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The role of *IL10* and *IL17* gene polymorphisms in treatment response in children and adolescents with severe asthma

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ABSTRACT

Objective: To determine whether polymorphisms of the *IL10* and *IL17* genes are associated with severe asthma control and bronchodilator reversibility in children and adolescents with severe asthma. **Methods:** This was a cross-sectional study, nested within a prospective cohort study of patients with severe asthma. Two outcomes were evaluated: asthma control and bronchodilator reversibility. We extracted DNA from peripheral blood and genotyped three single nucleotide polymorphisms: rs3819024 and rs2275913 in the *IL17A* gene; and rs3024498 in the *IL10* gene. For the association analyses, we performed logistic regression in three genetic models (allelic, additive, and dominant). **Results:** The rs3024498 C allele in the *IL10* gene was associated with failure to achieve asthma control despite regular treatment (p = 0.02). However, the G allele of the *IL17A* rs3819024 polymorphism was associated with failure to respond to stimulation with a β_2 agonist. The rs2275913 polymorphism of the *IL17A* gene showed no relationship with asthma control or bronchodilator reversibility. **Conclusions:** In pediatric patients with severe asthma, the *IL10* polymorphism appears to be associated with failure to achieve clinical control, whereas the *IL17A* polymorphism appears to be associated with a worse bronchodilator response. Knowledge of the involvement of these polymorphisms opens future directions for pharmacogenetic studies and for the implementation of individualized therapeutic management of severe asthma in pediatric patients.

Keywords: Polymorphism, genetic; Interleukin-10; Interleukin-17; Asthma.

INTRODUCTION

Although severe asthma affects only approximately 2% of all children with asthma, it is associated with a high rate of morbidity.[1,2] The refractory form of severe asthma is characterized by continued poor control despite maximum doses of asthma controller medications and a focus on modifiable factors such as treatment adherence, exposure to allergens, and exposure to smoking. One major reason for that lack of control is probably the heterogeneous nature of the disease, the pathogenesis of which involves the interaction of environmental factors and individual variability, together with a complex genetic basis.[3]

The heterogeneity of severe asthma can be explained by distinct molecular phenotypes comprising cytokines unrelated to the classical Th2 lymphocyte pathway, such as IL-10 and IL-17.[4] By inhibiting the production of proinflammatory cytokines, IL-10 reduces allergic inflammation; its failure to respond to increasing doses of inhaled steroids in patients with severe asthma is likely related to poor clinical control.[5] In contrast, IL-17 stimulates the production of Th17 inflammatory cytokines, thus promoting airway inflammation.[6]

Polymorphisms in the *IL10* and *IL17* genes were described in a meta-analysis of pediatric patients of different ethnicities, although only the risk of asthma was assessed.[6,7] Polymorphisms of these cytokine genes might explain the difficulty in achieving clinical and functional control in patients with severe asthma. However, to our knowledge, there have been no studies associating genetic variants in *IL10* and *IL17* with clinical control in cases of severe asthma. Given that polymorphisms of these cytokine genes can be useful biomarkers for adjusting treatment regimens, the objective of the present study was to evaluate whether polymorphisms in the *IL10* and *IL17* genes were associated with severe asthma control and bronchodilator responsiveness in a sample of pediatric patients.

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METHODS

Study design, study participants, and clinical characteristics

This was a cross-sectional study, nested within a prospective cohort study of patients with severe asthma,[8] carried out between 2021 and 2022 at the Multidisciplinary Center for Difficult-to-Control Asthma and the Institute of Biological Sciences of the Federal University of Minas Gerais, located in the city of Belo Horizonte, Brazil. The study was conducted in collaboration with the Laboratory of Immunopharmacology and Molecular Biology at the Health Sciences Institute of the Federal University of Bahia, located in the city of Salvador, Brazil. We selected patients who had severe asthma (defined as asthma confirmed by an objective measure of lung function), who had good adherence to treatment, and who, despite elimination or minimization of factors associated with poor disease control, required high doses of inhaled corticosteroids (ICS—budesonide ≥ 1,600 µg or equivalent) and a second controller medication—long-acting β_2 agonists (LABAs), long-acting muscarinic antagonists, leukotriene receptor antagonists, or any combination of the three—or oral corticosteroids ≥ 50% of the previous year in order to maintain disease control, as well as those in whom the disease remained uncontrolled because of its intrinsic severity.[9-11] Given that severe refractory asthma is an uncommon phenotype, sampling was convenience-based. We performed a posteriori sample size calculation using the free, Web-based, open-source program OpenEpi, version 3.01, and found that, for a statistical power of 80%, a sample size of 34 per group was required.[12]

Patients who were first-degree relatives (e.g., siblings) were excluded, as were those with other chronic lung diseases. Healthy individuals were not included in the present study.

