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Lung function and bronchodilator response are associated with the SNP rs1042714 in *ADRB2* gene

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ABSTRACT. Asthma is a complex respiratory disease and many genes and genetic variants have been investigated for asthma susceptibility, pathogenesis, severity, and response to therapy. We aimed to evaluate whether single nucleotide polymorphism (SNP) rs1042714 of the *ADRB2* gene is associated with clinical variables, laboratory tests, comorbidities, and pulmonary function parameters, including bronchodilator response (BDR) to salbutamol, in female asthma patients from a referral clinic. We analyzed anthropometric data, laboratory test results, clinical and severity data, spirometry results, and allergic skin tests. DNA was analyzed with

allele-specific polymerase chain reaction. We found a significant association between the genotypes of the SNP rs1042714 and the pulmonary function parameters ΔFEV_1 (p = 0.023), FVC (p = 0.012), % reversibility (p = 0.012), and BDR (p = 0.040). Among patients with the CC genotype, we observed lower ΔFEV_1 (4.9; ±4.8), ΔFVC (2.6; ±4.8), % reversibility (8.3; ±9.2), and a higher percentage of negative BDR response (N = 84; 38.7%). These association were also identified in the dominant model (ΔFEV_1: p = 0.023; ΔFVC: p = 0.014; % reversibility: p = 0.018) and in the recessive model (ΔFVC: p = 0.033; % reversibility: p = 0.025; BDR: p = 0.031). Besides, we found an association between the genotypes and the response to cockroach allergens (p = 0.017) in a codominant model. Our study supports the hypothesis that SNP rs1042714 of the *ADRB2* gene is associated with lung function and BDR to salbutamol in women with asthma.

Key words: Asthma; Beta-2-adrenergic receptor; Genetic association; Precision medicine; Therapy response; Short-acting β2-agonists.

INTRODUCTION

Asthma has a heterogeneous clinical presentation and is characterized by chronic inflammation of the airways and hyperresponsiveness (GINA, 2015). It is diagnosed by a history of respiratory symptoms such as dyspnea, chest tightness, cough, and wheezing, which vary over time and in intensity, along with the variable limitation of expiratory airflow (GINA, 2015). Their high prevalence is mainly related to urbanization and affects 235-334 million people worldwide (Fullman et al., 2017). In Brazil, during the decade 2011-20, asthma was responsible for around 24,000 deaths (6-7 deaths/day) and over 1 million hospitalizations, only in public hospitals (DATASUS, 2024).

The etiology of asthma is complex and characterized by the poorly understood interaction between environmental and genetic risk factors. Studies have sought to examine genetic variants associated with asthma in hopes of better understanding the underlying mechanisms of the disease and identifying predictive genetic markers of risk, severity, and therapy response. Asthma genetic architecture has also been studied to elucidate the disparity in asthma morbidity and mortality between genders, which shifts from predominantly male to female around puberty (Mersha et al., 2015).

The beta-2-adrenergic receptor (*ADRB2*) gene, located on 5q32 (Sayers et al., 2024), has been investigated for asthma susceptibility and therapeutic response (Khan et al., 2018). The *ADRB2* consists of a single exon of 2013 nucleotides that encodes a receptor (β2-receptor or β2-AR) containing 413 amino acid residues distributed in seven transmembrane regions (Cho et al., 2005; Toraih et al., 2019; Sayers et al., 2024). The β2-AR is widely expressed in airway smooth muscle (Thakkinstian et al., 2005; Liang et al., 2014; Sood et al., 2018) and plays a role in various physiological responses such as bronchodilation, vasodilation, mucociliary clearance, and anti-inflammatory actions (Toraih et al., 2019). The clinically relevant effect of β2-AR is the relaxation of the smooth muscle of the lower airways and consequent bronchodilation, which can be activated by drugs that are agonists of these receptors (β2A) (Hall et al., 1995; Thakkinstian et al., 2005; de Paiva et al., 2014).

The *ADRB2* gene has been studied in various populations, and out of the 803 Single Nucleotide Variants (SNVs) identified, at least 10 are common variations reported in association with asthma, allergy, or response to β2A (Sayers et al., 2024). Among these, the SNP rs1042714 (c.79G>C, p.Glu27Gln or p.E27Q) results in the change of a glutamic acid residue (Glu; "E") for a glutamine residue (Gln; "Q") at codon 27, due to the substitution of guanine with a cytosine at position 79 of the coding region of the *ADRB2* gene (E [GAA] > Q [CAA]) (Sherry et al., 2001). The SNP rs1042714 is located in the region flanking the receptor binding site, potentially leading to negative regulation and desensitization of β2-AR (Giubergia et al., 2008; Liang et al., 2014; Yang et al., 2019). Therefore, our study tested the hypothesis that this variant is associated with clinical variables and spirometric parameters in patients diagnosed with asthma, including bronchodilator response (BDR) to β2A salbutamol.

MATERIAL AND METHODS

Study participants

After agreeing to participate in the study and signing the Informed Consent Form, patients diagnosed with asthma attending in the asthma reference outpatient clinic at the Hospital Santa Casa de Misericórdia de Vitória (HSCMV), ES, Brazil, were randomly interviewed according to their arrival in outpatient care. Due to the low frequency of men attending and the implications of genetic differences concerning sex (Mersha et al., 2015), only women were included. Patients diagnosed with chronic obstructive pulmonary disease (COPD) were excluded from the study.

