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PAPEL DO GENE DA IL-16 NO CÂNCER E DOENÇAS CARDIOVASCULARES E DE *HLA/KIR* NA COVID-19

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Tese apresentada ao Programa de Pós-Graduação em Biociências e Fisiopatologia do Departamento de Análises Clínicas e Biomedicina, Centro de Ciências da Saúde da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Biociências e Fisiopatologia.

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Orientadora: Jeane Eliete Laguila Visentainer

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Aos vinte e quatro dias do mês de fevereiro de dois mil e vinte e três, às oito horas, na sala 112 B do bloco T20, realizou-se a Defesa Pública da Tese de Doutorado de VICTOR HUGO DE SOUZA sob título "Papel do gene da IL-16 no câncer e doenças cardiovasculares e de HLA/KIR na Covid-19", do Programa de Pós-Graduação em Biociências e Fisiopatologia, área de concentração: Biociências e Fisiopatologia Aplicadas à Farmácia. A Banca Examinadora foi constituída pelos professores: Dr.ª Jeane Eliete Laguila Visentainer (presidente/orientadora), Dr. Jorge Juarez Vieira Teixeira (UEM – Membro), Dr.ª Cristiane Maria Colli (UFGD – membro), Dr.ª Larissa Danielle Bahls Pinto (UEM – membro) e Dr. Ricardo Alberto Moliterno (UEM – membro), Concluídos os trabalhos de apresentação e arguição, o pós-graduando foi considerado:

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PAPEL DO GENE DA IL-16 NO CÂNCER E DOENÇAS CARDIOVASCULARES E DE *HLA/KIR* NA COVID-19

RESUMO

As doenças cardiovasculares e o câncer, são líderes mundiais na causa de morbidade e mortalidade. A doença causada pelo novo coronavírus (COVID-19) é responsável pela maior pandemia dos últimos anos e representa um desafio aos sistemas de saúde mundiais. Embora o câncer e as doenças cardiovasculares sejam entidades distintas, eles compartilham semelhanças e interações que podem sugerir alguma biologia compartilhada. Fatores de risco como idade, sexo, e doenças de base parecem afetar o desfecho dessas doenças. Para além disso, fatores genéticos relacionados ao funcionamento da resposta imune parecem ter importância na patogênese tanto de doenças não infecciosas como câncer e doenças cardiovasculares, como de doenças infecciosas, como a COVID-19. Assim, este estudo teve como objetivo realizar duas revisões sistemáticas para desvendar a existência de polimorfismos em genes que possam ser marcadores de suscetibilidade nessas doenças. Os genes objetos de estudo foram *IL16* (manuscrito I), *HLA* e *KIR* (manuscrito II). Os estudos de revisão sistemática foram realizados nas bases de dados Medline, Web of Science e Scopus, conforme os critérios propostos pelo PRISMA. As análises estatísticas para metanálise foram feitas por avaliação de gráficos forest, construídos no programa R 3.5.2. A primeira revisão sistemática compreendeu um total de 18 estudos com câncer e sete com doenças cardiovasculares. O SNP rs11556218, responsável por alterar um aminoácido na estrutura proteica da IL-16, foi significativamente associado a um risco aumentado para o câncer e para as doenças cardiovasculares em chineses, em diferentes modelos de herança genética. Além disso, a variante rs4778889 foi associada a um risco aumentado para o câncer de estômago. A segunda revisão sistemática compreendeu um total de 43 estudos de variantes *HLA* e três estudos em genes *KIR* na COVID-19. A metanálise indicou alguns alelos associados a um risco aumentado para a COVID-19 e suas formas clínicas. Os alelos *HLA-A*11:01*, *HLA-A*23*, *HLA-A*66*, *HLA-B*07*, *HLA-B*14*, *HLA-B*57* e *HLA-C*04:01* foram associados com risco para COVID-19 e são previstos em estudos *in-silico* como tendo ligantes de baixa afinidade para a proteína Spike do SARS-CoV-2. Por outro lado, os alelos *HLA-C*03*, *HLA-C*05*, *HLA-DRB1*01:01*, *HLA-DRB1*04:01*, *HLA-DRB1*10* e *HLA-DRB1*12* foram associados à proteção contra COVID-19 e à maior afinidade com a proteína viral. Este estudo traz evidências de que a variabilidade genética

relacionada às células NK e a resposta citotóxica podem desempenhar um papel importante na COVID-19. Assim, ambos os estudos apontam, por meio de métodos sistematizados e análises estatísticas, novas associações destes componentes da resposta imune com a infecção pelo SARS-CoV-2. Apesar disso, o poder estatístico e a complexidade/variabilidade da interação vírus-hospedeiro continuam sendo um importante desafio para os estudos de associação genética.

Palavras-chave: Coronavírus, Câncer, Doenças cardiovasculares, *HLA*, *KIR*, Citocinas.

ROLE OF THE IL-16 GENE IN CANCER AND CARDIOVASCULAR DISEASES AND *HLA/KIR* IN COVID-19

ABSTRACT

The new coronavirus disease (COVID-19) is responsible for the largest pandemic in years and represents a challenge to world health systems. On the other hand, cardiovascular disease and cancer are world leaders in the cause of morbidity and mortality. Although cancer and cardiovascular disease are distinct entities, they share similarities and interactions that may suggest some shared biology. Age, gender, and chronic diseases seem to be important risk factors. In addition, host immune genetic factors appear to be important in pathogenesis of both non-infectious diseases (cancer and cardiovascular disease) and infectious diseases, as COVID-19. Thus, this study aimed to perform two systematic revisions of the literature and meta-analysis to explore the existence of polymorphisms in candidates genes to be markers of susceptibility in these diseases. The objects of this study were *IL16* (manuscript I), *HLA* and *KIR* (manuscript II) genetic variants. Systematic review studies were carried out based on the criteria proposed by the PRISMA statement, selecting database (Medline, Web of Science and Scopus) entries according to the proposed themes. Statistical analyzes for meta -analysis were made by evaluation of Forest plots, built in R 3.5.2 software. The first systematic review comprised 18 studies with cancer and seven with cardiovascular diseases. The SNP rs11556218, a missense mutation that alter an amino acid in the IL-16 protein, was significantly associated with an increased risk for cancer in Chinese in different genetic inheritance models. This same SNP was associated with an increased risk of cardiovascular disease in Chinese, also, the rs4778889 variant was associated with an increased risk of gastric cancer. The second systematic review comprised a total of 43 *HLA* and three *KIR* studies in COVID-19. The meta-analysis study indicated some alleles associated with a higher risk for COVID-19 and its clinical forms. The *HLA-A*11:01*, *HLA-A*23*, *HLA-A*66*, *HLA-B*07*, *HLA-B*14*, *HLA-B*57* and *HLA-C*04:01* alleles were associated with risk for COVID-19 and are predicted to have low-affinity for Spike protein ligands. On the other hand, *HLA-C*03*, *HLA-C*05*, *HLA-DRB1*01:01*, *HLA-DRB1*04:01*, *HLA-DRB1*10* and *HLA-DRB1*12* alleles were associated with protection against COVID-19 and higher affinity to viral protein. This study may be an indication that genetic variability related to NK cells and cytotoxic response may play a role in COVID-19. Thus, both studies point, through systematic and statistical methods, to new immunogenetics

associations with SARS-CoV-2 infection. Despite this, the statistical power and complexity/variability of the virus-host interaction remain an important challenge for genetic association studies.

Keywords: Coronavirus, Neoplasms, Cardiovascular diseases, receptors, *KIR*, *HLA* Antigens, Cytokines.

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CAPÍTULO I

INTRODUÇÃO

Papel da interleucina-16 no câncer e nas doenças cardiovasculares

As duas principais causas de morbidade e mortalidade em todo o mundo são, em primeiro lugar, as doenças cardiovasculares (CVD, do inglês *Cardiovascular Disease*) e, em segundo lugar, o câncer. As CVDs são a principal causa de morbidade e mortalidade em todo o mundo, com mais de 423 milhões de casos estimados e mais de 18 milhões de mortes somente em 2015. A doença arterial coronariana é a CVD mais prevalente, seguida do Acidente Vascular Cerebral (AVC) (ROTH et al., 2017). O câncer é a segunda principal causa de morbidade e mortalidade em todo o mundo. A GLOBOCAN (*Global Cancer Statistics*) estimou para 2020 um total de 18,1 milhões de pessoas com câncer e 9,6 milhões de mortes por esta doença (BRAY et al., 2018). Os tipos de câncer mais frequentes diferem entre as populações devido ao estilo de vida e às diferenças socioeconômicas. De acordo com estimativa dos EUA de 2019, os três tipos de câncer mais prevalentes entre homens foram câncer de próstata (3,65 milhões de casos), câncer colorretal (776.000) e melanoma (684.000); entre as mulheres, foram câncer de mama (3,86 milhões), câncer cervical (807.000) e câncer colorretal (768.000) (MILLER et al., 2019).

Embora câncer e CVD sejam considerados entidades distintas, eles compartilham semelhanças e interações entre seus fatores de risco (por exemplo, tabagismo, sedentarismo, hábitos alimentares nocivos, obesidade e diabetes mellitus), fornecendo perspectivas de que possuem alguns componentes de uma biologia compartilhada (KOENE et al., 2016). Apesar de a taxa de mortes por câncer tenha diminuído nos últimos anos, o risco de CVD em sobreviventes de câncer é um problema de saúde que deve ser monitorado pelos médicos, pois indivíduos em tratamento prolongado de câncer podem desenvolver problemas circulatórios como a CVD, levando à morte ou piora de sua qualidade da vida ou tratamento (ARMENIAN et al., 2016, 2017; MEHTA et al., 2018).

A inflamação é um processo natural do sistema imunológico que ajuda a combater infecções e lesões no organismo. No entanto, quando a inflamação se torna crônica, pode levar a uma série de doenças crônicas, incluindo o câncer e as doenças cardiovasculares (KOENE et al., 2016). No caso do câncer, a inflamação crônica pode promover o

crescimento e a disseminação de células cancerígenas, além de suprimir a resposta imunológica do organismo contra o tumor. Já nas doenças cardiovasculares, a inflamação crônica pode causar a formação de placas de gordura nas artérias, aumentando o risco de doenças como aterosclerose e infarto do miocárdio (ARMENIAN et al., 2017; CURIGLIANO et al., 2012). As citocinas são proteínas mensageiras que desempenham um papel importante na resposta imune e na regulação da inflamação (WALDMANN, 2018). Citocinas produzidas em um processo inflamatório crônico podem estimular o crescimento e a proliferação de células mutantes, levando ao desenvolvimento de tumores (WALDMANN, 2018).

A Interleucina-16 (IL-16) é uma quimiocina (citocina quimiotática) descrita e nomeada inicialmente como um fator quimiotático para linfócitos (LCF) (CRUIKSHANK et al., 1994). Esta citocina é produzida por uma variedade de células como linfócitos T (especialmente T citotóxicos), fibroblastos, células epiteliais e células dendríticas, em resposta a agentes patogênicos e a substâncias liberadas pelo organismo, como a histamina (CENTER et al., 2000). Polimorfismos no gene que codifica essa citocina (ver Figura 1) possuem relação com a imunopatogênese de doenças com importante resposta inflamatória como reumatismo e asma (KLIMIUK; GORONZY; WEYAND, 1999; LABERGE et al., 1997), doenças predominantemente autoimunes como lúpus eritematoso sistêmico e esclerose múltipla (LARD et al., 2002; SKUNDRIC et al., 2006) e na patogênese de mecanismos de formação de tumor e do câncer (RICHMOND et al., 2014).

A IL-16 utiliza as moléculas de CD4 como seus receptores, se ligando aos domínios D4 dessas moléculas e provocando uma cascata intracelular que modifica o padrão de resposta. Na resposta desencadeada pela IL-16 prevalece um tipo de resposta geralmente associada ao aumento do processo inflamatório (LYNCH et al., 2003), embora padrões de resposta anti-inflamatórios também tenham sido descritos em algumas doenças (CRUIKSHANK; KORNFELD; CENTER, 2000; KLIMIUK; GORONZY; WEYAND, 1999; MCFADDEN et al., 2007). Quando se liga a linfócitos T CD4+, a IL-16 atua isolando a molécula de CD4 do complexo MHC-TCR, inativando temporariamente o TCR e provocando uma sinalização intracelular com resposta independente da ação de TCR (CRUIKSHANK et al., 1994).

A IL-16 está envolvida na resposta imune contra tumores e pode ser um alvo terapêutico para o tratamento do câncer (RICHMOND et al., 2014). Essa citocina tem

sido associada a CVDs, como aterosclerose, hipertensão e insuficiência cardíaca (TAMAKI et al., 2013). Níveis elevados de IL-16 estão associados a um aumento do risco de desenvolvimento de CVD, enquanto níveis reduzidos são associados a uma menor incidência dessas doenças (SCHERNTHANER et al., 2017). Portanto, a IL-16 pode ser um marcador importante da CVD e do câncer, e o conhecimento da relação de polimorfismos em seu gene com as doenças pode melhorar o entendimento de seu uso como alvo terapêutico para prevenir e tratar essas doenças.

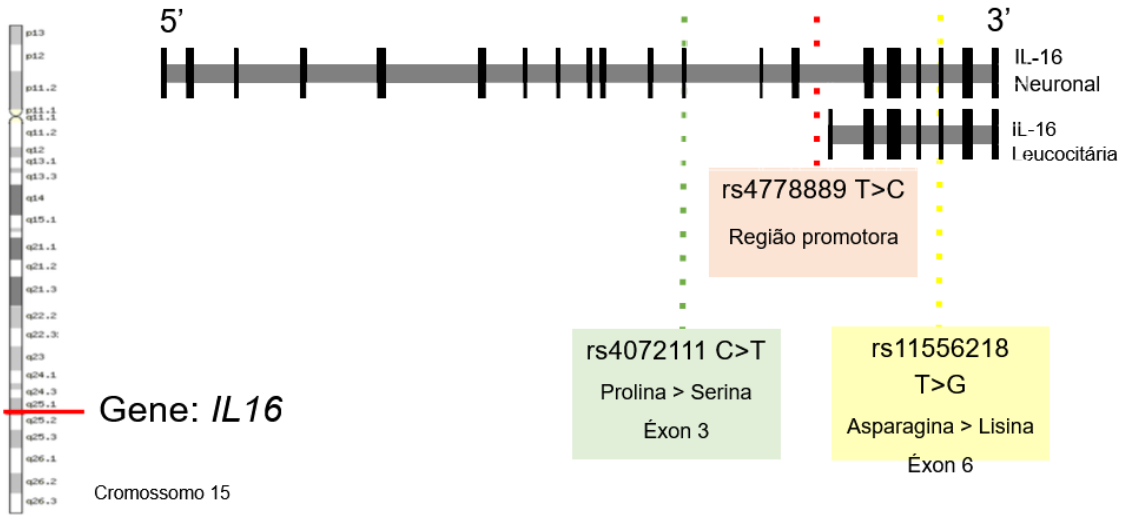

Figura 1. Localização cromossômica e polimorfismos de *IL16*. Quatro polimorfismos comumente estudados para o gene da IL-16 (*IL16*) são rs4778889 T>C (localizado na região promotora), rs11556218 T>G (mutação missense, com troca de aminoácido), rs4072111 C>T (mutação missense) e rs1131445 T>C (localizado na região 3'-UTR).

Papel de *HLA* e *KIR* na COVID-19

A doença causada pelo novo coronavírus (COVID-19) foi inicialmente caracterizada como uma síndrome respiratória aguda causada pelo vírus SARS-CoV-2. Essa doença foi inicialmente descrita em dezembro de 2019 (WANG et al., 2020a), se espalhando por todo o planeta. Os primeiros pacientes afetados em Wuhan, China, apresentavam uma ampla gama de sintomas desde sintomas mais brandos, passando por febre, tosse, fadiga, até sintomas mais severos como pneumonia e falta de ar (LI et al., 2020b). A COVID-19, porém, pode levar o paciente ao óbito por diversas causas sistêmicas que vão além dos quadros inicialmente descritos. Um dos fatores de risco mais

importantes para a doença tem sido descrito ser a idade, com indivíduos acima dos 65 anos apresentando grande risco e necessidade de cuidados intensivos (BRODIN, 2021). Outros fatores como sexo dos indivíduos, presença de doenças de base, como diabetes e doenças cardiovasculares, também parecem apresentar importância para determinar o desfecho da doença (BRODIN, 2021).

Os afetados por essa infecção possuem um novo tipo de betacoronavirus, que era desconhecido até então, mas como notável capacidade de transmissão entre pessoas assintomáticas e pré-sintomáticas (LIANG, 2020). O SARS-CoV-2 é um vírus envelopado, com RNA de fita simples, caracterizado por proteínas estruturais que tem relação direta com sua virulência (SONG et al., 2019; WAN et al., 2020). Os betacoronavírus também são conhecidos como causadores da síndrome respiratória aguda grave (ou SARS, causada pelo SARS-CoV). Mesmo com um percentual menor de indivíduos necessitando de cuidados intensivos, essa alta transmissibilidade permitiu com que a propagação do vírus permanecesse elevada, propiciando a contaminação de milhões de pessoas e colapsos de sistemas de saúde em muitos locais no mundo. De acordo com a Universidade John Hopkins, referência na divulgação de dados sobre a COVID-19, até fevereiro de 2023 mais de 675 milhões de casos da doença foram confirmados, com mais de 6,8 milhões de mortes no mundo (https://coronavirus.jhu.edu/map.html).

Modificações nas proteínas virais, que podem ser frequentes quando o vírus passa por adaptações evolutivas, podem levar a mudanças na imunopatogênese viral, favorecendo sua disseminação (ZHAO et al., 2020). A proteína superficial chamada Spike (S) é um dos principais componentes do vírus que se relacionam com a resposta imune. Essa estrutura tem um importante papel na entrada do vírus na célula do hospedeiro (KROKHIN et al., 2003). A virulência do SARS-CoV-2 pode ser atribuída a suas proteínas estruturais, assim, sua interação com o hospedeiro parece essencial para sua imunopatogênese (LIANG, 2020). Nesse sentido, novas variantes do vírus, como as que vem sendo observadas na África do Sul, no Reino Unido e no Brasil (SABINO et al., 2021), podem adicionar um novo nível de complexidade a esta interação, e podem ser capazes de evadir da resposta imune causada por vacinas ou por infecções anteriores (CALLAWAY, 2021) e serem ainda mais virulentas (SABINO et al., 2021).

A COVID-19 se apresenta como uma doença multifatorial. Uma série de fatores ou covariáveis podem influenciar no desfecho da doença em diferentes grupos

populacionais. Ainda não estão claros quais fatores atuam na COVID-19, ou qual o impacto deles na imunopatogênese dessa doença. Apesar disso, fatores como a idade do indivíduo, a presença de comorbidades como diabetes, doenças cardiovasculares, e algumas doenças pulmonares, parecem ser importantes para a COVID-19 (CARAMELO; FERREIRA; OLIVEIROS, 2020; GUAN et al., 2020; JAIN; YUAN, 2020). Cabe destacar que a maior parte dos expostos ao SARS-CoV-2 são assintomáticos ou desenvolvem sintomas leves (BRODIN, 2021). Apesar disso, uma parcela menor (mas importante) de pessoas pode desenvolver sintomas mais sérios, como falta de ar, com comprometimento importante dos pulmões, podendo levar a morte. Deste modo, conclui-se que fatores relacionados tanto com a variabilidade do próprio vírus, quanto com a biologia (incluindo a genética) do hospedeiro, podem influenciar no desenvolvimento de diferentes manifestações frente à infecção pelo SARS-CoV-2 (ITURRIETA-ZUAZO et al., 2020; LI et al., 2020c).

Em relação à imunidade adaptativa, a imunidade produzida por células (imunidade celular) tem um papel crucial no combate a COVID-19 em humanos (LE BERT et al., 2020). Ela protege o organismo por meio de ação de células, como linfócitos T citotóxicos, que são capazes de induzir apoptose em células infectadas por vírus. Ela atua também ampliando a ação de macrófagos e células assassinas naturais (NK, do inglês *natural killer*), facilitando a destruição dos patógenos via fagocitose e secreção de grânulos citotóxicos, respectivamente. A imunidade celular é responsável ainda por estimular a secreção de uma variedade de citocinas que influenciam na função de outras células. Para além desta, a resposta imune humoral é um dos principais mecanismos de defesa do organismo contra vírus. Ela envolve a produção de anticorpos pelas células B, que se ligam aos vírus e os neutralizam, impedindo que infectem outras células. Além disso, a resposta humoral ajuda a eliminar os vírus por meio da ativação do complemento, que destrói os vírus marcados pelos anticorpos. A produção de anticorpos também é importante para a formação da memória imunológica, que permitirá ao organismo reconhecer e responder mais rapidamente a infecções futuras (ABBAS; LICHTMAN; PILLAI, 2008; LE BERT et al., 2020).

Fatores genéticos apresentam importante relevância na patogênese da COVID-19 (PAIRO-CASTINEIRA et al., 2020). Diversos genes e seus polimorfismos podem ser associados com a infecção pelo coronavírus. Dentre os polimorfismos mais estudados

estão os chamados polimorfismos de nucleotídeo único (SNPs, do inglês *Single Nucleotide Polymorphism*), consistindo na troca de apenas um nucleotídeo na sequência do gene. A presença no gene desta troca pode interferir no produto a ser transcrito e em seu efeito biológico. A enzima conversora de angiotensina 2 (ACE2), por exemplo, é uma enzima ligada às membranas de várias células dos pulmões, tecidos cardiovasculares, rins e intestinos. A ACE2 diminui a pressão arterial e neutraliza a atividade da enzima conversora de angiotensina (ACE). A ACE2 chamou muita atenção, inicialmente em estudos com a SARS, mas também recentemente com a COVID-19, pois serve como uma porta de entrada nas células para alguns coronavírus, incluindo SARS-CoV e SARS-CoV-2 (CUERVO; GRANDVAUX, 2020). Polimorfismos no gene *ACE2* foram associados com a COVID-19 nas populações espanhola e italiana (BENETTI et al., 2020; GÓMEZ et al., 2020), e vários SNPs nesse gene foram correlacionados com o desfecho da COVID-19 (BELLONE; CALVISI, 2020; DARBANI, 2020) e com sintomas neurológicos (STRAFELLA et al., 2020). Outros genes do sistema imune humano, como *KIR* e *HLA*, têm sido identificados como importantes moduladores da resposta imune à infecção viral. Suas variantes genéticas podem influenciar a expressão de proteínas envolvidas na resposta imune, afetando a capacidade do hospedeiro de controlar a infecção. Portanto, o estudo de *KIR* e *HLA* na COVID-19 pode fornecer uma compreensão importante sobre a patogênese dessa doença.

A resposta imunitária à infecção pelo SARS-CoV é determinante para o desfecho da doença. Inicialmente, componentes da imunidade inata agem tentando conter a replicação do vírus. As células dendríticas plasmocitóides (pDCs) são um tipo de célula imunológica especializada em reconhecer e responder a infecções virais. Elas são capazes de produzir grandes quantidades de interferons tipo I, que são proteínas importantes para a defesa antiviral. Além disso, as pDCs também são capazes de apresentar antígenos virais para outros tipos de células imunológicas, como os linfócitos T, e assim contribuir para a ativação da resposta imunológica adaptativa (TAY et al., 2020). Essa resposta imune adaptativa contribui para a diminuição da carga viral por meio da resposta de linfócitos T e pela liberação de anticorpos (LI et al., 2020a). As células NK são também componentes cruciais do sistema imunológico inato e fornecem importante defesa inicial contra infecções virais (CAMPBELL; HASEGAWA, 2013). Até recentemente, as células NK eram descritas apenas como tendo função citotóxica no dano celular. No entanto, parece que essas células também desempenham um papel essencial na ativação e regulação de

uma resposta imune adaptativa, principalmente através da secreção de quimiocinas e citocinas inflamatórias, como interferon gama (IFN-γ), fator de necrose tumoral alfa (TNF-α) e fator estimulador de colônia de granulócitos-macrófagos (GM-CSF) (VIVIER et al., 2008). Além disso, um elemento básico no desenvolvimento das células NK é a interação constante dessas células com células doentes, infectadas, mas também com células saudáveis, o que ocorre principalmente por meio de moléculas de antígeno leucocitário humano (HLA) de classe I e receptores KIR inibitórios ou ativadores (FU; TIAN; WEI, 2014; PARHAM; GUETHLEIN, 2018).

Os receptores de células NK melhor caracterizados e estudados pertencem à família *killer immunoglobulin-like receptor* (KIR) e podem ser classificados, de acordo com seu efeito, em ativadores ou inibidores destas células (VILCHES; PARHAM, 2002). KIRs inibitórios são codificados por oito genes nomeados: *KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL5A*, *KIR2DL5B*, *KIR3DL1*, *KIR3DL2* e *KIR3DL3* (Figura 2). Enquanto KIR ativadores são codificados por outro conjunto de genes: *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, *KIR2DS5* e *KIR3DS1*. O produto do gene *KIR2DL4* pode atuar tanto como um ativador quanto como inibidor de NK. Além de todos estes genes, o cluster KIR também pode apresentar dois pseudogenes, chamados *KIR2DP1* e *KIR3DP1*, e quatro genes estruturais ou *framework* (*KIR3DL3*, *KIR3DP1*, *KIR2DL4*, *KIR3DL2*), que estão presentes em quase todos os indivíduos (CARRINGTON; MARTIN, 2005).

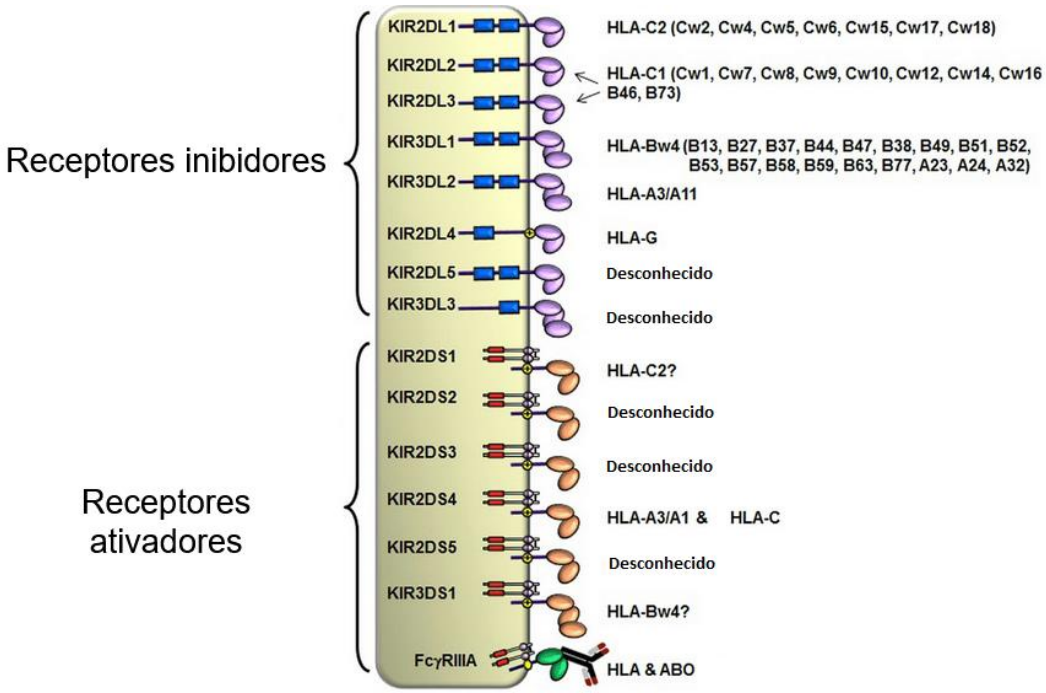

Figura 2. Receptores KIR e seus ligantes HLA conhecidos.

A atividade das células NK requer interação entre KIRs e seus ligantes HLA expressos na superfície das células. Essa interação pode ativar ou inibir a ação de NK sobre essas células. Portanto, a função citotóxica das células NK nas células alvo é regulada através de um complexo equilíbrio entre sinais ativadores e inibitórios resultantes da interação de KIRs e HLAs (FRANCESCHI et al., 2008). Assim, é possível que as moléculas KIR desempenhem um papel significativo no controle da resposta imune durante as infecções.

Por outro lado, o complexo principal de histocompatibilidade (MHC), desempenha um papel essencial no processo de reconhecimento de componentes virais e no desenvolvimento da resposta imune nas doenças causadas por eles. MHC, também conhecido como HLA, em humanos, é uma parte importante do sistema imunológico e seus genes codificam moléculas de superfície celular especializadas em apresentar peptídeos antigênicos ao receptor de células T (TCR) (ABBAS; LICHTMAN; PILLAI, 2008). As moléculas de MHC são divididas em duas classes principais: classe I e II. Enquanto as moléculas de MHC de classe I são apresentadas na superfície de todas as células nucleadas e apresentam antígenos para linfócitos T CD8, as moléculas de classe II geralmente são apresentadas em células mais especializadas na apresentação dos antígenos, apresentando-os para linfócitos T CD4 (ABBAS; LICHTMAN; PILLAI, 2008).

O sistema HLA possui genes com ações importantes na modulação do sistema imunitário contra infecções virais (Figura 3). A este respeito, a associação entre a infecção por SARS-CoV-2 e os alelos e genótipos HLA ainda é muito pouco esclarecida. No entanto, é conhecido que alelos HLA atuam na modulação da resposta imune a vários antígenos virais e podem ter uma influência significativa na capacidade de um indivíduo infectado de produzir uma resposta imune eficaz (HAMMER et al., 2015; TIAN et al., 2017).

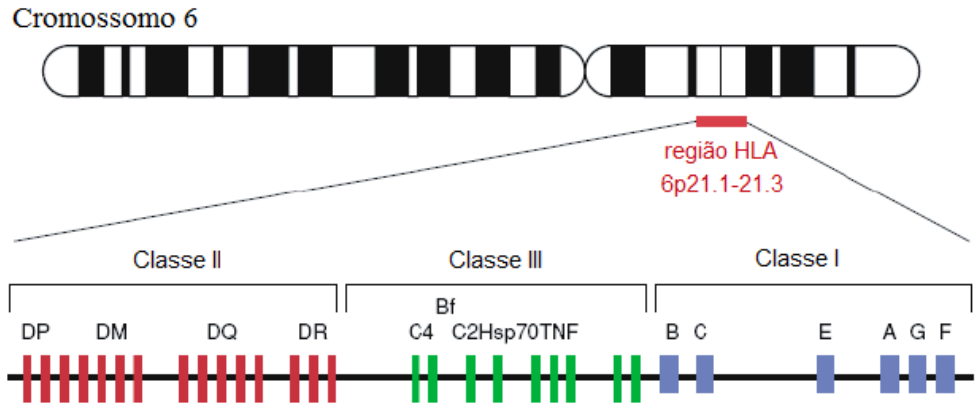

Figura 3. Localização cromossômica e classificação dos genes *HLA*. Tradicionalmente os genes HLA podem ser classificados em classe I e II, que codificam moléculas responsáveis pela apresentação de antígenos para linfócitos T CD8 (classe I) e T CD4 (classe II). Esta região também codifica outras proteínas como transportadores moleculares e proteínas acessórias.

Iniciativas como a base de dados HLACOVID-19 (http://www.hlacovid19.org/) estão sendo construídas para apoiar e combinar os esforços para estudar a associação entre HLA e COVID-19. Alguns estudos recentes permitem confirmar essa relação entre alelos HLA e infecções por coronavírus (SARS e COVID-19). Por exemplo, diferentes estudos encontraram associação de alelos do sorotipo HLA-B15 com essas doenças, dentre eles estão *HLA-B*46:01* (LIN et al., 2003; NGUYEN et al., 2020) *HLA-B*15:01* (CORREALE et al., 2020) *HLA-B*15:03* (ITURRIETA-ZUAZO et al., 2020; NGUYEN et al., 2020) e *HLA-B*15:27* (WANG et al., 2020b). A maior parte dos estudos se limitam as populações chinesa e europeia, sendo pouco conhecido o efeito que esses alelos podem ter em indivíduos afetados por COVID-19 em outras populações. Muitos destes estudos apresentam resultados obtidos por meio de avaliação de correlação entre dados de frequência de alelos e dados de frequência de casos e óbitos, não sendo desenvolvidos de acordo com um modelo controlado de estudos caso-controle e, portanto, sujeitos a efeito de fatores confundidores. Poucas evidências robustas advindas de resultados e limitações de estudos de metanálise foram observadas até o momento.

As moléculas HLA e os receptores KIR desempenham um papel importante na resposta imune a infecções virais, incluindo a COVID-19. O MHC pode apresentar peptídeos virais aos linfócitos T para que eles possam identificar e destruir células

infectadas, e receptores KIR, por sua vez, ficam responsáveis por regular a atividade das células NK. A variabilidade genética destes poderia, então, influenciar a forma como o hospedeiro responde à infecção pelo SARS-CoV-2. No entanto, ainda há muito a ser compreendido sobre o papel exato desses genes na COVID-19 e mais estudos são necessários para entender plenamente sua importância na resposta imunitária a essa doença.

Revisões sistemáticas e metanálises em estudos de associação genética com doenças

Para que um estudo de associação genética possa atingir o objetivo de compreender a influência de variantes genéticas em diferentes populações, uma formulação metodológica utilizando modelos específicos de estudo é necessária em sua construção. Neste sentido, os estudos do tipo caso-controle são extremamente úteis, principalmente por sua praticidade e menor custo financeiro e de tempo para serem concluídos, apesar dos vieses característicos e próprios de estudos dessa natureza. O objetivo de um estudo caso-controle é verificar se os casos (indivíduos afetados por uma doença) diferem significantemente dos controles (aqueles não afetados) em relação à exposição à um fator de risco (neste caso, uma variante genética). Da mesma forma, outros desenhos de estudos, como estudo de coorte, podem ser extremamente úteis para dar respostas a questões científicas. As revisões sistemáticas podem ser consideradas como um estudo representativo com evidências agrupadas para as respostas às perguntas científicas. Elas são revisões feitas com base em metodologias rígidas, validadas por especialistas, como a declaração de PRISMA (MOHER et al., 2009), dentre outras. Isso faz delas ferramentas particularmente úteis para embasar a tomada de decisão de gestores de saúde e, particularidade, na clínica médica.

