UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE BIOCIÊNCIAS
LABORATÓRIO DE IMUNOPATOLOGIA KEIZO ASAMI
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA APLICADA À SAÚDE

JEAN MOISÉS FERREIRA

REVISÃO SISTEMÁTICA, META-ANÁLISE E ANÁLISES EXPLORATÓRIAS SOBRE A INFLUÊNCIA DE VARIAÇÕES EM GENES DE QUIMIOCINAS E SEUS RECEPTORES NO DESENVOLVIMENTO DA FORMA CARDÍACA EM PACIENTES INFECTADOS PELO *TRYPANOSOMA CRUZI*

Recife

2024

JEAN MOISÉS FERREIRA

REVISÃO SISTEMÁTICA, META-ANÁLISE E ANÁLISES EXPLORATÓRIAS SOBRE A INFLUÊNCIA DE VARIAÇÕES EM GENES DE QUIMIOCINAS E SEUS RECEPTORES NO DESENVOLVIMENTO DA FORMA CARDÍACA EM PACIENTES INFECTADOS PELO *TRYPANOSOMA CRUZI*

Tese apresentada ao Programa de Pós-graduação em Biologia Aplicada à Saúde do Laboratório de Imunopatologia Keizo Asami – LIKA, do Centro de Biociências da Universidade Federal de Pernambuco, como parte dos requisitos parciais para obtenção do título de Doutor em Biologia Aplicada à Saúde.

Orientador: Dr. José Luiz de Lima-Filho

Coorientadora: Drª Elaine Virgínia Martins de Souza Figueiredo

Recife

2024

Catalogação na Fonte:
Bibliotecário:Marcos Antonio Soares da Silva
CRB4/1381

Ferreira,Jean Moisés.
Revisão sistemática, meta-análise e análises sobre a influencia de variações em genes de quimiocinas e seus receptores no desenvolvimento da forma cardíaca em pacientes infectados com Tryipanosoma Cruzi. / Jean Moisés Ferreira – 2024.

115 f. : il., fig.; tab.

Orientador: José Luiz de Lima-Filho.
Coorientadora: Elaine Virgínia Martins de Souza Figueiredo.

Tese (ddoutorado) – Programa de Pós-Graduação em Biologia Aplicada à Saúde do Laboratório de Imunopatologia Keizo Asami – LIKA, do Centro de Biociências da Universidade Federal de Pernambuco, 2024.

Inclui referências.

1. Doença de Chagas. 2. Polimorfismo genético. 3. Quimiocinas. 4. CCR5. 6. Meta-análise. Lima-Filho, José Luiz de. (Orient.). II .Figueiredo, Elaine Virgínia Martins de Souza. (Coorient.). III.Título.

616 CDD (22.ed.) UFPE/CB – 2024-021

JEAN MOISÉS FERREIRA

REVISÃO SISTEMÁTICA, META-ANÁLISE E ANÁLISES EXPLORATÓRIAS SOBRE A INFLUÊNCIA DE VARIAÇÕES EM GENES DE QUIMIOCINAS E SEUS RECEPTORES NO DESENVOLVIMENTO DA FORMA CARDÍACA EM PACIENTES INFECTADOS PELO *TRYPANOSOMA CRUZI*

Tese apresentada ao Programa de Pós-graduação em Biologia Aplicada à Saúde do Laboratório de Imunopatologia Keizo Asami – LIKA, do Centro de Biociências da Universidade Federal de Pernambuco, como parte dos requisitos parciais para obtenção do título de Doutor em Biologia Aplicada à Saúde.

Analisada/Aprovada em: 23/01/2024

BANCA EXAMINADORA

Profº Dr. José Luíz de Lima-Filho, Universidade Federal de Pernambuco

---

Profª Drª. Maria do Carmo Pimentel Batitucci, Universidade Federal do Espírito Santo

---

Profº Dr. Wilson Nadruz Junior, Universidade Estadual de Campinas

---

Profº Dr.Carlos Alberto de Carvalho Fraga, Universidade Federal de Alagoas

---

Profº Dr. Márcio Bezerra Santos, Universidade Federal de Alagoas

AGRADECIMENTOS

Tenho muito a agradecer, pois esse trabalho foi construído por alguém que recebeu o apoio de diversas pessoas, cada uma contribuindo de diferentes maneiras, desde uma única conversa de poucos minutos, à meses de colaboração e substancial.

Agradeço imensamente a toda minha família, em especial, minha mãe Gisleide, minha segunda mãe Maria Vencioneck e seu esposo João Carlos Dutra, e minhas irmãs, Laise e Luana, e minha sobrinha, Sofia, minhas estrelas! Assim como também ao meu companheiro Jean Carlos, uma pessoa maravilhosa. Em alguns momentos permanecem as palavras do cantor Roberto Carlos, “tenho tanto pra lhe falar, mas com palavras não sei dizer, como é grande o meu amor por você(s)!”.

Agradeço muitíssimo aos meus brilhantes amigos, pessoas mais que especiais, que tive a honra de conhecer, conviver e amar. Em especial, Wilson Silva, que mais um amigo, sempre foi um ser humano em todas as qualidades. Também à Bárbara Correia, uma lutadora e vencedora! E a tantos outros que tenho um carinho e orgulho!

Agradeço também ao professor e orientador José Luiz de Lima Filho, por aceitar mais esta empreitada juntamente à professora e coorientadora Elaine Virgínia. Pessoas incríveis, que tive a dádiva de conhecer!

Aos meus colegas do Laboratório Biotecnologia no LIKA, da Universidade Federal de Pernambuco, aos meus colegas do Laboratório Biologia Molecular e Expressão Gênica, da Universidade Federal de Alagoas (especialmente Bárbara Rayssa e Edilson Leite, sem os quais, este trabalho seria apenas um projeto). Aos meus colegas cientistas do Laboratório de Toxicologia Vegetal e Genética, da Universidade Federal do Espírito Santo, aos professores do Laboratório de Imunopatologia Keizo Asami, da Universidade Federal de Pernambuco, aos servidores, pois seus diversificados trabalhos, do mais simples ao mais complexo, possibilitaram esse estudo. Muito, muito, muito obrigado!

“... Você também quer saber a origem do universo? Então somos companheiros de viagem!”

Albedo Kreideprinz (Genshin Impact)

RESUMO

A doença de Chagas (DC) é uma doença tropical negligenciada (DTN), que afeta milhões de pacientes em todo o mundo, desencadeando milhares de mortes por ano. Muitas investigações têm associado os aspectos genéticos do paciente com a infecção e a evolução da DC. Há muita informação disponível na literatura sobre o papel das variantes genéticas e o desfecho da DC. Entre os genes ligados à imunidade já analisados, destacam-se as quimiocinas e seus receptores, como o *CCR5* humano, pelo seu papel funcional em processos imunológicos que pode ser influenciado por polimorfismos genéticos. Entretanto, os estudos apresentam resultados inconclusivos ou contraditórios sobre essas variantes, principalmente as do *CCR5*. Nosso trabalho objetivou realizar uma revisão sistemática e meta-análise para condensar as informações da literatura sobre a relação da DC e as variantes genéticas de quimiocinas e seus receptores e estimar a influência dessas variantes através de análises exploratórias. Nossa estratégia de busca baseada em PICOS foi usada para eleger os retornos nas bases de dados Periódicos CAPES, SciELO, PubMed, ScienceDirect, Web of Science e Scopus. A análise de qualidade dos artigos incluídos foi realizada usando o STREGA checklist. A meta-análise foi conduzida, juntamente às análises de componentes principais (PCA) para estimar as relações das variantes do *CCR5* humano e a DC. A heterogeneidade dos grupos foi avaliada através da inspeção dos gráficos do tipo *funnel plot* gerados. Os polimorfismos encontrados foram analisados pela ferramenta SNP2TFBS para identificar possíveis variantes que influenciam a interação com sítios de ligação do gene. Também utilizamos a ferramenta GTEx, associadas à base de dados de expressão genética. A revisão foi submetida à plataforma PROSPERO. Nossos resultados identificaram onze polimorfismos pertencentes ao *CCR5* (rs2856758, rs2734648, rs1799987, rs1799988, rs41469351, rs1800023, rs1800024, Δ32/rs333, rs3176763, rs3087253 e rs11575815) e analisados em onze diferentes trabalhos, além de 14 diferentes polimorfismos em genes de quimiocinas (*CCL2*, *CCL4*, *CCL5*, *CCL17*, *CCL19*, *CXCL8*, *CXCL9*, *CXCL10* e *CXCR3)*, e de outros receptores (*CCR1* e *CCR2)*. Os trabalhos foram performados na Argentina, Brasil, Espanha, Colômbia e Venezuela, incluindo pacientes Argentinos, Brasileiros, Colombianos, Peruanos e Venezuelanos, publicados entre 2001-2019. Dos SNPs pertencentes ao *CCR5* humano, oito foram passíveis de meta-análise, dos quais foram associados ao desenvolvimento da forma

cardíaca da DC rs1799987 (G/G e G/A, modelo dominante e G/G no modelo recessivo), rs2856758 (A/G em codominância), rs2734648 (T/T e T/G no modelo dominante), rs1799988 (T/T tanto em codominância como em recessividade), rs1800023 (alelo G, o genótipo G/G nos modelos de codominância e recessividade, o G/G e G/A no dominante) e rs1800024 (alelo T) em diferentes comparações. As análises de PCA foram capazes de indicar as relações entre os alelos e os genótipos dos polimorfismos em cada grupo de comparação. A SNP2TFBS identificou o rs1800023 como influenciador do fator de transcrição Spi1. Uma correlação foi estabelecida entre o os alelos associados à forma cardíaca da DC nesta revisão, integrantes do haplótipo C do gene *CCR5* (HHC-TGTG) e a forma cardíaca da DC. Em relação aos outros genes analisados, apenas três SNPs puderam ser meta-analisados. Nenhuma associação foi encontrada entre o rs1799864 (*CCR2*) e a DC. Entretanto, foram associados à forma cardíaca da DC os SNPs rs1024611 (*CCL2*, genótipo G/G no modelo de recessividade) e rs2107538 (*CCL5*, alelo T, o genótipo C/T no modelo de codominância e os carreadores T no modelo de dominância). O banco de dados GTEx indicou que as variantes rs1024611 (*CCL2*) e rs2107538 (*CCL5*) associadas à forma cardíaca são também responsáveis pelo quantitativo mais alto de transcrição dos respectivos genes. A ferramenta SNP2TFBS identificou quatro SNPs capazes de influenciarem fatores de transcrição: rs3136672 (CCR1) e rs2280964 (CXCR3), além dos já citados rs1024611 (CCL2) e rs2107538 (CCL5). Em suma, este segundo conjunto de dados, hipotetizamos que o volume de mRNA produzido pela influência dos genótipos G/G do SNP rs1024611 (*CCL2*), e C/T e T/T do SNP rs2107538 (*CCL5*) são consequentemente expressos, provocando um recrutamento exacerbado de células com capacidades inflamatórias, e que isto a longo prazo, permite o dano cardíaco nos pacientes.

Palavras-chave: Doença de Chagas; Polimorfismo genético; Quimiocinas; CCR5; Meta-análise.

ABSTRACT

Chagas disease (CD) is a neglected disease that affects millions of patients worldwide, causing thousands of deaths per year. Many investigations have associated the genetics of the patient with the infection and evolution of CD. There is much information available in the literature about the role of genetic variants and the outcome of CD. Among the genes linked to immunity already analyzed, chemokines and their receptors stand out, such as human CCR5, due to their functional role in immunological processes, which can be influenced by genetic polymorphisms. However, despite the numerous investigations performed, some studies have inconclusive or contradictory results on these variants, especially those of CCR5. Our work aimed to carry out a systematic review to condense the information in the literature on the relationship between CD and the genetic variants of chemokines and their receptors, as well as to estimate the influence of these variants through exploratory analyses. Our PICOS-based search strategy was used to select the returns in the CAPES, SciELO, PubMed, ScienceDirect, Web of Science and Scopus databases. The quality analysis of the included articles was performed using the STREGA checklist. A meta-analysis was conducted along with principal component analyzes (PCA) to estimate the relationships of human CCR5 variants and CD. The heterogeneity of the groups was assessed by inspecting the generated funnel plot graphs. The polymorphisms found were analyzed using the SNP2TFBS tool to identify possible variants that influence the interaction with gene binding sites. We also used the GTEx tool associated with the gene expression database. The review was submitted to the PROSPERO platform. Our results identified eleven studied polymorphisms belonging to CCR5 (rs2856758, rs2734648, rs1799987, rs1799988, rs41469351, rs1800023, rs1800024, Δ32/rs333, rs3176763, rs3087253 and rs11575815) analyzed in eleven different works, 14 different polymorphisms in chemokine genes (CCL2, CCL4, CCL5, CCL17, CCL19, CXCL8, CXCL9, CXCL10 and CXCR3), and other receptors (CCR1 and CCR2). The articles included in our work were performed in Argentina, Brazil, Spain, Colombia and Venezuela, including Argentine, Brazilian, Colombian, Peruvian and Venezuelan patients, published between 2001-2019. Among SNPs belonging to human CCR5, eight were amenable to meta-analysis, and the polymorphisms rs1799987 (G/G and G/A, dominant model and G/G in the recessive model), rs2856758 were associated with the development of the cardiac

form of CD (A/G in codominance), rs2734648 (T/T and T/G in the dominant model), rs1799988 (T/T in both codominance and recessiveness), rs1800023 (G allele, the G/G genotype in the codominance and recessive models , the G/G and G/A in the dominant) and rs1800024 (T allele) in different comparisons. PCA analyzes were able to indicate the relationships between the alleles and genotypes of the polymorphisms in each comparison group. SNP2TFBS identified rs1800023 as an influencer of the Spi1 transcription factor. A correlation was established between the alleles associated with the cardiac form of CD in this review, members of the C haplotype C of the CCR5 gene (HHC-TGTG) and the cardiac form of CD. Regarding the other genes, three SNPs were meta-analyzed, and although no association was found between rs1799864 (CCR2), the SNPs rs1024611 (CCL2, G/G genotype in the recessive model) were associated with the cardiac form of CD. and rs2107538 (CCL5, T allele, the C/T genotype in the codominance model, and the T carriers in the dominance model). The GTEx database indicated that the rs1024611 (CCL2) and rs2107538 (CCL5) variants associated with the cardiac form are also responsible for the highest amount of transcription of the respective genes. The SNP2TFBS tool identified four SNPs capable of influencing transcription factors: SNP rs3136672 (CCR1) and SNP rs2280964 (CXCR3), in addition to the aforementioned SNPs rs1024611 (CCL2) and rs2107538 (CCL5). In short, it was hypothesized that the mRNA volume influenced by the G/G genotypes of the CCL2 SNP rs1024611, and the C/T and T/T of the CCL5 SNP rs2107538 are consequently expressed, causing an exacerbated recruitment of cells with inflammatory capabilities, and in the long term leads to heart damage. We emphasize that these data must be accepted with caution and that new studies must be carried out in order to confirm these interpretations.

Keywords*: Chagas disease; Genetic polymorphism; Chemokines; CCR5; Meta-analysis.*

LISTA DE FIGURAS E ILUSTRAÇÕES

Revisão de Literatura

Resultados

Capítulo I

Capítulo II

LISTA DE TABELAS

Resultados

Capítulo I

Capítulo II

LISTA DE ABREVIATURAS E SIGLAS

DC	-Doença de Chagas (*Chagas disease*)
DTNs	-Doenças tropicais negligenciadas
T. cruzi	-Abreviação de *Trypanosoma cruzi*
ELISA	-Do inglês, *Enzyme Linked Immuno Sorbent Assay*
CCC	-Cardiomiopatia crônica de Chagas
CCR	-Do inglês, *CC chemokine receptor*
CCL	-Do inglês, *chemokine (C-C motif) ligand*
CP	-Agrupamento de pacientes de Chagas (*Chagas patients*)
nonCP	-Agrupamento de indivíduos não infectados com *T. cruzi* (*non-Chagas patients*)
SympCP	-Agrupamento de pacientes de Chagas sintomáticos (*Symptomatics Chagas patients*)
SNP	Do inglês, *Single Nucleotide Polymorphism*
UndCP	-Agrupamento de pacientes de Chagas assintomáticos (*Asymptomatic Chagas patients*)
CardCP	-Agrupamento de pacientes de Chagas com forma cardíaca (*Cardiac Chagas patients*)
DigCP	-Agrupamento de pacientes de Chagas com forma digestiva (*Digestive Chagas patients*)
PCA	-Análise de Componente Principal (*Principal component analysis*)
IHA	-Ensaio de Inibição da hemaglutinação (*Inhibition of hemagglutination*)
IIF	-Ensaio de imunofluorescência indireta (*Indirect immunofluorescence*)
HHC	-Haplótipo humano tipo C (haplótipo pertencente ao *CCR5*)
SARS-CoV-2	-Síndrome respiratória aguda grave do coronavirus 2 (*Coronavirus 2 severe acute respiratory syndrome*)

SUMÁRIO

1 INTRODUÇÃO

A doença de Chagas é uma doença tropical negligenciada (DTN), de evolução crônica e que pode levar à morte, sendo endêmica em todos os países onde os insetos transmissores do *Trypanosoma cruzi* (agente etiológico da doença) existem. A doença pode ser dividida entre fase aguda e crônica. Na fase aguda há o reconhecimento da infecção do parasita flagelado seguida de resposta imune inata. Após a fase aguda da infecção, o paciente pode entrar na fase crônica, a qual pode apresentar-se de diversas formas, conduzindo a problemas associados à cardiomiopatias, ou desordens do sistema digestório, ou até mesmo ambas concomitantemente, ou ainda não apresentar danos aos órgão/sistemas (indeterminados). Algumas hipóteses têm sido supostas e investigadas para entender os fatores determinantes que contribuem com cada um dos desfechos da fase crônica da doença de Chagas (KAYA, 2021; LANNES-VIEIRA *et al.*, 2017; PÉREZ-MOLINA; MOLINA, 2018).

Epidemiologicamente, os fatores mais evidentemente correlacionados com a infecção são os fatores socioeconômicos, enquanto o desfecho da doença aparentemente pode ser influenciado por vários agentes, como a variabilidade genética do parasito (influenciando seu tropismo); a modulação autoimune deflagrada pelo *Trypanosoma cruzi* (que poderia incitar uma resposta imune cruzada); e a própria genética do hospedeiro. Esta última, parece ter uma forte influência no curso da infecção em relação ao recrutamento de células efetoras da imunidade e produção de citocinas e quimiocinas, que atuam em microambientes inflamatórios, causado danos teciduais aos órgãos do hospedeiro, como por exemplo, o coração. Esta última hipótese tem sido analisada com entusiasmo no estudo da interação parasito-hospedeiro, e tem revelado importantes diferenças entre grupos de pacientes crônicos (com danos aos órgãos/sistemas) e assintomáticos (ACEVEDO; GIRARD; GOMEZ, 2018; ECHAVARRÍA *et al.*, 2021; NORMAN; LÓPEZ-VÉLEZ, 2019; SIMÕES *et al.*, 2018).

Por isso, diversos trabalhos analisaram múltiplas variações genéticas, como polimorfismos em genes associados à imunidade inata e à resposta imune adaptativa, como as variações genéticas presentes em genes de quimiocinas e seus receptores, fazendo com que um grande volume de informação sobre a influência de variantes genéticas fosse disponibilizado. Esses genes são fundamentais na integração de vários processos imunológicos. O gene *CCR5,* que decodifica a proteína "*CC chemokine receptor 5*", ou "CCR5", por exemplo, é expressa em linfócitos T, tendo função direta

no recrutamento de células efetoras. Diversas variantes de quimiocinas e seus receptores, incluindo o *CCR5* já foram associadas à doença de Chagas, entretanto algumas chamam atenção por apresentarem resultados inconclusivos ou contraditórios em diferentes estudos, tornando difícil concluir suas reais contribuições na doença. Análises de expressão gênica e bioinformática têm colocado ainda o sistema quimiocina-receptor como um elemento fundamental no desenvolvimento da doença de Chagas (ACOSTA-HERRERA *et al.*, 2019; CARVALHO *et al.*, 2019; DE OLIVEIRA *et al.*, BROCHET *et al.*, 2022; FERREIRA *et al.*, 2017; LAUGIER *et al.*, 2020).

Os resultados contraditórios ou inconclusivos dos trabalhos publicados podem ter sido influenciados por questões tais como populações com diferentes *backgrounds* genéticos, tamanho amostral insuficiente, e grupo controle inadequado (não infectados). Todas essas inconsistências podem ser sanadas agrupando populações com *backgroud* genético relacionado e utilizando pacientes crônicos indeterminados como controle, sendo este grupo compostos por indivíduos que mesmo com a confirmação da infecção não desenvolveram formas sintomáticas da doença (pacientes infectados sem danos aos órgão/sistemas) (BATISTA *et al.*, 2018; CALZADA *et al.*, 2001; FLÓREZ; MARTÍN; GONZÁLEZ, 2012; MACHUCA et al., 2014; FRADE *et al.*, 2013; LIMA *et al.*, 2018).

Uma vez que o perfil genético do paciente pode influenciar no desfecho da doença de Chagas, e há falta de clareza no envolvimento de variantes genéticas associadas à esta enfermidade, como é o caso dessas variantes em genes de quimiocinas e seus receptores, é necessária uma análise exploratória que estreite a influência dos achados disponibilizados na literatura. Assim, este trabalho objetivou localizar os dados sobre variáveis genéticas de quimiocinas e seus receptores de pacientes crônicos da doença de Chagas, compilar essas informações de maneira prospectiva e sintetizar novos conhecimentos, utilizando como ponto de partida esses dados através de ferramentas adequadas. Os dados gerados poderão esclarecer melhor os papéis dessas variantes genéticas no curso da doença de Chagas.

1.1 OBJETIVOS

1.1.1 Objetivo geral

Realizar uma revisão sistemática de literatura e meta-análise sobre variantes genéticas de quimiocinas e seus receptores associados à forma cardíaca da doença de Chagas (DC), bem como análises exploratórias do envolvimento destas variantes na doença.

1.1.2 Objetivos específicos

- Realizar uma revisão sistemática da literatura sobre variantes genéticas associadas à DC;
- Sintetizar os principais resultados dos artigos (agrupados) sobre variantes genéticas associadas à DC;
- Realizar meta-análises de variantes genéticas disponíveis nos estudos;
- Utilizar ferramentas de análises estatísticas para estreitar o conhecimento sobre a influências das variantes na DC.

2 REVISÃO DE LITERATURA

2.1 DOENÇAS TROPICAIS NEGLIGENCIADAS

As doenças tropicais negligenciadas (DTNs) são comuns entre as populações mais pobres do mundo, que vivem em países em desenvolvimento das regiões tropicais e subtropicais, com recursos limitados, muitas vezes refletidos nas infraestruturas médicas deficientes e saneamento abaixo do padrão (WENG; CHEN; WANG, 2018). Populações com DTNs endêmicas tendem a viver próximas a animais domésticos e gado, o que agrava a prevalência e a propagação dessas doenças (COHEN *et al.*, 2016; WHO, 2022). Esse grupo heterogêneo de doenças tende a compartilhar uma significante sobreposição geográfica com áreas das conhecidas "três grandes doenças": HIV/Aids, malária e tuberculose. Além disso, há evidências clínicas que apontam que DTNs podem aumentar a susceptibilidade a essas três doenças, ou alternativamente piorar o curso dessas doenças em pacientes já infectados (SIMON, 2016; VEALE, 2019).

Embora algumas organizações e especialistas em doenças infecciosas definem as DTNs de formas diferentes, a Organização Mundial da Saúde (OMS) identificou 20 DTNs prioritárias causadas majoritariamente por cinco classes de patógenos em 2011: vírus, bactérias, protozoários, fungos e helmintos. Essas doenças são a doença de Chagas, tripanossomíase africana (doença do sono), leishmaniose, úlcera de Buruli, lepra (hanseníase), tracoma, cisticercose/teníase, bouba (framboesia) e outras treponematoses endêmicas, dracunculíase (doença do verme de Guiné), equinococose, trematodiases de origem alimentar, filariose linfática, oncocercose (cegueira dos rios), esquistossomose, geohelmintíases, dengue e chikungunya, rabias (raivas), micetoma, cromoblastomicose e outras micoses profundas, escabiose e outras ectoparasitoses, bem como picada de cobra (envenenamento) (WHO, 2022). Estas três últimas foram adicionadas à listagem em 2017 (WENG; CHEN; WANG, 2018).

Além da doença em si, os infectados por DTNs também enfrentam situações decorrentes das infecções, como incapacidades, estigmatização, exclusão social e discriminação. A soma dessas condições pode colocar uma pressão financeira considerável sobre os pacientes e suas famílias, o que agrava a situação das famílias, uma vez que as DTNs se apresentam mais constantemente em comunidades empobrecidas. Além disso, a OMS indica que mais de um bilhão de pessoas sofrem de

uma ou mais DTNs em 149 países, o que causa cerca de 35.000 mortes por dia em todo o mundo (COHEN *et al.*, 2016; WHO, 2022).

Em termos de incapacidades de longo prazo, enfermidades e mortes, as DTNs impõem um fardo pesado com graves consequências sociais e econômicas no mundo em desenvolvimento. As DTNs custam às comunidades em desenvolvimento o equivalente a bilhões de dólares americanos a cada ano em custos diretos de saúde, perda de produtividade e redução dos níveis socioeconômico e educacional. Por isso, a falta de atenção dada a essas doenças é desproporcional à sua importância mundial. (WENG; CHEN; WANG, 2018; WHO, 2022). DTNs têm sido amplamente ignorados por fabricantes de medicamentos e formuladores de políticas públicas por décadas. Como resultado, o controle das DTNs ainda é inadequado e extremamente difícil hoje (WENG; CHEN; WANG, 2018).

A transmissão autóctone de doenças parasitárias e bacterianas negligenciadas têm se tornado cada vez mais relatada em algumas regiões do mundo. A ocorrência de tais DTNs nessas regiões não é por acaso, mas o reflexo de mudanças rápidas associadas às forças modernas e globalizantes que incluem rápidas expansões na população, urbanização, migrações humanas, alterações nos padrões de transportes, mudanças climáticas, taxas de vacinação em declínio acentuado e um novo paradigma de pobreza conhecido como “saúde de mármore”. Entre as DTNs que vêm se alastrando, principalmente nas Américas, estão doenças virais transmitidas por vetores e infecções parasitárias, incluindo doença de Chagas, tricomoníase e possivelmente leishmaniose e toxocaríase, bem como riquetsioses do grupo tifo (HOTEZ, 2018).

A OMS estabeleceu metas de eliminação de cinco DTNs (hanseníase, doença do sono, tracoma ofuscante, doença do verme da Guiné e filariose linfática) e controlar outras cinco (esquistossomose, geohelmintíases, leishmaniose visceral, oncocercose e doença de Chagas) até 2030 (WHO, 2007). No entanto, além da inadequação das condições de saneamento, a falta de medicamentos seguros, eficazes e acessíveis também é identificado como um fator-chave que pode dificultar o alcance dessas metas (WENG; CHEN; WANG, 2018).

Historicamente, as DTNs foram ignoradas pela indústria farmacêutica e cobertura de saúde pública em geral. A maioria das pessoas infectadas com DTNs são confrontadas com más condições sanitárias e têm nutrição e cuidados de saúde

inadequados. Eles são incapazes de pagar pelo tratamento, mesmo que disponível (WHO, 2022). Para a indústria farmacêutica, medicamentos para mitigar DTNs não trazem retorno econômico suficiente, de tal forma que existe pouco incentivo para estimular seu interesse comercial por pesquisa e desenvolvimento (P&D). Como resultado, poucos agentes terapêuticos novos foram lançados para DTNs nos últimos anos (WENG; CHEN; WANG, 2018).

2.2 PRINCIPAIS DOENÇAS TROPICAIS NEGLIGENCIADAS DA AMÉRICA LATINA E CARIBE

Segundo Fontecha e colaboradores, em sua publicação (FONTECHA; SÁNCHEZ; ORTIZ, 2021), o maior número de publicações científicas sobre DTNs da última década (2010-2019) advindas da América Latina e Caribe (LAC) foram pautadas principalmente em dengue (n=18.186), leishmaniose (n=16.589), e doença de Chagas (n=9.253), o que mostra a preocupação dos países desse bloco em relação a essas doenças. Além das três já citadas, outras doenças que foram indicadas foram febre da zika (n=9074), esquistossomose (n=7678), tracoma (n=5337) e febre da chikungunya (n=4969), respectivamente. Estes autores ainda destacam que os Estados Unidos da América são os maiores contribuidores em pesquisas sobre DTNs da última década, entretanto, o Brasil foi o país que mais contribuiu em pesquisas sobre a doença de Chagas, cromomicose, leishmaniose e hanseníase na última década.

Através desses dados é possível observar que há dois grandes grupos de doenças mais estudadas: arboviroses e doenças causadas por tripanossomatídeos. O grupo dos arbovírus (dengue, zika e chikungunya) foi o mais analisado na última década com mais de 32 mil publicações entre os sete tópicos mais presentes (FONTECHA; SÁNCHEZ; ORTIZ, 2021). As arboviroses tornaram-se o tipo mais destacado de doença infecciosa do mundo, com grande contribuição da febre da dengue, já que sua incidência e prevalência têm aumentado nas áreas endêmicas das regiões tropicais e subtropicais (LASERNA *et al.*, 2018; TORRES-SIGNES; DIP, 2021). Assim como o vírus da dengue (que possui quatro sorotipos: DEN-1, DEN-2, DEN-3 e DEN-4), os vírus causadores da febre da zika (ZIKV) e da chikungunya (CHIKV) podem ser transmitidos por mosquitos do gênero Aedes (*A. aegypti* e *A. albopictus*), e inclusive causar coinfecções nos pacientes (BORCHERING *et al.*, 2019; KAUR *et al.*, 2018).

Peculiarmente, com a recente pandemia de COVID-19, casos de coinfecção do SARS-CoV-2 e vírus da dengue têm sido notificados, e os pacientes têm apresentado trombocitopenia, linfocitopenia, transaminases e leucopenia, além disso, casos de coinfecção podem incluir choque séptico, síndrome respiratória aguda e falência de múltiplos órgãos, levando à morte (TSHETEN *et al.*, 2021). Esse tipo de coinfecção pode se tornar um grande desafio para as Américas até o fim da pandemia, já que a dengue está presente em áreas de infraestrutura precária e os recursos humanos e técnicos escassos (LASERNA *et al.*, 2018; MASYENI *et al.*, 2021; MIAH; HUSNA, 2021).

Além das arboviroses, os protozoários parasitários (Tripanosomatídeos) também é um grupo etiológico em destaque entre as DTNs. No mundo, a leishmaniose (causada por múltiplas espécies de *Leishmania*), a doença de Chagas, causada pelo *Trypanosoma cruzi* (*T. cruzi*) e a tripanossomíase humana africana (HAT - causada por *Trypanosoma brucei gambiense* ou *Trypanosoma brucei rhodesiense*) são as enfermidades que representam o maior número de mortes entre todas as doenças negligenciadas parasitárias advindas de protozoários (VARIKUTI *et al.*, 2018).

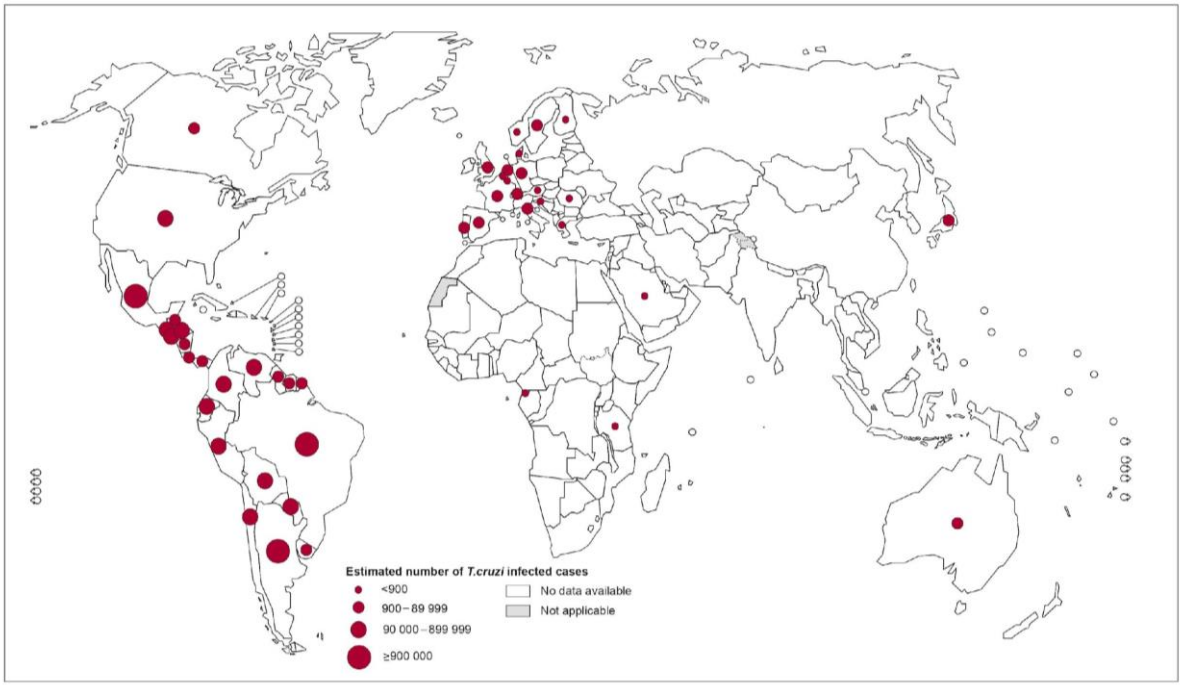
Os dados sobre esses dois grupos de DTNs são importantes para entender a gravidade dessas infecções. É provável que toda a população mundial que vive abaixo da linha de pobreza (delimitada pelo Banco Mundial pelo gasto por pessoa de US$ 1,90 ou menos por dia) esteja infectada com uma ou mais das 20 DTNs destacadas pela OMS, correspondendo a pelo menos 10% da população global (HOTEZ *et al.*, 2020). Vários países da América Latina se enquadram nesse aspecto (BARGAIN; AMINJONOV, 2021). Dessa maneira, concebe-se que o reconhecimento e tratamento em massa para as DTNs é importante alvo para atingir as metas da OMS para a cobertura universal de saúde (HOTEZ *et al.*, 2020).

2.3 ASPECTOS GERAIS SOBRE A DOENÇA DE CHAGAS (DC)

A Tripanossomíase americana, também conhecida como doença de Chagas (DC) é uma enfermidade causada pelo parasito protozoário *Trypanosoma cruzi* (*T. cruzi*). Apesar desse gênero possuir outras variedades capazes de infectar humanos, apenas a espécie *T. cruzi* é capaz de provocar a doença. A DC é endêmica em toda a América do Sul e Central, incluindo o México, mas não nas ilhas do Caribe ou em Porto Rico. A

Bolívia tem as maiores taxas de prevalência e incidência. Cerca de 28.000 novos casos incidem anualmente nas Américas, e, segundo a OMS, seis milhões de pessoas já são afetadas pela DC. Embora as infecções pelo *T. cruzi* ocorrerem principalmente nas zonas rurais das Américas Central e do Sul, devido a diferentes modos de transmissão, os Estados Unidos têm até 300.000 casos. A infecção torna-se crônica ao longo da vida do paciente, e todos os grupos etários são atingidos (ECHAVARRÍA et al., 2021; KAYA, 2021; NORMAN; LÓPEZ-VÉLEZ, 2019; WHO, 2022). Uma representação da distribuição global da DC está presente na Figura 1.

