat other diseases involving CCR5, especially some types of cancer (Miao et al., 2020). In Brazil, CCR5 blockers represent a good choice for HIV treatment, since most of the circulating viral strains show CCR5 tropism (Arruda et al., 2014; Pessôa et al., 2015; Avanzi et al., 2017). Based on the scenario presented above, Figure 1 shown an alluvial diagram representing the classic outcomes associated with the CCR5Δ32, including "desirable" and "undesirable" effects.

Another major achievement involving CCR5Δ32, and HIV infection was the sustained remission of the infection in the 'Berlin Patient', reported in 2009 (Hutter et al., 2009) and confirmed in 2011 (Allers et al., 2011), and in the 'London Patient', reported in 2019 (Gupta et al., 2019) and confirmed in 2020 (Gupta et al., 2020). Both individuals were HIV positive and developed hematological malignant diseases (acute myeloid leukemia and Hodgkin's lymphoma, respectively), requiring allogeneic hematopoietic stem-cell transplantations. After receiving cell transplantations from CCR5Δ32 homozygous donors, both showed sustained remission of HIV infection. Other cases similar to Berlin and London patients are being followed up, such as the 'Düsseldorf patient' (Kalidasan and Theva Das, 2020). The success of this strategy, although involving few cases, shows that sustained remission of HIV is possible to be achieved and subsequently maintained free of antiretroviral therapy. The Berlin patient, Timothy Ray Brown, passed away on September 29, 2020, due to the recurrence of acute myeloid leukemia, not HIV infection (UNAIDS, 2020; Watts, 2020). In addition to having collaborated enormously to advance research involving HIV, T. R. Brown created the Timothy Ray Brown Foundation and contributed significantly to the field of HIV/AIDS research, with a big and admirable impact on global society as an HIV activist (UNAIDS, 2019; Brown, 2020; Watts, 2020).

Currently, it is known that the influence of CCR5 and CCR5Δ32 goes beyond protection against HIV infection and is much broader than previously believed, influencing the susceptibility and outcome of different conditions, such as other different viral, bacterial, and parasitic diseases (Ellwanger et al., 2020a; Ellwanger et al., 2020c), as well as non-infectious inflammatory conditions (Chies and Nardi, 2001; Eri et al., 2004; Boiardi et al., 2011; Baltus et al., 2015). This occurs because the lack of CCR5 expression, in humans naturally due to CCR5Δ32, interferes with multiple aspects of inflammatory responses, including expression of immune system genes, levels of inflammatory markers, and activity of immune cells (Afzal et al., 2008; Hutter et al., 2011; Muntinghe et al., 2012; Joo et al., 2019; Kulmann-Leal et al., 2020; Martín-Leal et al., 2020; Matti and Legler, 2020). On the other hand, now looking at the undesirable aspects of CCR5Δ32, this genetic variant increases the risk of serious complications caused by the West Nile virus and Tick-borne encephalitis virus (Glass et al., 2006; Lim et al., 2008; Kindberg et al., 2008; Lim et al., 2010; Mickienė et al., 2014; Ellwanger and Chies, 2019).

Although Brazilians form a population of more than 210 million individuals, genetic studies in this population are still limited, with the majority of genetic studies focusing on populations with European ancestry (Giolo et al., 2012; Halagan et al., 2018). The Brazilian population can serve as a study case to understand the impact of genetic admixture on the frequency of genetic variants, such as CCR5Δ32, and its impacts on different conditions and pharmacogenomics (Suarez-Kurtz et al., 2014). Understanding the extent to which the CCR5Δ32 variant influences the health of different populations is critical since it indicates which individuals and ethnic groups are more likely to benefit from therapies focused on modulating CCR5 in the context of cancer, infections, and inflammatory diseases. Focusing on HIV, knowing the frequency of CCR5Δ32 in different human populations is the initial step to guide potential new attempts at sustained remission of HIV infection through stem cell transplantation with CCR5Δ32 homozygous genotype. Moreover, it is also essential to understand how CCR5Δ32 impacts the health of the Brazilian population.

Considering that (I) the frequency of CCR5Δ32 is quite varied among Brazilians from different country's regions and that (II) the role of CCR5Δ32 in various pathological conditions is an emerging topic with several knowledge gaps, the primary objective of this article is to review the effects of the genetic variant CCR5Δ32 on the Brazilian population,

considering several diseases and clinical conditions. The secondary objective of this article is to discuss the impacts of a European-derived variant, the CCR5Δ32, on a highly mixed population.

2. Methods

For the initial selection of articles, the terms “CCR5”, “CCR5 delta 32”, “CCR5Δ32” and “rs333”, used in combination with “Brazil” or “Brazilian”, were searched on PubMed (https://pubmed.ncbi.nlm.nih.gov/). Subsequently, the same search strategy was used on Scientific Electronic Library Online - SciELO (https://scielo.org/). The articles were initially selected based on the title and abstract. Only articles addressing CCR5Δ32 in Brazilian populations were included in this review. Articles published in English and Portuguese were considered in the evaluation, without restriction concerning the date of publication. On some specific occasions, the reference list of selected articles was also used as an additional source of published works involving CCR5Δ32 in the Brazilian population. Additional unstructured searches were performed on PubMed to select the articles cited in the introduction section and additional points of the review.

3. CCR5Δ32 frequency in Brazil

A study published in 2016 by Silva-Carvalho and collaborators presented a very complete meta-analysis regarding the CCR5Δ32 frequency in Brazil. In addition to original data from those authors, the meta-analysis included 29 articles reporting the CCR5Δ32 frequency in Brazil, encompassing populations from ten Brazilian States. The study found an overall allelic frequency of 4% in the country (Silva-Carvalho et al., 2016). The frequencies of the CCR5Δ32 allele in the Brazilian States, including data compiled by Silva-Carvalho et al. (2016), are summarized in Figure 2. Henceforward, we expand the information concerning the CCR5Δ32 frequency in Brazil, highlighting studies not included in the meta-analysis by Silva-Carvalho et al. (2016), and including data obtained from studies with indigenous populations and quilombola communities, as discussed below.

Leboute et al. (1999) reported the absence of the CCR5Δ32 allele in a sample of 300 Amerindians from four indigenous populations of the Brazilian Amazon region, namely: Tikuna (*n*=191), Baniwa (*n*=46), Kashinawa (*n*=29), and Kanamari (*n*=34). Based on such data, we can argue that, at least until the date of publication of that work (Leboute et al., 1999), the studied Amazonian tribes probably did not have a significant degree of miscegenation at a level sufficient for the introduction of the CCR5Δ32 allele into those indigenous groups. Alternatively, the allele could already be circulating in the groups, but it may not have been detected due to the small sample size.

Carvalhaes et al. (2004) also described the frequency of the CCR5Δ32 allele in different ethnic groups of the Brazilian Amazon region, specifically from Pará State. The sample groups investigated were composed of 394 individuals from Belém (capital of Pará), 67 Afro-Brazilian individuals, 89 Amerindian individuals, and 111 Japanese immigrants. The CCR5Δ32 allele was not observed in Amerindian individuals and Japanese immigrants. In the sample of Afro-Brazilian individuals, only one individual carrying the allele in heterozygous was found, with the allele frequency, in this case, being 0.75%. In the sample of random individuals from Belém, one homozygous individual for the gene deletion and 22 heterozygous individuals were found, resulting in a CCR5Δ32 allele frequency of 3.04% (Carvalhaes et al., 2004).

Hünemeier et al. (2005) evaluated the frequency of the CCR5Δ32 allele in Native American populations in Brazil and Paraguay: five Amazonian groups (Tiriyo, Mura, Cinta Larga, Gavião, and Zoró); a group from the Paraguayan Gran Chaco (Lengua); one from the Paraguayan forest (Aché); and one from southern Brazil (Kaingang). The CCR5Δ32 allele was found only in two groups: Mura (2%) and Kaingang (3%). The presence of the CCR5Δ32 allele in the samples of these two groups may be due to gene flow, which is explained by previous data showing that both populations have a degree of miscegenation. Thus, the CCR5Δ32 allele may have been introduced in American-native populations due to European miscegenation (Hünemeier et al., 2005).

Vargas et al. (2006) investigated the distribution of the CCR5Δ32 allele in individuals from Alegrete, a city in the western region of Rio Grande do Sul State. The population of Alegrete is highly admixed, with the genetic participation of Spanish, Portuguese, African, and Amerindian peoples. In the study, 103 healthy and unrelated individuals were analyzed, being divided into ‘white’ (*n*=59), ‘brown’ (*n*=31), and ‘black’

(*n*=13). No CCR5Δ32 homozygous individuals were found, and the frequency of heterozygotes was 14% in whites, 13% in browns, and 8% in blacks. Allele frequencies were 6.8%, 6.4%, and 3.8%, respectively (Vargas et al., 2006).

Ferreira-Fernandes et al. (2015) analyzed the CCR5Δ32 frequency in a sample of the population of the Piauí State. The sample consisted of 223 elderly individuals from the Network of Research on Frailty in Elderly Brazilians. The CCR5Δ32 allele was found only in heterozygous in the sample, with an allele frequency of 1.8%. In order to have a more robust investigation, the sample was also stratified according to sex and age (dividing the groups into individuals below or above 73 years old), but the frequencies were not statistically different between groups, ranging from 1.5% to 2.3%. The general CCR5Δ32 frequency observed is in accordance with other data presented by groups also from northeastern Brazil (Ferreira-Fernandes et al., 2015).

Carvalho et al. (2004) evaluated the CCR5Δ32 frequency in three quilombola communities in the states of Sergipe (Mocambo community) and Bahia (Rio das Rãs and São Gonçalo communities). The groups were founded about 150 years ago by individuals from Sub-Saharan Africa and/or their descendants. The study evaluated individuals born in quilombola communities and recent immigrants, with a total of 100 inhabitants from Rio das Rãs, 71 from Mocambo, and 53 from São Gonçalo. In these communities, 28 were recent immigrants from Rio das Rãs, 18 from Mocambo, and 15 from São Gonçalo. Thus, the total sample size was 224 individuals: 163 born in the quilombos and 61 recent immigrants. In most cases, the oldest person in each family was chosen to participate in the study. The CCR5Δ32 allele was found in the three communities evaluated, but only in heterozygosis, with allele frequencies of 5.6 % in Mocambo, 1% in Rio das Rãs, and 0.9% in São Gonçalo. According to the authors, the differences in allele frequencies can be due to several factors, including different proportions of parental populations in the founder's individuals, a founder-effect, and different patterns of inter-ethnic contact (Carvalho et al., 2004).

Finally, we summarized in Figure 2 the frequencies of CCR5Δ32 allele in thirteen Brazilian States, according to data of ten states compiled by Silva-Carvalho et al. (2016), and the frequencies observed by Hüneimeier et al. (2005) in the Mura population (Amazonas State), by Carvalho et al. (2004) in individuals from Mocambo community (Sergipe State), and Ferreira-Fernandes et al. (2015) in individuals from Piauí. To the best

of our knowledge, there are no data available in the literature on CCR5Δ32 in the other Brazilian States.

4. CCR5Δ32 in infectious diseases

CCR5 plays a critical role in the regulation of the immune response against infectious agents, controlling the traffic of immune cells [e.g., Natural Killer (NK) and T-regulatory (Treg) cells] towards inflammation sites. For instance, a recent study with mice showed that CCR5 has a pivotal role in the recruitment of NK cells to the kidney allowing an adequate neutrophil activity during systemic *Candida albicans* infection, acting as a fundamental molecule for a proper immune response. Actually, the absence of CCR5 expression resulted in uncontrolled inflammation and increased renal damage in face of *C. albicans* infection (Nguyen et al., 2020). Also, Treg cells play a fundamental role in resolving inflammatory conditions, providing an immunosuppressive activity. During infection by different pathogens (e.g., *Schistosoma* spp.), the poor recruitment of Treg cells to the inflammation sites due to CCR5 absence causes uncontrolled inflammation and related tissue damage (Souza et al., 2011; Ellwanger et al., 2020a). On the other hand, during Rocio virus infection, the CCR5 absence was associated with reduced brain inflammation and better prognosis in animals (Cháves et al., 2012). Taking together, imbalances in the CCR5-mediated immune responses due to CCR5Δ32 can cause both reduced or exacerbated inflammation, depending on the type of pathogen responsible for the infection (e.g., fungus, bacteria, virus), the infection site, or the immune cell type affected by the lack or reduction of CCR5 expression (Ellwanger et al., 2020a). In this context, studies addressing CCR5Δ32 and viruses in the Brazilian population will be discussed here, including HIV, Human T-lymphotropic virus (HTLV), Dengue, Influenza A, Hepatitis C virus (HCV), Hepatitis B virus (HBV), and Human papillomavirus (HPV).

As explained in the introduction section, CCR5Δ32 exerts its protective effect against HIV infection through two mechanisms: reduced expression of the CCR5 gene (gene-dosage effect; probably the most important mechanism) (Wu et al., 1997; Venkatesan et al., 2002) and sequestration of CCR5 and CXCR4 from the cell surface (Agrawal et al., 2004; Agrawal et al., 2007). Many studies that evaluated CCR5Δ32 in the Brazilian population corroborated the protective effect of the variant on susceptibility or

clinical aspects of HIV infection (e.g., Accetturi et al., 2000; Reiche et al., 2008; Rigato et al., 2008; Valverde-Villegas et al., 2017), although other studies have not evidenced these effects, in some cases probably due to the small sample size (e.g., Carvalhaes et al. 2005; Angelis et al., 2007). The main results of the studies involving CCR5Δ32 and HIV infection in Brazil are detailed in Table 1.

Experimental evidence indicated that the course of HTLV (type 1 and 2) infection and HIV/HTLV co-infection may be affected by CCR5 expression patterns, which can be modulated by such viruses (Barrios et al., 2011; Oo et al., 2015). The CCR5 and its ligands can also influence the course of Dengue infection (Sierra et al., 2014; Marques et al., 2015). CCR5Δ32 was associated with an increased risk of fatal Influenza virus infection in Spanish individuals (Falcon et al., 2015). However, CCR5Δ32 has a limited impact on these infections in the Brazilian population. Studying HTLV-1 infection, Pereira et al. (2000) no statistically significant association was found between CCR5Δ32 and susceptibility or presence/absence of a symptomatic infection. Also, no statistically significant association was observed when the frequencies of CCR5Δ32 were compared between severe Dengue cases and controls (Xavier-Carvalho et al., 2013). The CCR5Δ32 was not associated with hospitalization in individuals infected by Influenza A virus (2009 pandemic H1N1 strain) (Mastri et al., 2015). Subsequently, a study addressing the same virus also reported no significant effect of CCR5Δ32 on H1N1 infection severity (Matos et al., 2019).