Dentre as ferramentas modernas que podem ser integradas a uma metanálise estão os chamados estudos *in-silico*. Tais análises computacionais são uma importante ferramenta para a compreensão dos sistemas imunológicos, especialmente no que diz respeito a moléculas HLA. Os estudos *in-silico* permitem a análise de dados genéticos e imunológicos em larga escala, fornecendo uma compreensão mais profunda dos padrões de diversidade na apresentação de antígenos (CHEN et al., 2021). Eles podem ser usados para prever as interações entre HLA e diferentes epítopos, identificar potenciais ligantes ou não-ligantes, e avaliar o impacto de mutações genéticas (tanto do hospedeiro quanto do vírus) na apresentação de antígenos. A combinação de dados genômicos e

imunológicos com análise *in-silico* também tem sido útil na compreensão da complexidade do sistema imunológico, incluindo a personalização do tratamento de doenças autoimunes e infecciosas (CHEN et al., 2021).

Os SNPs são variantes genéticas de um único nucleotídeo que podem resultar em uma alteração funcional dos aminoácidos traduzidos por seu códon (sendo chamados de não sinônimos) ou podem ser silenciosos quando a troca do nucleotídeo gera um mesmo aminoácido (sinônimos), ou simplesmente podem ocorrer nas regiões não-codificadoras. Os SNPs podem influenciar a atividade do promotor de um gene (afetando a expressão gênica, a concentração de uma determinada proteína em um tecido ou local de lesão), a conformação e estabilidade do RNA mensageiro e a localização intracelular de diversas proteínas (SHASTRY, 2009). Portanto, a identificação e estudos de associação destes SNPs com doenças, bem como a análise de seus efeitos, podem levar a uma melhor compreensão de seu impacto na saúde dos indivíduos.

Em síntese, a utilização de revisões sistemáticas e metanálises em estudos sobre polimorfismos genéticos é uma prática altamente valiosa, pois permite a integração de resultados de diversos estudos individuais e análises complementares para produzir uma visão agregada, mais abrangente e conclusiva sobre o assunto em questão. Além disso, este tipo de estudo pode ajudar a elucidar incongruências ou dúvidas que possam surgir a partir de resultados conflitantes em estudos individuais. Portanto, revisões sistemáticas e metanálises são ferramentas valiosas que podem aumentar a confiança, a precisão das conclusões obtidas a partir de estudos sobre polimorfismos genéticos e gerar evidências para a comunidade científica internacional e prática da clínica médica.

JUSTIFICATIVA

Os estudos apresentados neste trabalho fazem uso de desenhos de pesquisa de revisão sistemática e metanálise, que são bastante reconhecidos e validados no meio científico. Assim, tendo em vista as demandas deste atual cenário de pandemia, novos estudos são necessários com o objetivo de avaliar o papel de variantes genéticas da resposta imunitária com a patogênese da COVID-19. Ademais, câncer e doenças cardiovasculares são as principais causas de óbitos no mundo moderno, transpassando cenários de pandemias de doenças infecciosas.

A COVID-19 é responsável por uma pandemia de ampla distribuição geográfica, que acomete grande parte da população mundial, e suas causas e consequências são desastrosas para a saúde e para a economia mundial. Assim, um dos objetivos deste estudo é ajudar a entender e caracterizar a COVID-19 e suas variadas formas, uma doença multifatorial e com muitos aspectos ainda pouco compreendidos, especialmente quanto a seus mecanismos imunológicos em relação aos componentes da resposta imune celular. Além disso, este estudo embasará uma série de novos estudos dos efeitos dos polimorfismos genéticos na resposta imune em indivíduos com COVID-19 que serão realizados pelo Laboratório de Imunogenética da Universidade Estadual de Maringá (LIG-UEM) nos próximos anos. O câncer e as doenças cardiovasculares são igualmente relevantes causas de problemas em sistemas de saúde no mundo todo. Estudos correlacionando essas doenças a componentes diversos da resposta imune, com reconhecida importância para a genética do hospedeiro, como os polimorfismos de genes *KIR*, *HLA* e de genes de citocinas, como a IL-16, podem trazer uma resposta para o comportamento variado dessas doenças em diferentes indivíduos.

O desenvolvimento de revisões sistemáticas e de metanálises é essencial para resolução de dúvidas científicas que devem vir com o acúmulo de evidências de estudos de associação. Esse tipo de estudo será uma ferramenta fundamental para este laboratório nos próximos anos, pois permite a construção de um corpo mais robusto de evidências para responder questões científicas. Assim, ainda existe muito espaço para que se esclareça as possíveis associações entre doenças infecciosas ou não infecciosas e variantes da genética do hospedeiro, e o uso de estudos de metanálise e análise computacional acerca desse tema deve ser de interesse médico em todo o mundo nos próximos anos.

OBJETIVOS

Objetivo geral

Avaliar a influência de polimorfismos genéticos de *HLA/KIR* na imunopatogênese da COVID-19 e de *IL16* no câncer e nas doenças cardiovasculares em publicações científicas.

Objetivos específicos

1. Avaliar o papel de polimorfismos do gene da citocina inflamatória IL-16 no desenvolvimento do câncer e de doenças cardiovasculares em publicações científicas por meio de revisão sistemática e metanálise.

2. Avaliar a correlação de alelos *HLA* e genes *KIR* com a COVID-19 em publicações científicas por meio de revisão sistemática e metanálise.

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CAPÍTULO II

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Association of functional *IL16* polymorphisms with cancer and cardiovascular disease: A meta-analysis

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Abstract

Introduction: Interleukin-16 (IL-16) is a chemotactic cytokine that is found to increase in Cancer and cardiovascular diseases (CVD). Single nucleotide polymorphisms (SNPs) in *IL16* were associated with diseases. Thus, we conducted a systematic review and meta-analysis to evaluate possible associations between *IL16* rs4778889, rs11556218, rs4072111 and rs1131445 SNPs and the risk for cancer or CVD. **Materials and methods:** This study was performed according to the PRISMA statement. Medline, Web of Science, and Scopus databases were systematically reviewed, and a meta-analysis was conducted. **Results:** The analysis comprised 6386 individuals with cancer and 2415 with CVD. The SNP rs11556218 was significantly associated with an increased risk for cancer in Chinese in different genetic inheritance models. Also, to the best of our knowledge, this is the first meta-analysis to show an association of rs4778889 with an increased risk of gastric cancer and rs11556218 with an increased risk of CVD in Chinese. **Conclusion:** Our meta-analysis suggested that the SNPs rs11556218 and rs4778889 of *IL16* were associated with an increased risk for cancer in Chinese and rs11556218 with increased risk for CVD in Chinese, highlighting the need for further studies on the impact of these polymorphisms on cancer treatment and surveillance.

Introduction

Cancer is the second leading cause of morbidity and mortality worldwide. GLOBOCAN (Global cancer statistics) estimated for 2018 a total of 18.1 million people with cancer and 9.6 million deaths from this disease [1]. The most frequent types of cancer differ among populations due to lifestyle and socioeconomic differences. According to a 2019 USA estimate, the three most prevalent types of cancer among men were prostate cancer (3.65 million cases), colorectal cancer (776,000), and melanoma (684,000); among women, these were breast cancer (3.86 million), cervical cancer (807,000), and colorectal cancer (768,000) [2]. In China, 4.3 million new cases and approximately 2.8 million cancer deaths were estimated in 2015. Lung, stomach, liver, esophageal, and colorectal cancer are the leading causes of cancer death among Chinese [3]. Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide, with over 422.7 million estimated cases and 17.92 million deaths in 2015 alone. Coronary artery disease is the most prevalent CVD, followed by stroke [4].

Although cancer and CVD are considered distinct entities, they have similarities and interactions among risk factors (e.g., smoking habits, physical activity, dietary habits, obesity, and diabetes mellitus), supporting a shared biology [5]. Although the rate of cancer death has been decreasing over the years, the risk for CVD in cancer survivors is a health problem that should be monitored by physicians, because individuals in long-term cancer treatment may develop CVD, leading to death or a worsening quality of life [6–8]. CVD in these patients is related to disruption in the organization of the cardiac tissues (such as those caused by chemotherapy, radiotherapy, and other drugs) [6,9] which can result in several heart problems, hypertension, and cerebrovascular accidents, among other complications. Although chronic inflammation is the link in the pathogenesis of cancer and CVD [10,11], additional factors may be shared among these diseases, including those that cannot be modified by individuals, such as age, gender, ethnicity, and genetic differences related to the immune response [5].

Cytokines are mediators of the immune response, and some, such as interferon-α (IFN-α), interleukin (IL)-2, and granulocyte macrophage colony stimulating factor (GM-GSF), are currently indicated in cancer treatment. Other cytokines, such as IL-12, -15, and -21, are undergoing clinical trials [12]. Cardiovascular immunotherapy is also being studied for therapeutic

use. One strategy is to target the immune system's ability to regulate arterial inflammation, for instance, neutralizing proinflammatory cytokines such as IL-1β to resolve inflammatory atherosclerosis. Maintaining tissue homeostasis and repairing lesions are also possibilities, which can be carried out through the action of cytokines such as IL-4 and IL-13 [13].

Interleukin-16 (IL-16) is a chemotactic cytokine whose gene (*IL16*) is located on chromosome 15q26.3. The receptor of IL-16 is a CD4 molecule initially described as inhibiting the interaction between CD4 and HIV-1 [14,15]. This cytokine is coded as a precursor molecule called pro-IL-16, which is cleaved by caspase-3, generating a smaller secreted molecule [16]. The caspase-3 cleavage site is located between the PDZ2 and PDZ3 domains of the precursor protein and allows processing of pro-IL-16 into its mature form [17]. Both pro-IL-16 and mature IL-16 are biologically active. In lymphocytes, pro-IL-16 acts as a transcriptional repressor in the cell, regulating cell cycle progression through its N-terminal domain [18]. Meanwhile, mature IL-16 is secreted by cells such as CD8+ T and B lymphocytes and is responsible for the processes of chemotaxis, cell growth, and differentiation (e.g., increases IL-2 receptor expression).

IL-16 participates in malignant cell proliferation and transformation, acting on a variety of cells involved in the immune response, alone or in conjunction with other cytokines [18–21]. IL-16 levels were directly correlated with gastrointestinal tumor progression [22] and multiple myeloma [23,24]; therefore, these malignant tumors are suggested targets for anti-IL-16 therapies [25]. Other studies also demonstrated increases in tissue expression and serum concentrations of IL-16 in malignant ovarian tumors [26] and cutaneous T-cell lymphoma [20,27]. In cardiovascular diseases, high IL-16 serum concentrations were associated with asymptomatic carotid plaques and reduced numbers of cardiovascular events after surgery, suggesting a protective profile in atherosclerosis and the risk of cardiovascular events [28–30]. However, increased levels of this cytokine were also associated with cardiac fibrosis and myocardial stiffening [31], production of proinflammatory cytokines such as IL-1β and IL-6 [32], as well as the risk for acute myocardial infarction [33], being a potential target for CVD treatments [34].

Single nucleotide polymorphisms (SNPs) are common genetic variations that can alter protein composition or gene expression and thus modify the functioning of immune response components such as cytokines. These modifications may lead to changes in the immune response, cell cycle regulation and metabolism, and DNA repair associated with cancer and CVD

susceptibility [35,36]. SNPs may be located in gene promoter regions (altering gene expression and epigenetic modifications), exons (affecting transcription and translation), introns (modifying splicing and regulation), as well as 5’ and 3’ untranslated regions (UTRs) (affecting translation through microRNA binding) [36]. When associations of SNPs with diseases are confirmed, this knowledge may offer greater understanding of disease prevention, prediction, prognosis and treatment. Four polymorphisms commonly studied for *IL16* are rs4778889 (located in the promoter region), rs11556218 (missense mutation), rs4072111 (missense mutation), and rs1131445 (located in the 3'-UTR).

A better understanding of the connections between cancer and CVD is crucial for identifying and preventing short- and long-term problems in the treatment and prevention of these diseases. In the study of the association of SNPs with diseases, a major analysis tool is meta-analysis. Meta-analysis is the statistical analysis of the evidence from different individual studies, with the aim of integrating them, combining and summarizing their results [37]. Its importance is given by reducing, for example, the standard deviation and the confidence interval, making the result more reliable, in addition to enabling the inclusion of future studies that may be published. The meta-analysis may show an effect that, individually, cannot be observed due to lack of statistical power (limited sample size). Meta-analysis also allows a synthesis of contradictory data even if the statistical power is small [38]. Thus, in this study we performed a systematic review and meta-analysis to evaluate and update information on possible correlations between the polymorphisms rs4778889 T>C, rs11556218 T>G, rs4072111 C>T, and rs1131445 T>C of *IL16* and the risk of cancer or CVD.

Results

This systematic review included a total of 6386 cancer subjects and 7395 controls in 19 studies for different types of cancer, as well as 2415 individuals with CVD and 2317 controls in 7 studies. The studied population consisted of Chinese and Iranian individuals. The results are shown in Table 2. SNP rs11556218 of *IL16* was statistically associated with the risk of cancer in Chinese (T vs. G; Pooled OR = 1.38; 95% CI 1.23-1.56; random model). Specifically, for gastric cancer in Chinese, the SNP rs4778889 was associated with risk factors for disease development (T vs. C; Pooled OR = 1.18; 95% CI 1.03-1.35; Fixed model). The SNP rs11556218 was also associated with the risk of CVD in Chinese (T vs. G; pooled OR = 1.51; 95% CI 1.07-2.14; random

model). Forest plots for all statistically significant comparisons are shown in Supplementary Figure 1. For the selected studies, no statistically significant associations were observed with rs1131445 and rs4072111 and the selected disease groups. For the Iranian population, significance was not found between *IL16* polymorphisms and cancer or CVD in this meta-analysis.

Table 2. Meta-analysis results for groups of diseases.

Comparison [a]	n	Association test		Model	Heterogeneity test			PBE [b]	Citation
		OR	CI 95%		τ^2	P_{het}	I^2		
Cancer - rs11556218 T>G (total case/control number=5022/5779)									
T vs G	14	1.38	(1.23 - 1.56)	R	0.04	<0.01	71	0.98	[40,43,44, 68,81–85,87–89,93,98]
G/G+T/T vs T/G	14	1.36	(1.19 - 1.55)	R	0.04	<0.01	62	0.92	
T/T vs T/G+G/G	14	1.49	(1.28 - 1.72)	R	0.05	<0.01	70	0.88	
T/T+T/G vs G/G	14	1.56	(1.33 - 1.84)	F	0.04	0.15	29	0.65	
T/T vs T/G	14	1.43	(1.24 - 1.66)	R	0.05	<0.01	65	0.90	
T/T vs G/G	14	1.77	(1.50 - 2.10)	F	0.07	0.06	41	0.93	
T/G vs G/G	14	1.29	(1.08 – 1.53)	F	0.00	0.72	0	0.20	
Gastric cancer - rs4778889 T>C (total case/control number=1046/1310)									
T vs C	3	1.18	(1.03 - 1.35)	F	0.01	0.15	47	0.35	[68,81,93]
T/T + T/C vs C/C	3	1.41	(1.02 - 1.95)	F	0.00	0.72	0	0.65	
T/T vs C/C	3	1.48	(1.06 - 2.06)	F	0.00	0.61	0	0.87	
Cardiovascular disease - rs11556218 T>G (total case/control number=1643/1685)									
T vs G	5	1.51	(1.07 – 2.14)	R	0.17	<0.01	90	0.07	[63,73,92, 95,96]
G/G+T/T vs T/G	5	1.87	(1.08 - 3.23)	R	0.43	<0.01	92	0.23	
T/T vs T/G+G/G	5	2.00	(1.11 - 3.63)	R	0.52	<0.01	94	0.24	
T/T vs T/G	5	2.00	(1.08 - 3.71)	R	0.55	<0.01	94	0.24	

n: Number of selected studies; R: Random model; F: Fixed Model; OR: Pooled *Odds Ratio*.
[a] for all comparisons P-value<0.05.
[b] PBE: P-value for the linear regression test of funnel plot asymmetry (Egger's test).
Only significant results of OR are presented.

To avoid a possible bias caused by studies whose genotype frequency distribution in the control groups was not in HWE (Hardy-Weinberg equilibrium), stratified analyses were performed including only studies that met this criterion (P ≥ 0.05), according to the goodness-of-fit test for HWE. These results are shown in Table 3. After these analyses, the *IL16* SNP rs11556218 remain associated with the risk of cancer in Chinese (T vs. G; pooled OR = 1.41, 95% CI 1.26-1.59; random model) in the genetic inheritance models described in Table 3. In addition, a subgroup analysis was carried out to separate individuals with gastric and renal cancer from other types of cancer (see Table 3 and Supplementary Figure 2). There was a significant association of rs11556218 with risk for carcinoma and osteosarcoma in all evaluated inheritance models (T vs. G; pooled OR = 1.53; 95% CI 1.34-1.73; random model), while no association was observed with gastric and renal cancer.

Table 3. Meta-analysis results for studies with control groups in the Hardy-Weinberg equilibrium.

Comparison [a]	n	Association test		Model	Heterogeneity test			PBE [b]	Citation
		OR	CI 95%		τ^2	P_{het}	I^2		
Cancer - rs11556218 T>G (total case/control number=4367/5675)									
T vs G	13	1.41	(1.26 - 1.59)	R	0.03	<0.01	68	0.99	[40,43,44,68,81–84,87–89,93,98]
G/G+T/T vs T/G	13	1.41	(1.23 - 1.61)	R	0.04	<0.01	61	0.68	
T/T vs T/G+G/G	13	1.54	(1.33 - 1.78)	R	0.05	<0.01	68	0.74	
T/T+T/G vs G/G	13	1.66	(1.39 - 1.98)	F	0.04	0.21	23	0.41	
T/T vs T/G	13	1.48	(1.28 - 1.71)	R	0.05	<0.01	64	0.69	
T/T vs G/G	13	1.92	(1.60 - 2.30)	F	0.05	0.15	29	0.66	
T/G vs G/G	13	1.31	(1.09 – 1.58)	F	0.00	0.62	0	0.13	
Cancer – Carcinoma and other types - rs11556218 T>G (total case/control number=3521/4398)									
T vs G	10	1.53	(1.34 – 1.73)	R	0.02	<0.01	62	0.63	[40,43,44,68,82–84,88,89,98]
G/G+T/T vs T/G	10	1.53	(1.32 – 1.77)	R	0.03	0.02	54	0.45	
T/T vs T/G+G/G	10	1.70	(1.46 – 1.97)	R	0.03	0.01	58	0.41	
T/T+T/G vs G/G	10	1.76	(1.44 – 2.16)	F	0.07	0.10	39	0.17	
T/T vs T/G	10	1.63	(1.40 – 1.89)	R	0.03	0.02	54	0.41	
T/T vs G/G	10	2.10	(1.71 – 2.58)	F	0.06	0.12	35	0.06	
T/G vs G/G	10	1.33	(1.08 – 1.65)	F	0.02	0.29	16	0.39	
Cancer – Gastric and renal cancer - rs11556218 T>G (total case/control number=1154/1515)									
T vs G	4	1.16	(1.02 – 1.32)	F	0.01	0.20	35	0.77	[68,81,85,87]
Cardiovascular disease - rs11556218 T>G (total case/control number=1405/1507)									
T vs G	4	1.70	(1.25 - 2.32)	R	0.10	<0.01	85	0.29	[63,92,95,96]
G/G+T/T vs T/G	4	2.03	(1.09 – 3.78)	R	0.47	<0.01	93	0.33	
T/T vs T/G+G/G	4	2.33	(1.24 – 4.41)	R	0.49	<0.01	94	0.49	
T/T+T/G vs G/G	4	1.77	(1.24 – 2.53)	F	0.18	0.10	49	0.09	
T/T vs T/G	4	2.25	(1.12 - 4.50)	R	0.59	<0.01	94	0.58	
T/T vs G/G	4	2.50	(1.19 – 5.25)	R	0.50	<0.01	72	0.39	

n: Number of selected studies; R: Random model; F: Fixed Model; OR: Pooled *Odds Ratio*.
[a] for all comparisons P-value<0.05.
[b] PBE: P-value for the linear regression test of funnel plot asymmetry (Egger's test).
Only significant results of OR are presented.

An association of *IL16* rs11556218 with the risk for CVD in Chinese was also found for different genetic inheritance models (T vs. G; pooled OR = 1.70; 95% CI 1.25-2.32; random model). An analysis of the covariates evaluated by CVD studies was carried out with the aim of finding a possible source of heterogeneity for the obtained results. These assessed covariates are described in Supplementary Table 2. In this sense, it was not possible to identify in this analysis which covariables could partially explain the qualitative heterogeneity observed in this CVD analysis.

The statistical power after HWE stratification results, considering a subtle genetic effect (1.25), showed a slight reduction for the association between rs11556218 and cancer (from 99.9% to 93.0% after stratification for HWE) but a substantial reduction for rs11556218 and CVD (90.7%

to 81.0%) and for rs4778889 and gastric cancer (72.0% to 54.3%). Forest plots for all statistically significant comparisons after stratification of studies with controls in HWE are shown in Supplementary Figure 2.

The mean minor allele frequencies (MAF) observed among the control groups of the selected studies were 21.5% for rs4778889 (C allele), 21.3% for rs11556218 (G allele), 21.5% for rs4072111 (T allele), and 32.3% for rs1131445 (C allele). These frequencies were similar to those described in the 1000Genomes database for East Asian populations (21.2%, 16.5%, 20.8%, and 31.2%, respectively), and the Fisher test did not indicate a statistically significant difference between the allele frequencies in these populations ($P > 0.05$).

Egger's tests did not indicate possible bias in the selection of publications ($P \geq 0.05$), and these results are shown in Tables 2 and 3. The funnel plot for visual assessment is available in Supplementary Figure 3. It is noteworthy that the bias in funnel plots should be interpreted carefully for a meta-analysis with a small number of studies (around 10 or less), since tests in a small number of studies have limited statistical power due to the great heterogeneity of these datasets [39]. The quality assessment of the studies selected by the NOS (Table 4) indicated a 95% score of the studies as reasonable or good (greater than 5).

Table 4. Newcastle-Ottawa Quality assessment scale for selected studies.

Study	Selection	Comparability [a]	Exposure	Total score
Shih *et al.* [43]	* * * *	**	* *	8
Wu *et al.* [44]	* * * *	**	* *	8
He *et al*. [81]	* * * *	**	* * *	9
Li *et al*. [82]	* * * *	**	* *	8
Yang *et al*. [73]	* *	*	* *	5
MaiMaiTiMin *et al*. [83]	* * * *	**	* *	8
Tang *et al*. [84]	* * * *	**	* *	8
Yang *et al*. [85]	* * *	**	* *	7
Yao *et al*. [86]	* * *	**	* *	7
Kashfi *et al*. [67]	* *	**	* *	6
Wang and Zhu [87]	* * * *	**	* *	8
Luo *et al*. [88]	* * * *	**	* *	8
Qin *et al*. [89]	* * * *	**	* *	8
Hai-Feng *et al.* [90]	* * * *	**	* * *	9
Huang *et al*. [91]	* * * *	**	* *	8
Liu *et al*. [63]	* * *	**	* *	7
Tong *et al*. [92]	* * * *	**	* *	8

Study	Selection	Comparability [a]	Exposure	Total score
Zhang and Wang [93]	* * * *	**	* * *	9
Azimzadeh *et al*. [72]	* * * *	**	* *	8
Azimzadeh *et al*. [94]	* * * *	**	* *	8
Chen *et al*. [95]*	* * * *	**	* *	8
Li *et al*. [40]	* * *	**	* *	7
Wu *et al*. [96]	* *	**	* *	6
Zhu *et al*. [97]	* * * *	**	* *	8
Gao *et al*. [98]	* * *	*	* *	6
Gao *et al*. [68]	* * * *	**	* *	8

Each category can be awarded with one point (*).
[a] Up to two points can be given to this category (**) when additional factors are controlled.

Discussion

This systematic review included a total of 6386 individuals with cancer and 2415 individuals with CVD, which confers high statistical power to verify weaker associations. The *IL16* rs11556218 polymorphism was significantly associated with the risk of cancer in Chinese, in different models of genetic inheritance. In addition, to the best of our knowledge, this is the first meta-analysis to show an association between the rs477889 polymorphism and the risk of gastric cancer and the first to demonstrate an association of rs11556218 and the risk of CVD in Chinese.

The association between *IL16* rs11556218 and cancer or CVD remained even after careful analysis of studies control groups according to HWE criteria, and perhaps the loss of association observed for rs4778889 and gastric cancer after stratification was due to the significant loss of statistical power when the sample size of the combined studies was reduced. We also see different results when different types of cancer are grouped together. In our meta-analysis, the subgroup of renal and gastric cancer was not associated with rs11556218. These different results in distinct subgroups can be explained by SNPs having different behaviors in different types of cancer [40]. In addition, sample size limitations should be considered in analyzes of smaller number of studies. Finally, selection bias should not be ruled out in the analysis of different studies.

In agreement with our results, two other meta-analyses published in 2014 did not find a significant association between rs4778889 and rs4072111 of *IL16* and cancer. However, these same studies observed a statistically significant association between rs11556218 and cancer, an

association observed in our study with the addition of new studies published after these meta-analyses results [41–44]. The SNP rs11556218, consisting of a substitution of the T nucleotide by a G, is located in the exon region of the gene and results in a modification of the pro-IL-16 PDZ2 domain (IL-16 isoform with 631 amino acids) and npro-IL-16 (neuronal IL-16 isoform with 1331 amino acids). This modification consists of a substitution of asparagine for lysine in the protein at positions 446 (pro-IL-16) and 1147 (npro-IL-16), as illustrated in Figure 1. This mutation may result in structural changes in the protein, consequently affecting its function in the immune response [45]. This SNP was also associated with prostate cancer in a replication GWAS study in African Americans [46]. It has also been associated with asparaginase-related thrombosis and pancreatitis in the treatment of acute lymphoid leukemia in Caucasians [47], diabetes mellitus [48], autoimmune diseases [49,50], endometriosis [45,51], and knee osteoarthritis [52,53].

A meta-analysis published in 2019 focusing only on renal cancer association studies also found no association between rs4778889 and rs11556218 and this disease [54]. A possible explanation for this lack of association may be related to the limited statistical power to verify subtle associations, due to the small number of individuals in the evaluated studies (total of 790 individuals).

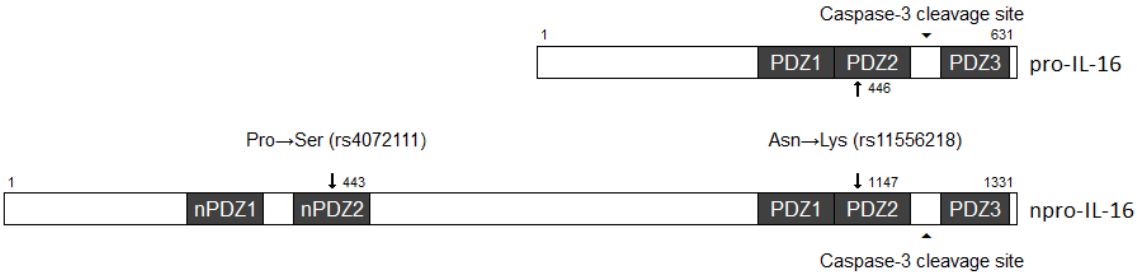

Figure 1. Schematic representation of two SNPs changes in PDZ domains of pro-IL-16 and npro-IL-16. The rs11556218 polymorphism alters the PDZ2 domain at positions 446 and 1147 of pro-IL-16 and n-pro-IL-16, respectively. This polymorphism will cause a substitution of an asparagine to a lysine at the protein, affecting protein recognition by its receptor. The rs4072111 polymorphism is present in the nPDZ2 domain of npro-IL-16 and will cause a substitution of a proline to serine in the protein precursor. Adapted from Bannert *et al.* [17].

Another meta-analysis, published in 2014, found no association between rs4778889 and cancer, which was confirmed in our study for the general cancer group [55]. However, we observed, for the first time, a statistically significant association of this polymorphism with gastric cancer in Chinese. This SNP in the promoter region of the *IL16* gene was related to changes in expression [56]. It was previously associated with precancerous gastric lesions [57], gestational

diabetes [58], autoimmune diseases [50,59], knee osteoarthritis [53], endometriosis [60], Crohn's disease [61] and allergic contact dermatitis [62].

The SNP rs4072111, consisting of a substitution of the C nucleotide by a T, is located in an intronic region and results in a modification of the nPro-IL-16 PDZ2 domain, with a serine to proline exchange at position 434 [45]. In our study, we found no association of this polymorphism with cancer. Moreover, it was not possible to perform a meta-analysis of the association between rs4072111 and CVD due to the limited number of studies, and no association was observed in the study of Liu *et al*. [63]. This SNP was previously identified as a marker candidate for aggressive prostate cancer [64], although this was not confirmed for an African American population [46,65]. This polymorphism was also correlated with better prognosis for chronic lymphoid leukemia in an English population [66], associated with the risk of liver cancer [40], colorectal and gastric cancer [67,68], autoimmunity [49,50], endometriosis [51] and knee osteoarthritis [52,53].

The polymorphism rs1131445 (T>C) is located in the 3'-UTR of the gene, and the mutation predicts disruption of the binding of a microRNA inhibitor (miR135b-mRNA) of the gene sequence, which would result in greater pro-IL-16 expression [69]. In our study, we did not observe any association of this SNP with cancer or CVD. Studies have found that this SNP has been associated with cervical cancer in Chinese and prostate cancer in African Americans [46,70]. Its mutant allele may also be informative about the time to prostate cancer diagnosis among African Americans [71]. It was also associated with gastric and colorectal cancer in Iranians [67,72], CVD in Chinese [73], Graves' disease in Chinese [59] and endometriosis in Iranian women [51].

Important limitations should be considered in our study. First, every systematic review presents some risk of bias, related to the difficulty of obtaining non-indexed studies, and the heterogeneity present in the selected studies. Moreover, the results obtained are limited to the populations surveyed and, therefore, lack information for a meta-analysis on the frequency distributions of these polymorphisms in other populations, although an attempt to broadly describe the literature has been made. Similarly, important covariates must be considered to explain the associations observed in these studies, thus multivariate methods of analysis can be useful, highlighting the need for detailed publication of the profile of the populations studied, although the

reliability of these methods depends on 10 or more studies. From the results of this review, further studies may be necessary to clarify the role of these polymorphisms in the immunopathogenesis of cancer and CVD, also helping to clarify the relationship between these diseases.

The SNP rs11556218 of *IL16* was significantly associated with an increased risk of cancer or CVD in Chinese. Additionally, the SNP rs4778889 was associated with an increased risk of gastric cancer, while the rs4072111 and rs1131445 SNPs were not associated with cancer or CVD in our study.

Materials and methods

The methodology of this study was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [74]. To identify studies investigating associations between *IL16* polymorphisms and cancer or CVD, the MEDLINE, Web of Science and Scopus databases were searched up to June 30, 2020, according to the strategy detailed in Supplementary Table 1. The language of publication was not defined as a search criterion, but only articles in the English language were included. All steps were performed by two pairs of reviewers in duplicate. Article screening procedures are included in Diagram 1.

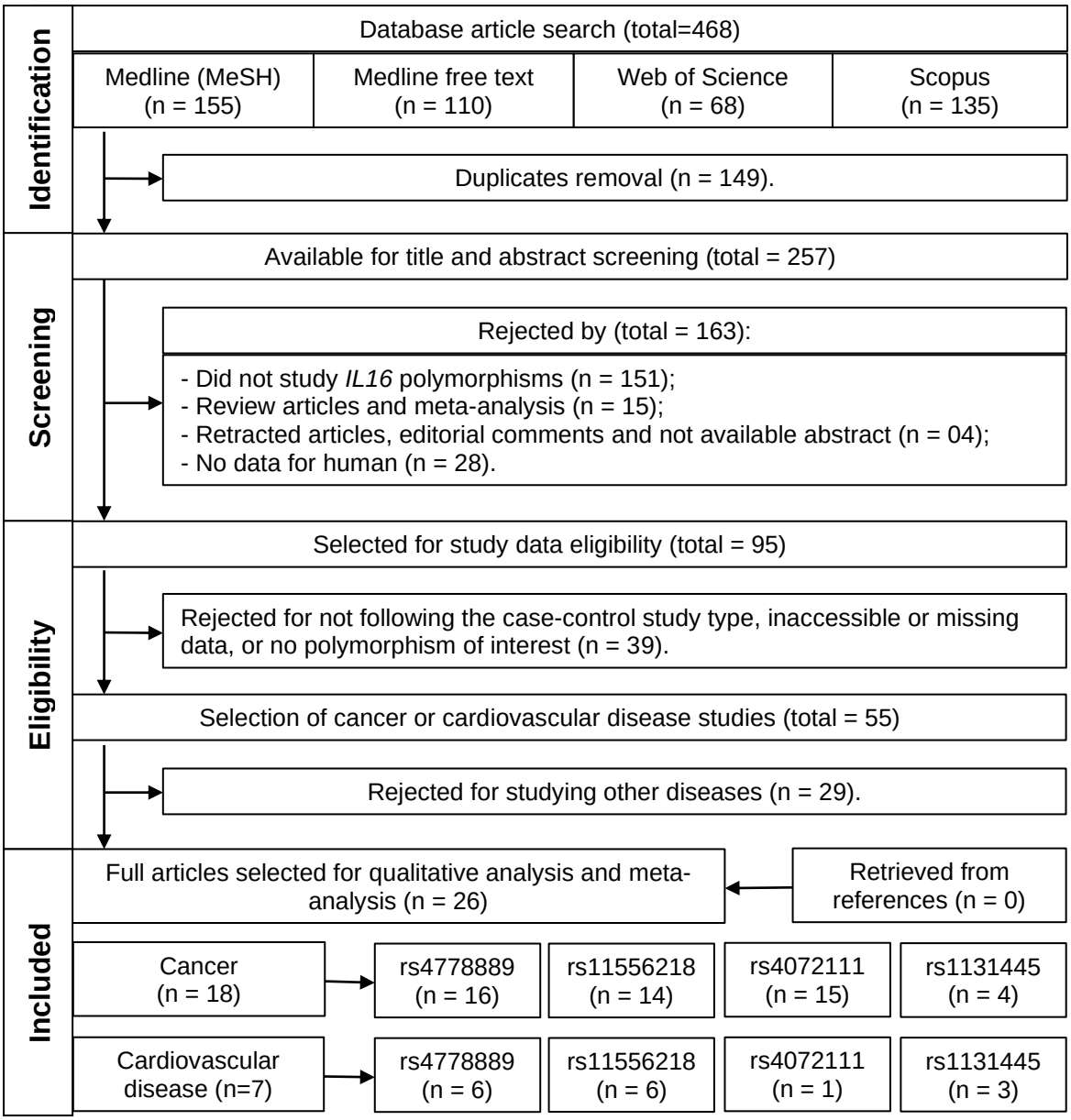

Diagram 1. Flowchart of the studies selection.