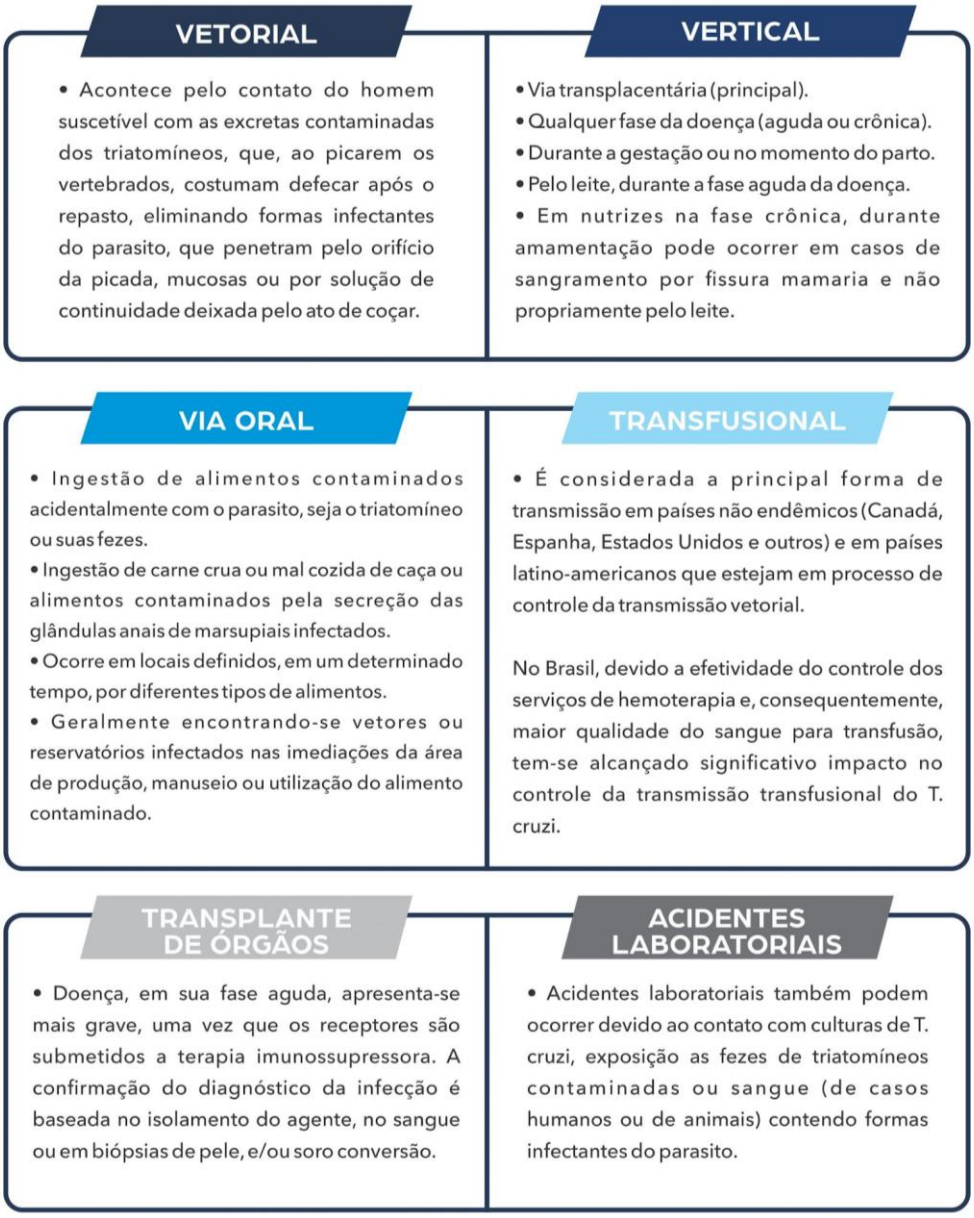
Figura 1 – Distribuição global dos casos de DC, estimativas oficiais de 2018.



Fonte: Adaptado de WHO (2022).

A doença de Chagas ocorre quando o *T. cruzi* é transmitido de alguma maneira ao hospedeiro humano (KAYA, 2021; NORMAN; LÓPEZ-VÉLEZ, 2019). A Figura 2 apresenta um resumo sobre os diferentes modos de transmissão do *T. cruzi*.

Figura 2 – Tipos e características de transmissão do *Tripanossoma cruzi*.



Fonte: Adaptado de: http://www.saude.pa.gov.br/a-secretaria/diretorias/dvs/chagas/doenca-de-chagas-2/; acesso em outubro de 2022.

A anamnese para a DC pode incluir perguntas sobre dificuldades do paciente em engolir e evacuar. O diagnóstico para novos casos de DC pode ser auxiliado com a verificação de inchaço ao redor da picada do inseto transmissor, como uma lesão na pele ou um inchaço arroxeado das pálpebras de um olho (o chamado sinal de Romaña). Exames cardíacos e pulmonares para insuficiência cardíaca congestiva são

recomendados. Já os exames laboratoriais para confirmação da infecção podem contar com a verificação da infecção no esfregaço de sangue na fase aguda (uma vez que pode ser vista com facilidade) e pode ser repetido após 15 dias, enquanto na fase crônica, testes sorológicos (ELISA – do inglês, *Enzyme Linked Immuno Sorbent Assay*) podem ser necessários (KAYA, 2021; NORMAN; LÓPEZ-VÉLEZ, 2019; WHO, 2022).

Em relação ao tratamento da DC, há dois medicamentos que são quase 100% eficazes na cura da doença se administrados logo após a infecção: benzonidazol e nifurtimox, podendo ser administrados inclusive nos casos de transmissão congênita. Entretanto, ambos os medicamentos antiparasitários têm suas eficácias diminuídas quanto mais tempo a pessoa está infectada, e as reações adversas desses medicamentos são mais frequentes em pacientes com idade avançada. O tratamento também é indicado para aqueles em que a infecção foi reativada devido à imunossupressão e para pacientes em fase crônica inicial (KAYA, 2021; NORMAN; LÓPEZ-VÉLEZ, 2019; WHO, 2022).

Segundo a OMS, adultos infectados, especialmente aqueles sem sintomas, devem receber tratamento porque o tratamento antiparasitário também pode prevenir ou conter a progressão da doença e prevenir a transmissão congênita em mulheres grávidas, assim como deve ser evitado por essas mulheres (pelo risco de teratonicidade) ou por pessoas com insuficiência renal ou hepática a administração de benzonidazol e nifurtimox. O nifurtimox também é contraindicado para pessoas com antecedentes de distúrbios neurológicos ou psiquiátricos. Em outros casos, os benefícios potenciais da medicação na prevenção ou retardo do desenvolvimento da doença de Chagas devem ser ponderados em relação à duração do tratamento (até 2 meses) e possíveis reações adversas (ocorrendo em até 40% dos pacientes adultos tratados). Além disso, pode ser necessário tratamento específico para diferentes manifestações da doença, como manifestações cardíacas, digestivas ou neurológicas (WHO, 2022).

2.4 AGENTE ETIOLÓGICO DA DC: *T. cruzi*, CLASSIFICAÇÃO E CICLO DE VIDA

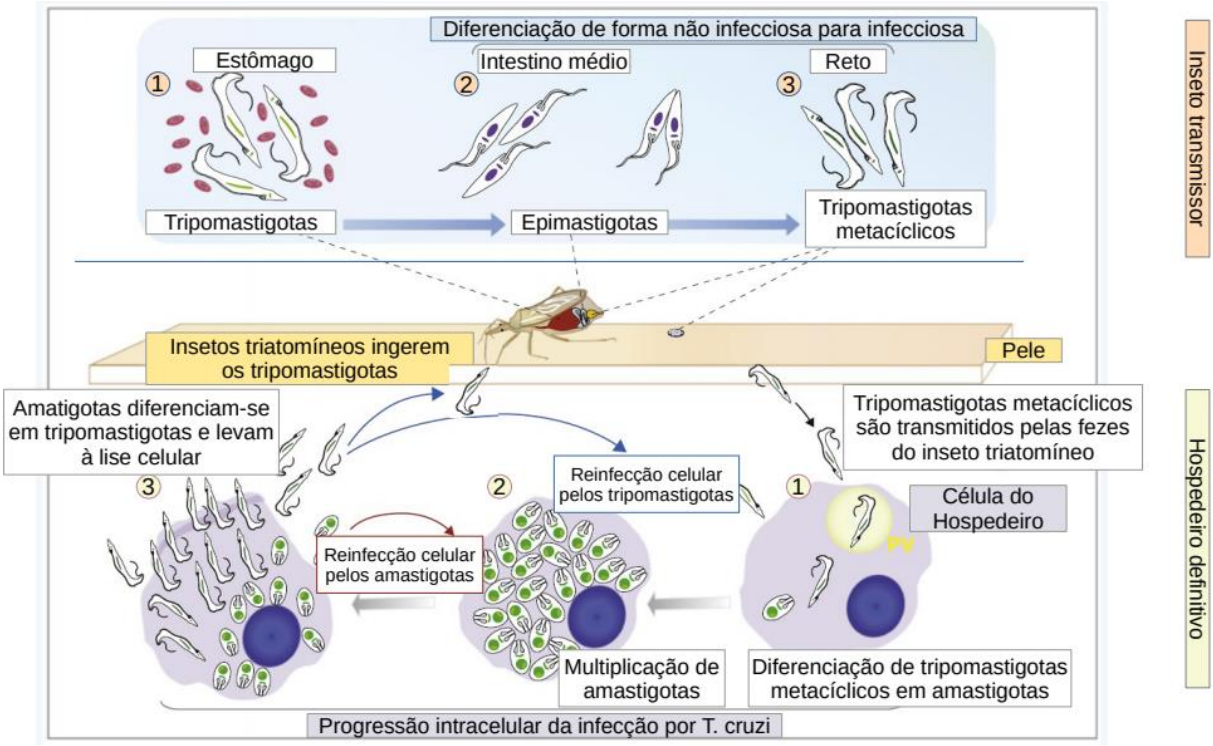
Brevemente, a taxonomia e classificação do *T. cruzi* é conhecida da seguinte maneira: REINO: Protozoa, FILO: Euglenozoa, CLASSE: Kinetoplastea, ORDEM: Kinetoplastida, FAMÍLIA: Trypanosomatidae, GÊNERO: *Trypanosoma*, ESPÉCIE: *T. cruzi*

O *T. cruzi* é transmitido principalmente por insetos reduvídeos (pertencentes à família Reduviidae, principalmente dos gêneros *Triatoma*, *Rhodnius* e *Panstrongylus*) hematófagos em países endêmicos. No ciclo biológico, o *T. cruzi* apresenta duas etapas (em hospedeiro invertebrado e hospedeiro vertebrado) em três formas evolutivas: amastigota, epimastigota e tripomastigota, e se desenvolve alternadamente em mamíferos de várias classes e insetos reduvídeos (ACEVEDO; GIRARD; GOMEZ, 2018; ARIAS-GIRALDO *et al.*, 2020; SALASSA; ROMANO, 2019). Os protozoários *T. cruzi* na forma de tripomastigotas metacíclicos são liberados nas fezes de reduvídeos durante o repasto sanguíneo, então esses tripomastigotas metacíclicos penetram em um hospedeiro mamífero através de feridas na pele (inclusive da picada) ou membranas mucosas e invadem as células circundantes (ACEVEDO; GIRARD; GOMEZ, 2018; MARTÍN-ESCOLANO *et al.*, 2022; MORETTI; MORTARA; SCHENKMAN, 2020).

Após a invasão celular, os tripomastigotas metacíclicos são contidos dentro de um vacúolo parasitóforo, de onde escapam, transformam-se em amastigotas e se multiplicam no citosol. Mais tarde, após a divisão binária, os amastigotas se diferenciam novamente em tripomastigotas com alta mobilidade, que são liberados após a lise celular (MORETTI; MORTARA; SCHENKMAN, 2020; SALASSA; ROMANO, 2019).

Após a lise celular ter ocorrido, os novos tripomastigotas podem então infectar células vizinhas, migrar para diferentes tecidos ou ser ingeridos por um inseto vetor. Os parasitos nos tecidos, associados a uma resposta imune, contribuem para os sintomas crônicos da doença. Espécies reativas de oxigênio (ROS), entre outros fatores, desempenham um papel importante durante a multiplicação do parasita e a transformação entre estágios (MARTÍN-ESCOLANO *et al.*, 2022; MORETTI; MORTARA; SCHENKMAN, 2020; SALASSA; ROMANO, 2019). Um resumo gráfico das fases e das transformações do *T. cruzi* no hospedeiro intermediário e definitivo, insetos e ser humano, respectivamente, pode ser visualizado na Figura 3.

Figura 3 – Formas do protozoário *T. cruzi* e suas diferenciações em cada hospedeiro (intermediário e definitivo).



Fonte: Adaptado de MORETTI; MORTARA; SCHENKMAN (2020).

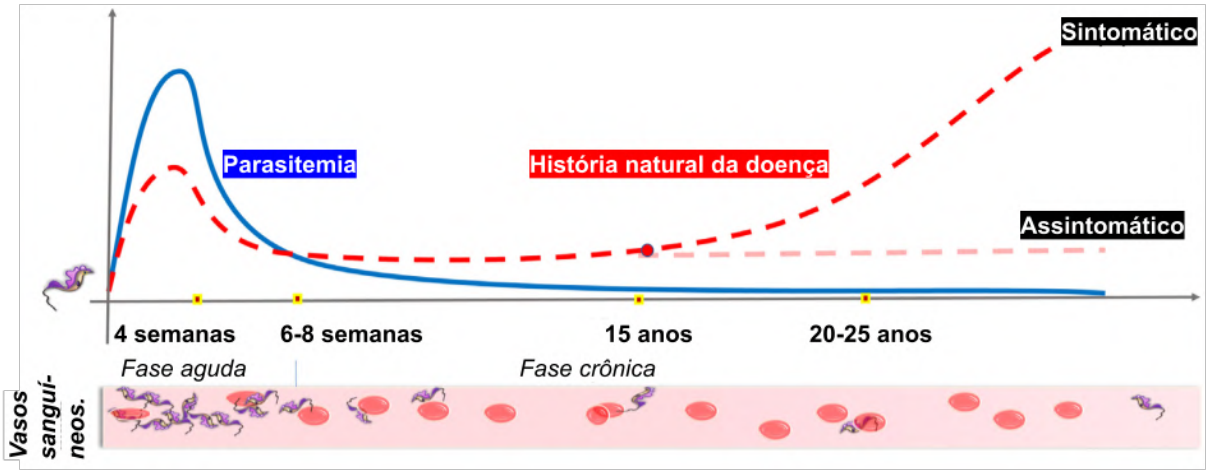
2.5 SINTOMATOLOGIA DA DOENÇA DE CHAGAS

2.5.1 Caracterização da fase aguda

A fase aguda começa entre uma a duas semanas após a picada do inseto, apresentando febre e linfadenopatia difusa (inchaço dos nódulos linfáticos, modificando sua consistência e/ou tamanho e/ou quantidade), e embora raros, podem acontecer casos letais de miocardite e meningoencefalite (KAYA, 2021; WHO, 2022). Na fase aguda da doença de Chagas, na maioria dos casos sintomáticos, observa-se febre, dor muscular, irritabilidade, conjuntivite, anorexia, vômitos, diarreia, linfadenopatia, hepatoesplenomegalia, miocardite, anemia, trombocitopenia, leucocitose com predominância de linfócitos, funcionamento anormal do fígado e níveis elevados de enzimas cardíacas (LANNES-VIEIRA *et al.*, 2017; PÉREZ-MOLINA; MOLINA, 2018).

Como resultado da infecção pelo *T. cruzi*, o hospedeiro desenvolve ao final de algumas semanas uma resposta imune específica que, no entanto, não é completamente eficaz para que os parasitas sejam inteiramente erradicados. Esta resposta imune forte e específica associada à resposta imune inata controla a infecção aguda evoluindo para a fase crônica (LANNES-VIEIRA *et al.*, 2017; PÉREZ-MOLINA; MOLINA, 2018). Uma representação da história natural da DC pode ser observada na Figura 4.

Figura 4 – Representação gráfica da história natural da DC *vs*. parasitemia.



Fonte: Adaptado de DE BONA et al. (2018).

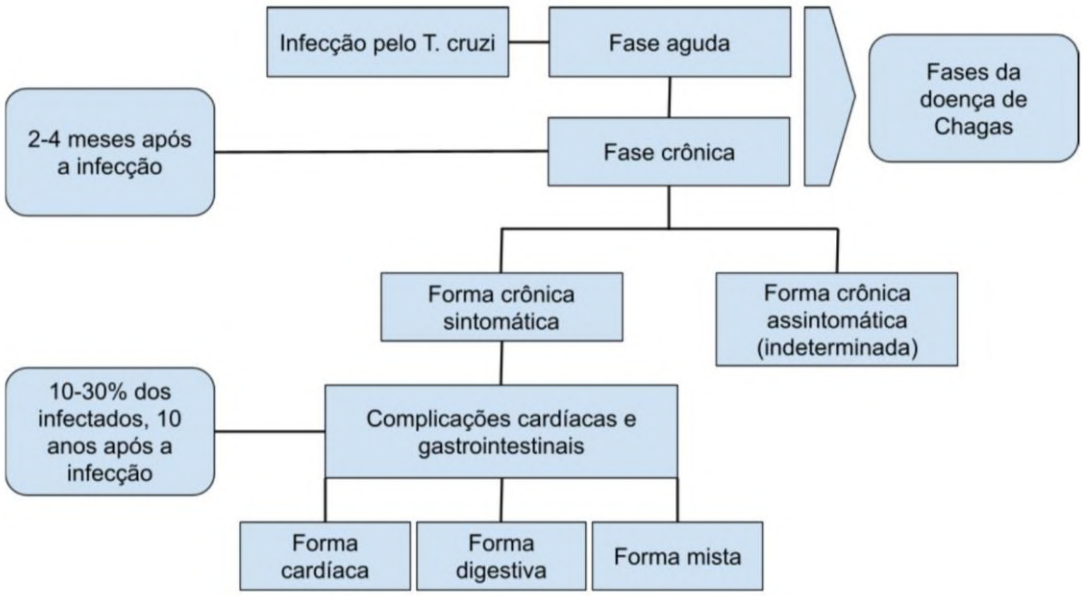
2.5.2 Caracterização da fase crônica

Cerca de 70% dos indivíduos infectados pelo *T. cruzi* não desenvolverão doença clínica e permanecerão na chamada forma indeterminada da doença (com ausência de sinais e sintomas) e terão bom prognóstico em longo prazo. O estágio indeterminado é frequentemente observado na maioria dos pacientes, pode durar entre 10 e 20 anos ou indefinidamente (KAYA, 2021; LANNES-VIEIRA et al., 2017; WHO, 2022).

Cerca de 30% dos indivíduos infectados apresentam danos aos órgãos/sistemas clínicos da fase crônica da doença de Chagas. As manifestações da fase crônica incluem manifestações digestivas (que podem se apresentar, não raros, casos de megaesôfago e megacólon), cardio-digestivas, neurológicas e cardíacas, sendo esta última a forma mais significante devido a frequência observada e gravidade (KAYA, 2021; LANNES-

VIEIRA *et al.*, 2017; WHO, 2022). Um esquema simplificado sobre as fases da doença pode ser visto na Figura 5.

Figura 5 – Fluxograma sobre as fases e as formas da DC.



Fonte: Autor (2023).

2.5.2.1 Forma clínica cardíaca da fase crônica

Na forma cardíaca da fase crônica da DC, a cardiomiopatia (miocardite) crônica é uma das principais manifestações associadas à morbidade, sendo possivelmente desencadeada pela interação parasito-hospedeiro que ocorre durante a fase aguda (ECHAVARRÍA *et al.*, 2021; LANNES-VIEIRA *et al.*, 2017; SIMÕES *et al.*, 2018). Pode também incluir: disfunção biventricular severa, insuficiência cardíaca progressiva, aneurismas apicais, distúrbios graves da condução atrioventricular e intraventricular, arritmias ventriculares complexas, fenômenos tromboembólicos e cardiomegalia com elevados índices de morbidade e mortalidade, seja por falência miocárdica ou por morte súbita (ECHAVARRÍA *et al.*, 2021; LANNES-VIEIRA *et al.*, 2017; SIMÕES *et al.*, 2018).

A morte súbita, que pode ocorrer mesmo em pacientes assintomáticos, representa a principal causa de morte em pacientes com cardiomiopatia chagásica, e a

insuficiência cardíaca de etiologia chagásica está associada à maior mortalidade do que a insuficiência cardíaca de outras causas. Histologicamente se verifica uma cardiomiopatia linfocitária difusa, escassos ninhos de parasitas e fibrose intersticial difusa, sendo a fibrose uma das manifestações mais significativas da cardiopatia chagásica crônica e encontra-se associada a cardiomiócitos em degeneração e infiltrados inflamatórios (ECHAVARRÍA *et al.*, 2021; LANNES-VIEIRA *et al.*, 2017; SIMÕES *et al.*, 2018). As respostas hemodinâmicas e neuro-hormonais em cardiomiopatia chagásica são semelhantes às de outras cardiomiopatias. A terapia medicamentosa deve ser iniciada com os mesmos regimes que na insuficiência cardíaca de outras causas (PÉREZ-MOLINA; MOLINA, 2018)

2.5.2.2 *Forma clínica digestiva da fase crônica*

Pacientes portadores da forma digestiva apresentam uma série de sintomas relacionados à obstrução desse sistema e seus órgãos, e é caracterizada pela incapacidade do esfíncter esofágico inferior de relaxar em resposta à deglutição, além da ausência de peristaltismo no próprio esôfago. Ambas as anormalidades motoras determinam a dilatação esofágica, levando a uma estagnação do processo digestivo (estase alimentar), que produzirá a maioria dos sintomas e complicações da doença. Os sintomas iniciais da forma digestiva na fase crônica podem ser bastante inespecíficos, como hipersalivação, tosse noturna, sensação de tosse após comer e perda de peso (OROZCO *et al.*, 2022; PÉREZ-MOLINA; MOLINA, 2018).

Tanto no megaesôfago quanto no megacólon, os órgãos exibem grande aumento do lúmen e hipertrofia da camada muscular. Histologicamente, os órgãos afetados demonstram lesões inflamatórias do sistema nervoso entérico, associadas com uma grande redução no número de neurônios. Não há tratamento específico para distúrbios digestivos leves (sintomas como disfagia ou constipação), já para casos graves (megassíndrome), o tratamento endoscópico ou cirúrgico é essencial (LANNES-VIEIRA *et al.*, 2017; PÉREZ-MOLINA; MOLINA, 2018).

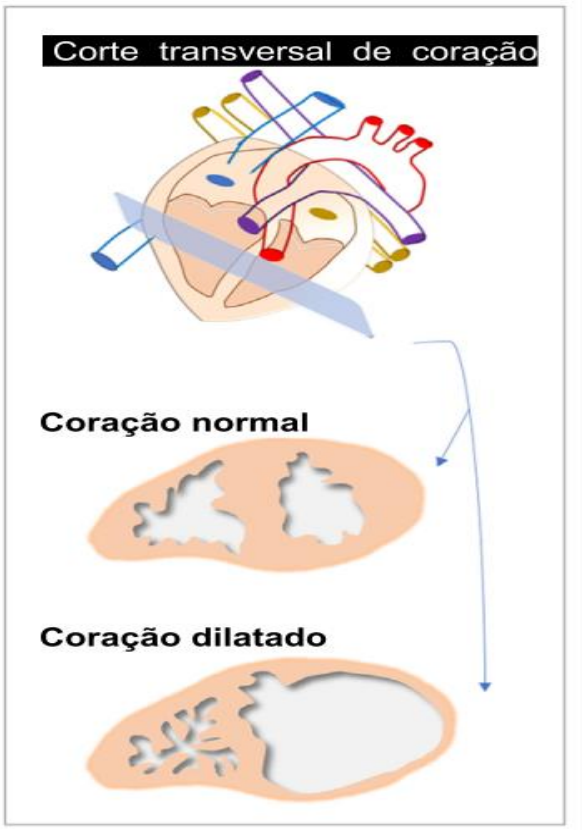
2.6 CARACTERIZAÇÃO DA MIOCARDITE NA DC

Sabe-se que há diferenças relevantes na apresentação de cardiomiopatias na fase aguda e na fase crônica da DC. A caracterização patológica e das alterações clínicas da miocardite da doença de Chagas são:

- Fase aguda: A miocardite aguda da infecção chagásica é caracterizada pela (i) destruição das células musculares cardíacas parasitadas, (ii) presença de intenso parasitismo e (iii) presença de infiltrado inflamatório mononuclear, sendo atribuída diretamente ao parasito e à resposta imune humoral e/ou celular dirigida aos seus antígenos (DE BONA *et al.*, 2018; LANNES-VIEIRA *et al.*, 2017).
- Fase crônica: a miocardite persistente da infecção chagásica pode levar à cardiomiopatia crônica de Chagas (CCC), que se distingue por apresentar (i) destruição de fibras miocárdicas no foco inflamatório, (ii) infiltrado inflamatório polifocal com células mononucleares, (iii) áreas de fibrose e (iv) baixa parasitemia (não havendo correlação entre parasitismo e a reação inflamatória) (DE BONA *et al.*, 2018; LANNES-VIEIRA *et al.*, 2017). É observado que a doença de Chagas, em especial a CCC apresenta o maior risco de morte quando comparada às outras cardiomiopatias cujas condições não apresentam o componente da cronicidade inflamatória, o que ocorre no caso da DC (LANNES-VIEIRA *et al.*, 2017; PÉREZ-MOLINA; MOLINA, 2018).

A CCC envolve o aparecimento de um quadro clínico de miocardiopatia dilatada, com disfunção ventricular esquerda global e de síndrome de insuficiência cardíaca (IC) (SIMÕES *et al.*, 2018). Uma representação de corte histológico de coração pode ser visualizada na Figura 6.

Figura 6 - Representação de corte anatômico do coração, demonstrando diferença entre coração normal e coração dilatado pela cardiomiopatia chagásica.



Fonte: adaptado de DE BONA et al. (2018).

A partir da classificação para IC proposta pela Sociedade Brasileira de Cardiologia, foi criado um estadiamento para a CCC para identificar cinco subgrupos distintos, baseados em alterações do eletrocardiograma (ECG), alterações do ecocardiograma (ECO) associadas ao valor da fração de ejeção ventricular esquerda (FEVE), sendo este último no valor de 45% o que delimita o nível de acometimento (SIMÕES *et al.*, 2018). A Figura 7 mostra, resumidamente, o estadiamento da CCC.

Figura 7 - Classificação clínica da disfunção ventricular esquerda na cardiopatia da doença de Chagas.

Fase aguda	Fase crônica				
	Forma indeterminada	Forma cardíaca sem disfunção ventricular	Forma cardíaca com disfunção ventricular		
	A	B1	B2	C	D
Pacientes com quadro compatível com Doença de Chagas aguda	Pacientes sob risco de desenvolver ICC. Possuem sorologia possitiva, não têm cardiopatia estrutural ou sintomas de ICC. Também não têm alterações digestivas	Pacientes com cardiopatia estrutural, evidenciada por alterações eletrocardiográficas, mas com função ventricular global normal e sem sinais e sintomas atuais ou prévios de ICC	Pacientes com cardiopatia estrutural, caracterizada por disfunção ventricular global, mas sem sinais e sintomas prévios ou atuais de ICC	Pacientes com disfunção ventricular e com sintomas prévios ou atuis de ICC. (NYHA, I, II, III ou IV)	Pacientes com sintomas refratários de ICC em repouso, apesar de tratamento clínico otimizado, necessitando intervenções especializadas

Legenda: ICC - insuficiência cardíaca de Chagas. NYHA - New York Heart Association, que classifica a extensão da insuficiência Cardíaca, relacionando-a dispneia a atividades físicas rotineiras: Classe I - sem sintomas e sem limitações em atividades cotidianas; Classe II - leves limitações e sintomas ao realizar atividades rotineiras, com dispneias habituais, porém com conforto no repouso; Classe III - com dispneia ao realizar esforços menores que as rotineiras (atividades simples do cotidiano), e limitações em atividades físicas; e Classe IV - importantes limitações, com dispneia até mesmo no repouso.

Fonte: Adaptado de SIMÕES et al., (2018).

2.7 PROPOSTAS EXPLICATIVAS SOBRE A FISIOPATOGENIA E EVOLUÇÃO DA CARDIOMIOPATIA CHAGÁSICA CRÔNICA (CCC)

Atualmente, acredita-se que as diferentes manifestações clínicas da doença de Chagas sejam consequência de múltiplos fatores ligados ao *T. cruzi*, tais como: (i) cepa, (ii) virulência, (iii) antigenicidade, (iv) tropismo e (v) tamanho do inóculo; como também ligadas ao hospedeiro, como por exemplo: (i) idade, (ii) características relacionadas a hormônios sexuais, (iii) características genéticas e (iv) status imune prévio e decorrente de coinfecções (LANNES-VIEIRA *et al.*, 2017; PÉREZ-MOLINA; MOLINA, 2018).

2.7.1 Teorias relacionadas a CCC e a imunidade na DC

Embora intensas pesquisas visando desvendar os mecanismos envolvidos na patogênese do dano tecidual da doença de Chagas tenham sido desenvolvidas por anos, permanece obscura a razão pela qual alguns pacientes permanecem assintomáticos enquanto outros evoluem para o quadro sintomático, incluindo a CCC. As duas principais hipóteses mais aceitas não são mutuamente exclusivas e ambas podem contribuir para o desfecho clínico da infecção através de autoimunidade: ativação do espectador (*bystander activation*) e mimetismo molecular (*molecular mimicry*) (ACEVEDO; GIRARD; GOMEZ, 2018; DE BONA *et al.*, 2018; LANNES-VIEIRA *et al.*, 2017).

Bystander activation propõe que o dano tecidual é uma consequência direta da presença de parasitas vivos, induzindo inflamação crônica, causada pela exposição de proteínas intracelulares do hospedeiro e do parasita, resultando em potentes estímulos imunológicos devido à liberação de autoantígenos que induzem a produção de autoanticorpos. Nessas circunstâncias, as células infectadas, as células T CD8+ e as células inflamatórias (macrófagos) dentro do foco inflamatório, liberam citocinas como fator de necrose tumoral (TNF), TNF-β, linfotoxina (LT) e óxido nítrico (NO), que podem levar à morte do "espectador" (células vizinhas não infectadas) (ACEVEDO; GIRARD; GOMEZ, 2018; DE BONA *et al.*, 2018; FUJINAMI *et al.*, 2006; LANNES-VIEIRA *et al.*, 2017).

Enquanto a segunda hipótese, *molecular mimicry*, baseia-se em uma resposta autorreativa, desencadeada por mimetismo molecular entre o parasita e as proteínas do hospedeiro. O mimetismo molecular ocorreria quando há semelhanças estruturais entre moléculas específicas do *T. cruzi* e as moléculas do hospedeiro, desencadeando a ativação das células T CD8+. Anticorpos específicos de células B podem participar do mecanismo de citotoxicidade (ADCC - mediado por células dependentes de anticorpos) nas células-alvo. Neutrófilos, eosinófilos e células *Natural Killers* (NK) interagem com esses anticorpos via CD16 (receptor Fc) e liberam moléculas líticas como enzimas, perforinas ou TNF nas células-alvo, independente do sistema complemento (ACEVEDO; GIRARD; GOMEZ, 2018; DE BONA *et al.*, 2018; FUJINAMI *et al.*, 2006; LANNES-VIEIRA *et al.*, 2017).

Independentemente dos mecanismos envolvidos na patologia, o principal fator subjacente é a resposta imune orquestrada pelo organismo hospedeiro e sua interação com o parasita. Entretanto, o entendimento geral da interação entre o parasita-hospedeiro é uma complexa rede de relações entre antígenos, anticorpos, células da imunidade, citocinas e quimiocinas (ACEVEDO; GIRARD; GOMEZ, 2018; CRISTOVÃO-SILVA *et al.*, 2021; DE BONA *et al.*, 2018; LANNES-VIEIRA *et al.*, 2017). É importante destacar que apesar de urgente, essa enorme quantidade de informações sobre a doença não tem tido aplicações concretas na área. Provavelmente a razão disso está na própria complexidade da interação entre o *T. cruzi* e o paciente, que assim como a maioria das infecções por protozoários, dificilmente podem ser compreendidas pelas estratégias reducionistas tradicionais (ACEVEDO; GIRARD; GOMEZ, 2018; MARDAHL; BORUP; NEJSUM, 2019). Brevemente, serão apresentados importantes aspectos envolvendo diferentes componentes da imunidade inata e a imunidade adaptativa e o *T. cruzi:*

2.7.1.1 *Imunidade, CCC e T. cruzi*

Na fase crônica da doença de Chagas, os sinais clínicos e os sintomas dos pacientes, ou a ausência destes, parecem estar relacionados à resposta imune tipo Th1 do indivíduo. Embora a resposta imune primária seja importante para conter a replicação do parasita durante a fase aguda, também pode estar envolvida no desenvolvimento de doença cardíaca grave. Na fase aguda, as células apresentadoras de antígenos apresentam os antígenos do parasito aos linfócitos T. Estes linfócitos T (CD4+) juntamente a monócitos/macrófagos, são consideradas as células responsáveis por iniciar e formar a resposta imune na doença de Chagas (destacando, portanto, o efeito dos fagócitos e da apresentação de antígenos no estabelecimento e no perfil da memória imunológica de longo prazo) (ACEVEDO; GIRARD; GOMEZ, 2018; ANDRADE *et al.*, 2017; CRISTOVÃO-SILVA *et al.*, 2021; DE BONA *et al.*, 2018; LANNES-VIEIRA *et al.*, 2017).

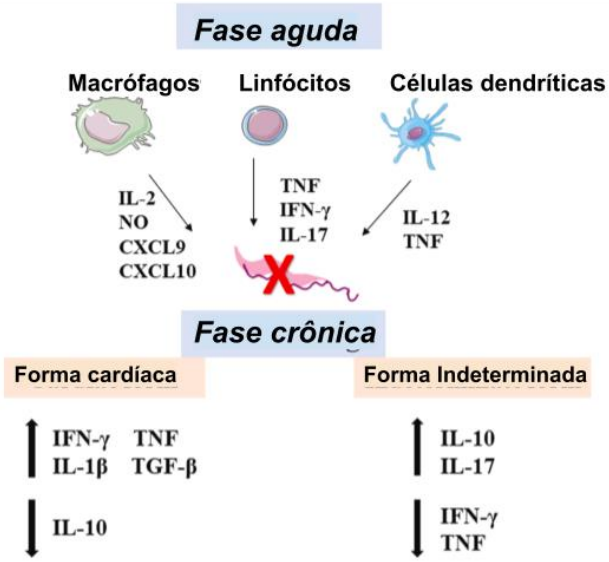
As células T CD8+ têm predominância na miocardite crônica chagásica, enquanto as CD4+ são as menos presentes. Em relação às células T CD4+, o subtipo T-helper 1 é o mais presente. Ambas, CD4+ e CD8+, parecem ter papéis contraditórios, sendo responsáveis por lesar células infectadas ou de tecidos vizinhos ao

desencadearem respostas mediadas (uma vez que haja uma constante presença de antígenos do parasita). Por outro lado, as células T CD4+ podem desenvolver um papel protetor através da produção de IFN-γ (com intenção de eliminar o agente infeccioso), além de contribuir para a indução de anticorpos, no auxílio de diferenciação de outros subtipos de células T (incluindo as CD8+) e na ativação de células fagocíticas (ACEVEDO; GIRARD; GOMEZ, 2018; ANDRADE *et al.*, 2017; CRISTOVÃO-SILVA *et al.*, 2021; DE BONA *et al.*, 2018).