Information was obtained on the presence of asthma in childhood, occurrence or absence of symptom remission (period in years without symptoms of asthma), occurrence of refractory asthma, presence of comorbidities such as adverse drug reactions (ADRs), allergic rhinitis, atopy, type 2 diabetes mellitus (DM2), gastroesophageal reflux disease (GERD), obesity and systemic arterial hypertension (SAH). Asthma severity was determined according to the Global Initiative for Asthma (GINA, 2015) in mild, moderate, and severe asthma. Data were obtained through interviews and medical records.

Anthropometry and laboratory analysis

The Body Mass Index (BMI) was calculated by the ratio between weight and height in kg/m^2. The measurements of total and specific Immunoglobulin E (IgE) were obtained in serum by FEIA (fluorescence enzyme immunoassay) using the ImmunoCAP® Phadia 250 system (Thermo Fisher Scientific, Brazil). The manufacturer's recommended reference range for total IgE in adults is less than 200.0 kU/L. The BMI, % of eosinophils and % total IgE were determined based on the averages of the last three measurement records.

Allergy skin tests

Data from biochemical analyses and allergy skin prick tests (SPT) was obtained from the patient's medical records. The SPT was performed, according to the manufacturer's instructions, using FDA Allergenic® (FDA Allergenic; Rio de Janeiro, RJ, BR) cockroach extracts (*Blattella germanica* and *Periplaneta americana*), cat and dog epithelia, fungi (*Alternaria alternata*, *Aspergillus*

fumigatus, and *Cladosporium herbarum*), and dust mites (*Blomia tropicallis*, *Dermatophagoides farinae*, and *D.pteronissynus*).

Lung function tests

Pulmonary function was evaluated by spirometry according to the guidelines of the American Thoracic Society and European Respiratory Society (ATS/ERS) (Stanojevic et al., 2022), using the Koko® spirometer (KoKo PFT; nSpire Health, Longont, CO, USA). The following parameters were obtained: Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC), FEV_1/FVC ratio, and Mean Forced Expiratory Flow in the middle range of FVC, i.e., between 25 and 75% of the FVC curve ($FEF_{25-75\%}$). These parameters were measured before and after using a short-acting β2A (400 mcg of salbutamol) to measure the absolute (Δ) and percentage variation. The data resulted from the averages of the parameters of the spirometries performed in the patients' last three consultations.

The BDR was estimated by the magnitude of reversibility, calculated as a variation in FEV_1 expressed as variability in both absolute volume and percentage of the baseline value, as indicated below:

$$\% \text{ Reversibility} = \frac{FEV_1 \text{ (after drug)} - FEV_1 \text{ (before drug)}}{FEV_1 \text{ (before drug)}} \times 100$$

A positive BDR was considered percentage reversibility ≥ 12% and ≥ 200 mL in absolute value (GINA, 2015).

DNA isolation and quantification

DNA was isolated from 3mL samples of whole blood using the "salting out" technique (Miller et al., 1988) or Puregene Blood Kit Qiagen® (Qiagen Inc., Redwood City, CA, USA) according to the manufacturer's instructions. DNA quantification was performed using a NanoDrop 1000® UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). A concentration of 100ng was used for genotyping.

Genotyping of the rs1042714

The analysis of the rs1042714 of the *ADRB2* gene was performed by allele-specific polymerase chain reaction (PCR) (Amplification-Refractory Mutation System, ARMS) (de Paiva et al., 2014). Two PCR reactions were performed per sample using the common primer: 5'-AGGCCCATGACCAGATCAGCACAGGCCAG-3'; the allele-specific primer C: 5'-GCCATGCGCCGGACCACGACGTCACGCATC-3'; and the allele-specific primer G: 5'-GCCATGCGCCGGACCACGACGTCACGCAAG-3'.

PCR reactions were performed with positive and negative controls. In each 10 **µ**L reaction, the following were used: 2.5 mM of each dNTP, 5.0 nM of $MgCl_2$, 0.4 U of Taq polymerase, 0.2 pmol of each primer, and 100 ng of genomic DNA. The amplification conditions were: 94 °C (5 min); 35 cycles of 94 °C (1 min.), 67 °C (1 min.); 72 °C (1 min.); 72 °C (10 min.). Amplified products underwent 12% polyacrylamide gel electrophoresis, followed by staining with 2% silver nitrate ($AgNO_3$).

STATISTICAL ANALYSIS

The continuous variables were described as absolute numbers, means, medians, or percentages when more appropriate. The normality of the distribution was assessed using the Kolmogorov-Smirnov test. The Hardy-Weinberg equilibrium (HWE) was assessed by allele and genotype counting using the chi-square test (χ^2). The association of genotypes and inheritance models with continuous variables following a normal distribution was tested using ANOVA for three genotypic classes, and the T-test was applied to genetic models with two categories, respectively. The Kruskal-Wallis and Mann-Whitney U tests were used for variables without normal distribution. When more appropriate, the χ^2 or Fisher tests were used to evaluate the association between genotypes and categorical variables. A value of $p<0.05$ was considered significant. Statistical analyses were performed using *SPSS*® software, version 25.0.

ETHICS

This study was approved by the Research Ethics Committee of the Escola Superior de Ciências da Santa Casa de Misericórdia (EMESCAM), under number 148/2010.