Articles with available abstracts were selected for title and abstract screening as to their relevance and fitness to the proposed theme. Only original human studies of *IL16* polymorphisms were included. In the next step, the 95 selected articles were evaluated by reading the full text, as to their suitability for inclusion in a meta-analysis (case-control study, evaluating at least one of the polymorphisms of interest, availability of genotype frequency data). Thus, 55 articles were obtained that met all these criteria. All doubts regarding the eligibility of a study or not (according to the criteria described above) were discussed among all authors and resolved after consensus. After a consensus was reached between two authors working in pairs, 26 studies were selected for data collection, according to the diseases of interest. Also, the references of these studies

were searched for additional studies. The studies and SNPs selected in this review are described in Table 1. The 26 selected studies were further reassessed by a third independent pair of authors, composed of authors with more experience in the field of cytokine polymorphisms and association studies, evaluating whether all the studies and results were in accordance with the proposed criteria for this review.

Genotype frequency data were collected from the selected studies. Allele frequencies were obtained by counting the total number of alleles in all genotypes, and the number of individuals used in the meta-analysis was obtained by summing the total number of described genotypes. Distinct groups of cases or controls within the same study were combined into a single group for distinct and unrelated groups of individuals. The cancer group was analyzed by combining all cancer types or individually for each type of cancer (when a comparison was possible). Statistical analyses were performed by using the program R, version 3.5.2, with the meta-analysis package “meta” [75].

The meta-analysis results are represented by the Forest plot. This chart aims to summarize the results of the pooled studies giving them a general trend. It represents the studies with the proportions of events of interest in each study for the case-control groups. The percentage values of weight and effect size (represented by the *Odds Ratio*, OR) of each study in the final result are presented in two possible models according to observed heterogeneity: fixed and random. Thus, for all comparisons performed, Forest plots were constructed with a heterogeneity test (I^2). When the value of I^2 was greater than 50% and the test showed statistically significant results, the random OR model was chosen. When I^2 was less than 50% with no evidence of significant heterogeneity, the fixed model was chosen. Six comparison groups of alleles and genotypes were used to verify the influence of genetic association with diseases. Considering “M” as the wildtype allele and “m” as the mutated allele, the combinations analyzed were (1) M vs. m alleles; (2) MM vs. Mm genotypes; (3) MM vs. mm and (4) Mm vs. mm; and also, genetic inheritance models (5) MM + Mm vs. mm (recessive); (6) MM vs. Mm + mm (dominant); and (7) MM + mm vs. Mm (overdominant).

Table 1. General characteristics of selected *IL16* polymorphisms studies.

HWEc	rs1131445			n	HWEc	rs4072111			n	HWEc	rs11556218			n	HWEc	rs4778889			n	Group	Disease	Population	Year	Study
	C/C	T/C	T/T			T/T	C/T	C/C			G/G	T/G	T/T			C/C	T/C	T/T						
					0.87	38	332	588	958	0.76	56	269	633	958	0.99	32	293	633	958	Case	Oral cancer	Taiwanese	2020	Shih *et al.* [43]
						41	346	571	958		26	239	693	958		35	302	621	958	Control				
					0.98	17	117	224	358	0.71	26	131	201	358	0.97	11	113	234	358	Case	Lung cancer	Taiwanese	2020	Wu *et al.* [44]
						28	247	441	716		19	161	536	716		21	239	456	716	Control				
0.98	47	211	221	479	0.97	19	126	334	479	0.99	22	151	306	479	0.87	30	182	267	479	Case	Gastric cancer	Chinese	2018	He *et al*. [81]
	51	222	210	483		16	122	345	483		18	157	308	483		25	192	266	483	Control				
					0.63	23	186	329	538	0.63	44	252	242	538	0.91	25	190	323	538	Case	Papillary thyroid carcinoma	Chinese	2018	Li *et al*. [82]
						27	241	357	625		28	247	350	625		35	217	373	625	Control				
										<0.01	34	71	133	238	0.45	29	107	102	238	Case	Acute coronary syndrome	Chinese	2018	Yang *et al*. [73]
											36	46	96	178		32	75	71	178	Control				
0.66	25	119	156	300	0.76	2	52	246	300											Case	Gastric cancer	Iranian	2016	Kashfi *et al*. [67]
	24	121	111	256		7	65	184	256											Control				
					0.99	29	95	106	230	0.05	45	67	118	230						Case	Breast cancer	Chinese	2016	MaiMaiTiMin *et al*. [83]
						22	98	110	230		18	50	162	230						Control				
					0.77	16	124	218	358	0.77	19	174	165	358	0.98	16	127	215	358	Case	Osteosarcoma	Chinese	2016	Tang *et al*. [84]
						15	158	229	402		16	151	235	402		22	140	240	402	Control				
										0.63	15	110	149	274	0.82	28	113	132	273	Case	Renal cell cancer	Chinese	2016	Yang *et al*. [85]
											12	107	155	274		14	84	176	274	Control				
					0.98	29	90	101	220						0.08	45	62	113	220	Case	Ovarian cancer	Chinese	2016	Yao *et al*. [86]
						17	93	110	220							13	45	162	220	Control				
										0.86	12	75	94	181	0.85	22	77	82	181	Case	Renal cell cancer	Chinese	2015	Wang and Zhu [87]
											15	108	155	278		12	106	160	278	Control				
					0.85	12	66	138	216	0.32	7	119	90	216	0.85	6	68	142	216	Case	Glioma	Chinese	2014	Luo *et al*. [88]
						12	101	162	275		9	114	152	275		11	99	165	275	Control				
					0.05	0	34	41	75	0.84	6	37	32	75	0.11	0	36	39	75	Case	Nasopharyngeal carcinoma	Chinese	2014	Qin *et al*. [89]
						0	31	44	75		3	26	46	75		0	26	49	75	Control				
0.05	50	90	120	260						**<0.01**	21	64	175	260	0.06	33	89	139	261	Case		Chinese	2013	

HWEc	rs1131445			n	HWEc	rs4072111			n	HWEc	rs11556218			n	HWEc	rs4778889			n	Group	Disease	Population	Year	Study
	C/C	T/C	T/T			T/T	C/T	C/C			G/G	T/G	T/T			C/C	T/C	T/T						
	50	95	137	282							28	63	191	282		39	91	151	281	Control	Coronary heart disease			Hai-Feng *et al*. [90]
0.98	57	295	299	651																Case	Coronary artery disease	Chinese	2013	Huang *et al*. [91]
	46	196	186	428																Control				
					0.89	10	63	125	198	0.52	8	102	88	198	0.85	8	66	124	198	Case	Ischemic stroke	Chinese	2013	Liu *et al*. [63]
						10	85	141	236		6	94	136	236		10	88	138	236	Control				
0.08	56	112	157	325						0.72	34	69	223	326	0.13	50	106	170	326	Case	Coronary heart disease	Chinese	2013	Tong *et al*. [92]
	54	116	171	341							12	80	248	340		33	107	201	341	Control				
0.02	68	114	165	347	**<0.01**	49	56	242	347	**0.03**	68	114	165	347	0.47	45	114	188	347	Case	Gastric cancer	Chinese	2013	Zhang and Wang [93]
	60	112	174	346		42	54	251	347		60	112	174	346		29	106	212	347	Control				
0.93	36	110	103	249																Case	Colorectal cancer	Iranian	2012	Azimzadeh *et al*. [72]
	34	159	201	394																Control				
					0.99	8	56	196	260	0.47	20	178	62	260	0.44	9	73	178	260	Case	Colorectal cancer	Iranian	2011	Azimzadeh *et al*. [94]
						4	77	324	405		55	226	124	405		19	112	274	405	Control				
										0.77	23	235	42	300	0.98	10	117	173	300	Case	Coronary artery disease	Chinese	2011	Chen *et al*. [95][a]
											16	149	232	397		18	138	241	397	Control				
										0.27	12	264	148	424						Case				
											10	144	178	332						Control				
					0.89	16	80	110	206	0.13	22	62	122	206	0.83	6	42	158	206	Case	Hepatocellular carcinoma	Chinese	2011	Li *et al*. [40]
						24	104	136	264		26	78	160	264		6	76	182	264	Control				
										0.48	4	92	61	157	0.91	7	55	95	157	Case	Coronary artery disease	Chinese	2011	Wu *et al*. [96]
											8	87	107	202		10	75	117	202	Control				
															0.89	14	122	199	335	Case	Renal cell cancer	Chinese	2010	Zhu *et al*. [97]
																34	135	171	340	Control				
					0.58	8	87	111	206	0.38	6	109	91	206	0.99	10	65	131	206	Case	Nasopharyngeal carcinoma	Chinese	2009	Gao *et al*. [98]
						13	139	221	373		12	151	210	373		17	128	228	373	Control				
					0.85	22	195	379	596	0.56	28	331	237	596	0.99	24	209	363	596	Case	Colorectal and gastric cancer	Chinese	2009	Gao *et al*. [68]
						18	179	283	480		18	197	265	480		22	164	294	480	Control				

n: Case/control number of individuals, given by the sum of genotypes in the collected data.
a This study consisted of two independent groups of cases and controls.
HWEc: P value for Hardy-Weinberg equilibrium goodness-of-fit test in control groups (P< 0.05).

Quality evaluation of the selected studies was performed according to the Newcastle-Ottawa (NOS) scale by two independent authors. Disagreements were resolved by consensus, and studies with a score higher than 5 were considered as good or reasonable [76]. Possible biases of published meta-analyses were verified by Egger's test analysis [77], funnel plot visual evaluation, and Begg's test [78]. All individual study results were expressed as ORs with a 95% confidence interval (CI). The Hardy-Weinberg equilibrium (HWE) of the control group of each study was checked according to the chi-square test for goodness-of-fit ($P < 0.05$), as recommended for assessing study quality [79]. After this, statistical power analysis was performed using the QUANTO software [80], to evaluate sample size effects on the statistical power. The reference population allele frequencies used for comparison were obtained from the 1000 Genomes Project (phase3 release V3+, available at: https://www.internationalgenome.org/) and the comparison was made using the exact Fisher test in R. For all tests, $P < 0.05$ was considered to indicate statistical significance.

Abbreviations

Global cancer statistics - GLOBOCAN

Cardiovascular disease - CVD

Interferon-α - IFN-α

Interleukin - IL

Granulocyte macrophage colony stimulating factor - GM-GSF

Single nucleotide polymorphism - SNP

Odds Ratio - OR

Confidence interval - CI

Hardy-Weinberg equilibrium - HWE

Newcastle-Ottawa Scale - NOS

Genome-wide association study - GWAS

Preferred Reporting Items for Systematic Reviews and Meta-Analyses – PRISMA

Author contributions

VHS conceived and designed the analysis, performed statistical analysis and wrote the manuscript; JBA, BTT and HVA participated in literature search, article selection and data

collection; ECLV, contributed with analysis of bias and quality assessment; JELV and AMS contributed with data analysis, critical review of the manuscript, and supervision. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Supporting Information

Supplementary Table 1. Search strategy in selected databases.

Medline (PubMed MeSH terms)
(((((("Polymorphism, Genetic"[Mesh]) OR "Genetic Techniques"[Mesh]) OR "Genotype"[Mesh]) OR "Alleles"[Mesh])) AND "Interleukin-16"[Mesh] . Free term: IL16 AND Polymorphism
Scopus (Article title, Abstract, Keywords)
"Polymorphism, Genetic" OR "Genetic Techniques" OR Genotype OR Alleles AND "Interleukin-16"
Web of Science (Topic)
(TS=(Polymorphism, Genetic OR Genetic Techniques OR Genotype OR Alleles) AND TS=Interleukin-16)

Supplementary Table 2. Covariables published in selected case-control studies of cardiovascular diseases.

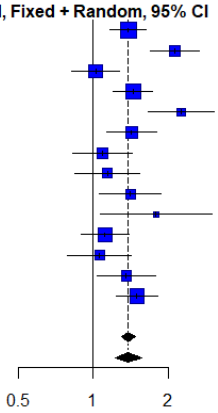
Study (group)	Age (Mean±SD)	*P*	Male (%)	*P*	Hypertension (%)	*P*	Diabetes (%)	*P*	Smoking (%)	*P*
Liu 2013 (Case)	57.2±11.8	0.11	122 (61.6)	0.38	119 (60.1)	**<0.01**	40 (20.2)	**<0.01**		
Liu 2013 (Control)	55.4±11.7		155 (65.7)		22 (9.3)		8 (3.4)			
Tong 2013 (Case)	61.4±8.7	0.26	243 (74.6)	**<0.01**	159 (48.8)	**<0.01**	136 (41.7)	**<0.01**	135 (41.4)	**<0.01**
Tong 2013 (Control)	60.6±9.6		210 (61.6)		128 (37.5)		67 (19.6)		93 (27.3)	
Chen 2011 (1) (Case)	57.2±7.8	**0.01**	243 (81.0)	**<0.01**	126 (42.0)	0.18	45 (15.0)	**<0.01**	150 (50.0)	**<0.01**
Chen 2011 (1) (Control)	59.1±11.6		263 (66.2)		147 (37.0)		24 (6.0)		147 (37.0)	
Chen 2011 (2) (Case)	61.9±10.9	0.07	340 (80.2)	**<0.01**	230 (54.2)	**<0.01**	94 (22.2)	**<0.01**	220 (51.9)	**<0.01**
Chen 2011 (2) (Control)	60.5±10.4		227 (68.4)		107 (32.2)		33 (9.9)		81 (24.4)	
Wu 2011 (Case)	62.8±11.6	0.30	95 (60.5)	0.76	50 (31.8)		26 (16.6)			
Wu 2011 (Control)	61.6±10.4		119 (58.9)							

Study (group)	Triglyceride (mmol/L)	*P*	Total Cholesterol (mmol/L)	*P*	HDL (mmol/L)	*P*	LDL (mmol/L)	*P*
Tong 2013 (Case)	2±1.1	**0.01**	4.2±1.0	**<0.01**	1.2±0.4	**<0.01**	2.5±0.9	**<0.01**
Tong 2013 (Control)	1.8±0.9		4.7±1.0		1.5±0.4		2.9±0.9	
Chen 2011 (1) (Case)	2.1±1.4	**<0.01**	4.1±1.4	**<0.01**	1.1±0.4	**<0.01**	2.5±1.1	**<0.01**
Chen 2011 (1) (Control)	1.6±1.0		4.8±0.9		1.7±0.4		2.9±0.8	
Chen 2011 (2) (Case)	1.9±1.1	0.99	4.1±1.1	**<0.01**	1.2±0.3	**<0.01**	2.5±1.0	**<0.01**
Chen 2011 (2) (Control)	1.9±1.3		4.7±1.0		1.5±0.4		2.8±0.8	

A - All cancer and T vs G comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Shih 2020	381	1916	291	1916	14.3%	8.8%	1.39 [1.17; 1.64]
Wu 2020	183	716	199	1432	6.1%	7.8%	2.13 [1.70; 2.66]
He 2018	195	958	193	966	9.4%	7.8%	1.02 [0.82; 1.28]
Li 2018	340	1076	303	1250	11.8%	8.5%	1.44 [1.20; 1.73]
MaiMaiTiMin 2016	157	460	86	460	3.5%	6.3%	2.25 [1.66; 3.05]
Tang 2016	212	716	183	804	7.5%	7.7%	1.43 [1.13; 1.80]
Yang 2016	140	548	131	548	6.0%	6.8%	1.09 [0.83; 1.44]
Wang 2015	99	362	138	556	4.9%	6.4%	1.14 [0.84; 1.54]
Luo 2014	133	432	132	550	4.9%	6.7%	1.41 [1.06; 1.87]
Qin 2014	49	150	32	150	1.3%	3.6%	1.79 [1.06; 3.01]
Zhang 2013	250	694	232	692	9.1%	7.8%	1.12 [0.89; 1.39]
Li 2011	106	412	130	528	5.2%	6.5%	1.06 [0.79; 1.43]
Gao 2009	121	412	175	746	5.4%	6.9%	1.36 [1.03; 1.78]
Gao 2008	387	1192	233	960	10.7%	8.4%	1.50 [1.24; 1.82]
Total (fixed effect, 95% CI)		**10044**		**11558**	**100.0%**	--	**1.38 [1.30; 1.47]**
Total (random effects, 95% CI)					--	**100.0%**	**1.38 [1.23; 1.56]**

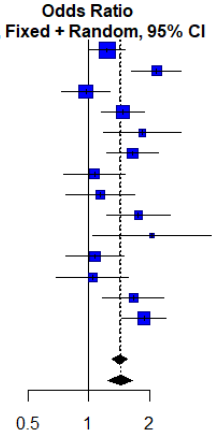
Heterogeneity: $Tau^2 = 0.0357$; $Chi^2 = 44.09$, df = 13 (P < 0.01); $I^2 = 71\%$



B - All cancer and TT vs TG comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Shih 2020	269	902	239	932	17.3%	9.4%	1.23 [1.00; 1.51]
Wu 2020	131	332	161	697	6.6%	8.1%	2.17 [1.64; 2.88]
He 2018	151	457	157	465	10.9%	8.2%	0.97 [0.74; 1.27]
Li 2018	252	494	247	597	11.5%	8.8%	1.48 [1.16; 1.88]
MaiMaiTiMin 2016	67	185	50	212	3.1%	5.7%	1.84 [1.19; 2.85]
Tang 2016	174	339	151	386	7.2%	7.8%	1.64 [1.22; 2.20]
Yang 2016	110	259	107	262	6.4%	6.9%	1.07 [0.75; 1.52]
Wang 2015	75	169	108	263	4.9%	6.3%	1.15 [0.77; 1.69]
Luo 2014	119	209	114	266	4.5%	6.7%	1.76 [1.22; 2.54]
Qin 2014	37	69	26	72	1.2%	3.3%	2.05 [1.04; 4.02]
Zhang 2013	114	279	112	286	6.9%	7.1%	1.07 [0.77; 1.50]
Li 2011	62	184	78	238	4.7%	6.0%	1.04 [0.69; 1.57]
Gao 2009	109	200	151	361	5.1%	7.0%	1.67 [1.18; 2.36]
Gao 2008	331	568	197	462	9.5%	8.7%	1.88 [1.47; 2.41]
Total (fixed effect, 95% CI)		**4646**		**5499**	**100.0%**	--	**1.42 [1.31; 1.55]**
Total (random effects, 95% CI)					--	**100.0%**	**1.43 [1.24; 1.66]**

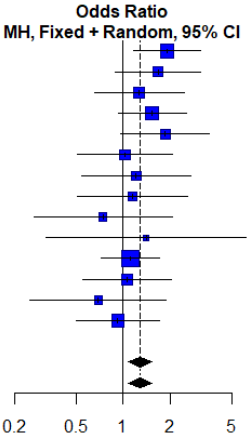
Heterogeneity: $Tau^2 = 0.0468$; $Chi^2 = 37.13$, df = 13 (P < 0.01); $I^2 = 65\%$



C - All cancer and TG vs GG comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Shih 2020	56	325	26	265	10.5%	12.4%	1.91 [1.16; 3.14]
Wu 2020	26	157	19	180	6.5%	7.6%	1.68 [0.89; 3.17]
He 2018	22	173	18	175	6.9%	7.0%	1.27 [0.66; 2.46]
Li 2018	44	296	28	275	10.9%	12.0%	1.54 [0.93; 2.55]
MaiMaiTiMin 2016	45	112	18	68	5.9%	7.0%	1.87 [0.97; 3.60]
Tang 2016	19	193	16	167	6.8%	6.2%	1.03 [0.51; 2.07]
Yang 2016	15	125	12	119	4.8%	4.7%	1.22 [0.54; 2.72]
Wang 2015	12	87	15	123	4.7%	4.6%	1.15 [0.51; 2.60]
Luo 2014	7	126	9	123	3.8%	2.9%	0.75 [0.27; 2.07]
Qin 2014	6	43	3	29	1.4%	1.4%	1.41 [0.32; 6.14]
Zhang 2013	68	182	60	172	17.0%	16.2%	1.11 [0.72; 1.72]
Li 2011	22	84	26	104	7.6%	7.0%	1.06 [0.55; 2.06]
Gao 2009	6	115	12	163	4.1%	3.0%	0.69 [0.25; 1.90]
Gao 2008	28	359	18	215	9.2%	8.0%	0.93 [0.50; 1.72]
Total (fixed effect, 95% CI)		**2377**		**2178**	**100.0%**	--	**1.29 [1.08; 1.53]**
Total (random effects, 95% CI)					--	**100.0%**	**1.28 [1.08; 1.53]**

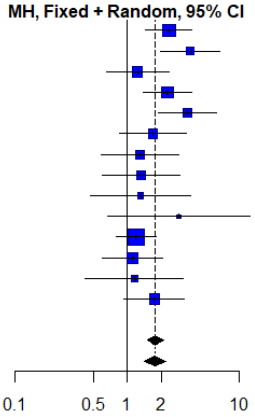
Heterogeneity: $Tau^2 = 0$; $Chi^2 = 9.72$, df = 13 (P = 0.72); $I^2 = 0\%$



D - All cancer and TT vs GG comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Shih 2020	56	689	26	719	11.2%	10.3%	2.36 [1.46; 3.80]
Wu 2020	26	227	19	555	4.7%	8.0%	3.65 [1.98; 6.74]
He 2018	22	328	18	326	8.1%	7.6%	1.23 [0.65; 2.34]
Li 2018	44	286	28	378	9.8%	9.8%	2.27 [1.38; 3.75]
MaiMaiTiMin 2016	45	163	18	180	5.9%	8.2%	3.43 [1.89; 6.23]
Tang 2016	19	184	16	251	5.8%	6.9%	1.69 [0.84; 3.39]
Yang 2016	15	164	12	167	5.2%	5.8%	1.30 [0.59; 2.87]
Wang 2015	12	106	15	170	4.9%	5.7%	1.32 [0.59; 2.94]
Luo 2014	7	97	9	161	3.0%	4.0%	1.31 [0.47; 3.65]
Qin 2014	6	38	3	49	1.1%	2.2%	2.88 [0.67; 12.35]
Zhang 2013	68	233	60	234	20.3%	11.7%	1.20 [0.80; 1.80]
Li 2011	22	144	26	186	9.2%	8.0%	1.11 [0.60; 2.05]
Gao 2009	6	97	12	222	3.3%	4.0%	1.15 [0.42; 3.17]
Gao 2008	28	265	18	283	7.5%	7.9%	1.74 [0.94; 3.23]
Total (fixed effect, 95% CI)		**3021**		**3881**	**100.0%**	**--**	**1.77 [1.50; 2.10]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.77 [1.40; 2.22]**

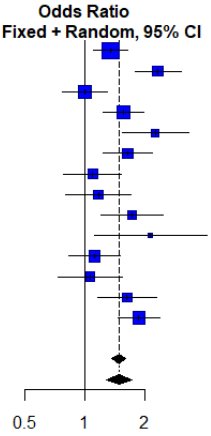
Heterogeneity: $Tau^2 = 0.0736$; $Chi^2 = 22.01$, df = 13 (P = 0.06); $I^2 = 41\%$



E - All cancer and TT vs TG + GG comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Shih 2020	325	958	265	958	17.0%	9.1%	1.34 [1.11; 1.63]
Wu 2020	157	358	180	716	6.5%	8.0%	2.33 [1.78; 3.04]
He 2018	173	479	175	483	10.8%	8.0%	1.00 [0.76; 1.29]
Li 2018	296	538	275	625	11.1%	8.5%	1.56 [1.23; 1.96]
MaiMaiTiMin 2016	112	230	68	230	3.4%	6.3%	2.26 [1.54; 3.32]
Tang 2016	193	358	167	402	7.0%	7.7%	1.65 [1.23; 2.19]
Yang 2016	125	274	119	274	6.3%	6.9%	1.09 [0.78; 1.53]
Wang 2015	87	181	123	278	4.9%	6.4%	1.17 [0.80; 1.70]
Luo 2014	126	216	123	275	4.4%	6.6%	1.73 [1.21; 2.48]
Qin 2014	43	75	29	75	1.2%	3.5%	2.13 [1.11; 4.09]
Zhang 2013	182	347	172	346	8.0%	7.5%	1.12 [0.83; 1.50]
Li 2011	84	206	104	264	5.2%	6.4%	1.06 [0.73; 1.54]
Gao 2009	115	206	163	373	5.0%	6.8%	1.63 [1.16; 2.29]
Gao 2008	359	596	215	480	9.2%	8.3%	1.87 [1.46; 2.38]
Total (fixed effect, 95% CI)		**5022**		**5779**	**100.0%**	**--**	**1.48 [1.37; 1.60]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.49 [1.28; 1.72]**

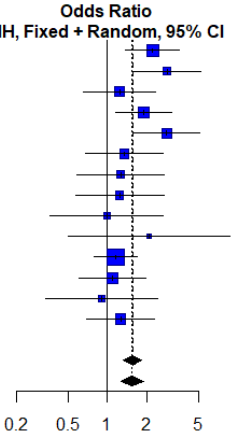
Heterogeneity: $Tau^2 = 0.0529$; $Chi^2 = 42.94$, df = 13 (P < 0.01); $I^2 = 70\%$



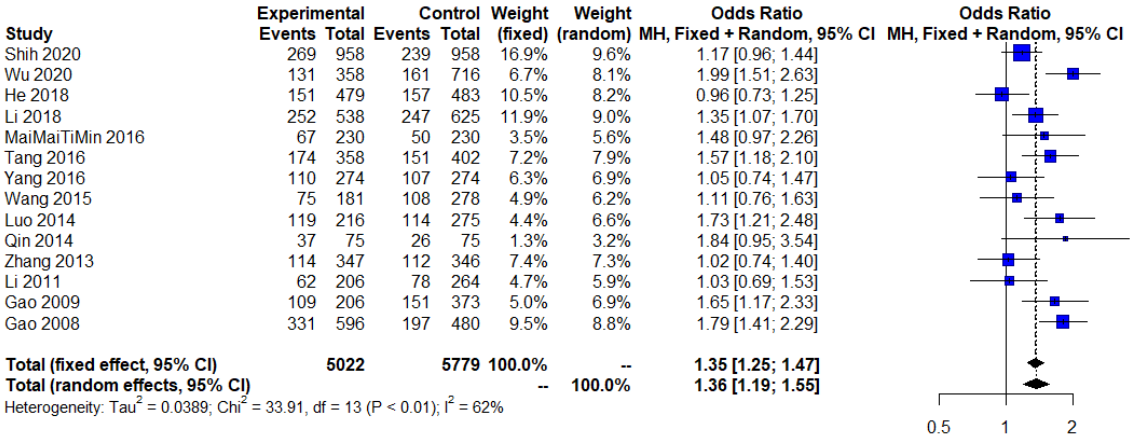
F - All cancer and TT + TG vs GG comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Shih 2020	56	958	26	958	10.4%	10.7%	2.23 [1.39; 3.58]
Wu 2020	26	358	19	716	5.0%	7.8%	2.87 [1.57; 5.27]
He 2018	22	479	18	483	7.3%	7.3%	1.24 [0.66; 2.35]
Li 2018	44	538	28	625	10.1%	10.3%	1.90 [1.17; 3.10]
MaiMaiTiMin 2016	45	230	18	230	6.2%	8.3%	2.86 [1.60; 5.12]
Tang 2016	19	358	16	402	6.1%	6.6%	1.35 [0.68; 2.67]
Yang 2016	15	274	12	274	4.8%	5.4%	1.26 [0.58; 2.75]
Wang 2015	12	181	15	278	4.7%	5.3%	1.24 [0.57; 2.72]
Luo 2014	7	216	9	275	3.3%	3.5%	0.99 [0.36; 2.70]
Qin 2014	6	75	3	75	1.2%	1.9%	2.09 [0.50; 8.67]
Zhang 2013	68	347	60	346	20.6%	13.4%	1.16 [0.79; 1.71]
Li 2011	22	206	26	264	8.7%	7.9%	1.09 [0.60; 1.99]
Gao 2009	6	206	12	373	3.5%	3.6%	0.90 [0.33; 2.44]
Gao 2008	28	596	18	480	8.1%	7.8%	1.27 [0.69; 2.32]
Total (fixed effect, 95% CI)		**5022**		**5779**	**100.0%**	**--**	**1.56 [1.33; 1.84]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.55 [1.26; 1.90]**

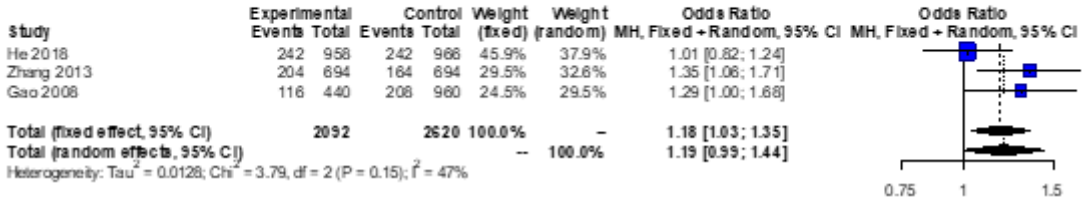
Heterogeneity: $Tau^2 = 0.0414$; $Chi^2 = 18.32$, df = 13 (P = 0.15); $I^2 = 29\%$



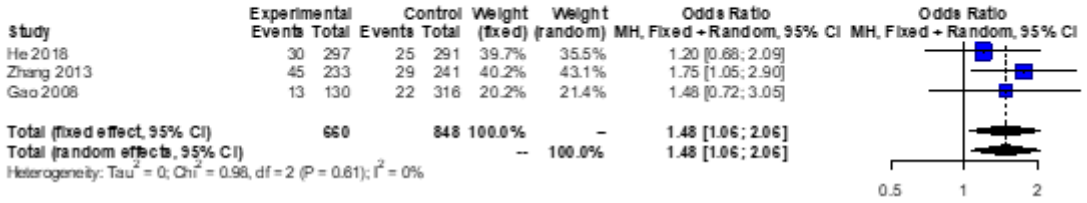
G - All cancer and TT + GG vs TG comparison for rs11556218.



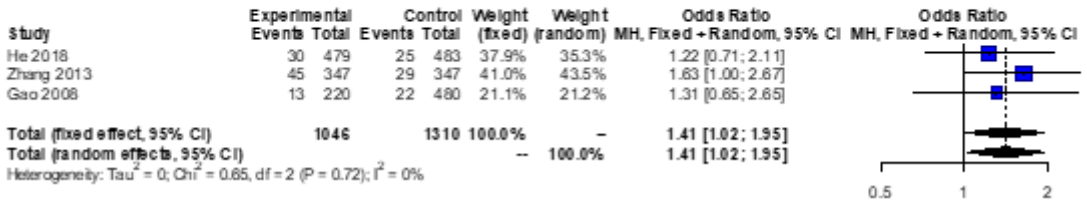
H - Gastric cancer and T vs C comparison for rs4778889.



I - Gastric cancer and TT vs CC comparison for rs4778889.



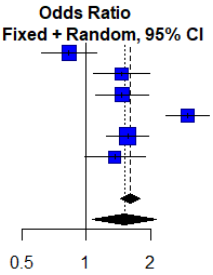
J - Gastric cancer and TT + TC vs CC comparison for rs4778889.



K - Cardiovascular disease and T vs G comparison for rs11556218

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Yang 2018	139	476	118	356	18.8%	16.4%	0.83 [0.62; 1.12]
Liu 2013	118	396	106	472	13.3%	16.3%	1.47 [1.08; 1.99]
Tong 2013	137	652	104	680	15.8%	16.6%	1.47 [1.11; 1.95]
Chen 2011 (1)	281	600	181	794	16.3%	17.3%	2.98 [2.37; 3.76]
Chen 2011 (2)	288	848	164	664	23.8%	17.3%	1.57 [1.25; 1.97]
Wu 2011	100	314	103	404	12.0%	16.0%	1.37 [0.99; 1.89]
Total (fixed effect, 95% CI)		**3286**		**3370**	**100.0%**	--	**1.61 [1.44; 1.79]**
Total (random effects, 95% CI)					--	**100.0%**	**1.51 [1.07; 2.14]**

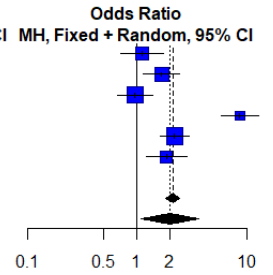
Heterogeneity: $Tau^2 = 0.1669$; $Chi^2 = 48.33$, df = 5 (P < 0.01); $I^2 = 90\%$



L - Cardiovascular disease and TT vs TG comparison for rs11556218

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Yang 2018	71	204	46	142	14.7%	16.3%	1.11 [0.71; 1.76]
Liu 2013	102	190	94	230	16.4%	16.7%	1.68 [1.14; 2.47]
Tong 2013	69	292	80	328	24.0%	16.8%	0.96 [0.66; 1.39]
Chen 2011 (1)	235	277	149	381	7.9%	16.7%	8.71 [5.91; 12.84]
Chen 2011 (2)	264	412	144	322	24.2%	17.1%	2.20 [1.64; 2.97]
Wu 2011	92	153	87	194	12.7%	16.4%	1.85 [1.21; 2.85]
Total (fixed effect, 95% CI)		**1528**		**1597**	**100.0%**	--	**2.13 [1.84; 2.47]**
Total (random effects, 95% CI)					--	**100.0%**	**2.00 [1.08; 3.71]**

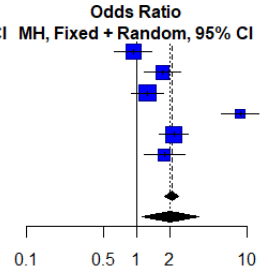
Heterogeneity: $Tau^2 = 0.5535$; $Chi^2 = 78.41$, df = 5 (P < 0.01); $I^2 = 94\%$



M - Cardiovascular disease and TT vs TG+GG comparison for rs11556218

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Yang 2018	105	238	82	178	19.6%	16.5%	0.92 [0.63; 1.37]
Liu 2013	110	198	100	236	15.2%	16.6%	1.70 [1.16; 2.49]
Tong 2013	103	326	92	340	23.1%	16.9%	1.25 [0.89; 1.74]
Chen 2011 (1)	258	300	165	397	7.4%	16.6%	8.64 [5.89; 12.66]
Chen 2011 (2)	276	424	154	332	22.6%	17.1%	2.16 [1.61; 2.89]
Wu 2011	96	157	95	202	12.1%	16.3%	1.77 [1.16; 2.71]
Total (fixed effect, 95% CI)		**1643**		**1685**	**100.0%**	--	**2.07 [1.80; 2.39]**
Total (random effects, 95% CI)					--	**100.0%**	**2.00 [1.11; 3.63]**

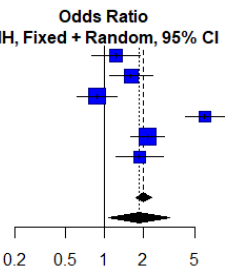
Heterogeneity: $Tau^2 = 0.5154$; $Chi^2 = 80.52$, df = 5 (P < 0.01); $I^2 = 94\%$



N - Cardiovascular disease and (TT+GG vs TG) comparison for rs11556218

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Yang 2018	71	238	46	178	14.2%	16.2%	1.22 [0.79; 1.89]
Liu 2013	102	198	94	236	16.0%	16.6%	1.61 [1.10; 2.35]
Tong 2013	69	326	80	340	23.7%	16.7%	0.87 [0.61; 1.26]
Chen 2011 (1)	235	300	149	397	10.7%	16.9%	6.02 [4.28; 8.47]
Chen 2011 (2)	264	424	144	332	23.4%	17.2%	2.15 [1.61; 2.89]
Wu 2011	92	157	87	202	12.1%	16.3%	1.87 [1.23; 2.85]
Total (fixed effect, 95% CI)		**1643**		**1685**	**100.0%**	--	**2.01 [1.74; 2.32]**
Total (random effects, 95% CI)					--	**100.0%**	**1.87 [1.08; 3.23]**

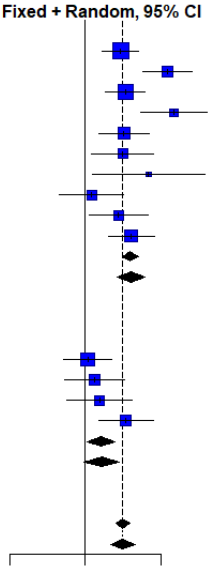
Heterogeneity: $Tau^2 = 0.4281$; $Chi^2 = 66.33$, df = 5 (P < 0.01); $I^2 = 92\%$



Supplementary Figure 1. Forest plot of comparisons for all polymorphisms associated with disease in the pooled studies.