Há evidências de que distintos perfis de citocinas são apresentados em diferentes formas clínicas da DC. Por exemplo, um perfil Th1 (IFN-γ, TNF, IL-2, IL-6, IL-9, IL-12) com baixo nível de um perfil Th2 (IL-4, IL- 5, IL-10, IL-13) é observado em pacientes com sinais clínicos cardíacos e digestivos, enquanto o oposto ocorre na forma indeterminada da DC. A IL-10 é mais elevada em indivíduos com a forma indeterminada quando comparados às outras formas (sugerindo um papel no impedimento da progressão dos danos aos órgãos/sistemas e controlando a morbidade da doença). É provável que o equilíbrio dessas citocinas desempenhe um papel fundamental no desenvolvimento/evolução da doença. TNF, IFN-γ e IL-6 (todas citocinas envolvidas em perfis pró-inflamatórios) estão diretamente envolvidas na patologia cardíaca crônica na DC, juntamente a uma maior quantidade de monócitos inflamatórios em comparação aos indivíduos indeterminados (ACEVEDO; GIRARD; GOMEZ, 2018; ANDRADE *et al.*, 2017; CRISTOVÃO-SILVA *et al.*, 2021; DE BONA *et al.*, 2018).

Ainda sobre a forma indeterminada, é observado que há aumento da expressão de citocinas e fatores de transcrição relacionados aos perfis Th2, Th9, Th22 e Treg, associado à redução da expressão de citocinas pró-inflamatórias como IFN-γ e TNF (ACEVEDO; GIRARD; GOMEZ, 2018; ANDRADE *et al.*, 2017; CRISTOVÃO-SILVA *et al.*, 2021; DE BONA *et al.*, 2018). Um resumo gráfico do papel das células da resposta imune tipo Th1 na fase aguda e da discrepância entre as respostas imunológicas da forma clínica cardíaca na DC e da forma indeterminada são apresentadas na Figura 8.

Figura 8 - Resumo gráfico do perfil de liberação de citocinas pelas células da resposta imune tipo Th1 e perfil de citocinas em pacientes na forma cardíaca e na forma indeterminada da DC.



Fonte: Adaptado de CRISTOVÃO-SILVA et al., (2021).

2.8 O PAPEL DE QUIMIOCINAS E SEUS RECEPTORES NA DC

Apesar de citocinas pró-inflamatórias como o IFN-γ e o TNF terem papéis centrais na DC, não pode ser negada a importância de moléculas como as quimiocinas, envolvidas no controle do recrutamento e da migração celular (LANNES-VIEIRA *et al.*, 2017). As quimiocinas e seus receptores têm sido estudados na DC justamente pela possibilidade dos seus envolvimentos na fase crônica da forma cardíaca, por permitirem a manutenção da inflamação crônica (BATISTA *et al.*, 2018; DE OLIVEIRA *et al.*, 2015).

Quimiocinas são um importante grupo de pequenas citocinas (7-15 kDa) capazes de recrutar com especificidade subgrupos de leucócitos. Há duas grandes subfamílias de quimiocinas baseadas na posição de seus resíduos de cisteína, CXC (quimiotática de neutrófilos) e CC (quimiotática de monócitos e subtipos de linfócitos) (QI *et al.*, 2020). Estudos apontam que na inflamação crônica da forma cardíaca da DC, os cardimiócitos produzem citocinas, quimiocinas e óxido nítrico que atraem leucócitos para o sítio inflamatório, para controlar a replicação intracelular parasitária (CALZADA *et al.*, 2001).

A expressão de receptores de quimiocinas em linfócitos T, juntamente a expressão de quimiocinas específicas em tecidos, são importantes fatores que controlam a infiltração de linfócitos em diferentes tipos de tecidos (CUNHA-NETO *et al.*, 2009; NOGUEIRA *et al.*, 2012). Por isso, entender os mecanismos de colonização de células da resposta inflamatória nos tecidos cardíacos pode revelar alvos moleculares que modulam a resposta inflamatória na forma cardíaca da DC (BATISTA *et al.*, 2018; DE OLIVEIRA *et al.*, 2015).

Entre os receptores de quimiocinas que podem contribuir efetivamente na colonização de células inflamatórias, e por fim, no desfecho da DC, está CCR5, descrito como um elemento fundamental no desenvolvimento da DC através de análises de expressão gênica e bioinformática (BROCHET *et al.*, 2022; FERREIRA *et al.*, 2017; LAUGIER *et al.*, 2020). A proteína CCR5 – do inglês *chemokine receptor 5* - é um receptor de quimiocinas do tipo CC, que se liga a moléculas como MIP1-α (CCL3), MIP1-β (CCL4) e RANTES (CCL5) (ALDINUCCI; BORGHESE; CASAGRANDE, 2020; MAURER; VON STEBUT, 2004), todas relacionadas à doenças inflamatórias, tornando o CCR5 um importante alvo molecular dessas doenças, e um ponto muitas vezes comum entre a imunidade e a fisiopatologia das mesmas (DONNINELLI *et al.*, 2021; PATTERSON *et al.*, 2020; ZENG *et al.*, 2021).

O CCR5 é expresso em monócitos, macrófagos e linfócitos como as células Th1 (conhecidas por auxiliarem na destruição de patógenos) (STONE *et al.*, 2017). Em casos graves de cardiomiopatia da DC, o infiltrado mononuclear geralmente é composto justamente por linfócitos T e macrófagos (CUNHA-NETO *et al.*, 2009; NOGUEIRA *et al.*, 2012), o que pode sugerir o envolvimento do CCR5 como um fator colaborativo na inflamação crônica (JIMÉNEZ *et al.*, 2019).

2.9 ESTUDOS GENÉTICOS DO GENE *CCR5* E SUA RELAÇÃO COM A DC

Muitas investigações têm associado a genética do paciente com a infecção e a evolução da DC (ACOSTA-HERRERA *et al.*, 2019; GOMES DOS SANTOS *et al.*, 2020), indicando que variações genéticas, como polimorfismos em genes de citocinas, quimiocinas e receptores celulares podem contribuir no desfecho da doença, uma vez

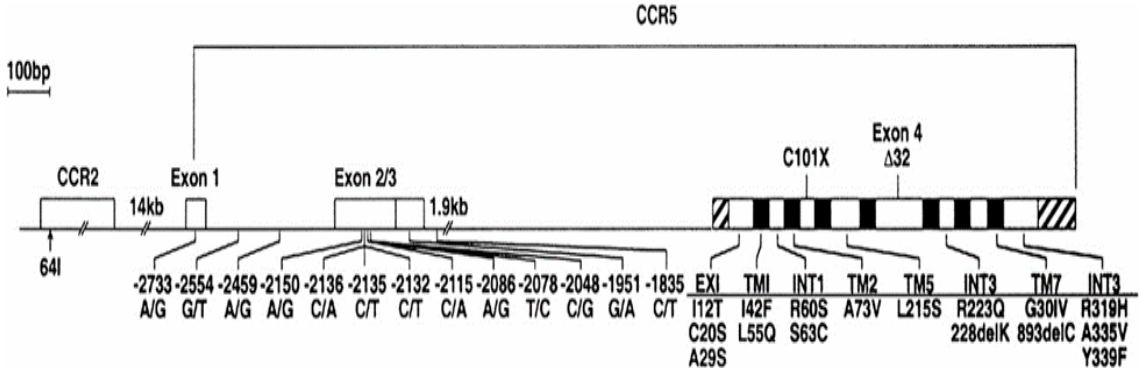
que estes genes têm papéis centrais na ativação e regulação do sistema imunológico (CHEVILLARD et al., 2018).

Não são raros os estudos que apontam associações da genética do paciente com seu quadro clínico, incluindo quadros de cardiomiopatias. Em relação à DC, diversas revisões de literatura já foram conduzidas, no esforço de condensar as informações sobre os achados dos envolvimentos de diversas variáveis genéticas com as formas clínicas da DC (ACOSTA-HERRERA *et al.*, 2019; CARVALHO *et al.*, 2019; DE OLIVEIRA *et al.*, 2016; DEL PUERTO *et al.*, 2013; GIGLI *et al.*, 2019; GOMES DOS SANTOS *et al.*, 2020).

O gene *CCR5* - localizado no cromossomo 21 (3p21.31) - é um dos mais estudados na DC (ACOSTA-HERRERA *et al.*, 2019; SHAW, 2017). Estudos *in vivo* têm demonstrado que camundongos deficientes para este gene não desenvolvem a forma cardíaca, indicando que a expressão do gene *CCR5* está ligada à forma crônica cardíaca da doença, levando à CCC (BATISTA *et al.*, 2018). Comparativamente, análises com pacientes cardiomiopáticos exibiram maior expressão desse gene, resultando no aumento da inflamação (DE OLIVEIRA *et al.*, 2015; NOGUEIRA *et al.*, 2012).

Os estudos sobre o *CCR5* são alguns dos mais replicados, assim como estudos das variantes genéticas desse gene, cujos resultados por vezes apresentam relação com a DC, principalmente com as formas crônicas da doença (DE OLIVEIRA *et al.*, 2016). Assim, entre as variantes genéticas de receptores celulares já estudados na DC, destaca-se o *CCR5*. Diversas variantes do *CCR5* já foram investigadas quanto aos seus envolvimentos na DC, entretanto ainda há estudos com resultados inconclusivos ou contraditórios (BATISTA *et al.*, 2018; CALZADA *et al.*, 2001; FLÓREZ; MARTÍN; GONZÁLEZ, 2012; FRADE *et al.*, 2013; LIMA *et al.*, 2018), tornando difícil concluir suas reais contribuições na doença. A Figura 9 ilustra as variantes identificadas nas regiões codificantes do gene CCR5 (e do gene vizinho CCR2).

Figura 9 – Mapa da estrutura genética do *CCR5*.



Legenda: O sistema de numeração usado designa o primeiro nucleotídeo do sítio de início da tradução de CCR5 como a posição 1, e o nucleotídeo imediatamente anterior deste como a posição -1.

Fonte: Adaptado de CARRINGTON et al., (1999).

Os resultados contraditórios ou inconclusivos dos trabalhos de associação genética publicados podem ter sido influenciados por questões tais como populações com diferentes backgrounds genéticos, tamanho amostral insuficiente, e grupo controle inadequado (não infectados). Todos estes erros podem ser sanados agrupando populações com background genético relacionado e utilizando pacientes assintomáticos como controle (uma vez que infectados, mesmo assim, não desenvolveram formas sintomáticas da doença, o que não ocorreria em não infectados) (BATISTA *et al.*, 2018; FLÓREZ; MARTÍN; GONZÁLEZ, 2012; MACHUCA *et al.*, 2014; NOGUEIRA *et al.*, 2012). Considerando o exposto acima, o presente trabalho conduziu uma revisão da literatura dos polimorfismos de genes de quimiocinas e seus receptores, em especial, os do gene *CCR5* envolvidos na DC, objetivando elucidar através de meta-análise e outras análises exploratórias a relação desses polimorfismos com a doença.

3. METODOLOGIA

3.1 ESTRATÉGIAS DE BUSCA

Esta revisão foi registrada na plataforma PROSPERO (https://www.crd.york.ac.uk/PROSPERO/). As buscas foram baseadas na estratégia PICOS (SANTOS; PIMENTA; NOBRE, 2007), onde os participantes (P) foram pacientes infectados pelo *T. cruzi*; a intervenção (I) foi a identificação de polimorfismos dos genes de quimiocinas e seus receptores nos pacientes; as comparações (C) foram feitas com quaisquer grupos (assintomáticos ou não infectados) ou subgrupos (de doença crônica de Chagas) de pacientes com perfis bem delimitados; o resultado (O) foi a presença ou ausência do polimorfismo em pacientes; e os tipos de estudos (S) foram estudos de associação genética.

3.2 SELEÇÃO DE TERMOS, ANÁLISE DE DECS E CONSTRUÇÃO DE *STRINGS*

Para eleger os grupos de palavras que foram utilizados nas buscas das bases de dados, foram primariamente lidos os guias e protocolos estabelecidos pela OMS e pela PAHO para o manejo de pacientes de DC (WHO, 2015; PAHO, 2022), além de artigos de especialistas e revisões (BERN, 2015; PÉREZ-MOLINA; MOLINA, 2018).

Após essas leituras, foram destacadas as frases que se referiam ou descreviam diretamente à infecção pelo T. cruzi, e dessas frases foram elencados os termos (1) “chagas disease”, (2) “Trypanosomiasis” e (3) “Trypanosoma cruzi infection”. Usando a ferramenta de Descritores em Ciências da Saúde - DeCS, do portal BVS (https://decs.bvsalud.org/), foram buscados os sinônimos gerais de cada um desses termos, cujos retornos foram: (1) “chagastic disease”, (2) “American trypanosomiasis”, “Trypanosome parasite”, “Trypanosome infection”, “Trypanosoma cruzi”, “Trypanosomosis”, e (3) “T. cruzi”, “Trypanosoma cruzi” e “cruzi’’ (references codes C01.610.752.300.900.200, C01.920.625 e SP4.012.148.149). O mesmo método foi usado para o termo “Genetic polymorphism”, cujos resultados foram “Polymorphic gene”; “Single nucleotide polymorphism”, “Genetic variation”, “Allelic variation” e “SNP’’ (references codes G05.365.795, G05.365.795.598, G05.365, G05.380 e SP4.102.118).

Após encontrados todos os sinônimos dos descritores acima, através do método de contagem direta, foram destacadas tanto as palavras comumente repetidas quanto as que apareceram uma única vez. Juntamente aos operadores booleanos, os termos destacados foram usados com variações nos sufixos para formar os *strings* de busca e suas variações: ‘Chaga* disease’ OR ‘Trypanosom*’ OR ‘cruzi’ AND ‘Polymorphi*’ OR ‘Gene*’ OR ‘Variation’ OR ‘SNP’.

3.3 BASES DE DADOS

As buscas foram realizadas sem restrições de tempo no modo avançado das bases de dados, utilizando o *string* citado acima e suas variações no campo “título”. As bases de dados usadas foram o Portal de periódicos da CAPES, SciELO, Scopus, Web of Science, PubMed e ScienceDirect. As buscas ocorreram entre janeiro e fevereiro de 2022. Foram usados como filtros para os retornos apenas artigos, escritos em inglês e avaliados por pares (quando disponíveis, na intenção de melhorar a qualidade dos retornos). Posteriormente, os artigos incluídos na síntese qualitativa tiveram suas referências verificadas para englobar artigos que não foram detectados durante as buscas nas bases de dados. Informações sobre os textos (número de repetições que o texto foi detectado nas buscas, língua usada na publicação, tipo de texto e tipo de estudo) foram organizadas usando o LibreOffice Calc.

3.4 CRITÉRIOS DE INCLUSÃO E EXCLUSÃO

Foram critérios de inclusão: (1) publicações originais; (2) estudos de associação genética e (3) artigos disponibilizados eletronicamente. Foram critérios de exclusão: (1) estudos duplicados, (2) estudos usando modelo animal, (3) estudos *in vitro*, (4) estudos de correlação entre a genética do parasita e o paciente, (5) estudos de análise genética de vetores, (6) estudos que não avaliaram polimorfismos genéticos de quimiocinas e seus receptores, (7) revisões de literatura e (8) resumos de congressos.

3.5 SELEÇÃO DE PUBLICAÇÕES E ANÁLISE DE QUALIDADE

As publicações foram inicialmente analisadas pelo título e pelo resumo utilizando os critérios de inclusão e exclusão nos retornos. Dois revisores independentes

(JMF e BRCS) analisaram todos os retornos, inconsistências entre os revisores foram resolvidas pela discussão da publicação juntamente a um terceiro revisor (ACMS).

A análise da qualidade dos estudos foi realizada após a seleção dos estudos. A ferramenta de análise de qualidade "STrengthening the REporting of Genetic Association Studies (STREGA)" foi utilizada. O STREGA consiste em um checklist do estudo genético, avaliando cada parte do artigo científico (título e resumo, introdução, métodos, resultado, discussão e outras informações), onde um total de 22 pontos pode ser alcançado no estudo. Usando o STREGA os estudos foram classificados como baixa qualidade (< 9), média qualidade (9-16) e alta qualidade (17-22).

3.6 EXTRAÇÃO DE DADOS

Foram extraídos os dados sobre o design do estudo, ano de publicação, país de origem do estudo (baseado no primeiro autor), a população estudada, tamanho da amostra, informações sobre o perfil dos pacientes do grupo analisado, polimorfismos analisados, principais resultados, método de confirmação laboratorial para a infecção e método de confirmação para o polimorfismo.

3.7 META-ANÁLISE

Os dados dos artigos eleitos foram organizados com ajuda do Planilhas Google. As frequências alélicas e genotípicas globais, utilizando os dados dos artigos incluídos, foram calculadas com o auxílio da mesma ferramenta. As meta-análises foram realizadas em todos os polimorfismos disponíveis no software RevMan 5.4, onde a força da associação foi calculada usando um intervalo de confiança de 95% e razão de chances (*odds ratio* - OR), sendo o valor gerado OR<1 considerado como fator (tendência) de proteção ou diminuição do risco, enquanto valores OR>1 foram considerados como fator (tendência) de risco, os resultados foram considerados significantes quando $p \leq 0.05$. A heterogeneidade dos estudos foi avaliada usando o teste padrão (Q^2 e I^2). Quando $I^2 \geq 50\%$ e $p \leq 0.05$, indicando uma heterogeneidade significativa, provavelmente causada por variações entre os estudos, optamos pelo uso do modelo de análise randômica (padronizado pelo software), para todos os outros casos, o modelo de análise fixado foi usado.

Foram avaliadas as influências dos alelos (mutado *vs.* selvagem) e dos genótipos em diferentes modelos de herança: codominância (homozigoto mutado *vs.* homozigoto selvagem, ou heterozigoto *vs.* homozigoto selvagem), recessividade (homozigoto mutado *vs.* heterozigoto + homozigoto selvagem) e dominância (homozigoto mutado + heterozigoto *vs.* homozigoto selvagem). Os gráficos do tipo *funnel-plot* foram inspecionados na procura de possíveis vieses de publicação.

3.8 GTEx (eQTL) E FERRAMENTA SNP2TFBS

Para identificar polimorfismos de nucleotídeo único (SNPs) que podem afetar a ligação do fator de transcrição e os sítios de ligação, usamos a interface da web Mapping SNPs to Transcription Factor Binding Sites (SNP2TFBS) (https://ccg.epfl.ch/snp2tfbs/). Para avaliar a relação entre as variantes genéticas associadas ao risco do desenvolvimento da forma cardíaca da DC e o perfil de expressão do gene, examinamos o *expression quantitative trait loci* (eQTL) do banco de dados GTEx (https://gtexportal.org/home/).

3.9 ENQUADRAMENTO DOS PACIENTES E ANÁLISE ESTATÍSTICA

Utilizando os dados disponíveis nas publicações, os pacientes foram agrupados em diferentes grupos: os indivíduos saudáveis e sem infecção pelo *T. cruzi* foram denominados como não infectados (nonCP), enquanto os pacientes infectados pelo *T. cruzi* foram tratados genericamente como pacientes crônicos de doença de chagas (CP). O grupo “CP” foi subdividido de acordo com a presença ou não de sintomas em dois grupos menores: Pacientes crônicos assintomáticos em forma indeterminada (UndCP) e pacientes crônicos sintomáticos (SympCP). Os pacientes do grupo “SympCP” foram separados em três grupos de acordo com o tipo de apresentação da forma crônica da DC: Aqueles que apresentaram qualquer tipo de alteração/complicações cardíacas como decorrente da DC foram agrupados como pacientes cardíacos (CardCP), enquanto aqueles com complicações associadas ao sistema digestório foram agrupados como pacientes digestivos (DigCP). Os pacientes que apresentaram tanto formas envolvendo problemas cardíacos quanto digestivos foram agrupados como de complicação mistas (CardDigCP). Em nossas comparações a presença de um paciente envolvido no grupo CardCP ou DigCp não o exclui da participação do grupo CardDigCP, e vice-versa.

3.10 ANÁLISE ESTATÍSTICA

Em ordem de avaliar a interação entre os dados da amostra, para os resultados das comparações globais (usando todos os estudos da comparação) em que houve significância, foi performada uma Análise de Componente Principal (PCA), usando o software Past 4.10 (https://www.nhm.uio.no/english/research/infrastructure/past/). Para as comparações significativas encontradas na síntese quantitativa, o poder amostral foi calculado usando o G*Power 3.1.9.7 (χ^2-Goodness-of-fit, Post hoc, α=0.05) (ERDFELDER, 2009; FAUL *et al.*, 2007), para determinar a representatividade do resultado na população, foram considerados como significativos os valores >80%. Análises estatísticas exploratórias foram realizadas para auxiliar na compreensão do desenvolvimento da forma cardíaca da DC. Para tanto, foram verificadas, por meio da correlação de Pearson, as possíveis correlações entre haplótipos e seus respectivos alelos constituintes. O teste do qui-quadrado foi utilizado para comparar a frequência do haplótipo HHC entre os grupos, adotando-se um nível de significância de 5%.

4 RESULTADOS

4.1 CAPÍTULO I

Estreitando a relação entre polimorfismos do gene CCR5 humano e a doença de Chagas: revisão sistemática e meta-análise

Artigo publicado na revista *life* - MDPI (ISSN 2075-1729):

Qualis 2017-2020: A2 (CIÊNCIAS BIOLÓGICAS I);

Fator de Impacto: 3.2 (2022);

Link: https://www.mdpi.com/2075-1729/13/8/1677.

life

MDPI

Article

Narrowing the Relationship between Human CCR5 Gene Polymorphisms and Chagas Disease: Systematic Review and Meta-Analysis

Jean Moisés Ferreira [1,2,*], Barbara Rayssa Correia dos Santos [3], Edilson Leite de Moura [3], Ana Caroline Melo dos Santos [3], Jean Carlos Vencioneck Dutra [2], Elaine Virgínia Martins de Sousa Figueiredo [3] and José Luiz de Lima Filho [1,*]

[1] Laboratório de Imunopatologia Keizo Asami—LIKA, Centro de Biociências, Universidade Federal de Pernambuco (UFPE), Recife 50670-901, Pernambuco, Brazil
[2] Secretaria de Estado de Educação do Espírito Santo (SEDU), Santa Lucia, Vitória 29056-085, Espírito Santo, Brazil; jeanvencioneck@gmail.com
[3] Laboratório de Biologia Molecular e Expressão Gênica—LABMEG, Departamento de Ciências Biológicas, Universidade Federal de Alagoas (UFAL), Campus Arapiraca, Arapiraca 57300-970, Alagoas, Brazil; brc.rayssa@gmail.com (B.R.C.d.S.); edilsonleite17@gmail.com (E.L.d.M.); anacaroline12305@gmail.com (A.C.M.d.S.); elaine.figueiredo@arapiraca.ufal.br (E.V.M.d.S.F.)
* Correspondence: jean.moises@hotmail.com (J.M.F.); joseluiz60@gmail.com (J.L.d.L.F.)

Abstract: Our aim was to carry out a qualitative and quantitative synthesis of the influence of CCR5 genetic variants on Chagas disease (CD) through a systematic review. A total of 1197 articles were analyzed, and eleven were included in the review. A meta-analysis was conducted along with principal component analyses (PCAs). The polymorphisms found were analyzed using the SNP2TFBS tool to identify possible variants that influence the interaction with gene binding sites. Eleven studied variants were identified: rs2856758, rs2734648, rs1799987, rs1799988, rs41469351, rs1800023, rs1800024, Δ32/rs333, rs3176763, rs3087253 and rs11575815. The studies analyzed were published between 2001 and 2019, conducted in Argentina, Brazil, Spain, Colombia and Venezuela, and included Argentine, Brazilian, Colombian, Peruvian and Venezuelan patients. Eight polymorphisms were subjected to the meta-analysis, of which six were associated with the development of the cardiac form of CD: rs1799987—G/G and G/A in the dominance model and G/G in the recessiveness model; rs2856758—A/G in the codominance model; rs2734648—T/T and T/G in the dominance model; rs1799988—T/T in both the codominance and recessiveness models; rs1800023—G allele and the G/G genotype in the codominance and recessiveness models, and the G/G and G/A genotypes in the dominance model; and rs1800024—T allele. The PCA analyses were able to indicate the relationships between the alleles and the genotypes of the polymorphisms. The SNP2TFBS tool identified rs1800023 as an influencer of the Spi1 transcription factor ($p < 0.05$). A correlation was established between the alleles associated with the cardiac form of CD in this review, members of the C haplotype of the gene (HHC–TGTG), and the cardiac form of CD.

Keywords: American trypanosomiasis; SNPs; chemokines

Citation: Ferreira, J.M.; Santos, B.R.C.d.; Moura, E.L.d.; Santos, A.C.M.d.; Vencioneck Dutra, J.C.; Figueiredo, E.V.M.d.S.; Lima Filho, J.L.d. Narrowing the Relationship between Human CCR5 Gene Polymorphisms and Chagas Disease: Systematic Review and Meta-Analysis. *Life* **2023**, *13*, 1677. https://doi.org/10.3390/life13081677

Academic Editors: Alba Abras and Anna Fernández-Arévalo

Received: 29 May 2023
Revised: 19 July 2023
Accepted: 21 July 2023
Published: 2 August 2023

1. Introduction

Chagas disease (CD)—known as American trypanosomiasis—is a neglected disease that affects seven million patients worldwide. About 12–20 thousand patients die every year and approximately 75 million people are exposed to CD, which mainly affects low-income populations. [1–3]. After infection by the etiologic agent (*Trypanosoma cruzi*), the patient enters the acute phase, followed by the chronic phase. The chronic phase can be asymptomatic (patients with an undetermined outcome), it can present symptoms related to cardiac complications or those associated with the digestive system, or a mixed form of cardiac and digestive system complications simultaneously [3].

Some investigations have associated the genetics of the patient with the infection and evolution of CD [4,5], indicating that polymorphisms in cytokines, chemokines and cellular receptor genes may contribute to the outcome of this disease, since these genes have central roles in activating and/or regulating the immune system [6]. CCR5 stands out among the cell receptor genes already analyzed. The CCR5 gene is located on chromosome 21 (3p21.31) and encodes the CC chemokine receptor 5 protein, or "CCR5", which is expressed in T cells and integrates various immune processes [7]. Several variants of CCR5 have been associated with CD; however, some of these genetic variants have called attention to inconclusive or contradictory results in different studies [8–13], making it difficult to understand the real contributions of this gene to CD.

Gene expression and bioinformatics analyses continue to list CCR5 as a fundamental element in the development of CD [14–16]. In order to elucidate the relationship between CCR5 polymorphisms and CD, this study aimed to carry out a literature review on the CCR5 polymorphisms involved in CD, as well as to establish the correlations between these polymorphisms and CD through meta-analyses.

2. Materials and Methods

2.1. Search Strategies

This review has been registered on the PROSPERO platform (https://www.crd.york.ac.uk/PROSPERO/, March 2023). The searches were based on the PICOS strategy [17], where the participants (P) were patients infected with *T. cruzi*; intervention (I) was the identification of CCR5 gene polymorphisms in patients; comparisons (C) were made with any groups (asymptomatic or uninfected) or subgroups (chronic Chagas disease) of patients with well-defined profiles; the outcome (O) was the presence or absence of the polymorphism in patients; and the types of studies (S) were genetic association studies.

2.2. Term Selection, DeCS Analysis and String Construction

To choose the groups of words used in the database searches, the guides and protocols established by WHO and PAHO for the management of CD patients were primarily read [1,18], as well as expert articles and reviews [2,19]. After these readings, the sentences that referred to or directly described the *T. cruzi* infection were highlighted and the terms (1) "chagas disease", (2) "Trypanosomiasis" and (3) "*Trypanosoma cruzi* infection" were listed. Using the Health Sciences Descriptors tool—DeCS, from the BVS portal (https://decs.bvsalud.org/, January 2022)—the general synonyms of each term were sought, whose returns were: (1) "chagasic disease", (2) "American trypanosomiasis", "Trypanosome parasite", "Trypanosome infection", "*Trypanosoma cruzi*", "Trypanosomosis", and (3) "*T. cruzi*", "*Trypanosoma cruzi*" and "*cruzi*" (references codes C01.610.752.300.900.200, C01.920.625 and SP4.012.148.149). The same method was used for the term "Genetic polymorphism", whose results were "Polymorphic gene", "Single nucleotide polymorphism", "Genetic variation", "Allelic variation" and "SNP" (reference codes G05.365.795, G05.365.795.598, G05.365, G05.380 and SP4.102.118).

After finding all the synonyms of each descriptor, through the direct counting method, both commonly repeated words and those that appeared only once were highlighted. Applying Boolean operators, the highlighted terms were used with variations in the suffixes to form the search strings and their variations: "Chaga* disease" OR "Trypanosom*" OR "cruzi" AND "Polymorphi*" OR "Gene*" OR "Variation" OR "SNP".

2.3. Data Base

The searches were carried out without time restrictions in the advanced mode of the databases, using the string mentioned above and its variations in the "title" field. The databases used were the CAPES Journal Portal, SciELO, Scopus, Web of Science, PubMed and ScienceDirect. The searches took place between January and February 2022. Only peer-reviewed articles written in English (when available, with the intention of improving the quality of returns) were used as filters for returns. Subsequently, the arti-

cles included in the qualitative synthesis had their references checked to include articles that were not detected during the database searches. Information about the manuscripts (number of repetitions of the manuscript detected the in searches, language used in the publication, type of text and type of study) were organized using LibreOffice Calc.

2.4. Inclusion and Exclusion Criteria

The following inclusion criteria were used: (1) original publications, (2) genetic association studies, and (3) electronically available articles. The following exclusion criteria were used: (1) duplicate studies, (2) studies using an animal model, (3) in vitro studies, (4) correlation studies between the genetics of the parasite and the patient, (5) genetic analysis studies of vectors, (6) studies that did not evaluate CCR5 genetic polymorphisms, (7) literature reviews, and (8) conference abstracts.

2.5. Selection of Publications and Quality Analysis

The publications were initially analyzed by title and abstract using the inclusion and exclusion criteria in the returns. Two independent reviewers (JMF and BRCS) reviewed all the returns; inconsistencies between reviewers were resolved by discussing the publication with a third reviewer (ACMS).

The analysis of the quality of the studies was performed after the selection of the studies. The quality analysis tool "Strengthening the Reporting of Genetic Association Studies (STREGA)" consists of a genetic study checklist, evaluating each part of the scientific article (title, abstract, introduction, methods, results, discussion, and other information), where a total of 22 points can be reached in the study. Using STREGA, studies were classified as low quality (<9), medium quality (9–16) and high quality (17–22).

2.6. Data Extraction

Data on the study design, year of publication, country of origin of the study (based on the first author), the studied population, sample size, information on the profile of the patients in the analyzed group, analyzed polymorphisms, main results, laboratory confirmation method for infection and confirmation method for polymorphism were extracted.

2.7. Meta-Analysis

Data from the selected articles were organized using Google Sheets. Global allele and genotype frequencies, using data from the included articles, were calculated using the same tool. Meta-analyses were performed on all polymorphisms available in the RevMan 5.4 software, where the strength of association was calculated using a 95% confidence interval and odds ratio (OR), with the generated value OR < 1 considered as a protective factor (trend) or risk reduction, while OR > 1 values were considered as a risk factor (trend), the results were considered significant when $p \leq 0.05$. Study heterogeneity was assessed using the standard test (Q test and I-squared). When $I^2 \geq 50\%$ and $p \leq 0.05$, indicating significant heterogeneity, probably caused by variations between studies, we chose to use the random analysis model (standardized by the software); for all other cases, the fixed analysis model was used [20]. All study designs (case–control, cohort and cross-sectional studies) were eligible for the meta-analysis [21].

The influences of the alleles (mutated vs. wild) and genotypes in different models of inheritance were evaluated: codominance (mutated homozygote vs. wild homozygote, or heterozygote vs. wild homozygote), recessiveness (mutated homozygote vs. heterozygous + wild homozygote) and dominance (mutated homozygote + heterozygote vs. wild-type homozygote). Funnel-plot graphs were inspected for possible publication bias.

2.8. SNP2TFBS

To identify single nucleotide polymorphisms (SNPs) that may affect transcription factor binding, we used the Mapping SNPs to Transcription Factor Binding Sites (SNP2TFBS) web interface (https://ccg.epfl.ch/snp2tfbs/, May 2022).

2.9. Patient Classification

Using the data available in the publications, patients were grouped into two main groups: healthy individuals without *T. cruzi* infection were termed as non-infected (nonCP), while patients infected with *T. cruzi* were generically termed as chronic patients with Chagas disease (CP). The "CP" group was subdivided according to the presence or absence of symptoms into two smaller groups: chronic asymptomatic patients in an undetermined form (UndCP) and chronic symptomatic patients (SympCP). Patients in the "SympCP" group were divided into three groups according to the clinical manifestation of the chronic form of CD: patients who presented any type of cardiac alteration/complications resulting from CD were grouped as cardiac patients (CardCP), while those with complications associated with the digestive system were grouped as digestive patients (DigCP). Patients who had both forms involving heart and digestive problems were grouped as mixed complications (CarDigCP). In our comparisons, the presence of someone involved in the CardCP or DigCp group did not exclude them from participating in the CarDigCP group, and vice versa.

2.10. Statistical Analysis

In order to evaluate the interaction between the sample data, for the results of the global comparisons (using all comparison studies) where there was significance, principal component analysis (PCA) was performed using the Past 4.10 software (https://www.nhm.uio.no/english/research/infrastructure/past/, May 2022). For significant comparisons found in the quantitative synthesis, sample power was calculated using G*Power 3.1.9.7 (χ^2-Goodness-of-fit, Post hoc, $\alpha = 0.05$) [22,23], to determine the representativeness of the results in the population, values >80% were considered significant.

Exploratory statistical analyses were performed to establish comparisons between genetic factors and the development of the cardiac form of CD. For this purpose, the possible correlations between haplotypes and their respective constituent alleles were verified using Pearson's correlation. The $\chi 2$ test was used to compare the frequency of the HHC haplotype between groups, adopting a significance level of 5%.

3. Results

3.1. Characterization of the Studies

Our work gathered the available information from the literature on the influence of CCR5 gene variants on CD. The flow of searches, analyses and the collection of articles is outlined in Figure 1. Table 1 summarizes the qualitative synthesis, with the characterization and main results of each of the eleven studies found in our search. One of the articles [24] proved to be a previous study of another with a larger sample [13]; therefore, we considered the most recent manuscript for the quantitative synthesis. Table 1 also presents the genetic variants used in the quantitative synthesis. The comparisons made between groups, the sample power found through G*Power, and the frequencies found are available in Supplementary Table S1.

Table 1. Data extracted from articles included in the systematic review. The data presented consist of: STREGA score, main results associated with CCR5 variants and overall frequency (calculated based on the sum of samples from all collected studies), inclusion in the quantitative synthesis (meta-analysis), types of groups compared in the quantitative synthesis, and number of participants in each group (NonCP, CP, SympCP, CardCP and UndCP).