RESULTS

Characterization of participants

The study analyzed data from 227 women, with a median age of 51 years (range 17-78 years). The anthropometric and laboratory data of the participants are described in Table 1, and clinical data is in Table 2.

Of the total patients, 133 (58.6%) reported onset of asthma in childhood, and in 94 (41.4%), the first crisis occurred in adulthood; 138 (61.1%) patients did not present asthma remission. According to the severity of asthma, the distribution of phenotypes was: 97 (42.7%) patients with severe asthma, 89 (39.2%) moderate, and 41 (18.1%) mild (Table 2).

ADRs were reported by 72 (31.7%) patients, rhinitis by 186 (81.9%), and atopy by 128 (73.6%). Among the non-allergic comorbidities, the most common was GERD (53.7%). Of the

Table 1. Demographic, anthropometric and laboratory analysis data of the study group.

Demographic and anthropometeric data	Mean (SD)	Median	Range (Min-Max)
Age	50,0 (1,0)	51	61 (17-78)
Age onset	16,5 (1,4)	7	71 (0-71)
BMI	30,6 (0,5)	29,4	41,31 (16,16-57,48)
Laboratory analysis			
Eosinophils (%)	4,6 (0,31)	3,0	25,99 (0,00-25,99)
Total IgE (kU/L)	413,5 (46,1)	195,2	2995,03 (4,97-3000)

SD: standard deviation; BMI (body mass index) reference values: underweight (<18.5), eutrophic (18.5-24.9), overweight (25-29.9), and obesity (≥ 30.0) (21); Reference values of total immunoglobulin E (IgE) as standardized by the manufacturer, for above 16 years and adults: < 200.0 kU/L.

Table 2. Clinical data of the study group.

Asthma severity	N (%)	
Mild	41 (18,1)	
Moderate	89 (39,2)	
Severe	97 (42,7)	
Disease characteristics	**Yes (%)**	**No (%)**
Asthma remission	88 (38,9)	138 (61,1)
Bronchodilator response (BDR)	64 (29,5)	153 (70,5)
Childhood asthma	133 (58,6)	94 (41,4)
Refractory asthma	41 (18,1)	186 (81,9)
Comorbidities	**Yes (%)**	**No (%)**
Adverse drug reactions (ADRs)	72 (31,7)	155 (68,3)
Allergic rhinitis	186 (81,9)	41 (18,1)
Atopy	128 (73,6)	46 (26,4)
Diabetes mellitus 2 (DM2)	42 (18,5)	185 (81,5)
Gastroesophageal reflux disease (GERD)	122 (53,7)	105 (46,3)
Obesity	106 (46,9)	120 (53,1)
Systemic arterial hypertension (SAH)	108 (47,6)	119 (52,4)
Allergy skin tests	**Positive (%)**	**Negative (%)**
Prick test (at least one allergen)	95 (60,5)	62 (39,5)
Cat epithelium	6 (3,9)	149 (96,1)
Cockroach	23 (14,7)	133 (85,3)
Dog epithelium	13 (8,4)	142 (91,6)
Fungus (any of the 3 *spp.*)	13 (8,3)	143 (91,7)
Alternaria alternata	8 (3,5)	148 (94,9)
Aspergillus fumigatus	8 (3,5)	148 (94,9)
Cladosporium herbarum	3 (1,9)	153 (98,1)
Mites (any of the 3 *spp.*)	91 (58,3)	65 (41,7)
Blomia tropicallis	76 (48,7)	80 (51,3)
Dermatophagoides farinae	76 (48,7)	80 (51,3)
Dermatophagoide pteronissynus	66 (42,3)	90 (57,7)

Fungus: corresponds to an allergic reaction to any of the three species of fungus tested. Mist: corresponds to an allergic reaction to any of the three species of mites tested.

total number of patients with allergic tests available (N = 157), 95 (60.5%) showed a response to one of the allergens tested in the SPT, with *B.tropicallis* and *D.farinae* antigens being responsible for the highest number of sensitizations (N = 76; 48.7%) (Table 2).

Lung function tests

The parameters of the pulmonary function tests (FEV_1, FVC, FEV_1/FVC, and $FEF_{25-75\%}$) before and after using β2A salbutamol, along with their respective Δ, are presented in Table 3. Positive BDR was observed in 64 patients (29.5%) in the study (Table 2).

Table 3. Pulmonary function test data of the study group.

Spirometry parameters	Mean (SD)	Median	Range (Min-Max)
Pre FEV_1	64,8 (1,4)	67,2	79,70 (24,00-103,70)
Pre FVC	77,5 (1,2)	78,4	78,00 (36,30-114,30)
Pre FEV_1/FVC	81,1 (1,0)	83,2	71,70 (34,00- 05,70)
Pre $FEF_{25-75\%}$	45,8 (1,8)	42,0	102,70 (10,00-112,70)
Post FEV_1	70,1 (1,5)	72,6	86,40 (26,30-112,70)
Post FVC	80,5 (1,2)	82,9	86,30 (37,00-123,30)
Post FEV_1/FVC	84,5 (1,0)	86,2	65,00 (43,00- 108,00)
Post $FEF_{25-75\%}$	54,9 (2,2)	51,3	126,00 (12,00-138,00)
ΔFEV_1	5,3 (0,4)	5,0	35,70 (-16,70-19,00)
ΔFVC	3,1 (0,4)	3,0	39,40 (-23,00-16,40)
Δ FEV_1/FVC	3,4 (0,3)	3,0	39,00 (-12,00-27,00)
Δ $FEF_{25-75\%}$	9,1 (0,7)	6,7	60,36 (-18,36-42,00)
%Reversibility	9,1 (0,6)	7,8	97,05 (-29,29-67,75)

SD: standard deviation. FEV_1: forced expiratory volume in the 1st second; FVC: forced vital capacity; FEV_1/FVC: the ratio between FEV_1 and FVC; $FEF_{25-75\%}$: mean forced expiratory flow in the intermediate range of FVC; Pre: before use of bronchodilator; Post: after use of bronchodilator; Δ: absolute variation of each parameter. Reversibility: variation in FEV_1 expressed as a percentage of the baseline value (23).

