UNIVERSIDADE FEDERAL DA BAHIA
INSTITUTO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA

DISSERTAÇÃO DE MESTRADO

RAQUEL BISPO DE SÃO PEDRO

Avaliação de genes auto inflamatórios em crianças com Síndrome Inflamatória Multissistêmica Pediátrica (SIM-P) associada à infecção pelo SARS-CoV-2

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AVALIAÇÃO DE GENES AUTOINFLAMATÓRIOS EM CRIANÇAS COM SÍNDROME INFLAMATÓRIA MULTISSISTÊMICA PEDIÁTRICA (SIM-P) ASSOCIADA À INFECÇÃO PELO SARS-COV-2

RAQUEL BISPO DE SÃO PEDRO

Dissertação de mestrado apresentada ao Programa de Pós-graduação em Imunologia, da Universidade Federal da Bahia, como requisito para obtenção do título de Mestre em Imunologia.

Orientador: Prof. Dr. Pablo Rafael Silveira Oliveira

Coorientador: Prof. Dr. Carlos E. Sampaio Guedes

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Nº23M/2023,
ATA DA SESSÃO PÚBLICA DO COLEGIADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA PARA JULGAMENTO DO TRABALHO DE DISSERTAÇÃO INTITULADO **"AVALIAÇÃO DE GENES AUTOINFLAMATÓRIOS EM CRIANÇAS COM SÍNDROME INFLAMATÓRIA MULTISSISTÊMICA PEDIÁTRICA (SIM-P) ASSOCIADA À INFECÇÃO PELO SARS-COV-2"** DA MESTRANDA **RAQUEL BISPO DE SÃO PEDRO.**

Aos treze dias do mês de dezembro de dois mil e vinte e três, na sala de reunião vinculada ao Programa de Pós-graduação em Imunologia da Universidade Federal da Bahia (PPGIm-UFBA), a Banca Examinadora composta pelos Professores: **Pablo Rafael Silveira Oliveira** (orientador)**, Dr. Thiago Luiz de Paula Castro** e **Dra. Vivian Botelho Lorenzo** se reuniram com a finalidade de discutir, avaliar e julgar o Trabalho de Dissertação intitulado: **"Avaliação De Genes Auto Inflamatórios em Crianças com Síndrome Inflamatória Multissistêmica Pediátrica (Sim-P) Associada à Infecção pelo Sars-Cov-2"** da mestranda **Raquel Bispo De São Pedro.** Após a apresentação, arguição e comentários dos membros da Banca Examinadora fica determinado que a Dissertação pode ser apresentada ao colegiado do PPGIm no formato atual. Portanto, a Pós-Graduanda está habilitada à obtenção do título de Mestre em Imunologia. Adicionalmente, os pareceres individuais dos membros da Banca Examinadora serão anexados à ata. Nada mais havendo a tratar se encerra a sessão da qual é lavrada a presente ata que após lida e aprovada vai assinada pelos componentes da Banca examinadora, pela mestranda e pela Coordenadora do Programa de Pós-Graduação. Salvador, treze de dezembro de dois mil e vinte e três.

Prof. Dr. Pablo Rafael Silveira Oliveira
Orientador

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Raquel Bispo de São Pedro
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Coordenadora do PPGIm
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RESUMO

Introdução: A Síndrome Inflamatória Multissistêmica Pediátrica (SIM-P) é uma condição inflamatória associada à infecção pelo SARS-CoV-2. É caracterizada por febre, sintomas gastrointestinais proeminentes, manifestações mucocutâneas, sintomas respiratórios e choque. A ocorrência de SIM-P pode estar associada a erros inatos da imunidade, que afetam seletivamente a resposta imune do hospedeiro contra o SARS-CoV-2. **Objetivo:** Identificar variantes em genes envolvidos em condições autoinflamatórias primárias que podem ser determinantes para a SIM-P. **Métodos:** Foram coletadas células e informações clínicas e laboratoriais de 21 pacientes pediátricos com SIM-P, recrutados em três hospitais públicos da região Nordeste do Brasil. Os casos de SIM-P foram classificados como graves ou moderados, considerando-se a necessidade ou não, respectivamente, de Ventilação por Pressão Positiva (PPV) e/ou medicação vasopressora. Em seguida, foi realizado sequenciamento total do exoma (WES) dos indivíduos e aplicada estratégia de priorização de Variantes de Nucleotídeo Único (SNVs) com potencial deletério, com foco em 56 genes previamente implicados em doenças autoinflamatórias (de acordo com o Comitê de Imunidade da União Internacional das Sociedades de Imunologia, 2022). **Resultados:** Nove indivíduos apresentaram a forma moderada da SIM-P, enquanto os outros 12 foram incluídos no grupo de SIM-P grave. Na abordagem de priorização de variantes, foram identificadas 6 SNVs em 5 genes diferentes (*ADAM17, CARD14*, *IKBKG, PSTPIP1* e *SH3BP12*). Todas essas variantes foram encontradas em crianças/adolescentes com a forma grave da SIM-P. Notavelmente, foram selecionadas duas variantes (rs1200631089 e rs144458353) no gene *ADAM17*. Tal gene codifica uma protease implicada no processamento do fator de necrose tumoral alfa (TNF-α) e desempenha papel fundamental na infecção pelo SARS-CoV-2, clivando a Enzima Conversora de Angiotensina 2 (ECA-2), o principal receptor humano para o SARS-CoV-2. **Conclusão:** Nossos dados sugerem que variantes deletérias raras em genes previamente implicados em condições autoinflamatórias, incluindo *ADAM17, IKBKG, PSTPIP1, SH3BP2 e CARD14,* podem explicar a ocorrência de SIM-P em crianças e adolescentes brasileiros previamente sadios.

Palavras-chave: SIM-P; SARS-CoV-2; Auto inflamatório; Genética.

ABSTRACT

Introduction: Multisystem inflammatory syndrome in children (MIS-C) is an inflammatory condition associated with SARS-CoV-2 infection. It is characterized by fever, prominent gastrointestinal symptoms, mucocutaneous manifestations, respiratory symptoms, and shock. The occurrence of P-MIS may be linked to innate immunological errors that selectively affect the host's immune response against SARS-CoV-2. **Ain:** Identify variants in genes involved in primary autoinflammatory conditions that may be implicated in MIS-C. **Methods:** Cells and clinical/laboratory information were collected from 21 pediatric patients with MIS-C recruited from three public hospitals in the Northeast region of Brazil. The MIS-C cases were categorized as severe or moderate based on the need for Positive Pressure Ventilation (PPV) and/or vasopressor medication, respectively. Subsequently, whole exome sequencing (WES) was performed on the individuals, and a strategy for prioritizing Single Nucleotide Variants (SNVs) with potential deleterious effects was applied. The focus was on 56 genes previously implicated in autoinflammatory diseases (according to the International Union of Immunological Societies Immunodeficiency Committee, 2022). **Results:** Nine individuals presented with the moderate form of MIS-C, while the remaining 12 were included in the severe MIS-C group. In the variant prioritization approach, six Single Nucleotide Variants (SNVs) were identified in five different genes (*ADAM17*, *CARD14*, *IKBKG*, *PSTPIP1*, and *SH3BP12*). All these variants were found in children/adolescents with the severe form of MIS-C. Notably, two variants (rs1200631089 and rs144458353) in the *ADAM17* gene were selected. This gene encodes a protease implicated in the processing of tumor necrosis factor-alpha (*TNF-α*) and plays a crucial role in SARS-CoV-2 infection by cleaving Angiotensin-Converting Enzyme 2 (*ACE2*), the primary human receptor for SARS-CoV-2. **Conclusion:** Our data suggest that rare deleterious variants in genes previously implicated in autoinflammatory conditions, including *ADAM17*, *IKBKG*, *PSTPIP1*, *SH3BP2*, and *CARD14*, may account for the occurrence of P-MIS in previously healthy Brazilian children and adolescents.

Keywords: MIS-C; SARS-CoV-2; Autoinflammatory; Genetics.

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LISTA DE SIGLAS E ABREVIAÇÕES

ABCC6	Transportadores ABC (Cassetes de ligação ATP)
ACE-2	Enzima Conversora de Angiotensina 2
ADAM17	Proteína contendo domínio de desintegrina e metaloproteinase 17
ALT	Alanina aminotransferase
Anti-N	Anti-Nucleocapsídeo
AP3B1	Subunidade beta-1 do complexo AP-3
AST	Aspartato aminotransferase
ATP4A	Subunidade de Transporte Alpha da ATPase H+/K+
BAM	Arquivo binário de mapa de alinhamento de sequência
BSCL2	Biogênese de gotículas lipídicas associada, Seipin
C	Proteína do Complemento
CADD	Depleção dependente de anotação combinada
CARD14	Membro da Família do Domínio de Recrutamento Caspase 14
CCL	Ligante da quimiocina (motivo C-C)
CD	Cluster de Diferenciação
CDC	Centro de Controle e Prevenção de Doenças
CDCP1	Proteína 1 contendo domínio CUB
COVID-19	Doença do coronavírus 2019
CPK	Creatinofosfoquinase
CSNK	Caseína quinase
CXCL	Ligante da quimiocina (motivo C-X-C)
CYBB	Cadeia Beta do Citocromo B-245
DHX58	DExH-box helicase 58
DK	Doença de Kawasaki
DNA	Ácido desoxirribonucleico
DOCK8	Dedicator da citocinese 8
EII	Erros Inatos da Imunidade
FREM1	Matriz Extracelular Relacionada ao FRAS1
GATK	ToolKit de Análise genômica
GDI	Índice de dano gênico
GnomAD	Banco de Dados de Agregação Genômica
HIV/AIDS	Vírus da imunodeficiência humana/ Síndrome da imunodeficiência adquirida
HLA	Antígeno Leucocitário Humano
HMG	Hospital Martagão Gesteira
HUOC	Hospital Universitário Oswaldo Cruz
IBGE	Instituto Brasileiro de Geografia e estatística
ICOM	Instituto Couto Maia
IDP	Imunodeficiência primária
IFIH1	Proteína Induzida por interferon com domínio C da helicase
IFN	Interferon
IFNAR1	Subunidade 1 do receptor do interferon alfa
IgA	Imunoglobulina A
IgG	Imunoglobulina G
IKBKG	Inibidor do Fator Nuclear Kappa B Quinase Subunidade Regulatória Gama
IL	Interleucina
IL-6R	Receptor da Interleucina 6
ILCs	Células linfoides inatas

IQR	Intervalo interquartil
IUIS	União Internacional de Sociedades Imunológicas
JAK - Janus quinase	Janus quinase
LDH	Desidrogenase Láctica
MAF	Frequência do alelo menor
MAP2K2	Proteína quinase ativada por mitógeno 2
MAP9	Proteína Associada a Microtúbulos 9
MAPK	Proteína quinase ativada por mitógeno
MCP-1	Proteína Quimiotática de Monócitos 1
mIL-6R	Receptor transmembrana IL-6
MPO	Mieloperoxidase
NEMO/IKK-γ	Modulador NF-kappa-B
NF-κB	Factor nuclear kappa B
NLRs	Receptor NOD-*like*
NOTCH1	Proteína homóloga de entalhe de locus neurogênico 1
OAS	Oligoadenilato sintase
OMS	Organização Mundial da Saúde
PAPA	Síndrome da Artrite piogênica, pioderma gangrenoso e acne
PCR	Proteína C Reativa
PDLIM5	Proteína 5 do domínio PDZ e LIM
pHLH	Linfo-histiocitose hemofagocítica primária
POLG	DNA polimerase nuclear codificada gama
PSTPIP1	Prolina-Serina-Treonina Fosfatase Proteína de Interação 1
RBM38	Proteína Motivo de Ligação ao RNA 38
RIG	Indutores de ácido retinóico
RNase L	Ribonuclease L
RR	Intervalo de referência
RT-PCR	Reação em Cadeia da Polimerase em Tempo Real
S100	Proteína ligadora de cálcio S100
SARS-CoV-2	Síndrome Respiratória Aguda Grave - Coronavírus 2
SDRA	Síndrome do Desconforto Respiratório Agudo
SH3BP2	Proteína 2 de ligação ao domínio SH3
SIFT	Separando Intolerantes de Tolerantes
sIL-6R	Receptor Solúvel da IL-6
SIM-P/MIS-C	Síndrome Inflamatória Multissistêmica Pediátrica
SNV	Variante de nucleotídeo simples
SOCS1	Supressor da sinalização de citocinas 1
STAT3	Transdutores de sinal e ativadores de transcrição 3
TCR	Receptor de células T
TLRs	Receptores Toll-*like*
TNF-α	Fator de Necrose tumoral alfa
TRADD	Proteína de domínio de morte associada a TNF-R1
TRBV11-2	Receptor de Células T Beta Variável 11-2
UNC13D	Proteína unc-13 homóloga D
UTI/ICU	Unidade de Terapia Intensiva
VEGF	Fator de Crescimento Endotelial Vascular
VHS	Velocidade de Hemossedimentação
VIF	Fator de inflação da variância
WBC	Leucócitos
WES	Sequenciamento total do exoma
XIAP	Inibidor da proteína da apoptose ligado ao X

SUMÁRIO

1. REVISÃO DE LITERATURA

1.1 COVID-19 em crianças versus Síndrome Inflamatória Multissistêmica Pediátrica (SIM-P)

A doença do coronavírus 2019 (COVID-19), causada pelo vírus da Síndrome Respiratória Aguda Grave Coronavírus 2 (SARS-CoV-2) não afeta crianças e adolescente do mesmo modo que afeta os adultos (Zachariah, P., 2022). Em adultos e idosos, a gravidade da doença causada pelo SARS-CoV-2 é amplamente influenciada por doenças preexistentes, como idade e sexo (Kurian, J. et al., 2022; Zhang, JJ., et al., 2023). Em um dos primeiros estudos sobre a COVID-19 grave, com 1.590 pacientes, foi visto que 399 (25,1%) pessoas apresentavam pelo menos uma comorbidade, enquanto 130 (8,2%) apresentavam duas ou mais comorbidades. As condições preexistentes mais comuns foram hipertensão (16,9%), diabetes (8,2%), doenças cardiovasculares (3,7%) e doença renal crônica (1,3%) (Guan, W.J., et al, 2020). Uma metanálise com 281.461 indivíduos mostrou que a idade média dos pacientes com COVID-19 grave foi de 60,4 anos e 61% desses indivíduos eram do sexo masculino (Li ,J., et al 2021).

Nos pacientes pediátricos infectados pelo SARS-CoV-2 a apresentação clínica geralmente possui um curso mais leve, com a maioria das crianças/adolescentes apresentando apenas febre, tosse seca, fadiga, cefaleia, odinofagia e/ou sintomas gastrointestinais (Rotulo, G., Palma, P., 2023). De dezembro de 2019 até fevereiro de 2020 na China, dos 73.314 casos de COVID-19 registrados em todas as faixas etárias, nenhum paciente pediátrico com 9 anos ou menos morreu, diferentemente dos grupos de pacientes com 70-79 anos e ≥ 80, cujas taxas de mortalidade foram de 8% e 14,8%, respectivamente (Wu, Z., McGoogan, J., 2020). São raros os casos de crianças com quadros clínicos graves causados pela infecção com o SARS-CoV-2, mas crianças obesas, com distúrbios neurológicos ou metabólicos, doença cardíaca congênita, doença falciforme ou imunossupressão evoluem mais frequentemente para formas graves (Kurian, J. et al., 2022). Ainda assim, é importante ressaltar que mesmo no grupo de crianças que apresentam tais comorbidades, as frequências de casos graves e críticos continuam substancialmente menores (2,5% e 0,6%, respectivamente) do que as apresentadas por indivíduos adultos (Rotulo, GA., Palma P., 2023).

Hipóteses foram levantadas para justificar as diferenças na suscetibilidade à infecção pelo SARS-CoV-2 e evolução da doença entre adultos e crianças. Dentre estas, a mais aceita é a de

que moléculas do hospedeiro envolvidas no processo de infecção pelo SARS-CoV-2 são menos expressas em jovens (Zachariah, P., 2022). A entrada do SARS-CoV-2 nas células humanas depende, dentre outras condições, da interação entre a proteína S (Spike) do vírus e receptores celulares, como a Enzima Conversora de Angiotensina 2 (ACE2) (Sharma C. et al., 2021). Logo, por apresentaram menor expressão de ACE2 nos tecidos pulmonares, crianças e adolescentes seriam menos suscetíveis à infecção pelo SARS-CoV-2 (Hoffmann, M. et al., 2020).

De modo geral, crianças infectadas pelo SARS-CoV-2 apresentam recuperação total em 1 a 2 semanas (Kurian, J., 2022). Entretanto, logo no início da pandemia de COVID-19 surgiram casos de uma síndrome semelhante à Doença de Kawasaki (DK), que foi considerada uma complicação da infecção pelo SARS-CoV-2, principalmente em crianças e adolescentes (Abuhammour, W., et al., 2022). Um relatório do *Royal College of Paediatrics and Child Health* (2020) do Reino Unido (UK) descreveu que a condição clínica citada não se tratava da DK, mas que representava uma condição pós-infecciosa relacionada ao SARS-CoV-2 (Waseem, M., et al., 2022), nomeando essa apresentação como Síndrome Inflamatória Multissistêmica Pediátrica (SIM-P) (Munblit, D., et al., 2022). Na mesma época, houve aumento substancial do número de casos de SIM-P nos Estados Unidos da América (EUA) e a descrição dos casos feita pelo Centro de Controle e Prevenção de Doenças (CDC) dos EUA coincidiu com os casos observados na Inglaterra e em diversos outros países (Lam, K.P., et al., 2022). Posteriormente, essa afecção rara também foi descrita em adultos (Patel, P., et al., 2021).

O diagnóstico da SIM-P, definido pela Organização Mundial da Saúde (OMS) (Lin, J., et al., 2023), considera crianças e adolescentes de 0 a 19 anos com febre ≥ 3 dias, e que possuam: ≥ 2 sinais clínicos de envolvimento multissistêmico (ex.: erupção cutânea e choque); marcadores elevados de inflamação; nenhuma outra inflamação com causa microbiana óbvia (sepse bacteriana) e confirmação de infecção pelo SARS-CoV-2 por Reação em Cadeia da Polimerase em Tempo Real (RT-PCR), sorologia, teste rápido ou história de contato com pessoa infectada (OMS, 2020).