Study Characterization									
References	**Study Country** [1]	**Study Design**	**Population/Sample (n)**	**Group Characterization** [2]	**STREGA Score**	**Confirmation Method of *T. cruzi* Infection**	**Confirmation Method of Polymorphism**	**Parasitemia Evaluation**	**Hardy-Weinberg Equilibrium (HWE)** [3]
Oliveira et al., 2014 [24]	Brazil	Cross-sectional	Brazilians/n = 168	CP = 168 (CardCP = 168)	8—Low quality	ELISA	PCR-RFLP	No	Nd
Frade et al., 2013 [11]	Brazil	Cohort	Brazilians/n = 433	CP = 433 (CardCP = 315 and UndCP = 118)	13—Medium quality	ELISA, IHA and IIF	Hybridization assay and qPCR	No	Yes [4]
Nogueira et al., 2012 [25]	Brazil	Cohort	Brazilians/n = 321	CP = 22 (CardCP = 171) and UndCP = 150	12—Medium quality	ELISA, IHA and IIF	qPCR	No	Yes
Juiz et al., 2019 [26]	Argentina	Case-control	Argentinians/n = 480	CP = 480 (CardCP= 215 and UndCP = 256)	14—Medium quality	Serologically	qPCR	No	Yes [5]
Calzada et al., 2001 [9]	Spain	Case-control	Peruvians/n = 172	CP = 172 (UndCP = 53; CardCP = 32) and nonCP = 87	14—Medium quality	ELISA, IHA and IIF	PCR-RFLP	No	Yes
Lima et al., 2018 [12]	Brazil	Observational study—case series	Brazilians/n = 6	CP = 6 (UndCP = 1 and CardCP = 5)	10—Medium quality	ELISA, IHA and PCR	PCR and PCR-RFLP	No	Nd
Batista et al., 2018 [8]	Brazil	Cross-sectional	Brazilians/n = 406	CP = 406 (CardCP = 296 and UndCP = 110)	17—High quality	ELISA and IIF	qPCR	Yes	Nd
De Oliveira et al., 2015 [13]	Brazil	Case-control	Brazilians/n = 412	CP = 240 (CardCP = 121 and DigCP = 98) and nonCP = 172	15—Medium quality	ELISA	PCR and PCR-RFLP	No	Yes
Flórez; Martín; González, 2012 [10]	Colombia	Case-control	Colombians/n = 260	CP = 260 (UndCp = 130 and CardCP = 130)	16—Medium quality	ELISA and IHA	PCR and PCR-RFLP	No	Yes
Fernandez-Mestre; Montagnani; Layrisse, 2004 [27]	Venezuela	Cross-sectional	Venezuelans n = 107	CP = 107 (UndCP = 34 and CardCP = 73)	11—Medium quality	ELISA, IHA and IIF	PCR-RFLP	No	Nd
Machuca et al., 2014 [28]	Colombia	Cohort	Colombians/n = 476	CP = 476 (UndCP = 206 and CardCP = 270)	13—Medium quality	ELISA and IHA	qPCR	No	Yes

Table 1. *Cont.*

CCR5 Polymorphisms			
Polymorphism	**Mutated Nucleotide Localization/Variation Type [and Change]/Consequence** [6]	**Main Finds of Studies**	**Quantitative Synthesis by Meta-Analysis (Yes/No)**
rs2856758	46370170/SNV [A > G]/intron variant, 5 prime UTR variant	Flórez et al., 2012 [10]: G allele increased in asymptomatic group in a comparison with the cardiomyopathy group, indicating the cardiomyopathy risk reduction (p = 0.021, OR = 0.51, 95% CI (0.29–0.91)). Juiz et al., 2019 [26]: not associated. Machuca et al., 2014 [28]: not associated.	Yes
rs2734648	46370349/SNV [G > C/G > T]/intron variant	Flórez et al., 2012 [10]: T allele associated with reduced risk of cardiomyopathy progression compared to symptomatic subgroups (group II vs. group III, p = 0.018, OR = 0.44, 95% CI (0.22–0.88)/and group III vs. group IV, p = 0.004, OR = 0.29, 95% CI (0.12–0.68)). Juiz et al., 2019 [26]: not associated. Machuca et al., 2014 [28]: not associated.	Yes
rs1799987	46370444/SNV [A > G]/intron variant	Batista et al., 2018 [8]: not associated. Calzada et al., 2001 [9]: G/A increased in asymptomatic group in a comparison with the cardiomyopathy group (p = 0.02, OR = 0.33, 95% CI (0.10–0.94)); G allele increased in asymptomatic group in a comparison with the cardiomyopathy group (p = 0.02, OR = 0.35, 95% CI (0.12–0.96)). Fernández-Mestre et al., 2004 [27]: not associated. Flórez et al., 2012 [10]: not associated. Juiz et al., 2019 [26]: not associated. Lima et al., 2018 [12]: not associated. Oliveira et al., 2015 [13]: A/A frequency was different among patients with digestive and cardiac forms, and health controls (p = 0.036, $\chi 2$ = 6.656 (DF = 2)); A/A frequency was different between patients with digestive and cardiac forms (p = 0.013, $\chi 2$ = 6.129 (DF = 1)); A/A frequency was different between patients with cardiac form and health controls (p = 0.077, $\chi 2$ = 3.128 (DF = 1)); Oliveira et al., 2014 [24]: not associated. Machuca et al., 2014 [28]: not associated.	Yes
rs1799988	46370768/SNV [C > T]/intron variant, 5 prime UTR variant	Flórez et al., 2012 [10]: not associated. Juiz et al., 2019 [26]: not associated. Machuca et al., 2014 [28]: not associated. Nogueira et al., 2012 [25]: C/C frequency was increased in CCC severe group than CCC moderate group (p = 0.01, $\chi 2$ = 5.55, OR = 2.31, 95% CI (1.14–4.67)); C allele was higher in CCC severe group than CCC moderate group (p = 0.01, $\chi 2$ = 6.15, OR = 0.58, 95% CI (0.37–0.89)).	Yes
rs41469351	46370771/SNV [C > T]/intron variant, 5 prime UTR variant	Flórez et al., 2012 [10]: not associated. Juiz et al., 2019 [26]: T allele frequency in a sample with Caucasian genetic background was increased in a demonstrated cardiomyopathy group than non-demonstrated cardiomyopathy group (p = 0.028, OR = 4.88, 95% CI (1.03–23.24)). Machuca et al., 2014 [28]: not associated.	Yes
rs1800023	46370817/SNV [A > G/A > T]/intron variant, 5 prime UTR variant	Flórez et al., 2012 [10]: not associated. Juiz et al., 2019 [26]: not associated. Machuca et al., 2014 [28]: G allele was more frequent in the group with lower CCC severity compared to symptomatic subgroups (group II vs. group III, p = 0.05, OR = 0.70, 95% CI (0.49–1.00)).	Yes

Table 1. *Cont.*

CCR5 Polymorphisms			
Polymorphism	**Mutated Nucleotide Localization/Variation Type [and Change]/Consequence** [6]	**Main Finds of Studies**	**Quantitative Synthesis by Meta-Analysis (Yes/No)**
rs1800024	46371068/SNV [C > T]/intron variant	Flórez et al., 2012 [10]: not associated. Juiz et al., 2019 [26]: T allele associated with reduced risk of cardiomyopathy in the group without symptoms of cardiomyopathy (asymptomatic group) when compared to the group with symptoms of cardiomyopathy (cardiomyopathy group) (p = 0.041, OR = 0.69, 95% CI (0.49–0.99)). Machuca et al., 2014 [28]: T allele associated with higher disease severity when compared to symptomatic subgroups (group II vs. group III, p = 0.02, OR = 1.70, 95% CI (1.06–2.71)).	Yes
rs3176763	46372790/SNV [G > A/G > T]/intron variant	Frade et al., 2013 [11]: C/C frequency was increased in CCC group than asymptomatic group when considered gender (p = 0.006, OR = 1.79, 95% CI (1.18–2.70)); C/A frequency was increased in CCC group than asymptomatic group when considered left ventricular ejection fraction under 0.4% values (p = 0.005, OR = 1.88, 95% CI (1.20–2.94)).	No
rs333[Δ32]	46373453–46373487/frameshift variant (Delins, length 35 pb), coding sequence variant, intron variant	Calzada et al., 2001 [9]: not associated. Fernández-Mestre et al., 2004 [27]: not associated. Flórez et al., 2012 [10]: not associated. Oliveira et al., 2015 [13]: not associated.	Yes
rs3087253	46377198/SNV [C > T]/intron variant	Frade et al., 2013 [11]: not associated.	No
rs11575815	46378679/SNV [A > T]/intron variant	Frade et al., 2013 [11]: A/T and A/A frequecy was increased in the CCC group than asymptomatic group (p = 0.030, OR = 1.41, 95% CI (1.03–1.92)).	No

ELISA—enzyme-linked immunosorbent assay; IHA—indirect hemagglutination assay; IIF—indirect immunofluorescence; [1] country of study is based on the first author; [2] the characterization of each study was performed by us in order to make comparisons in the quantitative synthesis. The categorizations originally made by the authors of each article are described in Table 2; [3] Nd = HWE not described by the authors; [4] HWE was presented only for the control group; [5] rs1799864 and 1800024 are not in HWE; [6] information provided by NCBI.

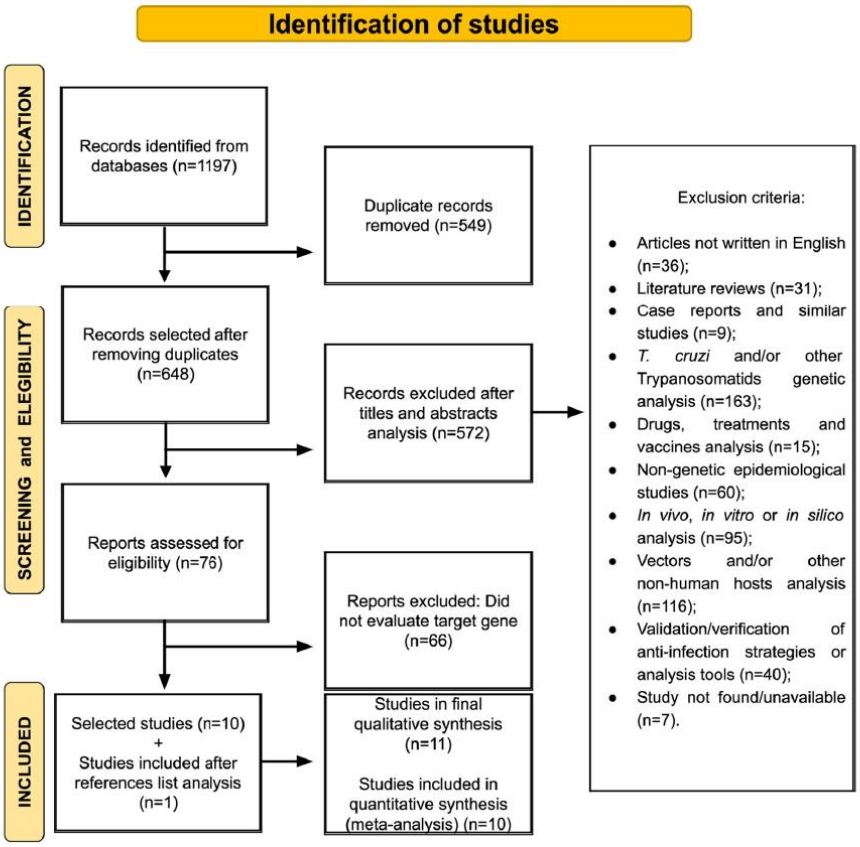


Figure 1. Flow of studies added in the systematic review. 1197 returns were collected from the databases, 11 studies were added in the qualitative synthesis, and 10 were used in the quantitative synthesis (meta-analysis).

3.2. Meta-Analysis and PCA

In our bibliographic survey, data on the genetic variants of CCR5 associated with CD were pooled, and eight genetic variants were subjected to a meta-analysis based on the number of studies: rs1799987, rs333, rs2856758, rs2734648, rs1799988, rs41469351, rs1800023 and rs1800024. The characteristics of the groups used in the studies that constituted the "CardCp" grouping are shown in Table 2. Only one study [13] analyzed patients with the digestive form of CD; therefore, comparisons were not possible using the "DigCP" grouping. During the analyses, two subgroups were formed based on the patients' genetic backgrounds: patients from countries emancipated from Spanish colonization (with a predominantly Amerindian and European background) and Brazilian patients (with a predominantly Amerindian, European and African background).

Table 2. Description of the groups that constitute the CardCP grouping.

Reference	Description of CardCP Group Patients
Batista et al., 2018 [8]	Group in stage B1 (with structural heart disease, evidenced by ECG or ECHO, but with normal global ventricular function and neither current nor previous signs or symptoms of congestive heart failure) and group in stage C (with ventricular dysfunction and current or previous symptoms of congestive heart failure)
Calzada et al., 2001 [9]	Cardiomyopathic group with cardiac symptoms, which were assessed by clinical and electrocardiographic (ECG) characteristics compatible with chagasic cardiomyopathy
Fernandez-Mestre, Montagnani and Layrisse, 2004 [27]	Group B (arrhythmia-related symptoms or embolic episodes as a first symptom, and in whom radiological size ranged from normal to severe cardiomegaly and various arrhythmias) and group C (with overt congestive heart failure, most with severe cardiomegaly and various arrhythmias)
Flórez, Martín and González, 2012 [10]	Group II (radiology indicative of light heart hypertrophy or minor ECG alterations), group III (moderate heart hypertrophy and considerable ECG alterations, mainly advanced conduction abnormalities) and group IV (severe cardiomegaly and marked ECG alterations, predominantly frequent and/or complex forms of ventricular arrhythmia)
Juiz et al., 2019 [26]	CD group (with clinical symptoms and electrocardiography alterations)
Lima et al., 2018 [12]	Patients that presented cardiac arrhythmia with stimulus conduction disorder by EKG, compatible with chagasic cardiopathy
Machuca et al., 2014 [28]	Symptomatic group (with minor symptoms and alterations) and cardiomyopathic group (with considerable symptoms and alterations)
Nogueira et al., 2012 [25]	CCC (moderate group and severe group)
Oliveira et al., 2015 [13]	Group of cardiac form (CCHD when presenting with electrocardiographic or echocardiographic abnormalities consistent with the disease)

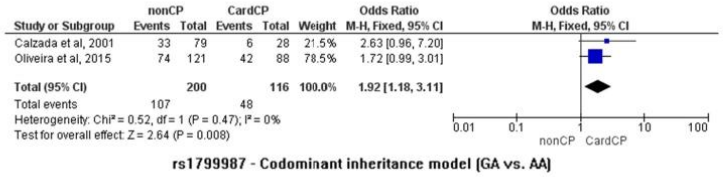
3.2.1. rs1799987

Eight studies evaluated the influence of the rs1799987 SNP on CD [8–10,12,13,26–28]. When comparing the groups of chronic vs. healthy controls (n = 558, CP = 304/nonCP = 254), symptomatic vs. asymptomatic (n = 1871, SympCP = 1031/UndCP = 840), healthy controls vs. asymptomatic (n = 370, nonCP = 254/UndCP = 116) and cardiac patients vs. asymptomatic (n = 1933, CardCP = 1093/UndCP = 840), no significant differences were found.

The comparison between patients in the healthy control group vs. cardiac patients (n = 407, nonCP = 254/CardCP = 153, [9,13]) showed, in the codominance model (A/G vs. A/A), an association of the heterozygous genotype with the risk of developing the cardiac form (p = 0.008, OR = 1.92, 95% CI (1.18–3.11), I^2 = 0%, fixed model). Similarly, in the dominance model (A/G + G/G vs. A/A), an association of G carriers with the risk of developing the cardiac form was also verified (p = 0.02, OR = 1.66, 95% CI (1.08–2.55), I^2 = 0%, fixed model). Both associations are summarized in Figure 2a,b, respectively.

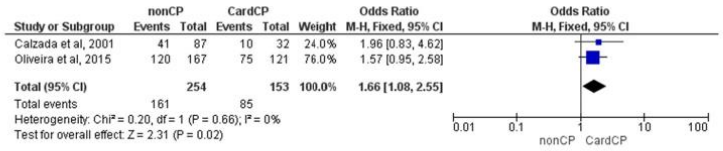
PCA was used to correlate the results obtained in the meta-analysis and establish inter-relationships between the variables, creating a smaller set of principal components (PCs). PC1 corresponded to 89.58% of the variance, while PC2 corresponded to 9.33%, totaling 98.91% of the variance. PC1 was dominated by the A and G alleles and the A/G genotype, while PC2 was predominantly dominated by the homozygous A/A and G/G genotypes. The analysis of the generated graphs suggested a correlation between the A allele and the A/A genotype, as well as suggesting a correlation between the G allele and the G/G genotype, while there was a distancing of the A/G genotype from these two groups (Figure 3a).

a



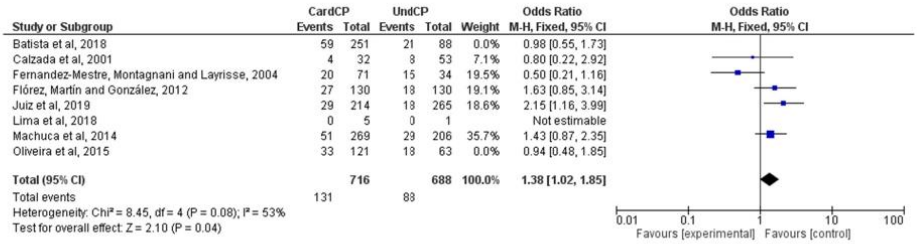
rs1799987 - Codominant inheritance model (GA vs. AA)

b



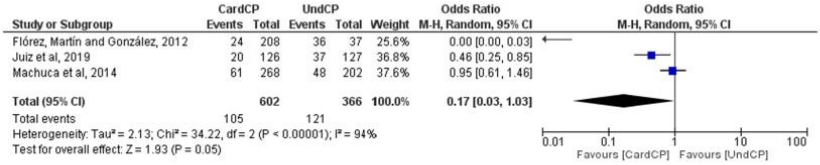
rs1799987 - Dominant inheritance model (GG+GA vs. AA)

c



rs1799987 - Recessive inheritance model (GG vs. GA+AA) - Samples from countries derived from Spanish colonization

d



rs2856758 - Codominant inheritance model (GA vs. AA)

Figure 2. Meta-analysis of CCR5 genetic polymorphisms (rs1799987 and rs2856758) associated with CD. Comparisons were performed using dominance, codominance and recessiveness models. In our study, some analyses showed new associations based on investigated subgroups. The Mantel–Haenszel (M–H) test is the statistical method that was applied to generate an estimate of the association between Chagas disease and the analyzed risk factor after calculation adjustments [(**a**) codominant inheritance model GA vs. AA, (**b**) dominant inheritance model GG+GA vs. AA, (**c**) recessive inheritance model GG vs. GA+AA, and (**d**) codominant inheritance model GA vs. AA]. References: Batista et al., 2018 [8]; Calzada et al., 2001 [9]; Flórez, Martín and González, 2012 [10]; Fernandez-Mestre, Montagnani and Layrisse, 2004 [27]; Juiz et al., 2019 [26]; Lima et al., 2018 [12]; Machuca et al., 2014 [28]; and Oliveira et al., 2015 [13].

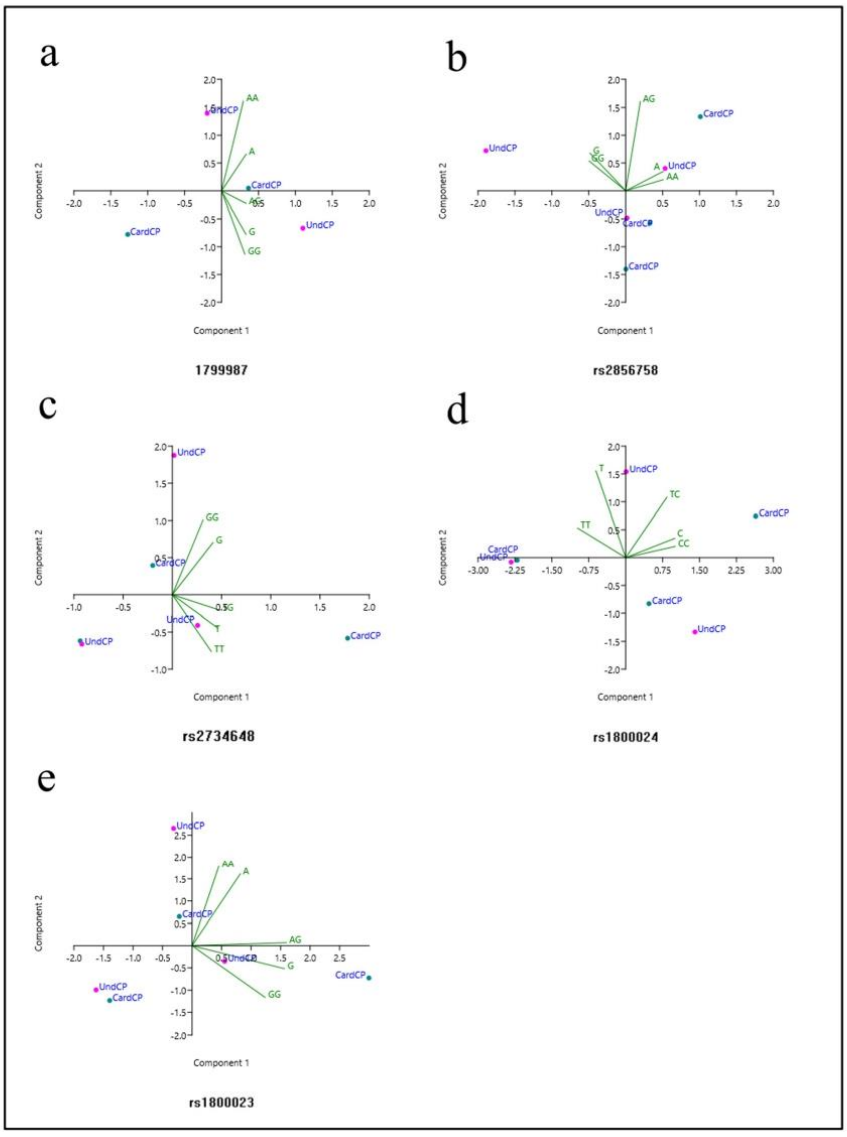


Figure 3. Principal component analysis (PCA) of genetic variants of CCR5 associated with CD. The established correlations were performed using the alleles and genotypes of each of the SNPs with statistical results on a global scale (without the need to analyze investigated subgroups). The green dots represent CD cardiac patient groups (CardCP), while the pink dots represent asymptomatic patients (UndCP). [(**a**) PCA between AA, AG and GG genotypes, and A and G alleles on SNP rs1799987; (**b**) PCA between AA, AG and GG genotypes, and A and G alleles on SNP rs2856758; (**c**) PCA between TT, TG and GG genotypes, and T and G alleles on SNP rs2734648; (**d**) PCA between TT, TC and CC genotypes, and T and C alleles on SNP rs1800024; and (**e**) PCA between AA, AG and GG genotypes, and A and G alleles on SNP rs1800023].

Although the global comparison between cardiac patients vs. asymptomatic patients showed no difference between the groups, when the analysis was limited to patients from countries originating from Spanish colonization, [9,10,26–28], n = 1404, CardCP = 716/UndCP = 688), in the recessiveness model (G/G vs. G/A + A/A), the G/G genotype was associated with the risk of developing the cardiac form ($p = 0.04$, OR = 1.38, 95% CI (1.02–1.85), $I^2 = 53\%$, fixed model), as shown in Figure 2c. Other statistical associations were not found.

3.2.2. rs333

Four studies analyzed the Δ32 deletion [9,10,13,27] in the groups of cardiac patients vs. undetermined (n = 583, CardCP = 366/UndCP = 217), chronic patients vs. healthy controls (n = 951, CP = 692/nonCP = 259), healthy vs. cardiac patients (n = 625, nonCP = 259/CardCP = 366) and healthy controls vs. undetermined (n = 476, nonCP = 259/UndCP217); however, due to the rarity of the Δ32 allele in the populations studied, some comparisons could not be performed. In addition, for viable comparisons, no differences were found between the analyzed groups.

3.2.3. rs2856758

The rs2856758 polymorphism was analyzed in three studies [10,26,28]. Only the comparison between cardiac patients vs. asymptomatic patients (n = 1255, CardCP = 654/UndCP = 601) was feasible. The codominance model (A/G vs. A/A) indicated an association between the heterozygous genotype and a decreased risk of developing the cardiac form of CD ($p = 0.05$, OR = 0.17, 95% CI (0.03–1.03), $I^2 = 94\%$, random model), as shown in Figure 2d. Other genetic models of association were not significant.

The PCA showed in our analysis that PC1 corresponded to 70.91% of the variance, while PC2 corresponded to 21.16%, totaling 92.07% of the variance (Figure 4b). The variables that dominated in PC1 were the A and G alleles, and the A/A and G/G genotypes. On the other hand, the variable that dominated in PC2 was the A/G genotype. According to the PCA, the A allele and the A/A genotype were correlated, while the G allele and the G/G genotype were correlated. The A/G genotype was not correlated with other data in the multivariate analysis. Other correlations were not possible to establish by PCA, since there was a high degree of heterogeneity in some of the investigated groups, as pointed out by the meta-analysis.

3.2.4. rs2734648

As shown in Figure 4a, three independent studies analyzed the relationship between the rs2734648 SNP and CD [10,24,28]. The comparison between cardiac patients vs. asymptomatic patients (n = 1347, CardCP = 712/UndCP = 635) was performed using these papers. Other comparisons were not possible. In the dominance model (T/T + T/G vs. G/G), an association was found between T carriers and the risk of developing the cardiac form ($p = 0.05$, OR = 1.24, 95% CI (1.00–1.55), $I^2 = 38\%$, fixed model). In the PCA, PC1 corresponded to 72.67% of the variance, while PC2 corresponded to 27%, totaling 99.67% of the variance (Figure 3c). The variables that dominated in PC1 were the T and G alleles, and the T/T and T/G genotypes. PC2 was dominated by the G allele and the T/T and G/G genotypes. It was observed that the T allele and the T/T and T/G genotypes were related, while the G allele and the G/G genotype were related, as shown in Figure 3.

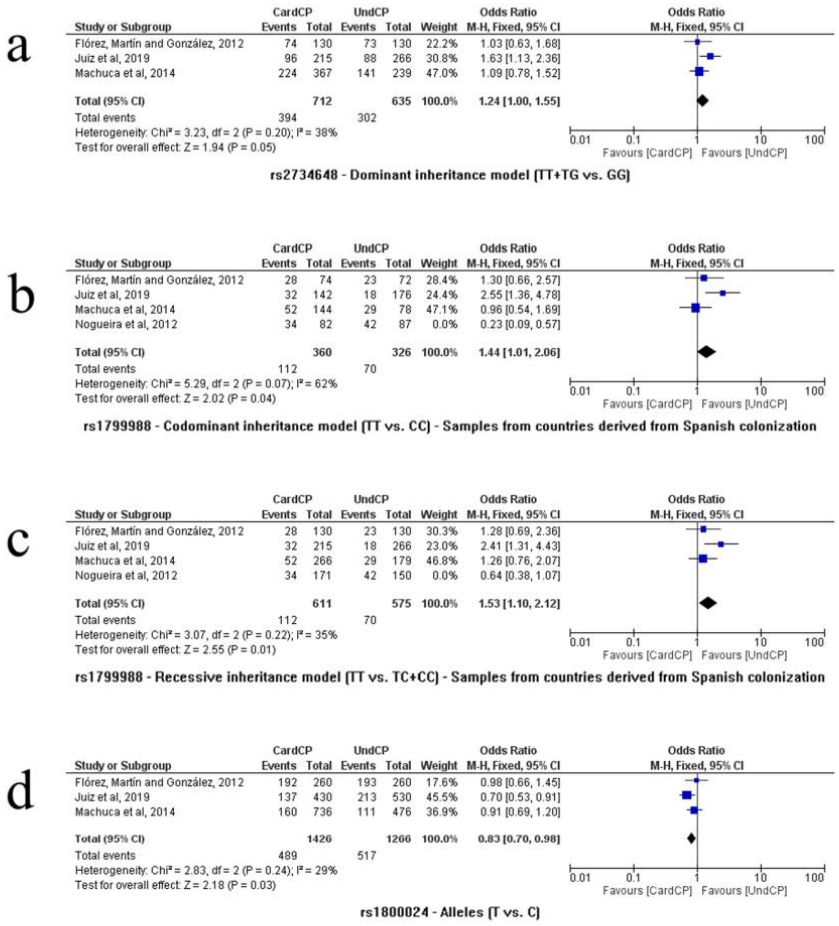


Figure 4. Meta-analysis of CCR5 genetic polymorphisms (rs2734648, rs1799988 and rs1800024) associated with CD. Comparisons were performed using dominance, codominance and recessiveness models. In our study, some analyses showed new associations based on investigated subgroups. The Mantel–Haenszel (M–H) test is the statistical method that was applied to generate an estimate of the association between Chagas disease and the analyzed risk factor after calculation adjustments [(**a**) dominant inheritance model TT+TG vs. GG, (**b**) codominant inheritance model TT vs. CC, (**c**) recessive inheritance model TT vs. TC+CC, and (**d**) alleles T vs. C]. References: Flórez, Martín and González, 2012 [10]; Juiz et al., 2019 [26]; Machuca et al., 2014 [28]; and Nogueira et al., 2012 [25].

3.2.5. rs1799988

Flórez, Martín and González, Juiz et al., Machuca et al., and Nogueira et al. [10,25,26] evaluated the influence of the rs1799988 polymorphism on CD, and these studies were used to compare cardiac patients vs. asymptomatic (n = 1507, CardCP = 782/UndCP = 725). No differences were found between the groups in any genetic model. However, when the analysis delimited only patients whose countries originated from Spanish colonization (Flórez, Martín and González, 2012 [10]; Juiz et al. [26] and Machuca et al. [28], n = 1186, CardCP = 575/UndCP = 611), the codominance model (T/T vs. C/C) showed an association between the homozygous T/T genotype and the risk of developing the cardiac form of CD ($p = 0.04$, OR = 1.44, 95% CI (1.01–2.06), $I^2 = 62\%$, fixed model); similarly, the recessiveness

model (T/T vs. T/C + C/C) also indicated an association of the homozygous T/T genotype with the same risk of developing the cardiac form of CD (p = 0.01, OR = 1.53, 95% CI (1.10–2.12), I^2 = 35%, fixed model), as shown in Figures 4b and 4c, respectively. Other statistical associations were not found.

3.2.6. rs41469351

Only three studies involving patients from Spanish colonization countries analyzed the SNP rs41469351 [10,24,28]. Cardiac vs. asymptomatic patients were compared using data from these studies (n = 1347, CardCP = 712/UndCP = 635); however, due to the rarity of the T allele and therefore the T/T genotype (not found in any of the studies), further comparisons could not be conducted. For viable comparisons, no differences were found between the analyzed groups.

3.2.7. rs1800024

Flórez, Martín and González, Juiz et al., and Machuca et al. [10,26,28] evaluated the influence of the SNP rs1800024 on CD. The comparison derived from these studies verified relationships between cardiac patients vs. asymptomatic (n = 1346, CardCP = 713/UndCP = 633). None of the evaluated genetic models showed any significant difference in the genotypic comparisons. However, the allele comparison (T vs. C) showed an association between the T allele and a decrease in the risk of developing the cardiac form of CD (p = 0.03, OR = 0.83, 95% CI (0.70–0.98), I^2 = 29%, fixed model) (Figure 4d). The PCA was used to analyze the relationships between the data, where PC1 and PC2 presented, respectively, 77.88% and 21.57% of the variances, totaling 99.45%. PC1 was dominated by the C allele and the homozygous genotypes C/C and T/T, whereas PC2 was dominated by the T allele and the T/C genotype. Two-dimensional analysis indicated a correlation between the T allele and the T/T genotype, while the C allele and the T/C and C/C genotypes were correlated (Figure 3d).

3.2.8. rs1800023

Only three studies analyzed the rs1800023 polymorphism [10,26,28] and genetic models comparing groups of cardiac vs. asymptomatic patients (n = 1216, CardCP = 615/UndCP = 601). The allele comparison (G vs. A) indicated an association of the G allele with the risk of developing the cardiac form of CD (p = 0.003, OR = 1.30, 95% CI (1.09–1.55), I^2 = 42%, fixed model). Significant differences were observed in almost all genetic models. The risk of developing the cardiac form of CD was associated with the G/G homozygote in the codominance model (G/G vs. A/A): p = 0.01; OR = 1.53; 95% CI (1.10–2.12); I^2= 35%; fixed model. The mutated G/G genotype was also associated in the recessiveness model (G/G vs. G/A + A/A): p = 0.01; OR = 1.60; 95% CI (1.10–2.34); I^2 = 42%; fixed model. In the dominance model (G/G + GA vs. A/A), genotypes associated with the G allele were also associated with the risk of developing the cardiac form of CD (p = 0.02, OR = 1.31, 95% CI (1.04–1.65), I^2 = 0%, fixed model). Representations of allelic and genotypic associations in the codominance, recessiveness and dominance models are shown in Figures 5a, 5b, 5c and 5d, respectively.

PCA indicated variances of 56.00% and 42.48% for PC1 and PC2, respectively, totaling 98.48%. The G allele and the G/A and G/G genotypes dominated PC1, while the A allele and the A/A genotype dominated PC2. The analysis of the graphs indicates a correlation between the A allele and the A/A genotype, as well as a correlation between the G allele and the genotypes carried by this allele (G/G and G/A) (Figure 3e).

Following our analyses, no other association between the analyzed variants and CD was verified. Considering that all the analyses showed a sample power >80% (good sample representativeness), and with the exclusion of the SNPs rs333 and rs41469351 (unfeasible meta-analysis), it is possible to suggest that the other alleles and genotypes that did not show any association with CD are not factors capable of influencing this infection.

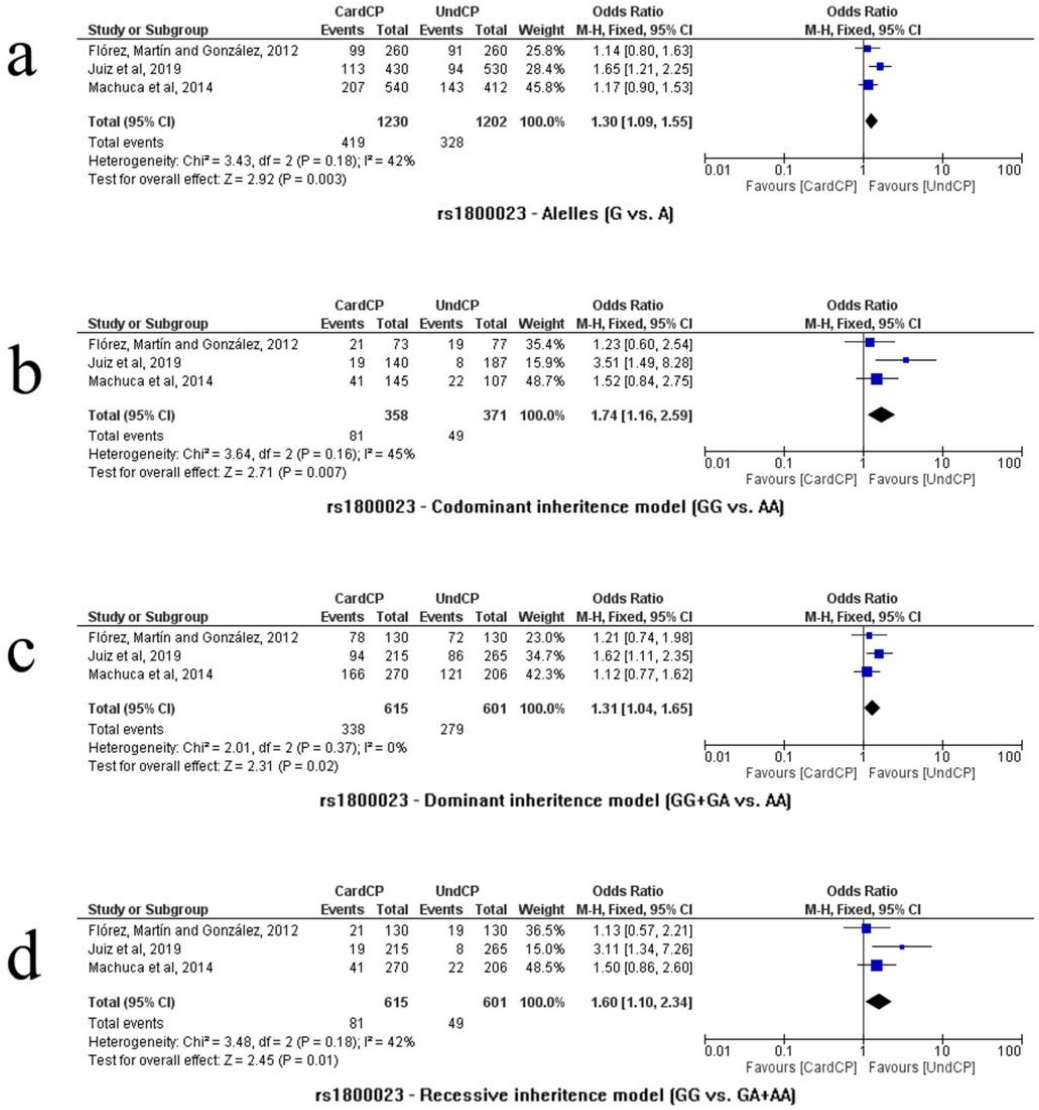


Figure 5. Meta-analysis of SNP rs1800023 (CCR5 genetic polymorphism) associated with CD. Analysis was performed on dominance, codominance and recessiveness models. The Mantel–Haenszel (M–H) test is the statistical method that was applied to generate an estimate of the association between Chagas disease and the analyzed risk factor after calculation adjustments. [(**a**) alleles G vs. A, (**b**) codominant inheritance model GG vs. AA, (**c**) dominant inheritance model GG + GA vs. AA, and (**d**) recessive inheritance model GG vs. GA + AA]. References: Flórez, Martín and González, 2012 [10]; Juiz et al., 2019 [26]; and Machuca et al., 2014 [28].