Table 4. Genotype and allele frequency of the SNP rs1042714 in *ABRB2* gene.

Genotype frequency (SNP rs1042714)	N (%)
CC	113 (49,8)
CG	106 (46,7)
GG	8 (3,5)
Allele frequency	
C	332 (0,73)
G	114 (0,27)
Genetic model	
Dominant model	
CC+CG	219 (96,5)
GG	8 (3,5)
Codominant model	
CC+GG	121 (53,3)
CG	106 (46,7)
Recessive model	
CG+GG	114 (50,2)
CC	113 (49,8)

N: number of subjects, except in calculating the frequency of alleles (in this case, it is given as the allele number in the study group); values are shown as number (%).

Table 5. Association of rs1042714 SNP genotypes of the *ADR*B2 gene and laboratory analysis, disease characteristics and pulmonar function tests data of the study group

	Genotypes			*p*	**Dominant model**		*p*	**Codominant model**		*p*	**Recessive model**		*p*
	CC	**CG**	**GG**		**CC+ CG**	**GG**		**CC+GG**	**CG**		**CG+GG**	**CC**	
Laboratory analysis	Mean (SD) N (%)												
Eosinophils (%)	4,8 (±4,6) 105 (52,0)	4,3 (±3,6) 90 (44,5)	9,1 (±12,5) 7 (3,5)	,436	4,5 (±4,1) 195 (96,5)	9,1 (±12,5) 7 (3,5)	,227	5,0 (±5,4) 112 (55,4)	4,3 (±3,6) 90 (44,6)	,517	4,6 (±4,8) 97 (48,0)	4,8 (±4,6) 105 (52,0)	,840
Total IgE (kU/L)	392,2 (±602,1) 103 (51,0)	418,6 (±595,9) 94 (46,5)	257,0 (±196,0) 5 (2,5)	,440	404,8 (±597,8) 197 (97,5)	257,0 (±196,0) 5 (2,5)	,991	386,0 (±589,7) 108 (53,5)	418,6 (±595,9) 94 (46,5)	,204	410,5 (±583,0) 99 (49,0)	392,2 (±602,1) 103 (51,0)	,207
Disease characteristics	N (%)												
Asthma severity	113 (49,8)	106 (46,7)	8 (3,5)		219 (96,5)	8 (3,5)		121 (53,3)	106 (46,7)		114 (50,2)	113 (49,8)	
Mild	22 (9,7)	18 (7,9)	1 (0,4)		40 (17,6)	1 (0,4)		23 (10,1)	18 (7,9)		19 (8,4)	22 (9,7)	
Moderate	40 (17,6)	44 (19,4)	5 (2,2)	,642[a]	84 (37,0)	5 (2,2)	,457[a]	45 (19,8)	44 (19,4)	,812	49 (21,6)	40 (17,6)	,519
Severe	51 (22,5)	44 (19,4)	2 (0,9)		95 (41,9)	2 (0,9)		53 (23,3)	44 (19,4)		46 (20,3)	51 (22,5)	
BDR	107 (49,3)	102 (47,0)	8 (3,7)		209 (96,3)	8 (3,7)		115 (53,0)	102 (47,0)		110 (50,7)	107 (49,3)	
Yes	23 (10,6)	36 (16,6)	5 (2,3)		59 (27,2)	5 (2,3)		28 (12,9)	36 (16,6)		41 (18,9)	23 (10,6)	
No	84 (38,7)	66 (30,4)	3 (1,4)	**,040[a]**	150 (69,1)	3 (1,4)	,130[a]	87 (40,1)	66 (30,4)	,193	69 (31,8)	84 (38,7)	**,031**
Childhood asthma	113 (49,8)	106 (46,7)	8 (3,5)		219 (96,5)	8 (3,5)		121 (53,3)	106 (46,7)		114 (50,2)	113 (49,8)	
Yes	64 (28,2)	67 (29,5)	2 (0,9)		131 (57,7)	2 (0,9)		55 (24,2)	67 (29,5)		69 (30,4)	64 (28,2)	
No	49 (21,6)	39 (17,2)	6 (2,6)	,086[a]	88 (38,8)	6 (2,6)	,069	66 (29,1)	39 (17,2)	,224	45 (19,8)	49 (21,6)	,591
Refractory asthma	113 (49,8)	106 (46,7)	8 (3,5)		219 (96,5)	8 (3,5)		121 (53,3)	106 (46,7)		114 (50,2)	113 (49,8)	
Yes	18 (7,9)	22 (9,7)	1 (0,4)		40 (17,6)	1 (0,4)		19 (8,4)	22 (9,7)		23 (10,1)	18 (7,9)	
No	95 (41,9)	84 (37,0)	7 (3,1)	,664[a]	179 (78,9)	7 (3,1)	1,00[a]	102 (44,9)	84 (37,0)	,388	91 (40,1)	95 (41,9)	,491
Remission	113 (50,0)	105 (46,5)	8 (3,5)		218 (96,5)	8 (3,5)		121 (53,5)	105 (46,5)		113 (50,0)	113 (50,0)	
Yes	42 (18,6)	43 (19,0)	5 (2,2)		85 (37,6)	5 (2,2)		45 (19,9)	43 (19,0)		46 (20,3)	42 (18,6)	
No	71 (31,4)	62 (27,4)	3 (1,3)	,872[a]	133 (58,8)	3 (1,3)	1,00[a]	76 (33,6)	62 (27,4)	,494[a]	67 (29,6)	71 (31,4)	,634[a]
Pulmonar function tests	Mean (SD) N (%)												
Pre FEV_1	65,2 (±19,4) 108 (49,3)	63,3 (±19,6) 103 (47,0)	62,5 (±23,6) 8 (3,7)	,718	64,3 (±19,5) 211 (96,3)	62,5 (±23,6) 8 (3,7)	,682	65,0 (±19,7) 116 (53,0)	63,3 (±19,6) 103 (47,0)	,537	63,2 (±19,8) 111 (50,7)	65,2 (±19,4) 108 (49,3)	,441
Pre FVC	77,7 (±15,9) 106 (48,8)	76,2 (±16,9) 103 (47,5)	79,1 (±24,6) 8 (3,7)	,751	77,0 (±16,4) 209 (96,3)	79,1 (±24,6) 8 (3,7)	,723	77,8 (±16,6) 114 (52,5)	76,2 (±16,9) 103 (47,5)	,469	76,4 (±17,4) 111 (51,2)	77,7 (±15,9) 106 (48,8)	,556
Pre FEV_1/FVC	81,0 (±13,0) 106 (48,8)	80,7 (±13,9) 103 (47,5)	78,3 (±7,6) 8 (3,7)	,600	80,9 (±13,4) 209 (96,3)	78,3 (±7,6) 8 (3,7)	,325	80,9 (±12,7) 114 (52,5)	80,7 (±13,9) 103 (47,5)	,968	80,5 (±13,5) 111 (51,2)	81,0 (±13,0) 106 (48,8)	,681
Pre $FEF_{25-75\%}$	45,8 (±25,2) 107 (49,1)	44,9 (±25,3) 103 (47,2)	38,6 (±20,4) 8 (3,7)	,679	45,4 (±25,2) 210 (96,3)	38,6 (±20,4) 8 (3,7)	,465	45,4 (±24,9) 115 (52,8)	44,9 (±25,3) 103 (47,2)	,728	44,5 (±25,0) 111 (50,9)	45,8 (±25,2) 107 (49,1)	,534
Post FEV_1	69,8 (±19,8) 107 (49,3)	68,6 (±20,2) 102 (47,0)	71,5 (±26,0) 8 (3,7)	,919	69,2 (±20,0) 209 (96,3)	71,5 (±26,1) 8 (3,7)	,993	69,9 (±20,1) 115 (53,0)	68,6 (±20,2) 102 (47,0)	,687	68,8 (±20,6) 110 (50,7)	69,8 (±19,8) 107 (49,3)	,685