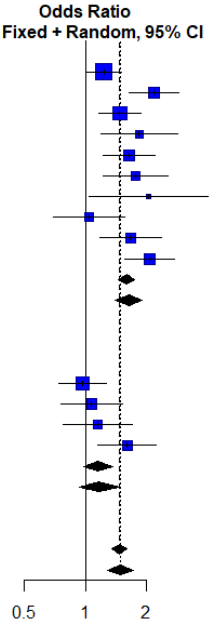
A - Cancer and T vs G comparison for rs11556218

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Type = Carcinoma and others							
Shih 2020	381	1916	291	1916	15.1%	9.0%	1.39 [1.17; 1.64]
Wu 2020	183	716	199	1432	6.4%	7.9%	2.13 [1.70; 2.66]
Li 2018	340	1076	303	1250	12.4%	8.7%	1.44 [1.20; 1.73]
MaiMaiTiMin 2016	157	460	86	460	3.7%	6.4%	2.25 [1.66; 3.05]
Tang 2016	212	716	183	804	7.9%	7.8%	1.43 [1.13; 1.80]
Luo 2014	133	432	132	550	5.2%	6.7%	1.41 [1.06; 1.87]
Qin 2014	49	150	32	150	1.4%	3.5%	1.79 [1.06; 3.01]
Li 2011	106	412	130	528	5.5%	6.5%	1.06 [0.79; 1.43]
Gao 2009	121	412	175	746	5.7%	6.9%	1.36 [1.03; 1.78]
Gao 2008 (CRC)	247	752	233	960	8.9%	8.1%	1.53 [1.23; 1.89]
Total (fixed effect, 95% CI)		7042		8796	72.2%	--	1.51 [1.40; 1.63]
Total (random effects, 95% CI)					--	71.4%	1.53 [1.34; 1.73]
Heterogeneity: Tau^2 = 0.0246; Chi^2 = 23.76, df = 9 (P < 0.01); I^2 = 62%							
Type = Gastric and renal cancer							
He 2018	195	958	193	966	9.9%	7.9%	1.02 [0.82; 1.28]
Yang 2016	140	548	131	548	6.3%	6.9%	1.09 [0.83; 1.44]
Wang 2015	99	362	138	556	5.1%	6.4%	1.14 [0.84; 1.54]
Gao 2008 (GC)	140	440	233	960	6.5%	7.4%	1.46 [1.14; 1.87]
Total (fixed effect, 95% CI)		2308		3030	27.8%	--	1.16 [1.02; 1.32]
Total (random effects, 95% CI)					--	28.6%	1.17 [0.99; 1.37]
Heterogeneity: Tau^2 = 0.0094; Chi^2 = 4.61, df = 3 (P = 0.20); I^2 = 35%							
Total (fixed effect, 95% CI)		**9350**		**11826**	**100.0%**	**--**	**1.41 [1.32; 1.51]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.41 [1.26; 1.59]**
Heterogeneity: Tau^2 = 0.0326; Chi^2 = 40.32, df = 13 (P < 0.01); I^2 = 68%							



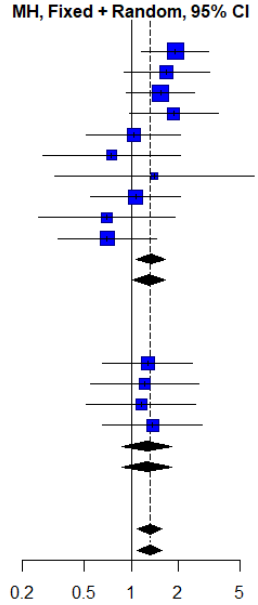
B - Cancer and TT vs TG comparison for rs11556218

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Type = Carcinoma and others							
Shih 2020	269	902	239	932	17.9%	9.5%	1.23 [1.00; 1.51]
Wu 2020	131	332	161	697	6.8%	8.1%	2.17 [1.64; 2.88]
Li 2018	252	494	247	597	11.9%	8.9%	1.48 [1.16; 1.88]
MaiMaiTiMin 2016	67	185	50	212	3.2%	5.7%	1.84 [1.19; 2.85]
Tang 2016	174	339	151	386	7.5%	7.9%	1.64 [1.22; 2.20]
Luo 2014	119	209	114	266	4.7%	6.7%	1.76 [1.22; 2.54]
Qin 2014	37	69	26	72	1.3%	3.3%	2.05 [1.04; 4.02]
Li 2011	62	184	78	238	4.9%	6.1%	1.04 [0.69; 1.57]
Gao 2009	109	200	151	361	5.3%	7.0%	1.67 [1.18; 2.36]
Gao 2008 (CRC)	219	362	197	462	7.4%	8.1%	2.06 [1.56; 2.73]
Total (fixed effect, 95% CI)		3276		4223	70.9%	--	1.59 [1.44; 1.75]
Total (random effects, 95% CI)					--	71.2%	1.63 [1.40; 1.89]
Heterogeneity: Tau^2 = 0.0301; Chi^2 = 19.77, df = 9 (P = 0.02); I^2 = 54%							
Type = Gastric and renal cancer							
He 2018	151	457	157	465	11.3%	8.3%	0.97 [0.74; 1.27]
Yang 2016	110	259	107	262	6.6%	7.0%	1.07 [0.75; 1.52]
Wang 2015	75	169	108	263	5.1%	6.3%	1.15 [0.77; 1.69]
Gao 2008 (GC)	112	206	197	462	6.0%	7.3%	1.60 [1.15; 2.23]
Total (fixed effect, 95% CI)		1091		1452	29.1%	--	1.15 [0.98; 1.36]
Total (random effects, 95% CI)					--	28.8%	1.17 [0.93; 1.46]
Heterogeneity: Tau^2 = 0.0244; Chi^2 = 5.56, df = 3 (P = 0.13); I^2 = 46%							
Total (fixed effect, 95% CI)		**4367**		**5675**	**100.0%**	**--**	**1.46 [1.35; 1.59]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.48 [1.28; 1.71]**
Heterogeneity: Tau^2 = 0.0463; Chi^2 = 36.38, df = 13 (P < 0.01); I^2 = 64%							



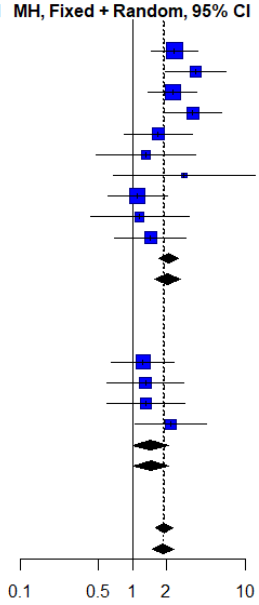
C - Cancer and TG vs GG comparison for rs11556218

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Type = Carcinoma and others							
Shih 2020	56	325	26	265	12.0%	14.2%	1.91 [1.16; 3.14]
Wu 2020	26	157	19	180	7.5%	8.7%	1.68 [0.89; 3.17]
Li 2018	44	296	28	275	12.6%	13.7%	1.54 [0.93; 2.55]
MaiMaiTiMin 2016	45	112	18	68	6.8%	8.1%	1.87 [0.97; 3.60]
Tang 2016	19	193	16	167	7.9%	7.1%	1.03 [0.51; 2.07]
Luo 2014	7	126	9	123	4.4%	3.4%	0.75 [0.27; 2.07]
Qin 2014	6	43	3	29	1.6%	1.6%	1.41 [0.32; 6.14]
Li 2011	22	84	26	104	8.7%	8.1%	1.06 [0.55; 2.06]
Gao 2009	6	115	12	163	4.8%	3.4%	0.69 [0.25; 1.90]
Gao 2008 (CRC)	14	233	18	215	8.9%	6.7%	0.70 [0.34; 1.44]
Total (fixed effect, 95% CI)		1684		1589	75.1%	--	1.33 [1.08; 1.65]
Total (random effects, 95% CI)					--	74.9%	1.30 [1.02; 1.66]
Heterogeneity: Tau^2 = 0.0243; Chi^2 = 10.74, df = 9 (P = 0.29); I^2 = 16%							
Type = Gastric and renal cancer							
He 2018	22	173	18	175	7.9%	8.0%	1.27 [0.66; 2.46]
Yang 2016	15	125	12	119	5.5%	5.4%	1.22 [0.54; 2.72]
Wang 2015	12	87	15	123	5.4%	5.3%	1.15 [0.51; 2.60]
Gao 2008 (GC)	14	126	18	215	6.0%	6.5%	1.37 [0.66; 2.86]
Total (fixed effect, 95% CI)		511		632	24.9%	--	1.26 [0.86; 1.82]
Total (random effects, 95% CI)					--	25.1%	1.26 [0.87; 1.83]
Heterogeneity: Tau^2 = 0; Chi^2 = 0.1, df = 3 (P = 0.99); I^2 = 0%							
Total (fixed effect, 95% CI)		**2195**		**2221**	**100.0%**	**--**	**1.31 [1.09; 1.58]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.31 [1.09; 1.58]**
Heterogeneity: Tau^2 = 0; Chi^2 = 10.91, df = 13 (P = 0.62); I^2 = 0%							



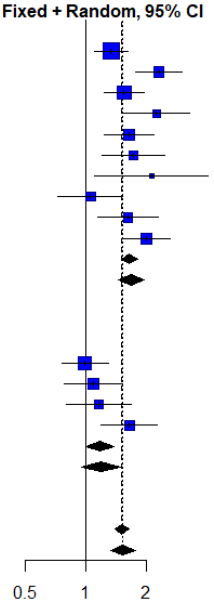
D - Cancer and TT vs GG comparison for rs11556218

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Type = Carcinoma and others							
Shih 2020	56	689	26	719	13.7%	11.7%	2.36 [1.46; 3.80]
Wu 2020	26	227	19	555	5.7%	8.6%	3.65 [1.98; 6.74]
Li 2018	44	286	28	378	11.9%	11.1%	2.27 [1.38; 3.75]
MaiMaiTiMin 2016	45	163	18	180	7.2%	9.0%	3.43 [1.89; 6.23]
Tang 2016	19	184	16	251	7.1%	7.3%	1.69 [0.84; 3.39]
Luo 2014	7	97	9	161	3.7%	4.0%	1.31 [0.47; 3.65]
Qin 2014	6	38	3	49	1.3%	2.1%	2.88 [0.67; 12.35]
Li 2011	22	144	26	186	11.3%	8.6%	1.11 [0.60; 2.05]
Gao 2009	6	97	12	222	4.0%	4.1%	1.15 [0.42; 3.17]
Gao 2008 (CRC)	14	157	18	283	6.8%	6.8%	1.44 [0.70; 2.98]
Total (fixed effect, 95% CI)		2082		2984	72.8%	--	2.10 [1.71; 2.58]
Total (random effects, 95% CI)					--	73.3%	2.04 [1.56; 2.67]
Heterogeneity: Tau^2 = 0.0644; Chi^2 = 13.93, df = 9 (P = 0.12); I^2 = 35%							
Type = Gastric and renal cancer							
He 2018	22	328	18	326	9.9%	8.1%	1.23 [0.65; 2.34]
Yang 2016	15	164	12	167	6.3%	6.0%	1.30 [0.59; 2.87]
Wang 2015	12	106	15	170	6.0%	5.9%	1.32 [0.59; 2.94]
Gao 2008 (GC)	14	108	18	283	5.1%	6.7%	2.19 [1.05; 4.58]
Total (fixed effect, 95% CI)		706		946	27.2%	--	1.45 [1.00; 2.09]
Total (random effects, 95% CI)					--	26.7%	1.46 [1.01; 2.11]
Heterogeneity: Tau^2 = 0; Chi^2 = 1.59, df = 3 (P = 0.66); I^2 = 0%							
Total (fixed effect, 95% CI)		**2788**		**3930**	**100.0%**	**--**	**1.92 [1.60; 2.30]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.87 [1.50; 2.34]**
Heterogeneity: Tau^2 = 0.0503; Chi^2 = 18.33, df = 13 (P = 0.15); I^2 = 29%							



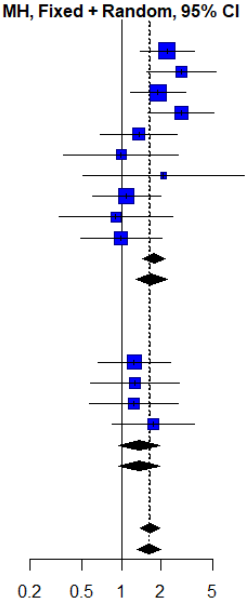
E - Cancer and TT vs TG+GG comparison for rs11556218

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Type = Carcinoma and others							
Shih 2020	325	958	265	958	17.8%	9.2%	1.34 [1.11; 1.63]
Wu 2020	157	358	180	716	6.9%	8.0%	2.33 [1.78; 3.04]
Li 2018	296	538	275	625	11.6%	8.6%	1.56 [1.23; 1.96]
MaiMaiTiMin 2016	112	230	68	230	3.5%	6.3%	2.26 [1.54; 3.32]
Tang 2016	193	358	167	402	7.4%	7.7%	1.65 [1.23; 2.19]
Luo 2014	126	216	123	275	4.6%	6.6%	1.73 [1.21; 2.48]
Qin 2014	43	75	29	75	1.3%	3.5%	2.13 [1.11; 4.09]
Li 2011	84	206	104	264	5.5%	6.5%	1.06 [0.73; 1.54]
Gao 2009	115	206	163	373	5.2%	6.9%	1.63 [1.16; 2.29]
Gao 2008 (CRC)	233	376	215	480	7.3%	7.9%	2.01 [1.53; 2.64]
Total (fixed effect, 95% CI)		3521		4398	71.1%	--	1.66 [1.51; 1.82]
Total (random effects, 95% CI)					--	71.3%	1.70 [1.46; 1.97]
Heterogeneity: Tau^2 = 0.0321; Chi^2 = 21.49, df = 9 (P = 0.01); I^2 = 58%							
Type = Gastric and renal cancer							
He 2018	173	479	175	483	11.3%	8.1%	1.00 [0.76; 1.29]
Yang 2016	125	274	119	274	6.6%	7.0%	1.09 [0.78; 1.53]
Wang 2015	87	181	123	278	5.1%	6.4%	1.17 [0.80; 1.70]
Gao 2008 (GC)	126	220	215	480	5.9%	7.2%	1.65 [1.20; 2.28]
Total (fixed effect, 95% CI)		1154		1515	28.9%	--	1.18 [1.01; 1.38]
Total (random effects, 95% CI)					--	28.7%	1.19 [0.95; 1.50]
Heterogeneity: Tau^2 = 0.0268; Chi^2 = 6.01, df = 3 (P = 0.11); I^2 = 50%							
Total (fixed effect, 95% CI)		**4675**		**5913**	**100.0%**	**--**	**1.52 [1.40; 1.64]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.54 [1.33; 1.78]**
Heterogeneity: Tau^2 = 0.0506; Chi^2 = 40.73, df = 13 (P < 0.01); I^2 = 68%							



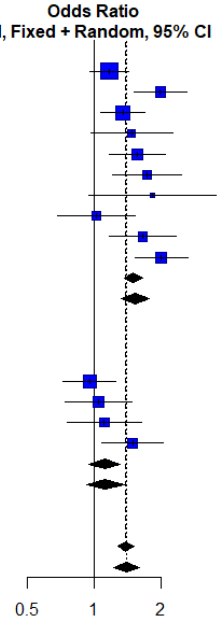
F - Cancer and TT+TG vs GG comparison for rs11556218

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Type = Carcinoma and others							
Shih 2020	56	958	26	958	12.7%	12.0%	2.23 [1.39; 3.58]
Wu 2020	26	358	19	716	6.1%	8.6%	2.87 [1.57; 5.27]
Li 2018	44	538	28	625	12.3%	11.6%	1.90 [1.17; 3.10]
MaiMaiTiMin 2016	45	230	18	230	7.5%	9.2%	2.86 [1.60; 5.12]
Tang 2016	19	358	16	402	7.4%	7.2%	1.35 [0.68; 2.67]
Luo 2014	7	216	9	275	4.0%	3.8%	0.99 [0.36; 2.70]
Qin 2014	6	75	3	75	1.4%	2.0%	2.09 [0.50; 8.67]
Li 2011	22	206	26	264	10.5%	8.7%	1.09 [0.60; 1.99]
Gao 2009	6	206	12	373	4.3%	3.8%	0.90 [0.33; 2.44]
Gao 2008 (CRC)	14	376	18	480	7.9%	6.7%	0.99 [0.49; 2.02]
Total (fixed effect, 95% CI)		3521		4398	74.1%	--	1.76 [1.44; 2.16]
Total (random effects, 95% CI)					--	73.7%	1.69 [1.28; 2.22]
Heterogeneity: Tau^2 = 0.0726; Chi^2 = 14.77, df = 9 (P = 0.10); I^2 = 39%							
Type = Gastric and renal cancer							
He 2018	22	479	18	483	8.9%	8.0%	1.24 [0.66; 2.35]
Yang 2016	15	274	12	274	5.9%	5.8%	1.26 [0.58; 2.75]
Wang 2015	12	181	15	278	5.7%	5.8%	1.24 [0.57; 2.72]
Gao 2008 (GC)	14	220	18	480	5.5%	6.7%	1.74 [0.85; 3.57]
Total (fixed effect, 95% CI)		1154		1515	25.9%	--	1.35 [0.94; 1.94]
Total (random effects, 95% CI)					--	26.3%	1.36 [0.95; 1.95]
Heterogeneity: Tau^2 = 0; Chi^2 = 0.62, df = 3 (P = 0.89); I^2 = 0%							
Total (fixed effect, 95% CI)		**4675**		**5913**	**100.0%**	**--**	**1.66 [1.39; 1.98]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.61 [1.31; 1.99]**
Heterogeneity: Tau^2 = 0.0351; Chi^2 = 16.86, df = 13 (P = 0.21); I^2 = 23%							



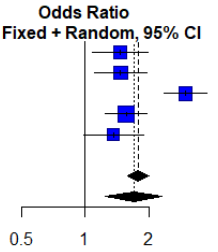
G - Cancer and TT+GG vs TG comparison for rs11556218

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Type = Carcinoma and others							
Shih 2020	269	958	239	958	17.5%	9.7%	1.17 [0.96; 1.44]
Wu 2020	131	358	161	716	6.9%	8.1%	1.99 [1.51; 2.63]
Li 2018	252	538	247	625	12.4%	9.0%	1.35 [1.07; 1.70]
MaiMaiTiMin 2016	67	230	50	230	3.6%	5.6%	1.48 [0.97; 2.26]
Tang 2016	174	358	151	402	7.5%	7.9%	1.57 [1.18; 2.10]
Luo 2014	119	216	114	275	4.6%	6.6%	1.73 [1.21; 2.48]
Qin 2014	37	75	26	75	1.3%	3.2%	1.84 [0.95; 3.54]
Li 2011	62	206	78	264	4.9%	6.0%	1.03 [0.69; 1.53]
Gao 2009	109	206	151	373	5.2%	6.9%	1.65 [1.17; 2.33]
Gao 2008 (CRC)	219	376	197	480	7.4%	8.2%	2.00 [1.52; 2.64]
Total (fixed effect, 95% CI)		3521		4398	71.3%	--	1.50 [1.36; 1.65]
Total (random effects, 95% CI)					--	71.3%	1.53 [1.32; 1.77]
Heterogeneity: Tau^2 = 0.0283; Chi^2 = 19.51, df = 9 (P = 0.02); I^2 = 54%							
Type = Gastric and renal cancer							
He 2018	151	479	157	483	10.9%	8.3%	0.96 [0.73; 1.25]
Yang 2016	110	274	107	274	6.5%	6.9%	1.05 [0.74; 1.47]
Wang 2015	75	181	108	278	5.1%	6.3%	1.11 [0.76; 1.63]
Gao 2008 (GC)	112	220	197	480	6.2%	7.3%	1.49 [1.08; 2.05]
Total (fixed effect, 95% CI)		1154		1515	28.7%	--	1.12 [0.95; 1.31]
Total (random effects, 95% CI)					--	28.7%	1.13 [0.92; 1.37]
Heterogeneity: Tau^2 = 0.0138; Chi^2 = 4.5, df = 3 (P = 0.21); I^2 = 33%							
Total (fixed effect, 95% CI)		**4675**		**5913**	**100.0%**	**--**	**1.39 [1.28; 1.51]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.41 [1.23; 1.61]**
Heterogeneity: Tau^2 = 0.0391; Chi^2 = 33.51, df = 13 (P < 0.01); I^2 = 61%							



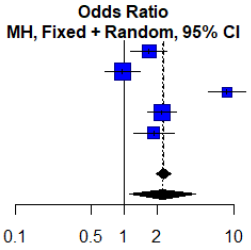
H - Cardiovascular disease and T vs G comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Liu 2013	118	396	106	472	16.4%	19.3%	1.47 [1.08; 1.99]
Tong 2013	137	652	104	680	19.4%	19.9%	1.47 [1.11; 1.95]
Chen 2001 (1)	281	600	181	794	20.0%	21.0%	2.98 [2.37; 3.76]
Chen 2001 (2)	288	848	164	664	29.3%	21.1%	1.57 [1.25; 1.97]
Wu 2011	100	314	103	404	14.8%	18.8%	1.37 [0.99; 1.89]
Total (fixed effect, 95% CI)		**2810**		**3014**	**100.0%**	**--**	**1.79 [1.59; 2.01]**
Total (random effects, 95% CI)					**--**	**100.0%**	**1.70 [1.25; 2.32]**
Heterogeneity: Tau^2 = 0.1043; Chi^2 = 26.29, df = 4 (P < 0.01); I^2 = 85%							



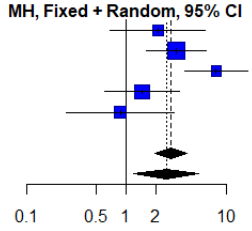
I - Cardiovascular disease and TT vs TG comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Liu 2013	102	190	94	230	19.3%	19.9%	1.68 [1.14; 2.47]
Tong 2013	69	292	80	328	28.1%	20.0%	0.96 [0.66; 1.39]
Chen 2001 (1)	235	277	149	381	9.3%	19.9%	8.71 [5.91; 12.84]
Chen 2001 (2)	264	412	144	322	28.4%	20.4%	2.20 [1.64; 2.97]
Wu 2011	92	153	87	194	14.9%	19.6%	1.85 [1.21; 2.85]
Total (fixed effect, 95% CI)		**1324**		**1455**	**100.0%**	**--**	**2.31 [1.97; 2.70]**
Total (random effects, 95% CI)					**--**	**100.0%**	**2.25 [1.12; 4.50]**
Heterogeneity: Tau^2 = 0.5907; Chi^2 = 70.53, df = 4 (P < 0.01); I^2 = 94%							



J - Cardiovascular disease and TT vs GG comparison for rs11556218.

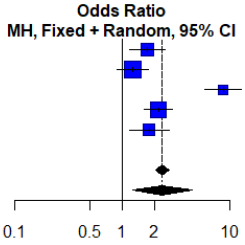
Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Liu 2013	8	96	6	142	13.4%	17.7%	2.06 [0.69; 6.14]
Tong 2013	34	257	12	260	31.4%	23.1%	3.15 [1.59; 6.24]
Chen 2001 (1)	23	65	16	248	13.0%	22.6%	7.94 [3.87; 16.28]
Chen 2001 (2)	12	160	10	188	25.8%	20.6%	1.44 [0.61; 3.43]
Wu 2011	4	65	8	115	16.4%	15.9%	0.88 [0.25; 3.03]
Total (fixed effect, 95% CI)		**643**		**953**	**100.0%**	**--**	**2.81 [1.94; 4.08]**
Total (random effects, 95% CI)					**--**	**100.0%**	**2.50 [1.19; 5.25]**
Heterogeneity: Tau^2 = 0.4991; Chi^2 = 14.11, df = 4 (P < 0.01); I^2 = 72%							



K - Cardiovascular disease and TT vs TG + GG comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Liu 2013	110	198	100	236	18.9%	19.9%	1.70 [1.16; 2.49]
Tong 2013	103	326	92	340	28.7%	20.2%	1.25 [0.89; 1.74]
Chen 2001 (1)	258	300	165	397	9.3%	19.9%	8.64 [5.89; 12.66]
Chen 2001 (2)	276	424	154	332	28.1%	20.5%	2.16 [1.61; 2.89]
Wu 2011	96	157	95	202	15.0%	19.6%	1.77 [1.16; 2.71]
Total (fixed effect, 95% CI)		**1405**		**1507**	**100.0%**	--	**2.35 [2.02; 2.74]**
Total (random effects, 95% CI)					--	**100.0%**	**2.33 [1.24; 4.41]**

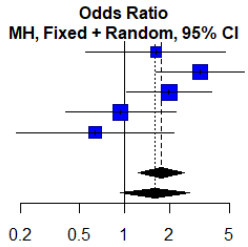
Heterogeneity: $Tau^2 = 0.4923$; $Chi^2 = 63.20$, df = 4 (P < 0.01); $I^2 = 94\%$



L - Cardiovascular disease and TT + TG vs GG comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Liu 2013	8	198	6	236	11.4%	15.6%	1.61 [0.55; 4.73]
Tong 2013	34	326	12	340	22.8%	25.1%	3.18 [1.62; 6.26]
Chen 2001 (1)	23	300	16	397	27.5%	25.7%	1.98 [1.03; 3.81]
Chen 2001 (2)	12	424	10	332	23.6%	20.4%	0.94 [0.40; 2.20]
Wu 2011	4	157	8	202	14.8%	13.2%	0.63 [0.19; 2.14]
Total (fixed effect, 95% CI)		**1405**		**1507**	**100.0%**	--	**1.77 [1.24; 2.53]**
Total (random effects, 95% CI)					--	**100.0%**	**1.60 [0.93; 2.73]**

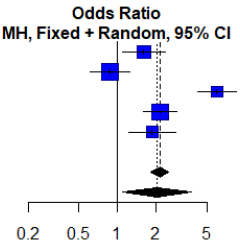
Heterogeneity: $Tau^2 = 0.1796$; $Chi^2 = 7.89$, df = 4 (P = 0.10); $I^2 = 49\%$



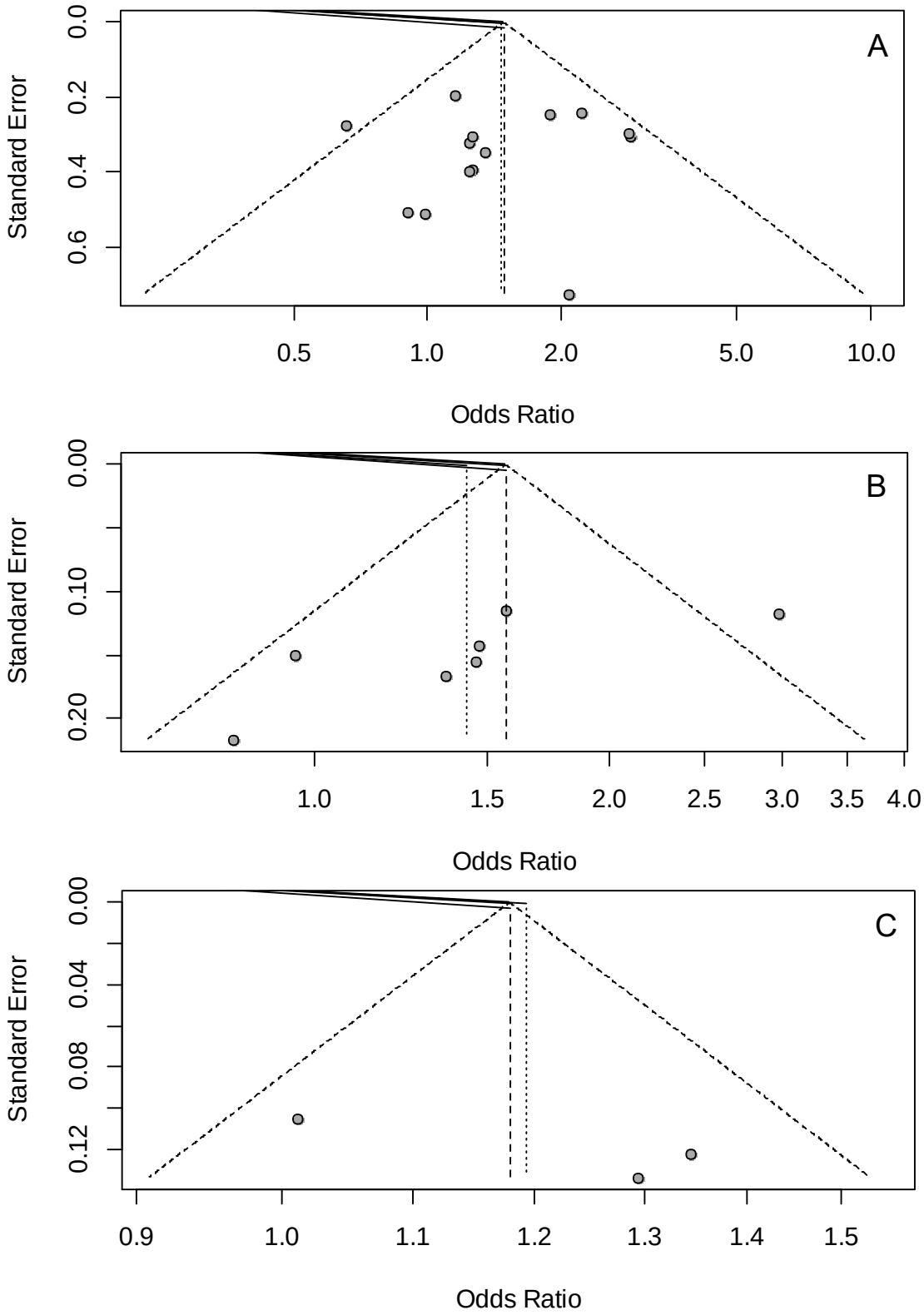
M - Cardiovascular disease and TT + GG vs TG comparison for rs11556218.

Study	Experimental Events	Experimental Total	Control Events	Control Total	Weight (fixed)	Weight (random)	Odds Ratio MH, Fixed + Random, 95% CI
Liu 2013	102	198	94	236	18.6%	19.9%	1.61 [1.10; 2.35]
Tong 2013	69	326	80	340	27.6%	20.0%	0.87 [0.61; 1.26]
Chen 2001 (1)	235	300	149	397	12.4%	20.2%	6.02 [4.28; 8.47]
Chen 2001 (2)	264	424	144	332	27.3%	20.5%	2.15 [1.61; 2.89]
Wu 2011	92	157	87	202	14.1%	19.5%	1.87 [1.23; 2.85]
Total (fixed effect, 95% CI)		**1405**		**1507**	**100.0%**	--	**2.14 [1.83; 2.49]**
Total (random effects, 95% CI)					--	**100.0%**	**2.03 [1.09; 3.78]**

Heterogeneity: $Tau^2 = 0.4683$; $Chi^2 = 60.92$, df = 4 (P < 0.01); $I^2 = 93\%$



Supplementary Figure 2. Forest plot of comparisons for all polymorphisms associated with disease in the pooled studies with control groups in Hardy-Weinberg equilibrium.



Supplementary Figure 3. Funnel plot of allele comparison of SNP rs11556218 and cancer (A), rs11556218 and CVD (B) and rs4778889 and gastric cancer (C).

Manuscrito II: "Association of *HLA* and *KIR* polymorphisms with different forms of COVID-19 *in-silico* study: a systematic review and meta-analysis."

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Association of *HLA* and *KIR* polymorphisms with different forms of COVID-19 *in-silico* study: a systematic review and meta-analysis

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Keywords: SARS-CoV-2; Genetic Variation; *HLA* antigens.