3.3. Publication Biases and Heterogeneity

As our analysis had less than ten studies in each comparison (K < 10), we considered inspecting the funnel-plot graphs to assess possible biases in the results of our comparisons. However, the scarcity of studies that analyzed the polymorphisms included in this review

did not allow for a distribution that was considered sufficient to infer the existence of publication bias. In addition, studies were found on both sides of the funnel-plot graphs (Supplementary Figures S1 and S2), which may indicate symmetry and a lack of bias.

In our investigation, only a significant comparison of the rs2856758 SNP (CardCP vs. UndCP, G/A vs. A/A, codominance model) indicated substantial heterogeneity (I^2 = 94%, $p < 0.00001$), as shown in Figure 2d. None of the other results showed significant heterogeneity.

3.4. SNP2TFBS

The prediction by the analysis tool indicated only one polymorphism (rs1800023, third intron) of the CCR5 gene capable of affecting the Spi1 transcription factor binding ($p < 0.05$), as represented in Figure 6.

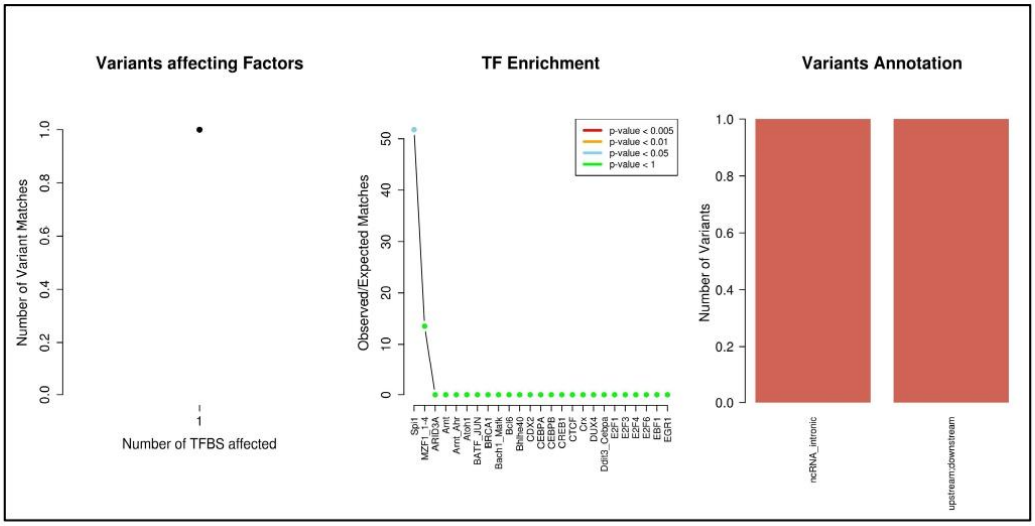


Figure 6. SNP2TFBS analysis of polymorphisms related to the CCR5 gene. Graphical representation requires at least two associations, and no other CCR5 polymorphisms have been listed as influencing any transcription factor. The rs1800629 SNP (TNF-α gene, upstream) was used for this purpose.

3.5. Correlation between HHC Haplotype, Alleles and CD

After the quantitative analyses, we observed that all alleles directly or indirectly associated with the cardiac form of CD (rs2734648 (T), rs1799987 (G), rs1799988 (T) and rs1800023 (G) were the same ones that constituted a haplotype described in the literature as HHC. To verify the possible correlations between the alleles associated with the development of the cardiac form of CD and the haplotype, an exploratory analysis was performed between HHC and the associated alleles. For the analysis, information from the studies included in this review was used [10,26,28]. Quantitative data were extracted from the haplotypes observed in cardiac patients, as well as data from alleles that formed the haplotypes in cardiac and indeterminate patients. The extracted data were used to construct a Pearson correlation. As alleles are structurally related to the haplotype, there is a possibility of error in interpreting the results. In this way, we used a second haplotype known as HHE as a control.

Two conditions were considered in order to assume the correlation between HHC and its alleles (HHC–TGTG) with the development of the cardiac form of CD as true. First, all correlations between the presented alleles and HHC should be strong (>0.800) and positive, and second, the results should be maintained when adding data from the alleles of

undetermined patients to the correlation. The inclusion of allelic data from undetermined patients in this second step aims to avoid misinterpretation of the correlation, given that the allele and HHC data came from the same groups of patients with the cardiac form. If both HHC–TGTG and HHE (HHE–GACA) are strongly positively correlated with their respective alleles, then it is suggested that the correlation between the alleles and haplotypes occurs due to structural dependence. However, if the pre-established correlations are observed only in HHC and its respective alleles, then a correlation with the development of the cardiac form of CD and HHC–TGTG is suggested. The correlation procedures were the same for both haplotypes.

All correlations between HHC and cardiac patient alleles were strongly and positively correlated (>0.950). These correlations were not found between HHE and its respective alleles. Considering the indeterminate patients in the analysis, all correlations between HHC and the alleles remained strong and positive: rs2734648-T (0.980, $p > 0.001$), rs1799987-G (0.935, $p > 0.01$), rs1799988-T (0.926, $p > 0.01$) and rs1800023-G (0.945, $p > 0.01$). For the tested conditions, the correlations between HHE and its respective alleles were classified as median or low (<0.700). Complementarily, using data from the articles, the total frequencies of HHC in cardiac and asymptomatic patients were compared using the χ2test. The results showed a significant difference between HHC frequencies between cardiac and asymptomatic patients ($\chi2 = 19.28$, df = 1, $p < 0.0001$; OR = 1.8, 95% CI (1.38–2.34); SP = 100%; n = 1338, CardCp = 898/UndCP = 440), which suggests a correlation between HHC–TGTG and the cardiac form of CD.

4. Discussion

Chemokines are an important group of small cytokines (7–15 kDa) capable of specifically recruiting subgroups of leukocytes. There are two major subfamilies of chemokines based on the position of their cysteine residues: CXC (neutrophil chemotactics) and CC (monocyte chemotactics and lymphocyte subtypes) [7]. The CCR5 protein is a CC-type chemokine receptor that binds to molecules such as MIP1-α (CCL3), MIP1-β (CCL4) and RANTES (CCL5) [29,30], all related to inflammatory diseases, making CCR5 an important molecular target of these diseases, and often a common point between immunity and their pathophysiology [31–33].

CCR5 is expressed in monocytes, macrophages and lymphocytes such as TH1 cells (known to aid in the destruction of pathogens) [34]. In severe cases of CD in the cardiac form, the patient develops chronic Chagas cardiomyopathy (CCC), which is characterized by persistent myocarditis, with histologically detected tissue fibrosis, the destruction of cardiomyocytes, and a mononuclear infiltrate (usually T cells and macrophages) [25,35]. In this regard, such events suggest the involvement of CCR5 as a collaborative factor in chronic inflammation [35]. Chemokines and their receptors have been studied in CD due to their possible involvement in the chronic phase of the cardiac form, since they allow the maintenance of chronic inflammation, which in turn is the result of the inability of the immune system to completely eliminate the *T. cruzi* parasite [8,13].

Studies indicate that the chronic inflammation of the cardiac form of CD results in tissue damage and is caused by the attempt to eliminate the *T. cruzi* parasite. Cardiomyocytes produce cytokines, chemokines and nitric oxide that attract leukocytes to the inflammatory site, and thus promote the control of parasitic intracellular replication [9]. The expression of chemokine receptors in T cells associated with the expression of specific chemokines in tissues is an important factor that controls lymphocyte infiltration in different types of tissues [25,36]. Therefore, understanding the colonization mechanisms of the inflammatory response cells in cardiac tissues may reveal molecular targets that modulate the inflammatory response in the cardiac form of CD [8,13].

The CCR5 gene is one of the most studied genes in CD [4,37]. In vivo studies have shown that mice deficient in this gene do not develop the cardiac form, indicating that the expression of CCR5 is linked to the chronic cardiac form of the disease, and to the progression to CCC [8]. Interestingly, this evidence is supported by patient studies, in

which analyses conducted with cardiomyopathic patients exhibited increased CCR5 expression, resulting in increased inflammation [13,25]. Studies on CCR5 are some of the most replicated, as well as studies on genetic variants of this gene, whose results are sometimes related to CD, especially with chronic forms of the disease [38].

In our work, we compiled the information present in the literature about the influence of genetic variants of the CCR5 gene on CD. In the qualitative synthesis, we observed that all studies were carried out with samples from South America, which points to the concern about the disease in that region. The patients' parasitemia was scarcely evaluated in the works found. The lack of verification of parasitemia leaves a gap in the influence of the studied genetic variants. There was also a shortage of studies that analyzed the influence of CCR5 and its genetic variants on the digestive form of the disease, since only one study analyzed this relationship [13].

For the construction of the groups used in the comparisons in the quantitative synthesis, we observed that the authors of the studies used different criteria to group the participants as chronic cardiac patients (Table 1). We observed that the main criteria used were the verification of mechanical and morphoanatomical abnormalities of the cardiac structures, and the electrocardiogram consistent with Chagas cardiomyopathy. We could assume that there was homogeneity in this group, so we presented the criteria used in the studies and generically classified them as the "CardCP" group.

The most notorious polymorphism studied in the works was rs1799987 (59029A/G). In the quantitative synthesis, by comparing the CardCp and nonCP groups, our analyses showed that the A/G genotype was associated with the risk of progression to the cardiac form. However, it is important to highlight that the comparison between infected patients and healthy controls presents a non-optimized experimental design, since the comparison is based on a control group that has no chance of evolving to the cardiac form, which would be optimized by the comparison with asymptomatic infected patients (UndCP), allowing a comparison between groups that are in the same infectious condition but presenting forms that evolve in different ways [11].

Our global comparison between CardCp and UndCP did not indicate an association with rs1799987; however, the subgroup of patients from countries originating from Spanish colonization indicated an association of the G/G genotype (recessive inheritance model) with the risk of developing the cardiac form, contradicting the study by Calzada et al. [9], which indicated that the G allele was more present in asymptomatic individuals. It is possible that the genetic background of the population used by Calzada and collaborators [9] was not similar to the populations of the other studies, and therefore they did not find these results. Another possibility is that the sample power of the study developed by Calzada and collaborators [9] was not ideal for comparison, resulting in a false positive. Our sample power in all positively associated results was >80%, indicating representativeness of the population (Table 1).

The rs2856758 SNP A/G genotype in the codominance model was associated with a decreased risk of developing the cardiac form of CD. This result was not observed in other studies [10,26,28];, however, Flórez et al. [10] observed an increased G allele in the asymptomatic group compared to the cardiomyopathic, associating this allele with a decrease in the risk of developing the cardiac form of CD. Our quantitative synthesis also showed an association between the rs2734648 T/T and T/G genotypes and the risk of developing the cardiac form of patients. Flórez et al. [10] have already observed an association of the T allele of this polymorphism with a reduction in the risk for the severity of Chagas cardiomyopathy, also corroborating our results.

Our global analysis of the rs1799988 polymorphism did not find differences between the analyzed groups, but in the analysis of the subgroup of countries formed after Spanish colonization [10,26,28], the codominance model showed an association between the homozygous T/T genotype and the cardiac form of CD, while the recessiveness model associated the same T/T genotype with the cardiac form. Unlike our findings, Nogueira et al. [25] identified an increased C/C genotype and C allele in the cardiac group (CCC)

compared to the moderate group. However, Nogueira et al. [25] used a Brazilian sample, while our subgroup results did not include Brazilians. It is important to emphasize that the countries of South America have populations with a high genetic mix, coming from native Amerindian, African and European populations. The Brazilian population, on the other hand, has a high contribution of European and African genomic inheritance, and a smaller Amerindian contribution. Thus, the samples from countries used in the quantitative synthesis of our study have greater contributions from European and Amerindian genomic heritages [39], which may result in different epidemiological scenarios.

The SNP rs1800023 indicated four different associations, all to some degree related to the G allele when comparing CardCP vs. UndCP (Figure 4). Machuca and others [28] have already established a relationship between the G allele and some degree of CCC severity, supporting our results. The rs1800024 SNP indicated the T allele as a risk factor for cardiomyopathy in CD, also previously suggested by Juiz and coauthors [26], although different from the results of Machuca et al. [28], which related the allele with the severity of the disease.

In this work, we used principal component analysis (PCA), an exploratory statistical analysis that establishes relationships between data without the prior definition of a model, unlike the meta-analysis, which was based on standardized models of genetic inheritance. These relationships are important in order to evaluate how the interaction of alleles and genotypes occurs in the samples. According to PCA, it is possible to observe data dispersion, which may not be fully represented in analyses using pre-established models, as in the meta-analysis performed in our study. Unfortunately, few inferences could be obtained using PCA due to the small number of available studies and, consequently, the limited data. In the PCA, the graphs (Figure 3) and/or three-dimensional representations eventually presented correlations using alleles and genotypes that grouped and curiously resembled the phenotypic groups, as well as referring to some patterns of genetic inheritance. However, these groupings did not necessarily reflect the possible phenotypic groups.

The PCA of rs1799987, based on the interaction between the genotypes, alleles and the condition of the participants (CardCp vs. nonCP), indicated that there was a correlation between the G carriers (G/A and G/G) and the G allele, as well as a correlation between the A allele and the AA genotype. The analysis performed using the SNP rs2856758 reported a cluster formed between the A allele and the A/A genotype, and a second cluster formed by the G allele and the G/G genotype, while the A/G genotype did not seem to be related to any of these groups. Additionally, it should be noted that in our study the A/G genotype obtained an association in the codominant inheritance model. Interestingly, this graphic representation was similar to the phenotypes that were presented in genetic inheritance in the codominance model, that is, the presentation of three different profiles.

The T/T and T/G genotypes of the rs2734648 SNP were both associated with the development of the cardiac form in the dominant inheritance model. In the multivariate analysis, these two genotypes were correlated with the T allele, while the G allele and the G/G genotype formed a second cluster in the PCA. This graphical representation is also similar to the phenotypic profiles in dominant/recessive-type inheritance (group with dominant allele trait and group with recessive allele trait). Our observations from the multivariate analysis of the rs1800023 SNP indicated a correlation between the A allele and the A/A genotype. Despite being relatively distant, a correlation was also observed between the G allele and the G/G and G/A genotypes (Figure 3). Finally, the PCA of the SNP rs1800024 indicated two clusters, one between the T allele and the T/T genotype, and another between the C allele and the T/C and C/C genotypes, similar to the phenotypic profiles observed in the inheritance of the dominant/recessive type.

The SNP2TFBS tool was used to verify the influence of polymorphisms in this review on transcription factors. The rs1800023 SNP was shown to be able to influence Spi1 factor activity. Spi1 is an important transcription factor that binds to a sequence known as PU-box (a purine-rich DNA sequence), located near the promoter region of target genes. Thus, Spi1 co-regulates the expression of target genes together with other transcription factors and

cofactors. The Spi1 protein can also bind to RNA to regulate the selective splicing of target genes [40].

Mummidi et al. [40] have already predicted through algorithms the action of Spi1 in the promoter region of CCR5; however, it is not exactly influenced by SNP rs1800023. Although they were unable to determine the specific binding of a transcription factor to rs1800023, they were able to determine that there was a factor that bound to the G allele but not the A allele, most likely Spi1. According to Mummidi and collaborators [40], the binding of the transcription factor to the altered regulatory regions can be an important selection pressure factor caused by the inter-relationship of the host with the parasite, which can determine the final result of the infection. In addition to their work evaluating the role of transcription factors in the promoter, the study by Mummidi et al. also determined the presence of nine human haplotypes (HHs) and evaluated their functionality in gene expression through their respective ability to bind to different transcription factors.

Many of the polymorphisms analyzed in this review are constituents of CCR5 haplotypes and capable of influencing its expression. Interestingly, all our findings involving an association with the risk of developing Chagas cardiomyopathy are within the C haplotype (HHC), according to Mummidi et al.: rs2734648 (T), rs1799987 (G), rs1799988 (T) and rs1800023 (G), this haplotype seems to induce a low transcriptional activity in Jukart cells (T lymphocyte lineage derived from leukemia), while in Nk cells (CD56+) and CD16+ it presents a high activity [41,42]. The studies by Flórez et al., Machuca et al. and Juiz et al. [10,26,28]—the latter with participants defined as creole and non-endemic—observed that HHC was the most common haplotype in their samples.

To verify possible correlations between HHC and its alleles (HHC–TGTG) and the cardiac form of CD, a Pearson correlation was performed. Our findings demonstrated that there was a very strong correlation between HHC and alleles. All the correlations were positive and significant (>0.800; $p < 0.05$). These results were not found in the correlation study using HHE as a control. Thus, we assume the relationship between HHC–TGTG and the development of the cardiac form of CD to be true. This result is supported by the fact that there was a significant difference in the frequency of HHC between the cardiac and indeterminate groups.

There are different hypotheses to explain the development of the cardiac form of CD in a certain group of patients, such as the influence of the genetic variability of the infecting parasite, molecular mimicry (between parasite antigens and host proteins, resulting in an autoimmune reaction by cross-reaction and reactivating cells), localized damage (induced by in situ expression of a tissue rich in infiltrate, producing chemokine receptor ligands), and finally, the patient's genetics [6,25,43].

Among the hypotheses described, we believe that the patient's genetics is the one that best explains the development of the cardiac form, since studies have indicated the lack of influence between the genetic variability of the parasite and the development of this form of CD [12,44]. Furthermore, any in situ reaction depends on molecular pathways that can be influenced by the patient's genotype, which makes this last hypothesis a result of the patient's genetic influence.

According to the authors of previous studies, it is possible that the course of CD in a patient is not the result of the mere presence or absence of a single genetic variant. It is believed that combinations between several different variants in several genes modestly contribute to the pathogenesis of the disease [10,26,28]. The CCR5 gene has a strong linkage disequilibrium in its shared loci with the CCR2 gene, which allowed us to verify the influence of multiple polymorphic changes in CCR5 expression on the cell surface as the CCR5 expression rate in blood cells changed [41,42]. Our work statistically contributes to this prerogative, indicating not only the individual influence of certain polymorphisms, but also the haplotypic configuration, which influences the migration and accumulation of cells, increasing the cellular infiltrate and leading to the development of the cardiac form of CD.

5. Conclusions

Some limitations of this study can be highlighted. Our quantitative synthesis presents results based on the included studies. These studies used different methodologies, as well as different comparison groups, which may imply inconsistencies in our results. It was also not possible to analyze the influence of environmental factors, such as the exposure time of patients, or factors intrinsic to the participants, such as age and gender. The scarcity of data was a limitation for our analyses and, consequently, our inferences. However, despite these limitations, carrying out this study had advantages, such as a better understanding of CD.

In short, we emphasize that meta-analysis is a tool capable of narrowing the relationship between the genetic profile of the hosts and the clinical outcome. In our analysis, the rarity of the Δ32 alleles of the SNP rs333 and the T allele of the SNP rs41469351 was evident in the studied samples from South American countries. In addition, the C allele (rs1800024) and the A/G heterozygote (rs2856758) were associated with a reduced risk of developing the cardiac form of CD. All alleles associated with an increased risk of developing the cardiac form of CD comprised human haplotype C (HHC). Our HHC–TGTG analysis was also shown to be correlated with the cardiac form of CD. In general terms, exploratory statistical analysis, such as PCA, made it possible to infer correlations between different factors without the need to establish genetic models. Based on our findings, PCA appears to be a strong tool that can help in the understanding of infectious diseases such as CD. Due to the heterogeneity of the data, we advise that the inferences indicated here be analyzed with caution.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life13081677/s1, Figure S1. Funnel plots obtained from comparisons with significant results ($p \leq 0.05$) of the SNPs rs1799987, rs2856758, rs2734648, rs1799988 and rs1800024. Figure S2. Funnel plots obtained from comparisons with significant results ($p \leq 0.05$) of SNP rs1800023. Table S1. Genetic variants, comparisons made between groups and their individual and total frequencies.

Author Contributions: J.M.F. and J.L.d.L.F. designed the paper; J.M.F., B.R.C.d.S., E.L.d.M., A.C.M.d.S., J.C.V.D., E.V.M.d.S.F. and J.L.d.L.F. performed the searchs and analyzed the data; J.M.F., J.C.V.D., E.V.M.d.S.F. and J.L.d.L.F. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES)—term of grant 05/2023.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Authors are grateful to Universidade Federal de Pernabuco (UFPE), Instituto Keizo Asami (iLIKA), Secretaria de Estado de Educação do Espírito Santo (SEDU) and Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES).

Conflicts of Interest: The authors declare no conflict of interest.

References

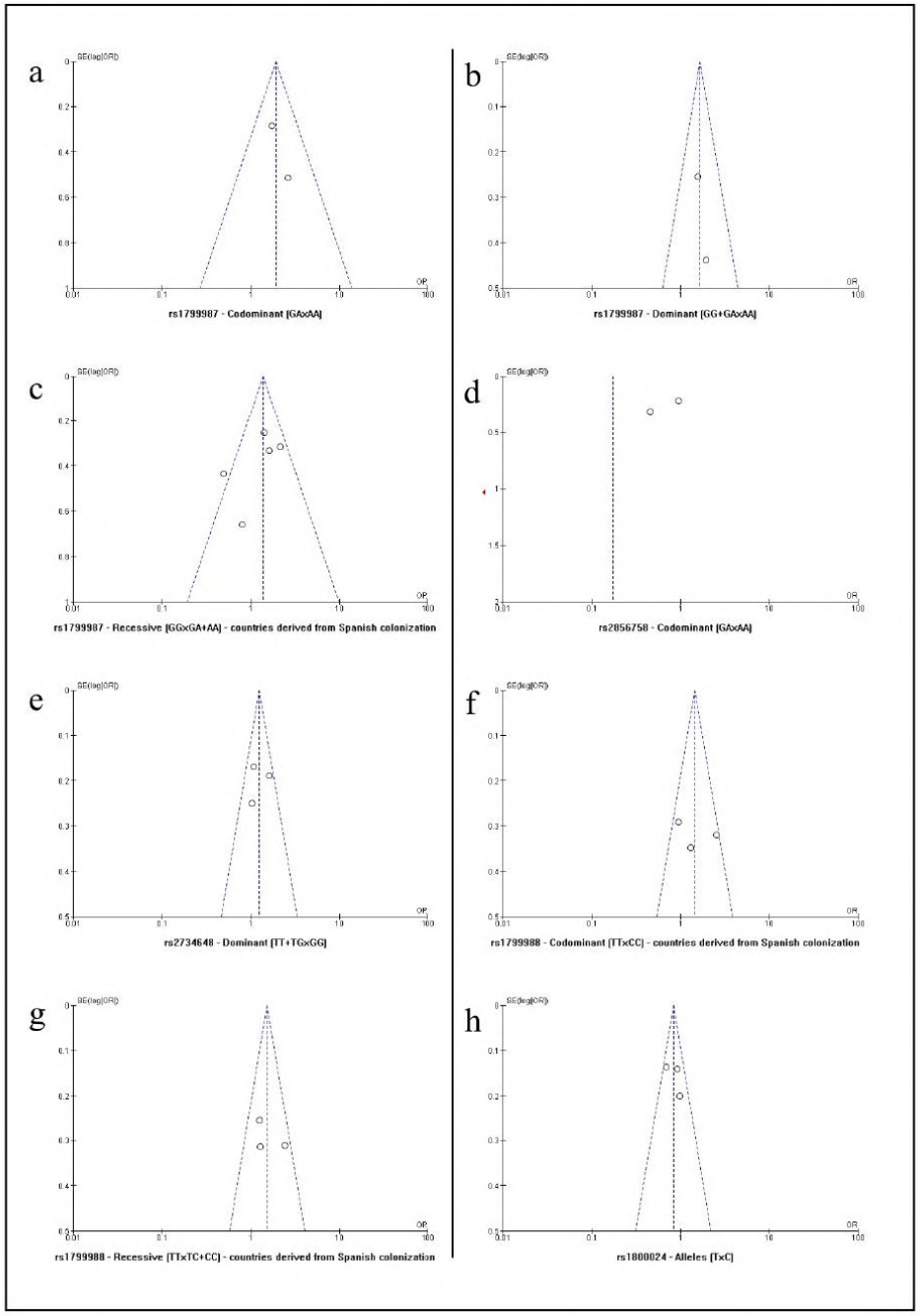
1. World Health Organization—WHO. Chagas Disease (American Trypanosomiasis). Available online: https://www.who.int/health-topics/chagas-disease#tab=tab_1 (accessed on 20 June 2022).
2. Pérez-Molina, J.A.; Molina, I. Chagas Disease. *Lancet* **2018**, *391*, 82–94. [CrossRef]
3. Suárez, C.; Nolder, D.; García-Mingo, A.; Moore, D.A.J.; Chiodini, P.L. Diagnosis and Clinical Management of Chagas Disease: An Increasing Challenge in Non-Endemic Areas. *Res. Rep. Trop. Med.* **2022**, *13*, 25. [CrossRef] [PubMed]
4. Acosta-Herrera, M.; Strauss, M.; Casares-Marfil, D.; Martín, J.; Network, C.G.C. Genomic Medicine in Chagas Disease. *Acta Trop.* **2019**, *197*, 105062. [CrossRef] [PubMed]

5. Gomes dos Santos, A.; Watanabe, E.H.; Ferreira, D.T.; Oliveira, J.; Nakanishi, É.S.; Oliveira, C.S.; Bocchi, E.; Novaes, C.T.G.; Cruz, F.; Carvalho, N.B. A Specific IL6 Polymorphic Genotype Modulates the Risk of *Trypanosoma Cruzi* Parasitemia While IL18, IL17A, and IL1B Variant Profiles and HIV Infection Protect against Cardiomyopathy in Chagas Disease. *Front. Immunol.* **2020**, *11*, 521409. [CrossRef] [PubMed]
6. Chevillard, C.; Nunes, J.P.S.; Frade, A.F.; Almeida, R.R.; Pandey, R.P.; Nascimento, M.S.; Kalil, J.; Cunha-Neto, E. Disease Tolerance and Pathogen Resistance Genes May Underlie *Trypanosoma Cruzi* Persistence and Differential Progression to Chagas Disease Cardiomyopathy. *Front. Immunol.* **2018**, *9*, 2791. [CrossRef] [PubMed]
7. Qi, B.; Fang, Q.; Liu, S.; Hou, W.; Li, J.; Huang, Y.; Shi, J. Advances of CCR5 Antagonists: From Small Molecules to Macromolecules. *Eur. J. Med. Chem.* **2020**, *208*, 112819. [CrossRef]
8. Batista, A.M.; Alvarado-Arnez, L.E.; Alves, S.M.; Melo, G.; Pereira, I.R.; de Souza Ruivo, L.A.; Da Silva, A.A.; Gibaldi, D.; do E S Protásio da Silva, T.; De Lorena, V.M.B. Genetic Polymorphism at CCL5 Is Associated with Protection in Chagas' Heart Disease: Antagonistic Participation of CCR1+ and CCR5+ Cells in Chronic Chagasic Cardiomyopathy. *Front. Immunol.* **2018**, *9*, 615. [CrossRef]
9. Calzada, J.E.; Nieto, A.; Beraun, Y.; Martin, J. Chemokine Receptor CCR5 Polymorphisms and Chagas' Disease Cardiomyopathy. *Tissue Antigens* **2001**, *58*, 154–158. [CrossRef]
10. Flórez, O.; Martín, J.; González, C.I. Genetic Variants in the Chemokines and Chemokine Receptors in Chagas Disease. *Hum. Immunol.* **2012**, *73*, 852–858. [CrossRef]
11. Frade, A.F.; Pissetti, C.W.; Ianni, B.M.; Saba, B.; Lin-Wang, H.T.; Nogueira, L.G.; de Melo Borges, A.; Buck, P.; Dias, F.; Baron, M. Genetic Susceptibility to Chagas Disease Cardiomyopathy: Involvement of Several Genes of the Innate Immunity and Chemokine-Dependent Migration Pathways. *BMC Infect. Dis.* **2013**, *13*, 587. [CrossRef]
12. Lima, A.P.B.; de Oliveira, M.T.; Silva, R.R.; Torres, R.M.; Veloso, V.M.; de Lana, M.; da Silva, G.N. Evaluation of Parasite and Host Genetics in Two Generations of a Family with Chagas Disease. *Parasitol. Res.* **2018**, *117*, 3009–3013. [CrossRef]
13. de Oliveira, A.P.; Bernardo, C.R.; da Silveira Camargo, A.V.; da Ronchi, L.S.; Borim, A.A.; Brandao de Mattos, C.C.; de Campos Junior, E.; Castiglioni, L.; Netinho, J.G.; Cavasini, C.E. Genetic Susceptibility to Cardiac and Digestive Clinical Forms of Chronic Chagas Disease: Involvement of the CCR5 59029 A/G Polymorphism. *PLoS ONE* **2015**, *10*, e0141847. [CrossRef]
14. Brochet, P.; Ianni, B.; Laugier, L.; Frade, A.; Teixeira, P.; Mady, C.; Ferreira, L.; Ferré, Q.; Santos, R.; Kuramoto, A. Epigenetic Regulation of Transcription Factor Binding Motifs Promotes Th1 Response in Chagas Disease Cardiomyopathy. *Front. Immunol.* **2022**, *13*, 4536. [CrossRef] [PubMed]
15. Laugier, L.; Ferreira, L.R.P.; Ferreira, F.M.; Cabantous, S.; Frade, A.F.; Nunes, J.P.; Ribeiro, R.A.; Brochet, P.; Teixeira, P.C.; Santos, R.H.B. MiRNAs May Play a Major Role in the Control of Gene Expression in Key Pathobiological Processes in Chagas Disease Cardiomyopathy. *PLoS Negl. Trop. Dis.* **2020**, *14*, e0008889. [CrossRef]
16. Ferreira, L.R.P.; Ferreira, F.M.; Laugier, L.; Cabantous, S.; Navarro, I.C.; da Silva Cândido, D.; Rigaud, V.C.; Real, J.M.; Pereira, G.V.; Pereira, I.R. Integration of MiRNA and Gene Expression Profiles Suggest a Role for MiRNAs in the Pathobiological Processes of Acute *Trypanosoma Cruzi* Infection. *Sci. Rep.* **2017**, *7*, 17990. [CrossRef]
17. Santos, C.M.d.C.; Pimenta, C.A.d.M.; Nobre, M.R.C. The PICO Strategy for the Research Question Construction and Evidence Search. *Rev. Lat. Am. Enferm.* **2007**, *15*, 508–511. [CrossRef] [PubMed]
18. Pan American Health Organization—PAHO. Chagas Disease—PAHO/WHO | Pan American Health Organization. Available online: https://www.paho.org/en/topics/chagas-disease (accessed on 20 June 2022).
19. Bern, C. Chagas' Disease. *N. Engl. J. Med.* **2015**, *373*, 456–466. [CrossRef] [PubMed]
20. Higgins, J.P.T.; Thompson, S.G.; Deeks, J.J.; Altman, D.G. Measuring Inconsistency in Meta-Analyses. *BMJ Br. Med. J.* **2003**, *327*, 557–560. [CrossRef]
21. Wijarnpreecha, K.; Werlang, M.; Panjawatanan, P.; Kroner, P.T.; Cheungpasitporn, W.; Lukens, F.J.; Pungpapong, S.; Ungprasert, P. Association between Sarcopenia and Hepatic Encephalopathy: A Systematic Review and Meta-Analysis. *Ann. Hepatol.* **2020**, *19*, 245–250. [CrossRef]
22. Erdfelder, E. Statistical Power Analyses Using G * Power 3.1: Tests for Correlation and Regression Analyses. *Behav. Res. Methods* **2009**, *41*, 1149–1160. [CrossRef]
23. Faul, F.; Erdfelder, E.; Lang, A.-G.; Buchner, A. G*Power 3: A Flexible Statistical Power Analysis Program for the Social, Behavioral, and Biomedical Sciences. *Behav. Res. Methods* **2007**, *39*, 175–191. [CrossRef]
24. Oliveira, A.P.; Bernardo, C.R.; Camargo, A.V.S.; Villafanha, D.F.; Cavasini, C.E.; de Mattos, C.C.B.; de Godoy, M.F.; Bestetti, R.B.; de Mattos, L.C. CCR5 Chemokine Receptor Gene Variants in Chronic Chagas' Disease. *Int. J. Cardiol.* **2014**, *176*, 520–522. [CrossRef]
25. Nogueira, L.G.; Santos, R.H.B.; Ianni, B.M.; Fiorelli, A.I.; Mairena, E.C.; Benvenuti, L.A.; Frade, A.; Donadi, E.; Dias, F.; Saba, B. Myocardial Chemokine Expression and Intensity of Myocarditis in Chagas Cardiomyopathy Are Controlled by Polymorphisms in CXCL9 and CXCL10. *PLoS Negl. Trop. Dis.* **2012**, *6*, e1867. [CrossRef]
26. Juiz, N.A.; Estupiñán, E.; Hernández, D.; Garcilazo, A.; Chadi, R.; Morales Sanfurgo, G.; Schijman, A.G.; Longhi, S.A.; González, C.I. Association Study between CCR2-CCR5 Genes Polymorphisms and Chronic Chagas Heart Disease in Wichi and in Admixed Populations from Argentina. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007033. [CrossRef] [PubMed]
27. Fernández-Mestre, M.T.; Montagnani, S.; Layrisse, Z. Is the CCR5-59029-G/G Genotype a Protective Factor for Cardiomyopathy in Chagas Disease? *Hum. Immunol.* **2004**, *65*, 725–728. [CrossRef] [PubMed]