HCV and HBV are associated with the development of hepatocarcinoma and other liver diseases (Perz et al., 2006). Similarly, HPV is strongly associated with the development of cervical cancer (Oyervides-Muñoz et al., 2018). CCR5 could affect both susceptibility to these viruses and associated diseases due to its regulatory role in inflammatory reactions. Our group evaluated the influence of CCR5Δ32 on susceptibility to HCV infection and HCV/HIV co-infection. In the same study, we also accessed the potential impact of the CCR5Δ32 on HCV-related fibrosis, cirrhosis, and hepatocarcinoma. In total, 1352 individuals were included in the study. No statistically significant associations of CCR5Δ32 with the evaluated criteria were observed (Ellwanger et al., 2018a). Similarly, Mangieri et al. (2019) observed no significant effect of CCR5Δ32 on susceptibility to HPV infection or cervical lesions. Also, the CCR5Δ32 was not associated with infection by a particular HPV genotype (Santos et al., 2016). More recently, we

evaluated the influence of CCR5Δ32 on susceptibility to HBV infection and HBV/HIV co-infection in a study involving 1113 individuals. We found no significant effect of CCR5Δ32 on susceptibility to HBV mono-infection. On the other hand, the CCR5Δ32 allele exerted a protective influence on HBV/HIV co-infection. Of note, this result was potentially due to the known protective effect of CCR5Δ32 on HIV infection (Ellwanger et al., 2020d).

The influence of CCR5Δ32 on parasitic diseases was also investigated in the Brazilian population, including Chagas disease, leishmaniasis, and toxoplasmosis. CCR5 can have two opposite effects on Chagas disease, a disease caused by the *Trypanosoma cruzi* infection. CCR5 mediates the control of acute infection, assuming a favorable role for the host. In opposition, the increased expression of CCR5 during Chagas disease is associated with exacerbated inflammation and related cardiac complications (Oliveira et al., 2016). Thus, the levels of CCR5 expression are critical in the outcome of Chagas disease. However, two other studies found no association between the CCR5Δ32 variant and cardiac or digestive manifestations on chronic Chagas disease (Oliveira et al., 2014a; Oliveira et al., 2015).

Oliveira et al. (2007) and Ribas et al. (2013) reported no statistically significant difference between *Leishmania*-infected individuals and controls concerning CCR5Δ32 frequencies. In the study performed by Oliveira et al. (2007), the CCR5Δ32 allele carriers showed a less severe spectrum of clinical manifestations, but without statistical significance. Ribas et al. (2013) observed a higher frequency of the CCR5Δ32 polymorphism among a subgroup of patients with recurrent lesion, but this specific result was based on a very small cohort.

The CCR5Δ32 wild-type genotype in association with AA or AG genotypes (from *CCR5* rs1799987 polymorphism) was associated with increased risk of ocular toxoplasmosis, potentially due to the persistent CCR5-mediated inflammation in individuals with normal CCR5 expression (Faria Junior et al., 2018). Also evaluating Brazilians, Vallochi et al. (2008) found no association between the CCR5Δ32 and ocular toxoplasmosis (based on a brief description; detailed data not described by such authors).

Based on the studies discussed above, with the exception of the protective effect of CCR5Δ32 on HIV infection, the impacts of CCR5Δ32 on viral and parasitic infections in Brazilian populations seem quite limited (details of each study detailed in Table 1 and

Table 2). However, considering the recognized role of CCR5 in the regulation of inflammation, it is possible that potential influences of CCR5Δ32 on non-HIV infections have not been detected due to the small number of studies carried out in Brazil on these topics, many of them involving a small sample size.

Finally, the impact of the CCR5Δ32 on fungal infections is unknown in Brazilian populations, and therefore research in this field is needed. Of note, Brazil is affected by several endemic mycoses, such as Dermatophytosis, Paracoccidioidomycosis, Histoplasmosis, and Cryptococcosis, among others (Costa et al., 2018). Understanding whether and how the CCR5Δ32 influences the susceptibility or clinical progression of these diseases can provide insights into the potential use of CCR5-based therapies for these diseases.

5. CCR5Δ32 in inflammatory conditions

Considering the critical role of CCR5 in the regulation of the inflammatory response, several authors have been investigating the effect of CCR5Δ32 on conditions that have their susceptibility or clinical course affected by different types (e.g., systemic, local) and intensity of inflammation. In this topic, we review the role of CCR5Δ32 on the following inflammatory diseases or inflammation-related clinical conditions: multiple sclerosis, systemic lupus erythematosus, preeclampsia, rheumatoid arthritis, juvenile idiopathic arthritis, periodontitis, osteomyelitis, transplant rejection, and sickle cell disease. Details of each study are described in Table 3 and discussed below.

Multiple sclerosis is an autoimmune, chronic, and inflammatory disease showing heterogeneity in clinical findings. Chemokines and chemokine receptors are molecules involved in the pathogenesis of multiple sclerosis (Kaimen-Maciel et al., 2007; Allanore et al., 2015), and the CCR5Δ32 can influence different aspects of this disease, as shown in studies with non-Brazilian individuals (Sellebjerg et al., 2000; Otaegui et al. 2007; van Veen et al., 2007). In Brazil, only two papers explored the possible impact of the CCR5Δ32 on multiple sclerosis. Based on magnetic resonance imaging, Kaimen-Maciel et al. (2007) observed a decreased disease progression in patients bearing the CCR5Δ32 allele. Subsequently, Troncoso et al. (2018) described a statistically significant higher CCR5Δ32 allele frequency in Euro-Brazilian controls (7.4%) compared to Euro-Brazilian

patients (3.3%), suggesting a protective role of the variant on the development of multiple sclerosis. Besides, the frequency of the CCR5Δ32 was higher in Euro-Brazilian patients with progressive multiple sclerosis than Euro-Brazilian patients with relapse remitting multiple sclerosis (Troncoso et al., 2018). Both studies carried out in Brazil show that the CCR5Δ32 variant can influence both the susceptibility and the clinical outcome of multiple sclerosis.

Systemic lupus erythematosus is a chronic inflammatory autoimmune disease characterized by the large production of autoantibodies, triggering generalized tissue damage. This disease has different clinical manifestations and a complex genetic influence, and chemokines and their receptors, such as CCR5, are implicated in the pathogenesis of lupus (Carvalho et al., 2013; Schauren et al., 2013; Cheng et al., 2014; Baltus et al., 2015). The CCR5Δ32 variant has already been studied in this context, being previously associated to protection against lupus development and, albeit in a contradictory manner, this polymorphism was also associated to susceptibility to nephritis in lupus patients (Carvalho et al., 2013; Cheng et al., 2014). In Brazil, two papers evaluated the CCR5Δ32 variant in lupus.

Schauren et al. (2013) investigated the role of the CCR5Δ32 in healthy patients and controls of Rio Grande do Sul State. A lower frequency of the CCR5Δ32 allele was found in Euro-Brazilian patients (2.7%) compared to Euro-Brazilian controls (7.5 %), suggesting a protective role of the variant against the development of systemic lupus erythematosus. However, in the same study, patients with the CCR5Δ32 allele had a greater predisposition to the development of class IV nephritis than patients without the allele, which suggests a more severe clinical outcome associated with the genetic variant (Schauren et al., 2013).

Baltus et al. (2015) evaluated the frequencies of the CCR5Δ32 in patients and controls in the Paraná State, also southern Brazil. Unlike the first study, the frequency of the CCR5Δ32 allele was statistically higher in patients (6.8%) than in controls (1.9%), suggesting the variant as a risk factor for systemic lupus erythematosus. Also, by stratifying the sample according to ethnicity, the researchers identified that Euro-Brazilian individuals carrying the CCR5Δ32 were more likely to develop systemic lupus erythematosus than Afro-Brazilian patients carrying the variant. In another analysis of the study, CCR5Δ32 carriers had a lower age of systemic lupus erythematosus onset and higher levels of anti-dsDNA antibodies. Thus, the CCR5Δ32 allele was associated with

increased susceptibility to the development of systemic lupus erythematosus and severity in clinical outcomes (Baltus et al., 2015).

Preeclampsia is a hypertensive gestational complication and an important cause of maternal-fetal mortality in Brazil. Relevant clinical findings of the disease, such as edema and proteinuria after the 20th week of pregnancy, are intricate with an excessive inflammatory process and endothelial dysfunction. In preeclampsia, increased systemic production of pro-inflammatory chemokines was observed, highlighting the role of the chemokine-ligand system in this condition (Telini et al., 2014; Michita et al., 2018; Kaminski et al., 2019). Two studies evaluating the CCR5Δ32 variant in preeclampsia were carried out in Brazil, both published by our group, but evaluating samples from different Brazilian regions. Firstly, Telini et al. (2014) evaluated the frequency of the CCR5Δ32 in Brazilian women who developed preeclampsia and women who did not develop this condition during their pregnancies. The group of healthy women had a higher frequency of the CCR5Δ32 allele (14%) when compared to the group of women who developed preeclampsia (7%). The analysis revealed a protective role of the variant on preeclampsia development (Telini et al., 2014). More recently, Kaminski et al. (2019) also investigated the role of CCR5Δ32 in women who developed preeclampsia and in women with healthy pregnancies. In accordance with the results of Telini et al. (2014), healthy pregnant women also showed an increased CCR5Δ32 allele frequency (4.5%) compared to the group of pregnant women with preeclampsia (1.6%). Thus, the study corroborated the protective role of the CCR5Δ32 variant on preeclampsia development, endorsing the hypothesis that a reduced inflammatory millieu may contribute to a lower risk of developing preeclampsia (Kaminski et al., 2019).

Rheumatoid arthritis is a systemic autoimmune disease characterized by progressive damage to the joints caused by chronic inflammation in the synovial fluid. Given the intense migration of immune cells to the inflammation sites, the role of CCR5 in rheumatoid arthritis appears to be of great importance (Kohem et al., 2007; Toson et al., 2017). In Brazil, two studies investigating the role of the CCR5Δ32 variant in rheumatoid arthritis were published. Kohem et al. (2007) evaluated the frequency of the allele in healthy patients and controls from the Rio Grande do Sul State, and no statistically significant difference was found between the groups. Of note, the sample group was relatively small, with 92 patients and 160 healthy controls (Kohem et al., 2007). Toson et

al. (2017) performed a similar study but evaluating the frequency of the CCR5Δ32 variant in different Brazilian populations, considering four different regions (south, southeast, northeast, and north). Two of the four sample groups, from southern and northern regions, showed a statistically significant difference between rheumatoid arthritis patients and healthy controls (4% *vs.* 7.5%; 1.1% *vs.* 3.4%, respectively), being precisely the groups with the largest sample sizes. The difference concerning the northeast region sample was not statistically significant but followed a similar trend to the groups in southern and northern. Only the southeastern sample deviated from the trend, with the small sample size possibly being the reason for the lack of statistical association. In sum, the study suggests a protective role for the CCR5Δ32 variant against the development of rheumatoid arthritis (Toson et al., 2017).

Juvenile idiopathic arthritis is a chronic inflammatory condition characterized in the synovial joints of young people up to 16 years of age (Scheibel et al., 2008; Veit et al., 2011). Scheibel et al. (2008) investigated the potential association of the CCR5Δ32 variant with juvenile idiopathic arthritis subtypes in a sample from Porto Alegre, southern Brazil. A statistically significant difference was found between patients (9.4%) and healthy controls (3.8%), especially considering the group of patients of the systemic juvenile idiopathic arthritis subtype (25%). The researchers conclude that the CCR5Δ32 variant, although not a risk factor for the development of juvenile idiopathic arthritis, contributes to the progression and clinical status of patients (Scheibel et al., 2008).

Periodontitis is an oral disease characterized by a chronic infection accompanied by inflammatory processes, causing irreversible and progressive destruction of dental support structures. The CCR5-mediated immune responses affect multiple aspects of periodontitis. For instance, not only CCR5 and its ligands are important in the context of disease protection, but also influence periodontal destruction and bone resorption (Gamonal et al., 2001; Garlet et al., 2003; Ferreira et al., 2011; Cavalla et al., 2017; Rossi et al., 2019). Cavalla et al. (2017) investigated the CCR5Δ32 variant and its possible influence on periodontitis development. The CCR5Δ32 allele was significantly more frequent in individuals classified in the group of chronic gingivitis (11.1%) than in individuals with chronic periodontal disease (5.8%) or aggressive periodontal disease (5.5 %). This result suggests a protective role of the variant concerning periodontitis (Cavalla et al., 2017).

Osteomyelitis is an infectious-inflammatory condition that can occur after bone trauma often following *Staphylococcus aureus* infection (Olson and Horswill, 2013; Souza et al., 2015). Souza et al. (2015) evaluated the CCR5Δ32 frequency in patients who were admitted to a hospital in Fortaleza, northeastern Brazil, with bone trauma. The patients were prospectively studied to assess a possible development of osteomyelitis. There was no statistically significant difference between individuals who developed and those who did not develop the disease, but all patients with closed fractures (type I or type II) and who carried the CCR5Δ32 variant did not developed the condition. The researchers conclude that the lack of statistical significance observed in their study was probably due to the low sample size (Souza et al., 2015).

The immune response and inflammatory processes that occur after an organ transplant are critical in the process of tissue rejection. Genetic variants related to the immune system can therefore influence the response to transplantation (Akalin and Murphy, 2001; Fischereder et al., 2001; Moench et al., 2004; Cilião et al., 2017). In Brazil, Cilião et al. (2017) evaluated the CCR5Δ32 frequency in transplanted individuals who had episodes of rejection comparing to individuals who did not have such episodes. A sample of 246 patients was collected in a referral hospital in Londrina, Paraná State. However, the frequency of the CCR5Δ32 variant did not vary significantly between the groups (Cilião et al., 2017).

Sickle cell disease is an inherited disorder caused by a single nucleotide substitution in the beta-globin gene. This mutation originated in Africa and is, therefore, more common in African populations and Afro-descendants. Sickle cell disease can be understood as a chronic inflammatory condition, which may be the cause of associated secondary complications. In this sense, high levels of inflammation in sickle cell disease patients are related to disease morbidity (Chies and Hutz, 2003; Vargas et al., 2005; Lopes et al., 2014; Nascimento et al., 2016). In Brazil, four studies investigated the influence of the CCR5Δ32 variant in sickle cell disease, all detailed below.

Chies and Hutz (2003) accessed the potential role of the CCR5Δ32 in severe and recurrent infections that could contribute to differentiated survival of sickle cell anemia patients. The study involved individuals from different ethnic groups and the frequencies of the CCR5Δ32 allele found were 4.4% in Euro-Brazilian controls, 1.3% in Afro-Brazilian controls, and 5.1% in sickle cell anemia patients. When comparing these

frequencies between the different groups, no statistically significant difference was found. However, it is important to note that, considering the same ethnic background of the groups of patients and Afro-Brazilian controls, a difference in the allele frequency was evidenced, being the CCR5Δ32 allele three times more present in the group of sickle cell anemia patients. Given the low frequency of the allele in the sample of Afro-Brazilian controls, a 3-fold increase in the group of patients is quite important. The researchers suggested that the CCR5Δ32 allele was more frequent in the group of patients for conferring some advantages concerning the clinical course of the disease (Chies and Hutz, 2003). As mentioned previously, sickle cell anemia can be considered a chronic inflammatory disease (Chies and Nardi, 2001), and patients with the CCR5Δ32 allele would benefit from developing inflammatory responses at low levels. According to this hypothesis, the CCR5Δ32 allele was associated with an improvement in the general health status of the patients (Chies and Nardi, 2001; Chies and Hutz, 2003).