SUMMARY

COVID-19 is a complex viral infection with a network of factors. *HLA* and *KIR* variants are essential host genetic components in orchestrate the immune response against viruses. We investigated possible *HLA/KIR* genetics associated with different forms of COVID-19 with the aid of *in-silico* analyses through a systematic review and meta-analysis. The methodology of this review is based on the PRISMA statement. A search was made in PubMed, Web of Science and Scopus databases. All statistical analyses were performed in R 4.2.0 (package “meta”). The meta-analysis results are presented in forest plots. The SARS-CoV-2 Spike protein were evaluated for predicted binding affinity with different *HLA* alleles using the MHC-Pan. The affinity results were also compared for different viral strains. This review comprised a total of 43 *HLA* and three *KIR* studies in COVID-19. The meta-analysis study indicated some alleles associated with a higher risk for COVID-19 and its

clinical forms. The *HLA-A*11:01*, *HLA-A*23*, *HLA-A*66*, *HLA-B*07*, *HLA-B*14*, *HLA-B*57* and *HLA-C*04:01* alleles were associated with risk for COVID-19 and predicted to have low-affinity ligands to protein Spike. On the other hand, *HLA-C*03*, *HLA-C*05*, *HLA-DRB1*01:01*, *HLA-DRB1*04:01*, *HLA-DRB1*10* and *HLA-DRB1*12* alleles were associated with protection against COVID-19 and higher affinity to viral protein. In this study, *KIR2DL2* and *KIR2DS3* genes are associated with increased risk to COVID-19. This study seems to indicate that genetic variability related to NK cells may play a role in COVID-19. Despite this, the statistical power and complexity/variability of host-virus interaction remains an important challenge for genetic association studies.

1. INTRODUCTION

The disease caused by the novel coronavirus (COVID-19) is a viral infection initially characterized as an acute respiratory syndrome [1] with high transmissibility. Until the beginning of the year 2023, more than 675 million cases have been confirmed worldwide, with more than 6.8 million deaths [2]. A complex network of factors related to host-virus interaction, as well as other aspects such as public health measures, may explain the different pandemic scenarios in the world [3,4]. Patients affected by COVID-19 present a wide range of symptoms, from mild ones, to more severe, such as pneumonia [5]. One of the most important risk factor for the disease appears to be advanced age [6]. However, other factors such as host genetic background are also important [6].

Host genetic diversity play a role on the modulation of the immune response in viral infections [7]. Particularly, the variability of the major histocompatibility complex (MHC) system, also known as human leukocyte antigen (HLA), may be determinant to the immune response against viral infections because its genes encode cell surface molecules specialized in presenting immunodominant epitopes to immunocompetent cells [8]. The *HLA* genes are located on chromosome 6p21, the most polymorphic region of the human genome [9], which reflects on distinct *HLA* alleles that may have different affinity to antigens peptides

that are presented to T lymphocytes and natural killer (NK) cells [10–12]. NK and T cells play an important role in fighting viruses. NK cells act under regulation by molecules expressed on their membrane, such as the killer cell immunoglobulin-like receptors (KIRs). KIRs are a family of highly polymorphic type 1 transmembrane glycoproteins that provide inhibitory or activating signals to NK cells after their interaction with HLA class I allotypes. While T lymphocyte subsets are activated by T cell receptor (TCR)/MHC-peptide interactions signaling [13].

Although many studies are being done with the aim of understanding the association of these genes with COVID-19, the main problem in association studies is the lack of statistical power due to the need for extremely large samples to detect subtle genetic effects. Increasing power represents greater accuracy in detecting subtle genetic effects in a population. As the frequency of genetic variants is proportional to the sample size needed to study it, a pool of multiple studies can be very useful to increase the detection of disease-associated genetic variants. In addition, *in-silico* studies with HLA peptides have favoured the understanding of the role of different *HLA* alleles in COVID-19. The binding affinity of an epitope to the MHC molecule plays an important role in determining its immunogenicity, and high affinity bindings tend to be associated with greater immune responsiveness, although this is not the only determining factor of immunogenicity [14]. The objective of this study was to evaluate the possible associations between genetic variants of *HLA/KIR* with the different forms of COVID-19 with the aid of systematic reviews and meta-analyses and *in-silico* analyses.

2. METHODS

2.1. Databases search

The methodology of this review is based on the PRISMA statement [15]. This study was registered on the systematic review platform PROSPERO (under id CRD42022367604). This review was performed at all stages by at least two independent authors (VHS, MB and

JMVZ) and disagreements were resolved between all authors. Our search was carried out in the PubMed, Web of Science and SCOPUS databases covering the period from 01/01/2019 to 10/31/2022. These searches and the databases were created using the R 4.2.0 software (packages “wosr” and “easyPubmed”). The articles were selected based on a filtering method (Diagram 1). Initially, we selected all the results of the search terms in the three databases (Supplementary Table 1). After that, we removed the duplicate results, leaving a total of 6000 records. These articles were evaluated by title and abstract for suitability to the proposed theme: original studies that, somehow, brought results about possible associations of host immune response genes with COVID-19. After remove duplicates, the studies were filtered by their type, excluding papers that were not original, such as reviews, retracted articles, letters, comments, news, technical report, congress, and editorials. Thus, we were able to evaluate 128 complete papers to identify association studies with *HLA* alleles or *KIR* genes, finding 43 original studies with these criteria. Finally, we separated the studies in the qualitative analyses group (all 43) and meta-analysis group (removed 10 studies that did not bring enough data to meta-analysis).

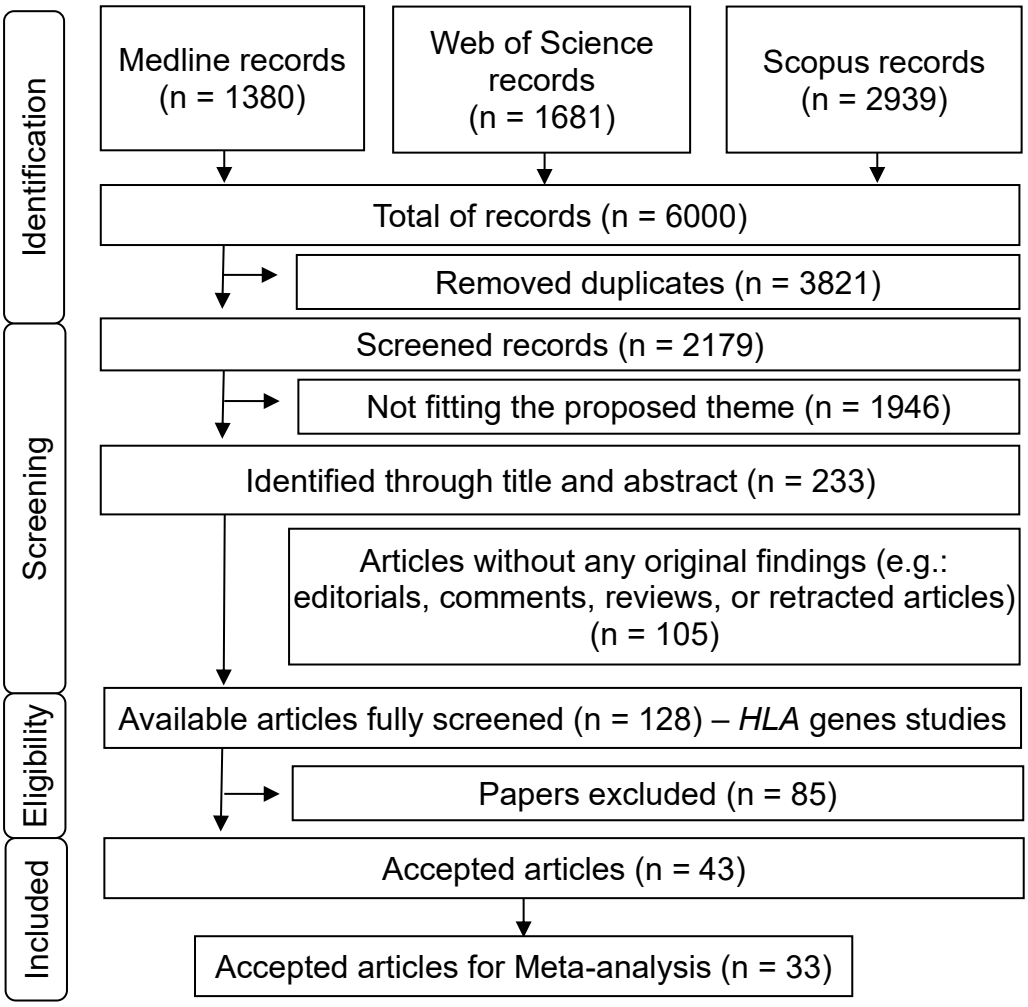

Diagram 1. Flowchart of the studies screening.

Supplementary Table 1. Search strategy of the review.

Search strategy	Base	records
(((Genotype [MeSH Terms]) OR (Alleles [MeSH Terms])) OR (Polymorphism, genetic [MeSH Terms]) OR (Histocompatibility Antigens [MeSH Terms])) AND ((((SARS-CoV-2[MeSH Terms]) OR (Betacoronavirus [MeSH Terms])) OR (severe acute respiratory syndrome [MeSH Terms])) OR (Coronavirus infections [MeSH Terms]))	Pubmed	1380
(Genotype OR Alleles OR Polymorphism, genetic OR Histocompatibility Antigens) AND (SARS-CoV-2 OR betacoronaviruses OR severe acute respiratory syndrome OR Coronavirus infections)	Web of Science	1681
(TITLE-ABS-KEY (genotype) OR TITLE-ABS-KEY (alleles) OR TITLE-ABS-KEY (polymorphism, AND genetic) OR TITLE-ABS-KEY (histocompatibility AND antigens) AND TITLE-ABS-KEY (sars-cov-2) OR TITLE-ABS-KEY (betacoronaviruses) OR TITLE-ABS-KEY (severe AND acute AND respiratory AND syndrome) OR TITLE-ABS-KEY (coronavirus AND infections)) AND PUBYEAR > 2018	Scopus	2939

2.2. Meta-analysis

Distinct allele count data were collected from the selected studies for *HLA-A*, *HLA-B*, *HLA-C* and *HLA-DRB1* loci and *KIR* genes. They were compared in a χ^2 test with the sum of the remaining alleles/genes, given by the formula y = (2 × N) - n, where “y” is the sum of remaining alleles/genes, “N” the total sample size, and “n” the number of the selected allele or KIR gene. Different groups of COVID-19 were subdivided into disease severity subgroups: asymptomatic; mild disease, when individuals do not require hospitalization; moderate disease, when requiring some degree of hospital care; and severe disease, when requiring intensive care or even dying.

All statistical analyses were performed in R software (package “meta”) [16]. The meta-analysis results are presented in forest plots. This plot summarizes the results in a pool of studies, combining individuals genetic effect size (*Odds Ratio*) with a 95% confidence interval (CI), resulting in two possible models according to the observed heterogeneity of the studies (I^2): fixed and random models. When the value of I^2 was greater than 50%, the random OR model was chosen. This model attempts to reduce the effect that very large or

small studies can have on the analysis. When I^2 was less than 50% with no evidence of significant heterogeneity, the fixed model was chosen.

Statistical power analysis was performed using the QUANTO software [17]. Different allele frequencies were used in a simulation with different OR sizes, to verify the available statistical power.

2.3. *In-silico* assays

The sequences of the different SARS-CoV-2 Spike protein variants were obtained from NCBI data (www.ncbi.nlm.nigh.gov/sars-cov-2). These sequences were evaluated in peptides of 9 (class I) and 13 (class II) amino acids. These peptides were evaluated for predicted binding affinity with different *HLA* alleles by the half maximal inhibitory concentration (IC50) using the MHC-Pan ("smm" methods for class I and "nn align" for class II). Peptides were considered "non-binders" when having an IC50 greater than 5000 nM. They were considered "weak binders" when their IC50 was between 500 and 5000 nM (class I) or between 1000 and 5000nM (class II). Furthermore, they were considered "regular ligands" when their IC50 was between 50 and 500nM (class I) or 50 and 1000nM (class II). Finally, those with IC50 less than 50 nM were considered "strong binders". These results were also compared for different viral strains, to see the differences that viral mutations would cause in affinity for *HLA* alleles.

2.4. *Quality analysis of the selected studies*

The quality assessment of the selected studies was originally based on the Newcastle-Ottawa scale (NOS) [18], performed with modifications and made by four independent authors (VHS, JMVZ, QALN and MB). We added topics to be qualitatively assessed within each study (Figure 1), with responses ranked according to the risk of bias they bring to our study conclusions. For example, when a study does not use asymptomatic controls, but only generic control groups (without confirmation of contact with the virus), it can be a high risk of bias for that specific assessment. Possible biases of published meta-

analyses were verified by funnel plot visual evaluation, and Begg's test [19] and these analyzes did not point to major risks of bias. The Hardy-Weinberg equilibrium (HWE) of each study was checked according to the chi-square test for goodness-of-fit, as recommended for assessing study quality [20].

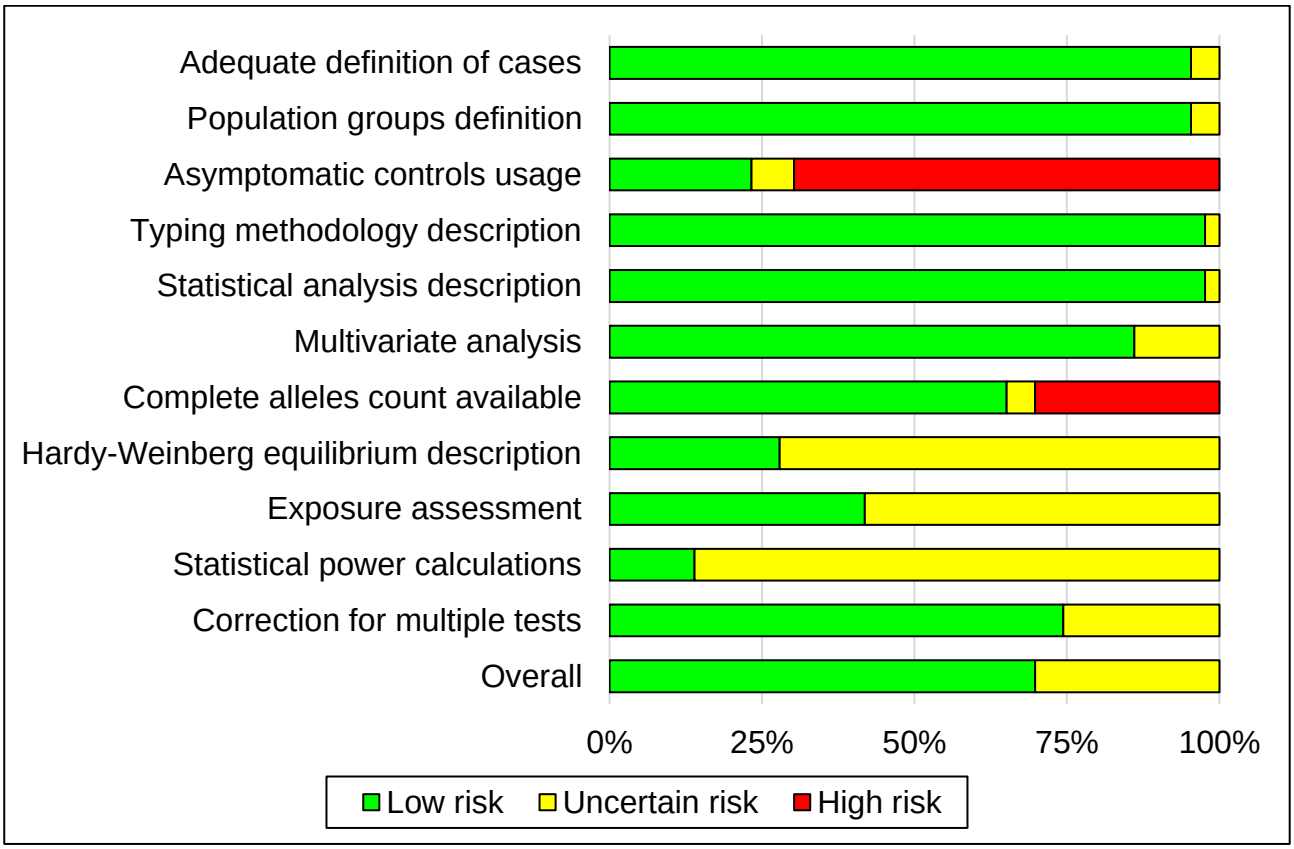

Figure 1. Quality analysis of the selected studies.

3. RESULTS

This review comprised a total of 43 original *HLA* association studies with COVID-19 (more details in Supplementary Table 2). Only three of these studies also evaluated the *KIR* genes. We observed that half of all studies evaluated white populations, while non-whites divided all other studies, especially Asian populations. A very low representation was observed for non-white populations in undeveloped countries, although these have also been greatly affected by COVID-19. The meta-analysis stage included a total of 33 studies, with more than 3000 individuals with COVID-19. The meta-analysis comprised the evaluation of 160 low-resolution and 313 high-resolution *HLA-A*, *HLA-B*, *HLA-C*, *HLA-*

DRB1, *HLA-DPA1*, *HLA-DPB1*, *HLA-DQA1* and *HLA-DQB1* alleles, and 16 *KIR* genes. In this study, different alleles were associated with COVID-19, especially class I alleles. The associated results are shown in Table 1.

Many of these associations at low resolution were repeated for some specific alleles at high resolution. Despite the accumulation of studies, their heterogeneity and the limitation of sample size was a limitation for statistical power. We observed important statistical power limitations for many *HLA* alleles and *KIR* genes. This means that even if the studies (or the pool of studies) have a good sample size, rare alleles may suffer from low statistical power for association studies.

Table 1. Meta-analysis results of *HLA* and *KIR* association with different groups of COVID-19.

Variant	Studies	Event	Control	OR	CI 95%	Model	I^2
Mild and moderate *vs.* severe COVID-19							
*A*30*	3	443	627	1.64	1.23 - 2.19	F	0.00
Mild vs. severe COVID-19							
C2 group	4	295	337	0.67	0.47 - 0.96	F	0.00
*A*03*	6	346	275	0.63	0.40 - 0.99	F	0.00
*A*11*	7	381	412	2.09	1.40 - 3.12	F	0.00
*A*11:01*	4	187	219	2.62	1.32 - 5.20	F	0.00
*A*23*	3	183	184	2.66	1.02 - 6.90	F	0.00
*DQA1*01*	3	133	196	1.77	1.15 - 2.72	F	31.50
*DQA1*05*	4	188	166	1.90	1.30 - 2.76	F	19.17
Mild *vs.* moderate COVID-19							
*A*02:01*	3	67	73	0.18	0.05 - 0.74	R	62.56
Symptomatic *vs.* asymptomatic COVID-19							
C2 group	3	4211	536	1.26	1.11 - 1.44	R	62.02
*B*07*	5	6346	888	1.21	1.03 - 1.43	F	30.45
*B*49*	4	6958	915	0.70	0.51 - 0.95	F	0.00
*DRB1*04*	5	5891	1055	0.85	0.74 - 0.97	F	38.48
*DRB1*10*	3	6191	1106	0.66	0.43 - 0.99	F	28.47
*DRB1*15*	5	5886	1112	1.25	1.08 - 1.46	F	25.68
Severe *vs.* asymptomatic COVID-19							
Bw4/A23/A24/A32	3	110	262	2.00	1.01 - 3.97	R	70.86
*DRB1*04*	3	110	273	1.80	1.20 - 2.69	F	0.00
*DRB1*14*	3	142	300	0.43	0.20 - 0.90	F	0.00
Mild COVID-19 *vs.* healthy controls							
KIR2DL2	3	359	516	1.36	1.10 - 1.68	F	37.31
Severe COVID-19 *vs.* healthy controls							
C2 group	3	316	687	0.76	0.59 - 0.99	F	0.00
*A*24*	3	184	1280	0.65	0.45 - 0.93	F	0.00

Variant	Studies	Event	Control	OR	CI 95%	Model	I^2
*B*14*	5	429	3109	1.53	1.19 - 1.95	F	24.42
*B*52*	3	256	1429	1.81	1.11 - 2.95	F	0.00
*B*55*	4	423	2686	1.68	1.01 - 2.78	F	30.65
KIR2DS3	3	295	580	1.29	1.01 - 1.65	F	0.00
Symptomatic COVID-19 *vs.* healthy controls							
C1 group	3	960	16082	1.55	1.28 - 1.87	F	34.07
*A*66*	3	729	5428	2.23	1.13 - 4.41	F	0.00
*B*14*	10	2333	7937	1.31	1.15 - 1.51	F	0.00
*B*18*	8	1656	6295	0.79	0.67 - 0.91	F	38.02
*B*40*	12	2364	34596	1.17	1.00 - 1.36	F	44.05
*B*51*	12	2326	34931	0.82	0.72 - 0.94	F	32.91
*B*51:01*	6	1225	29724	0.68	0.54 - 0.84	F	18.57
*B*56*	5	7060	4970	1.99	1.10 - 3.59	F	41.29
*B*57*	10	2020	14081	1.47	1.11 - 1.93	F	25.62
*C*03*	8	1511	28780	0.84	0.71 - 0.99	F	0.00
*C*04*	7	1222	27912	1.38	1.06 - 1.80	R	72.38
*C*04:01*	4	758	23732	1.53	1.14 - 2.05	R	69.56
*C*05*	7	1323	35335	0.77	0.65 - 0.92	F	49.69
*DRB1*01:01*	5	1036	26065	0.76	0.60 - 0.96	F	11.23
*DRB1*04*	12	1873	38386	0.80	0.67 - 0.96	R	50.00
*DRB1*04:01*	4	879	25739	0.67	0.47 - 0.96	F	0.00
*DRB1*12*	10	1904	27568	0.59	0.42 - 0.81	F	0.00
*DRB1*16*	9	1485	9557	1.29	1.06 - 1.57	F	0.92
*DQB1*02:01*	4	774	22583	1.29	1.10 - 1.51	F	35.28
*DQB1*04:02*	4	867	25834	1.39	1.04 - 1.86	F	0.00
*DQB1*05*	9	1408	36290	1.17	1.04 - 1.32	F	42.30
*DQB1*05:02*	3	638	4377	1.55	1.10 - 2.20	F	0.00
KIR2DL2	3	1260	1032	1.22	1.02 - 1.47	F	35.00

n: Number of selected studies; R: Random model; F: Fixed Model; OR: Pooled *Odds Ratio; I^2: Heterogeneity test*.
* The compared groups consisted of different subgroups of individuals with COVID-19 and a group of healthy controls. For all comparisons $P<0.05$.

The different alleles described in the studies were also analyzed via *in-silico* study for 21 variants of SARS-CoV-2. In this analysis, we selected different variants of the SARS-CoV-2 Spike protein. Viral variants were classified according to phylogenetic analysis of their similarity. Different proportions of predicted binding affinities of viral peptides with *HLA* alleles were observed. The results of these analyzes are described in Figure 2 and were classified from alleles that present these peptides with greater affinity to those with lower

affinity. In general, we noticed that *HLA-DRB1* and *HLA-C* loci have a greater affinity for binding to this viral protein than the other loci.

We also evaluated how many peptides changed their affinity for each allele in a new viral strain (Supplementary Figure 1). We observed that, as expected, viral genetic polymorphisms can change the affinity of these peptides to HLA molecules. This can be particularly noted in omicron variants and their subvariants. The virus appears to modify the binding efficiency of HLA alleles by changing viral protein's structure. Alleles that were efficient (or inefficient) can gradually lose this ability as the virus changes.

The meta-analysis study indicated some alleles associated with a higher risk for COVID-19 and its clinical forms. For example, the *HLA-A*11:01* allele was associated with increased risk for severe COVID-19 compared to mild forms (OR=2.62, CI=1.32-5.20). This allele was also predicted to have low affinity for SARS-CoV-2 protein S.

Other risk-associated class I allelic groups with low predicted binding affinity were: *HLA-A*23*, *HLA-A*66*, *HLA-B*07*, *HLA-B*14*, *HLA-B*57* and *HLA-C*04:01*. It is important to note that some of these alleles, for example the *HLA-C*04:01* (OR=1.53, CI=1.14-2.05), improved its affinity to omicron variants (Supplementary Figure 1-O).

We also observed that *HLA-DRB1* and *HLA-C* alleles were associated with protection against different forms of COVID-19. Many of these alleles also had a high affinity in the *in-silico* analysis. They were *HLA-C*03*, *HLA-C*05*, *HLA-DRB1*01:01*, *HLA-DRB1*04:01*, *HLA-DRB1*10* and *HLA-DRB1*12*. In this case, we can also observe that some alleles, such as *HLA-C*05:01*, have even more increased affinity in omicron variants, although this increase is not a rule to all alleles. Finally, although limited in number and small sample size, the meta-analysis of the *KIR* studies pointed to two genes related to risk for COVID-19 compared to healthy controls: *KIR2DL2* (OR=1.36, CI=1.10 – 1.68) and *KIR2DS3* (OR=1.29, CI= 1.01 – 1.65).

Supplementary Table 2. Selected studies of *HLA* and *KIR* variants association with different groups of COVID-19.

Reference	Studied genes	Main findings	Methodology HLA	Country
[40]	*HLA* (Class I and II)	HLA-B07 supertype was associated with a significant risk of severe COVID-19; on the other hand, the HLA-B27 and *HLA-C*12:02* alleles were protective to COVID-19. These associations were confirmed after multiple regression analyses.	PCR-SSP	Italy
[41]	*HLA* (Class I and II)	The *HLA-A*01:01* allele was reduced in a cohort of patients with severe bilateral pneumonia and could therefore be considered a protective factor for severe symptoms in COVID-19.	NGS	Russia
[42]	*HLA* (Class I and II)	*HLA-A*68* has been found to prevent COVID-19 severity and death. *HLA-DRB1*03* also appears to be a protective factor, but the low frequency of this allele in the study population limits statistical significance.	PCR-SSP	Mexico
[22]	*HLA* (Class I and II)	They observed age-dependent protection against severe COVID-19 by *HLA-B*51:01*. In contrast, the *HLA-C*04:01* allele was associated with a significant dose-dependent increase in disease risk.	NGS	Armenia
[43]	*HLA* (class I)	The alleles associated with severe COVID-19 were *HLA-B*41*, *HLA-B*42*, *HLA-C*16* and *HLA-C*17*, while *HLA-B*15*, *HLA-C*07* and *HLA- C*12* were significantly associated with protection from death. Regression analysis indicated that *HLA-B*15* was associated with death protection.	PCR-SSO	Egypt
[44]	*HLA* (Class I and II)	Alleles *HLA-B*51:01* and *HLA-A*26:01* showed an association between protection and severity of COVID-19; while *HLA-A*03:01*, *HLA-DRB1*15:01* and supertype B44 are associated with the risk to COVID-19.	NGS	United Arab Emirates
[35]	*HLA* (class II)	The presence of the *HLA-DRB1*04* allele was associated with protection against severe and critical COVID-19.	PCR-SSP	Iran

Reference	Studied genes	Main findings	Methodology HLA	Country
36	*HLA* (Class I and II)	The frequencies of *HLA-A*01*, *HLA-B*56* and *HLA-C*01* were significantly increased in COVID-19 patients. In addition, the frequencies of *HLA-A*03* and *HLA-C*06* were significantly increased in fatal cases. Regarding HLA class II, *HLA-DRB1*04* was significantly elevated in controls, whereas *HLA-DRB1*08* was significantly elevated in the COVID-19 group.	PCR-SSO	Saudi Arabia
45	*HLA* (Class I and II)	The alleles *HLA-A*11:01:01:01*, *HLA-C*12:02:02:01* and *HLA-B*52:01:01:02* were found to be associated with the severity of COVID-19. After multivariate analysis, the *HLA-A*11:01:01:01* association remained significant after accounting for other confounding factors and comorbidities.	PCR-SBT	Japan
37	*HLA* (Class I and II)	The study found a significant difference in the frequency of *HLA-DRB1*04:01* alleles in severe COVID-19 patients compared with the asymptomatic group. The frequencies *of HLA-DQA1*01:01*, *HLA-DQB1*05:01* and *HLA-DRB1*01:01* haplotypes were significantly lower in the asymptomatic group compared to the UK background population.	NGS	United Kingdom
46	*HLA* (Class I and II)	*HLA-B*51* and *HLA-DRB1*13* alleles were significantly associated with COVID-19 death and *HLA-B*35* was associated with mild infection (although not after Bonferroni correction).	PCR-rSSO	Pakistanis, Indians, and Bangladeshis
47	*HLA* (Class I and II)	The *HLA-DRB1*09:01* allele was significantly associated with the risk of severe COVID-19.	PCR-SSO	Japan
48	*HLA* (Class I)	The presence of the *HLA-A*01:01* allele was associated with a high risk, whereas *HLA-A*02:01* and *HLA-A*03:01* alleles were primarily responsible for a low risk of COVID-19.	NGS	Russia
23	*HLA* (Class I and II)	The data suggest that the extended haplotype *HLA-A*02:05-B*58:01-C*07:01-DRB1*03:01* is protective against COVID-19 in the Sardinian population. Genetic factors that negatively affected disease progression was the presence	PCR-SSP and NGS	Italy

Reference	Studied genes	Main findings	Methodology HLA	Country
		of the *HLA-DRB1*08:01* allele and G6PDH deficiency, but not the beta thalassemia trait.		
49	*HLA*-B	A significant positive association was found between the HLA-B22 group and COVID-19.	PCR-SBT	China (Hong Kong)
38	*HLA* (Class I and II)	Logistic regression analysis indicated that the presence of certain alleles was associated with higher mortality, such as *HLA-A*11*, *HLA-C*01*, and *HLA-DQB1*04.*	PCR-SSO	Spain (Canarias)
3	*HLA* (Class I)	Probably due to low statistical power, allele-specific analyses did not show significance.	PCR-SSO	Spain
50	*HLA* (Class I and II)	Association of *HLA-DQB1*06* with risk to COVID-19. Several alleles were associated with COVID-19 (especially in the HLA-DQ1 group) but without Bonferroni confirmation.	PCR-SSO and NGS	United Kingdom
32	*HLA* (Class I and II)	The study observed an association between *HLA-C*07:29* and *HLA-B*15:27* and COVID-19 risk. In the absence of confirmation by Bonferroni, they observed an association between COVID-19 and *HLA-B*40:06* and *HLA-DRB1*04:06* alleles.	NGS	China
34	*HLA* (Class I and II)	*HLA-B*15:01* is closely related to asymptomatic infection with SARS-CoV-2 and may be involved in the mechanism of early viral clearance.	-	United States (White population)
39	*HLA* (Class I and II) and *MICA*	This is a study with SNPs. They observed association of HLA alleles encoding Lys at residue 71 (mostly *HLA-DRB1*03:01* and *HLA-DRB1*04:01*) with symptomatic COVID-19. Associations in variants related to *MICA* also highlight the importance of NK cells in COVID-19.	WGS	Brazil
51	*HLA* (Class I and II)	The study shows *HLA-DRB1*01:01* and *HLA-B*35:01* alleles in association with reduced disease duration in mild and moderate COVID-19.	NGS	Germany
52	*HLA* (Class I and II)	No association was observed.	PCR-SSO	Spain

Reference	Studied genes	Main findings	Methodology HLA	Country
[53]	*HLA* (Class I and II)	No association was observed after strict adjustment for multiple tests.	NGS	Europe
[24]	*HLA* (Class I and II) and *KIR*	The functional unit *KIR2DS2/HLA-C1* is highly protective against the negative consequences of COVID-19.	NGS and PCR-SBT (HLA) and PCR-SSP (KIR)	Italy
[54]	*HLA* (Class I and II)	They identified two predisposing alleles, *HLA-DRB1*08:02* in the Hispanic group and *HLA-A*30:02* in young African Americans.	NGS and PCR-SBT	United States (midwestern)
[55]	*HLA* (Class I and II)	HLA-B53 group was associated with the risk to death to COVID-19 in Black Americans.	PCR-rSSO	United States (Black)
[25]	*HLA* (Class I and II)	*HLA-C*04:01:01:01*, *HLA-DRB5*01:01:01:02*, *HLA-DQA1*03:01:01:01*, *HLA-DPB1*04:01:01:41*, and *HLA-DPA1*01:03:01:02* were associated with severe COVID-19	NGS	India
[56]	*HLA* (Class I and II)	An influence of *HLA* on COVID-19 susceptibility is supported by the association with HLA-A homozygosity but there is no evidence for a role of HLA-A, -B, or -DRB1.	PCR-SSO	Brazil
[57]	*HLA* (Class I and II)	None of the 66 most common *HLA* loci (HLA-A, -B, -C, -DQB1, -DRB1) was found to be associated with COVID-19 or hospitalization in the Israeli population.	DNA-based techniques	Israel
[58]	*HLA* (Class I and II)	*HLA-DRB1*08* is associated with mortality in COVID-19 patients.	DNA-based techniques	Italy
[59]	*HLA* (Class I and II)	After Bonferroni's correction for multiple tests, a significant association was found for *HLA-DRB1*15:01*, *HLA-DQB1*06:02* and *HLA-B*27:07*.	NGS	Italy
[26]	*HLA* (Class I and II)	There is a potential association of *HLA-C*04:01* with severe COVID-19. Carriers of this allele had a higher risk of intubation when infected with SARS-CoV-2.	NGS	Germany, Spain, Sweden, United States
[33]	*HLA* (Class I and II)	The *HLA-B*15:27* and *HLA-DRB1*04:06* alleles were associated with COVID-19 susceptibility in China.	NGS	China
[60]	*HLA* (Class I and II)	They observed significant associations of *HLA-B*38*, *HLA-A*68*, *HLA-A*24* and *HLA-DRB1*01* alleles with risk for COVID-19.	PCR-SSO	Iran

Reference	Studied genes	Main findings	Methodology HLA	Country
61	*HLA* (Class I and II)	Frequency comparisons show alleles (*HLA-A*03*, *HLA-B*35*, *HLA-DRB1*16*, *HLA-A*32*, *HLA-B*58*, *HLA-B*55* and *HLA-DRB1*14*) possibly associated with COVID-19. Despite this, after correcting for multiple tests, only *HLA-A*03* remained significantly associated with disease.	PCR-SSP	Iran
62	*HLA-C and KIR*	The frequencies of *KIR2DL2*, *KIR2DS3*, *HLA-C1C1* and *KIR2DL2/HLA-C1C1* were associated with the risk to COVID-19. *KIR2DL3+KIR2DL2-/HLA-C1+Others+* was associated with protection to this disease.	PCR-SSP	China
63	*HLA-B* and *MICA*	The study results indicate that the STR polymorphisms in *MICA*A9* have an impact on the risk of contagion and COVID-19 severity. There was no significant association between HLA-B alleles and COVID-19.	PCR-SSO	Spain
64	*HLA* (Class I and II)	The presence of *HLA-DRB1*13:02* is associated with an increased risk of symptomatic COVID-19.	Imputation (SNPs associated with HLA)	United Kingdom
27	*HLA (*Class I) and *KIR*	They observed that *KIR3DL1*+HLA-Bw4+ and *KIR3DL2*+HLA-A3/11+ combinations were associated with protection against COVID-19. Furthermore, activating receptors *KIR2DS1+KIR2DS5+* are more frequent in patients with severe COVID-19.	NGS (HLA) and PCR-SSO (KIR)	United States
65	*HLA* (Class I and II)	Univariate analysis showed that HLA-A10, *HLA-B*13*, HLA-B22 and *HLA-B*55* alleles were associated with COVID-19, and *HLA-B*57*, *HLA-DRB1*11* and *HLA-DRB1*13* alleles associated in logistic regression analysis.	PCR-SSO	Turkey
66	*HLA* (Class I and II)	No *HLA* allele or group was significantly associated with COVID-19 in the entire cohort of patients, African Americans, or Caucasians.	NGS	United States
67	*HLA* (Class I and II)	The *HLA-A*24* allele was associated with severe COVID-19.	PCR-SSP	Iran

4. DISCUSSION

In this study, different *HLA* alleles were associated with COVID-19, especially class I alleles. It is important to note that, in addition to meta-analysis results being representative of the studied population groups, they are also a picture of the prevalent viral strains of the period that they were sampled. For instance, the *HLA-C*04:01* allele was associated with risk for COVID-19 comparing asymptomatic and symptomatic individuals. This allele was previously predicted, in *in-silico* studies, to bind a smaller number of SARS-CoV-2 peptides.