28. Machuca, M.A.; Suárez, E.U.; Echeverría, L.E.; Martín, J.; González, C.I. SNP/Haplotype Associations of CCR2 and CCR5 Genes with Severity of Chagasic Cardiomyopathy. *Hum. Immunol.* **2014**, *75*, 1210–1215. [CrossRef]
29. Aldinucci, D.; Borghese, C.; Casagrande, N. The CCL5/CCR5 Axis in Cancer Progression. *Cancers* **2020**, *12*, 1765. [CrossRef] [PubMed]
30. Maurer, M.; Von Stebut, E. Macrophage Inflammatory Protein-1. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 1882–1886. [CrossRef] [PubMed]
31. Donninelli, G.; Studer, V.; Brambilla, L.; Zecca, C.; Peluso, D.; Laroni, A.; Michelis, D.; Mantegazza, R.; Confalonieri, P.; Volpe, E. Immune Soluble Factors in the Cerebrospinal Fluid of Progressive Multiple Sclerosis Patients Segregate into Two Groups. *Front. Immunol.* **2021**, *12*, 633167. [CrossRef]
32. Patterson, B.K.; Seethamraju, H.; Dhody, K.; Corley, M.J.; Kazempour, K.; Lalezari, J.; Pang, A.P.S.; Sugai, C.; Francisco, E.B.; Pise, A. Disruption of the CCL5/RANTES-CCR5 Pathway Restores Immune Homeostasis and Reduces Plasma Viral Load in Critical COVID-19. *MedRxiv* **2020**. [CrossRef]
33. Zeng, Z.; Lan, T.; Wei, Y.; Wei, X. CCL5/CCR5 Axis in Human Diseases and Related Treatments. *Genes Dis.* **2021**, *9*, 12–27. [CrossRef] [PubMed]
34. Stone, M.J.; Hayward, J.A.; Huang, C.E.; Huma, Z.; Sanchez, J. Mechanisms of Regulation of the Chemokine-Receptor Network. *Int. J. Mol. Sci.* **2017**, *18*, 342. [CrossRef] [PubMed]
35. Jiménez, P.; Jaimes, J.; Poveda, C.; Ramírez, J.D. A Systematic Review of the *Trypanosoma Cruzi* Genetic Heterogeneity, Host Immune Response and Genetic Factors as Plausible Drivers of Chronic Chagasic Cardiomyopathy. *Parasitology* **2019**, *146*, 269–283. [CrossRef]
36. Cunha-Neto, E.; Nogueira, L.G.; Teixeira, P.C.; Ramasawmy, R.; Drigo, S.A.; Goldberg, A.C.; Fonseca, S.G.; Bilate, A.M.; Kalil, J. Immunological and Non-Immunological Effects of Cytokines and Chemokines in the Pathogenesis of Chronic Chagas Disease Cardiomyopathy. *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 252–258. [CrossRef] [PubMed]
37. Shaw, M.-A. Human Genetic Susceptibility to Chagas Disease. In *American Trypanosomiasis Chagas Disease*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 629–652.
38. de Oliveira, A.P.; Ayo, C.M.; Bestetti, R.B.; de Mattos, C.C.B.; Cavasini, C.E.; de Mattos, L.C. The Role of CCR5 in Chagas Disease-a Systematic Review. *Infect. Genet. Evol.* **2016**, *45*, 132–137. [CrossRef]
39. Rodrigues de Moura, R.; Coelho, A.V.C.; de Queiroz Balbino, V.; Crovella, S.; Brandão, L.A.C. Meta-analysis of Brazilian Genetic Admixture and Comparison with Other Latin America Countries. *Am. J. Hum. Biol.* **2015**, *27*, 674–680. [CrossRef]
40. Du, B.; Gao, W.; Qin, Y.; Zhong, J.; Zhang, Z. Study on the Role of Transcription Factor SPI1 in the Development of Glioma. *Chin. Neurosurg. J.* **2022**, *8*, 7. [CrossRef]
41. Mummidi, S.; Bamshad, M.; Ahuja, S.S.; Gonzalez, E.; Feuillet, P.M.; Begum, K.; Galvis, M.C.; Kostecki, V.; Valente, A.J.; Murthy, K.K. Evolution of Human and Non-Human Primate CC Chemokine Receptor 5 Gene and mRNA: Potential roles for haplotype and mRNA diversity, differential haplotype-specific transcriptional activity, and altered transcripition factor binding to polymorphic nucleotides. *J. Biol. Chem.* **2000**, *275*, 18946–18961. [CrossRef]
42. Mummidi, S.; Ahuja, S.S.; McDaniel, B.L.; Ahuja, S.K. The Human CC Chemokine Receptor 5 (CCR5) Gene: Multiple Transcripts with 5′-End Heterogeneity, Dual Promoter Usage, and Evidence for Polymorphisms within the Regulatory Regions and Noncoding Exons. *J. Biol. Chem.* **1997**, *272*, 30662–30671. [CrossRef]
43. De Bona, E.; Lidani, K.C.F.; Bavia, L.; Omidian, Z.; Gremski, L.H.; Sandri, T.L.; de Messias Reason, I.J. Autoimmunity in Chronic Chagas Disease: A Road of Multiple Pathways to Cardiomyopathy? *Front. Immunol.* **2018**, *9*, 1842. [CrossRef]
44. D'Ávila, D.A.; Macedo, A.M.; Valadares, H.M.S.; Gontijo, E.D.; de Castro, A.M.; Machado, C.R.; Chiari, E.; Galvão, L.M.C. Probing Population Dynamics of *Trypanosoma Cruzi* during Progression of the Chronic Phase in Chagasic Patients. *J. Clin. Microbiol.* **2009**, *47*, 1718–1725. [CrossRef] [PubMed]

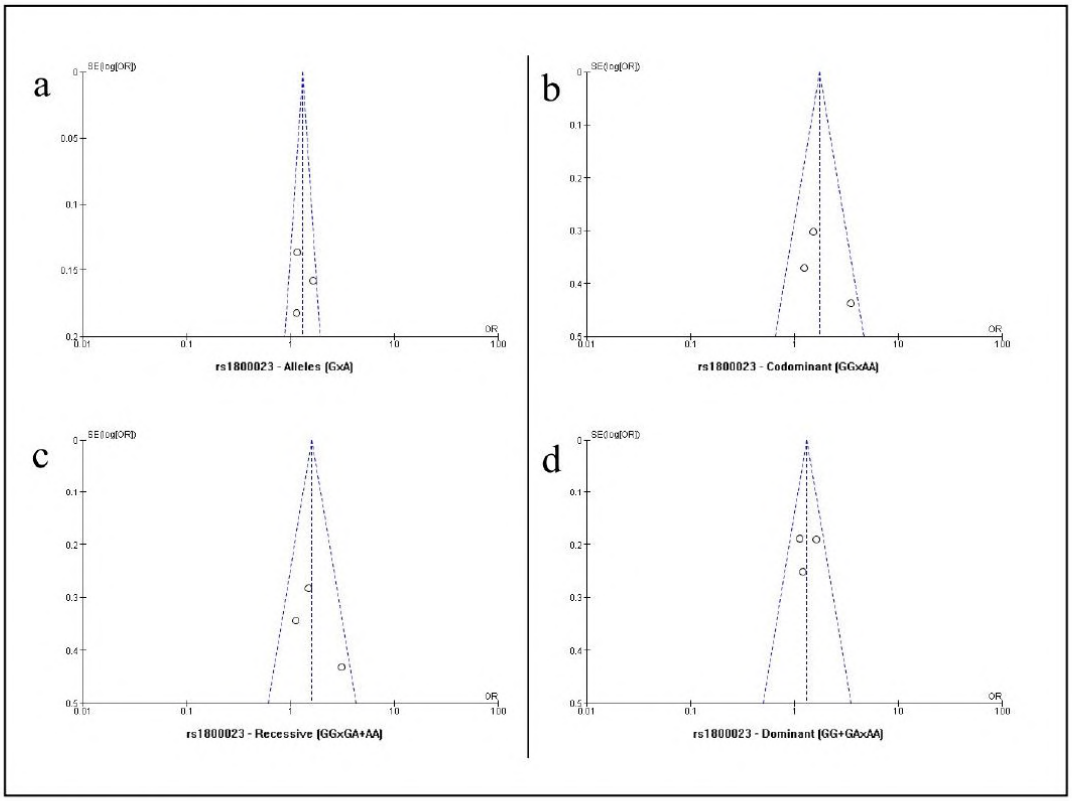
Supplementary Materials

Supplementary Figure S1. Funnel plots obtained from comparisons with significant results ($p \leq 0.05$) of the SNPs rs1799987, rs2856758, rs2734648, rs1799988 and rs1800024.



Source: Author, 2023.

Supplementary Figure S2. Funnel plots obtained from comparisons with significant results ($p \leq 0.05$) of SNP rs1800023.



Source: Author, 2023.

In all groups, the Hardy-Weinberg equilibrium (HWE) was analyzed. The Chi-square test (χ^2) was performed in all comparisons, and results with $p \leq 0.05$ were considered significant.

Supplementary Table S1. Genetic variants, comparisons made between groups and their individual and total frequencies.

Polymorphism	Comparison (group 1 *vs.* group 2), and sample power (SP%)*	Group 1 frequency**	Group 2 frequency**	Total frequency (group 1+ group 2)**
rs2856758	CardCP *vs.* UndCP, SP=100%	AA=0,7599388379 AG=0,1605504587 GG=0,07951070336 A=0,8402140673 G=0,1597859327	AA=0,4076539101 AG=0,2013311148 GG=0,391014975 A=0,5083194676 G=0,4916805324	AA=0,5912350598 AG=0,1800796813 GG=0,228685259 A=0,6812749004 G=0,3187250996
rs2734648	CardCP *vs.* UndCP, SP>99%	TT=0,1348314607 TG=0,4185393258 GG=0,4466292135 T=0,3441011236 G=0,6558988764	TT=0,09921259843 TG=0,3763779528 GG=0,5244094488 T=0,2874015748 G=0,7125984252	TT=0,1180400891 TG=0,3986636971 GG=0,4832962138 T=0,3173719376 G=0,6826280624
rs1799987	nonCp *vs.* CardCP, SP>96%	AA=0,3661417323 AG=0,4212598425 GG=0,2125984252 A=0,5767716535 G=0,4232283465	AA=0,4444444444 AG=0,3137254902 GG=0,2418300654 A=0,6013071895 G=0,3986928105	AA=0,4307856761 AG=0,4783538215 GG=0,2266167825 A=0,6699625869 G=0,4657936932
	CardCP *vs.* UndCP, SP>99%	AA=0,373284538 AG=0,4226898445 GG=0,2040256176 A=0,5846294602 G=0,4153705398	AA=0,4380952381 AG=0,4107142857 GG=0,1511904762 A=0,643452381 G=0,356547619	AA=0,4014485256 AG=0,4174857734 GG=0,181065701 A=0,6101914123 G=0,3898085877
	CardCP *vs.* UndCP, SP>99%**	AA**=0,4106145251 AG**=0,406424581 GG**=0,1829608939 A**=0,6138268156 G**=0,3861731844	AA**=0,4622093023 AG**=0,4098837209 GG**=0,1279069767 A**=0,6671511628 G**=0,3328488372	AA**=0,4358974359 AG**=0,4081196581 GG**=0,155982906 A**=0,639957265 G**=0,360042735

	CardCP *vs.* UndCP, SP>37%***	AA***=0,3023872679	AA***=0,3289473684	AA***=0,3100189036
		AG***=0,4535809019	AG***=0,4144736842	AG***=0,4423440454
		GG***=0,2440318302	GG***=0,2565789474	GG***=0,247637051
		A***=0,5291777188	A***=0,5361842105	A***=0,5311909263
		G***=0,4708222812	G***=0,4638157895	G***=0,4688090737
	CP *vs.* nonCp, SP >37%	AA=0,3717105263	AA=0,3661417323	AA=0,3691756272
		AG=0,3914473684	AG=0,4212598425	AG=0,4050179211
		GG=0,2368421053	GG=0,2125984252	GG=0,2258064516
		A=0,5674342105	A=0,5767716535	A=0,5716845878
		G=0,4325657895	G=0,4232283465	G=0,4283154122
	SympCP *vs.* UndCP, SP=100%	AA=0,3346265761	AA=0,4380952381	AA=0,3810796366
		AG=0,4296799224	AG=0,4107142857	AG=0,4211651523
		GG=0,2356935015	GG=0,1511904762	GG=0,1977552111
		A=0,5494665373	A=0,643452381	A=0,5916622127
		G0,4505334627	G0,356547619	G=0,4083377873
	nonCP *vs.* UndCP, SP>57%	AA=0,3661417323	AA=0,4224137931	AA=0,3837837838
		AG=0,4212598425	AG=0,3534482759	AG=0,4
		GG=0,2125984252	GG=0,224137931	GG=0,2162162162
		A=0,5767716535	A=0,599137931	A=0,5837837838
		G=0,4232283465	G=0,400862069	G=0,4162162162
rs1799988	CardCP *vs.* UndCP, SP>99%	TT=0,1867007673	TT=0,1544827586	TT=0,1712010617
		TC=0,4347826087	TC=0,4303448276	TC=0,4326476443
		CC=0,378516624	CC=0,4151724138	CC=0,396151294
		T=0,4040920716	T=0,3696551724	T=0,3875248839
		C=0,5959079284	C=0,6303448276	C=0,6124751161
	CardCP *vs.* UndCP, SP>99%**	TT**=0,1833060556	TT**=0,1217391304	TT**=0,1534569983
		TC**=0,410801964	TC**=0,4330434783	TC**=0,4215851602
		CC**=0,4058919804	CC**=0,4452173913	CC**=0,4249578415
		T**=0,3887070376	T**=0,3382608696	T**=0,3642495784

		C**=0,6112929624	C**=0,6617391304	C**=0,6357504216
rs41469351	CardCP *vs.* UndCP$^{\#}$	TT=0 TC=0,01966292135 CC=0,9803370787 T=0,009831460674 C=0,9901685393	TT=0 TC=0,01417322835 CC=0,9858267717 T=0,007086614173 C=0,9929133858	TT=0 TC=0,01707498144 CC=0,9829250186 T=0,00853749072 C=0,9914625093
rs1800023	CardCP *vs.* UndCP, SP=100%	AA=0,4504065041 AG=0,4178861789 GG=0,1317073171 A=0,6593495935 G=0,3406504065	AA=0,5357737105 AG=0,3826955075 GG=0,08153078203 A=0,7271214642 G=0,2728785358	AA=0,4925986842 AG=0,4004934211 GG=0,1069078947 A=0,6928453947 G=0,3071546053
rs1800024	CardCP *vs.* UndCP, SP>99%	TT=0,1795231417 TC=0,3267882188 CC=0,4936886396 T=0,3429172511 C=0,6570827489	TT=0,2353870458 TC=0,345971564 CC=0,4186413902 T=0,4083728278 C=0,5916271722	TT=0,205794948 TC=0,3358098068 CC=0,4583952452 T=0,3736998514 C=0,6263001486
rs333[Δ32]	CP *vs.* nonCp$^{\#}$	Del/Del=0 Del/Ins=0,06647398844 Ins/Ins=0,9335260116 Del=0,03323699422 Ins=0,9667630058	Del/Del=0,003861003861 Del/Ins=0,06177606178 Ins/Ins=0,9343629344 Del=0,03474903475 Ins=0,9652509653	Del/Del=0,001051524711 Del/Ins=0,06519453207 Ins/Ins=0,9337539432 Del=0,03364879075 Ins=0,9663512093
	CardCP *vs.* UndCP$^{\#}$	Del/Del=0 Del/Ins=0,07103825137 Ins/Ins=0,9289617486 Del=0,03551912568 Ins0,9644808743	Del/Del=0 Del/Ins=0,06912442396 Ins/Ins=0,930875576 Del=0,03456221198 Ins=0,965437788	Del/Del=0 Del/Ins=0,07032590051 Ins/Ins=0,9296740995 Del=0,03516295026 Ins=0,9648370497
	nonCP *vs.* UndCP$^{\#}$	Del/Del=0,003861003861 Del/Ins=0,06177606178 Ins/Ins=0,9343629344 Del=0,03474903475	Del/Del=0 Del/Ins=0,06912442396 Ins/Ins=0,930875576 Del=0,03456221198	Del/Del=0,002100840336 Del/Ins=0,06512605042 Ins/Ins=0,9327731092 Del=0,03466386555

	Ins=0,9652509653	Ins=0,965437788	Ins=0,9653361345
nonCp *vs.* CardCP[#]	Del/Del=0,003861003861 Del/Ins=0,06177606178 Ins/Ins=0,9343629344 Del=0,03474903475 Ins=0,9652509653	Del/Del=0 Del/Ins=0,07103825137 Ins/Ins=0,9289617486 Del=0,03551912568 Ins=0,9644808743	Del/Del=0,0016 Del/Ins=0,0672 Ins/Ins=0,9312 Del=0,0352 Ins=0,9648

*Sampling power >80 indicates a good representation of the population (effect expressed in the population). Polymorphisms not associated by the meta-analysis (increased or reduced risk) did not have SP calculated; ** Frequency found using all study participants; *** Analyses conducted only with samples from countries arising from Spanish Colonization; **** Analyses conducted with Brazilian samples only, # Analysis unable to be completed due to lack of data.

Source: Author, 2023.

4.2 CAPÍTULO II

Polimorfismos genéticos de quimiocinas e seus receptores na doença de Chagas: revisão sistemática e metanálise

Artigo submetido à revista ACTA TROPICA (0001-706X):

Qualis 2013-1016: B1 (CIÊNCIAS BIOLÓGICAS I);

Qualis 2017-2020: A2 (CIÊNCIAS BIOLÓGICAS I);

Fator de Impacto: 2.7 (2022).

Acta Tropica

Genetic polymorphisms of chemokines and their receptors in Chagas disease: Systematic review and meta-analysis

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Section/Category:	Protozoans(excl. Plasmodium spp), pathogenesis; diagnosis, eco-epidemiology, genetics, vectors, immu
Keywords:	Chagas disease; Genetic polymorphism; Chemokines; Meta-analysis
Corresponding Author:	Jean Carlos Vencioneck Dutra, Dr Federal University of Espirito Santo BRAZIL
First Author:	Jean Carlos Vencioneck Dutra, Dr
Order of Authors:	Jean Carlos Vencioneck Dutra, Dr
	Jean Moisés Ferreira
	Barbara Rayssa Correia dos Santos
	Edilson Leite de Moura
	Elaine Virgínia Martins de Sousa Figueiredo
	José Luiz de Lima Filho
Abstract:	Objective: Carry out a qualitative and quantitative synthesis of the influence of genetic variants of chemokines on Chagas disease (CD) through a systematic review. Methods: Searches were performed on CAPES Journal Portal, SciELO, Scopus, Web of Science, PubMed and ScienceDirect databases. A meta-analysis was conducted in order to narrow the understanding of the influence of the SNPs found in the disease, as well as using two tools for exploratory analyzes (database GTEx and SNP2TFBS). Results: 1197 articles were found, of which seven were included in this review. They were verified the variants of chemokine genes CCL2, CCL4, CCL5, CCL17, CCL19, CXCL8, CXCL9, CXCL10 and CXCR3, and CCR1 and CCR2 receptors, totaling 14 different SNPs. The meta-analysis indicates an association of the G/G genotype of the SNP rs1024611 (CCL2 -2518A/G) in the recessive model with the development of the cardiac form in CD. Three other associations were found between the SNP rs2107538 (CCL5 -403C/T) and the risk for the cardiac form, one of which was allelic, where the T allele (T vs. C) was associated, and two genotypic, one in the Codominance model (C/T vs. C/C), where the heterozygous genotype was associated, and another in the dominant inheritance model (T/T+C/T vs. C/C), where T carriers were associated. The GTEx database indicated that the associated variants in our meta-analysis are responsible for the highest amount of transcription of the respective genes. The SNP2TFBS tool identified four SNPs capable of influencing transcription factors: SNP rs3136672 (CCR1) and SNP rs2280964 (CXCR3), in addition to the already mentioned SNPs rs1024611 (CCL2) and rs2107538 (CCL5). Conclusions: In short, we hypothesized that the volume of mRNA caused by the G/G genotypes of the CCL2 SNP rs1024611, and the C/T and T/T of the CCL5 SNP rs2107538 are consequently expressed, causing an exacerbated recruitment of cells with inflammatory capacities, which in the long term, leads to damage cardiac.
Suggested Reviewers:	Sergio Sosa-Estani ssosa@dndi.org Specialist in Drugs for r Neglected Diseases, Epidemiology and Public Health Research.
	Jadel Kratz jkratz@dndi.org Specialist in Drugs for Neglected Diseases.
	Susan Wyllie

	s.wyllie@dundee.ac.uk Specialist in anti- infectives research.
	Tim Miles tim.j.miles@gsk.com Specialist in Global health Medicines .

Cover Letter

Dear Editor in Chief,

Please find enclosed our manuscript, "*Genetic polymorphisms of chemokines and their receptors in Chagas disease: Systematic review and meta-analysis*" by Jean Moisés Ferreira et al., which we would like to submit for publication as an original Research Article in the *Acta Tropica*. Chagas disease, known as American trypanosomiasis, is a neglected disease that affects seven million patients worldwide. The patient's genetics has been related to the evolution of Chagas disease, and some studies indicate that polymorphisms in cytokine genes, chemokines and cell receptors may contribute to the worsening of the disease. Hence, our aim was to carry out a qualitative and quantitative synthesis of the influence of chemiokines and their recptors genetic variants on Chagas disease through a systematic review. A total of 1197 articles were analysed, and the manuscripts included in our study were published between 2006-2019, conducted in Argentina, Brazil and Colombia, with Argentine, Brazilian and Colombian patients. Fourteen polymorphisms were found, and among the meta-analysed SNPs, *CCL2* and *CCL5* SNPs were associated with cardiac form of Chagas disease. Explorative analysis were conduced and showed thats G/G genotypes of the *CCL2* SNP rs1024611, and the C/T and T/T of the *CCL5* SNP rs2107538 are responsible for the highest amount of transcription of the respective genes. We hypothesized that the volume of mRNA caused by the G/G genotypes of the *CCL2* SNP rs1024611, and the C/T and T/T of the *CCL5* SNP rs2107538 are consequently expressed, causing an exacerbated recruitment of cells with inflammatory capacities, which in the long term, leads to damage cardiac. These findings can help in the implementation of therapeutic practices that improves the quality life of the patients. We confirm that this manuscript has not been published elsewhere and is not under consideration by another Journal. All authors have approved the manuscript and agree with its submission to the *Acta Tropica*.

We believe our findings would appeal to the readership of the *Acta Tropica*. Please address all correspondence to Jean Moisés Ferreira – jean.moises@hotmail.com.

Highlights

Genetic variations in chemokines on Chagas disease.

Chemokine and receptor genes SNPs associated with the Chagas disease by meta-analysis.

G/G genotype of SNP rs1024611 (CCL2 -2518A/G) is linked to the development of the cardiac form of Chagas disease.

SNP rs2107538 (CCL5 -403C/T) shows genetic and allelic associations with the risk of the cardiac form of the disease.

Exploratory analyses using GTEx and SNP2TFBS databases underscore the functional significance of these SNPs in gene expression and their interaction with transcription factors.

Genetic polymorphisms of chemokines and their receptors in Chagas disease: Systematic review and meta-analysis

Jean Moisés Ferreira[1*], Barbara Rayssa Correia dos Santos[2], Edilson Leite de Moura[2], Jean Carlos Vencioneck Dutra[3], Elaine Virgínia Martins de Sousa Figueiredo[2] and José Luiz de Lima Filho[1]

[1*]Laboratório de Imunopatologia Keizo Asami - LIKA, Centro de Biociências, Universidade Federal de Pernambuco (UFPE), 50670-901, Recife, Pernambuco, Brazil; jean.moises@hotmail;

[2]Laboratório de Biologia Molecular e Expressão Gênica - LABMEG, Departamento de Ciências Biológicas, Universidade Federal de Alagoas (UFAL), Campus Arapiraca, 57300-970, Arapiraca, Alagoas, Brazil;

[3]Secretaria de Estado da Educação (SEDU), Santa Lucia, 29056-085, Vitória, Espírito Santo, Brazil.

Abstract

Objective: Carry out a qualitative and quantitative synthesis of the influence of genetic variants of chemokines on Chagas disease (CD) through a systematic review.

Methods: Searches were performed on CAPES Journal Portal, SciELO, Scopus, Web of Science, PubMed and ScienceDirect databases. A meta-analysis was conducted in order to narrow the understanding of the influence of the SNPs found in the disease, as well as using two tools for exploratory analyzes (database GTEx and SNP2TFBS).

Results: 1197 articles were found, of which seven were included in this review. They were verified the variants of chemokine genes *CCL2*, *CCL4*, *CCL5*, *CCL17*, *CCL19*, *CXCL8*, *CXCL9*, *CXCL10* and *CXCR3*, and *CCR1* and *CCR2* receptors, totaling 14 different SNPs. The meta-analysis indicates an association of the G/G genotype of the SNP rs1024611 (*CCL2* -2518A/G) in the recessive model with the development of the cardiac form in CD. Three other associations were found between the SNP rs2107538 (*CCL5* -403C/T) and the risk for the cardiac form, one of which was allelic, where the T allele (T *vs.* C) was associated, and two genotypic, one in the Codominance model (C/T vs. C/C), where the heterozygous genotype was associated, and another in the dominant inheritance model (T/T+C/T *vs.* C/C), where T carriers were associated. The GTEx database indicated that the associated variants in our meta-analysis are responsible for the highest amount of transcription of the respective genes. The SNP2TFBS tool identified four SNPs capable of influencing transcription factors: SNP rs3136672 (*CCR1*) and SNP rs2280964 (*CXCR3*), in addition to the already mentioned SNPs rs1024611 (*CCL2*) and rs2107538 (*CCL5*).

Conclusions: In short, we hypothesized that the volume of mRNA caused by the G/G genotypes of the *CCL2* SNP rs1024611, and the C/T and T/T of the *CCL5* SNP rs2107538 are consequently expressed, causing an exacerbated recruitment of cells with inflammatory capacities, which in the long term, leads to damage cardiac.

Keywords: Chagas disease; Genetic polymorphism; Chemokines; Meta-analysis.

Introduction

American trypanosomiasis, also known as Chagas disease (CD) is one of the most important neglected diseases in the world, sometimes accompanied by other diseases, especially in less favored communities in developing countries. Chagas disease already affects about six million people worldwide, with approximately 28,000 new cases per year, causing thousands of deaths per year (ECHAVARRÍA *et al.*, 2021; NORMAN; LÓPEZ-VÉLEZ, 2019; WENG; CHEN; WANG, 2018).

The etiological agent of CD is the trypanosomid known as *Trypanosoma cruzi*. After infection, the host enters the acute phase, and after a few weeks it develops a specific immune response that is not completely effective for the parasites to be eradicated. After the acute phase of the infection, the patient enters the chronic phase, which can vary from patient to patient. About 70% of patients do not have symptoms, while 30% develop some symptomatic form, such as the digestive, cardiac or mixed forms of the disease (simultaneously presenting digestive and cardiac symptoms) (KAYA, 2021; PÉREZ-MOLINA; MOLINA, 2018; VARIKUTI et al., 2018) .

The cardiac form is more popularly known, and its manifestations may include progressive heart failure, complex ventricular arrhythmias, cardiomegaly and sudden death. It is a consensus that the cardiac form of CD presents a cardiac infiltrate characterized by mononuclear cells, a high concentration and production of chemokines and cytokines, which cause tissue damage and lead to cardiac tissue fibrosis. Thus, it is important to understand the role of chemokines and their receptors in this form of the disease, as they support the continuity of the inflammatory process, as well as the cellular infiltrate. For this reason, insight into the molecular mechanisms that influence these cytokines and receptors is valuable to clearly understand the pathophysiology of this form of the disease. (BATISTA *et*

al., 2018; DE OLIVEIRA *et al.*, 2016; ECHAVARRÍA *et al.*, 2021; SIMÕES *et al.*, 2018) .

Some studies have analyzed the influence of genetic variants on the outcome of the cardiac form in DC and on the expression of proteins, such as chemokines and their receptors. (ACOSTA-HERRERA *et al.*, 2019; CARVALHO *et al.*, 2019; GOMES DOS SANTOS *et al.*, 2020) . However, some results are inconclusive or contradictory, or may still present a non-optimized experimental design (based on a control group that has no chance of evolving to the cardiac form, i.e., not infected) (DE OLIVEIRA *et al.*, 2015; LIMA *et al.*, 2018) . Thus, this systematic review aimed to (1) gather the information present in the literature on the influence of these molecules and condense its main results, and (2) conduct exploratory analyzes using data from viable variables found in scientific articles, in order to narrow down the the relationship of these variables with the development of CD.

Methodology

Search strategies, term selection, DeCS analysis and string construction

The methodology used in this study was previously described in another manuscript published by our research group (data not shown). This review has been filed with PROSPERO (https://www.crd.york.ac.uk/PROSPERO/) and PICOS strategy was implemented to perform the searches (SANTOS; PIMENTA; NOBRE, 2007) , where: P - participants, were CD patients; I - intervention, was the identification of polymorphisms of chemokine genes and their receptors in patients; C - comparisons were made between the groups of asymptomatic patients and patients with the cardiac form; The - result was the presence/absence of the polymorphism in CD patients; and S - types of studies, were genetic association studies.

Guidelines and protocols established by WHO and PAHO for the management of CD patients (PAHO, 2022; WHO, 2022) , and expert articles and reviews (BERN, 2015; PÉREZ-MOLINA; MOLINA, 2018) were used to elect the groups of words used in searches. The following terms were listed: (1) “Chagas disease”, (2) “Trypanosomiasis” and (3) “Trypanosoma cruzi infection”. Through Health Sciences Descriptors - DeCS, from the BVS portal (https://decs.bvsalud.org/), synonyms of the terms were found: (1) “chagastic disease”, (2) “American trypanosomiasis”, “Trypanosome parasite”, “Trypanosome infection”, “*Trypanosoma cruzi*”, “Trypanosomosis”, and (3) “*T. cruzi*”, “*Trypanosoma cruzi*” and “cruzi” (reference codes C01.610.752.300.900.200, C01.920.625 and

SP4.012.148.149). The same method was used for the term “Genetic polymorphism”, whose results were “Polymorphic gene”; “Single nucleotide polymorphism”, “Genetic variation”, “Allelic variation” and “SNP’’ (reference codes G05.365.795, G05.365.795.598, G05.365, G05.380 and SP4.102.118). Using Boolean operators, the terms were used to form the search strings and their variations: 'Chaga* disease' OR 'Trypanosom*' OR 'cruzi' AND 'Polymorphi*' OR 'Gene*' OR 'Variation' OR 'SNP'.

Databases, inclusion and exclusion criteria

The formed string was used in the databases without time restrictions. The databases used in the searches were the CAPES Journal Portal, SciELO, Scopus, Web of Science, PubMed and ScienceDirect, and were performed between January and February 2022. The filters used in the searches were applied in the search for written scientific articles in English and evaluated by peers. Subsequently, the articles included in the qualitative synthesis had their references checked, in order to include articles that were not detected during searches in the databases. Generic information about texts was organized using LibreOffice Calc.

Inclusion criteria were: (1) original publications; (2) association studies of chemokine gene variants and/or chemokine receptors, and (3) electronically available articles. Exclusion criteria were: (1) duplicate studies, (2) studies using an animal model, (3) *in vitro* studies, (4) studies of correlation between the genetics of the parasite and the patient, (5) studies of genetic analysis of vectors, (6) studies that did not evaluate chemokine genetic polymorphisms, (7) literature reviews, and (8) conference abstracts..

Selection of publications, quality analysis and data extraction

Titles and abstracts were initially used to analyze the presence of inclusion and exclusion criteria. Independently, two reviewers (JMF and BRCS) analyzed the returns. Inconsistencies were resolved by discussing the publication with a third reviewer (ACMS). The quality of the studies was performed using the “STrengthening the REporting of Genetic Association Studies (STREGA)” tool. The STREGA tool consists of a checklist that evaluates each part of the scientific article, where a total of 22 points can be reached in the

checklist. Using this tool, studies were classified as low quality when the score was less than 9, medium quality when the score was between 9-16, and high quality when the score was between 17-22.

Data on the profile of the patients, analyzed polymorphisms, main results, laboratory confirmation method for the infection and confirmation method for the polymorphism, study design, year of publication, country of origin of the study (based on the first author), the studied population and sample size were extracted.

Meta-analysis and sample power

Global allele and genotype frequencies were calculated. Meta-analyses were performed on all polymorphisms available in the RevMan 5.4 software, where the strength of association was calculated using a 95% confidence interval and odds ratio (OR), with the generated value OR<1 considered as a protective factor (trend) or risk reduction, while OR>1 values were considered as a risk factor (trend), the results were considered significant when $p \leq 0.05$. Study heterogeneity was assessed using the standard test (Q^2 and I^2). When $I^2 \geq 50\%$ and $p \leq 0.05$, indicating significant heterogeneity, probably caused by variations between studies, we chose to use the random analysis model (standardized by the software), for all other cases, the fixed analysis model was used.

We evaluated the influence of alleles (mutated *vs*. wild-type) and genotypes on different inheritance models: recessiveness (mutated homozygote *vs*. heterozygote + wild-type homozygote), dominance (mutated homozygote + heterozygote *vs*. wild-type homozygote) and codominance (mutated homozygote *vs*. wild-type homozygote, or heterozygous *vs*. homozygous wild-type). Funnel-plot graphs were inspected for possible publication bias.

The sample power was calculated using G*Power 3.1.9.7 (χ^2-Goodness-of-fit, Post hoc, α=0.05) (ERDFELDER, 2009; FAUL *et al.*, 2007), for the significant comparisons found in the synthesis quantitative. Values >80% were considered significant.

GTEx (eQTL) and SNP2TFBS Tool

To identify single nucleotide polymorphisms (SNPs) that may affect transcription factor binding, we used the Mapping SNPs to Transcription Factor Binding Sites (SNP2TFBS) web interface (https://ccg.epfl.ch/snp2tfbs/). In order to assess the relationship between the genetic variants associated with the risk of developing the cardiac form of CD and the gene expression profile, we examined the quantitative expression trait loci (eQTL) from the GTEx database (https://gtexportal.org/home/).

Patient classification

Using data available in publications, patients were grouped into two different groups: asymptomatic chronic patients in an undetermined form, that is, asymptomatic patients were generically described as the UndCP grouping. While those who had any type of cardiac alteration/complications as a result of CD were grouped as cardiac patients (CardCP). The Original criteria used by the authors to classify the patients as a cardiac group are available in **Supplementary Table 1**.

Results

Characterization of included studies

After the article inclusion stage, in our analyzes we found publications occurred between 2006-2019, related to the chemokine genes CCL2, CCL4, CCL5, CCL17, CCL19, CXCL8, CXCL9, CXCL10 and CXCR3, in addition to CCR1, CCR2 and CCR5 receptors. However, data related to the *CCR5* gene were forwarded in another publication (data not shown), so we framed articles strictly related to CCR5 as "did not assess target genes". Thus, the articles included in this review were published between 2006-2018, analyzed 14 different SNPs, and were conducted using samples from Brazil, Colombia and Argentina: BATISTA et al., 2018; FLÓREZ; MARTÍN; GONZÁLEZ, 2012; FRADE et al., 2013; JUIZ et al., 2019; MACHUCA et al., 2014; NOGUEIRA et al., 2012; RAMASAWMY et al., 2006. The graphical summary of the searches and inclusions of these publications are shown in **Figure 1**. The characterization of the studies and the main results of the publications are available in **Table 1**.

Figure 1 – Flow of studies added in the systematic review. 1197 returns were identified from the databases, 7 met the criteria and were included in the qualitative synthesis, and 6 of these studies were used in the meta-analysis.