Subsequently, Vargas et al. (2005) evaluated CCR5Δ32 in sickle cell anemia patients from Porto Alegre, Rio Grande do Sul State. No statistically significant difference was observed in the study but, interestingly, the CCR5Δ32 allele was present only in the group of patients with a severe clinical course (when the pain rate was considered). Such data may indicate a trend towards the development of a severe clinical course associated with the CCR5Δ32 allele in sickle cell anemia patients (Vargas et al., 2005). Lopes et al. (2014) compared the CCR5Δ32 frequencies of two groups of patients (pediatric and adult) and between sick adults and healthy controls from Pernambuco, northeastern Brazil. There were no statistically significant differences in any of the comparisons made in the study (Lopes et al., 2014). Finally, Nascimento et al. (2016) evaluated the CCR5Δ32 frequency in sickle cell anemia patients from Bahia State. However, the CCR5Δ32 allele was not found in the study (Nascimento et al., 2016).

6. CCR5Δ32 in cancer

Chemokines and chemokine receptors have fundamental participation in both antitumor response and pathogenesis of cancer. The migration of regulatory immune cells to tumor sites can create an immunosuppressor environment proper for cancer development. Also, cancer cells can subvert the anti-tumor action of chemokine-ligand

interactions (Aoki et al., 2009; Oliveira et al., 2014b; Banin-Hirata et al., 2015; Derossi et al., 2019; Vieira-Filho et al., 2020). Of note, CD4+ T cells are important modulators of the immune response, acting as drivers for the action of effector cells. Some CD4+ regulatory T cells express the CCR5 molecule, being this a key receptor of the cellular response against tumor development. The presence of the CCR5Δ32 variant can impair the action of CCR5+/CD4+ T cells, influencing the risk of cancer development. In brief, chemokine receptors can assume multiple roles in different tumoral processes, and more investigation is needed to unravel the connections between CCR5 and cancer (Magnani et al., 2012; Kulmann-Leal et al., 2020).

The action of CD8+ cytotoxic T cells is very important in the antitumor immune response. The use of immunomodulators in antitumor treatment is increasingly common, with carboxymethyl-glucan (CM-G) being one of the best-described immunostimulators (Magnani et al., 2012; Farhood et al., 2018). Magnani et al. (2012) evaluated the CD3+, CD4+ and CD8+ cell populations of patients with advanced prostate cancer and compared this data with the CCR5 genotype, associating it with the administration of oral CM-G for 28 days. The CCR5Δ32 variant was found only in a heterozygous genotype, in six patients, at an allelic frequency of 10%. Five patients reported a family history of prostate cancer, two of whom had affected first-degree relatives. Both patients carried the CCR5Δ32 allele. In general, CCR5Δ32 non-carriers had higher counts on CD3+ and CD4+ cells when comparing respectively after and before treatment with CM-G, as well as higher counts of CD8+ cells when comparing to CCR5Δ32 carriers only after treatment with CM-G. In addition, the average CD4+/CD8+ cell ratio showed a worsened antitumor response after treatment in CCR5Δ32 allele carriers (Magnani et al., 2012). Zambra et al. (2013) also evaluated the CCR5Δ32 frequency in Brazilian prostate cancer patients, comparing to individuals affected by benign prostatic hyperplasia and healthy subjects. No association was found considering the variant and risk to both conditions, nor with clinical outcomes (Zambra et al., 2013).

Aoki et al. (2009) assessed the CCR5Δ32 frequency in individuals with breast cancer and healthy women. However, no significant difference was observed between groups. The impact of p53 genotypes, a known tumor suppressor gene, together with the CCR5Δ32 genotypes, was also evaluated revealing a higher frequency of individuals with the p53 Arg homozygous genotype and the CCR5Δ32 wild-type genotype amongst

controls as compared to patients (Aoki et al., 2009). Banin-Hirata et al. (2015) also evaluated whether the CCR5Δ32 variant was associated with susceptibility, response to treatment, and clinical course of breast cancer. No association was found between CCR5Δ32 and the features analyzed (Banin-Hirata et al., 2015). In accordance, Derossi et al. (2019) did not found an association between the CCR5Δ32 and CCL5 levels in breast cancer.

HPV infection is the main cause of cervical cancer. However, factors other than HPV infection, including genetic, immune, and environmental factors, also affect tumorigenesis (Santos et al., 2016; Yang et al., 2017; Ellwanger et al., 2018b). In this context, Santos et al. (2016) evaluated the CCR5Δ32 frequency in HPV+ women with and without cervical neoplastic lesions. No association was found between the variant and the presence of cancer or lesions severity (Santos et al., 2016).

In addition to the multiple roles of CCR5 in tumorigenesis and antitumor response, this molecule is also an important modulator of neuroinflammation (Ubogu et al., 2006; Sorce et al., 2011; Martin-Blondel et al., 2016), potentially affecting the development of brain-related diseases. In this sense, Vieira-Filho et al. (2020) found an association between the presence of the CCR5Δ32 allele and susceptibility to neuroblastoma. Lastly, Oliveira et al. (2014b) investigated the role of the CCR5Δ32 variant in acute lymphoblastic leukemia, but no association was found between the variant and the disease development (Oliveira et al., 2014b). In conclusion, the CCR5 has varied influences in different types of cancer.

7. Impacts of CCR5Δ32 on a highly admixed population – A critical look

At a population level, the effects of CCR5Δ32 on European populations may be different than those potentially observed in highly admixed populations. However, the population-specific effects of CCR5Δ32 are not only due to its frequency, but also due to its interaction with different alleles. There are nine widely known *CCR5* haplotypes, which are formed by combinations of eight *CCR5* polymorphisms (including CCR5Δ32) and one polymorphism located in the *CCR2* gene (Mehlotra, 2019; Ellwanger et al., 2020a). The impact of the *CCR5* haplotypes on HIV disease progression differs between African Americans and Caucasians since the effects of the CCR5Δ32 can be modulated by other

alleles heterogeneously distributed among the populations (Gonzales et al., 1999). In a broader perspective, this information indicates that the effect of the CCR5Δ32 observed in Europeans (or other non-Brazilian populations) may be modified by further genetic traits circulating in Brazilians, which may also vary in different regions of the country. In fact, the detection of the real effect of CCR5Δ32 on different health and disease conditions in the Brazilian population is not a simple task. Of note, gene-disease association studies performed with admixed populations can be difficult due to differential linkage disequilibrium patterns (Liu et al., 2013).

Pharmacogenomic approaches, including the use of CCR5 modulators based on the CCR5Δ32 genotyping, must be considered at an individual level, especially in highly admixed populations, where the frequency of polymorphisms may be very different from those observed in populations with greater genetic homogeneity (Suarez-Kurtz, 2005). The CCR5Δ32 genotyping could be taken into account in pharmacological treatments involving CCR5 blockade in the context of inflammatory diseases or particular types of cancer. The use of CCR5 modulators in individuals with the CCR5Δ32 genotype probably has a limited effect due to the natural absence of CCR5 expression on the cell surface. Although the number of individuals with this genotype is very low in an admixed population such as the Brazilian population, the cost-benefit of this strategy must be considered on a case-by-case basis. Despite the limitations, the area of pharmacogenomics involving CCR5Δ32 genotyping is expected to progress in the next years, especially considering the increasing use of CCR5 modulators to treat other diseases not associated with HIV infection. Some important advances have already been made. For instance, the CCR5Δ32 genotyping can help clinicians to predict the progression of human enteroviral cardiomyopathy, also helping the decision making concerning the early use of antiviral interferon-β therapy in such condition (Lassner et al., 2018).

8. Conclusions

The CCR5Δ32 allele frequency is quite variable in Brazil, being extremely low in some regions (e.g., 0.6% in Rondônia), but high in others (e.g., up to 9.3% in Paraná and 7.4% in Rio Grande do Sul). In Native American populations, the allele is absent or occurs

at low frequencies. In Brazil, CCR5Δ32 is not uncommon in non-Caucasian populations, as a result of the miscegenation that has occurred in the country.

Many studies corroborated the protective effect of the CCR5Δ32 on susceptibility or clinical aspects of HIV infection in the Brazilian population. On the other hand, there is no evidence pointing to a relevant role for CCR5Δ32 on Cutaneous leishmaniasis, Chagas disease, HTLV-1, Dengue virus, Influenza A, HPV, HBV and HCV infections, or HCV-HIV co-infection in Brazilians. Limited evidence indicates a potential involvement of CCR5Δ32 wild-type genotype in ocular toxoplasmosis and a protective effect of the variant on HBV/HIV co-infection.

Considering inflammatory conditions, the CCR5Δ32 can influence both the susceptibility and the clinical outcome of multiple sclerosis. Of note, CCR5Δ32 reduces the risk of preeclampsia and periodontitis development, potentially due to the CCR5Δ32-associated reduced inflammation. Moreover, CCR5Δ32 can reduce the risk of rheumatoid arthritis, but contributes to the progression and clinical status of juvenile idiopathic arthritis patients. CCR5Δ32 can also influence sickle cell anemia-related immune conditions. However, the impact of CCR5Δ32 on systemic lupus erythematosus is controversial. Concerning tumoral development, the CCR5Δ32 has varying influences on the development of different types of cancer, including prostate cancer and breast cancer. It is not possible to generalize the impact of the variant on cancer development, especially in the Brazilian population.

Understanding the real impact of the CCR5Δ32 variant in different conditions is essential to indicate in which diseases the use of CCR5 modulators may be relevant. This knowledge is fundamental for the advancement of CCR5-based therapies, especially in populations with a complex genetic structure. Finally, CCR5Δ32 influences should be assessed within the context of each particular population, since genetic admixture and interactions with other alleles may alter the expected phenotypic effects attributed to CCR5Δ32.

Conflicts of interest

The authors declare no conflicts of interest.

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Author contributions

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Table 1. Impacts of CCR5Δ32 on HIV infection.

Population	Sample	Main findings	Reference
Brazilian HIV+ individuals	177 ARV-naive individuals	Heterozygous individuals for CCR5Δ32 have a better response to ARV treatment than wild-type homozygotes	Accetturi et al. (2000)
Brazilian individuals from different regions	1162 individuals (133 with HIV+ status)	CCR5Δ32 heterozygous cells (PBMCs) showed partial resistance to R5-HIV-1 *in vitro*; No significant differences in CD4+ T-cell counts between HIV+ individuals heterozygous and wild-type homozygous for CCR5Δ32; HIV load in heterozygous individuals is significantly lower than in wild-type individuals	Grimaldi et al. (2002)
Individuals from São Paulo State, Brazil	129 HIV+ individuals and 26 blood donors	CCR5Δ32 heterozygous genotype was associated with reduces RANTES/CCL5 levels	Mikawa et al. (2002)
Individuals from São Paulo State, Brazil	183 HIV+ individuals and 115 controls	The frequency of the CCR5Δ32 heterozygous genotype was lower in HIV+ individuals (11.5%) than in controls (13.0%)	Munerato et al. (2003)
Individuals from Pará, Brazil	110 HIV+ and 139 uninfected individuals	Similar frequencies of the CCR5Δ32 allele were observed in the two groups: 2.7% in HIV+ individuals and 2.2% in the controls	Carvalhaes et al. (2005)
Children from Pernambuco State, Brazil	106 HIV+ and 70 uninfected children exposed to infection risk and 104 controls	No significant influence of the CCR5Δ32 in the risk of HIV vertical transmission	Souza et al. (2006)
HIV+ children from São Paulo State, Brazil	51 HIV+ children divided into rapid, moderate and slow progressors	No influence of the CCR5Δ32 in disease progression (limited sample size)	Angelis et al. (2007)
Individuals from southern Brazil	134 blood donors; 145 HIV-exposed seronegative individuals; 152 HIV+ asymptomatic individuals; 478 HIV+ individuals with AIDS	CCR5Δ32 homozygous genotype was significantly associated with reduced risk of HIV infection	Reiche et al. (2008)
Individuals from São Paulo State, Brazil	200 HIV+ (155 on pre- and post-ART) and 82 uninfected individuals	CCR5Δ32 heterozygous genotype was associated with better CD4+ T cell recovery after ART initiation	Rigato et al. (2008)
Injecting drug users from Rio de Janeiro State, Brazil	48 HIV+ and 558 uninfected injecting drug users	No significant impact of the CCR5Δ32 on susceptibility or protection to HIV infection	Teixeira et al. (2009)
Individuals from Bahia State, Brazil	506 HIV+ individuals (155 divided into rapid, typical and slow progressors)	CCR5Δ32 allele was more frequent in typical than in rapid progressors (without statistical significance)	Abe-Sandes et al. (2010)
HIV+ individuals from Rio Grande do Sul State, Brazil	249 HIV+ individuals	CCR5Δ32 heterozygous genotype was associated with reduced risk of CD4+ T cell depletion (univariate analysis) and with increased risk of death after AIDS diagnosis (multivariate analysis; potentially due to the emergence of CXCR4-tropic	Vieira et al. (2011)

		HIV strains); CCR5Δ32 was a protective factor on disease progression in survival curve analysis	
Serodiscordant couples from Santa Catarina State, Brazil	9 HIV-exposed seronegative individuals; 9 ART-treated HIV+ individuals; 12 healthy controls	The CCR5Δ32 heterozygous genotype was observed in two HIV-exposed seronegative individuals, two ART-treated HIV+ individuals, and one control; In one serodiscordant couple, both individuals had CCR5Δ32 heterozygous genotype and the CXCR4 viral tropism was observed in the infected individual	Santos et al. (2015)
Individuals from Roraima State, Brazil	117 HIV+ individuals	CCR5Δ32 heterozygous genotype was found in 11 individuals (9.4%); CCR5Δ32 allele frequency estimated at 4.6%	Corado et al. (2016)
Individuals from Pernambuco State, Brazil	213 HIV+ and 234 uninfected individuals	CCR5Δ32 frequency was reduced in HIV+ individuals compared to controls; Stratification of data according to CCR5Δ32 genotypes did not modify the results of *TRIM5* polymorphisms observed in the study	Silva et al. (2016)
Individuals from São Paulo State, Brazil	66 HIV+ individuals with recent infection	CCR5Δ32 heterozygous genotype was detected in two individuals (one infected by R5-tropic HIV strain and other by CXCR4-tropic HIV strain); No significant association between CCR5Δ32 and tropism switch	Arif et al. (2017)
Individuals from Paraná State, Brazil	35 individuals with HIV/HBV or HIV/HCV co-infection	CCR5Δ32 allele was not observed in the sample	Avanzi et al. (2017)
Individuals from Pará State, Brazil	30 HIV+ individuals (divided into viremia controllers and non-controllers)	CCR5Δ32 heterozygous genotype was detected in one non-viremia controller	Gomes et al. (2017)
Individuals from Paraná State, Brazil	81 perinatally infected HIV+ adolescents and young adults (61 genotyped for CCR5Δ32)	CCR5Δ32 heterozygous genotype was detected in one individual (1.6%); This patient was infected by an R5 HIV strain	Martin et al. (2017)
Individuals from Pernambuco State, Brazil	266 HIV+ and 223 uninfected individuals	CCR5Δ32 frequency was reduced in HIV+ individuals compared to controls (without statistical difference); CCR5Δ32 along with other polymorphisms did not show statistically significant influence on plasma viral load	Silva et al. (2017)
Individuals from Rio Grande do Sul State, Brazil	294 uninfected individuals and 206 HIV+ individuals (divided into 40 rapid progressors and 166 non-rapid progressors)	Plasma viral load was lower among CCR5Δ32 heterozygous individuals as compared to wild-type homozygous individuals	Valverde-Villegas et al. (2017)
Individuals from Pernambuco State, Brazil	248+ individuals divided into immunological recovery profiles	CCR5Δ32 heterozygous genotype was statistically associated with immunological recovery failure (result from logistic regression analysis)	Carvalho-Silva et al. (2020)

	during ART (222 of the 248 HIV+ individuals were genotyped for CCR5Δ32)		

ART: antiretroviral therapy.