This was confirmed in our analysis, in addition, we also observed that this allele tends to bind even less peptides of SARS-CoV-2 new variants, such as Omicron. This suggests a limited ability to present these antigens and build a strong viral immune response for individuals carrying this allele, and this risk may be exacerbated in omicron subvariants. [21].

Given the role of *HLA-C* in antigen presentation to NK cells, *HLA-C*04:01* molecules may cause NK cell hyporesponsiveness. This response may delay SARS-CoV-2 viral recognition and, subsequently, generate an inadequate immune response [22]. In this way, studies in the Italian population report a significantly high prevalence of the *HLA-C*04:01* allele and NK-cell inhibitor *KIR2DL1* in COVID-19 [23,24]. Finally, *HLA-C*04:01:01:01* was associated with the severity of COVID-19 in Indians [25] and *HLA-C*04:01* with COVID-19 in Europeans [26].

Our meta-analysis points to KIR genes associated with risk for COVID-19 (*KIR2DL2* and *KIR2DS3*). The individual results of articles on KIR and ligand association with COVID-19 are conflicting, sometimes placing KIR activators as protective against this disease [24], sometimes placing them as a risk [27]. To resolve this, we hypothesize that the KIR associations are related to a haplotype of KIR genes that correspond to a general balance in the NK cell response, which is practically impossible to observe in association studies without a good sample size.

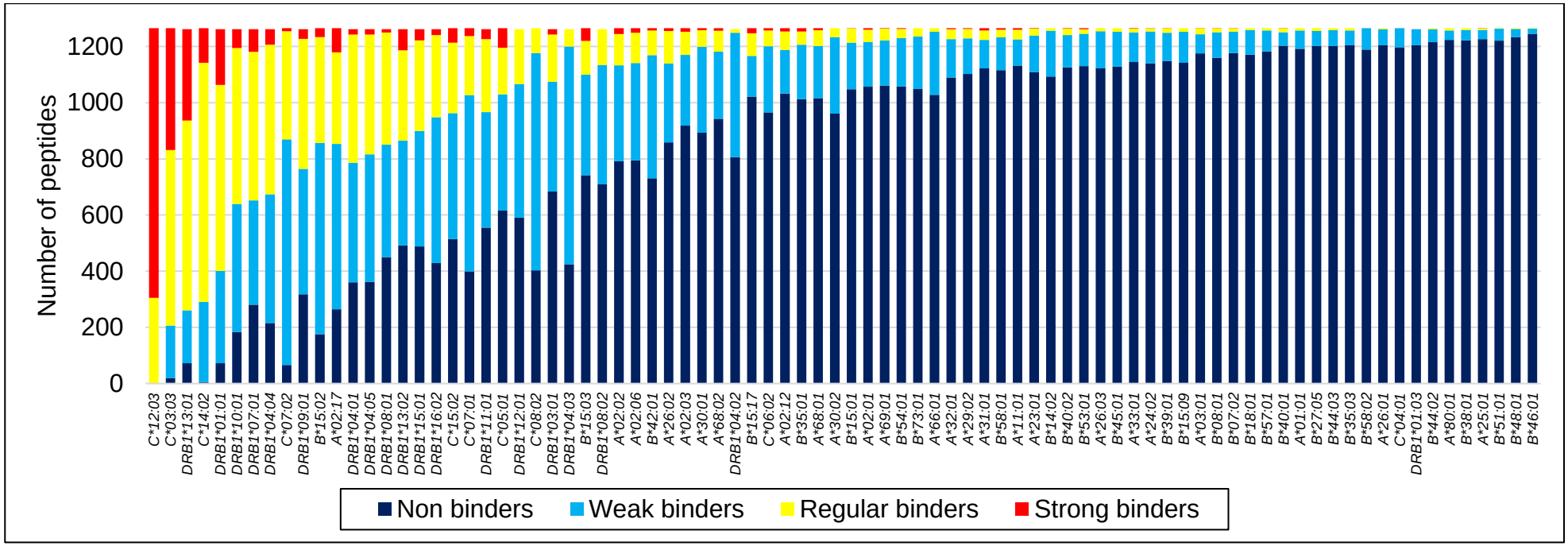

Figure 2. *In-silico* chart analysis of *HLA* alleles binds with SARS-CoV-2 Spike protein.

In our review, the *HLA-B*15:01* allele was associated with protection from symptomatic COVID-19. Interestingly, at the *HLA-B* locus, a large number of studies have observed an association of HLA-B15 serotype alleles with coronavirus infection, such as *HLA-B*46:01* [28], *HLA-B*15:01* [29,30], *HLA-B*15:02* [31], *HLA-B*15:03* [3] and *HLA-B*15:27* [32,33]. The *HLA-B*15:01* allele was also associated with symptomatic COVID-19 in the Spanish population, when associated with KIR3DS1 activating receptor, present in NK cells [29]. On the other hand, a study demonstrated that individuals with the *HLA-B*15:01* allele would be protected from COVID-19 symptoms. This effect was enhanced when in the presence of *HLA-DRB1*01:01*. In addition, *HLA-B*15:01* was found to bind more easily to the SARS-CoV-2 specific epitope, generating potent CD8+ T cell response, which may lead to better initial disease control [34]. Among *in-silico* studies, the *HLA-B*46:01* molecule appears to have low binding to SARS-CoV-2 epitopes, suggesting that individuals expressing this molecule may be more vulnerable to COVID-19 [21]. In our study, *HLA-B*46:01* was present in only 1.6% of individuals with COVID-19, which may indicate a lack of statistical power to detect an association of this allele with the disease. Thus, it seems that some allelic groups such as *HLA-B*15* (or *HLA-B*46*) may be interesting targets for association studies on COVID-19.

Among class II, *HLA-DRB1*04* was associated with protection from symptomatic COVID-19 in this pool analysis. Nevertheless, contradictory results were observed in the literature. In the Iranian population, the *HLA-DRB1*04* allelic group was associated with protection against COVID-19 [35], while *HLA-DRB1*04:06* was associated with risk in Chinese individuals [33]. Among Saudi Arabians, the frequencies of *HLA-DRB1*04* were increased in healthy controls [36]. In a study performed in the United Kingdom, *HLA-DRB1*04:01* had a higher frequency in patients with severe disease [37] and, In a Spanish population, *HLA-DQB1*04* had an association with high mortality [38]. A study in Brazil observed an association between *HLA-DRB1*04:01* and with symptomatic COVID-19 groups [39].

Our study shows that the complexity of HLA and KIR participation in disease susceptibility cannot be underestimated. New studies are needed to improve understanding of the relationship between HLA and COVID-19. Due to the great importance of HLA molecules in antigen presentation to T lymphocytes and NK cells, we believe that many HLA antigens must be associated with greater or lesser susceptibility to COVID-19, depending on the type of response that this molecule can trigger and allele distribution among populations. However, many rare alleles association may be missed in population studies due to the limited sample size of these studies. Furthermore, other components of the immune response that act in this disease, such as cytokines, have just begun to be studied. In the design of the studies, we believe that it is necessary to analyse the viral strain of the sample to be studied. Finally, a better design of control groups with proof of exposure to risk is needed to build case-control studies.

Therefore, some limitations should be considered in our study. The results obtained in systematic reviews are limited to the quality of available studies and to the profile of populations analysed. In this way, important covariates must be considered in future studies to explain their heterogeneity. Finally, although a pool of studies is carried out to overcome this problem, low statistical power remains an important limiting factor in the observation of subtle genetic effects in studies on COVID-19. The lack of information is another important and frequent issue, mainly characterized by lack of description of non-response rates; incomplete genotype or allele frequency data; covariates of interest not evaluated or described; and incomplete or vague descriptions of statistical methods, group selection, or genetic variants studied. Despite this, we believe that this study contributes to the understanding the *HLA/KIR* role in genetic susceptibility to COVID-19 as it aggregates the largest possible number of publications and follows a rigorous systematic review methodology. Our study also contributes to the improvement of *in-silico* analysis that can contribute to understanding viral proteins role in COVID-19.

5. CONCLUSIONS

This study may be an indication that genetic variability related to NK and T cells response variability may play a role in COVID-19. It appears that *HLA* alleles associated with risk for COVID-19 also have molecules with lower *in-silico* affinity for SARS-CoV-2 proteins. Despite this, the statistical power and complexity/variability of host-virus interaction remains an important challenge for genetic association studies in COVID-19.