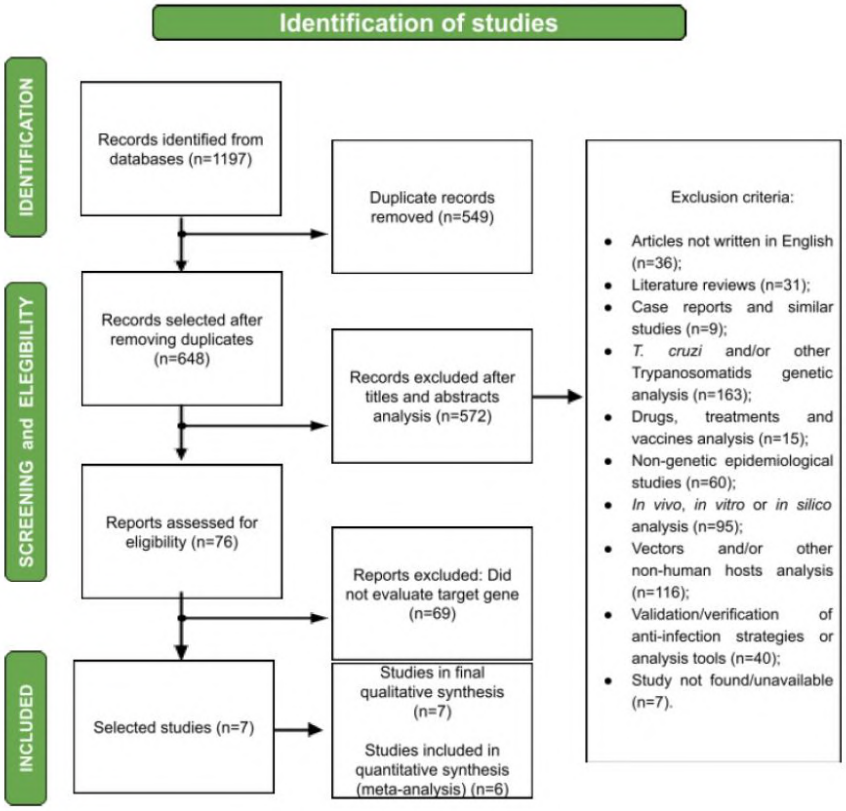


Table 1 - Data extracted from the included articles, STREGA score, main results associated with variants of the genes *CCL2*, *CCL4*, *CCL5*, *CCL17*, *CCL19*, *CXCL8*, *CXCL9*, *CXCL10*, *CXCR3*, *CCR1* and *CCR2*, and inclusion in the quantitative synthesis (meta-analysis).

	Study characterization		**Laboratorial proceeds**			**Genetics observations**	
References	**Country1; Study design**	**Sample (n); Population; Group characterization2)**	**Confirmation method of *T. cruzi* infection**	**Parasitemia evaluation**	**Confirmation method of Polymorphism**	**Hardy-Weinberg equilibrium (HWE)3**	**STREGA score**
Batista et al., 2018	Brazil Cross-sectional	n=406 / Brazilians (CardCP=296 and UndCP=110)	ELISA and IIF	Yes	qPCR	Nd	17 - High quality
Ramasawmy et al, 2006	Brazil Case-control	n=231 / Brazilians (CardCP=155 and UndCP=76)	ELISA, IHA and IIF	No	PCR-RFLP	Yes	13 - Medium quality
Flórez et al, 2012	Colombia Case-control	n=260 / Colombians (UndCp=130 and CardCP=130)	ELISA and IHA	No	PCR and PCR-RFLP	Yes	16 - Medium quality
Machuca et al, 2014	Colombia Cohort	n=476 / Colombians (UndCP=206 and CardCP=270)	ELISA and IHA	No	qPCR	Yes	13 - Medium quality
NOGUEIRA et al. , 2012	Brazil Cohort	n=325 / Brazilians (CardCP=174 and UndCP=151)	ELISA, IHA and IIF	No	qPCR	Yes	12 - Medium quality
Juiz et al, 2019	Argentina Case-control	n=480 / Argentinians (CardCP= 215 and UndCP=256)	Serologically	No	qPCR	Yes (rs1799864 and 1800024 are not in HWE)	14 - Medium quality

Frade et al., 2013	Brazil Cohort	n=433 / Brazilians (CardCP=315 and UndCP=118)	ELISA, IHA and IIF	No	Hybridization assay and qPCR	Yes (HWE was presented only for the control group)	13 - Medium quality

Gene	**Polymorphism**	**Nucleotide sequence localization / Variation type [and change] / Consequence6**	**Main finds of studies**	**Quantitative synthesis by Meta-analysis (Yes/No)**
CCL2	rs1024611	34252769 / SNV [A/G] / not reported;	Batista et al., 2018 - not associated; Ramasawmy et al, 2006 - When comparing the asymptomatic and cardiomyopathic (CCC) groups, a higher frequency of genotypes with the A allele (AA and AG) was observed in the CCC group ($\chi 2$=9.4; p=0.009) / A lower frequency of the GG genotype was observed in the CCC group when compared to the asymptomatic group (p=0.001, OR=4.1, 95%CI [1.7–11]) / A lower frequency of the AG genotype was observed in the CCC group when compared to the asymptomatic group (p=0.026, OR=2.7 , 95%CI [0.97–7.2]) / An association between the A allele and the risk of developing CCC was found (p=0.001, OR=1.9, 95%CI, [1.3-2.9]);	Yes
	rs3760396	34254422 / SNV [G/C, T] / Upstream variant; upstream transcript variant;	Frade et al, 2013 - not associated;	No
	rs2857656	34254988 / SNV [G/A, C] / Upstream variant; upstream transcript variant;	Frade et al, 2013 - not associated;	No
	rs4586	34256250 / SNV [T/A, C] / Stop gained; coding sequence variant; synonymous variant;	Frade et al, 2013 - When comparing the asymptomatic group with the CCC group, a difference in the frequency of the TT genotype was observed in the univariate analysis (TT *vs* TC + CC, covariate with gender), (p=0.032, OR=1.30, 95%CI, [1.02 -1.65]) / This association was also observed when comparing the asymptomatic and severe CCC	No

			groups (p=0.034, OR=1.34, 95%CI, [1.02-1.75]);	
	rs3917891	34258668 / SNV [C/T] / not reported;	Frade et al, 2013 - When comparing the asymptomatic group with the group with CCC, a difference in the frequency of the C/C genotype was observed in the univariate analysis (CC *vs* CT + TT, covariate with gender), (p=0.037, OR=1.56, 95%CI [1.03-2.37]) / This association was also observed when comparing the asymptomatic and severe CCC groups (p=0.053, OR=1.55, 95%CI, [1.00-2.41]);	No
	rs2530797	34259075 / SNV [C/T] / not reported;	Frade et al, 2013 - When comparing the asymptomatic group with the CCC group, a difference in the frequency of the A/A genotype was observed in the univariate analysis (AA *vs* AG + GG, covariate with gender), (p=0.028, OR=1.28, 95%CI, [1.03-1.60]) / This association was also observed when comparing the asymptomatic and severe CCC groups (p=0.005, OR=1.42, 95%CI, [1.11-1.82]);	No
	rs991804	34260706 / SNV [C/G, T] / not reported;	Frade et al, 2013 - not associated;	No
CCL4	rs1719153	36105836 / SNV [A/T] / Downstream transcript variant; downstream variant;	Nogueira et al, 2012 - not associated;	No
CCL5	rs2107538	35880776 / SNV [C/T] / Upstream variant; intron variant; upstream transcript variant;	Batista et al, 2018 - CT heterozygotes (p=0.04, OR=0.5) and T carriers (p=0.01, OR=0.5) were associated with protection against CCC; Flórez et al, 2012 - not associated; Nogueira et al, 2012 - not associated;	Yes
	rs2280788	35880401 / SNV [G/C] / Upstream variant; intron variant; upstream transcript variant;	Batista et al, 2018 - not associated; Flórez et al, 2012 - not associated;	Yes
CCL17	rs223827	57412095 / SNV [C/A, T] / Intron variant; genic upstream transcript variant; upstream transcript variant;	Nogueira et al, 2012 - not associated;	No
CCL19	rs3136658	34690403 / SNV [C/T] / Intron variant; genic upstream transcript variant;	Nogueira et al, 2012 - not associated;	No

CCR1	rs3181077	46209161 / SNV [C/T] / Upstream variant; upstream transcript variant;	Batista et al, 2018 - not associated;	No
	rs1491961	46208857 / SNV [T/A, C, G] / Upstream variant; upstream transcript variant;	Batista et al, 2018 - not associated;	No
	rs3136672	46201294 / SNV [T/C, G] / Downstream transcript variant; downstream variant;	Batista et al, 2018 - not associated;	No
CCR2	rs1799864	46357717 / SNV [G/A] / Coding sequence variant; missense variant;	Flórez et al, 2012 - not associated; Juiz et al, 2018 - not associated; Machuca et al, 2014 - The A allele was more frequent in patients with an aggravated state of cardiomyopathy, being associated with the severity of the disease (p=0.03, OR=1.63, 95%CI, [1.03–2.60]. P adjusted =0.02, OR adjusted=1.91, 95% CI, [1.10–3.30]);	Yes
	rs3138042	46359541 / SNV [A/G] / 3 prime UTR variant; intron variant;	Juiz et al, 2018 - not associated; Machuca et al, 2014 - not associated;	No
CXCL8	rs4073	73740307 / SNV [A/C, G, T] / Upstream transcript variant; upstream variant;	Flórez et al, 2012 - The distribution of the A allele in patients with more severe cardiomyopathy was significantly higher and associated with a risk of developing this form (p=0.024, OR=3.17, 95%CI [1.16–8.67]. P adjusted=0.026, OR adjusted=3.18, 95 %CI adjusted [1.15–8.81]), however, these associations were lost after corrections by multiple tests.	No
CXCL9	rs10336	76001835 / SNV [A/G, T] / 3 prime UTR variant; intron variant;	Nogueira et al, 2012 - The CC genotype was significantly less frequent among patients with severe cardiomyopathy than in the group of patients with moderate cardiomyopathy ($\chi 2$=5.70, p=0.01, OR=0.47, 95%CI, [0.25–0.87]) / The C allele was significantly less frequent among patients with severe cardiomyopathy than in the group of patients with moderate cardiomyopathy ($\chi 2$=5.81, p=0.002, OR=0.59, 95%CI, [0.37–0.93]);	No

CXCL10	rs3921	76021790 / SNV [C/A, G, T] / 3 prime UTR variant; non coding transcript variant; intron variant; genic upstream transcript variant;	Nogueira et al, 2012 - The GG genotype was significantly less frequent among patients with severe cardiomyopathy than in the group of patients with moderate cardiomyopathy ($\chi2$=7.36, p=0.006, OR=0.41, 95%CI, [0.22–0.79]) / The G allele was significantly less frequent among patients with severe cardiomyopathy than in the group of patients with moderate cardiomyopathy ($\chi2$=8.30, p=0.003, OR=0.51, 95%CI, [0.32–0.81]);	No
CXCR3	rs2280964	71618204 / SNV [C/A, G, T] / Intron variant.	Nogueira et al, 2012 - not associated.	No

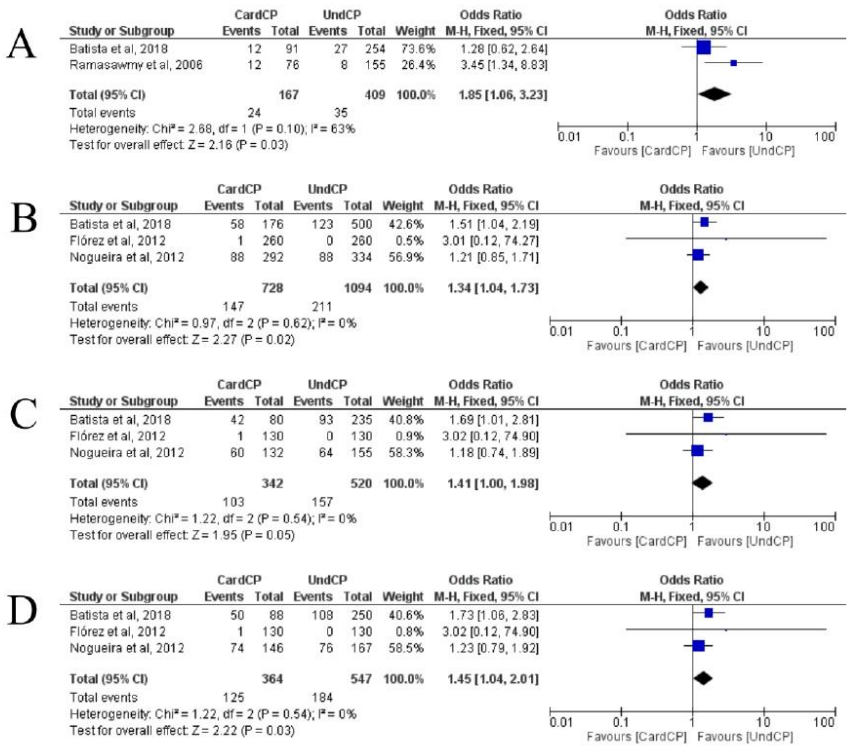
Legend: [1]Country of study based on the first author; [2]The characterization of each study was done by us in order to perform comparisons in the quantitative synthesis. The categorizations originally made by the authors of each article are described in Table 1.; [3]Nd = HWE not described by the authors; [4]Information provided by NCBI.

Meta-analysis

CCL2 - rs1024611

Two independent studies evaluated the influence of the SNP rs1024611 (CCL2 -2518A/G) on the evolution of the cardiac form of CD (Batista et al, 2018 e Ramasawmy et al, 2022). The sample power of the combined studies was SP= 88.2% (n=576, UndCP=167/CardCP=409). The meta-analysis indicated an association in the recessive pattern (GG *vs* AA+AG) of the GG genotype with the development of the cardiac form in CD (p=0.03, OR=1.85, 95% CI [1.06-3.23], I^2= 63 %, fixed model - Figure 2, part A). No other associations were found.

Figure 2 - Meta-analysis of the SNPs rs1024611 (CCL2 -2518A/G) and rs2107538 (CCL5 -403C/T)



CCL5 - rs2107538

The rs2107538 (CCL5 -403C/T) SNP was analyzed in three different studies (Batista et al, 2018, Flórez et al, 2012 and Nogueira et al, 2012). The meta-analysis (n=911, UndCP=364/CardCP=547) indicated an allelic association (T *vs*. C), where the T allele was indicated as a risk factor for the cardiac form of CD (p=0.02, OR =1.34, 95%CI [1.04-1.73], I^2= 0%, fixed model - Figure 2, part B). In the Codominance model (CT *vs*. CC), the heterozygous genotype was also indicated as a risk factor for the development of the cardiac form in CD (p=0.05, OR=1.41, 95%CI [1.00-1.98], I^2= 0 %, fixed model - Figure 2, part C). In the dominant inheritance model (TT+CT *vs*. CC), another association was obtained, pointing to genotypes with T carriers as risk factors for the development of the cardiac form

(p=0.03, OR=1.45, 95%CI [1.04-2.01], I^2= 0%, fixed model - Figure 2, part D). However, the sample power of the combined studies in the meta-analysis was only SP= 21.06%.

CCL5 - rs2280788

Only two studies involving CD patients analyzed the influence of the rs2280788 SNP (CCL5 -28G/C): Batista et al, 2018 and Flórez et al, 2012. Using data from these studies, cardiac patients *vs.* asymptomatic (n=496, CardCP=202/UndCP=164), however, due to the rarity of the C allele, and therefore the C/C genotype (not found in any of the studies), some comparisons could not be conducted. Finally, among the viable comparisons, no differences were found between the analyzed groups.

CCR2 - rs1799864

Flórez et al, 2012, Juíz et al, 2019 and Machuca et al, 2014 independently analyzed the influence of the SNP rs1799864 (CCR2 +190G/A) on CD. Comparisons between cardiac and asymptomatic groups (n=1226, CardCP=625/UndCP=601) did not show any type of association between alleles or genotypes with increased or reduced risk in CD. The sample power found was SP=90.34%.

CCR2 - rs3138042

Although two studies have independently evaluated the CCR2 SNP rs3138042 (Juíz et al, 2019 and Machuca et al, 2014), the meta-analysis was not possible to be conducted due to the non-presentation of genotypic and allelic data of asymptomatic patients in the studies (Machuca et al, 2014).

Publication bias, heterogeneity and overall frequency of results

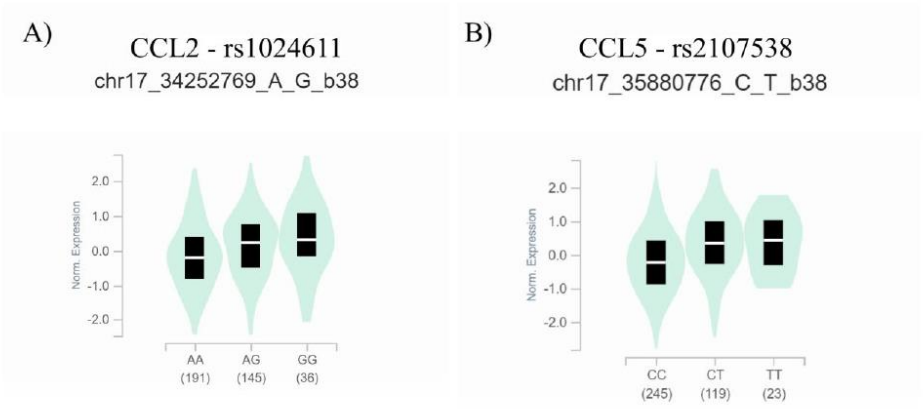
As our analyses had less than ten studies in each comparison (K<10), we considered

inspecting the funnel plot graphs to assess possible biases in the results of our comparisons. However, due to the reduced number of works that analyzed the polymorphisms included in this review, the data distribution was considered insufficient to infer the existence of publication bias. In addition, studies are found on both sides of the graphs **(Supplementary Figure 1)**, which may indicate symmetry and lack of bias. No significant comparisons in our results indicated substantial heterogeneity (I^2>50% and p<0.05), indicating homogeneity of studies. The global frequencies obtained in the analyses are available in **Supplementary Table 2**.

GTEx portal database and expression quantitative trait loci (eQTL)

Regarding the CCL2 SNP rs1024611, the medians presented for each genotype obtained by GTEx-eQTL were: AA=-0.1927; AG=0.2202; and GG=0.3355 (https://gtexportal.org/home/snp/rs1024611). It was observed that carriers of the variant genotypes (AG *vs*. AA and GG *vs*. AA) have a significantly higher expression of CCL2 mRNA (p=0.0000015; Normalized Effect Size - NES=0.28) in cardiac tissue than carriers of the wild type genotype. The eQTL-Violin plot is represented in **Figure 3**, part A.

Figure 3 - GTEx-eQTL data of SNP variants rs1024611 (CCL2) and rs2107538 (CCL5).

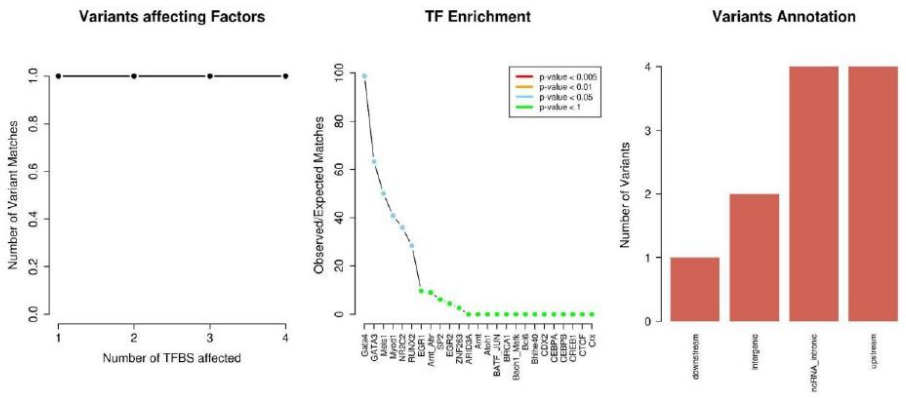


The CCL5 SNP rs2107538 the medians presented for each genotype were: CC=-0.2214; CT=0.3357; and TT=0.4189 (https://gtexportal.org/home/snp/rs2107538). Carriers of the T allele (CT *vs*. CC and TT *vs*. CC) were observed to have significantly higher expression of CCL5 mRNA (p=0.0000000000034; NES=0.38) in cardiac tissue than carriers of the wild-type genotype. The eQTL-Violin plot representing the activity of rs2107538 is represented in **Figure 3**, part B. The tissue analyzed in the expression of variants of rs1024611 (CCL2) was "Atrial Appendage", while for rs2107538 of CCL5 "Artery - Aorta" was used.

SNP2TFBS

The analysis of the SNP2TFBS tool identified four of the SNPs found in this review capable of influencing the binding of transcription factors to gene transcription sites. The SNP rs3136672 (*CCR1*) showed the ability to influence the transcription factor RUNX2 (p-value<0.05). The analysis also indicated that the SNP rs1024611 (*CCL2*) was able to significantly influence two transcription factors (Meis1 and Myod1), p-value<0.05 for both. The SNP rs2107538 (*CCL5*) was shown to be able to influence three transcription factors: GATA3, Gata4 and ZNF263, however only the first two were significantly influenced (both with p-value<0.05). Finally, the SNP rs2280964 (*CXCR3*) apparently influences the transcription factors EGR1, EGR2, SP2 and NR2C2, but only the latter significantly (p-value<0.05). The influence of the cited SNPs are represented in **Figure 4**.

Figure 4 - SNP2TFBS analysis results.



Discussion

Chemokines are directly involved in the control of cell recruitment and migration (GROOM, 2019) , allowing the maintenance of chronic inflammation (BATISTA et al., 2018; NOGUEIRA et al., 2012) . The expression of chemokine receptors on T lymphocytes, together with the expression of specific chemokines in tissues, are important factors that control lymphocyte infiltration into different types of tissues. (CUNHA-NETO *et al.*, 2009; NOGUEIRA *et al.*, 2012) .

Chemokines and their receptors have been studied in the chronic cardiac form of DC precisely in search of their contributions in this form of the disease in relation to maintaining the continuity of inflammation that leads to tissue damage (BATISTA et al., 2018; DE OLIVEIRA et al., 2015) . Studies indicate that to control parasitic intracellular replication in the chronic inflammation of the cardiac form of CD, cardiomyocytes produce nitric oxide, cytokines and chemokines that attract leukocytes to the inflammatory site (CALZADA et al., 2001) . Thus, understanding the colonization mechanisms of inflammatory response cells in cardiac tissues may reveal targets that modulate the development of the cardiac form of the disease (BATISTA et al., 2018; DE OLIVEIRA et al., 2015) .

There are two major subfamilies of chemokines based on the position of their cysteine

residues, CC (chemotactics of monocytes and lymphocyte subtypes) and CXC (chemotactics of neutrophils) (QI et al., 2020) . In our analyses, two SNPs belonging to CC-type cytokines were associated with the development of the cardiac form in CD (rs1024611/CCL2 and rs2107538/CCL5 -403C/T). For CCL2, the GG genotype was associated in the recessive model, and according to data obtained from the GTEx portal, there is a greater amount of CCL2 mRNA in GG genotypes genotypes with carriers with the A allele in cardiac tissues. This may indicate that patients with the homozygous GG genotype have a greater risk of developing the cardiac form caused by the greater volume of transcription of this gene and consequent expression, which allows an exacerbated recruitment of effector cells, which provide opportunities for chronic inflammation and, consequently, tissue damage.

Our results on the CCL2 SNP rs1024611 are different from the results found in previous works that analyzed this polymorphism (Batista et al, 2018 and Ramasawmy et al, 2022). Perhaps the aforementioned studies did not obtain the same results as the present study due to the lack of sample power of the works, or even because they used subdivisions for their patients in their respective methodologies, whereas in our work we assumed only two groups in the analyzes (asymptomatic and symptomatic cardiac).

In our results, the CCL5 SNP rs2107538 obtained an allelic association (T allele) and two genotypic associations (CT genotype in the Codominance model, and the TT and CT genotypes in the dominance model). Our results indicate a strong influence of T carriers on the cardiac form of CD. Data obtained from the GTEx portal indicate that genotypes with T carriers have a greater volume of *CCL5* gene expression than the CC genotype in cardiac tissues. And similar to the results obtained in the CCL2 SNP rs1024611, we hypothesized that this volume of mRNA caused by the CT and TT genotypes in the CCL5 SNP rs2107538 are consequently expressed, causing the recruitment of cells with inflammatory capacities and that in the long term allow cardiac damage.

Previous studies that analyzed the influence of the CCL5 SNP rs2107538 (Batista et al, 2018, Flórez et al, 2012 and Nogueira et al, 2012) did not obtain these results. Batista et al, 2018, after using logistic regression analyzes with adjustment for gender and ethnicity covariates, found associations between T carriers (TT and CT) with protection against the development of the most severe form of the cardiac form of CD (CCC - Chronic Chagas cardiomyopathy). It is possible that these results have been strongly influenced by the

covariates used. It is also possible that, similar to what was observed in the investigation of the CCL2 SNP rs1024611, the difference between the results was caused by the sample power of the works, or even, by using them in subdivisions for their patients. However, we emphasize that the sample power (SP) found here for the analysis of the CCL5 SNP rs2107538 was not ideal, which suggests a sample expansion to repeat the analyses.

Although our hypothesis attributes pathophysiological participation in mechanisms associated with the presented SNPs, the influence of more than one factor must be considered, such as genetic factors that predispose the development of the cardiac form in DC. According to authors of previous works, the simple presence or absence of a single genetic variant is not enough to influence the course of CD in a patient, so that combinations between different variants in several genes contribute modestly in the pathogenesis of the disease (FLÓREZ; MARTÍN; GONZÁLEZ, 2012; JUIZ et al., 2019; MACHUCA et al., 2014).

The analysis of the SNP2TFBs tool indicated that both polymorphisms already mentioned are capable of influencing certain transcription factors (TFs). We observed that Meis1 and Myod1 TFs can bind to CCL2 SNP rs1024611 (-2518A/G) in the promoter region of the gene, as well as GATA3 and GATA 4 TFs seem to be able to bind to CCL5 SNP rs2107538 (-403C/T). In this hand, it is possible that there is a regulatory pathway for the expression of these two genes, which promotes an increase or decrease in the levels of transcripts and their consequent proteins, contributing to the risk of CD progression.

Interestingly, the TFs influenced by the presented SNPs have diverse physiological functions. Meis1 belongs to the “homeobox”, which contains the transcriptional regulatory network (HOX), and is involved in several physiological and pathophysiological processes, as well as being remarkably related to cell migration, apoptosis and metabolism, in addition to apparently being involved in cell proliferation of cancer cells (SARAYLOO; DION; ROULEAU, 2019; YAO et al., 2021) . Myod1 is one of the basic transcription factors specific for skeletal muscles (helix-loop-helix, BHLH), which are also master factors and regulators in skeletal muscle development (MORITA; HAYASHI, 2022; PLINER et al., 2018) . GATA3 is essential for the development, maintenance, survival and proliferation of early T cell progenitors (ZAIDAN; OTTERSBACH, 2018) . And finally, GATA4 is known as a transcription factor that can induce cardiac hypertrophy and heart failure when acetylated (SHIMIZU et al., 2022) . It is not known whether these factors may have a deeper

contribution to the development of CD.

Pro-inflammatory (such as IFN-γ and TNF) and anti-inflammatory (such as IL-10) cytokines play a central role in the development of tissue damage caused in the cardiac form of the chronic phase of CD (ACEVEDO; GIRARD; GOMEZ, 2018 ; CRISTOVÃO-SILVA et al., 2021; DE BONA et al., 2018) . Added to this, molecules such as chemokines have been constantly investigated since they provide opportunities for cell colonization and infiltration in tissues, being a vital mechanism in the immune fight against cell parasites. In this work, we gather the knowledge available in the literature about the influence of genetic variants on chemokines and their receptors, as well as narrowing down the relationship between these variables and disease through exploratory analyses.

Our work established new associations between genetic polymorphisms and the cardiac form of DC, as well as established possible influences of SNPs on chemokine genes in TFs in the cardiac form of DC, both with considerable sample power. However, some points must be considered: a limited number of articles analyzed the influence of genetic variants on chemokine genes and their receptors in DC, few exploratory analyzes could be performed, and experimental analyzes were not conducted in order to corroborate our findings. We also indicate that the results obtained through our exploratory analyzes should be analyzed with caution. Finally, analyzes of other chemokine gene variables should be considered to understand the connection between these molecules and the pathophysiology of the disease.

References

ACEVEDO, Gonzalo R; GIRARD, Magali C; GOMEZ, Karina A. The unsolved jigsaw puzzle of the immune response in Chagas disease. **Frontiers in immunology**, [*s. l.*], v. 9, p. 1929, 2018.

ACOSTA-HERRERA, Marialbert *et al*. Genomic medicine in Chagas disease. **Acta tropica**, [*s. l.*], v. 197, p. 105062, 2019.

BATISTA, Angelica Martins *et al.* Genetic polymorphism at CCL5 is associated with protection in Chagas' heart disease: antagonistic participation of CCR1+ and CCR5+ cells in chronic chagasic cardiomyopathy. **Frontiers in immunology**, [*s. l.*], v. 9, p. 615, 2018.

BERN, Caryn. Chagas' disease. **New England Journal of Medicine**, [*s. l.*], v. 373, n. 5, p. 456–466, 2015.

CALZADA, J E *et al*. Chemokine receptor CCR5 polymorphisms and Chagas' disease cardiomyopathy. **Tissue antigens**, [*s. l.*], v. 58, n. 3, p. 154–158, 2001.

CARVALHO, Thaysa Buss *et al*. ACAT-1 gene rs1044925 SNP and its relation with different clinical forms of chronic Chagas disease. **Parasitology research**, [*s. l.*], v. 118, n. 8, p. 2343–2351, 2019.

CRISTOVÃO-SILVA, Ana Catarina *et al*. Chagas disease: Immunology of the disease at a glance. **Cytokine & Growth Factor Reviews**, [*s. l.*], v. 62, p. 15–22, 2021.

CUNHA-NETO, Edecio *et al*. Immunological and non-immunological effects of cytokines and chemokines in the pathogenesis of chronic Chagas disease cardiomyopathy. **Memorias do Instituto Oswaldo Cruz**, [*s. l.*], v. 104, p. 252–258, 2009.

DE BONA, Elidiana *et al.* Autoimmunity in chronic Chagas disease: a road of multiple pathways to cardiomyopathy?. **Frontiers in immunology**, [*s. l.*], v. 9, p. 1842, 2018.

DE OLIVEIRA, Amanda Priscila *et al.* Genetic susceptibility to cardiac and digestive clinical forms of chronic Chagas disease: involvement of the CCR5 59029 A/G polymorphism. **PLoS One**, [*s. l.*], v. 10, n. 11, p. e0141847, 2015.

DE OLIVEIRA, Amanda P *et al.* The role of CCR5 in Chagas disease-a systematic review. **Infection, Genetics and Evolution**, [*s. l.*], v. 45, p. 132–137, 2016.

ECHAVARRÍA, Natalia Giraldo *et al.* Chagas disease: chronic Chagas cardiomyopathy. **Current problems in cardiology**, [*s. l.*], v. 46, n. 3, p. 100507, 2021.

ERDFELDER, E. Statistical power analyses using G * Power 3 . 1 : Tests for correlation and regression analyses. [*s. l.*], v. 41, n. 4, p. 1149–1160, 2009.

FAUL, Franz *et al*. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. **Behavior research methods**, [*s. l.*], v. 39, n. 2, p. 175–191, 2007.

FLÓREZ, Oscar; MARTÍN, Javier; GONZÁLEZ, Clara Isabel. Genetic variants in the chemokines and chemokine receptors in Chagas disease. **Human Immunology**, [*s. l.*], v. 73, n. 8, p. 852–858, 2012.

FRADE, Amanda Farage *et al*. Genetic susceptibility to Chagas disease cardiomyopathy: involvement of several genes of the innate immunity and chemokine-dependent migration pathways. **BMC infectious diseases**, [*s. l.*], v. 13, n. 1, p. 1–17, 2013.

GOMES DOS SANTOS, Alexandra *et al.* A specific IL6 polymorphic genotype modulates the risk of Trypanosoma cruzi parasitemia while IL18, IL17A, and IL1B variant profiles and HIV infection protect against cardiomyopathy in Chagas disease. **Frontiers in immunology**, [*s. l.*], v. 11, p. 521409, 2020.

GROOM, Joanna R. Regulators of T- cell fate: Integration of cell migration, differentiation and function. **Immunological Reviews**, [*s. l.*], v. 289, n. 1, p. 101–114, 2019.

JUIZ, Natalia Anahí *et al*. Association study between CCR2-CCR5 genes polymorphisms and chronic Chagas heart disease in Wichi and in admixed populations from Argentina. **PLoS neglected tropical diseases**, [*s. l.*], v. 13, n. 1, p. e0007033, 2019.

KAYA, Mahmut. Trypanosomiasis. *In*: UVEITIS. [*S. l.*]: Springer, 2021. p. 225–228.

LIMA, Ana Paula Braga *et al*. Evaluation of parasite and host genetics in two generations of a family with Chagas disease. **Parasitology research**, [*s. l.*], v. 117, n. 9, p. 3009–3013, 2018.

MACHUCA, Mayra Alejandra *et al*. SNP/haplotype associations of CCR2 and CCR5 genes with severity of chagasic cardiomyopathy. **Human immunology**, [*s. l.*], v. 75, n. 12, p. 1210–1215, 2014.

MORITA, Tsuyoshi; HAYASHI, Ken'ichiro. Actin-related protein 5 functions as a novel modulator of MyoD and MyoG in skeletal muscle and in rhabdomyosarcoma. **Elife**, [*s. l.*], v. 11, p. e77746, 2022.

NOGUEIRA, Luciana Gabriel *et al*. Myocardial chemokine expression and intensity of myocarditis in Chagas cardiomyopathy are controlled by polymorphisms in CXCL9 and CXCL10. **PLoS neglected tropical diseases**, [*s. l.*], v. 6, n. 10, p. e1867, 2012.

NORMAN, Francesca F; LÓPEZ-VÉLEZ, Rogelio. Chagas disease: comments on the 2018 PAHO Guidelines for diagnosis and management. **Journal of travel medicine**, [*s. l.*], v. 26, n. 7, p. taz060, 2019.

PAN AMERICAN HEALTH ORGANIZATION - PAHO. **Chagas disease - PAHO/WHO | Pan American Health Organization**. [*S. l.*], 2022. Disponível em: https://www.paho.org/en/topics/chagas-disease. Acesso em: 20 jun. 2022.

PÉREZ-MOLINA, José A; MOLINA, Israel. Chagas disease. **The Lancet**, [*s. l.*], v. 391, n. 10115, p. 82–94, 2018.

PÉREZ-MOLINA, José A; MOLINA, Israel. Chagas disease cardiomyopathy treatment remains a challenge–Authors' reply. **The Lancet**, [*s. l.*], v. 391, n. 10136, p. 2209–2210, 2018.

PLINER, Hannah A *et al.* Cicero predicts cis-regulatory DNA interactions from single-cell chromatin accessibility data. **Molecular cell**, [*s. l.*], v. 71, n. 5, p. 858–871, 2018.

QI, Baowen *et al*. Advances of CCR5 antagonists: From small molecules to macromolecules. **European Journal of Medicinal Chemistry**, [*s. l.*], v. 208, p. 112819, 2020.

RAMASAWMY, Rajendranath *et al*. The monocyte chemoattractant protein–1 gene polymorphism is associated with cardiomyopathy in human Chagas disease. **Clinical Infectious Diseases**, [*s. l.*], v. 43, n. 3, p. 305–311, 2006.