Post FVC	80,3 (±15,8) 105 (48,8)	79,6 (±16,4) 102 (47,4)	85,5 (±24,6) 8 (3,7)	,623	80,0 (±16,1) 207 (96,3)	85,5 (±24,6) 8 (3,7)	,354	80,7 (±16,5) 113 (52,6)	79,6 (±16,4) 102 (47,4)	,642	80,0 (±17,0) 110 (51,2)	80,3 (±15,8) 105 (48,8)	,910
Post FEV_1/FVC	83,9 (±13,2) 105 (48,8)	84,0 (±14,4) 102 (47,4)	82,4 (±9,0) 8 (3,7)	,802	84,0 (±13,8) 207 (96,3)	82,4 (±9,0) 8 (3,7)	,513	83,8 (±12,9) 113 (52,6)	84,0 (±14,4) 102 (47,4)	,813	83,9 (±14,0) 110 (51,2)	83,9 (±13,2) 105 (48,8)	,991
Post $FEF_{25-75\%}$	52,7 (±29,4) 106 (49,1)	53,5 (±30,1) 102 (47,2)	48,5 (±26,9) 8 (3,7)	,943	53,1 (±29,7) 208 (96,3)	48,5 (±26,9) 8 (3,7)	,740	52,4 (±29,1) 114 (52,8)	53,5 (±30,1) 102 (47,2)	,881	53,1 (±29,8) 110 (50,9)	52,7 (±29,4) 106 (49,1)	,981
ΔFEV_1	4,9 (±4,8) 107 (49,3)	5,4 (±5,2) 102 (47,0)	8,9 (±4,7) 8 (3,7)	**,023**	5,2 (±5,0) 209 (96,3)	8,9 (±4,7) 8 (3,7)	**,023**	5,2 (±4,8) 115 (53,0)	5,4 (±5,2) 102 (47,0)	,276	5,7 (±5,3) 110 (50,7)	4,9 (±4,8) 107 (49,3)	,052
ΔFVC	2,6 (±4,8) 105 (48,8)	3,4 (±4,9) 102 (47,4)	6,4 (±2,9) 8 (3,7)	**,012**	3,0 (±4,9) 207 (96,3)	6,4 (±2,9) 8 (3,7)	**,014**	2,9 (±4,8) 113 (52,6)	3,4 (±4,9) 102 (47,4)	,230	3,6 (±4,9) 110 (51,2)	2,6 (±4,8) 105 (48,8)	**,033**
ΔFEV1/FVC	3,0 (±4,3) 105 (49,3)	3,4 (±3,2) 102 (47,0)	4,1 (2,7) 8 (3,7)	,400	3,2 (±3,8) 207 (96,3)	4,1 (±2,7) 8 (3,7)	,330	3,1(±4,2) 113 (52,6)	3,4 (±3,2) 102 (47,4)	,458	3,4 (±3,1) 110 (51,2)	3,0 (±4,3) 105 (48,8)	,267
$\Delta FEF_{25-75\%}$	7,6 (±8,9) 106 (49,1)	9,0 (±8,6) 102 (47,2)	9,9 (±7,6) 8 (3,7)	,270	8,3 (±8,8) 208 (96,3)	9,9 (±7,6) 8 (3,7)	,403	7,8 (±8,8) 114 (52,8)	9,0 (±8,6) 102 (47,2)	,228	9,1 (±8,5) 110 (50,9)	7,6 (±8,9) 106 (49,1)	,128
% Reversibility	8,3 (±9,2) 107 (49,3)	9,5 (±9,6) 102 (47,0)	14,6 (±6,5) 8 (3,7)	**,012**	8,9 (±9,4) 209 (96,3)	14,6 (±6,5) 8 (3,7)	**,018**	8,8 (±9,1) 115 (53,0)	9,5 (±9,6) 102 (47,0)	,176	9,8 (±9,5) 110 (50,7)	8,3 (±9,2) 107 (49,3)	**,025**