1.2 Aspectos epidemiológicos, clínicos e laboratoriais da SIM-P

Em 2020 no Brasil, a incidência da SIM-P foi de 1,1/100 mil habitantes com menos de 20 anos (Relvas-Brandt, L., et al., 2020). Uma das primeiras séries de casos de SIM-P no Brasil diagnosticou a condição em 23 crianças, com tempo médio de 15 dias entre a exposição ao vírus e o início dos sintomas (Farias, E., et al., 2020). Um outro estudo brasileiro, com 66 pacientes, relatou 06 (seis) indivíduos com SIM-P grave, sendo que 04 (quatro) desses pacientes morreram: 01 (um) adolescente previamente sadio, 01 (um) paciente com imunodeficiência primária com fibrose hepática concomitante e 02 (dois) com câncer (Pereira, M., et al., 2020). No mesmo ano, entre 1º de março e 10 de maio, um estudo focado na população de Nova York (EUA) relatou incidência de 02 (dois) casos de SIM-P a cada 100 mil indivíduos com idade <21 anos, em uma população com uma incidência de infecção confirmada por SARS-CoV-2 de 322/100 mil (Sancho-Shimizu, V., et al., 2021). Na população de Israel, a incidência de casos de SIM-P foi mais alta, com 54,5, 49,2 e 3,8 a cada 100 mil pessoas infectadas pelo SARS-CoV-2, seguindo respectivamente as ondas das variantes Alpha (2020-2021), Delta (2021) e Ômicron (2021-2022) (Levy, N., et al., 2022). Na Suécia, foi observado que dentre 2.117.443 crianças e adolescentes, 253 desenvolveram SIM-P, correspondendo a uma incidência de 6,8/100 mil (Rhedin, S., et al., 2022).

Ainda não há consenso sobre se o risco à SIM-P é influenciado por fatores genéticos ligados à ancestralidade. Mesmo assim, foi evidenciado que indivíduos com ancestralidade africana na França e no Reino Unido são desproporcionalmente mais afetados pela SIM-P do que indivíduos de outros grupos étnicos (Waseem, M., et al., 2022). Ao comparar a distribuição de etnias em pacientes com SIM-P nos EUA com a de crianças na população em geral, a SIM-P foi mais frequente entre crianças hispânicas e crianças negras e menos frequente entre crianças brancas e crianças asiáticas (Stierman, B., et al., 2021; Waseem, M., et al., 2022; Lin, J., et al., 2023). Esses achados se mantêm mesmo após a aplicação de correções estatísticas que levam em consideração as inequidades socioeconômicas e de acesso à saúde dessas populações. Avaliand-se crianças com diferentes origens étnicas nos EUA, a incidência da SIM-P foi maior entre negros (9,26/1 milhão), seguido por hispânicos o/latinos (8,92/1 milhão) e asiáticos ou das ilhas do Pacífico (2,94/1 milhão) (Payne, A.B., et al., 2021).

A SIM-P é caracterizada por desregulação sistêmica da resposta imune (Panaro, S, Cattalini, M., 2021). De modo geral, os indivíduos com SIM-P apresentam sorologia positiva para o SARS-CoV-2 e, em um terço destes, são observados sorologia e RT-PCR positivos (Panaro, S, Cattalini, M., 2021; Hosseini, P., et al., 2022). Diversas linhas de evidência indicam que os primeiros sintomas da SIM-P surgem cerca de 2 a 6 semanas após a infecção pelo SARS-CoV-2, sendo caracterizada, principalmente, por febre persistente e sinais inflamatórios sistêmicos (Panaro, S, Cattalini, M., 2021). Segundo revisão sistemática desenvolvida por Radia e colaboradores (2021), os sintomas mais comuns da SIM-P são: os gastrointestinais (71%), com incidência de vômitos (25%), diarreia (27%) e dor abdominal (36%); e os mucocutâneos (42%) (Figura 1).

As manifestações clínicas da SIM-P são influenciadas pela idade do paciente (Molloy, E.J., et al., 2022). Crianças de 0 a 4 anos desenvolvem com mais frequência conjuntivite bilateral não purulenta, rash cutânea e dor abdominal. Já os adolescentes (15 a 19 anos) apresentam mais sintomas respiratórios, como dispneia e tosse. Adicionalmente, a magnitude da resposta inflamatória na SIM-P se correlaciona com sua gravidade (Sacco, K., et al., 2022; Rhedin, S., et al., 2022). White e colaboradores (2020) relataram a admissão de 70 crianças com diagnóstico de SIM-P em um hospital em Londres (Inglaterra), e a complicação mais frequente nessas crianças foi lesão cardíaca. Tais resultados relacionados ao acometimento cardíaco na SIM-P não ficaram limitados aos pacientes de Londres. Em um estudo de Molloy e colaboradores (2022), foi observado que pacientes com SIM-P apresentaram evidências de disfunção cardíaca (40,6%), choque (35,4%), miocardite (22,8%) e dilatação de coronárias ou aneurisma (18,6%) (Molloy, E.J., et al., 2022)(Figura 1). Foi observado em outro estudo que uma parcela de indivíduos com SIM-P admitidos em unidade de terapia intensiva (UTI) apresentou fração de ejeção sistólica reduzida, pericardite, disfunção valvular, derrame pericárdico, arritmia, dilatação ventricular e taquicardia (Hosseini, P., et al., 2022). Embora as complicações mais prevalentes da SIM-P sejam disfunção ventricular e dilatação de coronárias, os mecanismos subjacentes a essas condições permanecem incompreendidos (Lin J., et al., 2023).

Os achados laboratoriais mais comuns na SIM-P são leucocitose com neutrofilia, marcadores inflamatórios elevados e anemia leve, que podem acometer até 90% dos pacientes (Panaro, S, Cattalini, M., 2021) (Figure 1). A lista de marcadores inflamatórios elevados inclui: proteína C reativa (PCR), ferritina, D-dímero e troponina I. Além disso, são

observados linfocitopenia e baixos níveis de vitamina D (White, M., et al., 2020). Nessa direção, um estudo com 45 crianças do Oriente Médio com SIM-P evidenciou elevação plasmática desses mesmos marcadores de inflamação, além do aumento da taxa de sedimentação de eritrócitos e da concentração plasmática de Interleucina-6 (IL-6) (Abuhammour, W., et al., 2022). Além desses marcadores, ainda são observados aumento da velocidade de hemossedimentação (VHS), elevação da desidrogenase láctica (LDH), aumento de triglicerídeos, tempo de protrombina prolongado e hipoalbuminemia (Hosseini, P., et., 2022).

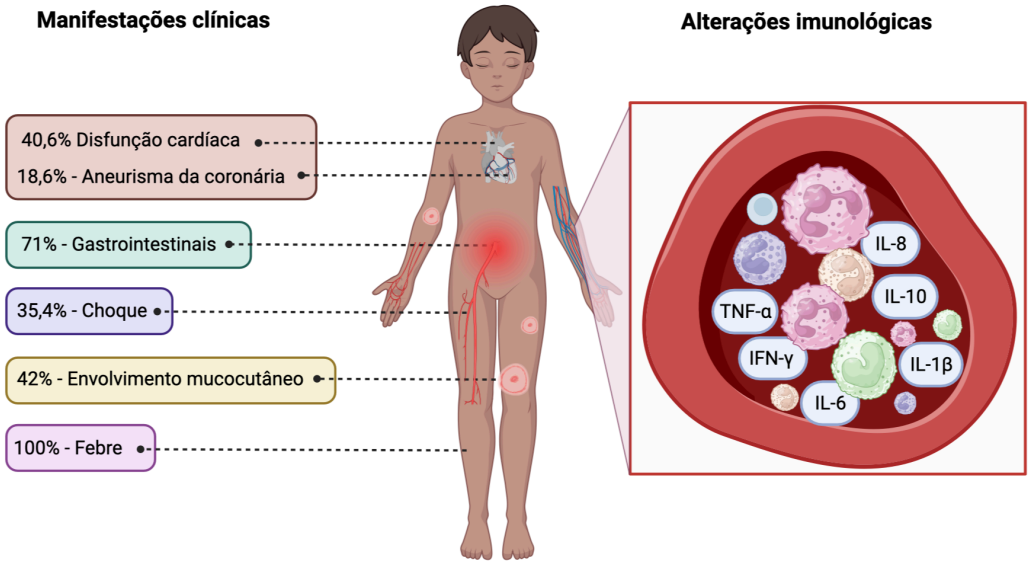

Figura 1. Manifestações clínicas e laboratoriais em crianças com SIM-P. As manifestações mais comuns no quadro clínico são: febre (100%); envolvimento gastrointestinal (71%); envolvimento mucocutâneo (42%); disfunção cardíaca (40,6%); choque (35,4%) e aneurisma da coronária (18,6%). Nas alterações imunológicas ocorrem leucocitose com neutrofilia, elevação de níveis de IL-8, IL-10, IL-6, TNF, IFN-γ e IL-1β. Baseado em: Molloy, E.J., et al., 2022/ Panaro, S, Cattalini, M., 2021; White, M., et al., 2020 e Abuhammour, W., et al., 2022.

Como dito inicialmente, a expressão de *ACE2* aumenta conforme a idade do paciente (Zachariah, P., 2022). Porém, também é fundamental salientar que o gene *ACE2* se situa no cromossomo X, sendo regulado negativamente pelo estrogênio, o que pode predispor indivíduos do sexo masculino à infecção pelo SARS-CoV-2 e/ou à SIM-P (Lin, J., et al., 2023). Com isso, foi proposta uma hipótese de que a SIM-P seria mais prevalente em crianças do sexo masculino, e que estas teriam maior risco de complicações cardíacas (Lin, J., et al., 2023; Rotulo, GA., Palma, P., 2023). Entretanto, séries de casos de SIM-P publicadas até o momento

não revelaram predominância do sexo masculino (Waseem M., et al., 2022). Embora acometa pessoas de 0 a 19 anos, crianças de 6 a 12 anos são mais suscetíveis à SIM-P (Lin, J., et al., 2023; Feldstein, L.R., et al., 2021).

1.3 Patofisiologia da SIM-P

1.3.1 Sistema Imune Inato

As células do sistema imune inato são fundamentais para o processo de infecção pelo SARS-CoV-2. As células dendríticas, por exemplo, expressam a molécula *CD147* que interage com a proteína Spike do SARS-CoV-2 e favorece a infecção (Panaro, S., Cattalini, M., 2021). Em um estudo publicado no final de 2022, foi observado que indivíduos com SIM-P apresentam redução nas contagens de monócitos e células dendríticas no sangue periférico, bem como diminuição da expressão de Antígeno Leucocitário Humano (*HLA-DR*) e aumento de monócitos não clássicos (Rajamanickam, A., et al., 2022).

Avaliando genes diferencialmente expressos em neutrófilos e monócitos, com o intuito de investigar as contribuições de componentes da resposta imune inata na SIM-P, Ramaswamy e colaboradores (2021) observaram que há aumento da expressão dos genes *S100A8, S100A9* e *S100A12,* que codificam alarminas. Dentre as funções dessas alarminas têm-se: influência na migração celular e quimiotaxia, além de indução do reparo tecidual (Zhang, Y., et al., 2021). Adicionalmente, foi evidenciado que monócitos de indivíduos com SIM-P têm expressão reduzida de moléculas apresentadoras de antígeno classe II (*HLA-DP*, *DQ* e *DR*) e *CD86,* que são cruciais para a ativação de células T (Ramaswamy, A., et al., 2021). Outrossim, foi observado menor número de Células Linfoides Inatas (ILCs) em crianças com SIM-P em comparação com controles sadios (Rotulo, G., Palma, P., 2023). As ILCs participam da resposta do hospedeiro contra a infecção pelo SARS-CoV-2 e promovem reparo tecidual através da ação da anfirregulina (Rotulo, G., Palma, P., 2023). Por fim, foi observado aumento do número de neutrófilos $CD64^{+}$ em indivíduos com SIM-P (Hosseini, P., et al., 2022).

1.3.2 Citocinas

A tempestade de citocinas, fenômeno relacionado com o desenvolvimento de quadro clínico grave em indivíduos com COVID-19, também está presente na SIM-P (Hosseini, P., et

al., 2022). Este evento é caracterizado por resposta imunológica excessiva, que pode culminar em falência de múltiplos órgãos e coagulopatia (Zanza, C., et al., 2022; Lin, J., et al., 2023). Um estudo prospectivo de 14 pacientes com SIM-P evidenciou que a tempestade de citocinas nessa síndrome tem como mediador central o IFN-γ, em oposição aos IFNs do tipo I, que, são implicados na COVID-19 aguda grave (Hoste, L., et al., 2022). Esse achado é corroborado pela observação de que as expressões de CXCL9 e CXCL10, quimiocinas induzidas por IFN-γ, estão elevadas na SIM-P (Hoste, L., et al., 2022; Lin, J., et al., 2023).

Identificou-se a existência de variantes genéticas com perda de função em crianças do Oriente Médio com SIM-P, localizadas nos genes *IL22RA2* e *IFIH1,* que possuem, respectivamente, funções de sinalização e indução de citocinas (Abuhammour, W., et al., 2022). As falhas nesses mecanismos podem levar à desordem imunológica, causando uma reação sistêmica (Zanza, J., et al., 2022; Rotulo, G., Palma, P., 2023). Na SIM-P, há elevação da expressão de diversas citocinas como: Interleucina (IL)-1β, IL-1RA, IL-6, IL-8, IL-10, IL-17, IL-18, IFN-γ e fator de necrose tumoral alfa (TNF)-α (Hoste, L., et al., 2022; Lin, J., et al., 2023). Notavelmente, esse aumento é mais comum em indivíduos com quadros mais graves de SIM-P e que apresentam miocardite (Panaro, S., Cattalini M., 2021).

Em uma análise comparativa entre a fase aguda da SIM-P e a fase pós recuperação, foi observada elevação da expressão de diversas quimiocinas (CCL1, CCL3, CCL11, CXCL1, CXCL10 e CXCL11) na fase aguda, mas que 6 a 9 meses após a resolução da SIM-P há diminuição significativa da produção dessas moléculas (Kumar, N.P., et al., 2022). Por outro lado, uma análise de biomarcadores solúveis na fase aguda da SIM-P revelou concentrações reduzidas de CCL22, uma quimiocina homeostática que promove a migração e função das células T reguladoras, o que pode favorecer o descontrole da resposta inflamatória (Sacco, K., et al., 2022).

Outras quimiocinas já foram descritas como elevadas na SIM-P, como CCL4, CCL20, CCL28 e CDCP1 (Kumar, N.P., et al., 2022; Lin, J., et al., 2023). A IL-6 destaca-se como importante marcador do perfil inflamatório da SIM-P através de suas vias de sinalização *cis* e *trans*. Na sinalização *cis*, a ligação da IL-6 com seu receptor transmembrana (mIL-6R) resulta na dimerização da gp130, fosforilação da *STAT3* e ativação de *Akt/mTOR* e *MAPK*. Já na via de sinalização *trans*, há ativação da via JAK/STAT3 através da formação do complexo da IL-6 com

o seu receptor solúvel (sIL-6R) ligados à gp130. Esse processo desencadeia mais produção de IL-6, IL-8, proteína quimiotática de monócitos 1 (MCP-1), fator de crescimento endotelial vascular (VEGF) e redução da expressão de E-caderina em células endoteliais, gerando um ciclo de dano endotelial derivado do aumento do nível dessas citocinas (Lin J., et al., 2023). O dano endotelial gerado pode, potencialmente, explicar a ocorrência de insuficiência renal em crianças com SIM-P (Panaro, S., Cattalini M., 2021).

Através de análises transcriptômica e proteômica, Sacco e colaboradores (2022) identificaram assinaturas moleculares em indivíduos com SIM-P, incluindo a ativação de vias dependentes de IFN tipo II e NF-κB, ativação do matrissomo, que engloba proteínas associadas à matriz extracelular (incluindo o endotélio), e níveis aumentados da proteína S do SARS-CoV-2 na circulação sanguínea (Sacco, K., et al., 2022). O NF-κB é um importante fator de transcrição envolvido na indução de genes pró-inflamatórios, elevação da produção de citocinas/quimiocinas e regulação do inflamassoma (Sacco, K., et al., 2022; Lin, J., et al., 2023). A assinatura do NF-κB visto na SIM-P pode possuir relação com o TNF-α, que também tem níveis elevados nessas crianças, uma vez que a ativação dessa última citocina pró-inflamatória induz o recrutamento de TRADD (proteína de domínio de morte associada a TNF-R1) e o TRADD está envolvido na ativação de NF-κB (Lin, J., et al., 2023).

1.3.3 Imunidade celular

Em estudo conduzido por Rajamanickam e colaboradores (2022), foi realizada análise de imunofenotipagem para identificar subconjuntos de células T na SIM-P. Como resultado, foi evidenciado que indivíduos com SIM-P apresentam baixas contagens de leucócitos, como linfócitos e monócitos. Por outro lado, esses pacientes apresentam números aumentados de eosinófilos. A infecção persistente pelo SARS-CoV-2 pode explicar a linfopenia dos indivíduos com SIM-P através do esgotamento das células *natural killer* e T (Rotulo, G., Palma P., 2023). Por outro lado, indivíduos com SIM-P podem exibir números aumentados de linfócitos B *naïve*, imaturos e/ou de memória em comparação com crianças com COVID-19 aguda (Rajamanickam, A., et al., 2022). A linfopenia observada na SIM-P além de abranger células $CD4^+$ e $CD8^+$, envolve também as células T γδ, que possuem propriedades antivirais (Rotulo G., Palma P., 2023). Foram observadas elevações das expressões de CD38 e do receptor HLA-DR em células imunes de indivíduos com SIM-P, indicando ativação de células T (Lin, J., et al.,

2023; Hosseini, P., et al., 2022). Foi evidenciado também que pacientes com SIM-P possuem depleção de células NK, que as impede de realizar suas funções, como, por exemplo, regular a atividade de células $CD8^+$ (Lin, J., et al., 2023).

Para investigar as respostas das células T na SIM-P, Lam e colaboradores (2022) avaliaram o repertório do receptor de células T (TCR) e viram que as células T $CD4^+$ e $CD8^+$ desses indivíduos exibem uma maior expansão do gene Receptor de Células T Beta Variável 11-2 (*TRBV11–2*), comparado a pacientes adultos e pediátricos com tempestade de citocinas ou COVID-19 aguda. Corroborando esse resultado, dados de sequenciamento do repertório de TCRβ (*TRB*) mostraram frequência aumentada de clonótipos *TRBV11–2* em amostras de SIM-P que foram coletadas logo após admissão hospitalar dos pacientes com SIM-P, seguida do declínio desses clonótipos durante a hospitalização (Sacco, K., et al., 2022). Embora possa funcionar como um biomarcador específico da SIM-P, ainda não se sabe qual o significado da expansão de células *TRBV11–2* (Lam, KP., et al., 2022). Entretanto, a expansão dessas células T observada nos casos de SIM-P pode indicar o papel de superantígenos em sua patogênese (Lin, J., et al., 2023).