SANTOS, Cristina Mamédio da Costa; PIMENTA, Cibele Andrucioli de Mattos; NOBRE, Moacyr Roberto Cuce. The PICO strategy for the research question construction and evidence search. **Revista latino-americana de enfermagem**, [*s. l.*], v. 15, n. 3, p. 508–511, 2007.

SARAYLOO, Faezeh; DION, Patrick A; ROULEAU, Guy A. MEIS1 and restless legs syndrome: a comprehensive review. **Frontiers in neurology**, [*s. l.*], v. 10, p. 935, 2019.

SHIMIZU, Satoshi *et al*. Multimerization of the GATA4 transcription factor regulates transcriptional activity and cardiomyocyte hypertrophic response. **International Journal of Biological Sciences**, [*s. l.*], v. 18, n. 3, p. 1079, 2022.

SIMÕES, Marcus Vinicius *et al*. Chagas disease cardiomyopathy. **International Journal of Cardiovascular Sciences**, [*s. l.*], v. 31, p. 173–189, 2018.

VARIKUTI, Sanjay *et al.* Host-directed drug therapies for neglected tropical diseases caused by protozoan parasites. **Frontiers in Microbiology**, [*s. l.*], v. 9, p. 2655, 2018.

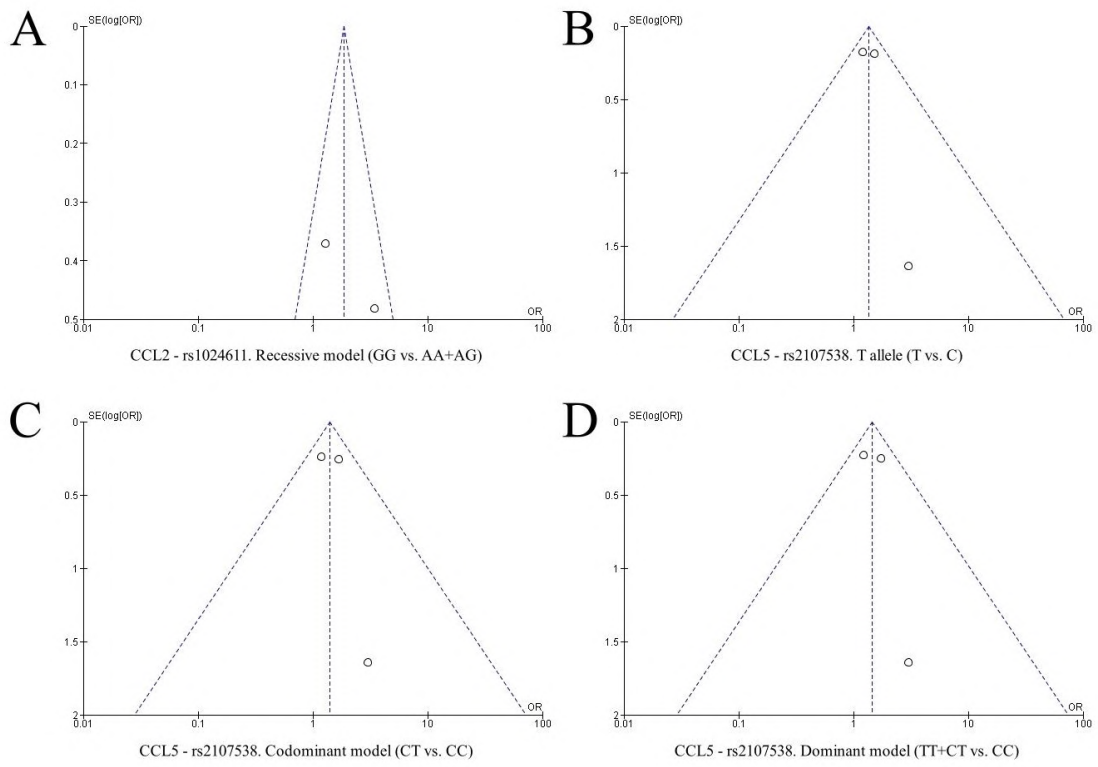
WENG, Hong-Bo; CHEN, Hai-Xia; WANG, Ming-Wei. Innovation in neglected tropical disease drug discovery and development. **Infectious diseases of poverty**, [*s. l.*], v. 7, n. 1, p. 1–9, 2018.

WORLD HEALTH ORGANIZATION - WHO. **Chagas disease (American trypanosomiasis)**. [*S. l.*], 2022. Disponível em: https://www.who.int/health-topics/chagas-disease#tab=tab_1. Acesso em: 20 jun. 2022.

YAO, Maozhong *et al.* MEIS1 and its potential as a cancer therapeutic target. **International Journal of Molecular Medicine**, [*s. l.*], v. 48, n. 3, p. 1–9, 2021.

ZAIDAN, Nada; OTTERSBACH, Katrin. The multi-faceted role of Gata3 in developmental haematopoiesis. **Royal Society Open Biology**, [*s. l.*], v. 8, n. 11, p. 180152, 2018.

Supplementary Figure 1 – Funnel plots obtained through comparisons with significant results (p≤0.05) of the SNPs rs1024611 (CCL2 −2518A/G) and rs2107538 (CCL5 −403C/T).



Supplementary Table 1 - Description of the groups included in the formation of the CardCP grouping.

Estudy	Description of the groups included in the formation of the CardCP grouping
Batista et al, 2018	"Group on stage B1 (with structural heart disease, evidenced by ECG or ECHO, but with normal global ventricular function and neither current nor previous signs and symptoms of congestive heart failure) and group on stage C (with ventricular dysfunction and current or previous symptoms of congestive heart failure)";
Juiz et al, 2019	"DC-group (with clinical symptoms and electrocardiography alterations)";
Machuca et al, 2014	"Symptomatic group (withminor sympthoms and alterations) and cardiomyopathics group (with considerable sympthoms and alterations)";
Flórez, Martín and González, 2012	"Group II (radiology indicative of light heart hypertrophy or minor ECG alterations), group III (moderate heart hypertrophy and considerable ECG alterations, mainly advanced conduction abnormalities) and group IV (severe cardiomegaly and marked ECG alterations, predominantly frequent and/or complex forms of ventricular arrhythmia)";
Nogueira et al, 2012	"CCC (moderate group and severe group)";
Ramasawmy et al, 2006	"Patients with CCC presented with abnormal echocardiograph findings that ranged from typical conduction abnormalities to severe arrhythmia. Some patients also presented varying degrees of ventricular dysfunction classified on the basis of left ventricular ejection fraction, with all other causes of ventricular dysfunction/heart failure excluded".

Supplementary Table 2 – Genetic variants and comparisons made between groups and their individual and total frequencies.

Comparison (group 1 *vs.* group 2), and sample power (SP%)*	Gene - Polymorphism	Group 1 frequency**	Group 2 frequency**	Total frequency (group 1 + group 2)**
UndCP vs. CardCP (SP= 88.2%)	*CCL2* - rs1024611	GG=0,1437125749; GA=0,377245509; AA=0,4790419162; G=0,3323353293; A=0,6676646707.	GG=0,08557457213; GA=0,4009779951; AA=0,5134474328; G=0,2860635697; A=0,7139364303.	GG=0,1024305556; GA=0,3940972222; AA=0,5034722222; G=0,2994791667; A=0,7005208333
(SP=21,06%)	*CCL5* - rs2107538	CC=0,6565934066; CT=0,282967033; TT=0,06043956044; C=0,7980769231; T=0,2019230769.	CC=0,6636197441; CT=0,2870201097; TT=0,04936014625; C=0,8071297989; T=0,1928702011.	CC=0,6608122942; CT=0,2854006586; TT=0,0537870472; C=0,8035126235; T=0,1964873765.
#	*CCL5* - rs2280788	GG=0,9817073171; GC=0,01829268293; CC=0; G=0,9908536585; C=0,009146341463.	GG=0,9702970297; GC=0,0297029703; CC=0; G=0,9851485149; C=0,01485148515.	GG=0,9717741935; GC=0,02822580645; CC=0; G=0,9858870968; C=0,01411290323.
(SP=90,34%)	*CCR2* - rs1799864	GG=0,53078203; GA=0,3427620632; AA=0,1264559068; G=0,7021630616; A=0,2978369384.	GG=0,5824; GA=0,3088; AA=0,1088; G=0,7368; A=0,2632.	GG=0,557096248; GA=0,3254486134; AA=0,1174551387; G=0,7198205546; A=0,2801794454.

Sample power >80 indicates a good representation of the population (effect expressed in the population); ** Frequency found with all study participants; #Polymorphism unable to complete sampling power effect analysis due to lack of data (SP not calculated). Source: Author 2023.

5. CONCLUSÃO

Neste trabalho, foi reunido o conhecimento disponível na literatura sobre a influência de variantes genéticas em quimiocinas e seus receptores na doença de Chagas, bem como foi afunilada a relação entre essas variáveis e esta doença por meio de análises estatísticas exploratórias, obtendo-se novas associações com considerável poder amostral. Foram analisadas as influências de SNPs sobre fatores de transcrição e a existências de correlações entre as variáveis alélicas e os genótipos formados por elas. Em suma, as informações de onze polimorfismos (rs2856758, rs2734648, rs1799987, rs1799988, rs41469351, rs1800023, rs1800024, Δ32/rs333, rs3176763, rs3087253 e rs11575815) pertencentes ao gene CCR5 foram analisadas. Destes SNPs, oito foram passíveis de meta-análises, e, ao fim das investigações, foi possível correlacionar a forma cardíaca da DC aos alelos do haplótipo C desse gene (HHC-TGTG).

Também foram coletados dados relacionados aos genes *CCL2*, *CCL4*, *CCL5*, *CCL17*, *CCL19*, *CXCL8*, *CXCL9*, *CXCL10* e *CXCR3*, e dos receptores *CCR1* e *CCR2*. Em relação a estes últimos dados, três SNPs puderam ser meta-analisados, e duas associações foram estabelecidas com o desenvolvimento da forma cardíaca da DC em pacientes infectados. Em consulta ao portal GTEx, verificou-se que as variantes rs1024611 (CCL2) e rs2107538 (CCL5) estão relacionadas com os mais altos níveis de transcritos dos respectivos genes, enquanto a ferramenta SNP2TFBS indicou que estas duas variantes também são capazes de influenciar certos fatores de transcrição. Em suma, presumimos que os volumes de mRNA produzidos pelas influências dessas variantes são expressos, contribuindo com o recrutamento exacerbado de células inflamatórias, permitindo o dano tecidual cardíaco de longo prazo nos pacientes.

Entretanto, alguns pontos devem ser considerados: um quantitativo limitado de artigos foi usado nesta revisão e nosso trabalho não contemplou verificações experimentais a fim de corroborar com os resultados obtidos. Por isso, indicamos que nossos achados devem ser interpretados com relativa cautela.

6. PERSPECTIVAS E CONSIDERAÇÕES FINAIS

Considerando o exposto, e que ainda há muitas lacunas no entendimento da relação entre a genética do paciente a DC, é viável que futuros trabalhos (1) utilizem este estudo como ponto de partida para futuras investigações ou como modelo para investigação da epidemiologia de outras doenças infecciosas; (2) analisem outras variantes, inclusive de genes centrais da imunidade, como citocinas e receptores celulares; (3) utilizem outras análises exploratórias para continuar afunilando a relação das variantes genéticas e a DC; (4) executem análises experimentais a fim de corroborar os achados deste trabalho e (5) possam contribuir na construção de um portal/banco de dados voltado para o armazenamento de dados de amostras de pacientes, incluindo dados socioeconômicos.

REFERÊNCIAS

ACEVEDO, G. R.; GIRARD, M. C.; GOMEZ, K. A. The unsolved jigsaw puzzle of the immune response in Chagas disease. **Frontiers in immunology**, v. 9, p. 1929, 2018.

ACOSTA-HERRERA, M.; STRAUSS, M.; CASARES-MARFIL, D.; MARTÍN, J.; NETWORK, C. G. C. Genomic medicine in Chagas disease. **Acta tropica**, v. 197, p. 105062, 2019.

ALDINUCCI, D.; BORGHESE, C.; CASAGRANDE, N. The CCL5/CCR5 axis in cancer progression. **Cancers**, v. 12, n. 7, p. 1765, 2020.

ANDRADE, Z. A.; ARAUJO-JORGE, T. C. de; LANNES-VIEIRA, J.; HIGUCHI, M. de L.; RASSI, A.; KROPF, S. P.; ROCHA, M. O. da C.; BARROS, M. V. L.; TOSTES, V. T. V.; JUNQUEIRA, L. L.; RIBEIRO, A. L. P.; REZENDE, J. M. de; SILVA, A. A. da. **Patologia**. Disponível em: <http://chagas.fiocruz.br/doenca/patologia/>. Acesso em: 11 jan. 2023.

ARIAS-GIRALDO, L. M.; MUÑOZ, M.; HERNÁNDEZ, C.; HERRERA, G.; VELÁSQUEZ-ORTIZ, N.; CANTILLO-BARRAZA, O.; URBANO, P.; CUERVO, A.; RAMÍREZ, J. D. Identification of blood-feeding sources in Panstrongylus, Psammolestes, Rhodnius and Triatoma using amplicon-based next-generation sequencing. **Parasites & vectors**, v. 13, n. 1, p. 1–14, 2020.

BARGAIN, O.; AMINJONOV, U. Poverty and COVID-19 in Africa and Latin America. **World development**, v. 142, p. 105422, 2021.

BATISTA, A. M.; ALVARADO-ARNEZ, L. E.; ALVES, S. M.; MELO, G.; PEREIRA, I. R.; RUIVO, L. A. de S.; DA SILVA, A. A.; GIBALDI, D.; DA SILVA, T. do E. S. P.; DE LORENA, V. M. B. Genetic polymorphism at CCL5 is associated with protection in Chagas' heart disease: antagonistic participation of CCR1+ and CCR5+ cells in chronic chagasic cardiomyopathy. **Frontiers in immunology**, v. 9, p. 615, 2018.

BERN, C. Chagas' disease. **New England Journal of Medicine**, v. 373, n. 5, p. 456–466, 2015.

BORCHERING, R. K.; HUANG, A. T.; MIER-Y-TERAN-ROMERO, L.; ROJAS, D. P.; RODRIGUEZ-BARRAQUER, I.; KATZELNICK, L. C.; MARTINEZ, S. D.; KING, G. D.; CINKOVICH, S. C.; LESSLER, J. Impacts of Zika emergence in Latin America on endemic dengue transmission. **Nature communications**, v. 10, n. 1, p. 1–9, 2019.

BROCHET, P.; IANNI, B.; LAUGIER, L.; FRADE, A.; TEIXEIRA, P.; MADY, C.; FERREIRA, L.; FERRÉ, Q.; SANTOS, R.; KURAMOTO, A. Epigenetic regulation of transcription factor binding motifs promotes Th1 response in Chagas disease Cardiomyopathy. **Frontiers in immunology**, v. 13, 2022.

CALZADA, J. E.; NIETO, A.; BERAUN, Y.; MARTIN, J. Chemokine receptor CCR5 polymorphisms and Chagas' disease cardiomyopathy. **Tissue antigens**, v. 58, n. 3, p. 154–158, 2001.

CARRINGTON, M.; DEAN, M.; MARTIN, M. P.; O'BRIEN, S. J. Genetics of HIV-1 infection: chemokine receptor CCR5 polymorphism and its consequences. **Human molecular genetics**, v. 8, n. 10, p. 1939–1945, 1999.

CARVALHO, T. B.; PADOVANI, C. R.; DE OLIVEIRA JÚNIOR, L. R.; LATINI, A. C. P.; KUROKAWA, C. S.; PEREIRA, P. C. M.; DOS SANTOS, R. M. ACAT-1 gene rs1044925 SNP and its relation with different clinical forms of chronic Chagas disease. **Parasitology research**, v. 118, n. 8, p. 2343–2351, 2019.

CHEVILLARD, C.; NUNES, J. P. S.; FRADE, A. F.; ALMEIDA, R. R.; PANDEY, R. P.; NASCIMENTO, M. S.; KALIL, J.; CUNHA-NETO, E. Disease tolerance and pathogen resistance genes may underlie Trypanosoma cruzi persistence and differential progression to Chagas disease cardiomyopathy. **Frontiers in immunology**, v. 9, p. 2791, 2018.

COHEN, J. P.; SILVA, L.; COHEN, A.; AWATIN, J.; STURGEON, R. Progress report on neglected tropical disease drug donation programs. **Clinical therapeutics**, v. 38, n. 5, p. 1193–1204, 2016.

CRISTOVÃO-SILVA, A. C.; BRELAZ-DE-CASTRO, M. C. A.; HERNANDES, M. Z.; PEREIRA, V. R. A. Chagas disease: Immunology of the disease at a glance. **Cytokine & Growth Factor Reviews**, v. 62, p. 15–22, 2021.

CUNHA-NETO, E.; NOGUEIRA, L. G.; TEIXEIRA, P. C.; RAMASAWMY, R.; DRIGO, S. A.; GOLDBERG, A. C.; FONSECA, S. G.; BILATE, A. M.; KALIL, J. Immunological and non-immunological effects of cytokines and chemokines in the pathogenesis of chronic Chagas disease cardiomyopathy. **Memorias do Instituto Oswaldo Cruz**, v. 104, p. 252–258, 2009.

D'ÁVILA, D. A.; MACEDO, A. M.; VALADARES, H. M. S.; GONTIJO, E. D.; DE CASTRO, A. M.; MACHADO, C. R.; CHIARI, E.; GALVÃO, L. M. C. Probing population dynamics of Trypanosoma cruzi during progression of the chronic phase in chagasic patients. **Journal of Clinical Microbiology**, v. 47, n. 6, p. 1718–1725, 2009.

DE BONA, E.; LIDANI, K. C. F.; BAVIA, L.; OMIDIAN, Z.; GREMSKI, L. H.; SANDRI, T. L.; MESSIAS REASON, I. J. de. Autoimmunity in chronic Chagas disease: a road of multiple pathways to cardiomyopathy? **Frontiers in immunology**, v. 9, p. 1842, 2018.

DE OLIVEIRA, A. P.; AYO, C. M.; BESTETTI, R. B.; DE MATTOS, C. C. B.; CAVASINI, C. E.; DE MATTOS, L. C. The role of CCR5 in Chagas disease-a systematic review. **Infection, Genetics and Evolution**, v. 45, p. 132–137, 2016.

DE OLIVEIRA, A. P.; BERNARDO, C. R.; CAMARGO, A. V. da S.; RONCHI, L. S.; BORIM, A. A.; BRANDAO DE MATTOS, C. C.; DE CAMPOS JUNIOR, E.; CASTIGLIONI, L.; NETINHO, J. G.; CAVASINI, C. E. Genetic susceptibility to cardiac and digestive clinical forms of chronic Chagas disease: involvement of the CCR5 59029 A/G polymorphism. **PLoS One**, v. 10, n. 11, p. e0141847, 2015.

DEL PUERTO, F.; KIKUCHI, M.; NISHIZAWA, J. E.; ROCA, Y.; AVILA, C.; GIANELLA, A.; LORA, J.; VELARDE, F. U. G.; HIRAYAMA, K. 21-Hydroxylase gene mutant allele CYP21A2* 15 strongly linked to the resistant HLA haplotype B* 14: 02-DRB1* 01: 02 in chronic Chagas disease. **Human Immunology**, v. 74, n. 6, p. 783–786, 2013.

DONNINELLI, G.; STUDER, V.; BRAMBILLA, L.; ZECCA, C.; PELUSO, D.; LARONI, A.; MICHELIS, D.; MANTEGAZZA, R.; CONFALONIERI, P.; VOLPE, E. Immune Soluble Factors in the Cerebrospinal Fluid of Progressive Multiple Sclerosis Patients Segregate Into Two Groups. **Frontiers in Immunology**, v. 12, p. 633167, 2021.

DU, B.; GAO, W.; QIN, Y.; ZHONG, J.; ZHANG, Z. Study on the role of transcription factor SPI1 in the development of glioma. **Chinese Neurosurgical Journal**, v. 8, n. 1, p. 1–10, 2022.

ECHAVARRÍA, N. G.; ECHEVERRÍA, L. E.; STEWART, M.; GALLEGO, C.; SALDARRIAGA, C. Chagas disease: chronic Chagas cardiomyopathy. **Current problems in cardiology**, v. 46, n. 3, p. 100507, 2021.

ERDFELDER, E. Statistical power analyses using G * Power 3 . 1 : Tests for correlation and regression analyses. v. 41, n. 4, p. 1149–1160, 2009.

FAUL, F.; ERDFELDER, E.; LANG, A.-G.; BUCHNER, A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. **Behavior research methods**, v. 39, n. 2, p. 175–91, maio 2007.

FERREIRA, L. R. P.; FERREIRA, F. M.; LAUGIER, L.; CABANTOUS, S.; NAVARRO, I. C.; DA SILVA CÂNDIDO, D.; RIGAUD, V. C.; REAL, J. M.; PEREIRA, G. V.; PEREIRA, I. R. Integration of miRNA and gene expression profiles suggest a role for miRNAs in the pathobiological processes of acute Trypanosoma cruzi infection. **Scientific reports**, v. 7, n. 1, p. 1–12, 2017.

FLÓREZ, O.; MARTÍN, J.; GONZÁLEZ, C. I. Genetic variants in the chemokines and chemokine receptors in Chagas disease. **Human Immunology**, v. 73, n. 8, p. 852–858, 2012.

FONTECHA, G.; SÁNCHEZ, A.; ORTIZ, B. Publication trends in neglected tropical diseases of Latin America and the Caribbean: a bibliometric analysis. **Pathogens**, v. 10, n. 3, p. 356, 2021.

FRADE, A. F.; PISSETTI, C. W.; IANNI, B. M.; SABA, B.; LIN-WANG, H. T.; NOGUEIRA, L. G.; DE MELO BORGES, A.; BUCK, P.; DIAS, F.; BARON, M. Genetic susceptibility to Chagas disease cardiomyopathy: involvement of several genes of the innate immunity and chemokine-dependent migration pathways. **BMC infectious diseases**, v. 13, n. 1, p. 1–17, 2013.

FUJINAMI, R. S.; VON HERRATH, M. G.; CHRISTEN, U.; WHITTON, J. L. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. **Clinical microbiology reviews**, v. 19, n. 1, p. 80–94, 2006.

GIGLI, M.; MERLO, M.; GRAW, S. L.; BARBATI, G.; ROWLAND, T. J.; SLAVOV, D. B.; STOLFO, D.; HAYWOOD, M. E.; DAL FERRO, M.; ALTINIER, A. Genetic risk of arrhythmic phenotypes in patients with dilated cardiomyopathy. **Journal of the American College of Cardiology**, v. 74, n. 11, p. 1480–1490, 2019.

GOMES DOS SANTOS, A.; WATANABE, E. H.; FERREIRA, D. T.; OLIVEIRA, J.; NAKANISHI, É. S.; OLIVEIRA, C. S.; BOCCHI, E.; NOVAES, C. T. G.; CRUZ, F.; CARVALHO, N. B. A specific IL6 polymorphic genotype modulates the risk of Trypanosoma cruzi parasitemia while IL18, IL17A, and IL1B variant profiles and HIV infection protect against cardiomyopathy in Chagas disease. **Frontiers in immunology**, v. 11, p. 521409, 2020.

HOTEZ, P. J. The rise of neglected tropical diseases in the" new Texas". **PLOS neglected tropical diseases**, v. 12, n. 1, p. e0005581, 2018.

HOTEZ, P. J.; AKSOY, S.; BRINDLEY, P. J.; KAMHAWI, S. **World neglected tropical diseases dayPLoS neglected tropical diseases**Public Library of Science, 2020.

JIMÉNEZ, P.; JAIMES, J.; POVEDA, C.; RAMÍREZ, J. D. A systematic review of the Trypanosoma cruzi genetic heterogeneity, host immune response and genetic factors as plausible drivers of chronic chagasic cardiomyopathy. **Parasitology**, v. 146, n. 3, p. 269–283, 2019.

JUIZ, N. A.; ESTUPIÑÁN, E.; HERNÁNDEZ, D.; GARCILAZO, A.; CHADI, R.; MORALES SANFURGO, G.; SCHIJMAN, A. G.; LONGHI, S. A.; GONZÁLEZ, C. I. Association study between CCR2-CCR5 genes polymorphisms and chronic Chagas heart disease in Wichi and in admixed populations from Argentina. **PLoS neglected tropical diseases**, v. 13, n. 1, p. e0007033, 2019.

KAUR, M.; SINGH, K.; SIDHU, S. K.; DEVI, P.; KAUR, M.; SONEJA, S.; SINGH, N. Coinfection of chikungunya and dengue viruses: A serological study from North Western region of Punjab, India. **Journal of laboratory physicians**, v. 10, n. 04, p. 443–447, 2018.

KAYA, M. Trypanosomiasis. *In*: **Uveitis**. [s.l.] Springer, 2021. p. 225–228.

LANNES-VIEIRA, J.; WAGHABI, M. C.; CORREA-OLIVEIRA, R.; DA SILVEIRA, A. B. M.; REIS, D. d'Ávila; SCHARFSTEIN, J. **Portal da doença de Chagas**. Disponível em: <http://chagas.fiocruz.br/doenca/patogenia/>. Acesso em: 4 jan. 2023.

LASERNA, A.; BARAHONA-CORREA, J.; BAQUERO, L.; CASTAÑEDA-CARDONA, C.; ROSSELLI, D. Economic impact of dengue fever in Latin America and the Caribbean: a systematic review. **Revista Panamericana de Salud Pública**, v. 42, p. e111, 2018.

LAUGIER, L.; FERREIRA, L. R. P.; FERREIRA, F. M.; CABANTOUS, S.; FRADE, A. F.; NUNES, J. P.; RIBEIRO, R. A.; BROCHET, P.; TEIXEIRA, P. C.; SANTOS, R. H. B. miRNAs may play a major role in the control of gene expression in key pathobiological processes in Chagas disease cardiomyopathy. **PLoS neglected tropical diseases**, v. 14, n. 12, p. e0008889, 2020.

LIMA, A. P. B.; DE OLIVEIRA, M. T.; SILVA, R. R.; TORRES, R. M.; VELOSO, V. M.; DE LANA, M.; DA SILVA, G. N. Evaluation of parasite and host genetics in two generations of a family with Chagas disease. **Parasitology research**, v. 117, n. 9, p. 3009–3013, 2018.

MACHUCA, M. A.; SUÁREZ, E. U.; ECHEVERRÍA, L. E.; MARTÍN, J.; GONZÁLEZ, C. I. SNP/haplotype associations of CCR2 and CCR5 genes with severity of chagasic cardiomyopathy. **Human immunology**, v. 75, n. 12, p. 1210–1215, 2014.

MARDAHL, M.; BORUP, A.; NEJSUM, P. A new level of complexity in parasite-host interaction: The role of extracellular vesicles. **Advances in Parasitology**, v. 104, p. 39–112, 2019.

MARTÍN-ESCOLANO, J.; MARÍN, C.; ROSALES, M. J.; TSAOUSIS, A. D.; MEDINA-CARMONA, E.; MARTÍN-ESCOLANO, R. An Updated View of the Trypanosoma cruzi Life Cycle: Intervention Points for an Effective Treatment. **ACS Infectious Diseases**, 2022.

MASYENI, S.; SANTOSO, M. S.; WIDYANINGSIH, P. D.; ASMARA, D. G. W.; NAINU, F.; HARAPAN, H.; SASMONO, R. T. Serological cross-reaction and coinfection of dengue and COVID-19 in Asia: Experience from Indonesia. **International Journal of Infectious Diseases**, v. 102, p. 152–154, 2021.

MAURER, M.; VON STEBUT, E. Macrophage inflammatory protein-1. **The international journal of biochemistry & cell biology**, v. 36, n. 10, p. 1882–1886, 2004.

MIAH, M. A.; HUSNA, A. Coinfection, coepidemics of COVID-19, and dengue in dengue-endemic countries: A serious health concern. **Journal of Medical Virology**, v. 93, n. 1, p. 161, 2021.

MONDIALE DE LA SANTÉ, O.; ORGANIZATION, W. H. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. **Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire**, v. 90, n. 06, p. 33–44, 2015.

MORETTI, N. S.; MORTARA, R. A.; SCHENKMAN, S. Trypanosoma cruzi. **Trends Parasitol**, v. 36, n. 4, p. 404–405, 2020.

MUMMIDI, S.; AHUJA, S. S.; MCDANIEL, B. L.; AHUJA, S. K. The human CC chemokine receptor 5 (CCR5) gene: multiple transcripts with 5′-end heterogeneity, dual promoter usage, and evidence for polymorphisms within the regulatory regions and noncoding exons. **Journal of Biological Chemistry**, v. 272, n. 49, p. 30662–30671, 1997.

MUMMIDI, S.; BAMSHAD, M.; AHUJA, S. S.; GONZALEZ, E.; FEUILLET, P. M.; BEGUM, K.; GALVIS, M. C.; KOSTECKI, V.; VALENTE, A. J.; MURTHY, K. K. Evolution of Human and Non-human Primate CC Chemokine Receptor 5 Gene and mRNA: POTENTIAL ROLES FOR HAPLOTYPE AND mRNA DIVERSITY, DIFFERENTIAL HAPLOTYPE-SPECIFIC TRANSCRIPTIONAL ACTIVITY, AND ALTERED TRANSCRIPTION FACTOR BINDING TO POLYMORPHIC NUCLEOTIDES. **Journal of Biological Chemistry**, v. 275, n. 25, p. 18946–18961, 2000.

NOGUEIRA, L. G.; SANTOS, R. H. B.; IANNI, B. M.; FIORELLI, A. I.; MAIRENA, E. C.; BENVENUTI, L. A.; FRADE, A.; DONADI, E.; DIAS, F.; SABA, B. Myocardial chemokine expression and intensity of myocarditis in Chagas cardiomyopathy are controlled by polymorphisms in CXCL9 and CXCL10. **PLoS neglected tropical diseases**, v. 6, n. 10, p. e1867, 2012.

NORMAN, F. F.; LÓPEZ-VÉLEZ, R. Chagas disease: comments on the 2018 PAHO Guidelines for diagnosis and management. **Journal of travel medicine**, v. 26, n. 7, p. taz060, 2019.

OLIVEIRA, A. P.; BERNARDO, C. R.; CAMARGO, A. V. S.; VILLAFANHA, D. F.; CAVASINI, C. E.; DE MATTOS, C. C. B.; DE GODOY, M. F.; BESTETTI, R. B.; DE MATTOS, L. C. CCR5 chemokine receptor gene variants in chronic Chagas' disease. **International journal of cardiology**, v. 176, n. 2, p. 520–522, 2014.

ORGANIZATION, W. H. **Global plan to combat neglected tropical diseases 2008-2015**. [s.l.] World Health Organization, 2007. .

OROZCO, V. H. G.; NAVARRO, J. E. V.; AGUIRRE, C. S.; OCAMPO, C. M. I.; SANDOVAL, C. I. D.; GALLEGOS, C. A. O.; CAMACHO, D. J. O.; BUCIO, A. N. Digestive Disorders in Chagas Disease: Megaesophagus and Chagasic Megacolon. 2022.

PAN AMERICAN HEALTH ORGANIZATION - PAHO. **Chagas disease - PAHO/WHO | Pan American Health Organization**. Disponível em: <https://www.paho.org/en/topics/chagas-disease>. Acesso em: 20 jun. 2022.

PATTERSON, B. K.; SEETHAMRAJU, H.; DHODY, K.; CORLEY, M. J.; KAZEMPOUR, K.; LALEZARI, J.; PANG, A. P. S.; SUGAI, C.; FRANCISCO, E. B.; PISE, A. Disruption of the CCL5/RANTES-CCR5 pathway restores immune homeostasis and reduces plasma viral load in critical COVID-19. **MedRxiv**, 2020.

PÉREZ-MOLINA, J. A.; MOLINA, I. Chagas disease. **The Lancet**, v. 391, n. 10115, p. 82–94, 2018.

QI, B.; FANG, Q.; LIU, S.; HOU, W.; LI, J.; HUANG, Y.; SHI, J. Advances of CCR5 antagonists: From small molecules to macromolecules. **European Journal of Medicinal Chemistry**, v. 208, p. 112819, 2020.

RODRIGUES DE MOURA, R.; COELHO, A. V. C.; DE QUEIROZ BALBINO, V.; CROVELLA, S.; BRANDÃO, L. A. C. Meta-analysis of Brazilian genetic admixture and comparison with other Latin America countries. **American Journal of Human Biology**, v. 27, n. 5, p. 674–680, 2015.

SALASSA, B. N.; ROMANO, P. S. Autophagy: a necessary process during the Trypanosoma cruzi life-cycle. **Virulence**, v. 10, n. 1, p. 460–469, 2019.

SANTOS, C. M. da C.; PIMENTA, C. A. de M.; NOBRE, M. R. C. The PICO strategy for the research question construction and evidence search. **Revista latino-americana de enfermagem**, v. 15, n. 3, p. 508–511, 2007.

SHAW, M.-A. Human genetic susceptibility to Chagas disease. *In*: **American Trypanosomiasis Chagas Disease**. [s.l.] Elsevier, 2017. p. 629–652.

SIMÕES, M. V.; ROMANO, M. M. D.; SCHMIDT, A.; MARTINS, K. S. M.; MARIN-NETO, J. A. Chagas disease cardiomyopathy. **International Journal of Cardiovascular Sciences**, v. 31, p. 173–189, 2018.

SIMON, G. G. Impacts of neglected tropical disease on incidence and progression of HIV/AIDS, tuberculosis, and malaria: scientific links. **International Journal of Infectious Diseases**, v. 42, p. 54–57, 2016.

STONE, M. J.; HAYWARD, J. A.; HUANG, C.; E. HUMA, Z.; SANCHEZ, J. Mechanisms of regulation of the chemokine-receptor network. **International journal of molecular sciences**, v. 18, n. 2, p. 342, 2017.

TORRES-SIGNES, A.; DIP, J. A. A Bayesian functional methodology for dengue risk mapping in Latin America and the Caribbean. **Acta Tropica**, v. 215, p. 105788, 2021.

TSHETEN, T.; CLEMENTS, A. C. A.; GRAY, D. J.; ADHIKARY, R. K.; WANGDI, K. Clinical features and outcomes of COVID-19 and dengue co-infection: a systematic review. **BMC infectious diseases**, v. 21, n. 1, p. 1–9, 2021.

VARIKUTI, S.; JHA, B. K.; VOLPEDO, G.; RYAN, N. M.; HALSEY, G.; HAMZA, O. M.; MCGWIRE, B. S.; SATOSKAR, A. R. Host-directed drug therapies for neglected

tropical diseases caused by protozoan parasites. **Frontiers in Microbiology**, v. 9, p. 2655, 2018.

VEALE, C. G. L. Unpacking the Pathogen Box—an open source tool for fighting neglected tropical disease. **ChemMedChem**, v. 14, n. 4, p. 386–453, 2019.

WENG, H.-B.; CHEN, H.-X.; WANG, M.-W. Innovation in neglected tropical disease drug discovery and development. **Infectious diseases of poverty**, v. 7, n. 1, p. 1–9, 2018.

WHO. **Neglected tropical diseases**. Disponível em: <https://www.who.int/health-topics/neglected-tropical-diseases#tab=tab_1>. Acesso em: 4 set. 2022.

WORLD HEALTH ORGANIZATION - WHO. **Chagas disease (American trypanosomiasis)**. Disponível em: <https://www.who.int/health-topics/chagas-disease#tab=tab_1>. Acesso em: 20 jun. 2022.

ZENG, Z.; LAN, T.; WEI, Y.; WEI, X. CCL5/CCR5 axis in human diseases and related treatments. **Genes & diseases**, 2021.