Clinical characteristics such as age (in years), age at initiation of treatment with ICS (in months), maternal smoking during pregnancy, reported passive smoking, previous ICU admission for asthma, severe exacerbations in the last 12 months, weight (in kg), height (in cm), and BMI (in kg/m^2) were assessed.

The dose of ICS was evaluated in terms of its equivalence with that of budesonide. The ICS adherence rate was considered optimized if it was above 80%. It was calculated as the proportion of the total recommended dose, by checking the dose counter of a pressurized metered-dose inhaler or by counting the capsules that had been used in a dry-powder inhaler.[9] Some patients were using dry-powder inhalers that delivered a combination of budesonide and formoterol (Alenia; *Aché Laboratórios Farmacêuticos S/A*, Guarulhos, Brazil), whereas others were using pressurized metered-dose inhalers that delivered a combination of fluticasone and salmeterol (Seretide; GlaxoSmithKline, Stevenage, United Kingdom) or omalizumab only (Xolair; *Novartis Biociências S/A.*, São Paulo, Brazil). The use of leukotriene receptor antagonists and biologic agents was also evaluated.

The diagnosis of allergic rhinitis was made on the basis of patient clinical history and a nasal symptom questionnaire, as well as a positive skin prick test for aeroallergens.[9] In addition to rhinitis, other comorbidities were considered: atopic dermatitis, mouth breathing, gastroesophageal reflux disease, behavioral disorders, and emotional disorders.[10]

Procedures

The level of asthma control was assessed by applying the GINA criteria.[10] Patients were asked whether in the last four weeks they had experienced symptoms of asthma during the day more than twice a week; woken up at night because of asthma; used a short-acting β_2 agonist (SABA) to relieve asthma symptoms more than twice a week; and had any activity limitations because of asthma. Controlled asthma was defined as a negative answer to all four questions, whereas uncontrolled asthma was defined as an affirmative answer to any one of the four questions. On the basis of their answers, patients were divided into two groups: controlled severe asthma and uncontrolled severe asthma.

Skin prick tests were performed and were considered positive if the wheal was at least 3 mm larger than that of the negative control.[13] We tested the following allergens, all obtained from the same supplier (Imunotec, São Paulo, Brazil): *Dermatophagoides pteronyssinus*; *Dermatophagoides farinae*; *Blomia tropicalis*; dog and cat dander; *Aspergillus* sp.; *Penicillium* sp.; *Periplaneta americana*; and *Cladosporium* sp. Peripheral blood eosinophils were also measured, as were serum cytokine levels. However, the latter were found to be no higher than the lower limit of detection.

All patients underwent pulmonary function tests, which were performed with a KoKo spirometer (KoKo PFT, Longmont, CO, USA) and in accordance with the recommendations of the American Thoracic Society.[14] FEV_1, FVC, and the FEV_1/FVC ratio were evaluated before and after administration of 400 µg of albuterol by pressurized metered-dose inhaler. Significant postbronchodilator variation, or bronchodilator reversibility, was defined as a 200 mL or 12% increase in FEV_1.[15]

Genomic DNA extraction, genotyping, and in silico *analysis*

Peripheral blood samples were collected under vacuum in 10-mL tubes containing the anticoagulant ethylenediaminetetraacetic acid (Vacutainer; Becton Dickinson, Sparks, MD, USA) and were centrifuged in a Kasvi centrifuge (K14-0815A; Kasvi, São José dos Pinhais, Brazil) at 3,000 rpm for 10 min at 4°C. The plasma and buffy coat were separated, after which they were placed in Eppendorf tubes and stored at −30°C. For DNA extraction, we employed a commercial blood kit (Gentra Puregene; QIAGEN, Hilden, Germany). All

genotyped samples were standardized at a concentration of 5 ng/μL and stored at −30°C until use.