1.3.4 Anticorpos e imunocomplexos

A apresentação da SIM-P semanas após a infecção pelo SARS-CoV-2 sugere que a resposta imune adaptativa desempenha um papel crucial no desenvolvimento desse distúrbio (Lam, KP., et al., 2022). Essa característica tardia da SIM-P indica que o SARS-CoV-2 induz uma resposta imunológica intensa e duradoura, mesmo em pacientes que não apresentaram sintomas no início da infecção (Rotulo, GA., Palma P., 2023). As crianças com SIM-P exibem uma resposta restrita de anticorpos, limitada a anticorpos anti-S, com menor atividade neutralizante geral. Além disso, foi observado que casos de SIM-P apresentam baixos níveis de anticorpos Anti-Nucleocapsídeo (Anti-N) do SARS-CoV-2, produzindo, portanto, uma resposta imune limitada (Weisberg, SP., et al., 2021).

Consiglio e colaboradores (2020) analisaram os alvos de autoanticorpos em amostras de pacientes com SIM-P e viram que seus principais focos eram antígenos de tecidos gastrointestinal (ATP4A), cardiovascular (PDLIM5), cerebral (MAP9) e músculo esquelético (RBM38). Detectaram também que quase todas as crianças com SIM-P, quando comparadas a controles não infectados, possuíam autoanticorpos contra endoglina, uma glicoproteína

expressa por células endoteliais, imprescindível para a manutenção da integridade estrutural do endotélio vascular e do músculo cardíaco. É possível que esses autoanticorpos não sejam a causa do dano tecidual, mas sim uma consequência dele (Consiglio, CR., et al., 2020; Lin, J., et al., 2023).

Outro estudo observou autoanticorpos contra MAP2K2 e contra outros três membros da família das caseína quinases (CSNK1A1, CSNK2A1, CSNK1E1) somente em indivíduos com SIM-P, e não em crianças sadias ou com DK (Consiglio, CR., et al., 2020). A função da via caseína-quinase 2 está relacionada à replicação viral, e os anticorpos contra componentes dessa via podem estar relacionados à imunopatogênese da SIM-P (Consiglio, CR., et al., 2020). Gruber e colaboradores (2020) testaram a hipótese de que a infecção pelo SARS-CoV-2 leva a uma resposta humoral auto reativa secundária e como resultado, observaram que os pacientes com SIM-P tinham autoanticorpos IgG e IgA contra antígenos cardiovasculares, gastrointestinais e endoteliais. Além disso, foi identificada a presença de autoanticorpos anti-La, frequentemente encontrados em doenças autoimunes e que podem interagir com o CD64, desencadeando inflamação e lesão tecidual (Gruber, C., et al., 2020). Em um estudo multicêntrico, retrospectivo, que envolveu indivíduos diagnosticados com SIM-P em cinco hospitais diferentes na Alemanha e Espanha, foram identificados autoanticorpos específicos anti-IL-1RA (Pfeifer, J., et al., 2022). Os autores do estudo descrevem que a produção de autoanticorpos anti-IL-1RA pode ser desencadeada por uma isoforma hiperfosforilada atípica do receptor da IL-1, sugerindo um componente autoimune na SIM-P (Pfeifer, J., et al., 2022; Lin, J., et al., 2023), como visto no estudo de Gruber e colaboradores (2020).

Finalmente, existe uma teoria baseada na presença de um motivo semelhante ao superantígeno na proteína S do SARS-CoV-2, que pode se ligar com alta afinidade à cadeia α e à região variável da cadeia β do TCR, levando à cascata inflamatória vista na SIM-P (Cheng, MH., et al., 2020; Rotulo G., Palma P., 2023).

1.4 Imunodeficiências primárias no contexto das doenças infecciosas

1.4.1 Erros inatos da imunidade e a susceptibilidade à SIM-P

As Imunodeficiências Primárias (IDPs) são desregulações imunológicas que fazem parte do espectro fenotípico causados por mutações em genes do sistema imunológico, denominados de Erros Inatos da Imunidade (EII) (Sogkas, G., Witte, T., 2023). As IDPs representam um grupo heterogêneo de EII germinativos monogênicos, que aumentam a suscetibilidade a infecções, autoimunidade, distúrbios autoinflamatórios, atopia, falência da medula óssea, e/ou malignidades (Olbrich P., Vinh DC., 2023; Tangye, SG., et al., 2022). A primeira IDP foi descrita em 1952 através de um relato de caso realizado por pesquisadores suíços, quando observaram que algumas crianças possuíam infecções pneumocócicas invasivas e infecções bacterianas piogênicas recorrentes e/ou múltiplas graves (BRUTON, O., 1952). Posteriormente, foi nomeada de agamaglobulinemia de Bruton, uma condição recessiva ligada ao cromossomo X. Atualmente, já foram relatados 485 EIIs pelo Comitê de Especialistas da União Internacional das Sociedades de Imunologia, que desde 1970 realiza atualizações da lista desses erros (Tangye, SG., et al., 2022). Os EIIs são divididos em diferentes grupos de distúrbios, totalizando 10 grupos, como por exemplo, o grupo de doenças de desregulação imune (grupo IV) ou de distúrbios autoinflamatórios (grupo VII) (Witte, T., Sogkas, G., 2023).

Variantes patogênicas presentes em alguns pacientes destacam a individualidade dos papéis fundamentais de genes e proteínas que contribuem para a homeostase imunológica e defesa do hospedeiro (Tangye, SG., et al., 2022). Através de 80 anos de pesquisa sobre o vírus influenza, cientistas descobriram que, embora raros, existem casos de crianças que após a infecção desenvolvem quadro clínicos graves (Casanova, JL., 2015). Um paciente de apenas 4 anos, sadio, desenvolveu Síndrome do Desconforto Respiratório Agudo (SDRA) ao ser infectado pelo vírus influenza. Em uma análise genética, observou-se que essa criança possuía duas mutações com perda de função no gene que codifica o fator regulatório 7 do IFN (*IRF7*), um fator de transcrição necessário para a amplificação de respostas antivirais coordenadas pelos IFN-α/β (Casanova, JL., 2015). Esses achados fornecem prova de princípio para a teoria monogênica de doenças infecciosas primárias com risco de vida (Rotulo, GA., Palma, P., 2023).

Baseando-se na hipótese de que erros congênitos monogênicos na imunidade a vírus podem explicar a ocorrência da SIM-P, Lee e colaboradores (2022) realizaram sequenciamento total do exoma de 558 crianças e adolescentes com esse distúrbio. Foram identificadas deficiências autossômicas recessivas nos genes *OAS1, OAS2* ou *RNase L* em 1%

dos indivíduos. Os autores concluíram, portanto, que erros inatos recessivos de gene único da via OAS-RNase L desencadeiam a produção de citocinas inflamatórias em fagócitos mononucleares infectados pelo SARS-CoV-2, o que pode ocasionar a SIM-P nesses indivíduos.

Em um outro estudo retrospectivo realizado na Polônia com 105 crianças infectadas pelo SARS-CoV-2 e com EII, foram identificados dois pacientes (1,9%) que com diagnóstico para SIM-P: uma menina de 1 ano com imunodeficiência combinada grave e uma outra paciente de 6 meses com hipogamaglobulinemia (Kołtan, S., et al., 2022). Abolhassani e colaboradores (2022), em análise genética da SIM-P, propuseram que EIIs que prejudicam a resposta imune ao SARS-CoV-2 e desregulam a imunidade dos interferons tipo I (IFN) podem explicar a ocorrência da SIM-P em algumas crianças. Nesse estudo, foi identificado um paciente com SIM-P que apresentou deleção homozigótica com perda de função no gene *IFNAR1* (Abolhassani, H., et al., 2022).

1.4.2 Outras evidências do envolvimento de fatores genéticos na SIM-P

A descoberta de uma mutação monogênica no gene supressor da sinalização de citocinas 1 (*SOCS1*) em um paciente que teve infecção pelo SARS-CoV-2 e desenvolveu SIM-P incentivou estudos adicionais nesta área, visto que esse gene funciona como um regulador negativo da sinalização de interferons tipo I e tipo II (Lee et al., 2020). Em um estudo prospectivo, incluindo 18 pacientes com SIM-P, foi identificado que erros inatos nos genes *SOCS1, XIAP* ou *CYBB* induzem alterações principalmente na atividade de IL-18, oncostatina M e fator nuclear κB, explicando a ocorrência de SIM-P em alguns indivíduos (Chou et al., 2021).

Em um estudo com sequenciamento total do exoma de 45 casos de SIM-P de origens principalmente árabes e asiáticas, foi observado enriquecimento de variantes genéticas raras relacionadas ao sistema imunológico, como: *TLR3, TLR6, IFNB1, IFNA6* e *IL22RA2* (Abuhammour, W., et al., 2022). Em outra análise de interação de proteínas realizada por Abuhammour e colaboradores (2022), foram observadas variantes patogênicas em genes envolvidos na sinalização de receptores Toll-*like* (TLRs), na sinalização de genes indutores de ácido retinóico tipo I (RIG-I), na resposta imune mediada por interferon e na sinalização do receptor NOD-*like* (NLRs).

Em outro estudo, foi observado que pacientes com COVID-19 possuíam variantes em quatro genes de IDPs (*UNC13D, AP3B1, RNF168, DHX58)* (Luo et al., 2021). Os autores propuseram que as variantes em *UNC13D* e *AP3B1* são associadas ao desenvolvimento de tempestades de citocinas, e parecem predispor crianças a desenvolver manifestações como a SIM-P. O desenvolvimento da SIM-P também foi ligado a mutações heterozigóticas não-silenciosas em genes já implicados na linfo-histiocitose hemofagocítica primária (pHLH) e no gene dedicador da citocinese 8 (*DOCK8*) (Vagrecha A., et al., 2022; Lin, J., et al., 2023). Ao realizar um estudo epigenômico, Davalos e colabores (2022), identificaram que os pacientes com SIM-P exibem um conjunto bem definido de *loci* epigenéticos que estão associados ao diagnóstico do distúrbio e suportam um papel direto de uma resposta imune hiperativada nas características de hiperinflamação e envolvimento de múltiplos órgãos (Davalos, V., et al., 2022). Tal perfil poderia ainda servir para a construção de uma assinatura epigenômica, associada à SIM-P (Davalos, V., et al., 2022). Santos-Rebouças e colaboradores (2022) realizaram o primeiro estudo genético de SIM-P no Brasil, com o objetivo de identificar os possíveis fatores de risco para essa síndrome. Como principal resultado, identificaram dez variantes raras (5 não-sinônimas, 2 sem sentido, 1 *splicing*, 1 deleção e 1 duplicação) em oito genes, sendo que eventuais defeitos em *FREM1, MPO, POLG, C6, C9, ABCA4, ABCC6* e *BSCL2* poderiam produzir respostas imunes menos efetivas contra a infecção pelo SARS-CoV-2 ou desencadear resposta imune retardada ao vírus (Santos-Rebouças, CB., et al., 2022).

A identificação de variantes genéticas determinantes para a SIM-P é fundamental para a melhor compreensão da arquitetura da doença e para definir estratégias de prevenção, como na detecção de indivíduos com alto risco de desenvolvimento da doença, e de tratamento, ao restabelecer parcialmente, por exemplo, uma função imunológica deficiente.

2. OBJETIVOS

2.1 Objetivo geral

Identificar variantes em genes previamente implicados em condições autoinflamatórias que possam estar envolvidos no desenvolvimento da Síndrome Inflamatória Multissistêmica Pediátrica (SIM-P).

2.2 Objetivos específicos

- Caracterizar, através de análises clínicas, laboratoriais e genéticas, casos de SIM-P em indivíduos previamente sadios, recrutados na Bahia e em Pernambuco, Brasil;

- Identificar variantes em regiões codificantes de genes relacionados a doenças autoinflamatórias primárias que podem ser implicadas na SIM-P;

- Analisar *in silico* os efeitos funcionais de alelos candidatos a causadores da SIM-P.

4. Manuscrito

Genetic screening of autoinflammatory genes in Latin American Individuals with Multisystem Inflammatory Syndrome in Children (MIS-C) associated with SARS-CoV-2 infection.

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4.1 Introduction

During the early stages of the COVID-19 pandemic, caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), cases of a Kawasaki Disease (KD)-like syndrome emerged in children and adolescents[1]. A report by the Royal College of Paediatrics and Child Health (2020) in the United Kingdom described that this clinical condition was not linked to KD, but rather represented a post-infectious condition related to SARS-CoV-2[2]. This presentation was subsequently named Multisystem Inflammatory Syndrome in Children (MIS-C)[3]. MIS-C is a rare life-threatening condition, with an incidence rate of 2/100,000 individuals under 21 years of age in New York, in 2020 (Sancho-Shimizu, V., et al., 2021). In the same year, 1.1/100,000 children and adolescent were diagnosed with MIS-C in Brazil[4].

MIS-C is characterized by systemic dysregulation of the immune response[5]. Importantly, individuals with MIS-C exhibit positive serology for SARS-CoV-2, and in one-third of these cases, positive serology and RT-PCR tests are observed[5,6]. Multiple lines of evidence suggest that the initial symptoms of MIS-C manifest 2 to 6 weeks following SARS-CoV-2 infection[5,7]. These symptoms are mainly characterized by persistent fever, diarrhea, vomiting, abdominal pain, bilateral non-purulent conjunctivitis, rash, cardiac complications, acute kidney injury, shock, sepsis, ventricular dysfunction and coronary artery aneurysms[8,9].

Laboratory findings commonly observed in MIS-C include intense leukocytosis with neutrophilia, lymphopenia, increased levels of C-reactive protein (CRP), ferritin, D-dimer, and troponin, affecting up to 90% of cases[5]. Additionally, MIS-C patients often exhibit increased levels of lactic dehydrogenase (LDH), triglycerides, erythrocyte sedimentation rate, prolonged prothrombin time, and hypoalbuminemia[9,10].

Primary Immunodeficiencies (PIDs) encompass a range of immune dysregulations that result from mutations in genes associated with the immune system, known as Inborn Errors of Immunity (IEI)[11]. Considering the hypothesis that monogenic congenital immune errors may contribute to the occurrence of MIS-C, Lee and colleagues (2022)[12] identified autosomal recessive deficiencies in the *OAS1*, *OAS2* or *RNase L* genes that may account for MIS-C in approximately 1% of individuals. Furthermore, it has been proposed that IEIs that impair the

immune response to SARS-CoV-2 and lead to dysregulation of type I interferon (IFNI) immunity are also implicated in the pathogenesis of MIS-C[13].

Macrophage Activation Syndrome (MAS) is a potentially fatal systemic complication in individuals with primary autoinflammatory diseases, characterized mainly by persistent fever and cytokine storm[14]. Interestingly, evidence has been reported of MIS-C patients with clinical complications of MAS and cytokine storm[15,16]. It is possible that the pathological mechanisms driving MIS-C are linked to IEI, specifically in genes implicated in autoinflammatory conditions, and that these mutations can selectively affect the immune response against SARS-CoV-2. Therefore, the present study aims to identify IEI potentially involved in MIS-C, thereby consolidating our understanding on the genetic risk factors for this condition.

4.2 Methods

4.2.1 Study design and sample selection

This is a cross-sectional study with a convenience sample of 21 unrelated children and adolescents (0 to 15 years of age), diagnosed with Multisystem Inflammatory Syndrome in Children (MIS-C) after infection with SARS-CoV-2. It was adopted the case definition for MIS-C described by the Centers for Disease Control and Prevention (CDC) of the United States of America, which includes: fever ≥ 38 °C for ≥ 24 hours; ≥ 1 laboratory evidence of inflammation (elevated C-reactive protein, d-dimer, ferritin, lactic acid dehydrogenase, elevated neutrophils, reduced lymphocytes and/or low albumin); evidence of clinically severe illness requiring hospitalization, with ≥ 2 organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological); positivity for SARS-CoV-2 infection by RT-PCR or serology; and no alternative plausible diagnoses. MIS-C was classified as moderate or severe, based on the CDC case definition[17]: moderate cases do not require vasopressor drugs or positive pressure ventilation; severe cases require vasopressors and/or positive pressure ventilation. None of the individuals had previously been vaccinated against COVID-19 and had no comorbidities, including, but not limited to overweight, obesity, cancer, diabetes, hypertension, HIV/AIDS, asthma, tuberculosis, or other systemic diseases. No patient died. No tests have been performed to diagnose other viruses that cause respiratory, exanthematic/febrile diseases.

4.2.2 Ethical considerations

This study was approved by the research ethics committee of the Instituto Aggeu Magalhães, Fundação Oswaldo Cruz, Brazil (resolution number: 4263871). All methods and protocols were performed in accordance with the principles of the Declaration of Helsinki. The parents or legal guardians of the children/adolescents agreed with the study and signed an informed consent form.

4.2.3 Clinical, laboratory and demographic data

Data were extracted from electronic medical records of three public hospitals in the Northeast region of Brazil [Hospital Martagão Gesteira (HMG - Salvador, BA), Instituto Couto Maia (ICOM - Salvador, BA) and Hospital Universitário Oswaldo Cruz (HUOC - Recife, PE)], from May 2020 to March 2021. At hospital admission, patients underwent serum (ELISA) and molecular (RT-PCR) testing for SARS-CoV-2, and the main clinical symptoms associated with SARS-CoV-2 infection were collected. During hospitalization, data on complications, medical interventions and laboratory findings were also collected.

4.2.4 Genomic DNA and Whole Exome Sequencing (WES)

The genomic DNA was extracted from oral mucosal cells using the ReliaPrep™ Blood gDNA Miniprep System kit (Promega Corp., Madison, WI, USA), according to the manufacturer's instructions. Samples were processed on the same day of collection and stored at -20°C until use. DNA concentrations were evaluated with the Qubit® DNA Assay kit, using a Qubit® 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA).

One microgram of genomic DNA was used for WES library preparation with the Illumina DNA Prep with Enrichment kit (Illumina, San Diego, CA, USA). The libraries were subjected to next-generation sequencing (2×150 bp) on the NovaSeq 6000 platform, using the NovaSeq SP v1 Reagent kit (Illumina, San Diego, CA, USA). Data for each sample were analyzed using the Genome Analysis ToolKit (GATK, gatk.broadinstitute.org). On average, 1% of the reads representing off-target regions were excluded. The remaining sequences were aligned to the reference human genome, version GRCh38, and the mapping files (BAM) were obtained using the SAMTools program (v1.15). Only target regions with 10 or more reads were retained in the study.

4.2.5 Variant detection and filtering strategy

Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were detected through the GATK Best Practices, using the GATK HaplotypeCaller program.

Functional annotations were carried out with ANNOVAR (v. 2019Oct24)[18]. The present study was focused on rare variants, with a highest population MAF < 0.001 (gnomAD v2.1.1), from 54 genes associated with autoinflammatory disorders, reported in the Human Inborn Errors of Immunity (IEI) 2022 list, from the International Union of Immunological Societies (IUIS)[19]. The combined annotation dependent depletion (CADD) score (>20)[20] and Gene Damage Index (GDI) standard cutoff (<13.83)[12] were used to prioritize potentially deleterious SNVs and INDELs in coding regions[21]. The genes encoding human proteins related to SARS-CoV-2 infection, evaluated in a previous study from our group[22] were removed from this analysis.