Table 2. Impacts of the CCR5Δ32 on infectious diseases.

Disease/Infection	Population (Brazilian state)	Sample	Main findings	Reference
HTLV-I infection	Individuals from Minas Gerais State, Brazil	229 blood donors (50 HTLV-I seronegative individuals; 179 HTLV-I-infected individuals)	No statistically significant association was observed concerning CCR5Δ32 and HTLV-I infection	Pereira et al. (2000)
Cutaneous leishmaniasis (*Leishmania* infection)	Individuals from Paraná State, Brazil	100 individuals with cutaneous leishmaniasis and 100 healthy controls	No statistically significant difference regarding CCR5Δ32 frequency between the two groups	Oliveira et al. (2007)
Cutaneous leishmaniasis (*Leishmania* infection)	Individuals from Paraná State, Brazil	111 individuals with cutaneous leishmaniasis and 218 controls	No statistically significant difference of the CCR5Δ32 frequency was observed between cases and controls	Ribas et al. (2013)
Dengue virus infection	Individuals from Rio de Janeiro State, Brazil	87 severe children's cases of Dengue and 326 controls	No statistically significant difference regarding CCR5Δ32 frequency between the two groups	Xavier-Carvalho et al. (2013)
Chagas disease (*Trypanosoma cruzi* infection)	Individuals from São Paulo State, Brazil	85 Chagas disease patients with normal left ventricular systolic function; 43 Chagas disease patients with mild to moderate left ventricular systolic dysfunction; 40 Chagas disease patients with severe left ventricular systolic dysfunction	No statistically significant association between CCR5Δ32 and Chagas disease-related left ventricular systolic dysfunction	Oliveira et al. (2014a)
Chagas disease (*Trypanosoma cruzi* infection)	Individuals from São Paulo State, Brazil	109 patients with digestive form of Chagas disease; 131 patients with cardiac form of Chagas disease; 172 controls	No statistically significant influence of the CCR5Δ32 on digestive or cardiac form of Chagas disease, including left ventricular systolic dysfunction	Oliveira et al. (2015)
Influenza A infection (2009 pandemic H1N1)	Individuals from northern and northeastern regions of Brazil	174 non-hospitalized Influenza-infected individuals and 156 hospitalized Influenza-infected individuals	No statistically significant impact of the CCR5Δ32 on infection severity	Maestri et al. (2015)
HPV infection	Individuals from Pernambuco State, Brazil	139 HPV-infected women with cervical lesions and 151 HPV-infected women without cervical lesions	No statistically significant influence of the CCR5Δ32 on HPV-related cervical lesions or infection by specific HPV genotype	Santos et al. (2016)
HCV infection, HCV/HIV co-infection and HCV-related hepatic diseases	Individuals from Rio Grande do Sul State, Brazil	674 HCV-infected individuals (stratified between 124 individuals without hepatic manifestation, 268 individuals with fibrosis, 190 individuals with cirrhosis and 92 individuals with	No statistically significant influence of the CCR5Δ32 on susceptibility to HCV infection, HCV/HIV co-infection or HCV-related hepatic manifestations	Ellwanger et al. (2018a)

		hepatocarcinoma); 104 HCV/HIV co-infected individuals; 300 HIV-infected individuals; 274 controls		
Ocular toxoplasmosis (*Toxoplasma gondii* infection)	Individuals from São Paulo State, Brazil	160 individuals with ocular toxoplasmosis; 160 individuals with non-ocular toxoplasmosis; 160 controls	In association with AA or AG genotypes (from *CCR5* 59029 A/G SNP - rs1799987), the CCR5Δ32 wild-type genotype was associated with increased risk of ocular toxoplasmosis (based on multivariate logistic regression analysis)	Faria Junior et al. (2018)
HPV infection	Individuals from Paraná State, Brazil	164 HPV-infected women and 185 control women	No statistically significant influence of the CCR5Δ32 on susceptibility to HPV infection or cervical lesions associated with HPV infection	Mangieri et al. (2019)
Influenza A infection (2009 pandemic H1N1)	Individuals from South, Southeast and Northeast Brazilian regions (nine states in total)	153 individuals with influenza like illness; 173 individuals with severe acute respiratory infection; 106 fatal influenza-infection cases	No significant effect of the CCR5Δ32 on severity of Influenza virus infection or Influenza-linked mortality	Matos et al. (2019)
HBV infection and HBV/HIV co-infection	Individuals from Rio Grande do Sul State, Brazil	335 HBV-infected individuals; 144 HBV/HIV co-infected individuals; 300 HIV-infected individuals; 334 controls	No significant effect of the CCR5Δ32 on susceptibility to HBV mono-infection; CCR5Δ32 was a protective factor on HBV/HIV co-infection	Ellwanger et al. (2020d)

Table 3. Impacts of the CCR5Δ32 on inflammatory conditions.

Disease/Condition	Population (Brazilian State)	Sample		Main findings	Reference
		Cases	Controls		
Multiple sclerosis (MS)	Paraná State	124 MS patients	127 healthy individuals	There was no statistically significant difference regarding the CCR5Δ32 allele between patients and controls, and no association was also found regarding clinical course and CCR5 variants; A decreased disease progression was observed in patients bearing the CCR5Δ32 allele, with carrier presenting lower Expanded Disability Status Scale (EDSS) values	Kaimen-Maciel et al. (2007)
	São Paulo State and Rio Grande do Sul State	261 MS patients	435 healthy individuals	Considering only Euro-Brazilians, the CCR5Δ32 allele frequency was significantly higher in healthy individuals than in MS patients (p=0.013). Also, there was a higher frequency of Δ32 homozygous and heterozygous individuals in controls than in patients (p=0.033)	Troncoso et al. (2018)
Juvenile idiopathic arthritis (JIA)	Rio Grande do Sul State	101 JIA patients and 203 rheumatoid arthritis patients	104 healthy individuals	The frequency of the CCR5Δ32 variant was significantly higher (p=0.028) in JIA patients (0.094) than in controls (0.038)	Scheibel et al. (2008)
Osteomyelitis	Ceará State	39 bone trauma with osteomyelitis cases	114 bone trauma without osteomyelitis cases	The frequency of the CC5Δ32 variant did not vary significantly, but patients with type I or type II fractures that carried the allele did not develop the disease	Souza et al. (2015)
Periodontitis	São Paulo State	197 chronic periodontitis cases and 91 aggressive periodontitis cases	218 healthy individuals and 193 chronic gingivitis cases	The frequency of the CCR5Δ32 variant was significantly higher in patients with chronic gingivitis (0.11) than in chronic (0.058) (p=0.01) or aggressive periodontitis (0.055) (p=0.03)	Cavalla et al. (2017)
Preeclampsia	Rio Grande do Sul State and Rio de	155 preeclampsia pregnancies	144 healthy pregnancies	The frequency of the CCR5Δ32 variant was significantly higher	Telini et al. (2014)

	Janeiro State			(p=0.047) in healthy women (0.14) than in pre-eclamptic women (0.07)	
	Minas Gerais State	156 preeclampsia pregnancies	213 healthy pregnancies	The frequency of the CCR5Δ32 variant was significantly higher (p=0.047) in healthy women (0.045) than in pre-eclamptic women (0.016)	Kaminski et al. (2019)
Rheumatoid arthritis (RA)	Rio Grande do Sul State	92 RA patients	160 healthy individuals	The frequency of the CCR5Δ32 variant did not vary significantly between the groups	Kohem et al. (2007)
	Pará State	186 RA patients	206 healthy individuals	The frequency of the CCR5Δ32 variant was significantly higher in healthy individuals (0.075) than in RA patients (0.040) (p=0.016)	Toson et al. (2017)
	Rio Grande do Sul State	361 RA patients	233 healthy individuals	The frequency of the CCR5Δ32 variant was significantly higher in healthy individuals (0.034) than in RA patients (0.011) (p=0.022)	
	Pernambuco State	104 AR patients	154 healthy individuals	The frequency of the CCR5Δ32 variant did not vary significantly between groups	
	São Paulo State	89 AR patients	83 healthy individuals	The frequency of the CCR5Δ32 variant did not vary significantly between groups	
Sickle cell disease (SCD)	Rio Grande do Sul State and Pernambuco State	79 SCD patients	112 healthy afro-Brazilian individuals and 102 healthy euro-Brazilian individuals	The comparison of the CCR5Δ32 frequency between afro-Brazilian healthy individuals (0.013) and SCD patients (0.051) was of borderline significance (p=0.05)	Chies and Hutz (2003)
	Rio Grande do Sul State	73 SCD patients	58 healthy individuals	The frequency of the CCR5Δ32 variant did not vary significantly between groups	Vargas et al. (2005)
	Pernambuco State	483 pediatric SCD patients and 312 adult SCD patients	247 healthy individuals	The frequency of the CCR5Δ32 variant did not vary significantly between the groups	Lopes et al. (2014)
	Bahia State	20 SCD patients	-	The CCR5Δ32 variant was not found in any patient evaluated	Nascimento et al. (2016)

Systemic lupus erythematosus (SLE)	Rio Grande do Sul State	280 euro-Brazilian SLE patients and 87 afro-Brazilian patients	235 euro-Brazilian healthy individuals and 200 afro-Brazilian healthy individuals	The frequency of the CCR5Δ32 variant was significantly higher in healthy euro-Brazilian controls (0.075) than in euro-Brazilian SLE patients (0.027) (p=0.002); Patients carrying the CCR5Δ32 variant were predisposed to the development of class IV nephritis (p=7E-6)	Schauren et al. (2013)
	Paraná State	169 SLE female patients	132 female healthy controls	The frequency of the CCR5Δ32 variant was significantly higher in patients (0.068) than in healthy controls (0.019) (p=0.0047). Euro-Brazilian individuals carrying the allele had a higher predisposition to the development of SLE than in afro-Brazilian individuals carrying the same variant (p=0.0286). Patients with heterozygous genotype presented a lower age of SLE onset and higher levels of anti-dsDNA antibodies when compared to individuals homozygous for the wild type allele (p=0.0293 and p=0.0255, respectively).	Baltus et al. (2015)
Transplant rejection	Paraná State	86 kidney transplant patients with rejection episodes	160 kidney transplant patients without rejection episodes	No statistically significant difference was found in the CCR5Δ32 frequency between the groups (8.3% for individuals with rejection episodes; 6.3% for transplant recipients without rejection)	Cilião et al. (2017)

Table 4. Impacts of the CCR5Δ32 on cancer.

Cancer type	Population (Brazilian state)	Sample	Main findings	Reference
Acute lymphoblastic leukemia (ALL)	Paraná State	79 ALL patients and 80 healthy controls	No statistically significant differences regarding CCR5Δ32 between ALL patients and controls	Oliveira et al. (2014)
Breast cancer (BC)	Paraná State	72 BC patients and 90 healthy women	The allelic frequency estimated in patients was of 3.47% and 7.78% in healthy women; However, no statistically significant difference was found between these groups	Aoki et al. (2009)
Breast cancer (BC)	Paraná State	118 BC patients and 180 healthy women	No statistically significant differences between groups regarding susceptibility, clinical outcome, or treatment response.	Banin-Hirata et al. (2015)
Breast cancer (BC)	Paraná State	94 samples from 47 BC patients (47 tumoral tissues and 47 adjacent tissues)	No impact of CCR5Δ32 on CCL5 levels considering tumoral or normal tissues	Derossi et al. (2019)
Cervical intraepithelial neoplasia (CIN)	Pernambuco State	290 HPV+ women (151 without cervical lesions and 139 with cervical lesions, divided in 12 women with cervical cancer (CC), 40 women with CIN I and 87 with CIN II or III)	No statistically significant differences regarding CCR5Δ32 between CIN or CC patients and HPV+ women without lesions	Santos et al. (2016)
Neuroblastoma (NB)	Paraná State	28 tissue samples from NB patients and 80 cancer-free children	CCR5Δ32 was more frequent in the group of NB patients than in healthy controls ($p<0.05$)	Vieira-Filho et al. (2020)
Prostate cancer (PCa)	Paraná State	30 advanced PCa patients	Significant increase in CD3+ and CD4+ cells was observed in CCR5Δ32 non-carriers; The average CD4+/CD8+ cell ratio decreased in CCR5Δ32 non-carriers after treatment	Magnani et al. (2012)
Prostate cancer (PCa)	Rio Grande do Sul State	119 healthy individuals, 136 PCa patients and 130 benign prostatic hyperplasia (BPH)	CCR5Δ32 allele was not statistically associated with risk of developing BPH or PCa or clinical outcomes of both conditions	Zambra et al. (2013)

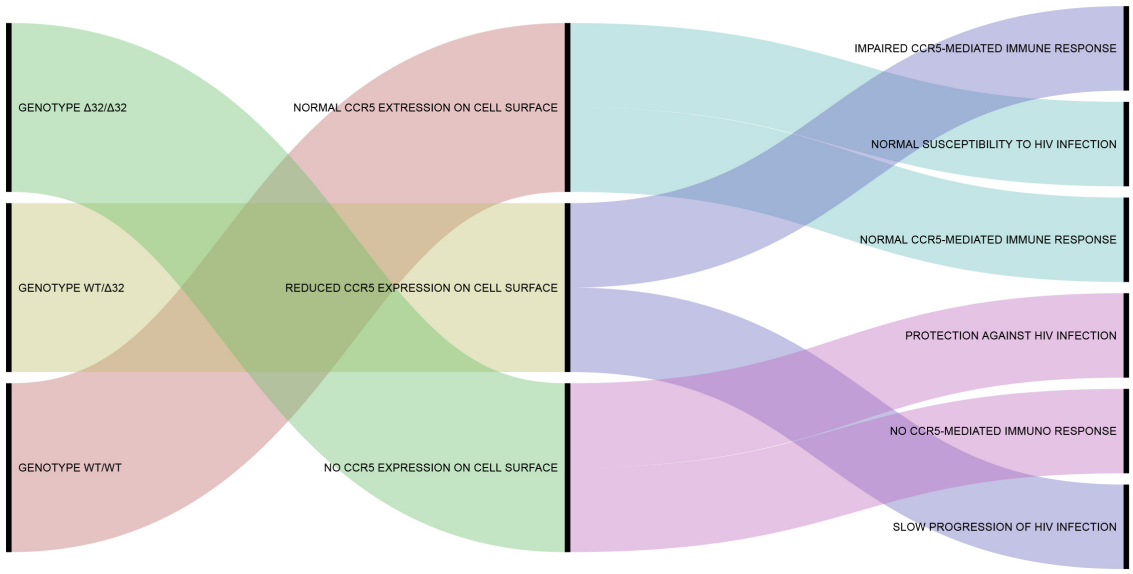

Figure 1. Alluvial diagram representing the classic outcomes associated with the CCR5Δ32. The CCR5Δ32 genotypes are shown in the left part of the diagram. The phenotypic effects of each genotype are shown in the center. The more classical consequences associated with each phenotype are shown in the right part of the diagram. This figure was created using RAWGraphs (https://rawgraphs.io/) (Mauri et al., 2017).