On the basis of previous association studies of asthma,[16-19] we selected three genotyped single nucleotide polymorphisms (SNPs) that have been associated with asthma: rs3819024 and rs2275913 in the *IL17A* gene; and rs3024498 in the *IL10* gene. Genotyping was performed with TaqMan probe-based 5'-nuclease assays (Applied Biosystems, Foster City, CA, USA) on the QuantStudio 12K Flex real-time polymerase chain reaction system (Applied Biosystems). In our analysis, we included only SNPs with a call rate of at least 93%. As negative controls, we used blank wells to evaluate nonspecific amplification.

Information regarding the function of each single nucleotide variant was obtained from the U.S. National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). Additionally, RegulomeDB was used in order to identify potential regulatory and functional variants through computational predictions and manual annotations.[20] The database assigns a score ranging from 1 to 6, where lower scores indicate increasing evidence of a variant being located in a functional region.[20]

HaploReg (Broad Institute, Cambridge, MA, USA) is a tool that allows researchers to explore annotations of the noncoding genome at variants on haplotype blocks. It specifically focuses on identifying candidate regulatory single nucleotide variants at disease-associated loci. HaploReg is designed to assist researchers in developing mechanistic hypotheses regarding the impact of noncoding variants on clinical phenotypes and normal variation.[21]

The U.S. National Institutes of Health Genotype-Tissue Expression (GTEx) Project (www.gtexportal.org) has provided valuable insights into the association between gene expression, genetic variation, and other molecular phenotypes across various human tissues.[22] Through expression quantitative trait loci mapping, we can effectively investigate the genetic factors responsible for changes in gene expression. Using this tool, we extracted information specifically related to the influence of variants within the gene of interest on its expression in whole blood samples.

Statistical analysis

The distribution of continuous variables was analyzed by using the Shapiro-Wilk test. Data are expressed as mean ± standard deviation, as median [interquartile range], or as absolute and relative frequencies, depending on the type of variable. For comparisons between groups (patients vs. controls or genotype vs. genotype), we used the unpaired Student's t-test or the Mann-Whitney test, as appropriate. Values of $p < 0.05$ were considered significant.

Association analyses were performed by logistic regression in three genetic models (allelic, additive, and dominant), adjusted for the covariates sex and age, using PLINK software, version 1.9.[23] To reduce the chance of associations with false-positive values of p,[23] only the SNP associations with values of $p < 0.05$, which were revalidated by permutation procedures, were considered statistically significant.

The study was approved by the Research Ethics Committee of the Federal University of Minas Gerais (Protocol no. 4.048.940). Written informed consent was obtained from all participants or their legal guardians.

RESULTS

Initially, 62 patients with severe asthma were eligible. However, 6 were excluded because they were first-degree relatives of other selected patients. Therefore, the sample comprised 56 patients with severe asthma: 19 in the controlled asthma group and 37 in the uncontrolled asthma group. The demographic, clinical, and functional characteristics of the study population are shown in Table 1.

We observed no significant difference between the two severe asthma groups in terms of functional variables and biological markers, showing that these markers do not discriminate between controlled and uncontrolled asthma. There was no difference between the two groups in terms of the BMI. However, the mean height was lower in the uncontrolled asthma group. Both groups of patients used high doses of ICS in combination with high doses of other controller medications, and there was no significant difference between the two groups regarding doses or treatment adherence.

Table 2 presents the characteristics of the SNPs studied. The minor allele frequency was greater than 10% for all three of the SNPs genotyped. No variants were excluded by the Hardy-Weinberg equilibrium or on the basis of a low call rate. The *IL17A* variants were in an untranslated region 2 kb upstream of the gene, and the *IL10* variant was in the 3'-untranslated region.

As can be seen in Table 3, patients with at least one rs3024498 C allele in the *IL10* gene were found to be at a greater risk of having uncontrolled asthma despite regular treatment. Neither of the two other variants was associated with failure to control severe asthma in any of the genetic models tested.

Severe asthma patients with the AG or GG genotype of rs3819024 had a lower bronchodilator response than did those with the AA genotype (Figure 1). Neither of the two other variants was associated with a lack of bronchodilator reversibility in any of the genetic models tested. The rs2275913 SNP of the *IL17A* gene showed no relationship with disease control or bronchodilator reversibility. Using the GTEx browser we found that the TC and CC genotypes of rs3024498 had higher expression of IL-10 in whole blood samples than did the TT genotype (Figure 2; $p = 0.000053$).