4.2.6 Individual ancestry analysis

Individual ancestry of the MIS-C cases was estimated with the ADMIXTURE software[23]. This analysis was conducted under an unsupervised mode, using the EUR, AFR and AMR samples of the 1000 Genomes Project[24]. This 1000 Genomes panel was merged with the genetic data of BR-MIS-C, using autosomal variants with a minor allele frequency (MAF) > 0.1 that were common to both datasets. Then, the merged data was pruned using PLINK 1.9 software[25] with a window size of 50 markers, a step size of 5 and a variance inflation factor (VIF) threshold of 1.5. A K = 3 was assumed based on the results of the principal components 1 and 2 (PC1 and PC2) and considering that the main continental parental groups that contributed to the formation of the Brazilian population are Europeans, Africans, and Native Americans[26].

4.2.7 Docking analysis

The 3D structures of wild-type ADAM17 (P78536), TNF (P01375), ACE2 (Q9BYF1), IL-6R (A0N0L5), and NOTCH1 (P46531) were downloaded from the Alphafold protein structure database (https://alphafold.ebi.ac). The SWISS-MODEL structure assessment online server was used to model the structure of wild-type ADAM17 or this protein with variants rs144458353 (P18L) or rs1200631089 (T663N) (https://swissmodel.expasy.org/assess/)[27]. The UCSF Chimera software package version 1.17 was used for molecular visualization and structural analysis[28]. Docking simulations were performed using the ClusPro protein-protein

docking server (https://cluspro.bu.edu/)[29], and the results were visualized in LigPlot+ v.2.2 to display the confirmed binding positions of ADAM17 proteins with receptors[30].

4.2.8 Statistical analysis

The Mann-Whitney U test was applied to evaluate quantitative variants, such as individual ancestry (EUR, AFR and AMR), length of hospital stay in days [total hospitalization and Intensive Care Unit (ICU)], duration of fever and symptoms before admission, and laboratory findings [white blood cells, neutrophils, lymphocytes, D-dimer, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), ferritin and C-reactive protein (CPR)]. Qualitative data (sore throat, cough, dyspnea, headache, heart involvement, rash, abdominal pain, diarrhea, vomiting, bilateral non-purulent conjunctivitis, pneumonia, shock, sepsis, and acute respiratory distress syndrome) was evaluated using Fisher exact test. All statistical analyzes were conducted in the GraphPad Prism software (version 8.4.2). $P < 0.05$ was considered statistically significant.

4.3 Results

4.3.1 Demographic, clinical and laboratory data from patients with moderate or severe MIS-C

In this study, we recruited 21 children and adolescents, wherein 9 individuals exhibited a moderate form of MIS-C, while 12 individuals presented with a severe manifestation (Supplementary Figure 1). In the moderate MIS-C group, the median age was 6 years [interquartile range (IQR): 0.5-15], whereas the severe MIS-C group had a median age of 8 years (IQR: 2-15) ($p = 0.61$) (Figure 2A). The two groups exhibited similar sex distributions, with males accounting for the majority of moderate (55%) and severe (58%) cases ($p = 0.18$) (Figure 2A). Individual ancestry analysis confirmed the admixture nature of the MIS-C cases (Figure 2B). The average global European and African ancestries were significantly different ($p < 0.02$) between groups, with severe cases showing lower European and higher African ancestries than those with moderate MIS-C [Severe group: 0.39 (IQR: 0.17-0.56) European and 0.45 (IQR: 0.30-0.76) African ancestries; Moderate group: 0.56 (IQR: 0.35-0.77) European and 0.29 (IQR: 0.14-0.53) African ancestries].

The average total hospitalization time was significantly different ($p = 0.03$) in moderate (8 days) or severe (21 days) MIS-C (Figure 2C). Otherwise, median length of stay in the pediatric intensive care unit (PICU) was similar between groups ($p = 0.09$). In moderate and severe MIS-C cases, respectively, the median duration of fever was 8 and 6 days ($p = 0.27$) and the duration of symptoms before hospitalization was 8 and 7 days ($p = 0.31$) (Figure 2). Finally, as shown in Figure 2D and Supplementary Figure 2, the frequencies of clinical manifestations were similar ($p > 0.05$) in moderate and severe cases.

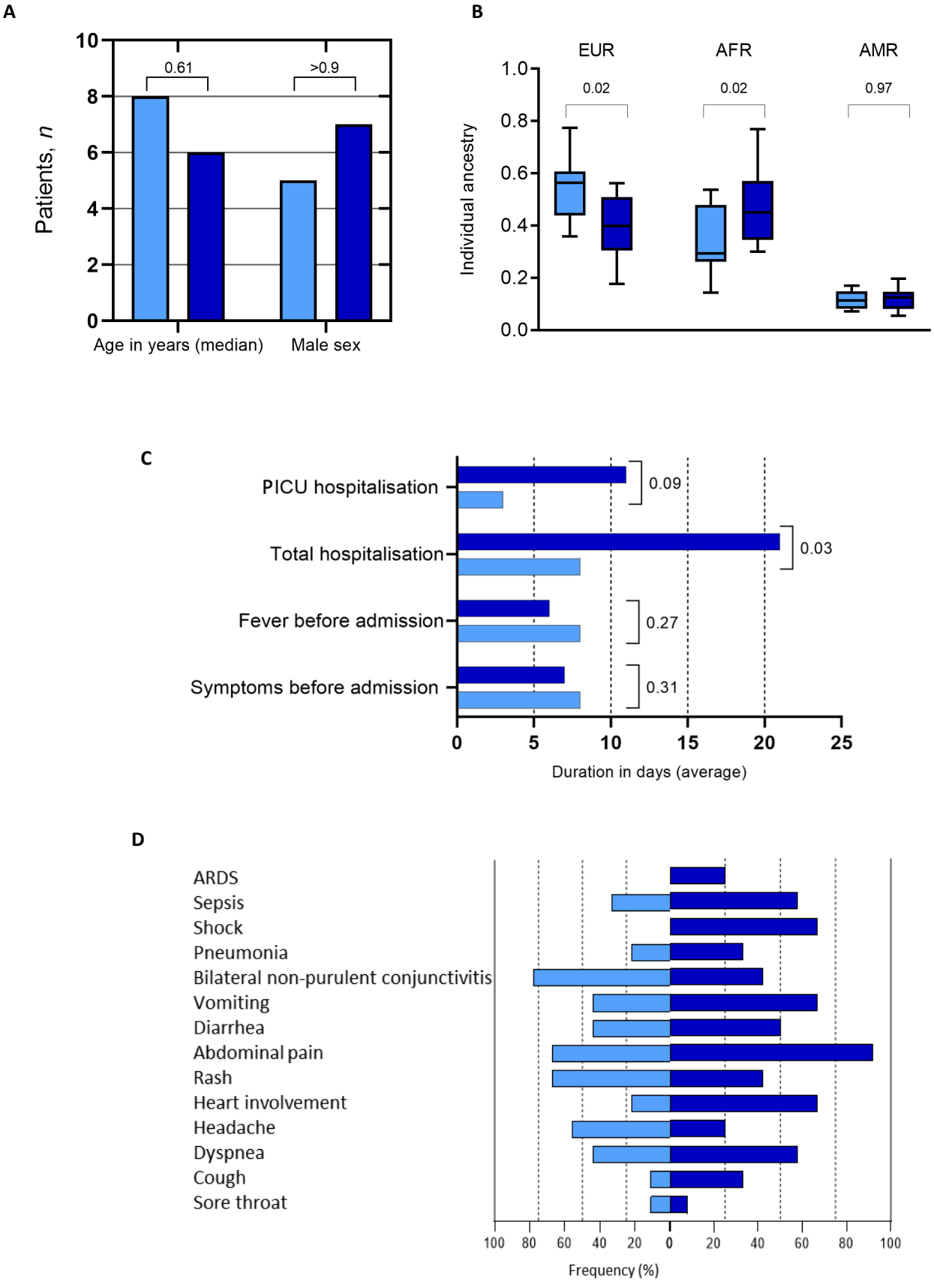

A
Patients, n
0.61
>0.9
Age in years (median)
Male sex
B
EUR
AFR
AMR
0.02
0.02
0.97
Individual ancestry
C
PICU hospitalisation
Total hospitalisation
Fever before admission
Symptoms before admission
0.09
0.03
0.27
0.31
Duration in days (average)
D
ARDS
Sepsis
Shock
Pneumonia
Bilateral non-purulent conjunctivitis
Vomiting
Diarrhea
Abdominal pain
Rash
Heart involvement
Headache
Dyspnea
Cough
Sore throat
Frequency (%)

Figure 2. Epidemiological and clinical characteristics of children and adolescents with severe or moderate MIS-C. MIS-C groups: moderate in light blue and severe in dark blue. **A)** Median age in years and sex**. B)** Individual ancestry boxplot of moderate and severe MIS-C (K = 3). **C)** Hospitalization time [Peadiatric Intensive Care Unit (PICU) or total length of stay in hospital in days], duration of fever and symptoms before admission in days. **D)** Frequency of clinical manifestations and complications during hospitalization. Abbreviation, ARDS: Acute respiratory distress syndrome. p-values for association tests (Mann-Whitney or Fisher exact test for quantitative or qualitative variables, respectively).

Results of laboratory tests during hospitalization are presented in Figure 3 and Supplementary Table 2. Most patients developed leukocytosis [moderate median = 17,870 cells/mm^3 (IQR: 13,950-19,950) *vs* severe median = 15,885 cells/mm^3 (IQR: 13,005-24,450)], p = 0.83] and neutrophilia [moderate median = 10,773 cells/mm^3 (IQR: 9,170-15,190) *vs* severe median = 11,525 cells/mm^3 (IQR: 7,766-21,428), *p* = 0.65]. Both moderate [median = 3,950 cells/mm^3 (IQR: 1,425-5,655)] and severe [median= 3,107 cells/mm^3 (IQR= 1,129-4,562)] MIS-C had lymphocyte counts within the reference range (RR) 900-4,000 cells/mm^3 and showed no discrepant values between groups (*p* = 0.59). Inflammatory markers such as C-reactive protein (CRP) [moderate: median = 75 mg/L (IQR: 24.8-146); severe: median = 164 mg/L (IQR: 7.3-188.3) (RR: <5 mg/L)], d-dimer [moderate: median = 4.3 mg/L (IQR: 2.4-5.8); severe: median = 6.3 mg/L (IQR: 3.8-8.3) (RR: <0.5 mg/L)] and ferritin [moderate: median = 469 ng/mL (IQR: 245-766); severe: median = 695 ng/mL (IQR: 34-1500) (RR: 7-140 ng/mL)] were elevated in almost all patients, despite not identifying statistical differences between the two groups (*p* = 0.70, p = 0.12 and *p* = 0.78, respectively). Lactic Dehydrogenase (LDH) values were higher than the reference range in both MIS-C groups [median = 422 U/L (IQR: 224.2-540.5); severe: median = 278 U/L (IQR: 242-671) (RR: 100 - 295 U/L) (*p* = 0.94)]. The non-observance of statistical significances in these results does not indicate that they do not exist, but it can indicate that this study has no power to detect it.

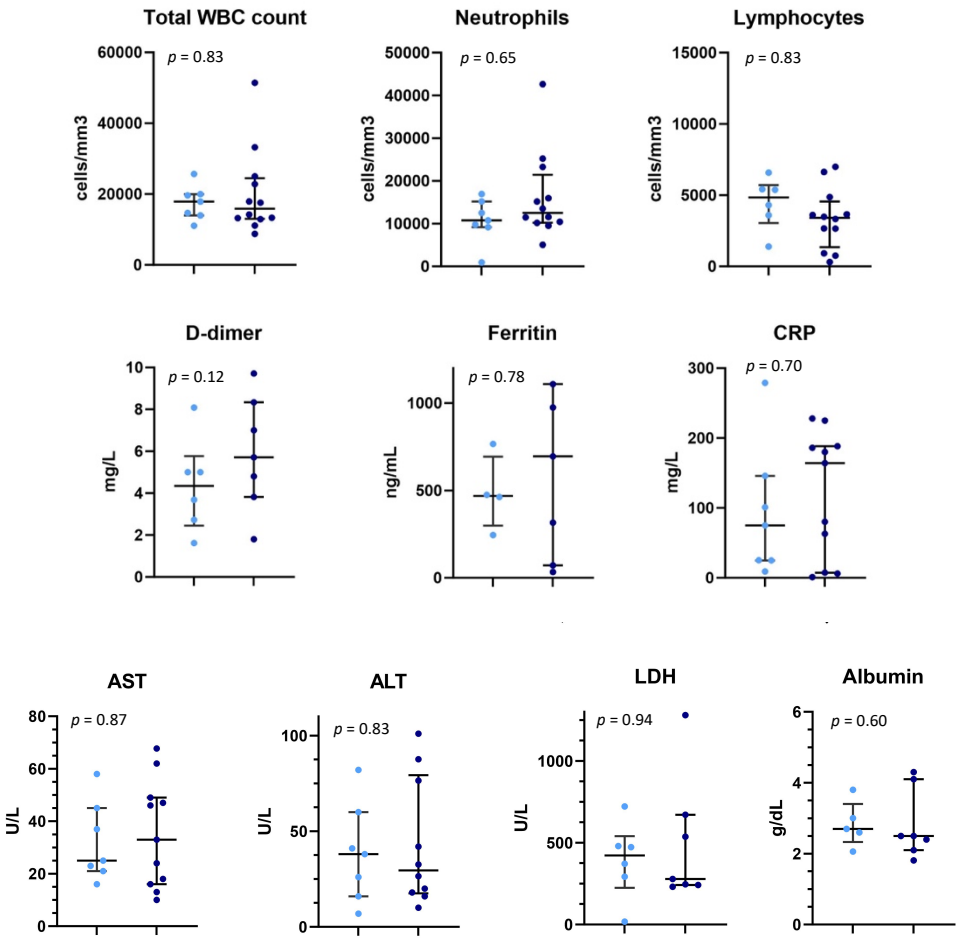


Figure 3. Laboratory findings in children and adolescents hospitalized with MIS-C. MIS-C groups: moderate in light blue and severe in dark blue. The data is represented by the median and interquartile range. Only parameters available for more than 50% of the individuals are shown. Abbreviations, RR: reference range - for ages 6 to 12 years. WBC: white blood cell. AST: aspartate aminotransferase. ALT: alanine aminotransferase. LDH: lactic dehydrogenase. CRP: C-reactive protein. p-values were obtained by Mann-Whitney test.

4.3.2 Sequencing data and variant prioritization

It was found 264,308 unique short variants in the 21 WES evaluated here. As shown in Supplementary Figure 3, in the first step of the variant prioritization approach, only SNVs present in 54 genes implicated in autoinflammatory primary immunodeficiencies were selected (*n* = 630 SNVs). Assuming that MIS-C is a rare condition, only variants with minor allele frequency (MAF) ≤ 0.001 in the GnomAD database (all dataset) were selected (*n* = 20

SNVs). Finally, we restricted our analysis to potentially deleterious variants, with CADD score ≥ 20 and located in genes with GDI < 13.83.

This filtering strategy revealed the presence of rare variants potentially implicated in MIS-C in 5 (24%) patients, all of them presenting severe MIS-C (Table 1). All variants are in heterozygous state. Two nonsynonymous variants [rs1200631089 (CADD = 24) and rs144458353 (CADD = 21.8)] were identified in Disintegrin and Metalloproteinase Domain-Containing Protein 17 (*ADAM17*) gene, which has a GDI = 4.35. Four others SNVs in 4 different genes were also found. Two of these variants were identified in 2 different patients, one in the Inhibitor of Nuclear Factor Kappa B Kinase Regulatory Subunit Gamma (*IKBKG*) [rs1215029143 (CADD = 23; GDI = 0.09)] and another in the Proline-Serine-Threonine Phosphatase Interacting Protein 1 (*PSTPIP1*) gene [rs77026017 (CADD = 22.6; GDI = 3.21)]. Additionally, this prioritizing strategy revealed 2 variants in patient 10 (P10), the first one [rs139934973 (CADD = 22.7)] in the SH3 domain binding protein 2 (*SH3BP2*) gene (GDI = 2.23), and the second one [rs1054063201 (CADD = 23.1)] in the Caspase Recruitment Domain Family Member 14 (*CARD14*) gene (GDI = 9.49).

Table 1. Variants potentially implicated in MIS-C

							Frequency
Gene	**Patient**	**MIS-C**	**SNV(ID)** Consequence	**Genotype**	**CADD**	**GDI**	**GnomAD exome all**
ADAM17							
	P12	Severe	rs1200631089 missense	G/T	24	4.35	0
	P18	Severe	rs144458353 missense	G/A	21.8		2×10^{-4}
IKBKG							
	P1	Severe	rs1215029143 missense	G/T	23	0.09	0
PSTPIP1							
	P5	Severe	rs77026017 missense	C/T	22.6	3.21	8×10^{-4}
SH3BP2							
	P10		rs139934973 missense	C/A	22.7	2.23	1×10^{-4}
CARD14		Severe					
	P10		rs1054063201 missense	C/T	23.1	9.49	0

*All heterozygous

Global databases from Genome Aggregation Database (GnomAD, v2.1.1). Human genome reference: GRCh38.

Abbreviations, SNV(ID): Single Nucleotide Variant identifier. CADD: Combined Annotation-Dependent Depletion, v1.5 [CADD Phread]). GDI: Gene Damage Index.

4.3.3 Docking analysis

The docking analyses in this study were focused on ADAM17 as it is the most relevant protein involved in the pathophysiology of MIS-C based on the literature, compared to the mutations in the other proteins identified[31]. Docking simulation was carried out to compare whether the presence of the variants identified in the ADAM17 protein would affect its interaction with its ligands (Table 2). It was proposed by Zunke and Rose-John (2017) that ADAM17 had more than 80 substrates involved in the immune system, growth and development factors and receptors such as Angiotensin Converting Enzyme 2 (ACE2)[31]. Four receptors (TNF-α, ACE2, IL-6R and NOTCH1) were selected to represent the main pathways regulated by ADAM17 and which may be involved in mechanisms underlying the hyperinflammation observed in MIS-C. In addition, dysregulations in these four pathways caused by the sheddase alteration of ADAM17 have already been reported[31]. These molecules directly interact with ADAM17 and are involved in relevant pathophysiological pathways for SARS-CoV-2 infection.

ADAM17 has 824 amino acid residues, divided into signal sequence (1–17 aa), prodomain (18–214 aa), catalytic domain (215–473 aa), a disintegrin domain (474–572 aa), a cysteine-rich domain (603–671 aa), followed by a transmembrane domain (672–694 aa) and a cytoplasmic domain (695–824 aa)[32] (Figure 4). Here, the structure of the wild-type ADAM17 was modeled, along with those carrying the variants rs144458353 (P18L, in the prodomain) or rs1200631089 (T663N, in the cysteine-rich domain).