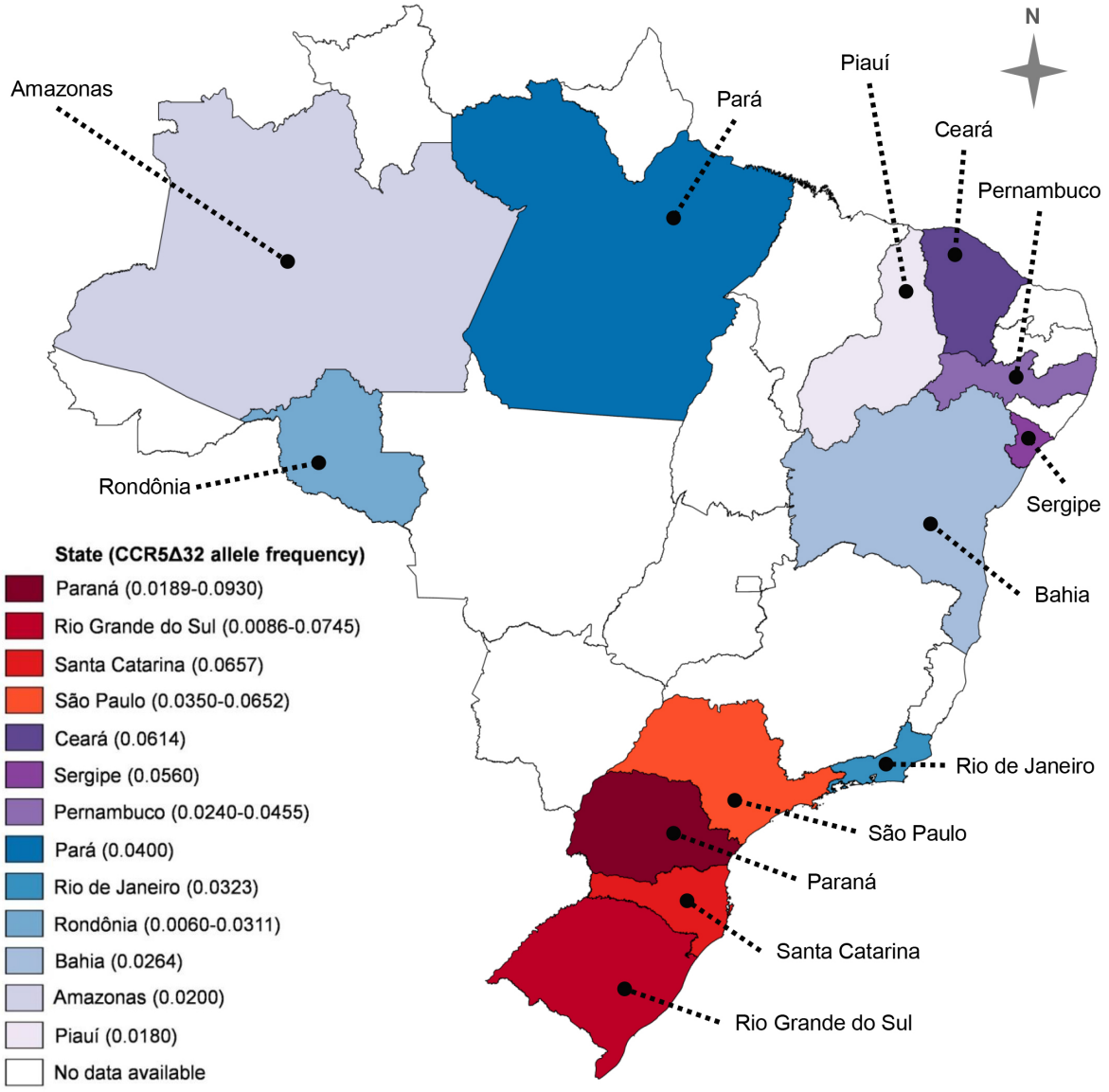

Figure 2. CCR5Δ32 allele frequency in thirteen Brazilian states. Two values in parentheses represent the lowest and the highest frequency observed in a given state. Data from Silva-Carvalho et al. (2016), Hüneimeier et al. (2005) (Mura population; Amazonas State), Carvalho et al. (2004) (Mocambo community; Sergipe State), and Ferreira-Fernandes et al. (2015) (Piauí State). The map was created with the help of MapChart (https://mapchart.net/), licensed under a Creative Commons Attribution-ShareAlike 4.0 International License.

CAPÍTULO 2

O presente capítulo traz um artigo de hipótese que contempla o terceiro objetivo específico desta dissertação, publicado no periódico *Hematology, Transfusion and Cell Therapy:*

KULMANN-LEAL, Bruna; ELLWANGER, Joel Henrique; CHIES, José Artur Bogo. **A functional interaction between the CCR5 and CD34 molecules expressed in hematopoietic cells can support (or even promote) the development of cancer**. Hematol., Transfus. Cell Ther., São Paulo, v. 42, n. 1, p. 70-76, Mar. 2020.

HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Special article

A functional interaction between the CCR5 and CD34 molecules expressed in hematopoietic cells can support (or even promote) the development of cancer

Bruna Kulmann-Leal, Joel Henrique Ellwanger, José Artur Bogo Chies *

Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

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ABSTRACT

Inflammation and angiogenesis are linked to the development of cancer since both can support the establishment of a tumor-prone microenvironment. The CCR5 is a major regulatory molecule involved in inflammation. The CD34 molecule is commonly described as a hematopoietic stem cell marker, and $CD34^+$ cells are involved in the regulation of distinct physiological processes, including angiogenesis. CCR5 participates in the development of various types of cancer, and recently, a reduced CCR5 expression was associated with low $CD34^+$ cell counts in human cord blood. A naturally occurring genetic variant of the *CCR5* gene, the so-called CCR5Δ32 polymorphism, consists of a 32 base-pair deletion in the DNA, interfering in the CCR5 protein levels on the cell surface. When in homozygosis, this variant leads to a total absence of CCR5 expression on the cell surface. In heterozygous individuals, CCR5 surface levels are reduced. Based on these key findings, we hypothesize that a functional interaction can connect CCR5 and CD34 molecules (giving rise to a "CCR5-CD34 axis"). According to this, a CCR5-CD34 interaction can potentially support the development of different types of cancer. Consequently, the lack of CCR5 in association with reduced $CD34^+$ cell counts could indicate a protective factor against the development of cancer. It is required to characterize in detail the functional relationship between CCR5 and CD34 proteins, as well as the real influence of both molecules on the susceptibility and development of cancer at population level. If our hypothesis is confirmed, the CCR5-CD34 axis may be a potential target in the development of anti-cancer therapies.

* *Corresponding author at*: Laboratório de Imunobiologia e Imunogenética (Prédio 43323, Laboratório 212), Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul – UFRGS. Av. Bento Gonçalves, 9500, Campus do Vale, Porto Alegre RS, Brazil.
E-mail address: jabchies@terra.com.br (J.A. Chies).
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Background

CD34⁺ cells – stem cells which could turn bad

The CD34 molecule is a commonly targeted antigenic determinant used as a characteristic marker to isolate and analyze hematopoietic stem cells.[1–9] This protein is expressed by all hematopoietic stem cells and its expression is lost during the cell differentiation. Therefore, mature hematopoietic cells are usually CD34 negative.[1] On the other hand, this molecule is also present in a variety of mature cell types, as mesenchymal cells, muscle satellite cells, corneal keratocytes, interstitial cells, epithelial progenitors, vascular endothelial progenitors, and activated endothelial cells.[10] Of note, CD34 expression has been already correlated to vasculogenic and angiogenic processes, the mesenchymal CD34⁺ stem cells being closely associated to vascularization.[1,4–6,8,9,11] Importantly, both vasculogenic and angiogenic processes are essential to the development of cancer.[12–17]Additionally, there are numerous studies describing CD34 expression in tumor cells,[2,5,6,9,18] although the loss of this molecule has been associated with an invasive malignant phenotype.[8,19] In this context, the CD34 molecule can be viewed in neoplastic cells as a dedifferentiation marker. Also, CD34 could be expressed by endothelial cells and endothelial progenitors as a potential tumor vascularization marker.

CCR5: a chemokine receptor with multiple inflammatory functions

Chemokines are fundamental regulators of the development, differentiation, and migration of leukocytes.[20–24] In addition, chemokines participate in the angiogenic process.[21,25] The CC Chemokine Receptor 5 (CCR5) is a well-known example of such molecules, being especially involved in leukocyte migration.[26] Furthermore, CCR5 is well-known due to its classic role as a co-receptor molecule used by the HIV type I viruses.[21,25,27–31] In addition, CCR5 acts in Th1 immune responses and the lack of signaling through this molecule leads to a shift in the Th2 responses.[20,21] Several chemokines were described as efficient agonists of this receptor, such as RANTES (CCL5), MIP-1α (CCL3) and MIP-1β (CCL4). On the other hand, some chemokines interact less efficiently with CCR5[20–22,25,27,32] or are classified as CCR5 antagonists, which is the case of CCL7.[25,29] Of note, the impact of CCR5 on the development of cancer is an emerging topic, with several studies suggesting that CCR5 plays an important role in the establishment of the tumor microenvironment and progression of different types of cancer. Notably, the CCR5 molecule has been related to the migration and spread of tumor cells, and therefore, to metastasis. Moreover, the presence of the receptor in neoplasms has been associated with the migration of regulatory cells and generation of an immunosuppressor tumor-prone environment. Detailed examples and other effects of CCR5 on tumorigenesis are shown in Table 1.[22–24,33–44]

As mentioned above, the CCR5 protein has a regulatory effect on inflammatory cells,[26,27] thus it must have an enhancing function in the migration of pivotal cells for tumorigenesis, explaining at least partially its connections with the development of cancer. Importantly, the CCR5 expression was already observed in some regulatory T (Treg) cell subsets.[45–50] In certain circumstances and types of cancer, Treg cells were linked with a better prognosis.[51] Nevertheless, we are facing a two-edged sword, since Treg cells can also be subverted by tumor cells in order to generate a tolerogenic environment, allowing the proliferation and establishment of neoplasms.[52–55] Thus, we can suppose that the absence of CCR5 in Treg cells may be a protective factor in the context of tumorigenesis.

Inflammation and the development of cancer

The role of the immune system in the development of cancer is a field of extensive debate. Even though inflammatory cells could eliminate tumor cells, inducing their death, they are also important components of the tumor microenvironment, sometimes favoring tumor growth and proliferation, also promoting neoangiogenesis.[36–38] In this sense, some immune cell-derived factors, when in disbalance, can promote tumor progression. Adding to the complexity of the interactions happening in the tumor, there is a wide variety of immune cells in a neoplastic environment, including lymphocytes, macrophages, neutrophils and dendritic cells, which can produce cytokines and other mediators that enhance the tumor development.[56] The immune surveillance and the individual responses to cancer therapy are also affected by different inflammatory patterns.[57]

CCRΔ32 and the CD34⁺ stem cell repertoire in the cord blood - a puzzling observation

Recently, a study performed by Enrich et al.[58] raised intriguing results. A cohort of Spanish cord blood donors was genotyped for the CCR5Δ32 polymorphism (rs333), a genetic variant which consists of a 32 base-pair deletion in the open reading frame of the *CCR5* gene. This deletion causes a premature stop-codon, which leads to the formation of a truncated protein that is not expressed on the cell surface.[30,31] Heterozygous and homozygous individuals for CCR5Δ32 show respectively reduced CCR5 expression and no expression of the CCR5 molecule on the cell surface.[59,60] As previously mentioned, CCR5 is an HIV-I co-receptor and this genetic variant became widely known due to its association, when in homozygous, to resistance against HIV infection.[61,62] The main objective of Enrich et al.[58] was to identify the CCR5Δ32 homozygous cord blood units, which could be used as donor cells to be transplanted to HIV+individuals. The idea was that the CCR5Δ32 homozygous cord blood units would repopulate the host with a set of cells which would not allow HIV infection, avoiding the maintenance of the virus infection, eventually leading to viral clearance. A similar approach using bone marrow CCR5Δ32 homozygous cells has been successfully applied in one patient, who remains with sustained suppression of the HIV infection.[63–65] Nevertheless, among the results of Enrich et al.,[58] one was surprising and unprecedented: a smaller amount of CD34⁺ cells was found in the samples from the CCR5Δ32 homozygous donors, as compared to both heterozygous and CCR5 wild-type homozygous. This situation represents a drawback to the main objective of the authors since a) fewer cells would be available from these donors and b)

Table 1 – Roles of CCR5 in tumorigenesis.

Type of tumor/cell line	CCR5 role in tumorigenesis/key findings	References
Reed Sternberg (RS) primary cells and Hodgkin Lymphoma (HL)-derived cell line	Presence of CCL5/CCR5 axis is related to tumor proliferation and microenvironment formation.	Aldinucci et al.[43]
Human breast cancer cell line	CCR5 activation by CCL5 leads to cancer proliferation in a mTOR (mammalian Target of Rapamycin) dependent manner.	Murooka et al.[41]
Cervical cancer cells	Greater expression of CCR5 was found in cancer tissues. With downregulation of the *CCR5* gene, tumor proliferation and cell invasion were diminished.	Che et al.[44]
Metastasis of colon rectal cancer in liver	CCR5 was expressed by metastatic tumor cells, lymphocytes, and myeloid cells. The probable interaction between CCR5 and its ligand CCL5 produced by T lymphocytes surrounding the tumor microenvironment promotes cell invasion and metastasis.	Halama et al.[36]
Metastasis of mammary carcinoma in lungs	Regulatory cells expressing high levels of CCR5 accumulate in metastatic mammary carcinomas in mice, suggesting an immunosuppressor role of CCR5, which could favor tumor development.	Halvorsen et al.[40]
Melanoma in mice	CCR5 expression by myeloid-derived suppressor cells leads to migration to primary tumors and metastatic tissues and therefore to tumor progression.	Umansky et al.[42]

potentially cord blood units from those CCR5Δ32 homozygous donors would present a lower reconstitution potential of the host leukocyte repertoires. In this same study,[58] Enrich et al. suggested that CCR5 and its agonist MIP-1α (CCL3) play a critical role in the hematopoietic stem cell function. Specifically, MIP-1α possibly regulates cytokine-induced stem-cell proliferation and the lack of CCR5 in Δ32/Δ32 individuals might disrupt the MIP-1α signaling pathway and explain the lower $CD34^+$ cell counts.[20,58,66–68] From this unexpected result, a new point emerged: a connection between CCR5 and $CD34^+$ cells.