DISCUSSION

In pediatric patients, airway inflammation related to the allergic process is typically eosinophilic and orchestrated by Th2 cells. However, eosinophilia is

Table 1. Demographic, clinical, and functional characteristics of the study participants.[a]

Variable	Group		p
	CSA (n = 19)	USA (n = 37)	
Age, years	14.6 ± 3.74	12.2 ± 5.48	0.06
Male sex	10 (52.6)	18 (48.6)	0.89
Age at initiation of ICS, months (median)	34	24	0.56
Maternal smoking during pregnancy	1 (5.3)	3 (8.1)	0.69
Reported passive smoking	6 (31.6)	17 (45.9)	0.30
Previous ICU admission for asthma	7 (36.8)	9 (24.3)	0.27
Severe exacerbations in the last 12 months	1 (5.3)	6 (16.2)	0.23
Comorbidities			
Allergic rhinitis	9 (47.4)	15 (40.5)	0.69
Rhinitis + ≥ 1 other comorbidity	10 (52.6)	22 (59.5)	0.69
Weight, kg	51.00	42.15	0.027
Height, cm	164.0	147.4	0.003
BMI, kg/m^2	20.8	19.0	0.37
Medications			0.44
ICS dose,[b] µg:	800 [800-1,200]		
associated with an LABA or other controller medication		800 [800-1,600]	
Measured adherence rate > 80%	9 (47.4)	22 (59.5)	0.25
Positive skin prick test	19 (100.0)	18 (81.8)	0.05
Peripheral blood eosinophils, %	8.3 [5.8-12.1]	7.4 [4.0-10.5]	0.41
Baseline spirometry parameters			
Pre-BD FEV_1, mL	2.82 [2.13-3.22]	2.57 [1.74-3.11]	0.68
Pre-BD FEV_1, %	86.0 [79.8-97.5]	89.5 [77.5-101.2]	0.49
FEV_1/FVC ratio	82.0 [76.5-86.5]	82.0 [79.15-88.5]	0.73
Post-BD FEV_1, mL	2.88 [2.35-3.6]	2.74 [1.96-3.26]	0.59
Post-BD FEV_1, %	89.0 [85.5-101.5]	94.0 [84-103.5]	0.49

CSA: controlled severe asthma; USA: uncontrolled severe asthma; LABA: long-acting β_2 agonist; ICS: inhaled corticosteroid(s); and BD: bronchodilator. [a]Values expressed as n (%), mean ± SD, or median [IQR], except where otherwise indicated. [b]Dose equivalent to that of budesonide.

not synonymous with activation of a Th2-mediated response.[4,24] This was demonstrated in a study of pediatric patients with severe asthma that was refractory to treatment, in which no significant levels of IL-4, IL-5, or IL-13 were observed in sputum samples or endobronchial biopsy samples.[25] Therefore, it remains unclear which immune mechanism would explain the conversion of eosinophilia into asthma and the severity phenotype. In the present study, we demonstrated that polymorphisms in the *IL10* and *IL17* genes were associated with failure to achieve asthma control and with the bronchodilator response in patients with severe asthma. Poor asthma control was associated with the presence of at least one C allele of the *IL10* rs3024498 polymorphism in patients under regular treatment with high doses of ICS associated with LABAs or other controller medications. It is known that one of the objectives of asthma treatment is to achieve symptom control with the lowest possible dose of inhaled medication.

On the basis of the data provided by the GTEx Project, individuals with at least one C allele of the *IL10* rs3024498 polymorphism show increased expression of IL-10 in the blood. Although we were unable to measure IL-10 levels in our population, Rogers et al.[26] showed higher IL-10 levels in children with uncontrolled asthma than in those with controlled asthma. The variant form of this gene likely contributes to this disparity.[26]

In view of the fact that high doses of ICS have major adverse effects, the knowledge that a lack of asthma control might be associated with the rs3024498 polymorphism in the *IL10* gene could be used in order to guide medical management, thus minimizing the risks associated with the continuous use of ICS and progressive increases in the dosage.