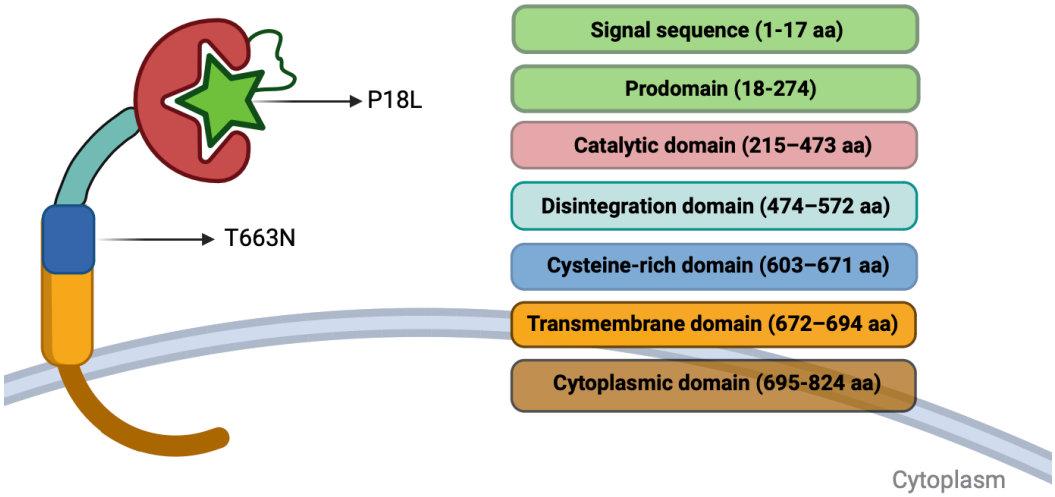


Figure 4. ADAM17 domains. The ADAM17 protein has 7 domains: signal sequence (1–17 aa); prodomain (18–214 aa); catalytic domain (215–473 aa); disintegrin domain (474–572 aa); cysteine-rich domain (603–671 aa); transmembrane domain (672–694 aa) and a cytoplasmic domain (695–824 aa). The P18L and T663N variants of ADAM17 are in the prodomain and cysteine-rich domain respectively. Based on: Gooz, M., 2010; AlAjmi M., et al., 2022).

It was observed similar docking affinities between the TNF-α protein and the wild-type ADAM17 protein (ADAM17-WT) (Figure 5) or the ADAM17 protein carrying the T663N mutation (ADAM17-T663N) (Figure 6), with binding energies of -1950.5 and -1946.8, respectively. However, the docking energy of the ADAM17 carrying the P18L variant (ADAM17-P18L) with the TNF-α molecule (energy: -1911.6) was higher than that with the ADAM17-WT protein (energy: -1950.5) (Figure 7). Notably, the TNF-α binding complex with the ADAM17-T663N protein exhibited three hydrogen bonds, representing fewer interaction regions when compared to the TNF-α/ADAM17-P18L complex, which displayed five hydrogen bonds. This pattern of H-bond formation in the ADAM17-P18L variant is similar to the binding characteristics observed in the TNF-α/ADAM17-WT complex. These findings can suggest that ADAM17-P18L and ADAM17-T663N mutations may influence molecular interactions with TNF-α, potentially causing a deficiency in the TNF-α immune response and leading to autoinflammatory dysregulation, as seen in autoimmune diseases.

The docking energies of ADAM17-P18L or ADAM17-T663N with the ACE2 protein were -1634.4 or -1630.0, respectively. These docking energies were higher than those found to the ACE2 interaction with the ADAM17-WT protein (energy: -1638.5). Each of these interactions formed 5 hydrogen bonds (Supplementary Figure 4, 5 and 6). Additionally, the docking energies of the IL-6R with the ADAM17-P18L and ADAM17-T663N mutant proteins was higher than the observed with ADAM17-WT, -1713.3, -1705.6 or -1716.3, respectively. Importantly, each of these complexes formed 11 hydrogen bonds (Supplementary Figure 7, 8 and 9). These findings collectively highlight a consistent pattern wherein specific mutations like P18L and T663N in ADAM17 may influence the stability of interactions with both ACE2 and IL-6R receptors.

It was found a substantial disparity in binding affinities among the ADAM17 molecules and the NOTCH1 receptor, with ADAM17-WT interaction exhibiting markedly lower energy (-

2472.1) compared to the ADAM17-P18L (-2379.1) and ADAM17-T663N (-2374.1) energies. In addition, differences were observed in the number of hydrogen bonds formed in the dockings. The NOTCH1/ADAM17-T663N interaction presented 25 hydrogen bonds (Figure 10), while the NOTCH1/ADAM17-P18L displayed 31 hydrogen bonds (Figure 9). In contrast, the NOTCH1/ADAM17-WT complex exhibited the highest number of interactions, with a total of 38 hydrogen bonds (Figure 8). Details on these clusters of interactions are described in Table 2.

Table 2. Binding interactions between wild-type ADAM17, ADAM17-P18L or ADAM17-T663N and 4 ligands

Protein Complex		Cluster	Members	Energy	Interacting Residues	
					Number of H-bonds	Residues
TNF	WT	0	76	-1946.8	5	Lys87-Pro710, Ser85-His709, Arg28-Ser660, Arg28-Thr663, Arg28-Leu659
	P18L	0	78	-1911.6	5	Ser85-His709, Lys87-Ser711, Lys87-Pro710, Arg28-Ser660, Arg28-Thr663
	T663N	0	80	-1950.0	3	Lys87-Pro710, Ser85-His709, Arg28-Asn663
ACE2	WT	0	45	-1638.5	5	Glu778-Asn712, Arg768-Gln701, Arg768-Asp695, Arg775-Tyr702
	P18L	0	46	-1634.4	5	Arg768-Asp695, Arg768-Gln701, Glu778-Asn712, Arg775-Tyr702
	T663N	0	48	-1630.0	5	Glu778-Asn712, Arg775-Tyr702, Arg768-Gln701, Arg768-Asp695
IL6R	WT	0	40	-1716.3	11	His405-Asn702, Ser403-Val713, Glu302-Arg802, Met404-Glu714, Glu399-Tyr702, Trp392-Asp695, Arg395-Asp695, Lys398-Asp720, Lys398-Ser718, Ala11-Lys700
	P18L	0	40	-1713.3	11	Lys398-Met719, Lys395-Asp720, Lys398-Ser718, Lys401-Leu716, His405-Asn712, Ser403-Asn712, Met404-Glu714, Glu399-Tyr702, Trp392-Asp695, Arg395-Asp695, Ala11-Lys700
	T663N	0	46	-1705.6	11	Glu302-Thr801, Gly301-Arg802, Gly301-Arg802, Arg395-Asp695, Arg395-Asp695, Glu399-Tyr702, Thr402-Asn712, Ser156-Lys700, Val4-Lys696, Lys398-Ser717, Lys398-Leu716

NOTCH1	WT	0	40	-2472.1	38	Gln2444-Arg816, Val2443-Arg816, Ser497-Lys805, Ser497-Arg802, Cys499-Arg802, Asn502-Arg802, Leu2468-Asp657, His2454-Ser808, His2454-Lys810, Val2453-Lys810, Ala2452-Lys810, Gln2459-Arg77, Leu2446-Arg813, His2378-Arg813, Asn2496-Lys697, Asp2495-Lys697, Ser2364- Met715, Gln2361-Ser711, Gln2361-His709, Ser2360-Leu707, Gln2381-Arg813, Gln2381-Trp684, Ser2491-Cys693, Ser2491-His692, Leu2359-Leu707, Glu483-Ile685, Ser2357-Ser704, Ser2357-Gln701, Gln2388-Arg725, Gln2386-Ile726, Gln2386-Arg725, Glu455-Arg725, Gly472-Arg725, Gly472-Ser723
	P18L	0	28	-2379.1	31	Phe2509-Gln734, Gln2444-Gln658, Thr2375-Gln814, Thr2375-Arg813, Gln2376-Gln814, Gln2376-Arg813, Leu2373-Arg816, Leu2502-Arg816, Gln2501-Arg816, Arg2372-Arg816, Ser2449-Ser808, Gln2440-Lys753, Glu2437-Lys753, Glu2437-Ala751, Gly2430-Ser747, Leu2434-Pro749, Leu2434-Ala750, Gly2427-Val744, Ser2357-Lys728, Ser2357-Arg725, Gln2384-Asn662, Gln2459-Lys697, Gly484-Asn671, Gln475-Asp670, Glu473-Lys666, Glu483-Gly674
	T663N	0	29	-2374.1	25	Pro2508-Gln734, Leu2373-Arg816, Leu2502-Arg816, Gln2501-Arg816, Gln2444-Gln658, Glu2437-Lys753, Glu2437-Lys753, Ser2449-Ser808, Thr2375-Gln814, Thr2375-Gln814, Thr2375-Arg813, Ser2435-Ala750, Leu2434-Pro749, Gln2376-Arg813, Gln2376-Arg813, Gly2427-Val744, Ser2357-Arg725, Ser2357-Lys728, Gln2384-Asn662, Glu473-Lys666, Gln475-Lys666, Glu483-Val673, Gly484-Asn671

WT: WILD-TYPE

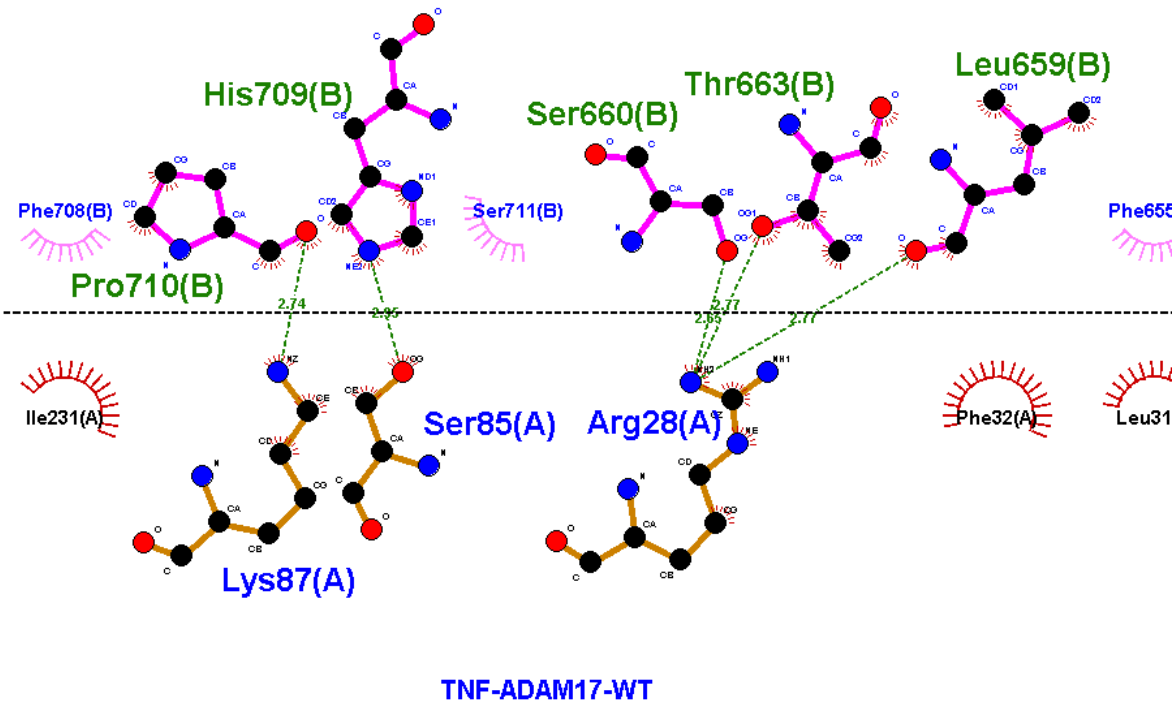


Figure 5. **The binding interaction between TNF-α and ADAM17-WT.** The complex was formed by 5 H-bonds: Lys87-Pro710, Ser85-His709, Arg28-Ser660, Arg28-Thr663, Arg28-Leu659. Chain A: TNF-α; Chain B: ADAM17-WT.

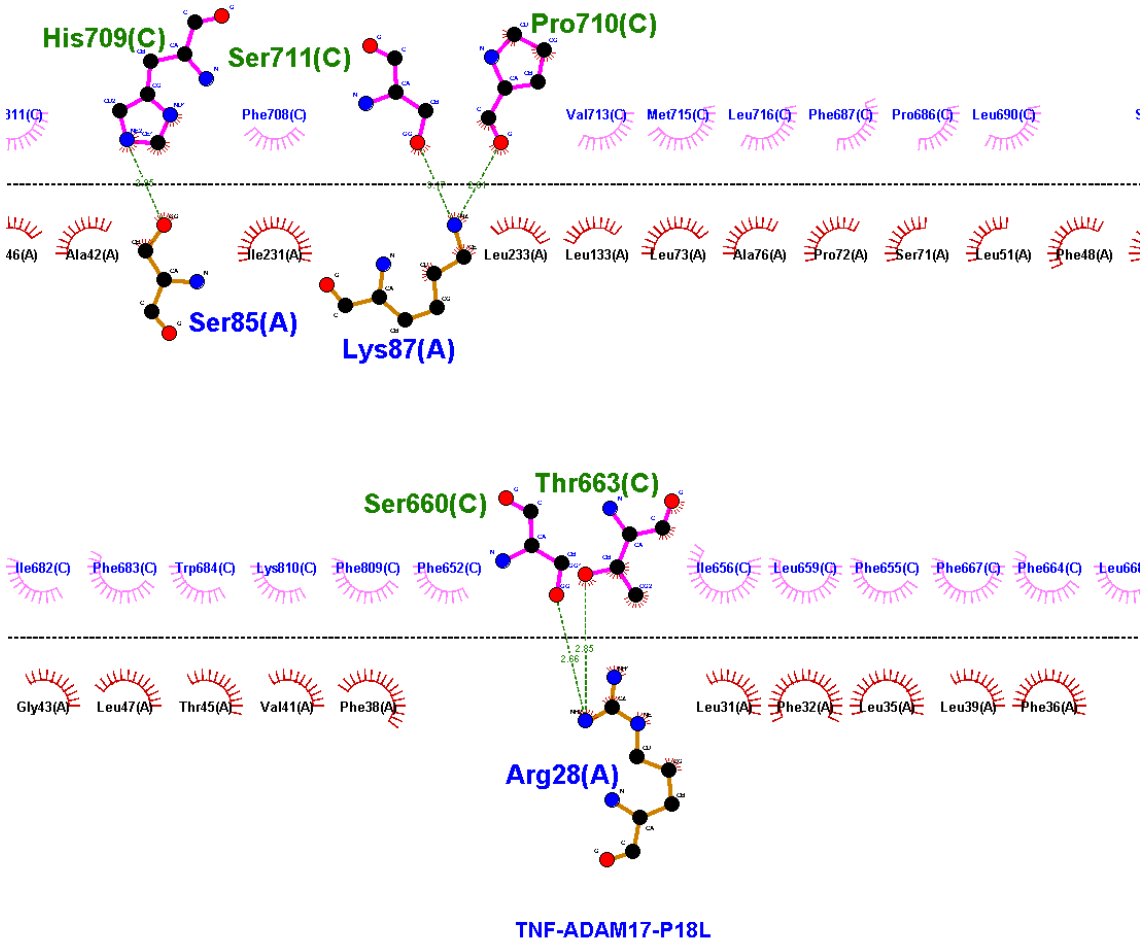


Figure 6. **The binding interaction between TNF-α and ADAM17-P18L.** The complex was formed by 5 H-bonds: Ser85-His709, Lys87-Ser711, Lys87-Pro710, Arg28-Ser660, Arg28-Thr663. Chain A: TNF-α; Chain C: ADAM17-P18L.

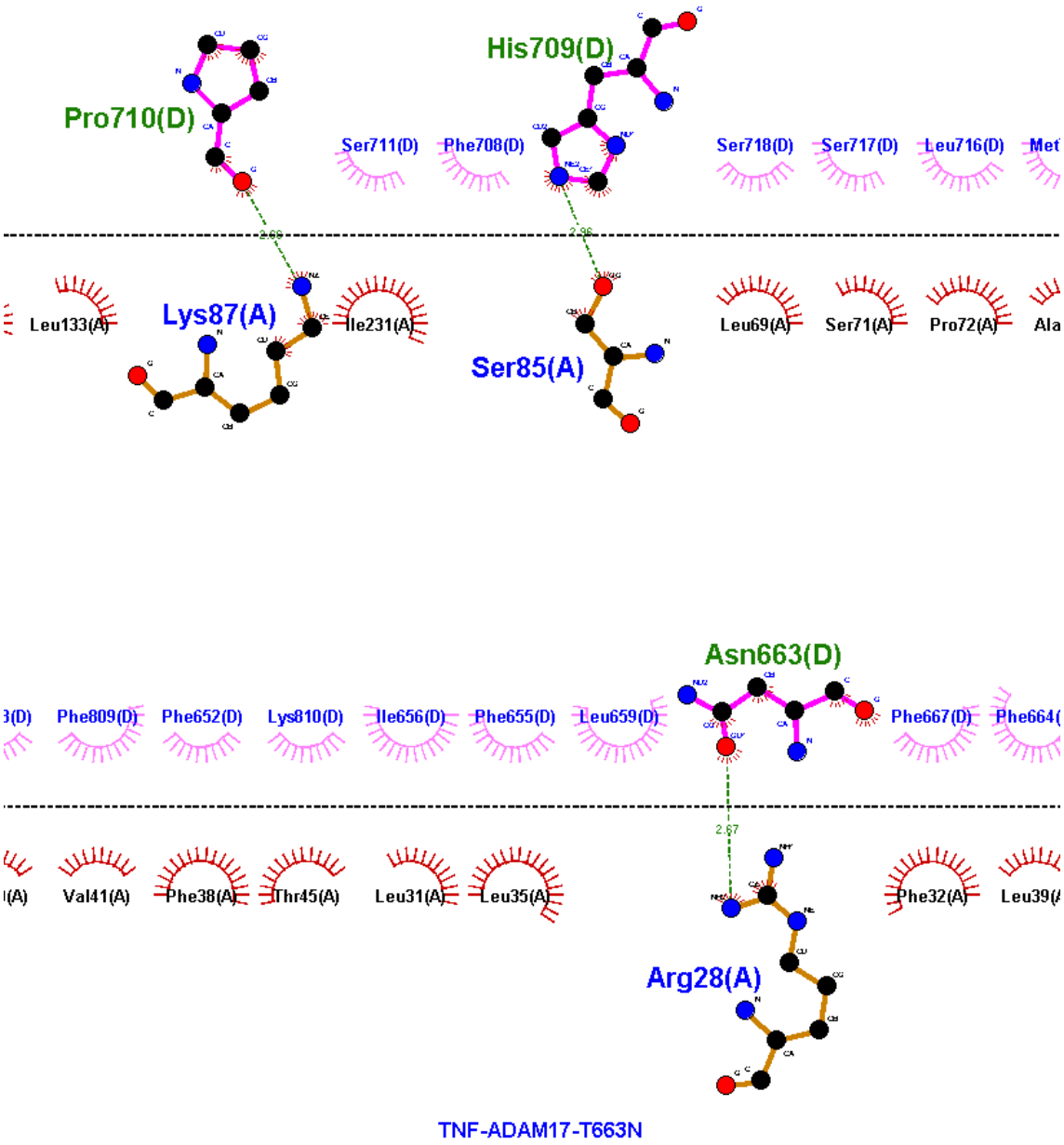


Figure 7. **The binding interaction between TNF-α and ADAM17-T663N.** The complex was formed by 3 H-bonds: Lys87-Pro710, Ser85-His709, Arg28-Asn663. Chain A: TNF-α; Chain D: ADAM17-T663N.