Linking $CD34^+$ cells, the $CCR5^+$ repertoire and cancer - the hypothesis

Considering that $CD34^+$ cells were already associated with tumor development, and taking into account that the absence of $CCR5^+$ cells (which happens in homozygous individuals of the CCR5Δ32 variant) is linked with a lower proportion of $CD34^+$ stem cells, it is possible to infer that a functional interaction between $CCR5^+$ and $CD34^+$ cells exists and that this interaction can give support to cell proliferation and expansion and perhaps, to the establishment/development of different types of cancer. Consequently, the reduced expression of CCR5, in association with low $CD34^+$ cell counts, would protect against the establishment of a tumor microenvironment and the development of cancer (Fig. 1). Additionally, data from previous studies show that the CCR5 agonist MIP-1α has a direct effect on $CD34^+$ cells,[32,58,67,68] suggesting a functional connection between CCR5 and CD34. Thereafter, this functional correlation between CCR5 and the $CD34^+$ cells will be referred to as the "CCR5-CD34 axis". Importantly, our hypothesis mainly addresses the role of CCR5 in the migration of inflammatory cells to the tumor environment (CCR5 as a mediator of inflammation). However, as previously mentioned, CCR5 and its ligands play an important role in Treg cell recruitment and migration of tumor cells. These different roles of CCR5 in tumorigenesis needed to be considered in our hypothesis. Taken together, an association between CCR5 and CD34 may support tumorigenesis.

Consequences of the hypothesis

The unravelling of the mechanisms of tumorigenesis is crucial for the development of new drugs and effective treatments against different types of cancer. Here we hypothesize on the existence of a not yet described interaction between two important molecules of the immune system that may act together as tumorigenesis promoters. Potentially, the CCR5-CD34 axis could be a new target for cancer treatments, pharmacologically, or as part of gene therapy strategies. However, prior to this, the interactions between CCR5 and CD34 need to be characterized at both cellular and functional levels, and their real importance in the tumor biology needs to be understood in detail.

In general, the lack of CCR5 observed in homozygous individuals of the CCR5Δ32 variant is considered not to be associated with any severe essential physiological alterations. Although it is generally accepted that homozygous individuals of this variant have no severe immunological or clinical deficiencies,[30,69] exacerbated inflammatory responses have already been reported in association with the CCR5Δ32 variant.[70] Interestingly, a recent report has linked CCR5 absence with defective bone development.[71] Thus, it is crucial to consider that CCR5 mediates different immune functions and it is possible that its absence promotes changes of difficult detection, but with medical significance in a specific environment or context. For example, homozygous individuals of CCR5Δ32 are more susceptible to develop symptomatic West Nilo virus infection.[72,73] Nevertheless, CCR5Δ32 is a pleiotropic variant, promoting different outcomes in different situations.[74] Of note, especially considering the recent alleged report of CCR5 editing in humans, the pros and cons of the absence of CCR5 were addressed in a recent publication by our group.[75] If our hypothesis is confirmed, the

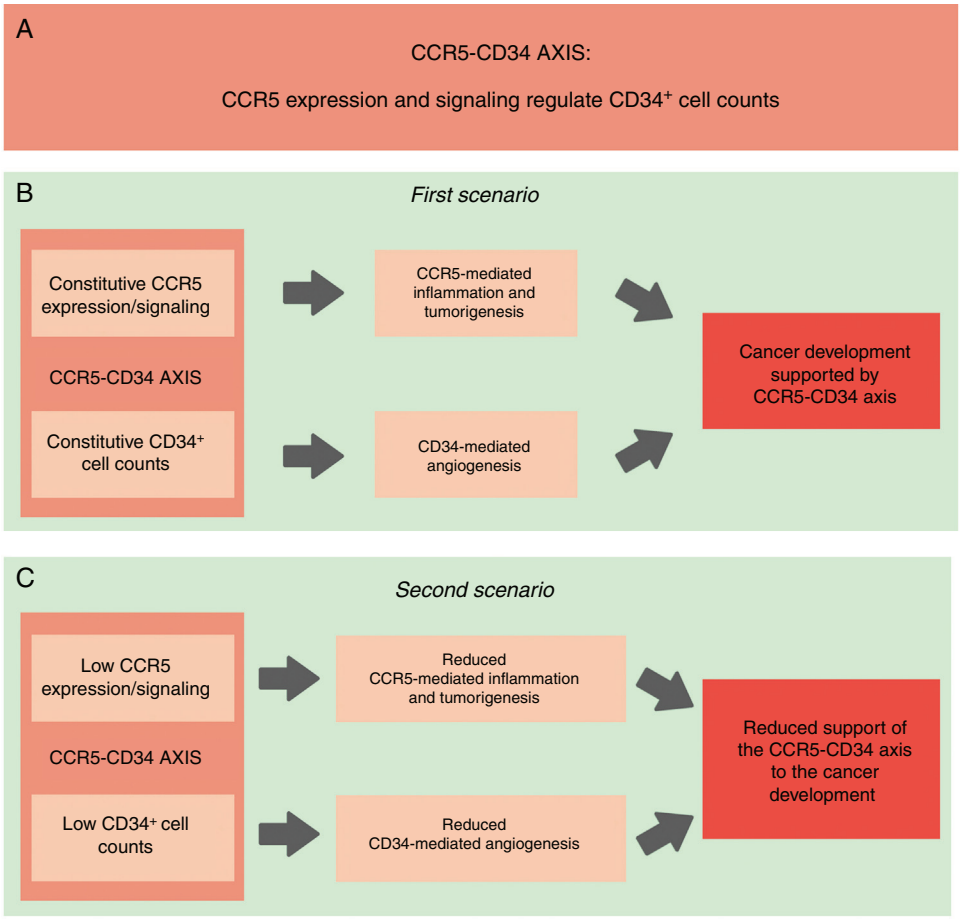

Fig. 1 – Schematic representation of our hypothesis. (A) CCR5-CD34 axis: functional interactions between CCR5 and CD34. (B) CCR5-CD34 axis supports the development of different types of cancer. Constitutive: normal/expected levels of CCR5 and CD34⁺ cells. (C) Reduced CCR5 expression in association with lower CD34⁺ cell counts would protect against the establishment of the tumor microenvironment and the development of cancer.

physiological significance of the absence of CCR5 needs to be reviewed. Although the chemokine-ligand system is robust and redundant,[76] which ensures its functioning in the absence of some specific chemokine or chemokine receptor, alterations in such a balanced system may have a significant impact on other diseases (in a favorable or unfavorable way). Looking at the hypothesis described here, the lack of CCR5 could be considered a protective factor against the development of cancer.

Testing our hypothesis

We suggest the following initiatives to test our hypothesis:

- First, it is necessary to investigate the CD34⁺ cell counts in individuals with different CCR5Δ32 genotypes in different populations with the aim of confirming the correlation between the CCR5 expression and CD34⁺ cell counts.
- Second, functional studies must be performed to evaluate the potential interactions between CCR5 and CD34⁺ cells, helping to understand the biological significance of the CCR5-CD34 axis and identify in which contexts such interactions occur.
- Third, it is necessary to evaluate the influence of the lack of CCR5 and the reduced CD34⁺ cell counts (separately and in association) on the development of cancer at a populational level, as well as in in vitro strategies.
- Fourth, it would be interesting to evaluate tumor growth in *CCR5* and *CD34* knockout animals and controls, aiming to compare the tumor progression between the groups.
- Finally, we suggest investigating the frequency of CCR5Δ32 and the cancer incidence at the populational level (in different geographic regions), in order to verify the correlation between these two data sets.

Conclusion and perspectives

Each cancer type comprises specific cellular and molecular environments. However, migration of inflammatory cells to the tumor site and angiogenesis are processes found in different types of solid tumors. Thus, if the influence of the CCR5-CD34 axis on the development of cancer is confirmed, it is possible that both molecules become targets for the development of new therapeutics against tumors. In addition, pharmacological strategies focusing on modulating both

molecules together may be quite promising. Currently, the use of CCR5 blockers is already being suggested for the treatment of different pathologies, besides HIV infection, including cancer.[23,24,36–38,77] The above-mentioned scenario shows that our hypothesis deserves to be investigated, once (I) it will contribute to the understanding of the factors that support the establishment of the tumor microenvironment and modify the susceptibility to the development of cancer and (II) it has the potential to reveal important implications for cancer treatment.

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Meeting of ethical standards

We declare that this study was developed according to all rules and meeting all ethical standards.

Conflicts of interest

The authors declare no conflicts of interest.

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CAPÍTULO 3

O presente capítulo apresenta um artigo de dados originais que contempla o quarto, o quinto e o sexto objetivos específicos desta dissertação, a ser submetido no periódico *Human Immunology:*

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Genotyping of the CCR5Δ32 variant in Brazilians from REDOME as an initial support strategy for sustained viral suppression in HIV-infected individuals

Bruna Kulmann-Leal[1,2], Joel Henrique Ellwanger[1,2], Ana Cristina Arend[3], Luiz Fernando Job Jobim[3], Mariana Jobim[3], Rafael Tomoya Michita[4], José Artur Bogo Chies[1,2]

1. Laboratório de Imunobiologia e Imunogenética, Departamento de Genética, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil

2. Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil

3. Serviço de Imunologia, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Rio Grande do Sul, Brazil.

4. Laboratório de Genética Molecular Humana, Universidade Luterana do Brasil (ULBRA), Canoas, Rio Grande do Sul, Brazil.

Corresponding author: Dr. José Artur Bogo Chies. Laboratório de Imunobiologia e Imunogenética (Prédio 43323, Laboratório 212), Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul – UFRGS. Av. Bento Gonçalves, 9500, Campus do Vale, Porto Alegre, Rio Grande do Sul, Brazil. Phone: +5551 33086737. E-mail: jabchies@terra.com.br

Abstract

CCR5 is a critical chemokine receptor in the context of HIV infection. As the main co-receptor used by the virus to penetrate host cells, reduced expression or absence of CCR5 dramatically impact the infection process. The genetic variant CCR5Δ32 consists of a 32 base-pair deletion in the coding region of the *CCR5* gene, producing a truncated protein that is not transported to the cell surface. Thus, individuals bearing the CCR5Δ32 homozygous genotype do not express CCR5 properly and are highly resistant to HIV infection. In HIV-infected individuals who develop malignant hematological diseases, transplantation of stem cells derived from donors with CCR5Δ32 homozygous genotype is a viable strategy of sustained remission of the HIV infection, as exemplified by the 'Berlin Patient' and the 'London Patient'. Based on these results, researchers in several countries are looking for CCR5Δ32 homozygous donors to replicate such strategy. In line with this view, the aim of this study was genotyping the CCR5Δ32 variant in individuals enrolled in the Registry of Voluntary Bone Marrow Donors (*Registro Nacional de Doadores de Medula Óssea* - REDOME) in the Rio Grande do Sul State, Brazil. Also, three different HLA loci (HLA-A, HLA-B, and HLA-DR) were assessed to verify a possible association between specific HLA alleles and the CCR5Δ32 variant. In a total of 1,186 donors, the CCR5Δ32 allelic frequency was 7.1%, and the frequency of the Δ32/Δ32 homozygous genotype was 0.76%. The HLA-B *44, *15 and *50 alleles were initially associated with CCR5Δ32, but the statistical significance was lost after the *p*-values were corrected. No other association between HLA alleles and the CCR5Δ32 variant was detected ($p>0.05$ in all analyses). Considering the large number of REDOME-registered individuals in Brazil and the allelic frequency observed in our study, there is a considerable amount of potential bone marrow CCR5Δ32 homozygous donors in Brazil. Thus, finding a donor who might donate bone marrow to HIV-infected individuals who need bone marrow transplantation is a possible and viable task, justifying the continuation of CCR5Δ32 genotyping in a larger number of donors.

Keywords: CCR5Δ32; immunogenetics; HIV; HLA; viral suppression

Introduction

The Human Immunodeficiency Virus (HIV) is responsible for the AIDS development and despite intense research involving HIV/AIDS since the 1980s, there is still no effective cure for the disease. Scientific efforts are still extremely necessary to reduce HIV-associated conditions, including AIDS and the chronic inflammation observed in patients on antiretroviral therapy (ART). In the clinical course of HIV infection, there is a large depletion of CD4+ T cells since CD4 is the main binding receptor which is used by HIV to penetrate cells through gp120 viral surface protein interaction. After the binding, the gp120 protein is displaced, leaving the gp41 viral protein free, which binds to a cellular co-receptor, more frequently CCR5, a chemokine receptor involved in inflammatory reactions. Because of its direct role in this pathogenic process, multiple aspects of CCR5 have been studied in the context of HIV infection and AIDS progression [1,2,3,4,5,6].

In 1996 a 32 base-pair deletion was described in the *CCR5* gene, which was subsequently named as CCR5Δ32 [7]. This variant was originally found in homozygosity in two individuals exposed to, but not infected with, the HIV, being investigated from the beginning as a HIV resistance factor. The CCR5Δ32 variant consists of a frameshift mutation in the coding region of the *CCR5* gene (exon 3), which generates a premature stop codon and the consequent expression of a non-functional protein, which is not transported to the cell surface to fulfill the original function of this chemokine receptor. Considering that individuals who present this variant in homozygosity do not express the molecule in the surface of their cells, CCR5-tropic HIV-1 strains are prevented from successfully interacting with the host cell, blocking the process of viral penetration [7,8,9,10,11,12,13].

The CCR5Δ32 has a heterogeneous distribution around the world. This variant has a European origin and is practically absent in African, Asian, and Amerindian populations [14,15,16]. The Brazilian population is highly admixed, with an important genetic contribution of European populations. The presence of Euro-Brazilian individuals is greater in the southern region of Brazil, which reflects the higher frequencies of the CCR5Δ32 allele in this region within the Brazilian territory. The CCR5Δ32 frequency is around 4% in Brazil, but it can be as high as 9% in specific southern populations [17,18].

Since its discovery, the CCR5Δ32 variant has been the subject of numerous studies in the context of HIV infection, and in 2009, Hütter et al. (2009) published an innovative and striking study. A 40-year-old HIV-infected man suffering from acute myeloid leukemia was followed by the researchers, being lately known as the 'Berlin Patient'. As a treatment for the neoplasia, the patient underwent an allogeneic stem cell transplant from a HLA compatible donor homozygous for the CCR5Δ32 allele. The donor was chosen in order to carry out a possible combined therapy for leukemia and HIV infection, since the donor's cells without CCR5 expression could not be infected by the patient's CCR5-tropic strains. After the transplant, the patient no longer received ART and his viral load was followed for the next 20 months. Remarkably, active and replicating viral particles were not detected in that period. Three years after the procedure Hütter and Thiel (2011) updated the Berlin Patient's clinical situation, reporting the continuous absence of replicative viral particles still without ART need [19,20].

Gupta et al. (2019) presented an assay describing a second successful case of sustained remission of HIV infection. Following a similar protocol described by Hütter and colleagues in 2009, a patient diagnosed with HIV-1 infection and IVb stage Hodgkin's lymphoma underwent an allogeneic stem cell transplant with CCR5Δ32 homozygous cells. ART was discontinued at day 510, and in the same way of the first reported case, the presence of HIV was no longer observed after the transplant. Subsequently, Gupta et al. (2020) reinforced the success of the procedure 30 months after the transplant, describing more details regarding the case. This second individual to achieve sustained remission of HIV infection became known as the 'London Patient' [21,22].