To our knowledge, there have been no studies associating the *IL10* rs3024498 polymorphism with failure to control pediatric asthma. However, this polymorphism has been reported to be associated with various other clinical conditions accompanied by an exuberant inflammatory process, such as systemic lupus erythematosus, rheumatoid arthritis, and chronic hepatitis.[27,28] In addition, other *IL10* polymorphisms (rs1800896, rs1800871, rs302109, rs1800872, and rs3024491) have been associated with pediatric asthma.[7,29]

In assessing the bronchodilator response, we found that patients with the AA homozygous genotype of rs3819024 (*IL17A*) were more likely to respond to the acute stimulation of an SABA than were those with the AG or GG genotype. Although we evaluated the response to an SABA, it should be borne in mind that

Table 2. Characteristics of the polymorphisms studied.

SNP	Gene	Chromosome	Position	A1/A2[a]	MAF	Functional annotation	RegulomeDB	HaploReg
rs3024498	*IL10*	1	206768184	C/T	0.1429	3'-untranslated variant	1f	Enhancer histone marks/ Dnase
rs3819024	*IL17A*	6	52185988	G/A	0.2987	2-kb upstream variant	1f	Enhancer histone marks
rs2275913	*IL17A*	6	52186235	A/G	0.2208	2-kb upstream variant	1f	Enhancer histone marks

SNP: single nucleotide polymorphism; and MAF: minor allele frequency. [a]The first is the alternative allele, and the second is the reference allele (1/2).

Table 3. Association of *IL10* and *IL17* gene variants with asthma control[a] in children and adolescents with severe asthma.[b]

Variant	Genotype	Group		Model	OR	95% CI	p	PPerm
		CSA (n = 19)	USA (n = 37)					
rs3024498 (*IL10*)	TT	17 (89.5)	26 (70.3)	Allelic	4.2	0.90-19.55	0.08	0.12
	CT	2 (10.5)	8 (21.6)	Additive	5.9	0.95-36.84	0.05	0.03
	CC	0 (0.0)	3 (8.1)	Dominant	6.9	1.003-47.9	0.04	0.02
rs3819024 (*IL17*)	AA	9 (47.4)	18 (48.6)	Allelic	0.8	0.38-1.99	0.83	0.92
	AG	7 (36.8)	15 (40.5)	Additive	0.6	0.27-1.56	0.33	0.70
	GG	3 (15.8)	4 (10.8)	Dominant	0.6	0.18-2.12	0.44	0.57
rs2275913 (*IL17*)	GG	13 (68.4)	18 (48.6)	Allelic	2.4	0.88-6.55	0.11	0.11
	GA	6 (31.6)	15 (40.5)	Additive	2.1	0.74-6.41	0.16	0.20
	AA	0 (0.0)	4 (10.8)	Dominant	2.0	0.60-6.82	0.25	0.29

CSA: controlled severe asthma; USA: uncontrolled severe asthma; and PPerm: permutation p value. [a]In accordance with the GINA criteria. [b]Values expressed as n (%), except where otherwise indicated.

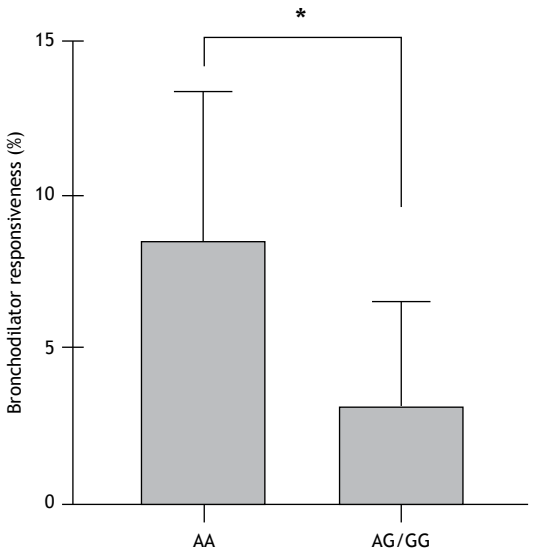

Figure 1. Bronchodilator responsiveness of rs3819024 genotypes. Note: Comparison of median percent change in percent predicted FEV_1 after bronchodilator use, by rs3819024 genotype. Asthma patients with the AG or GG genotype showed less bronchodilator responsiveness than did those with the AA genotype ($p < 0.05$; Mann-Whitney test).