SD: standard deviation. BDR: bronchodilator response; FEV_1: forced expiratory volume in the 1st second; FVC: forced vital capacity; FEV_1/FVC: the ratio between FEV_1 and FVC; $FEF_{25-75\%}$: mean forced expiratory flow in the intermediate range of FVC; Pre: before use of bronchodilator; Post: after use of bronchodilator; Δ: absolute variation of each parameter. Reversibility: variation in FEV_1 expressed as a percentage of the baseline value (23). [a]Result by Fisher's exact test.

Genotypes of the SNP rs1042714 and asthma phenotypes

The genotypic distribution for the SNP rs1042714 was 113 (49.8%) CC, 106 (46.7%) CG, and 8 GG (3.5%) and is not in HWE (χ^2 = 8.03; p = 0.0046) (Table 4). No significant association was found between the genotypes in the different inheritance models (dominant, codominant, and recessive) and the phenotypes of asthma severity, clinical characteristics, and laboratory test results (Table 5) or with the presence of comorbidities (ADRs, allergic rhinitis, atopy, DM2, GERD, obesity and SAH) (Supplementary table).

Regarding the SPT, a significant association was found between the distribution of genotypes (CC, CG, and GG) and the response to cat (p = 0.019) and dog epithelium allergens (p = 0.014). Associations were also observed in the dominant model (CC+CG x GG) with cat (p = 0.042) and dog epithelium (0.021) and in the codominant model (CC+GG x CG) with the response to cockroach allergens (p = 0.017) (Supplementary table).

In pulmonary function tests, a significant association was found between the genotypes of the SNP rs1042714 and the following parameters: ΔFEV_1 (p = 0.023), ΔFVC (p = 0.012), and % reversibility (p = 0.012). Patients with genotype CC showed lower FEV_1 (4.9; ±4.8), ΔFVC (2.6; ±4.8), and % reversibility (8.3; ±9.2) compared to patients with genotype GG (ΔFEV_1: 8.9; ±4.7; FVC: 6.4; ±2.9; % reversibility: 14.6; ±6.5) (Table 5). The association was also found in the dominant model with these three pulmonary function test parameters: ΔFEV_1 (p = 0.023), ΔFVC (p = 0.014), and % reversibility (p = 0.018). In the recessive model, the association was found for ΔFVC (p = 0.033) and % reversibility (p = 0.025) (Figure 1).

A higher percentage of negative BDR was found among patients with genotype CC (N = 84; 38.7%) compared to patients with genotypes CG (N = 66; 30.4%) and GG (N = 3; 1.4%) (p = 0.040). In the recessive model, an association with BDR was also observed, showing a higher percentage of patients with negative bronchodilator response among those with genotype CC (N = 84; 38.7%) compared to CG+GG (N = 69; 31.8%) (p = 0.031) (Table 5). The remaining lung function parameters are not associated with the genotypes or genetic models analyzed.

DISCUSSION

We investigated the association between the SNP rs1042714 of the *ADRB2* gene and clinical variables of women with asthma attended in a reference outpatient clinic, including disease severity, lung function parameters, and comorbidities. Our main finding was that the CC genotype was associated with a reduced response to the bronchodilator salbutamol.