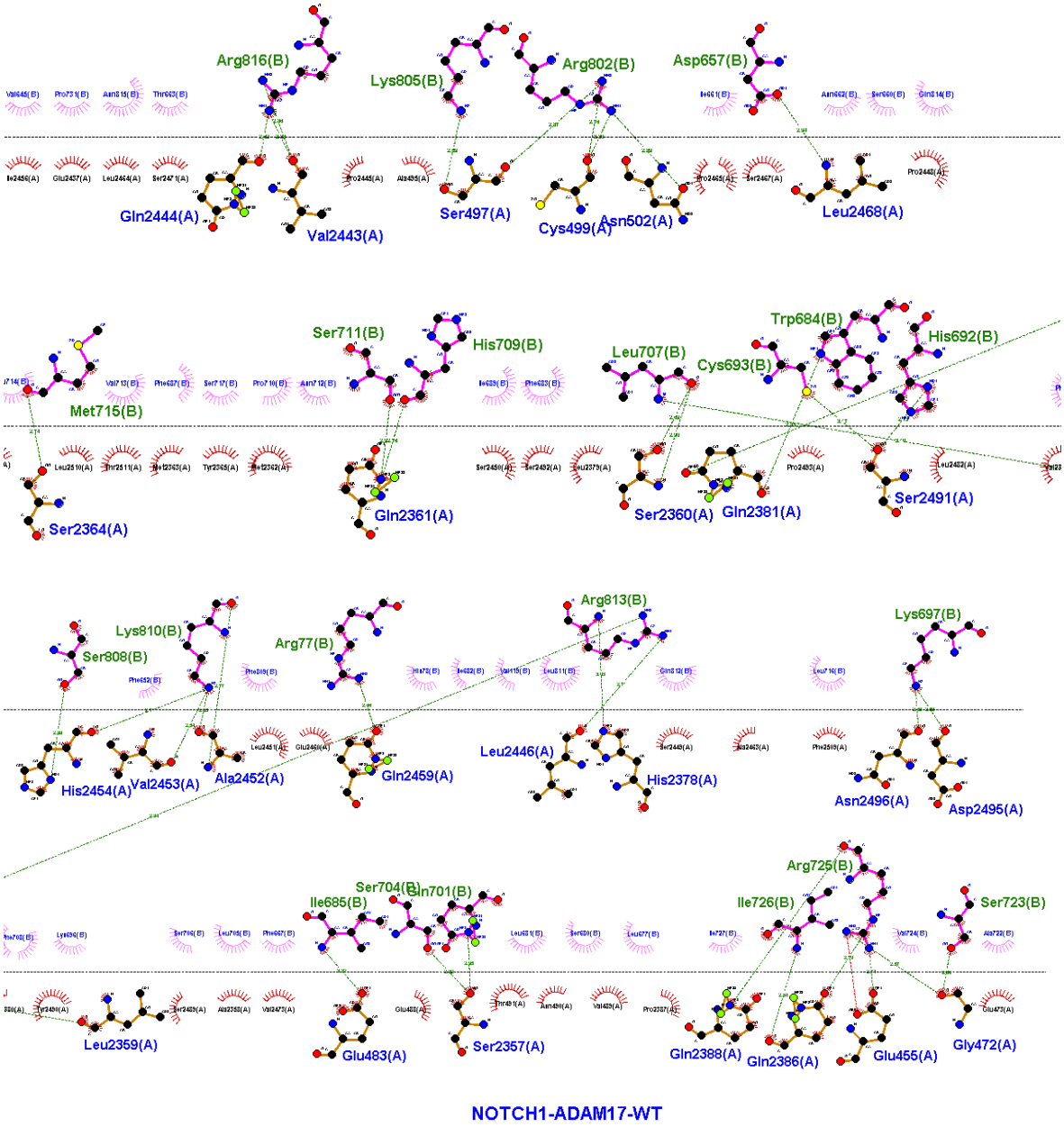
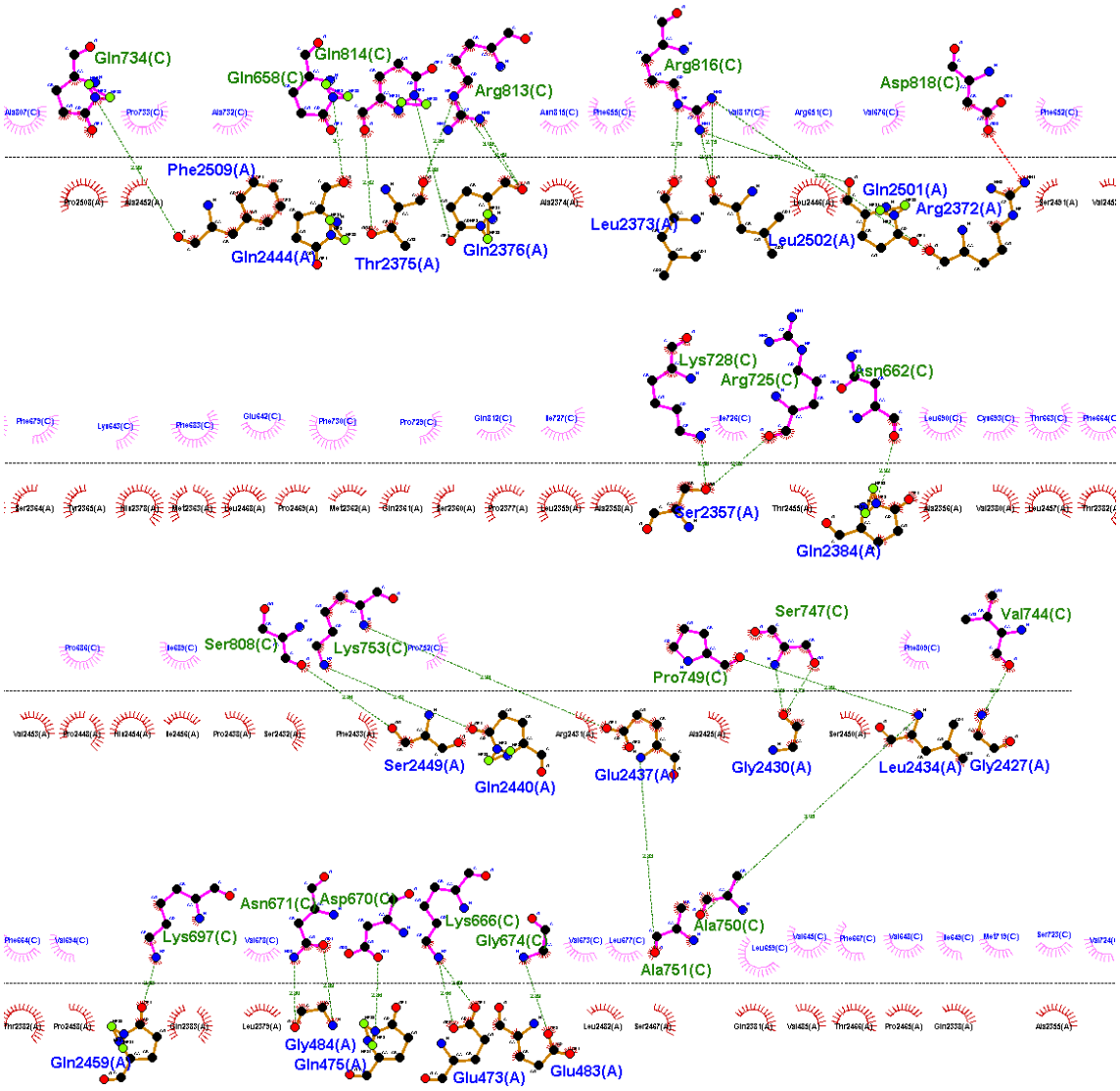


Figure 8. **The binding interaction between NOTCH1 and ADAM17-WT.** The complex was formed by 38 H-bonds: Gln2444-Arg816, Val2443-Arg816, Ser497-Lys805, Ser497-Arg802, Cys499-Arg802, Asn502-Arg802, Leu2468-Asp657, His2454-Ser808, His2454-Lys810, Val2453-Lys810, Ala2452-Lys810, Gln2459-Arg77, Leu2446-Arg813, His2378-Arg813, Asn2496-Lys697, Asp2495-Lys697, Ser2364-Met715, Gln2361-Ser711, Gln2361-His709, Ser2360-Leu707, Gln2381-Arg813, Gln2381-Trp684, Ser2491-Cys693, Ser2491-His692, Leu2359-Leu707, Glu483-Ile685, Ser2357-Ser704, Ser2357-Gln701, Gln2388-Arg725, Gln2386-Ile726, Gln2386-Arg725, Glu455-Arg725, Gly472-Arg725, Gly472-Ser723. Chain A: NOTCH1; Chain B: ADAM17-WT.



NOTCH1-ADAM17-P18L

Figure 9. **The binding interaction between NOTCH1 and ADAM17-P18L.** The complex was formed by 31 H-bonds: Phe2509-Gln734, Gln2444-Gln658, Thr2375-Gln814, Thr2375-Arg813, Gln2376-Gln814, Gln2376-Arg813, Leu2373-Arg816, Leu2502-Arg816, Gln2501-Arg816, Arg2372-Arg816, Ser2449-Ser808, Gln2440-Lys753, Glu2437-Lys753, Glu2437-Ala751, Gly2430-Ser747, Leu2434-Pro749, Leu2434-Ala750, Gly2427-Val744, Ser2357-Lys728, Ser2357-Arg725, Gln2384-Asn662, Gln2459-Lys697, Gly484-Asn671, Gln475-Asp670, Glu473-Lys666, Glu483-Gly674. Chain A: NOTCH1; Chain C: ADAM17-P18L.

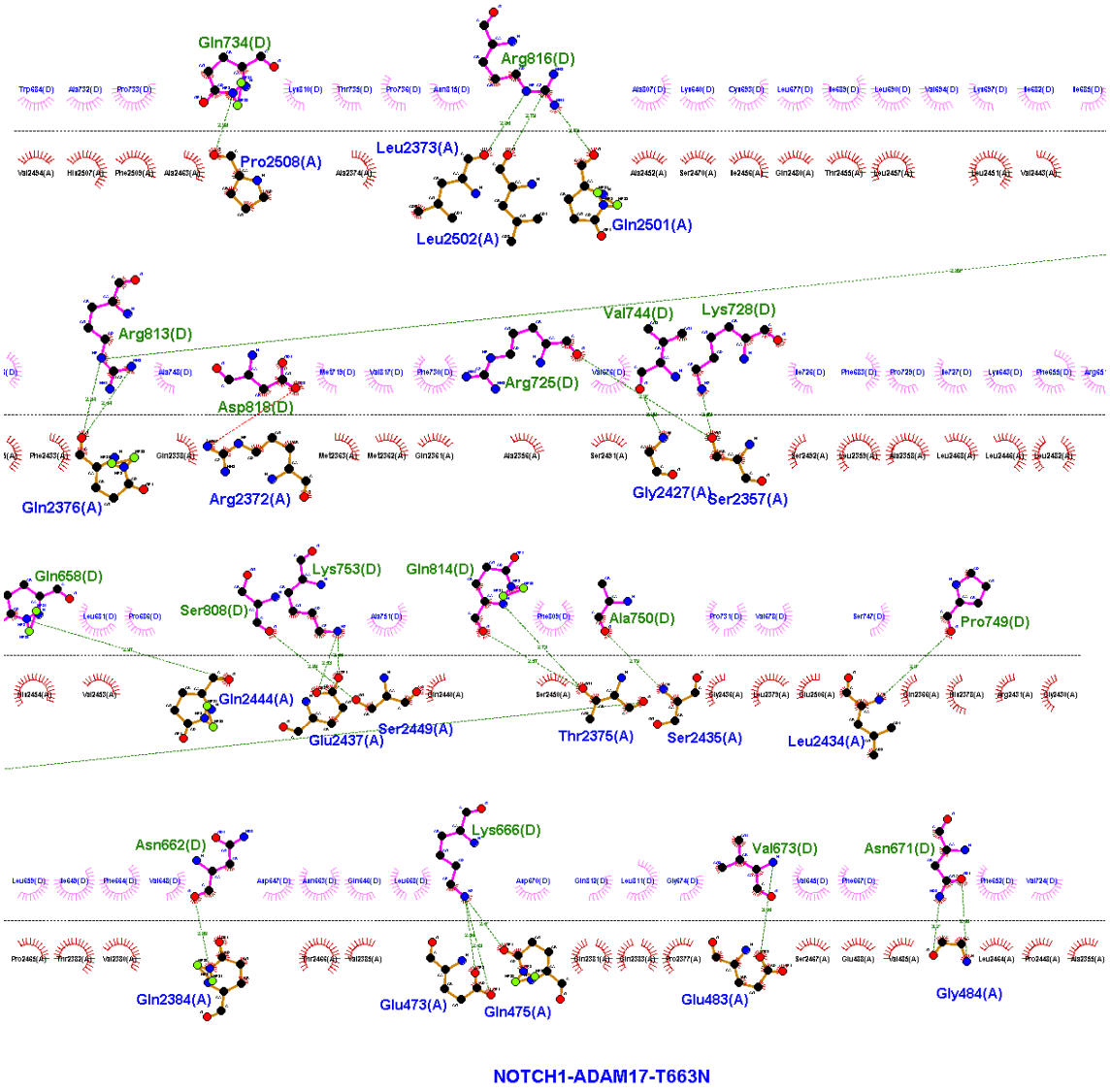


Figure 10. **The binding interaction between NOTCH1 and ADAM17-T663N.** The complex was formed by 25 H-bonds: Pro2508-Gln734, Leu2373-Arg816, Leu2502-Arg816, Gln2501-Arg816, Gln2444-Gln658, Glu2437-Lys753, Glu2437-Lys753, Ser2449-Ser808, Thr2375-Gln814, Thr2375-Gln814, Thr2375-Arg813, Ser2435-Ala750, Leu2434-Pro749, Gln2376-Arg813, Gln2376-Arg813, Gly2427-Val744, Ser2357-Arg725, Ser2357-Lys728, Gln2384-Asn662, Glu473-Lys666, Gln475-Lys666, Glu483-Val673, Gly484-Asn671. Chain A: NOTCH1; Chain D: ADAM17-T663N.

4.4 Discussion

In the present study, we conducted a comprehensive analysis of 21 Brazilian individuals who have been clinically diagnosed with MIS-C. Our primary objective was to identify rare deleterious genetic variants that could potentially predispose these individuals to MIS-C. The search for risk factors involved in MIS-C susceptibility or severity is crucial to define prevention strategies, such as the detection of individuals at high risk of developing the syndrome and to delineate new treatment protocols, for example, by partially re-establishing a deficient immunological function.

Since our patients were hospitalized between May 2020 and March 2021, we assume that the majority of them were infected with the SARS-CoV-2 strains B.1.1.28 and B.1.1.33, which were the most prevalent variants in Brazil at this period[33,34,35]. It is important to note, however, that no definitive correlations have been established between the different SARS-CoV-2 strains and the incidence of MIS-C. The epidemiology, clinical and laboratory findings for these MIS-C cases were consistent with previous results found by other authors[1,17,35]. It is important to note that patients with severe MIS-C presented longer hospitalization time than those with moderate MIS-C.

There is no consensus on whether susceptibility to MIS-C is influenced by individual ancestry. However, this condition seems to be more prevalent in Africans and Latinos than in Europeans[2,9,36]. In the present study, focused on Latin American admixed individuals, severe MIS-C was associated with higher African ancestry and lower European ancestry, when compared to moderate MIS-C. MIS-C is a heterogeneous condition that may occurs after SARS-CoV-2 infection for those with genetic predisposition. Notably, the individuals recruited here had no preexisting health problems, and had no previous diagnosis of PIDs. Genetic predisposition to severe SARS-CoV-2 infection has been reported in young patients with IEI, and it is possible that a genetic susceptibility to MIS-C occurs in individuals with IEI[12,13,19].

Here, we reported 6 heterozygous nonsynonymous SNVs in 5 autoinflammatory genes (*ADAM17*, *IKBKG*, *PSTPIP1*, *SH3BP2* and *CARD14*), found in 5 (~24%) patients. Although we performed variant prioritization analysis with the 21 MIS-C cases together, all candidate MIS-C-causing variants selected here were found in patients with the severe form (5 cases from

12; ∼42%), indicating a genetic enrichment in this group. Two variants [rs1200631089 (CADD = 24) and rs144458353 (CADD = 21.8)] in the *ADAM17* gene have been identified in patients P12 and P18, respectively. As a membrane "sheddase" protease, ADAM17 removes membrane proteins ectodomains from more than 80 substrates, including IL-6R and L-selectin[37]. This sheddase activity can regulate autoinflammatory processes, tumor growth and metastatic progression[31,38]. This metalloprotease is mainly expressed in the lung, kidney, thymus, and heart[31]. The inflammatory role of *ADAM17* is exerted by the cleavages of the ACE2 and TNF-α. The hyperactivation of *ADAM17* may reduce the function of *ACE2* in the tissues, causing hyperinflammation and tissue damage due to excess of Angiotensin I[37]. *ADAM17* hyperactivation in SARS-CoV-2 infection may contribute to the cytokine storm caused by the cleavage of *TNF-α* and *IL-6*, favoring hyperinflammatory pathological complications, vascular permeability, and multiple organ failure seen in COVID-19 and MIS-C[39,40].