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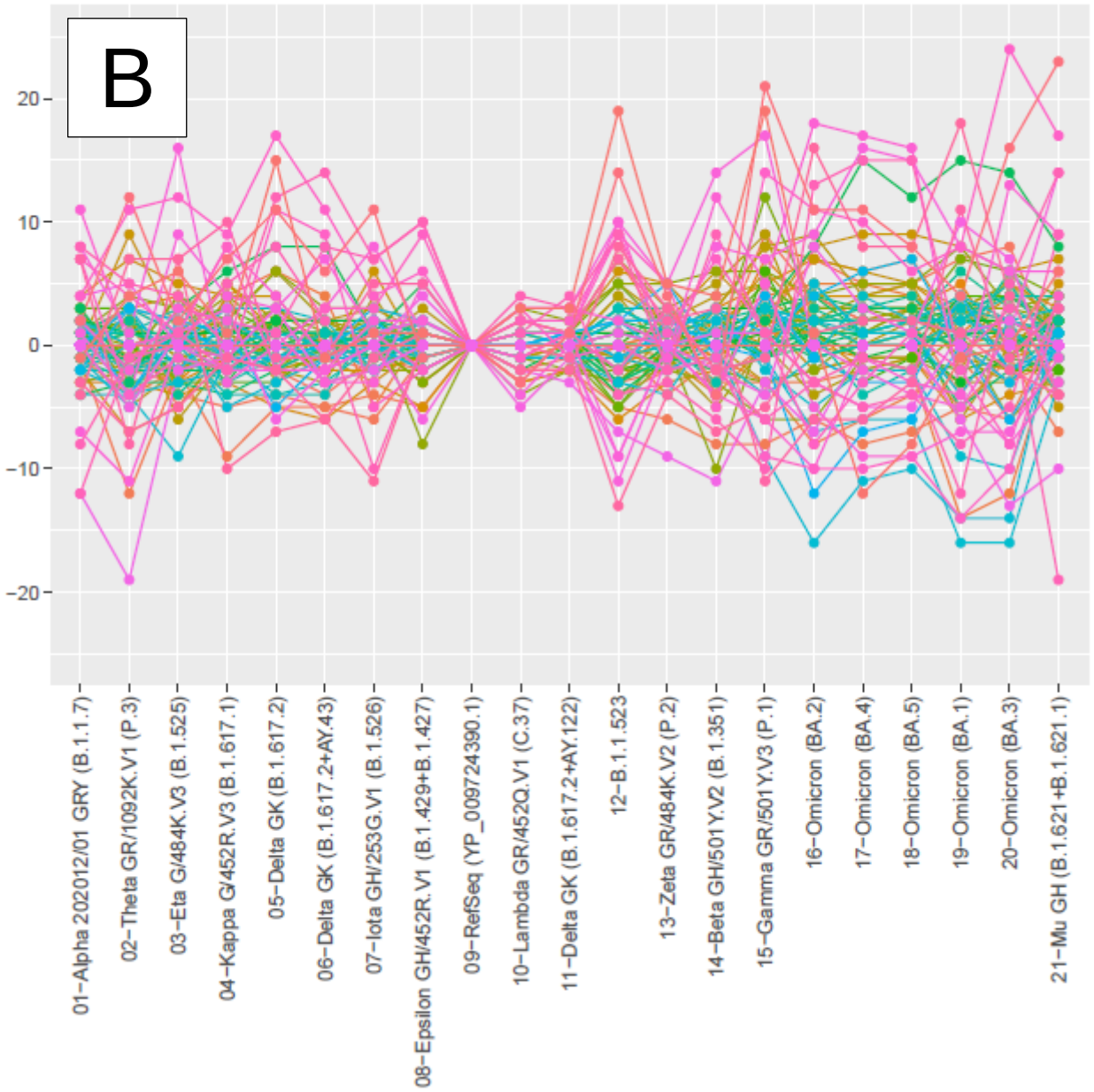
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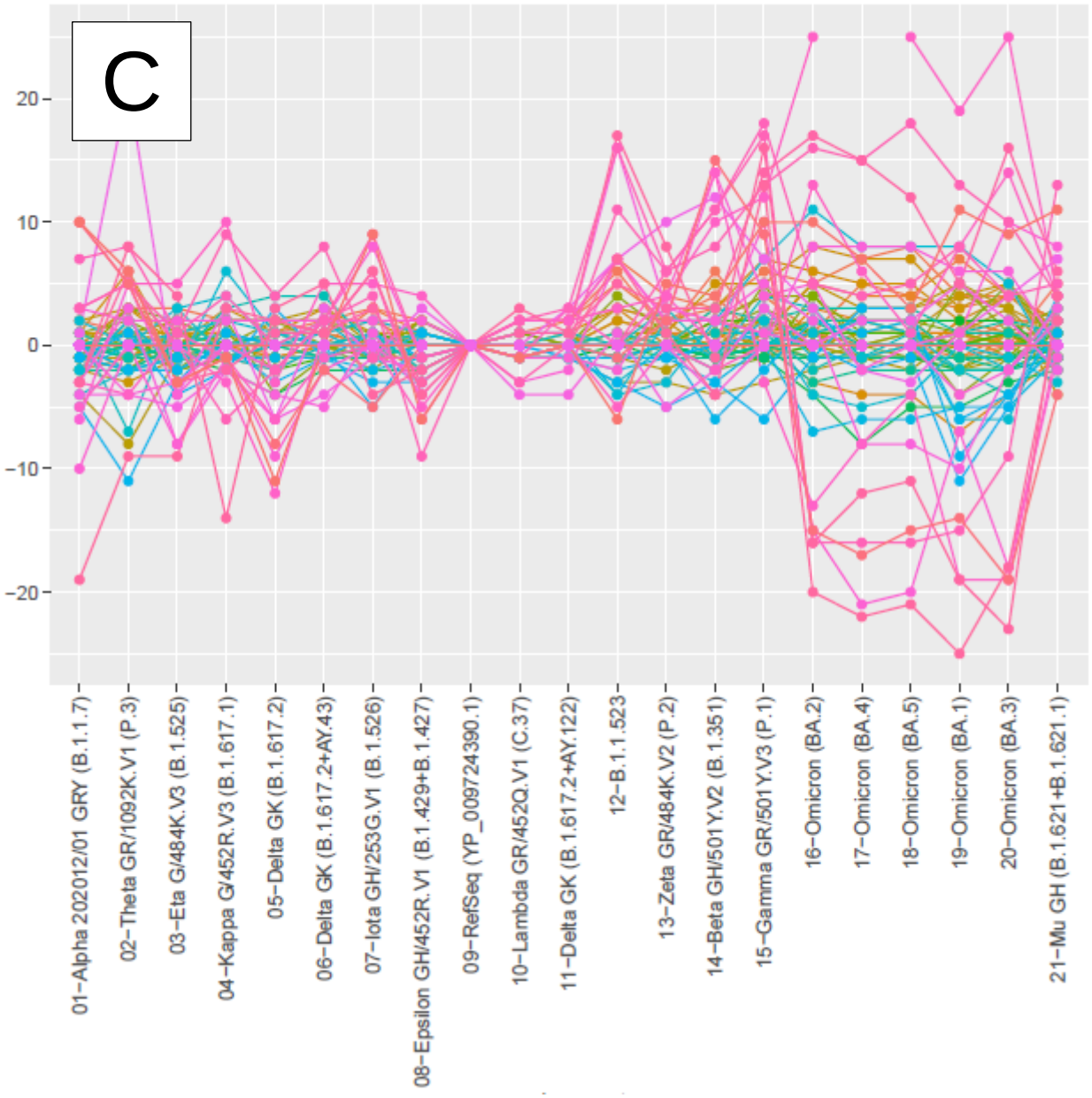
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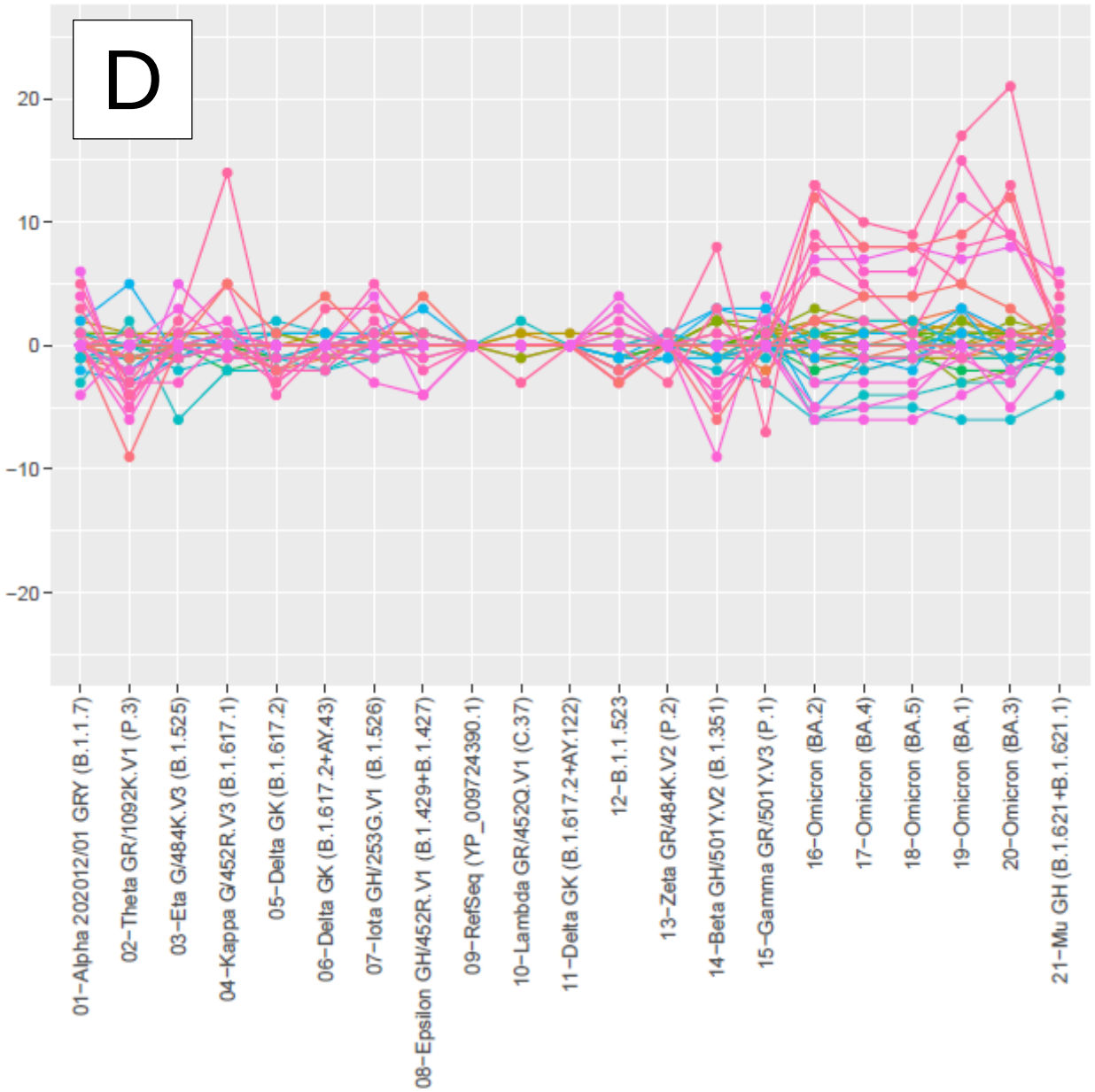
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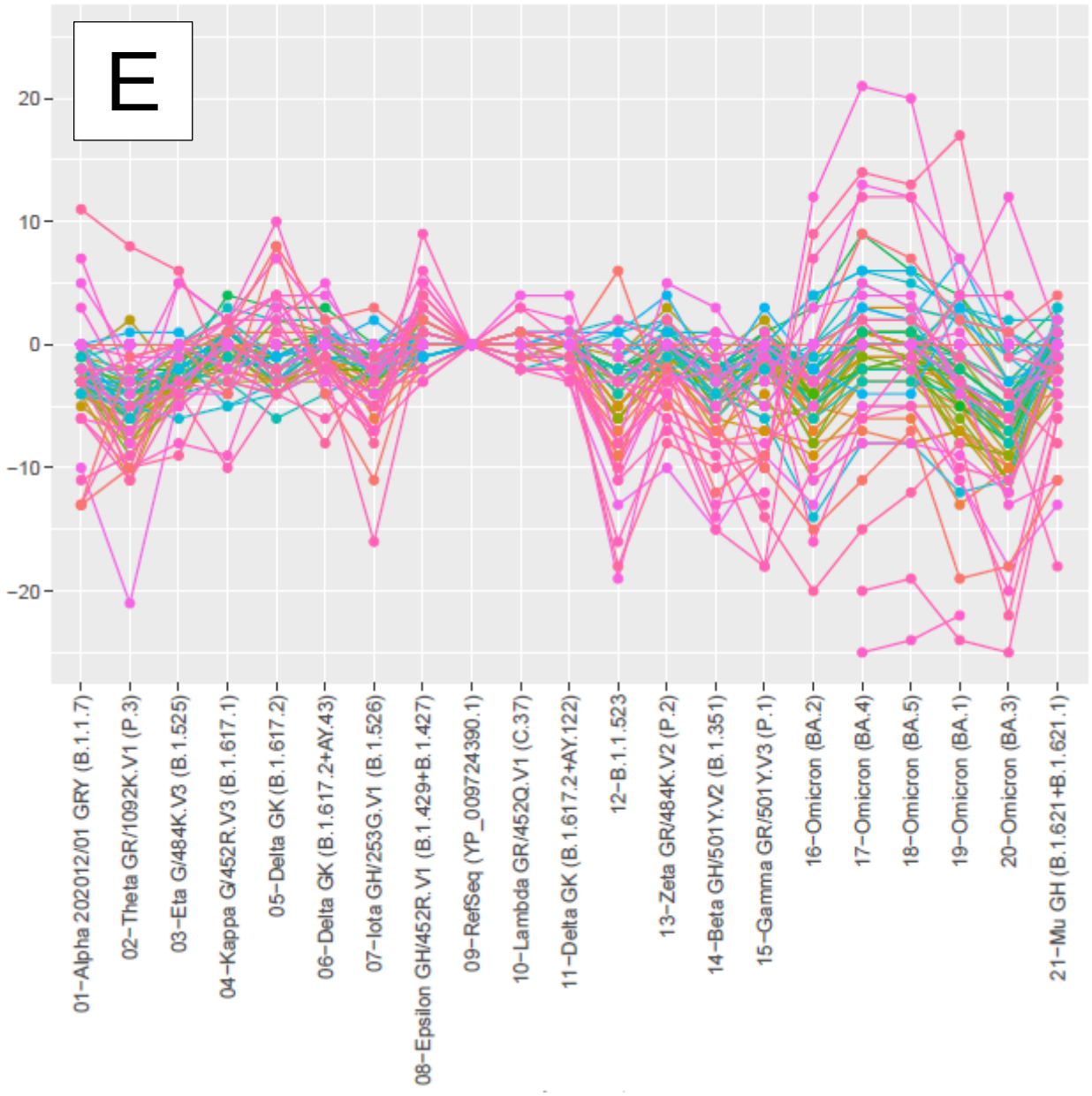
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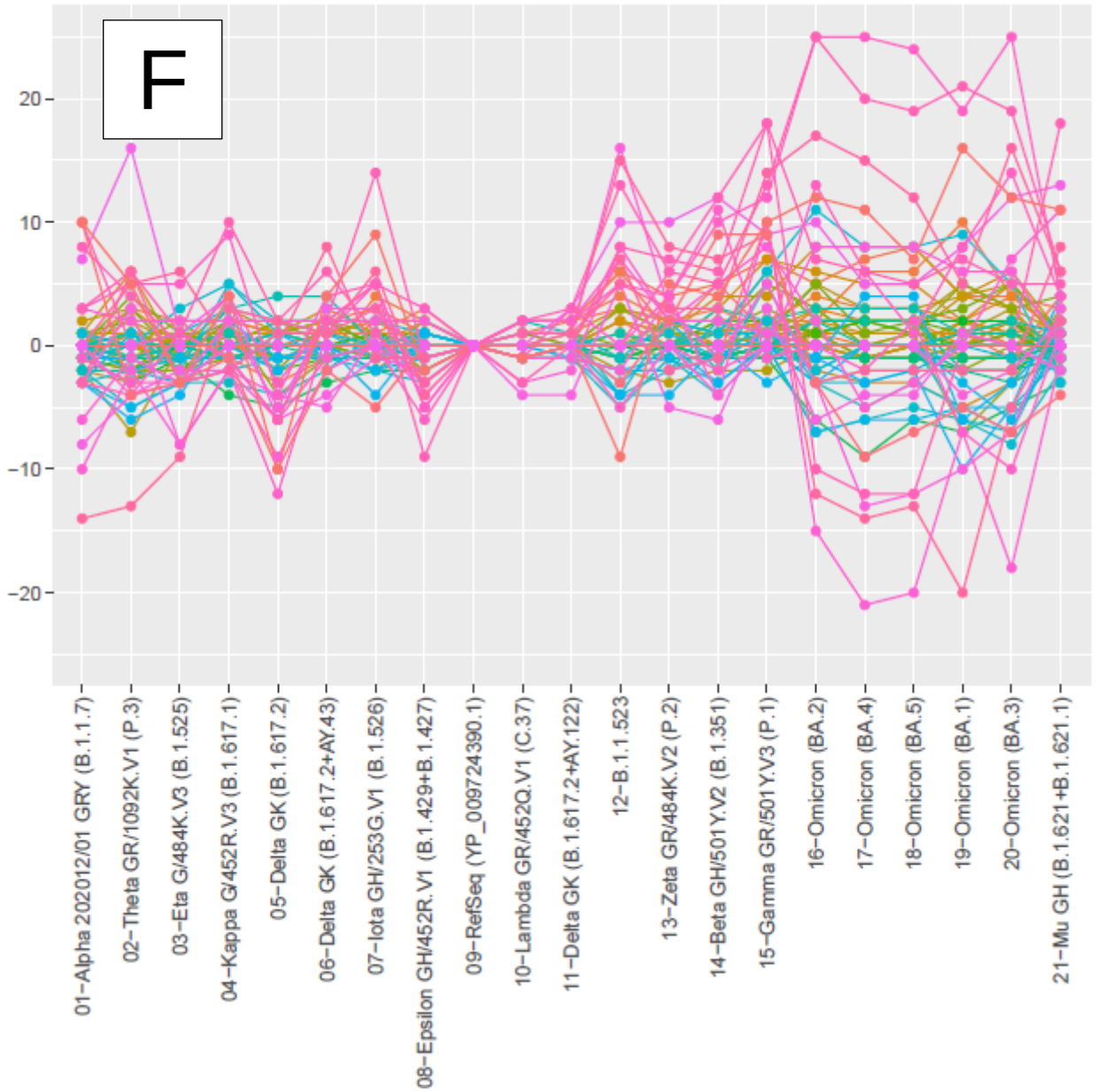
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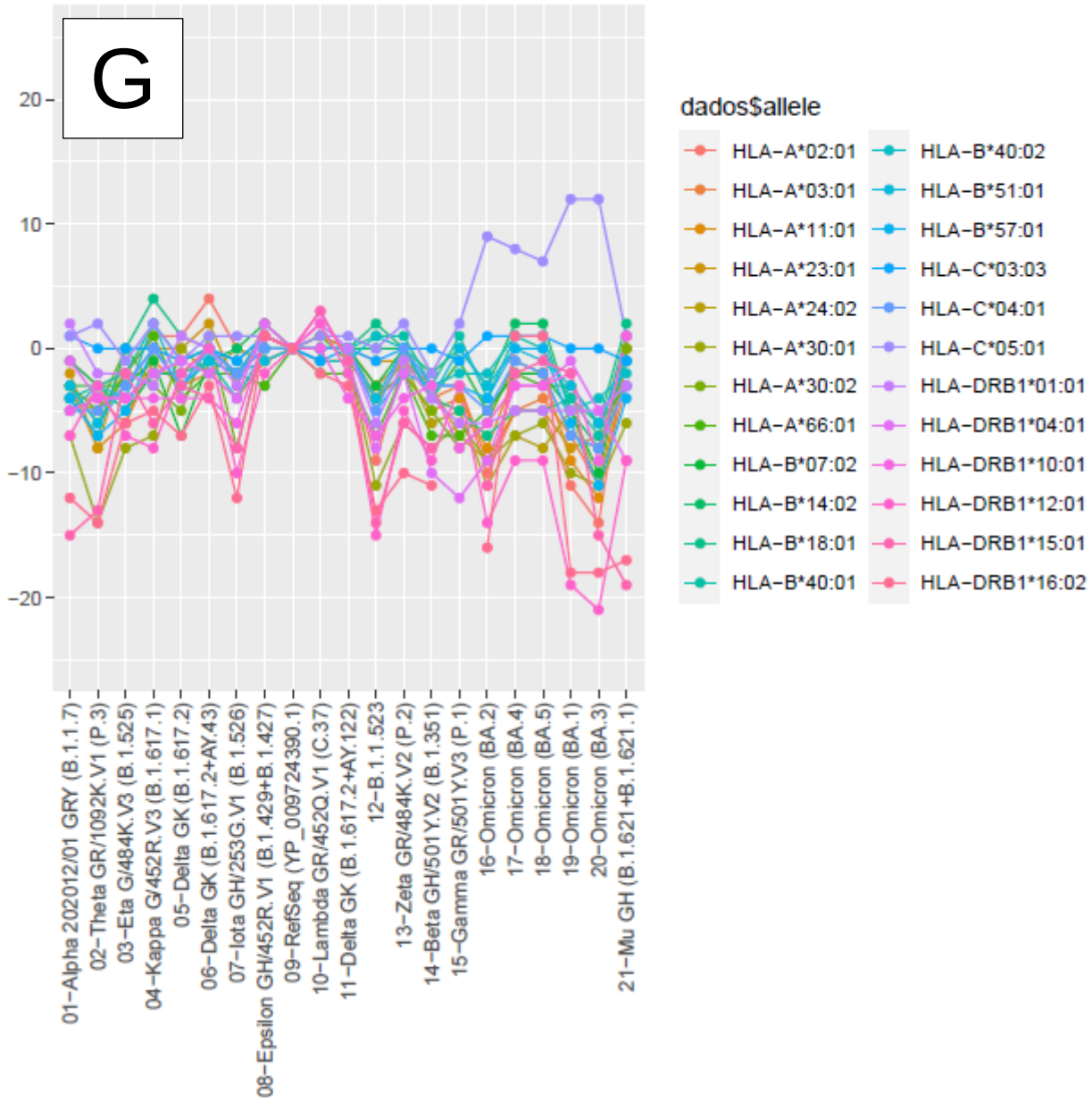
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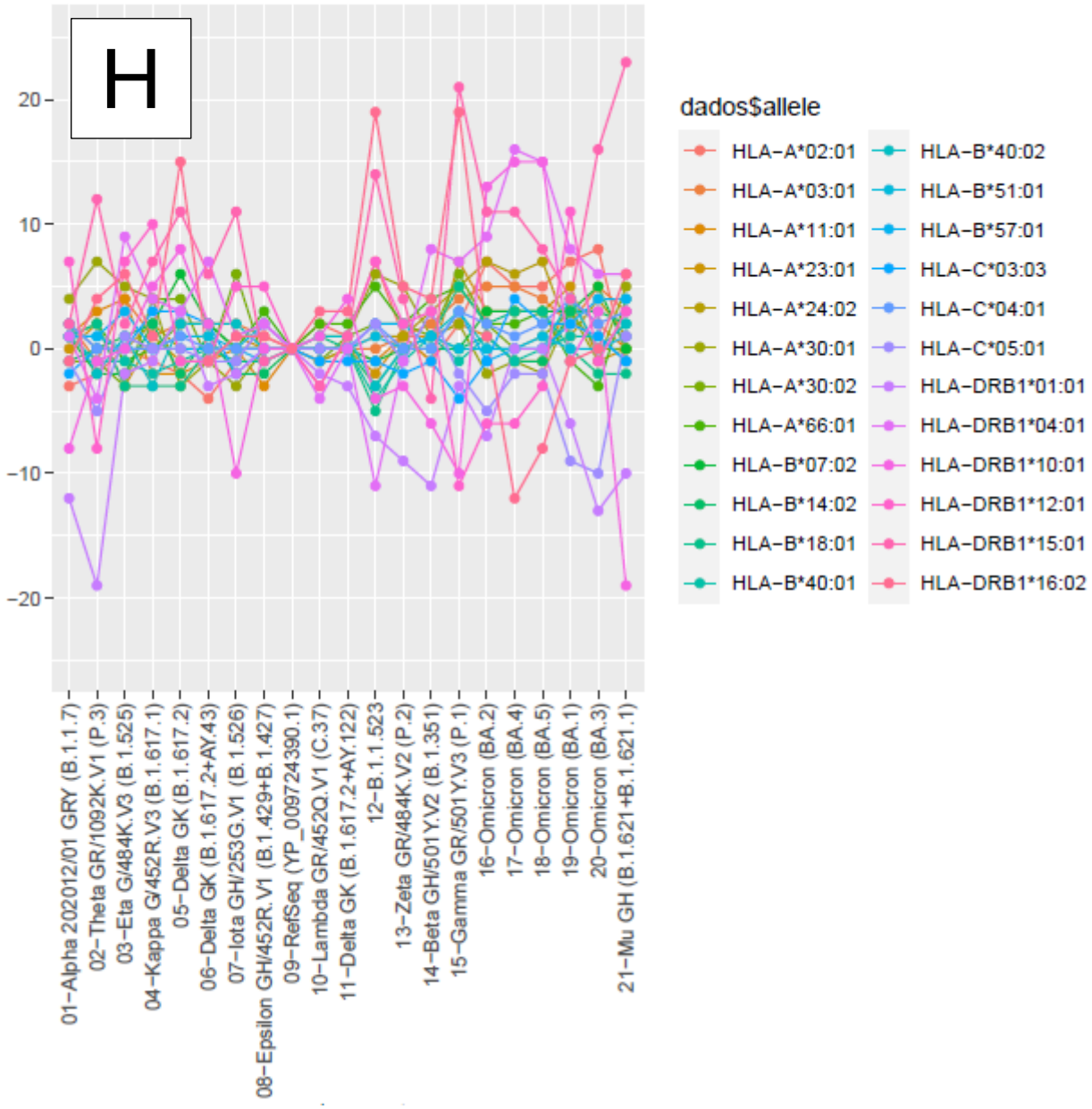
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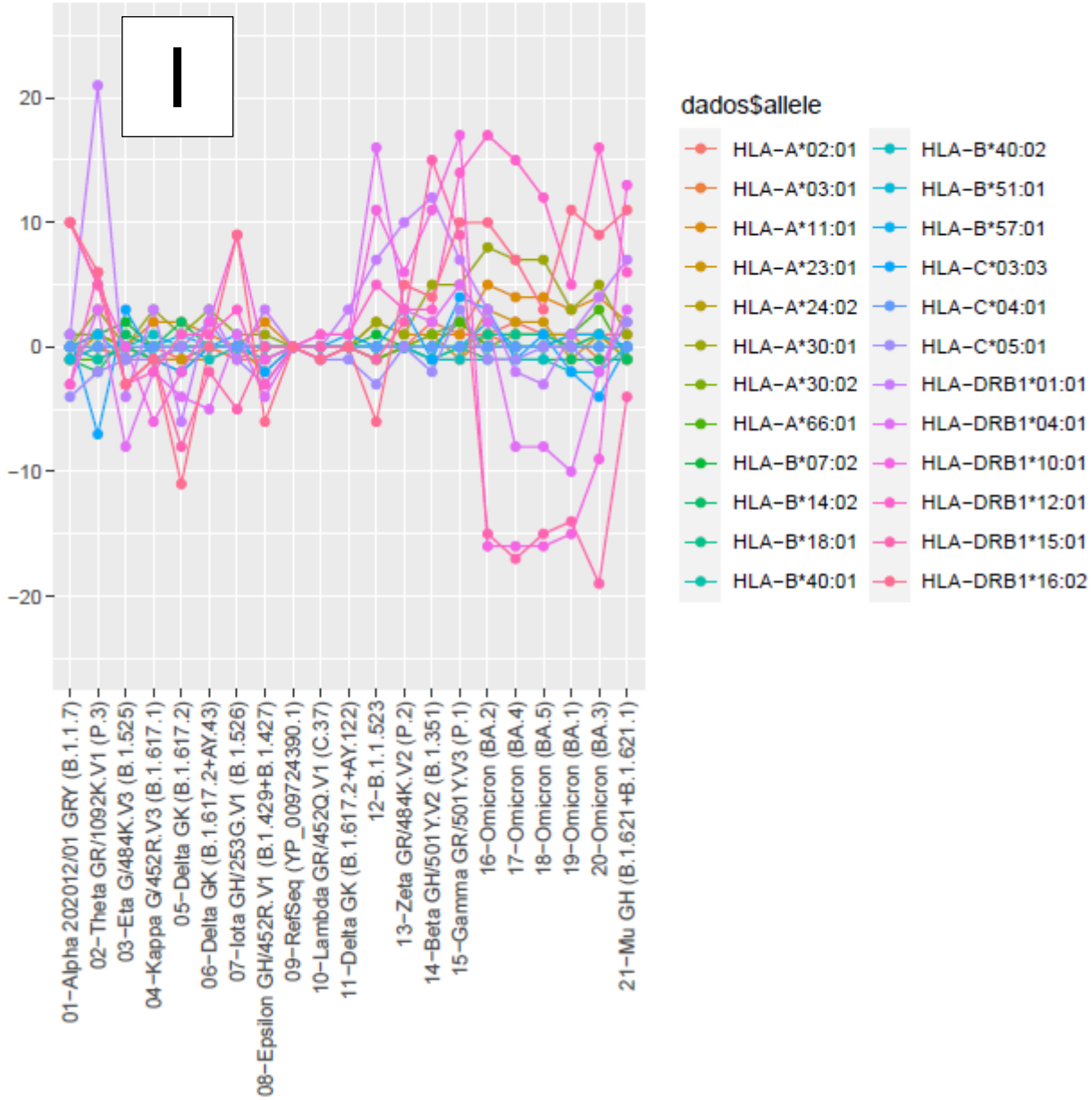
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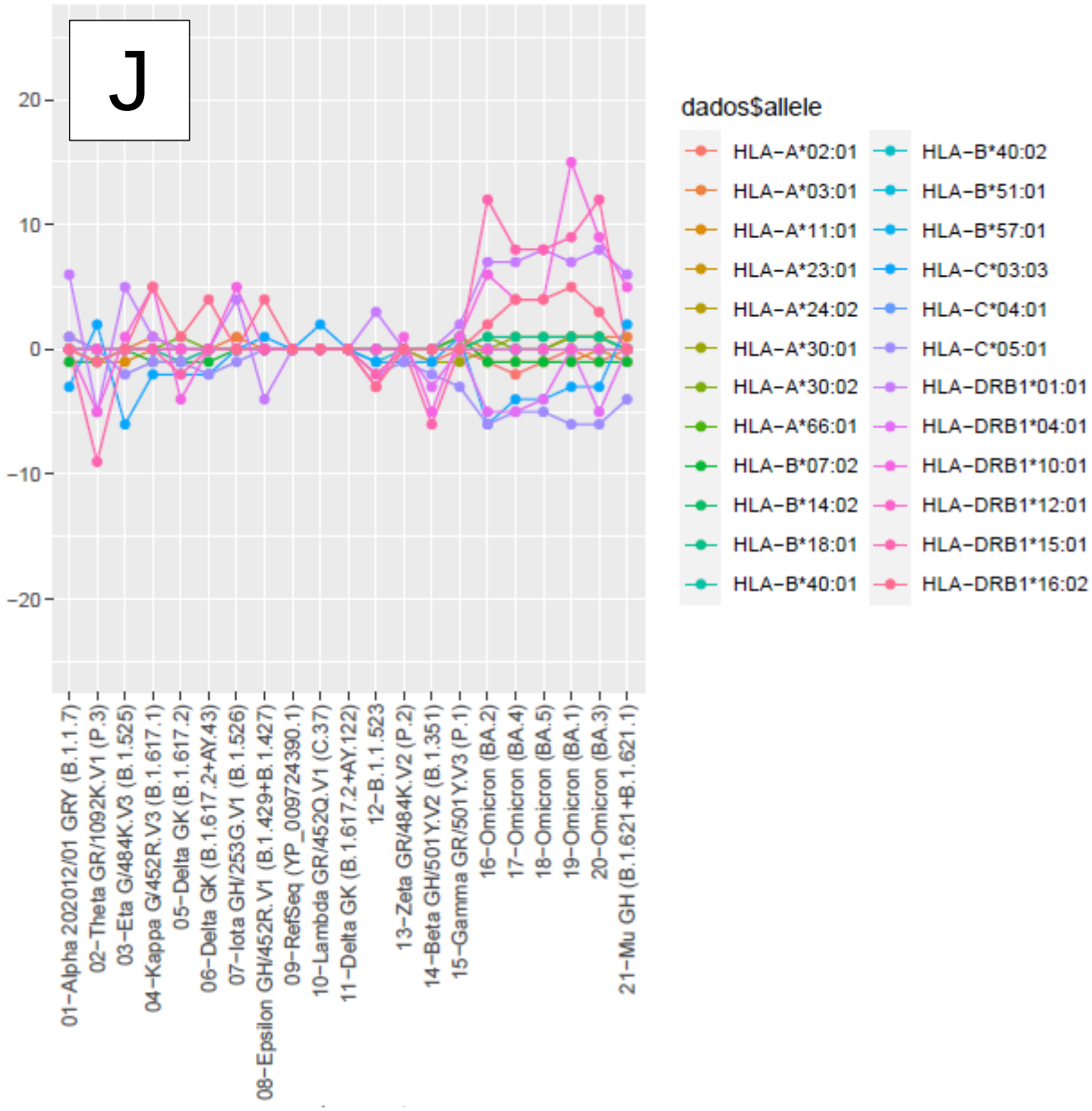
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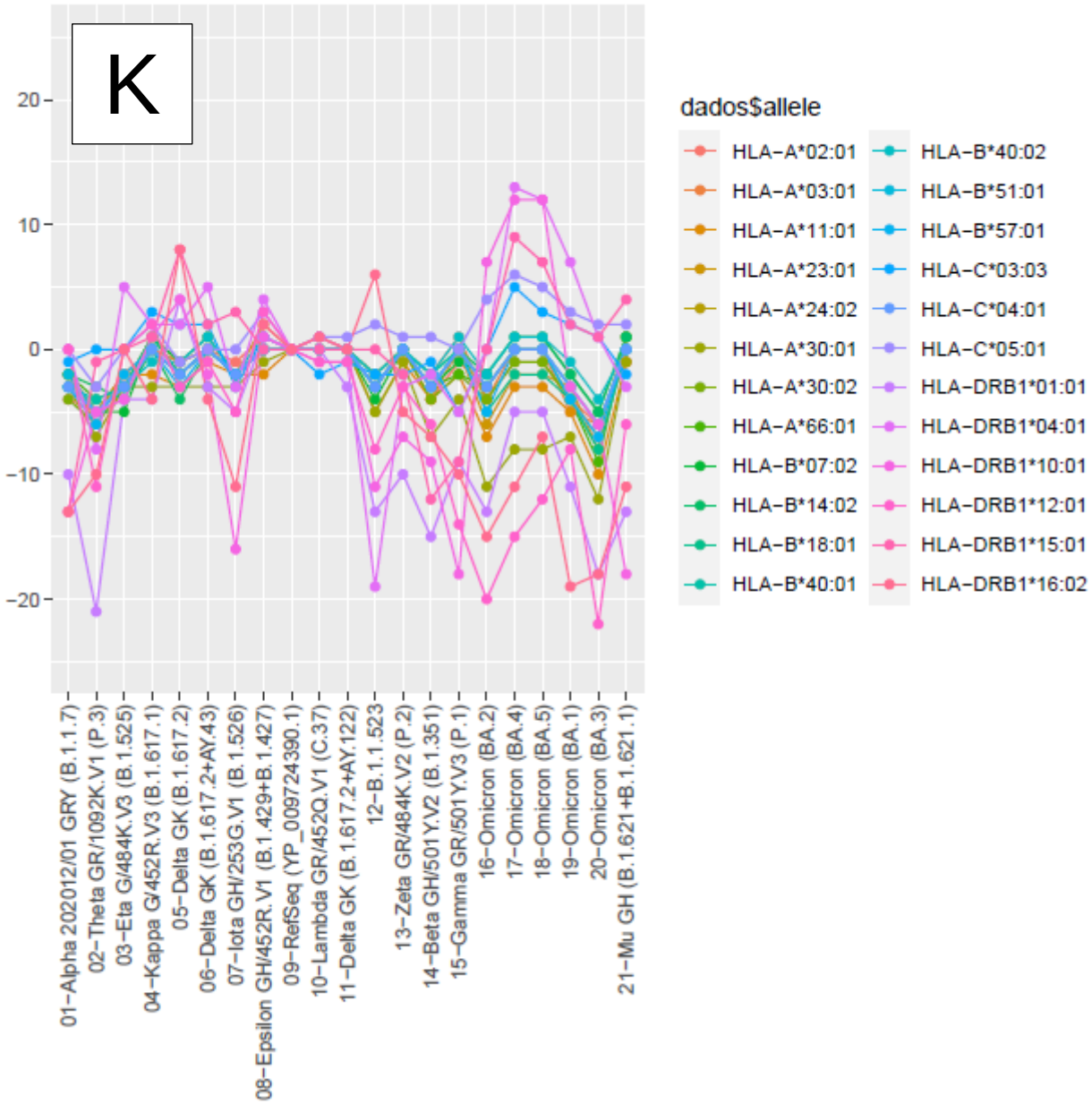
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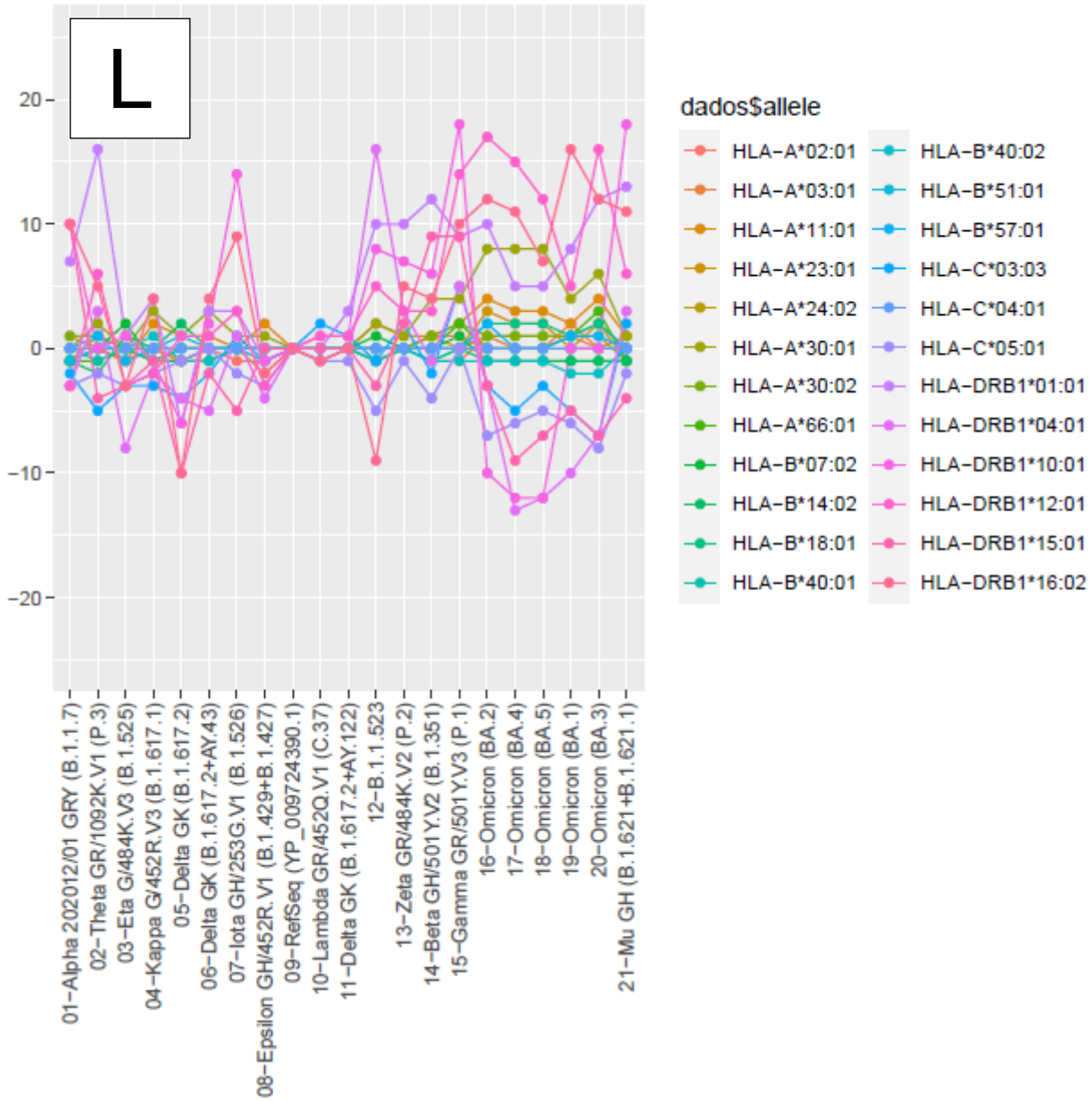
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05−Delta GK (B.1.617.2)
06−Delta GK (B.1.617.2+AY.43)
07−Iota GH/253G.V1 (B.1.526)
08−Epsilon GH/452R.V1 (B.1.429+B.1.427)
09−RefSeq (YP_009724390.1)
10−Lambda GR/452Q.V1 (C.37)
11−Delta GK (B.1.617.2+AY.122)
12−B.1.1.523
13−Zeta GR/484K.V2 (P.2)
14−Beta GH/501Y.V2 (B.1.351)
15−Gamma GR/501Y.V3 (P.1)
16−Omicron (BA.2)
17−Omicron (BA.4)
18−Omicron (BA.5)
19−Omicron (BA.1)
20−Omicron (BA.3)
21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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HLA−A*03:01
HLA−A*11:01
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HLA−B*40:01
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HLA−B*51:01
HLA−B*57:01
HLA−C*03:03
HLA−C*04:01
HLA−C*05:01
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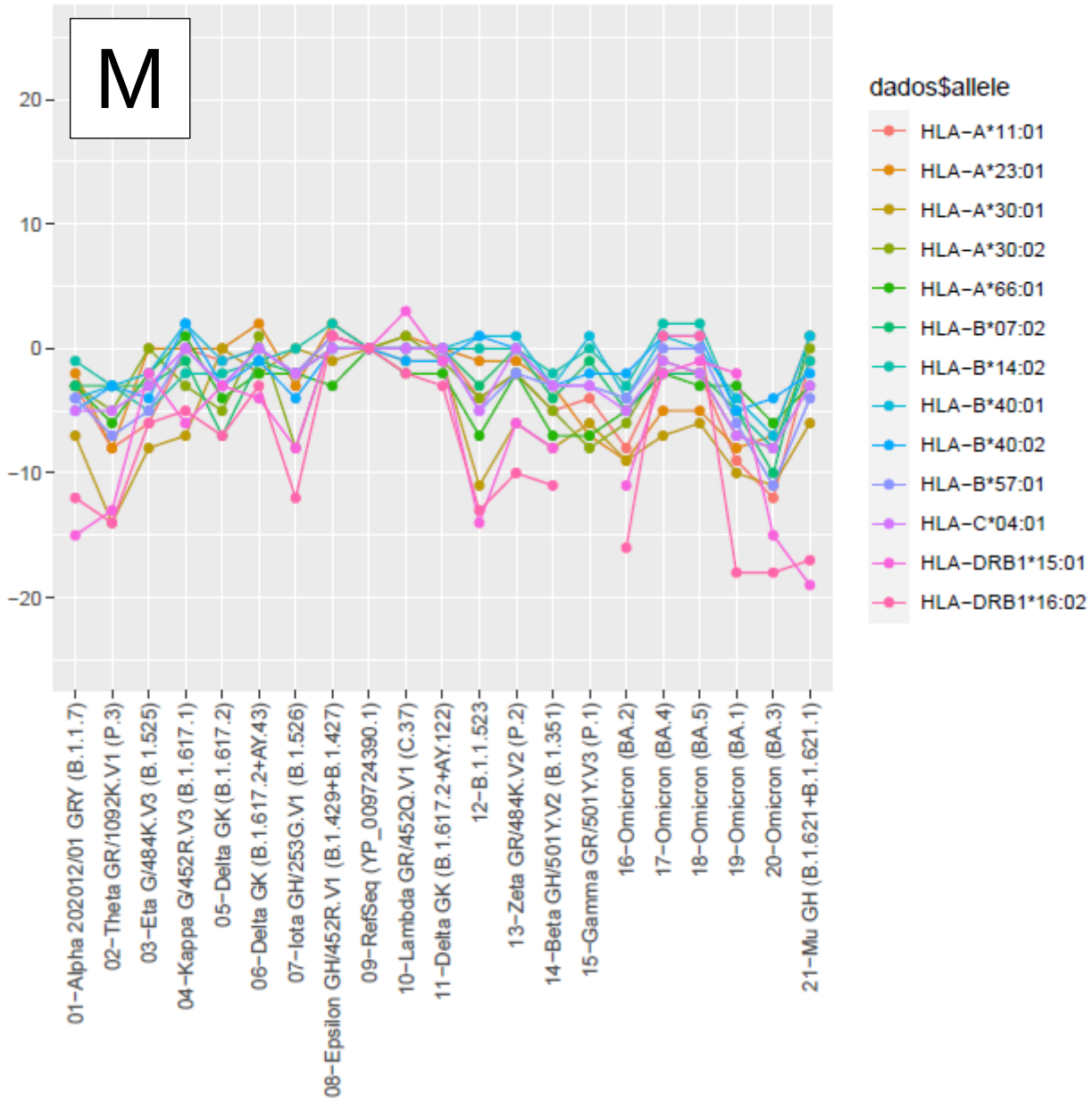
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06−Delta GK (B.1.617.2+AY.43)
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12−B.1.1.523
13−Zeta GR/484K.V2 (P.2)
14−Beta GH/501Y.V2 (B.1.351)
15−Gamma GR/501Y.V3 (P.1)
16−Omicron (BA.2)
17−Omicron (BA.4)
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19−Omicron (BA.1)
20−Omicron (BA.3)
21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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HLA−B*40:01
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HLA−DRB1*15:01
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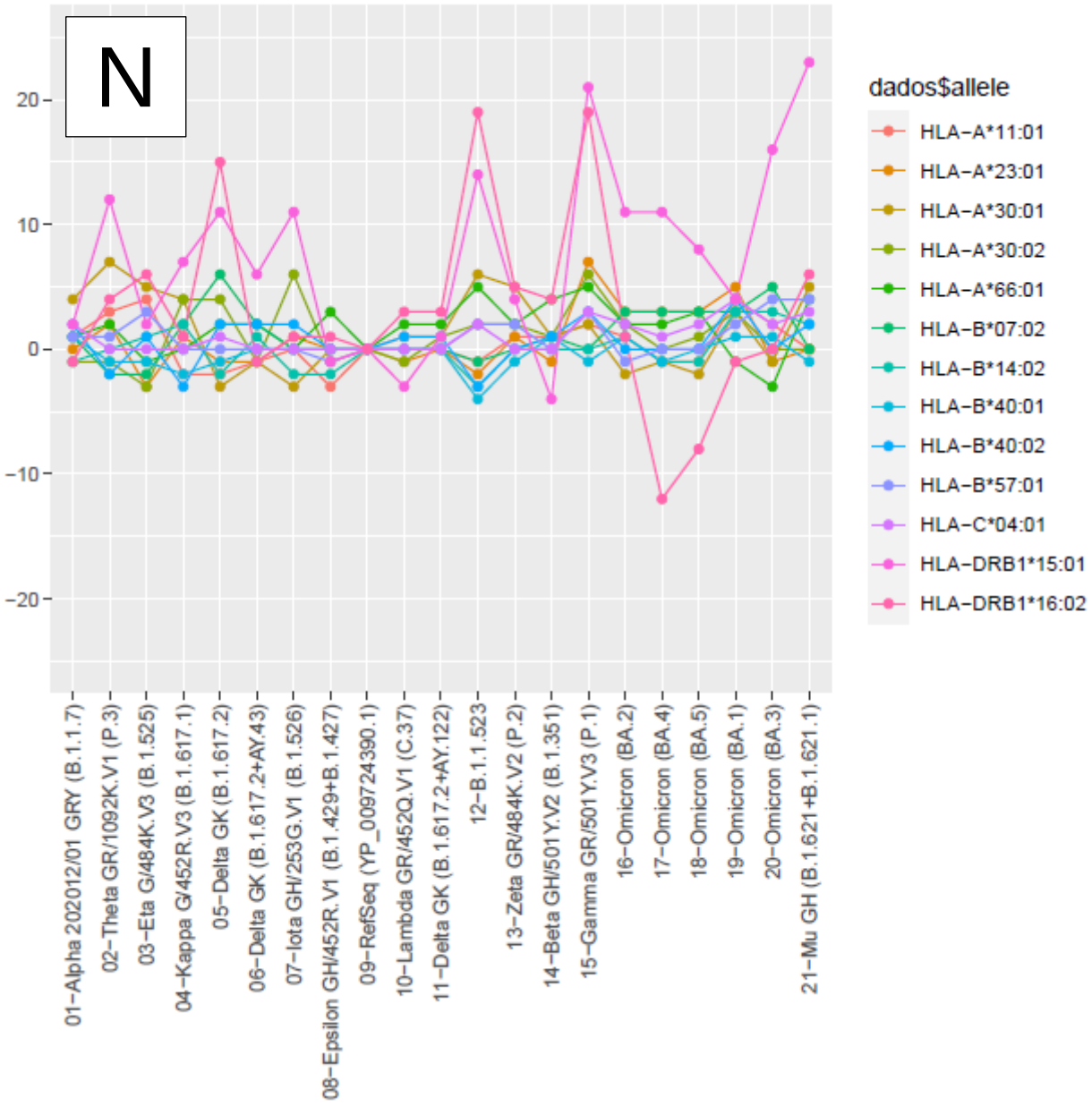
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05−Delta GK (B.1.617.2)
06−Delta GK (B.1.617.2+AY.43)
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08−Epsilon GH/452R.V1 (B.1.429+B.1.427)
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11−Delta GK (B.1.617.2+AY.122)
12−B.1.1.523
13−Zeta GR/484K.V2 (P.2)
14−Beta GH/501Y.V2 (B.1.351)
15−Gamma GR/501Y.V3 (P.1)
16−Omicron (BA.2)
17−Omicron (BA.4)
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19−Omicron (BA.1)
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21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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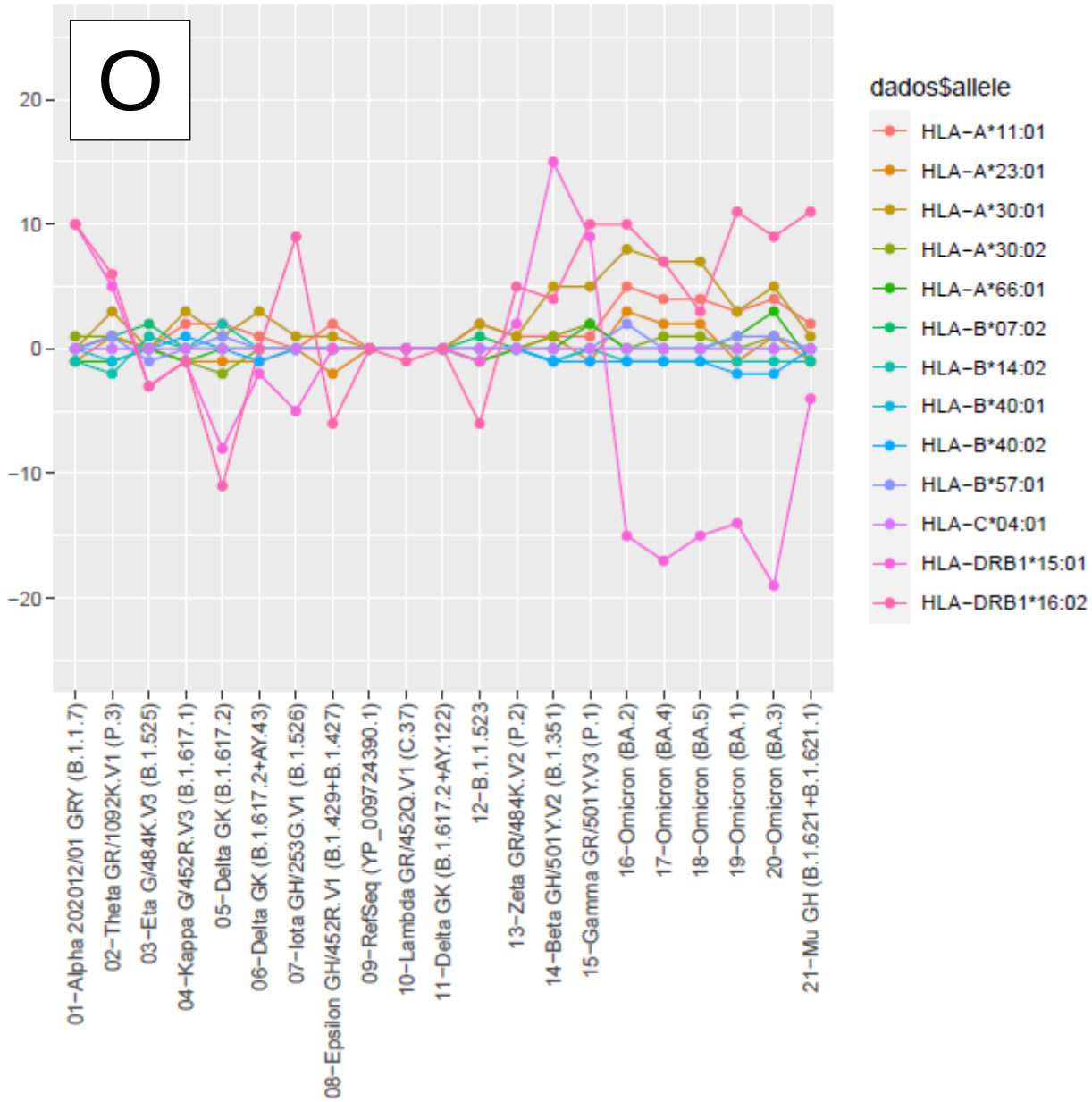
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05−Delta GK (B.1.617.2)
06−Delta GK (B.1.617.2+AY.43)
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08−Epsilon GH/452R.V1 (B.1.429+B.1.427)
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12−B.1.1.523
13−Zeta GR/484K.V2 (P.2)
14−Beta GH/501Y.V2 (B.1.351)
15−Gamma GR/501Y.V3 (P.1)
16−Omicron (BA.2)
17−Omicron (BA.4)
18−Omicron (BA.5)
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20−Omicron (BA.3)
21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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HLA−A*30:01
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HLA−B*40:01
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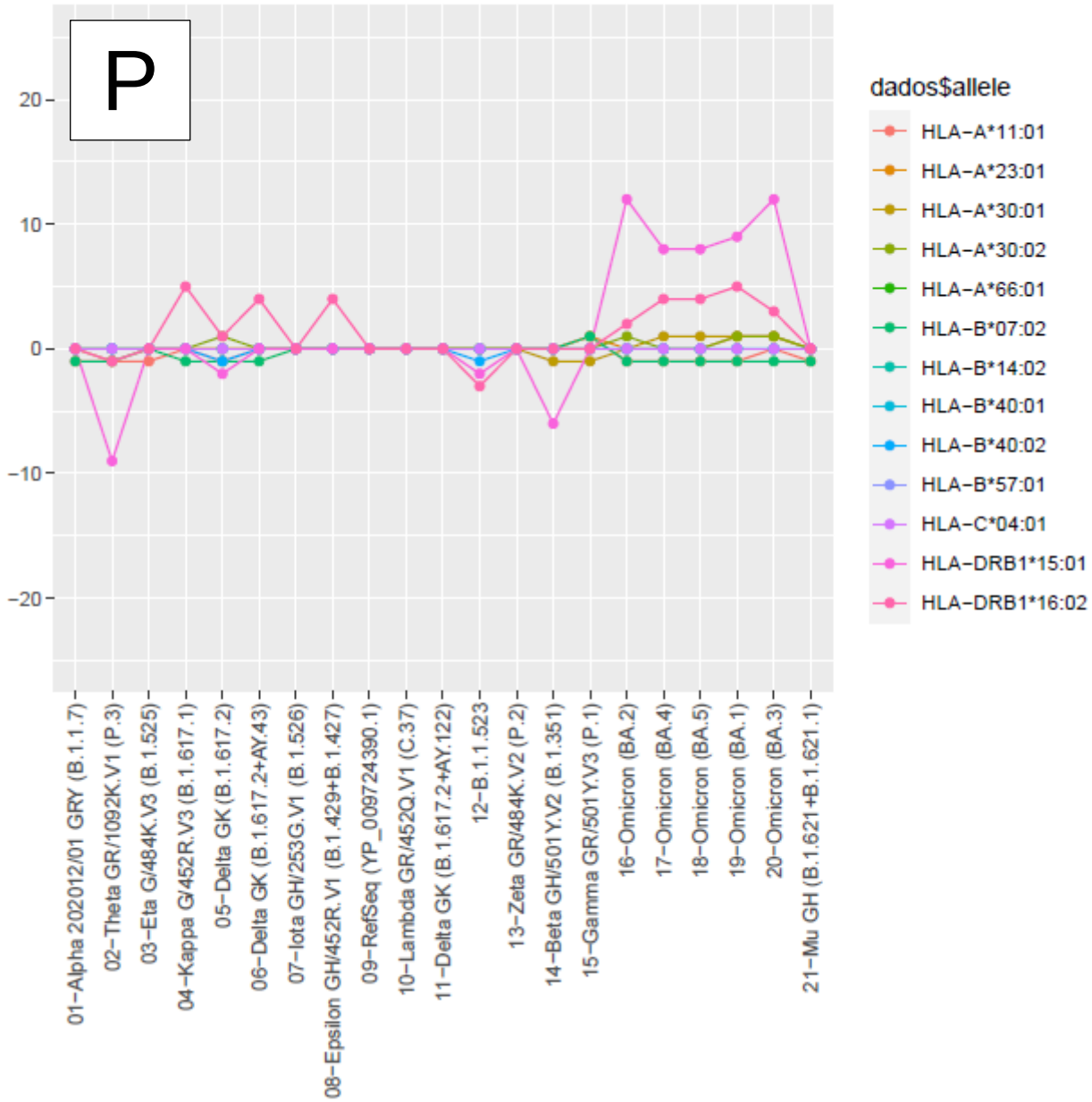
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05−Delta GK (B.1.617.2)
06−Delta GK (B.1.617.2+AY.43)
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09−RefSeq (YP_009724390.1)
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11−Delta GK (B.1.617.2+AY.122)
12−B.1.1.523
13−Zeta GR/484K.V2 (P.2)
14−Beta GH/501Y.V2 (B.1.351)
15−Gamma GR/501Y.V3 (P.1)
16−Omicron (BA.2)
17−Omicron (BA.4)
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21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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HLA−A*30:01
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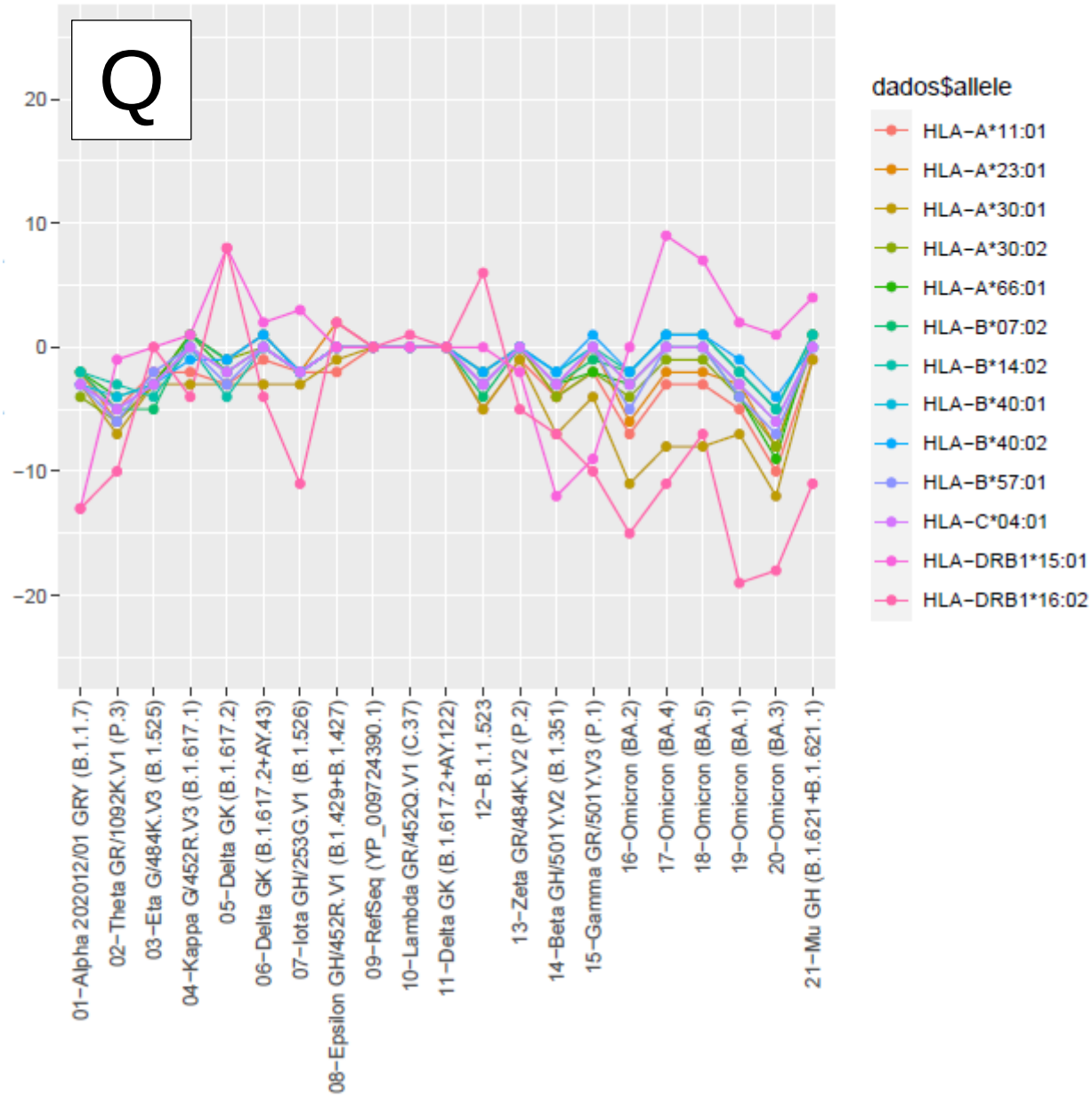
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05−Delta GK (B.1.617.2)
06−Delta GK (B.1.617.2+AY.43)
07−Iota GH/253G.V1 (B.1.526)
08−Epsilon GH/452R.V1 (B.1.429+B.1.427)
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10−Lambda GR/452Q.V1 (C.37)
11−Delta GK (B.1.617.2+AY.122)
12−B.1.1.523
13−Zeta GR/484K.V2 (P.2)
14−Beta GH/501Y.V2 (B.1.351)
15−Gamma GR/501Y.V3 (P.1)
16−Omicron (BA.2)
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21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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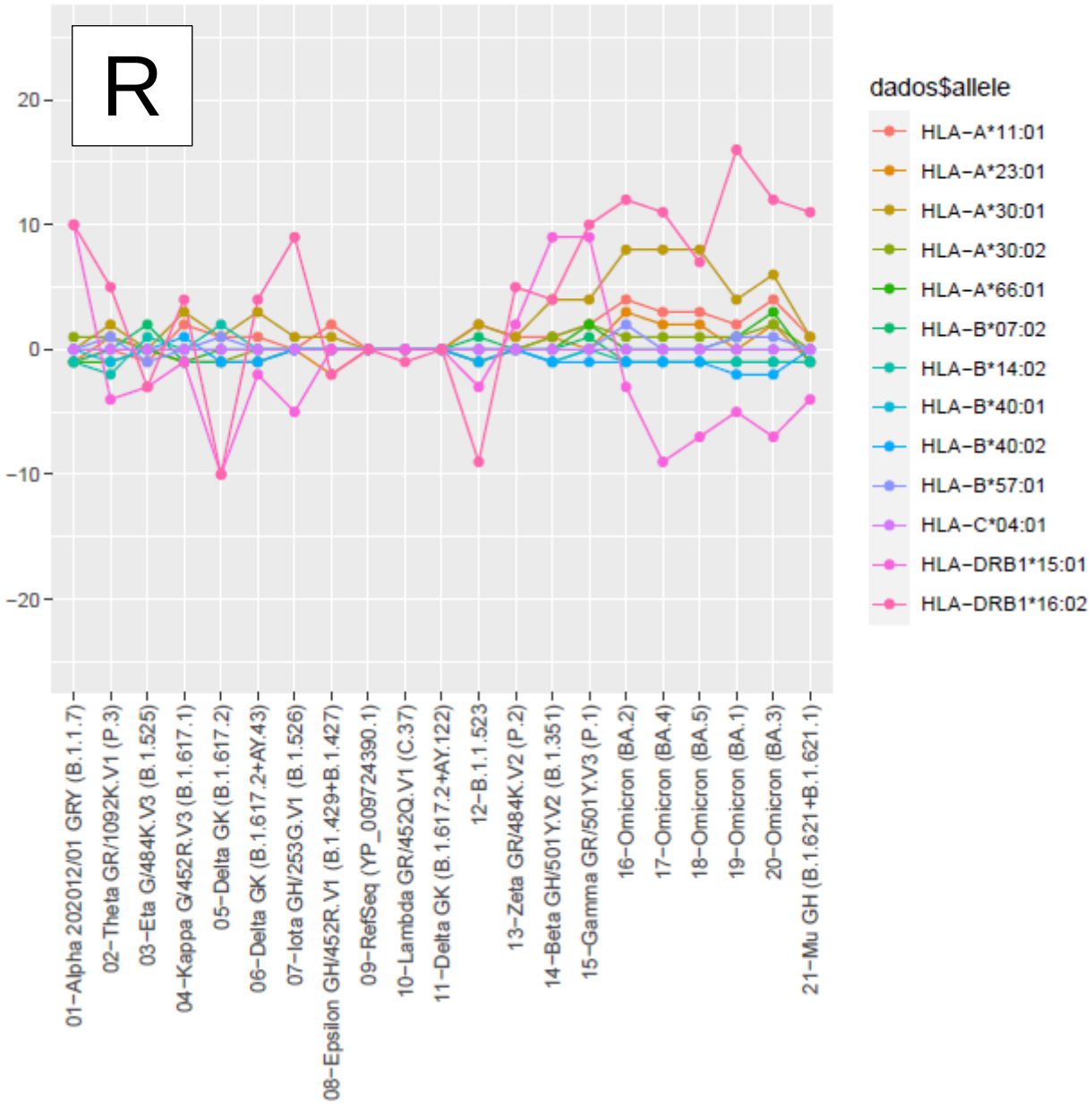
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05−Delta GK (B.1.617.2)
06−Delta GK (B.1.617.2+AY.43)
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16−Omicron (BA.2)
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21−Mu GH (B.1.621+B.1.621.1)
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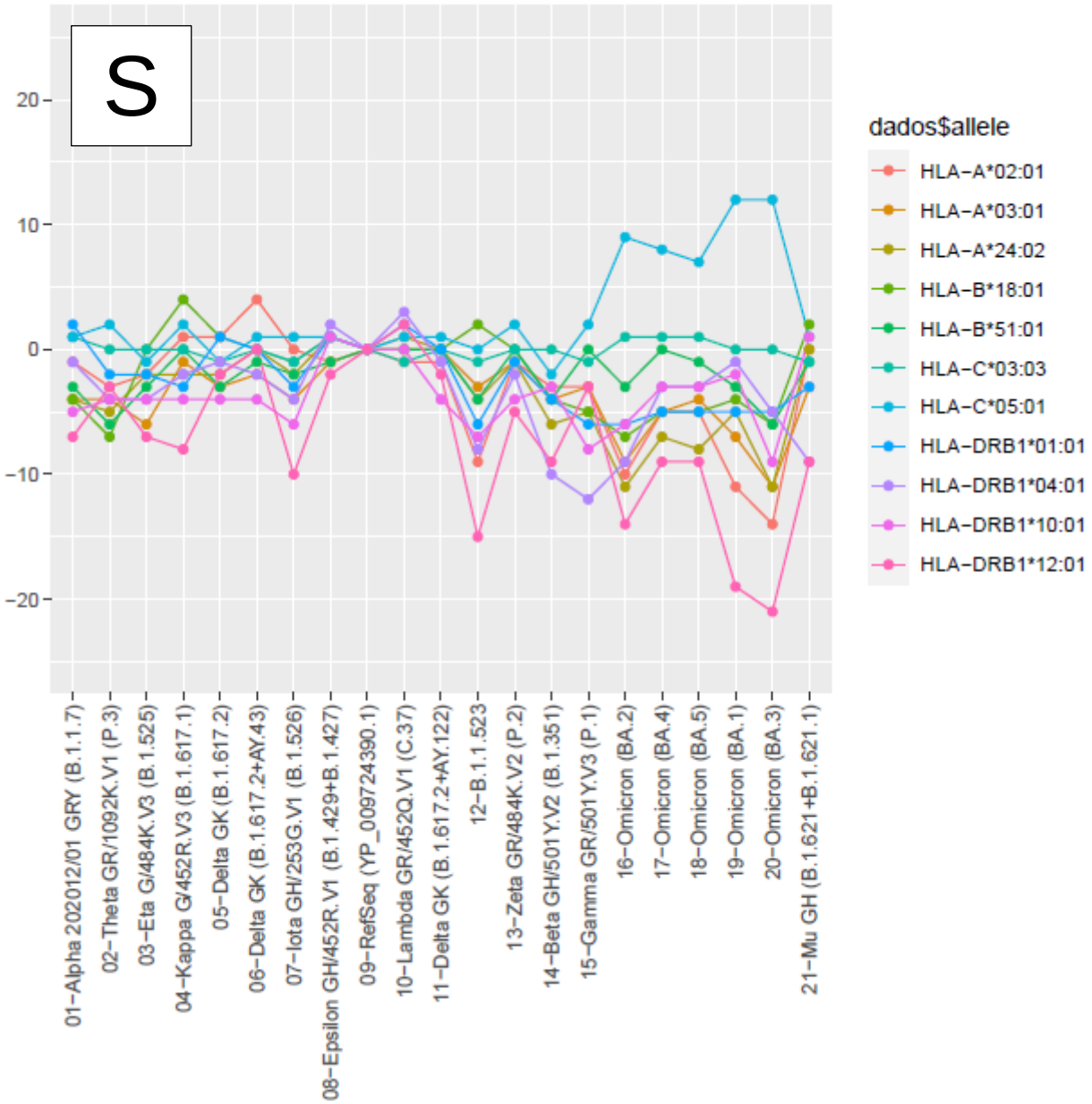
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14−Beta GH/501Y.V2 (B.1.351)
15−Gamma GR/501Y.V3 (P.1)
16−Omicron (BA.2)
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21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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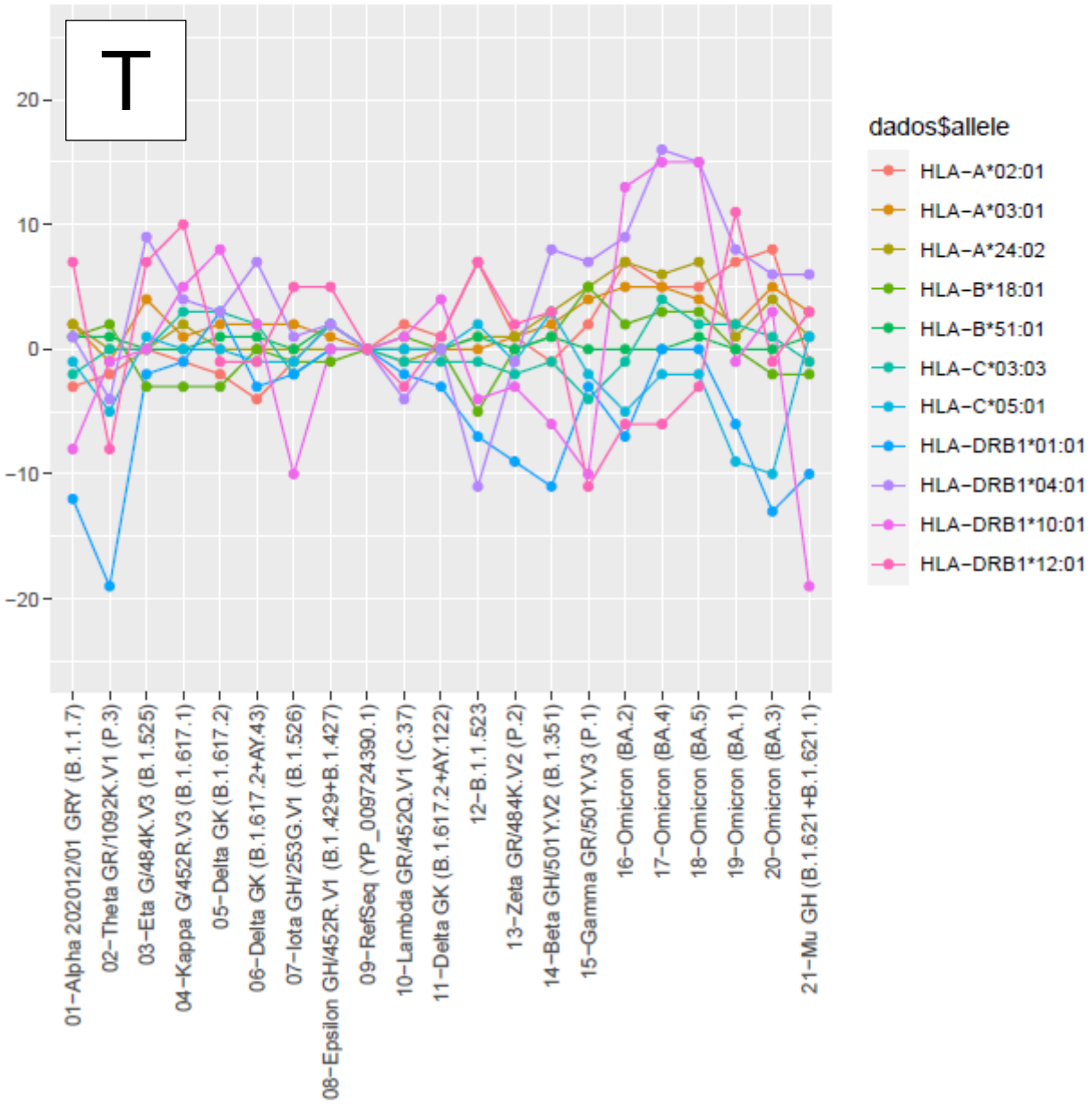
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17−Omicron (BA.4)
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19−Omicron (BA.1)
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21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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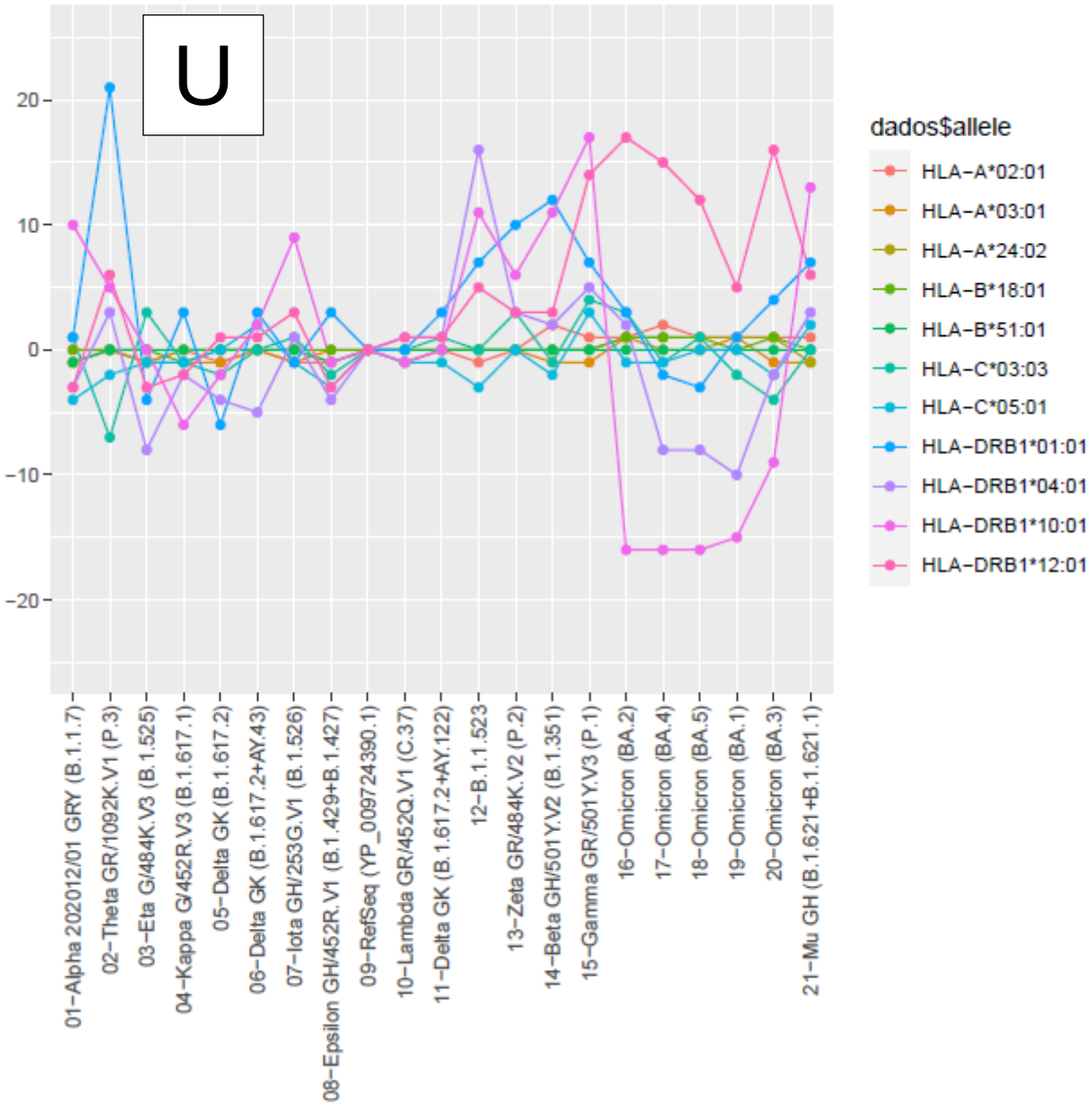
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05−Delta GK (B.1.617.2)
06−Delta GK (B.1.617.2+AY.43)
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17−Omicron (BA.4)
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21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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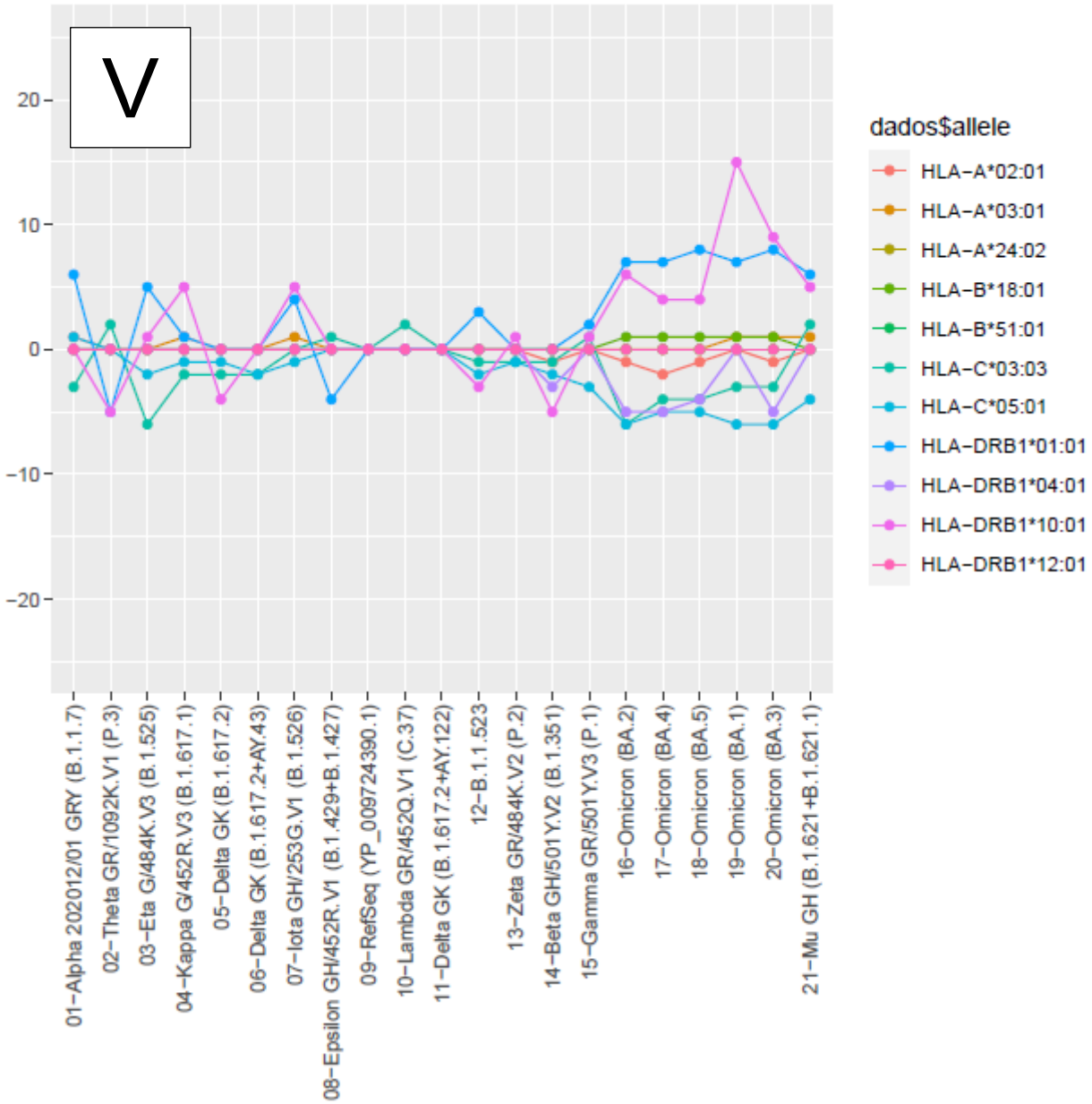
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21−Mu GH (B.1.621+B.1.621.1)
dados$allele
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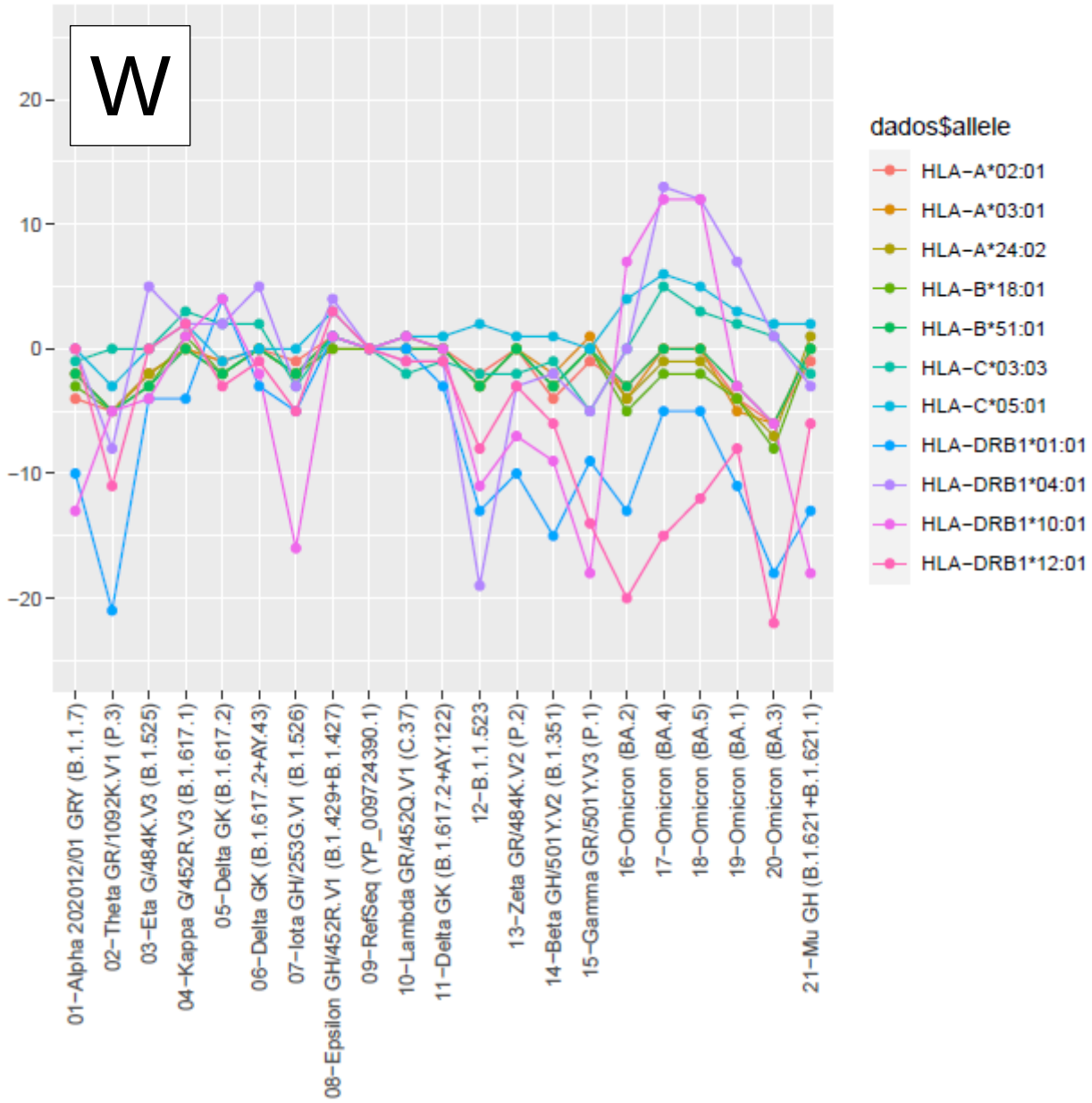
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05−Delta GK (B.1.617.2)
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21−Mu GH (B.1.621+B.1.621.1)
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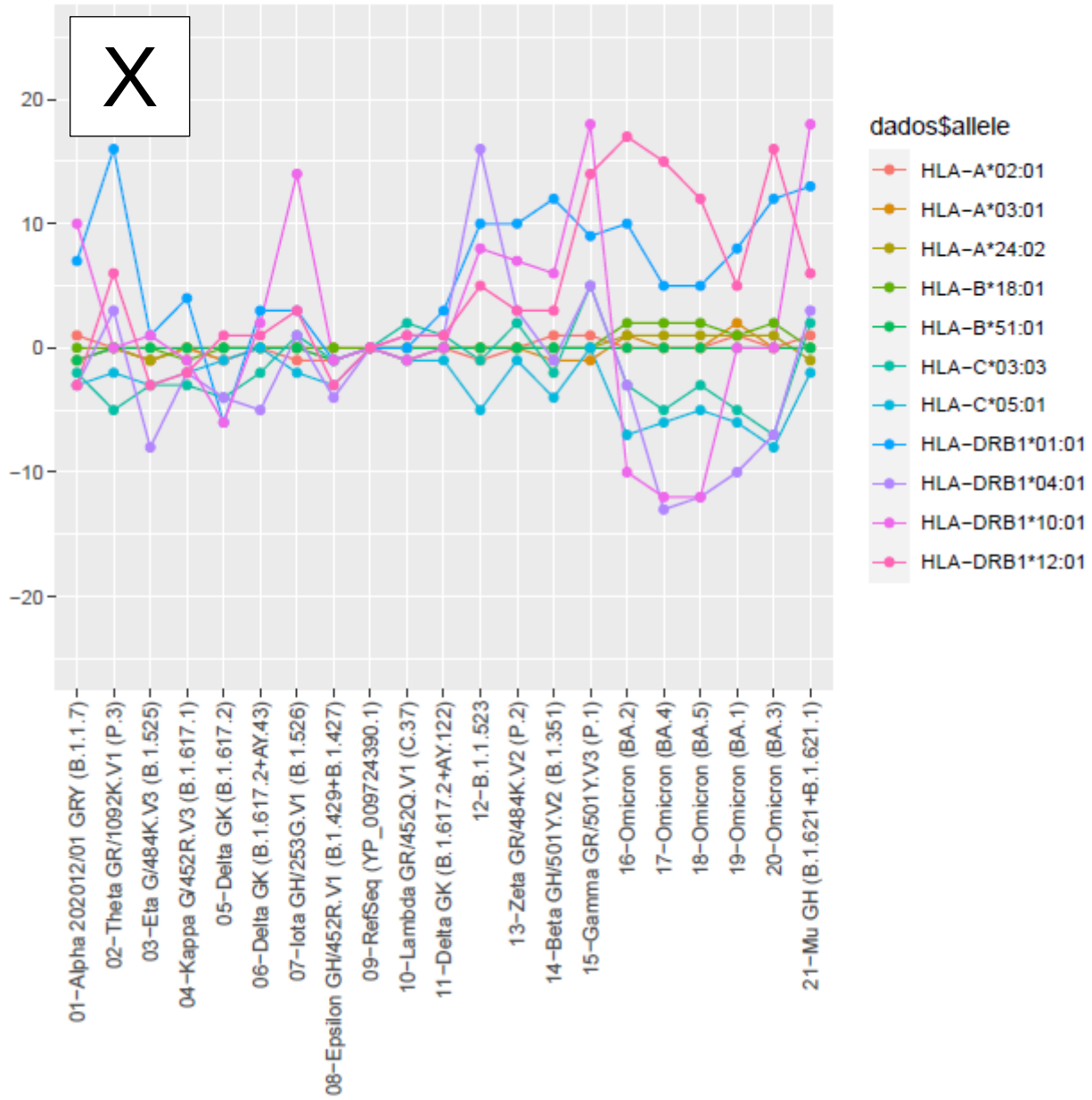
HLA−DRB1*12:01