Asthma is a heterogeneous disease, and the age of symptom onset is a critical factor in identifying different phenotypes. In our study, more than half of the patients exhibited asthma symptoms during childhood, with the first crisis occurring around the age of 7y. The decline in lung function in early-onset adult asthma tends to be more pronounced than in late-onset adult asthma. A recent study that explored *UK Biobank* data reported prevalences of childhood-onset adult asthma of approximately 25% (Pividori et al., 2019) for both sexes, while in a *Genome-Wide Association Study* (GWAS), 77% of patients (mean age 35 years) reported this early onset of asthma (Nieuwenhuis et al., 2016).

In our study over 60% of the patients did not refer to asthma remission, while the severe phenotype was predominant. The prevalence of asthma remission varies widely in previous

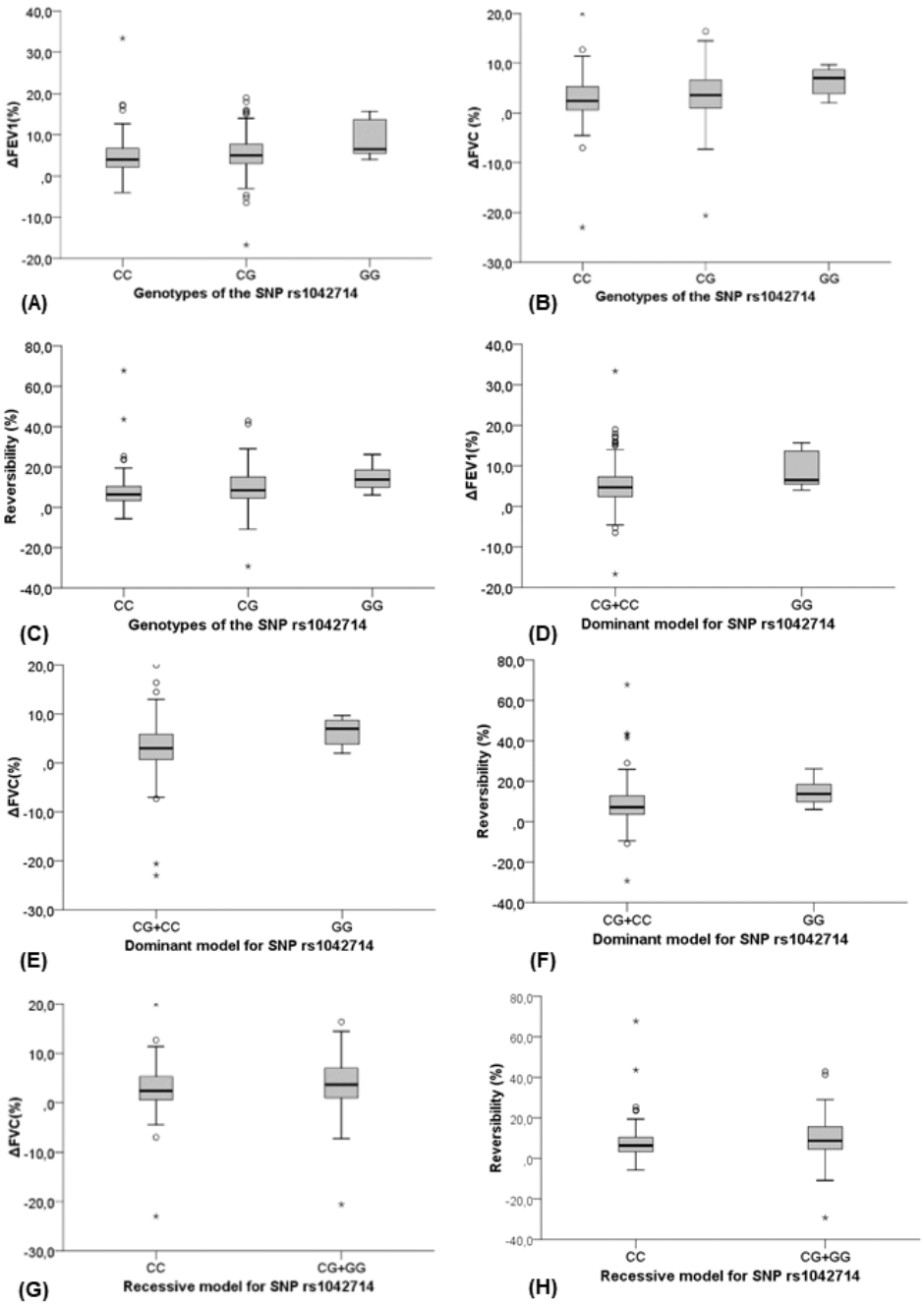

Figure 1. Significative associations between SNP rs1042714 and pulmonary function tests: **(A)** genotypes of the SNP rs1042714 and ΔFEV_1 (p = 0.023); **(B)** genotypes of the SNP rs1042714 and ΔFVC (p = 0.012); **(C)** genotypes of the SNP rs1042714 and % reversibility (p = 0.012); **(D)** Dominant model and ΔFEV_1 (p = 0.023); **(E)** Dominant model and ΔFVC (p = 0.014); **(F)** Dominant model and % reversibility (p = 0.018); **(G)** Recessive model and ΔFVC (p = 0.033); **(H)** Recessive model and %reversibility (p = 0.025).

studies, ranging from 2% to 52% (Carpaij et al., 2019). Evidence supports the inverse association between remission, female gender, and asthma severity (Andersson et al., 2013). Our findings, in an exclusively female cohort from a specialized service, confirm this trend, with lower remission and a higher prevalence of severe asthma.