The variant rs1215029143 (CADD = 23), identified in patient 1, is located in the gene *IKBKG* with a GDI of 0.09, indicating that mutations in this gene are more likely to be associated with more severe monogenic diseases[41]. The *IKBKG* gene, located on chromosome Xq28, encodes the essential modulator of NF-kappa-B (NEMO/IKK-γ). NEMO/IKK-γ plays a central role in innate and adaptive immunity by transducing signals in response to stimulation of pattern recognition receptors, the TNF-α receptor superfamily, and lymphocyte antigen receptors[19,42]. Lee and colleagues (2022)[42] reported that mutations involved in overexpression of an isoform of the NEMO protein cause inhibition of IFN type I expression and, consequently, reduced antiviral responses, leaving patients susceptible to viral and bacterial infections. These authors observed that individuals with NEMO mutations developed a pediatric autoinflammatory syndrome due to disorganization of post-infection inflammatory responses, a scenario similar to MIS-C.

The rs77026017 variant found in patient 5 is located at *PSTPIP1*, which encodes a protein that regulates T lymphocyte activation, cytoskeletal organization, and release of interleukin-1 by macrophages[43]. Pyogenic arthritis, pyoderma gangrenosum, and acne syndrome (PAPA) is an autoinflammatory disorder resulting from genetic variants in *PSTPIP1*[44]. Two variants (A230T and E250Q) in *PSTPIP1* have been identified in patients with PAPA syndrome and have been predicted to markedly increase the binding of *PSTPIP1*

to pyrine[45]. Resulting in a dominant negative effect on pyrine and inhibiting its anti-inflammatory activity, leading to increased secretion of IL-1β by macrophages in patients with PAPA autoinflammatory syndrome[46]. PAPA Syndrome and MIS-C, in common, are recognized by skin involvement in most patients[46,47]. However, in MIS-C, the inflammatory pattern in the skin consists of erythema, rash and scarlatiniform, morbilliform and urticarial eruptions[48].

Two variants potentially implicated in MIS-C at *SH3BP12* [rs139934973 (CADD: 22.7; GDI: 2.23)] and *CARD14* [rs1054063201 (CADD: 23.1; GDI: 9.49)] were found in patient 10. SH3BP12 protein acts on hematopoietic cells and induces B-cell receptor activation, NK-cell-mediated cytotoxicity, and basophilic cell degranulation[49]. Genetic variants in *SH3BP2* lead to increased bone resorption in the jaws of patients with cherubism[50]. The *CARD14* protein activates the signaling pathways of the inflammatory transcription factor NF-kappa-B[51]. It has been suggested that skin trauma, viral, and bacterial infections in individuals with hypomorphic/null mutations in the *CARD14* gene may act as triggers for psoriasis[52,53].

Based on the genetic findings in the MIS-C cases in this study, it was observed that the ADAM17 protein, according to the literature, was more likely to be involved in the immune dysregulation and pathophysiology of this syndrome[31,37,39]. To determine whether the P18L and T663N variants destabilized the protein and altered its function, docking analyses were carried out to compare the wild-type protein and its mutant types (ADAM17-P18L and ADAM17-T663N).

Although ADAM17 has more than 80 substrates, TNF-α, ACE2, IL-6R and NOTCH1 were selected because they have been reported in the literature to alter the function of the pathway due to the abnormal behavior of ADAM17[31]. These binding instability scenarios could justify the occurrence of MIS-C in patients carrying the P18L and T663N variants.

It was observed that P18L variant destabilizes the docking with the *TNF,* causing significant implications for the function and regulation of these cellular processes. The weak binding of ADAM17-P18L with TNF-a may be related to a deficiency in the pathway influenced by this cytokine and may lead to a reduced capacity to resolve inflammation, resulting in long-lasting inflammation, as in the pathophysiology of MIS-C[39,54,55]. In addition, TNF-α is involved in tissue repair and healing processes and low levels of this cytokine caused by reduced ADAM17

function can negatively affect the body's ability to respond effectively to injuries and regenerate tissues[39].

The ADAM17-P18L and ADAM17-T663N variants showed more binding instability with ACE2 and IL-6R than when compared with wild-type ADAM17. These changes may reflect a scenario of loss of sheddase function, disrupting ADAM17 affinities with its ligands or inducing structural destabilization of the ADAM17 protein, which in turn may trigger disruption of the homeostasis of the Renin-Angiotensin System and the IL-6 signaling pathway[31,37,56].

In Tregs of children with MIS-C, alterations in NOTCH 1 receptor signaling have already been observed, which were predicted to induce CD22, leading to destabilization of Tregs in a mTORC1-dependent manner and to the promotion of systemic inflammation[57,58]. Here, we observed that the ADAM17-P18L or ADAM17-T663N mutant proteins interact through fewer binding residues and have lower interaction affinities with NOTCH-1 than the ADAM17-WT protein[57,59.]

The genetic panorama of our study reinforces the possibility that IEIs are implicated in MIS-C by selectively affecting immunity against SARS-CoV-2. In addition, we suggest that variants of genes related to autoinflammatory diseases, such as *ADAM17*, *IKBKG*, *PSTPIP1*, *SH3BP2* and *CARD14,* may be implicated in MIS-C. Specifically, we identified that nonsynonymous variants in *ADAM17* can cause loss of function in the protein and lead to the immune dysregulation seen in MIS-C.

We found clinical and laboratory findings in our cohort that were similar to those found in previous studies. However, as we collected medical records from different hospitals, some data were not available for all patients. Because of that, most comparisons between moderate and severe patients resulted in a lack of statistical power. Analyses with a larger sample size and inclusion of patients from different ethnic groups are fundamental to identify genetic factors for MIS-C.

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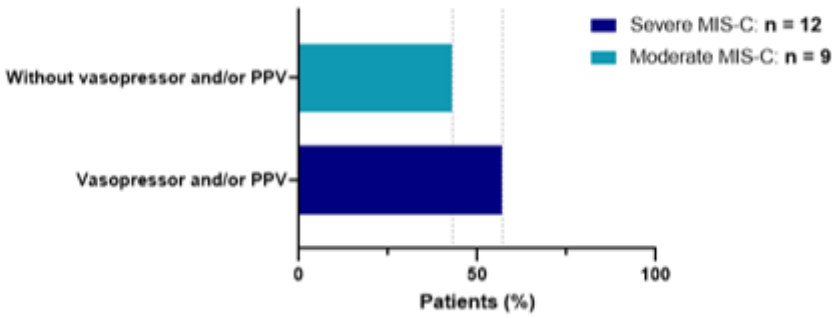
4.6 Supplementary Figures and Tables

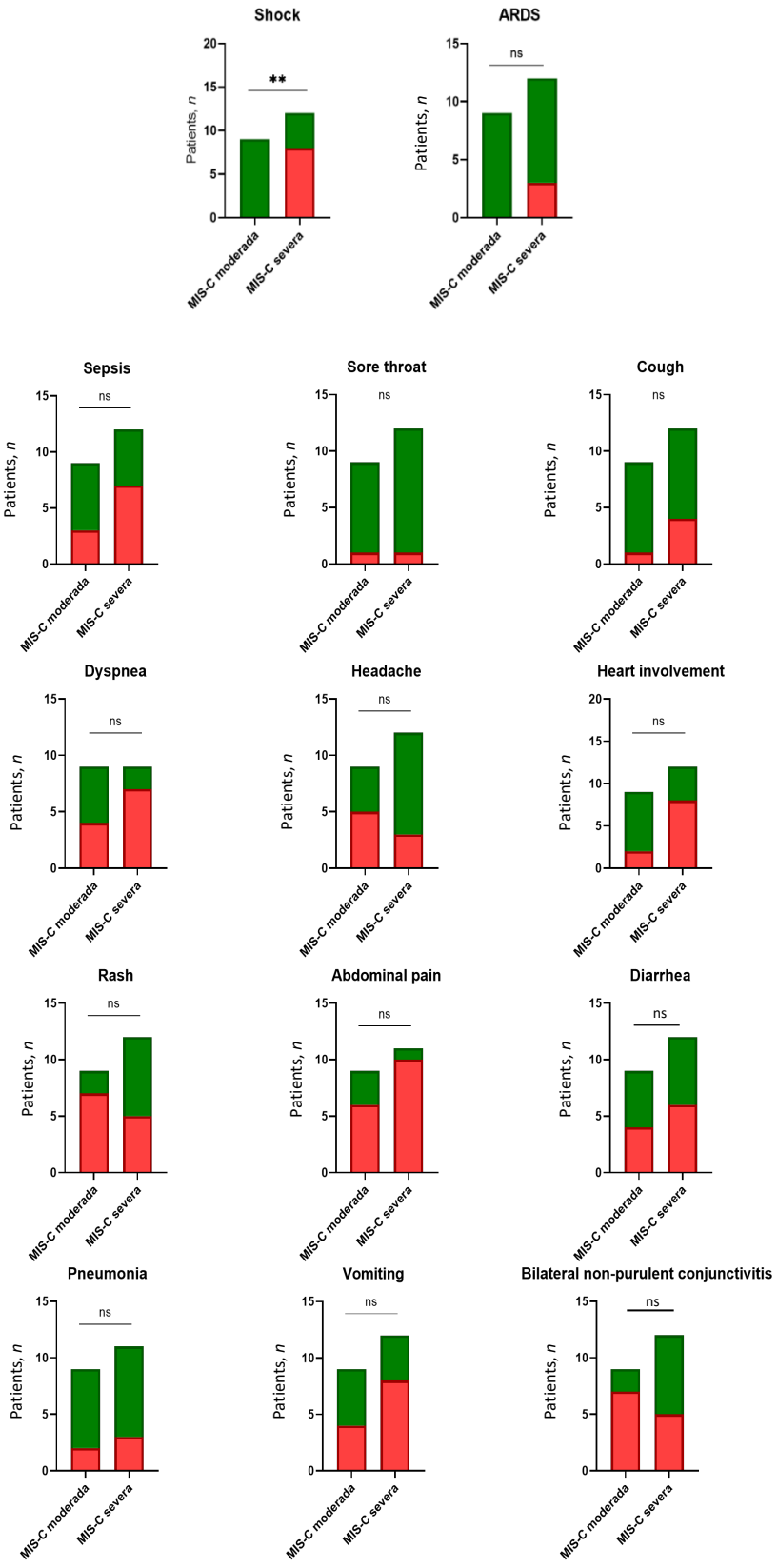
Supplementary Table 1. List of genes previously implicated in autoinflammatory diseases

ACP5, ADA2, ADAM17, ADAR1, ALPI, AP1S3, ATAD3A, C2orf69, CARD14, CDC42, COPA, DNASE1L3, DNASE2, HAVCR2, HCK, IFIH1, IKBKG, IL1RN, IL36RN, LPIN2, LSM11, MEFV, MVK, NCKAPIL, NLRC4, NLRP1, NLRP12, NLRP3, NOD2, OAS1, OTULIN, PLCG2, POLA1, PSMB8, PSMB9, PSMG2, PSTPIP1, RIPK1, RNASEH2A, RNASEH2B, RNASEH2C, RNU7-1, SAMHD1, SH3BP2, SLC29A3, STAT2, SYK, TBK1, TMEM173, TNFAIP3, TNFRSF1A, TREX1, TRIM22, USP18.

Described in the 2022 list published by The International Union of Immunological Societies (IUIS). DOI:10.1007/s10875-022-01352-z.



Supplementary Figure 1. Classification of MIS-C cases. Based on the U.S. Centers for Disease Control and Prevention (CDC). Abbreviation, PPV: positive prevention ventilation. 12 (57%) patients in MIS-C severe and 9 (43%) in MIS-C moderate group.


Shock
**
ARDS
ns
Sepsis
ns
Sore throat
ns
Cough
ns
Dyspnea
ns
Headache
ns
Heart involvement
ns
Rash
ns
Abdominal pain
ns
Diarrhea
ns
Pneumonia
ns
Vomiting
ns
Bilateral non-purulent conjunctivitis
ns
Patients, n
MIS-C moderada
MIS-C severa

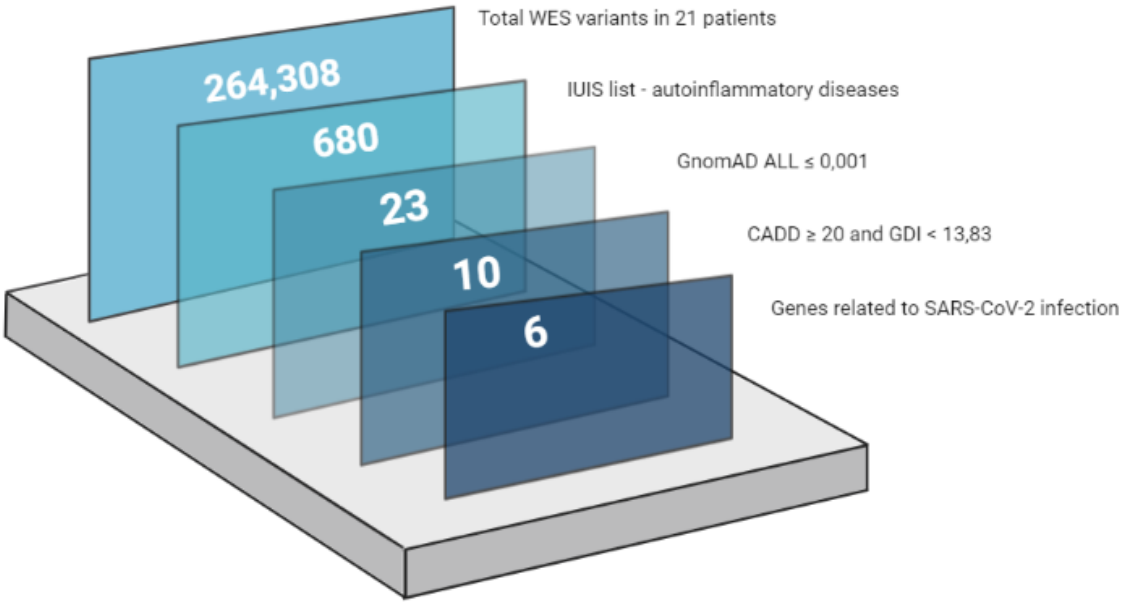
Supplementary Figure 2. **Clinical manifestations affected in patients with moderate and severe MIS-C**. Analyses of these categorical variables were performed with the Fisher exact test (GraphPad Prims 8.4.2). Red: Yes; Green: No. Y-axis represents the total number of affected patients in each group. Abbreviations, n: total number of patients. ARDS: Acute Respiratory Distress Syndrome. Ns: Not Statistically Significant. ** $P < 0.01$.

Supplementary Table 2. Laboratory findings in children and adolescent with moderate or severe MIS-C

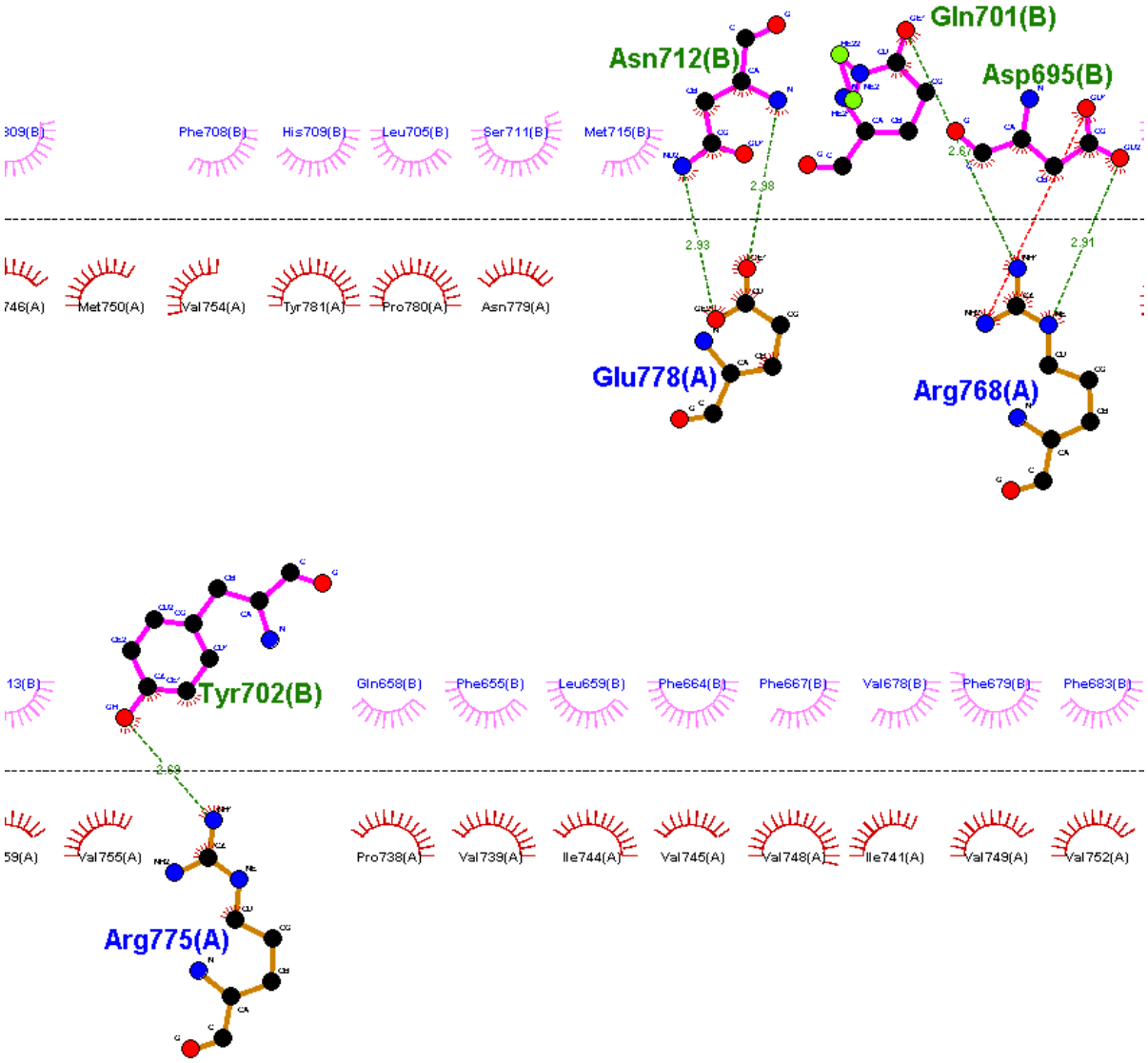
	Moderate MIS-C	Severe MIS-C		
	Median (IQR)		Reference range	*p* value
Total WBC count (cells/mm3)	17,870 (13,950-19,950)	15,885 (13,005-24,450)	5,000 - 13,000	0.83
Neutrophils (cells/mm3)	10,773 (9,170-15,190)	11,525 (7,766-21,428)	1,500 - 8,500	0.65
Lymphocytes (cells/mm3)	3,950 (1,425-5,655)	3,107 (1,129-4,562)	900 - 4,000	0.59
NLR	3.81 (1.45-10.6)	6.04 (0.84-37,82)	1 - 3	0.36
D-dimer (mg/L)	4.3 (2.4-5.8)	6.3 (3.8-8.3)	< 0.5	0.12
Albumin (g/dL)	2.7 (2.3-3.4)	2.5 (2.1-4.1)	3.4 - 5.4	0.60
AST (U/L)	25 (21-45)	33 (16-49)	15 - 60	0.87
ALT (U/L)	38 (16-60)	27 (16-76.5)	7 - 56	0.83
CPK (U/L)	65 (16.45-113.5)	53.5 (43.4-153.8)	24 - 200	0.79
LDH (U/L)	422 (224.2-540.5)	278 (242-671)	110 - 295	0.94
Ferritin (ng/mL)	469 (245-766)	695 (34-1500)	7 - 140	0.78
CRP (mg/L)	75 (24.8-146)	164 (7.3-188.3)	< 5	0.70

Moderate: *n*=9*;* Severe: *n*=12; Only findings available for more than 50% of the individuals enrolled in the study are shown. Laboratory findings (median with interquartile range). *p*-value was obtained using the nonparametric Mann-Whitney test. Indicates the statistical significance of severe MIS-C *vs* moderate MIS-C. NLR: number of neutrophils divided by the number of lymphocytes.