Considering the possibility of obtaining a sustained remission of HIV infection, different research groups are investigating the feasibility of this treatment in various populations. Solloch et al. (2017) summarized CCR5Δ32 frequencies for 87 countries, also describing the CCR5Δ32 genotypes for over 1.3 million individuals from Germany, Poland, and UK. The genotypes were from potential hematopoietic stem cell donors from a European stem cell bank (DKMS). The researchers estimated at 28.7% the possibility that a German HIV+ individual who needs a stem cell transplant in find a homozygous donor compatible for both CCR5Δ32 and HLA. Also, Enrich et al. (2017) genotyped 20,236 cord blood units (CBUs) from The Spanish Bone Marrow Registry (REDMO), and found a total of 130 CBUs homozygous for the CCR5Δ32 variant. Both studies show that, although the

CCR5Δ32 allele is not highly prevalent in most countries, it is possible to find homozygous individuals who are already registered in hematopoietic stem cell donor banks that could be useful in new attempts of HIV sustained suppression [16,23].

Altogether, the search for CCR5Δ32 homozygous individuals as potential donors to HIV+ patients in bone marrow transplants represents a cutting-edge initiative in scientific research involving HIV/AIDS. In Brazil, the National Registry of Bone Marrow Volunteer Donors (*Registro Nacional de Doadores de Medula Óssea* - REDOME) is responsible for gathering information from people willing to donate bone marrow to anyone who needs a transplant, having more than 5 million registered members. Considering this scenario, the pilot objective of this study was genotyping the CCR5Δ32 variant in a sample of Brazilians from REDOME as an initial support strategy for sustained viral suppression in HIV-infected individuals. Potential associations between CCR5Δ32 and HLA alleles were also investigated. The potential clinical implications of this research include benefits for HIV+ individuals, strengthening the proof-of-concept described by Hütter et al. (2009) and Gupta et al. (2019).

Methods

DNA samples and ethical aspects

The genomic DNA samples used in the study come from REDOME donors and were provided by the Immunology Service of *Hospital de Clínicas de Porto Alegre* (HCPA, Porto Alegre, Brazil), which is responsible for the REDOME in Porto Alegre, the capital of Rio Grande do Sul, the southernmost state of Brazil. A total of 1,186 samples were investigated in this study, of whom HLA-A, HLA-B and HLA-DR genotypes were genotyped previously. Only individuals recently registered on REDOME were included. All potential donors registered in REDOME sign a consent form allowing the genetic analysis of antigens of tissue compatibility for transplantation purposes, in which *CCR5* genotyping is included. This study was approved by the research ethics committees of the *Hospital de Clínicas de Porto Alegre* and *Universidade Federal do Rio Grande do Sul* (# 20460719.6.0000.5327).

CCR5Δ32 genotyping

The CCR5Δ32 variant (rs333) was genotyped according to Chies and Hutz (2003) and minor adaptations by Ellwanger et al. (2018) [24,25]. The fragments of interest were amplified by conventional PCR and the primers used in the reaction are as follows: CCR5a 5'-GGTCTTCATTACACCTGC-3'; CCR5b 5'-AGGATTCCCGAGTAGCAGATG-3'. Genotyping was performed by analyzing the amplicons on a 3% agarose gel under UV light. A single 137 base-pair band represents the wild-type homozygous genotype; a 137 base-pair band associated with a 105 base-pair band indicates heterozygous genotype; a single 105 base-pair band indicates the variant homozygous genotype.

Statistical analysis

Allelic and genotypic frequencies were initially calculated by counting, and the Hardy-Weinberg equilibrium was accessed using the chi-square test. Individuals were also evaluated grouped as CCR5Δ32 carriers (CCR5Δ32 homozygous individuals plus heterozygous individuals) and CCR5Δ32 non-carriers (individuals with wild-type homozygous genotype). Subsequently, a possible association between the CCR5Δ32 polymorphism and HLA-A, HLA-B and HLA-DR alleles were assessed. Individuals with uncertain HLA genotypes were excluded from the HLA-related analysis but maintained on CCR5Δ32 genotypic and allelic frequencies general counting. The comparisons of the HLA allele frequencies between CCR5Δ32 carriers and non-carriers were made by performing Pearson's chi-square with Yates's correction. For cells with expected values less than 5, the Wald chi-square test was chosen. Specifically, using a 2x2 matrix, the number of individuals with a particular HLA allele among CCR5Δ32 carriers and non-carries was compared to the number of individuals with other HLA alleles, also stratified as CCR5Δ32 carriers and non-carriers. The Benjamini-Hochberg step-up false discovery rate was performed to posterior adjustment of *p*-values for multiple comparisons. *P*-values <0.05 were considered statistically significant. All analyses were performed using the WinPEPI version 11.65 [26].

Results

Table 1 shows the CCR5Δ32 genotypic and allelic frequencies in the individuals from REDOME genotyped in this study. Most voluntary donors do not carry the CCR5Δ32 allele. However, we obtained an allelic frequency of 7.1%, which is considerably high in comparison with the average Brazilian frequency of 4-5%. In total, nine Δ32/Δ32 homozygous individuals were found (0.76%). The sample showed genotype distribution according to the expected for the Hardy-Weinberg equilibrium (p >0.05).

Table 2 shows the allelic frequencies of the three HLA loci investigated in the study. However, some donors with available CCR5Δ32 genotype had uncertain HLA genotypes due to limitations in the genotyping methodology. Thus, individuals in this situation were removed from the analyses in Table 2 onwards. Of note, 22 HLA-A, 36 HLA-B, and 15 HLA-DR alleles were found in REDOME donors. To assess the possible association between HLA alleles and the CCR5Δ32 variant, in Table 3 and Table 4 the HLA alleles were stratified among CCR5Δ32 carriers and non-carriers, respectively.

Finally, a comparison between CCR5Δ32 carriers and non-carriers regarding the HLA alleles was performed and Table 5 summarizes the chi-square results. At first, one allele frequency in HLA-B locus (allele 44) was significantly higher (p<0.05) in non-carriers, and two alleles (15 and 50) were significantly lower in the same group. However, after adjustment for multiple comparisons, no statistically significant result was maintained (p>0.05). No statistically significant difference was observed regarding the HLA-A and HLA-DR alleles (p>0.05).

Discussion

The works of Hütter et al. (2009) and Gupta et al. (2019) were pioneers in achieving sustained remission of HIV infection without the need for continuous use of ART. By deliberately looking for donors of hematopoietic stem cells who were also homozygous to the CCR5Δ32 variant, they achieved the functional cure of HIV infection in the ‘Berlin’ and ‘London’ patients. After such cutting-edge results, studies performed in

Spain by Enrich et al. (2017) and Germany, Poland and the UK by Solloch et al. (2017) reaffirm the importance of searching for CCR5Δ32 homozygous individuals already registered as donors in hematopoietic stem cell banks. Here, we conducted a similar investigation regarding Brazilian donors.

The CCR5Δ32 variant has a very heterogeneous distribution in different populations, even in the same country, which is the case in Brazil. According to Silva-Carvalho et al. (2016), the average Brazilian CCR5Δ32 frequency is around 4-5%. In our study, we found a frequency of 7.1%, which may be due to the high density of European-descendent individuals in the south region of Brazil. Also, according to Solloch et al. (2017), the average Brazilian allelic frequency of Δ32/Δ32 homozygous individuals is around 0.35%. Here, we found a frequency twice as high (0.76%). When extrapolating these results to the Brazilian population in general, it is important to note that the frequency of individuals carrying the allele and especially of homozygotes for the variant should be slightly lower [16,18].

Nevertheless, more than 5 million Brazilians are currently registered to REDOME. Considering a minimum frequency of 0.35% and a maximum frequency of 0.76% in 5 million, we could expect between 17,500 and 38,000 voluntary donors homozygous for the variant and who could therefore participate in transplants for HIV-infected individuals However, for this to be possible, it would be required to standardize the genotyping of the CCR5Δ32 variant in REDOME.

Considering the analysis regarding the HLA loci, no statistically significant difference was found after adjustment of *p*-values for multiple comparisons. However, even though the significance was lost after the adjustment, three alleles in HLA-B locus were initially different between CCR5Δ32 carriers and non-carriers (HLA-B*15, HLA-B*44, and HLA-B*50). The HLA-B loci are very polymorphic, presenting an allelic diversity superior to other HLA class I loci [27]. We can observe this difference by comparing the alleles found in the present work concerning the HLA-A and HLA-B loci.

This high HLA diversity may be due to a molecular evolution that could be happening faster in this specific locus. The observed faster evolution in HLA-B suggests that this molecule plays a critical role, more important than others, in immune responses against pathogens involving immune responses mediated by CD8+ cells, such as the antiviral response against HIV [27]. In this context, it might be interesting to investigate

the possible role of the HLA-B*15, HLA-B*44, and HLA-B*50 alleles in HIV infection and their potential association to the presence or absence of the CCR5Δ32 variant. In fact, the absence of association of a given HLA allele with the CCR5Δ32 variant could be regarded as advantageous since it would suggest that CCR5Δ32 homozygous donors are highly diversified in terms of their HLA haplotypes. This situation of great diversity keeps wide the possible range of compatible HLA/CCR5Δ32 homozygous matchings.

Conclusion

In the present study, the genotypic and allelic frequencies of the CCR5Δ32 in voluntary donors of bone marrow cells registered in the Brazilian REDOME were reported, as well as allelic frequencies of three HLA loci in the same sample. Considering the large number of individuals registered in REDOME, here we show that it is possible to find a considerable amount of CCR5Δ32 homozygous donors who might donate bone marrow to HIV patients that need bone marrow transplantation. Lastly, it was not possible to detect an association between HLA-specific alleles and the presence of the CCR5Δ32 variant, although the HLA-B*15, HLA-B*44, and HLA-B*50 alleles should be considered in further studies.

Conflicts of interest

The authors declare no conflicts of interest regarding this study.

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Author contributions

Bruna Kulmann-Leal performed the genotyping, data analysis and wrote the first version of the manuscript. **Joel Henrique Ellwanger** and **Rafael Tomoya Michita** analyzed the data and edited the manuscript. **Ana Cristina Arend**, **Luiz Fernando Job Jobim** and **Mariana Jobim** provided the samples. **Luiz Fernando Job Jobim** coordinated the work at *Hospital de Clínicas de Porto Alegre.* **José Artur Bogo Chies** revised and edited the manuscript and coordinated the work at *Universidade Federal do Rio Grande do Sul*. All authors approved the final version of the manuscript.

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Table 1. CCR5Δ32 genotypic and allelic frequencies among REDOME donors.

CCR5Δ32 profile	**REDOME donors (n=1186)**
wt/wt, n (%)	1026 (86.51%)
wt/Δ32, n (%)	151 (12.73%)
Δ32/Δ32, n (%)	9 (0.76%)
Δ32 carrier, n (%)	160 (13.49%)
Δ32 non-carrier, n (%)	1026 (86.51%)
Δ32 allele frequency	0.071

n, sample number. wt/wt, wild homozygous genotype. wt/Δ32, heterozygous genotype. Δ32/Δ32, variant homozygous genotype. Δ32 Allelic frequency: [(2x n individuals Δ32/Δ32) + (wt/Δ32 individuals)] / (2x n total individuals).

Table 2. HLA diversity of REDOME donors (n=1013).

HLA-A			*HLA-B*			*HLA-DR*		
Allele	**n**	**Frequency**	**Allele**	**n**	**Frequency**	**Allele**	**n**	**Frequency**
2	566	0.2794	44	245	0.1209	7	287	0.1416
24	218	0.1076	35	229	0.1130	11	274	0.1352
1	209	0.1032	15	182	0.0898	13	261	0.1288
3	194	0.0958	51	172	0.0849	4	240	0.1185
29	112	0.0553	7	168	0.0829	15	205	0.1012
11	111	0.0548	8	117	0.0577	1	202	0.0997
68	102	0.0503	40	107	0.0528	3	185	0.0913
32	89	0.0439	14	104	0.0513	8	108	0.0533
31	82	0.0405	18	104	0.0513	14	95	0.0469
23	81	0.0400	27	60	0.0296	16	72	0.0355
33	65	0.0321	38	58	0.0286	9	43	0.0212
26	62	0.0306	13	54	0.0267	10	28	0.0138
30	62	0.0306	57	52	0.0257	12	24	0.0118
25	26	0.0128	49	50	0.0247	27	1	0.0005
66	15	0.0074	58	36	0.0178	52	1	0.0005
74	15	0.0074	39	35	0,0173			
34	6	0.0030	50	35	0.0173			
36	5	0.0025	52	26	0.0128			
7	2	0.0010	45	25	0.0123			
69	2	0.0010	53	25	0.0123			
8	1	0.0005	55	25	0.0123			
9	1	0.0005	41	24	0.0118			
			48	23	0.0114			
			37	20	0.0099			
			42	12	0.0059			
			56	10	0.0049			
			47	9	0.0044			
			3	4	0.0020			
			81	4	0.0020			
			16	3	0.0015			
			1	2	0.0010			
			11	2	0.0010			
			4	1	0.0005			
			9	1	0.0005			
			46	1	0.0005			
			73	1	0.0005			

n, number of alleles. Of note, the total number of alleles will be twice the number of individuals analyzed. Frequencies of the HLA alleles presented here are among all REDOME donors, regardless the presence or absence of the CCR5Δ32 variant.

Table 3. HLA diversity in CCR5Δ32 carriers of REDOME (n=141).

HLA-A			*HLA-B*			*HLA-DR*		
Allele	**n**	**Frequency**	**Allele**	**n**	**Frequency**	**Allele**	**n**	**Frequency**
2	81	0.2872	15	37	0.1312	11	40	0.1418
1	33	0.1170	35	31	0.1099	13	39	0.1383
24	26	0.0922	51	26	0.0922	15	35	0.1241
3	22	0.0780	7	23	0.0816	7	33	0.1170
32	19	0.0674	44	22	0.0780	1	31	0.1099
29	17	0.0603	8	15	0.0532	4	31	0.1099
68	17	0.0603	18	15	0.0532	3	27	0.0957
11	14	0.0496	14	14	0.0496	8	17	0.0603
33	11	0.0390	27	12	0.0425	14	11	0.0390
23	9	0.0319	38	12	0.0425	16	11	0.0390
26	9	0.0319	40	9	0.0319	9	3	0.0106
31	8	0.0284	50	9	0.0319	10	2	0.0071
30	6	0.0213	13	8	0.0284	12	2	0.0071
25	5	0.0177	57	8	0.0284			
74	3	0.0106	39	6	0.0213			
66	2	0.0071	49	6	0.0213			
			45	5	0.0177			
			53	5	0.0177			
			41	4	0.0142			
			52	3	0.0106			
			55	3	0.0106			
			37	2	0.0071			
			42	2	0.0071			
			58	2	0.0071			
			47	1	0.0035			
			48	1	0.0035			
			56	1	0.0035			

n, number of alleles. Frequency of the HLA alleles presented here are among CCR5Δ32 carriers of REDOME, which are defined as individuals who carry the CCR5Δ32 variant in heterozygous or homozygous.