Supplementary Figure 1. This figure contains graphs with all selected alleles. In this figure these alleles are compared among different viral strains in relation to the increase or decrease in affinity (IC50) with the SARS-CoV-2 Spike protein ("x" axis). The "y" axis describes the total number of peptides that changed their affinity according to each virus variant. When evaluating all *HLA* alleles at the same time we have: **A**: count of **non-binding** viral peptides; **B**: **weak binders**; **C**: **regular binders**; **D**: **strong binders**; **E**: **non-binders + weak binders**; **F**: **regular binders + strong binders**. We then evaluated only the alleles

associated with COVID-19 in this meta-analysis: **G**: count of **non-binding** viral peptides; **H**: **weak binders**; **I**: **regular binders**; **J**: **strong binders**; **K**: **non-binders + weak binders**; **L**: **regular binders + strong binders**. We then split these alleles into two groups, separately assessing those that were at risk for the disease: **M**: count of **non-binding** viral peptides; **N**: **weak binders**; **O**: **regular binders**; **P**: **strong binders**; **Q**: **non-binders + weak binders**; **R**: **regular binders + strong binders**. Finally, we evaluated the alleles that were associated with protection from COVID-19: **S**: count of **non-binding** viral peptides; **T**: **weak binders**; **U**: **regular binders**; **V**: **strong binders**; **W**: **non-binders + weak binders**; **X**: **regular binders + strong binders**.

CAPÍTULO III

CONCLUSÕES

1. A presença do SNP rs11556218 de *IL16* foi associado, na metanálise, com um aumento do risco tanto para o câncer, quanto para doenças cardiovasculares. Além disso, o SNP rs4778889 foi associado a um risco aumentado de câncer gástrico. Portanto, este estudo indica um possível envolvimento de variantes genéticas da *IL16* com a patogênese do câncer e das doenças cardiovasculares.

2. Diferentes alelos *HLA* e genes *KIR* foram associados a COVID-19 na metanálise. Estudos *in-silico* sugerem que muitas das moléculas produzidas por esses alelos podem ter imunogenicidade correlacionada ao acréscimo de risco para a doença observado. Isso pode ser uma indicação de que a variabilidade das células NK e da resposta citotóxica pode desempenhar um papel importante na infecção pelo SARS-CoV-2. Apesar disso, o poder estatístico e a complexidade/variabilidade da interação hospedeiro-vírus continuam sendo um importante desafio neste tópico de estudo.

PERSPECTIVAS

As metodologias aplicadas neste estudo podem ser utilizadas para produzir novos conhecimentos nos estudos de associação genética com doenças. Os métodos podem ser replicados para inúmeros outros marcadores. O desenvolvimento desse estudo permitiu o aperfeiçoamento de métodos estatísticos e de técnicas de bioinformática parcial ou completamente automatizadas, o que possibilitou trabalhar com números altos de variantes genéticas e de estudos.

Para além disso, este estudo possibilita novos caminhos para o desenho de projetos de estudos caso-controle, permitindo elucidar problemas e superá-los e naturalmente explorar novos candidatos a marcadores genéticos.