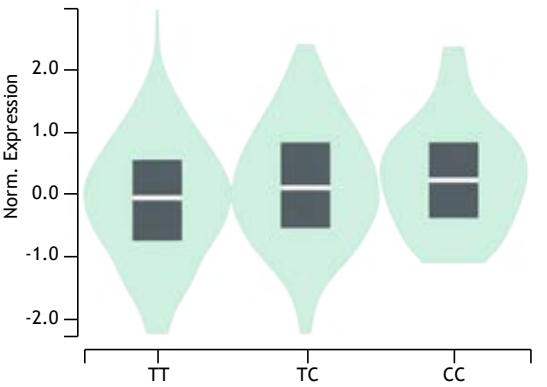

Figure 2. IL-10 expression for rs3024498 in whole blood samples, as assessed by the U.S. National Institutes of Health Genotype-Tissue Expression Project. Note: The TC and CC genotypes of rs3024498 have higher expression of IL-10 in whole blood samples than does the TT genotype ($p = 0.000053$).

formoterol, despite being an LABA and recommended in all severe asthma scenarios, has an onset of action similar to that of albuterol.[10] This is consistent with the findings of studies indicating that IL-17A acts directly on the airway smooth muscle, increasing contractility.[4,30] In clinical practice, blocking *IL17* signaling might be an attractive target for treating asthma with a Th17 phenotype.[4]

Although the specific effect of the rs3819024 polymorphism on IL-17A expression remains unknown, it is plausible that this variant may enhance the production

of this cytokine. This assumption is supported by its 2-kb upstream location with high regulatory potential, as indicated by a score of 1f in the RegulomeDB database, with the potential to impact the interaction with histones in the promoter (H3K4me3) and enhancer (H3K4me1) regions of Th17 lymphocytes, as described in HaploReg.

It is noteworthy that poor symptom control is not always attributable to poor adherence to treatment with ICS and LABAs or other controller medications. In our population, the treatment adherence rate was good in the controlled asthma and uncontrolled asthma groups, with no significant difference between the two groups. However, the phenotypic expression depends on the interaction of environmental factors and the genetic predisposition of an individual, assuming that genes do not operate in isolation but rather within their environment (which includes other genes around them), which can modify and even completely reverse their effects.[3] Therefore, given the high cost for society as a whole, studies that generate evidence from phenotyping, endotyping, and genotyping should be encouraged in order to identify the target patients and expend resources more rationally, as has been done in other chronic diseases.

Our study has some limitations. One is the small sample size, which is attributable to the fact that refractory severe asthma is an uncommon phenotype. The sample size calculation was carried out a posteriori with OpenEpi and showed that, for the study outcomes, with a statistical power of 80%, the minimum sample size would be 34 per group.[12] Our patients were selected from a cohort of patients who had a well-established diagnosis of severe asthma and who were under regular long-term follow-up, in which several methods were used in order to quantify adherence, minimize exposure to allergens, and manage comorbidities, thus limiting our sample size. Therefore, there is a need for studies involving larger samples of patients in order to replicate our findings and identify other genes that may explain the lack of disease control in patients with severe asthma. Future studies should also be carried out with the objective of analyzing the immunological profile, including other cytokines, which will help to understand the complex and heterogeneous pathophysiology of severe asthma. Another limitation of the present study is the lack of quantification of IL-10 and IL-17. This was due to the fact that the samples went through thermal variability, which could have denatured the cytokine proteins.

In conclusion, polymorphisms of the *IL10* and *IL17* genes appear to be involved in complex modulation pathways related to a lack of control and bronchodilator response in treatment-refractory severe asthma in children and adolescents. Functional studies should be carried out to characterize the molecular impact of such variants, which could facilitate the implementation of personalized treatment and management of asthma.

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AUTHOR CONTRIBUTIONS

MIRV: investigation, methodology, project administration, and writing of the original draft. LMLBFL and MVNPQ: conceptualization, data curation, formal analysis, investigation, methodology, resources, supervision, writing of the original draft, and reviewing and editing of the manuscript. RSC: conceptualization, formal analysis, investigation, methodology, resources, supervision, writing of the original draft, and reviewing and editing of the manuscript. MBRS, HSS, AO, and CAVF: methodology, formal analysis, writing of the original draft, and reviewing and editing of the manuscript. EMTS: data curation, investigation, methodology, formal analysis, validation, writing of the original draft, and reviewing and editing of the manuscript.

CONFLICTS OF INTEREST

None declared.

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