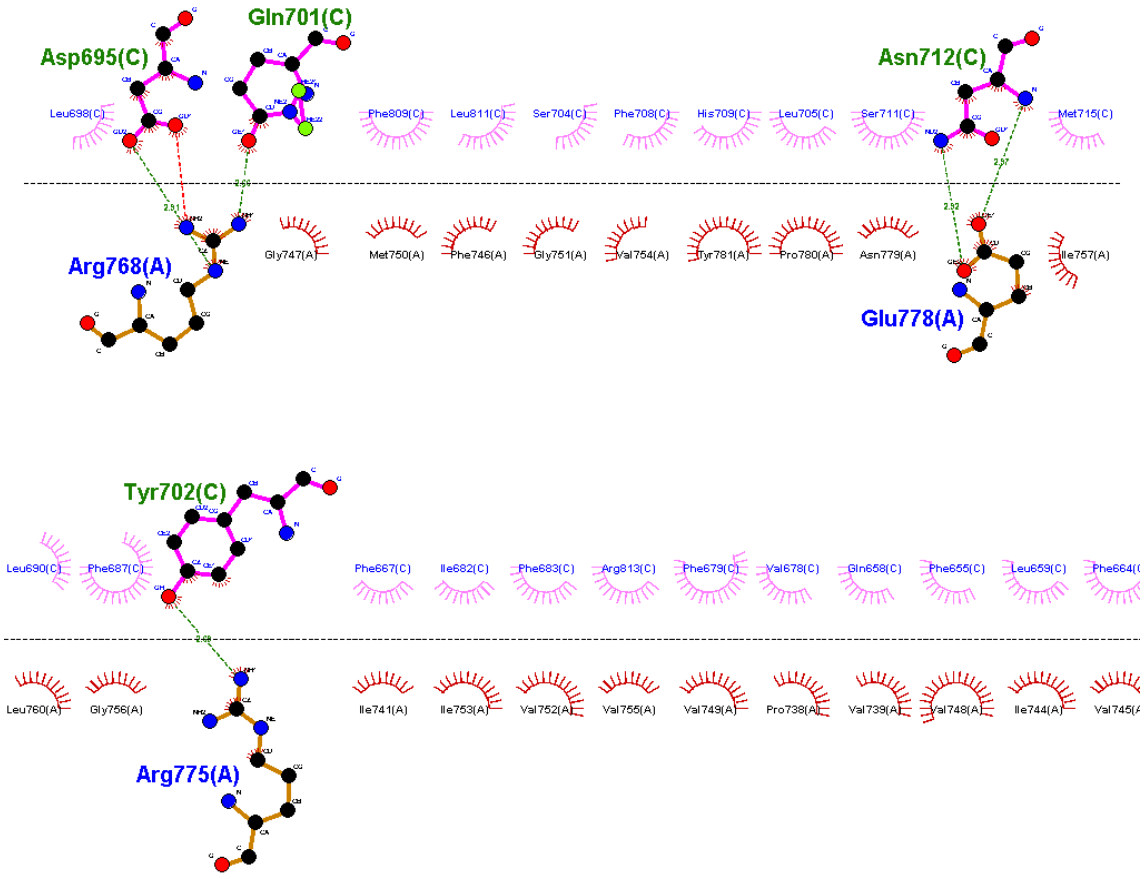
Abbreviations, IQR: interquartile range (first-third quartiles). RR: reference range - for ages 6 to 12 years. WBC: white blood cell. NLR: Neutrophil-to-lymphocyte ratio. AST: aspartate aminotransferase. ALT: alanine aminotransferase. CPK: creatine phosphokinase. LDH: lactic dehydrogenase. CRP: C-reactive protein.



Supplementary Figure 3. Strategy for prioritizing rare deleterious variants in genes previously implicated in autoinflammatory disorders. The numbers indicated at each step are the remaining unique small nucleotide variants (SNVs) found after filter application. The 2022 list of inborn errors of immunity of autoinflammatory diseases was obtained from the International Union of Immune Societies (IUIS). doi:10.1007/s10875-022-01352-z. Abbreviations, WES: whole exome sequencing. IUIS, International Union of Immune Societies. GnomAD: Genome Aggregation Database, v2.1.1. CADD, combined annotation dependent depletion algorithm. GDI, gene damage index.

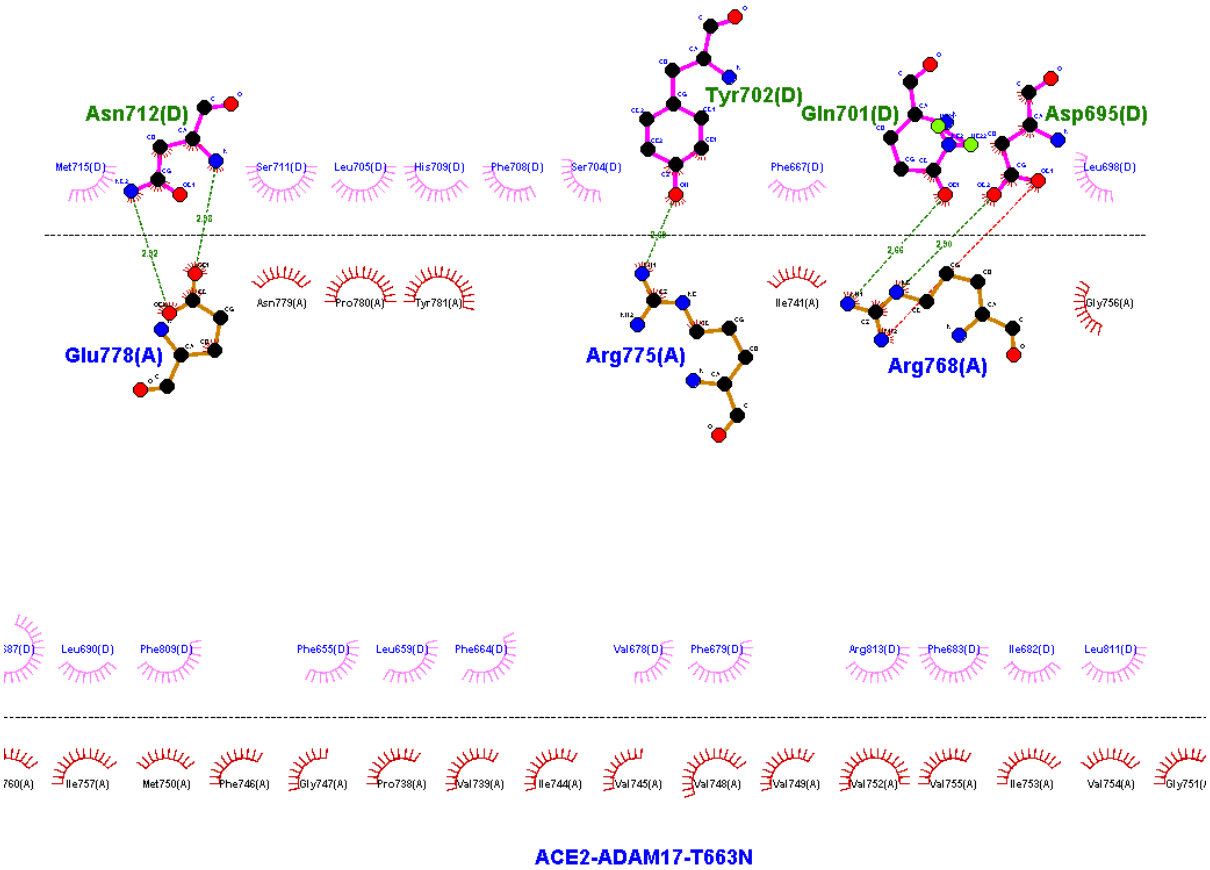


Supplementary Figure 4. **The binding interaction between ACE2 and ADAM17-WT.** The complex was formed by 5 H-bonds: Glu778-Asn712, Arg768-Gln701, Arg768-Asp695, Arg775-Tyr702. Chain A: NOTCH1; Chain B: ADAM17-WT.

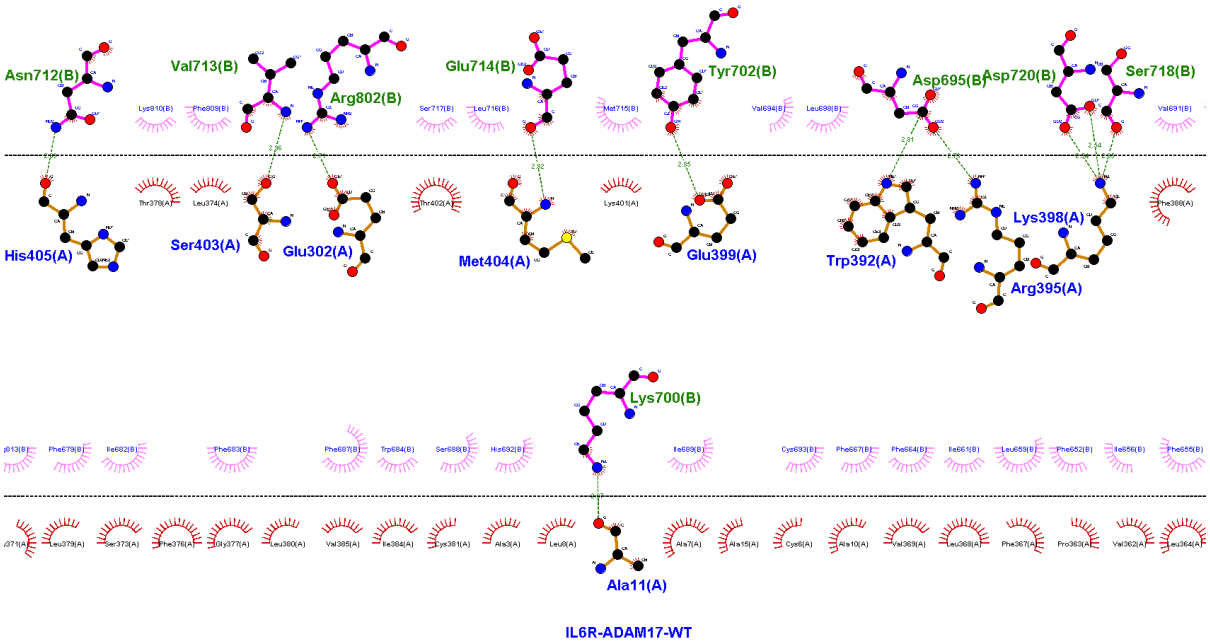


ACE2-ADAM17-P18L

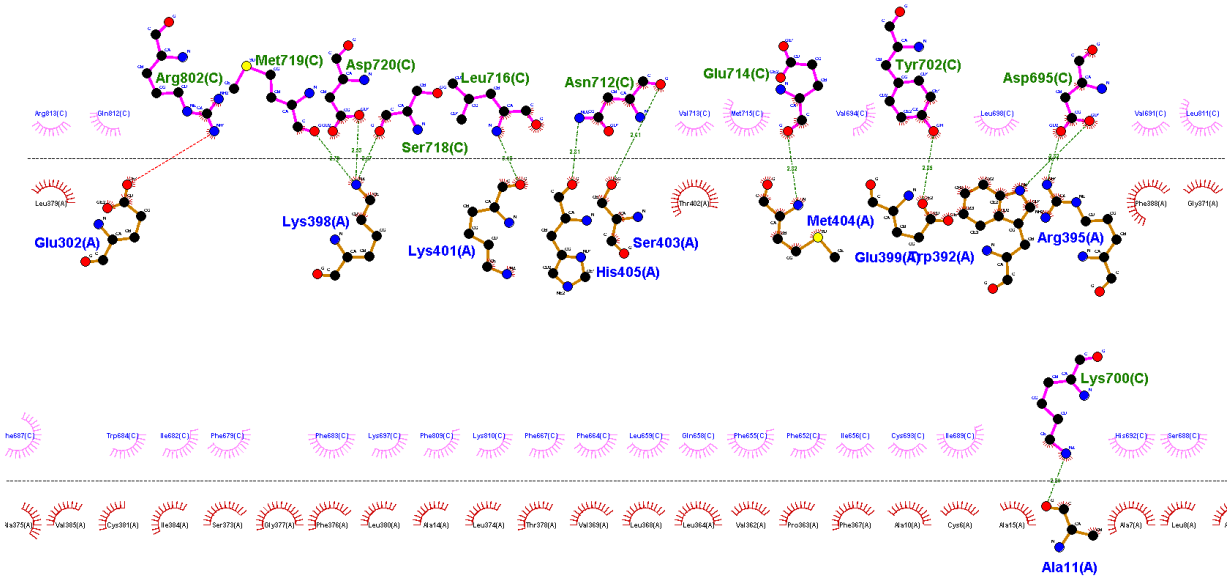
Supplementary Figure 5. **The binding interaction between ACE2 and ADAM17-P18L.** The complex was formed by 5 H-bonds: Arg768-Asp695, Arg768-Gln701, Glu778-Asn712, Arg775-Tyr702. Chain A: NOTCH1; Chain C: ADAM17-P18L.



Supplementary Figure 6. **The binding interaction between ACE2 and ADAM17-T663N.** The complex was formed by 5 H-bonds: Glu778-Asn712, Arg775-Tyr702, Arg768-Gln701, Arg768-Asp695. Chain A: NOTCH1; Chain D: ADAM17-T663N.

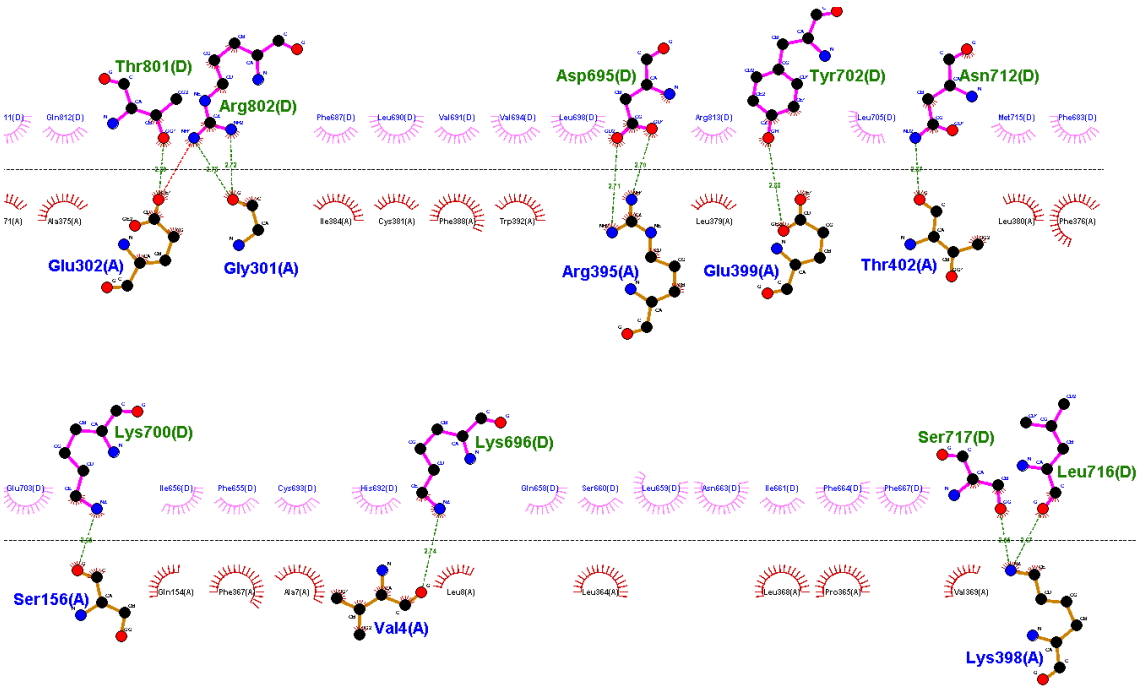


Supplementary Figure 7. **The binding interaction between IL6R and ADAM17-WT.** The complex was formed by 11 H-bonds: His405-Asn702, Ser403-Val713, Glu302-Arg802, Met404-Glu714, Glu399-Tyr702, Trp392-Asp695, Arg395-Asp695, Lys398-Asp720, Lys398-Ser718, Ala11-Lys700. Chain A: NOTCH1; Chain B: ADAM17-WT.



IL6R-ADAM17-P18L

Supplementary Figure 8. **The binding interaction between IL6R and ADAM17-P18L.** The complex was formed by 11 H-bonds: Lys398-Met719, Lys395-Asp720, Lys398-Ser718, Lys401-Leu716, His405-Asn712, Ser403-Asn712, Met404-Glu714, Glu399-Tyr702, Trp392-Asp695, Arg395-Asp695, Ala11-Lys700. Chain A: NOTCH1; Chain C: ADAM17-WT.



IL6R-ADAM17-T663N

Supplementary Figure 9. **The binding interaction between IL6R and ADAM17-T663N.** The complex was formed by 11 H-bonds: Glu302-Thr801, Gly301-Arg802, Gly301-Arg802, Arg395-Asp695, Arg395-Asp695, Glu399-Tyr702, Thr402-Asn712, Ser156-Lys700, Val4-Lys696, Lys398-Ser717, Lys398-Leu716. Chain A: NOTCH1; Chain D: ADAM17-T663N.

5 CONCLUSÕES

Variantes raras e potencialmente deletérias nos genes *ADAM17*, *IKBKG*, *PSTPIP1*, *SH3BP2* e *CARD14* podem estar envolvidas no desenvolvimento da SIM-P em crianças e adolescentes brasileiros previamente sadios. Especialmente, análises funcionais *in silico* sugerem que variantes não-sinônimas em *ADAM17* podem causar perda de função na proteína e levar à desregulação imunológica observada na SIM-P.

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