Table 4. HLA diversity in CCR5Δ32 non-carriers of REDOME (n=873).

HLA-A			*HLA-B*			*HLA-DR*		
Allele	**n**	**Frequency**	**Allele**	**n**	**Frequency**	**Allele**	**n**	**Frequency**
2	485	0.2778	44	223	0.1277	7	254	0.1455
24	192	0.1100	35	199	0.1140	11	234	0.1340
1	175	0.1002	51	147	0.0842	13	223	0.1277
3	174	0.0996	7	145	0.0830	4	209	0.1197
11	97	0.0556	15	145	0.0830	1	172	0.0985
29	95	0.0544	8	101	0.0578	15	170	0.0974
68	85	0.0487	40	98	0.0561	3	158	0.0905
31	74	0.0424	14	90	0.0515	8	91	0.0521
23	72	0.0412	18	89	0.0510	14	84	0.0481
32	70	0.0401	27	48	0.0275	16	61	0.0349
30	56	0.0321	13	46	0.0263	9	40	0.0229
33	54	0.0309	38	46	0.0263	10	26	0.0149
26	53	0.0304	49	44	0.0252	12	22	0.0126
25	21	0.0120	57	44	0.0252	27	1	0.0006
66	13	0.0074	58	34	0.0195	52	1	0.0006
74	12	0.0069	39	29	0.0166			
34	6	0.0034	50	26	0.0149			
36	5	0.0029	52	23	0.0132			
7	2	0.0011	48	22	0.0126			
69	2	0.0011	55	22	0.0126			
8	1	0.0006	41	20	0.0114			
9	1	0.0006	45	20	0.0114			
			53	20	0.0114			
			37	18	0.0103			
			42	10	0.0057			
			56	9	0.0052			
			47	8	0.0046			
			3	4	0.0023			
			81	4	0.0023			
			16	3	0.0017			
			1	2	0.0011			
			11	2	0.0011			
			4	1	0.0006			
			9	1	0.0006			
			46	1	0.0006			
			73	1	0.0006			

n, number of alleles. Frequency of the HLA alleles presented here are among CCR5Δ32 non-carriers of REDOME, which are defined as individuals who do not carry the CCR5Δ32 variant (homozygous to the wild-type allele).

Table 5. Comparisons of the HLA loci allele frequencies between CCR5Δ32 carriers and non-carriers.

Locus	Allele	O.R., C.I. 95%	*p*-value	Adjusted *p*-value
HLA-A	2	0.95, 0.72 - 1.26	0.797	0.9741
	24	1.22, 0.79 - 1.87	0.429	0.9741
	1	0.84, 0.57 - 1.25	0.449	0.9741
	3	1.31, 0.82 - 2.08	0.302	0.9741
	11	0.89, 0.50 - 1.58	0.792	0.9741
	29	1.11, 0.65 - 1.90	0.795	0.9741
	68	1.25, 0.73 - 2.14	0.496	0.9741
	31	0.66, 0.31 - 1.38	0.344	0.9741
	23	0.77, 0.38 - 1.55	0.563	0.9741
	32	1.73, 1.02 - 2.92	0.055	0.9741
	30	1.52, 0.65 - 3.57	0.429	0.9741
	33	0.79, 0.41 - 1.52	0.594	0.9741
	26	0.95, 0.46 - 1.95	1.000	1.0000
	25	0.67, 0.25 - 1.80	0.432*	0.9741
	66	1.05, 0.24 - 4.68	0.949*	0.9942
	74	0.64, 0.18 - 2.30	0.497*	0.9741
	34	2.11, 0.12 - 37.56	0.611*	0.9741
	36	1.78, 0.10 - 32.36	0.695*	0.9741
	7	0.81, 0.04 - 16.91	0.892*	0.9812
	69	0.81, 0.04 - 16.91	0.892*	0.9812
	8	0.49, 0.02 - 11.95	0.658*	0.9741
	9	0.49, 0.02 - 11.95	0.658*	0.9741
HLA-B	44	1.73, 1.10 - 2.73	**0.023**	0.4140
	35	1.04, 0.70 - 1.56	0.922	1.0000
	51	0.91, 0.58 - 1.40	0.740	1.0000
	7	1.02, 0.64 - 1.61	1.000	1.0000
	15	0.60, 0.41 - 0.88	**0.012**	0.4140
	8	1.09, 0.63 - 1.91	0.862	1.0000
	40	1.80, 0.90 - 3.61	0.123	1.0000
	14	1.04, 0.58 - 1.85	1.000	1.0000
	18	0.96, 0.54 - 1.68	0.991	1.0000
	27	0.64, 0.33 - 1.21	0.232	1.0000
	13	0.93, 0.43 - 1.98	1.000	1.0000
	38	0.61, 0.32 - 1.16	0.186	1.0000
	49	1.19, 0.50 - 2.82	0.851	1.0000
	57	0.89, 0.41 - 1.90	0.913	1.0000
	58	2.78, 0.66 - 11.64	0.223	1.0000
	39	0.78, 0.32 - 1.89	0.578*	1.0000
	50	0.46, 0.21 - 0.99	**0.047***	0.5640
	52	1.24, 0.37 - 4.16	0.726*	1.0000
	48	3.59, 0.48 - 26.71	0.213*	1.0000
	55	1.19, 0.35 - 3.99	0.782*	1.0000
	41	0.81, 0.27 - 2.37	0.695*	1.0000
	45	0.64, 0.24 - 1.72	0.379*	1.0000
	53	0.64, 0.24 - 1.72	0.379*	1.0000
	37	1.46, 0.34 - 6.32	0.614*	1.0000
	42	0.81, 0.18 - 3.70	0.782*	1.0000
	56	1.46, 0.18 - 11.54	0.722*	1.0000
	47	1.29, 0.16 - 10.38	0.809*	1.0000

	3	1.46, 0.08 - 27.17	0.800*	1.0000
	81	1.46, 0.08 - 27.17	0.800*	1.0000
	16	1.13, 0.06 - 22.02	0.934*	1.0000
	1	0.81, 0.04 - 16.91	0.892*	1.0000
	11	0.81, 0.04 - 16.91	0.892*	1.0000
	4	0.49, 0.02 - 11.95	0.658*	1.0000
	9	0.49, 0.02 - 11.95	0.658*	1.0000
	46	0.49, 0.02 - 11.95	0.658*	1.0000
	73	0.49, 0.02 - 11.95	0.658*	1.0000
HLA-DR	7	1.28, 0.87 - 1.89	0.238	0.8660
	11	0.94, 0.65 - 1.34	0.793	0.8660
	13	0.91, 0.63 - 1.32	0.692	0.8660
	4	1.10, 0.74 - 1.64	0.710	0.8660
	1	0.88, 0.59 - 1.33	0.627	0.8660
	15	0.76, 0.52 - 1.12	0.202	0.8660
	3	0.94, 0.61 - 1.44	0.863	0.8660
	8	0.86, 0.50 - 1.46	0.672	0.8660
	14	1.25, 0.66 - 2.36	0.603	0.8660
	16	0.89, 0.46 - 1.72	0.866	0.8660
	9	2.18, 0.67 - 7.10	0.269	0.8660
	10	2.12, 0.50 - 8.97	0.309*	0.8660
	12	1.79, 0.42 - 7.64	0.434*	0.8660
	27	0.49, 0.02 - 11.95	0.658*	0.8660
	52	0.49, 0.02 - 11.95	0.658*	0.8660

*When any cell has an expected frequency of <5, Wald chi-square test was used. Statistically significant *p*-values are shown in bold. Adjustment of *p*-values by the Benjamini-Hochberg step-up false discovery rate.

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CONSIDERAÇÕES FINAIS

Ao longo desta dissertação, diversos aspectos acerca do gene e da molécula CCR5 e da variante CCR5Δ32 foram abordados nos **Capítulos 1** e **2**. A **Figura 3** apresenta as frequências do alelo Δ32 em diversas populações mundiais, com base em dados compilados por Solloch et al. (2017). Para fins de comparação, foi acrescentada na Figura 3 a frequência da variante CCR5Δ32 encontrada nos resultados do **Capítulo 3** da presente dissertação. Percebe-se que a frequência encontrada na amostra aqui estudada é mais alta que a frequência geral observada na população brasileira, e isso pode ser explicado pela configuração da população do Rio Grande do Sul. Considerando que a variante CCR5Δ32 é de origem Europeia, é coerente ter sido observada uma frequência aumentada em uma amostra da região sul em comparação com a frequência média apresentada por Solloch et al. (2017).

Esta dissertação também explorou os possíveis papéis da variante CCR5Δ32 em diferentes infecções ao revisar os trabalhos publicados sobre o tema no Brasil. A partir dos dados analisados, além da já descrita influência deste polimorfismo na infecção pelo HIV-1, foram evidentes possíveis atuações do CCR5Δ32 na toxoplasmose e coinfecção por HIV/HBV (**Capítulo 1**).

Além disso, o papel da variante em condições inflamatórias também foi explorado, ressaltando sua importância na susceptibilidade e desfecho clínico da esclerose múltipla e risco de desenvolvimento de periodontite, pré-eclâmpsia e artrite reumatoide. Ademais, a influência do alelo Δ32 na artrite juvenil idiopática foi revisada, ficando evidente a influência da variante sobre a progressão e desfecho clínico piorados em portadores do alelo. Outros contextos também abordados se mostraram complexos e com resultados conflitantes (**Capítulo 1**).

Este trabalho também explorou o possível papel do CCR5 no desenvolvimento de câncer. Revisando trabalhos brasileiros, verificou-se que a variante CCR5Δ32 atua de maneira diversa dependendo do tipo de câncer investigado. Na população brasileira, não foi possível constatar um papel homogêneo do alelo no desenvolvimento tumoral (**Capítulo 1**). No entanto, ao revisitar uma bibliografia internacional, sugerimos esse

importante receptor como um promotor tumoral e instigamos a investigação do eixo CCR5-CD34 para melhor entendimento da interação funcional entre as moléculas e a possível elaboração de terapias tendo tais alvos (**Capítulo 2**).

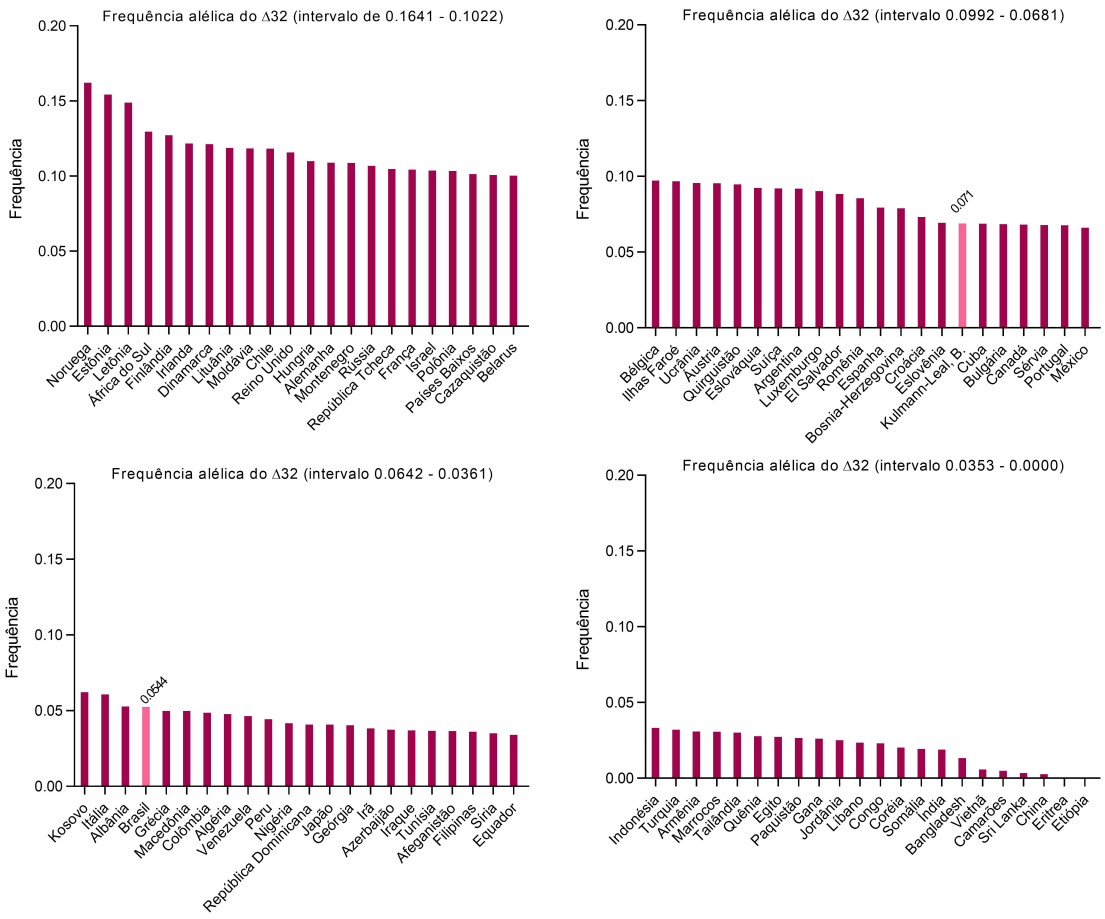

Figura 3. Frequências do alelo CCR5Δ32 ao redor do mundo. Figura da autora desta dissertação, com base em dados descritos por Solloch et al. (2017). Destacadas em rosa, a frequência de 0,071 no sul do Brasil (descrita por este trabalho no **Capítulo 3**) e a frequência média de 0,0544 do alelo na população brasileira em geral (com base em Solloch et al. 2017).

Por fim, esta dissertação avaliou a disponibilidade de doadores de medula ósseos devidamente registrados no REDOME que possuem o genótipo Δ32/Δ32, obtendo uma frequência de 0.76%. Com base nesta frequência, nosso trabalho estimou que pelo menos cerca de 17.500 indivíduos brasileiros estejam aptos a serem doadores para uma possível estratégia de obtenção de remissão sustentada da infecção pelo HIV. Por fim, não foi

encontrada uma associação robusta entre alelos específicos de HLA e a variante Δ32 (valores de *p* perderam a significância após correção por múltiplas comparações). Porém, é possível sugerir que o locus *HLA-B* e seus diferentes alelos possam apresentam uma associação com o CCR5Δ32 em avaliações envolvendo um maior número amostral (**Capítulo 3**).

A presente dissertação perpassou por diversos contextos envolvendo o CCR5 e introduziu novos dados a respeito da disponibilidade de doadores voluntários passíveis de participar de transplante de medula óssea para indivíduos HIV+. Como perspectivas, pretende-se (i) ampliar o número de indivíduos do REDOME genotipados para o alelo Δ32, (ii) realizar análises adicionais acerca do locus *HLA-B* e CCR5Δ32, e (iii) continuar revisando aspectos interessantes a respeito deste receptor de grande importância para o sistema imune e diferentes contextos clínicos.

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ANEXO - APROVAÇÃO EM COMITÊ DE ÉTICA

O trabalho apresentado no **Capítulo 3** desta dissertação teve aprovação no Comitê de Ética do Hospital de Clínicas de Porto Alegre, sob o número: CAAE 20460719.6.0000.5327.