Comorbidities are determinants in the prognosis and control of asthma (GINA et al., 2015), leading to increased healthcare requests and decreased quality of life. Rhinitis was the most common comorbidity, accounting for ~80%, as observed in other studies (Peters, 2007), highlighting the possible common etiology of both diseases. Indeed, respiratory comorbidities are estimated to occur five times more frequently in asthma patients compared to those without asthma (Su et al., 2016). The least common comorbidity was DM2, despite its association with increased asthma incidence and worsened clinical outcomes and morbidity (Su et al., 2016). Roughly half of the patients have GERD, obesity, and SAH, all recognized as risk factors for asthma and potential causal associations (Su et al., 2016).

To date, according to data extracted from HuGE Navigator (Yu et al., 2008), at least 1554 genes have been reported in studies focused on asthma. Genetic variants have been investigated as risk factors for asthma and as determinants of response to disease control therapies. *ADRB2* is the most studied gene in asthma (Yu et al., 2008), with a focus on the SNPs rs1042713 and rs1042714. The SNP rs1042714 has been associated with bronchial reactivity (Hall, 1995), a risk factor for development (de Paiva et al., 2014), control (Almomani et al., 2019) and severity of asthma (de Paiva et al., 2014; Mohamed-Hussein et al., 2018), the frequency of exacerbations (Sood et al., 2018), response to bronchodilators or therapy (Cho et al., 2005; Giubergia et al., 2008; Martin et al., 2008; Mohamed-Hussein et al., 2018;), wheezing in atopic individuals (Kim et al., 2002), and serum IgE levels (Dewar et al.,1997).

We did not find an association between the rs1042714 genotypes under different inheritance models, asthma severity phenotypes, disease characteristics (onset age, remission, childhood asthma, and refractory asthma), and laboratory tests (% of eosinophils and total IgE) or comorbidities. These results are consistent with other studies (Holloway et al., 2000; Kim et al., 2002; Birbian et al, 2012; Toraih et al., 2019; Zheng et al., 2023).

The association of the SNP rs1042714 and clinical variables in asthma is still controversial, especially when comparing studies from different population samples, age groups, ethnicities, and clinical parameters. In a study with an Arab cohort, predominantly composed of women (~77%), the C allele was found to be associated with uncontrolled asthma (Almomani et al., 2019). MOHAMED-HUSSEIN et al. (2018) reported the association of SNPs rs1042713 and rs1042714 with asthma severity. This contrast is also observed in meta-analysis studies with similar (Cho et al., 2005; Contopoulos-Ioannidis et al., 2005) and different results (Thakkinstian et al., 2005; Liang et al., 2014) from our findings. Thus, evaluation of each population is crucial, especially for precision medicine purposes.

An association, apparently not described in the literature, was found between SNP genotypes rs1042714 (also in the dominant model) and response to cat and dog epithelial allergens. We also found an association with the response to cockroach allergens in the codominant model. Although these data are promising, the findings should be explored in future studies addressing the role of SNP rs1042714 in immediate hypersensitivity, including to other allergens used in SPT.

Pulmonary function tests are essential in evaluating patients with respiratory symptoms (GINA, 2015; Stanojevic et al.,2022). Typically, asthma causes intermittent airway obstruction,

which is most often reversible spontaneously or with the use of rescue inhaled bronchodilators (GINA, 2015), such as salbutamol, a short-acting β2A (*Short-acting beta-agonist*, SABA). Due to their immediate action, SABAs are drugs used to induce bronchodilation during asthma exacerbations, however, it is estimated that ~60% of the interindividual variation in the response to salbutamol may be attributable to genetic factors (Yang et al., 2018).

We found association of the SNP rs1042714 with four parameters of the pulmonary function test (BDR, ΔFEV_1, ΔFVC, and % reversibility), thus supporting the hypothesis of association of this polymorphism with the patient's response to the β2A salbutamol. The CC genotype was associated with a lower response to the bronchodilator salbutamol, which agrees with previous studies. Since the 1990s, the G allele (p.27Glu) has been identified as a protective factor against receptor desensitization to β2A bronchodilators (Hall, 1995) and as an important factor in reducing receptor downregulation after prolonged exposure to β2A (Giubergia et al., 2008). This allele has also been associated with a better response to β2A in cohorts of different age groups and ethnicities, such as in children in Korea (Cho et al., 2005), Argentina (Giubergia et al., 2008), and Australia (Martin et al., 2008).

Variants that alter the function of β2-receptors could increase the risk of disease or reduce the response to endogenous and inhaled β2-agonists in asthma (Hall, 1995). The glutamic acid residue (Glu27) in the N-terminal region alters the network of electrostatic interactions of this region with the binding site (Bhosale et al., 2019), and, thus, the receptor with the Glu27 variant tends to bind better to β2A than the receptor with the Gln27 variant (Hall, 1995). Therefore, the pharmacogenetic evaluation of β2A and BDR have as the main target the *ADRB2* gene (Bhosale et al., 2019).

Other studies point in the opposite direction to our results. A case-control study in an adult and predominantly female cohort found an association between SNP rs1042714 and the bronchodilator response to β2A drugs (Mohamed-Hussein et al., 2018). However, the G allele was more frequent in patients who respond poorly to bronchodilators, and the C allele was more frequent in patients who respond well to the use of these medications (Mohamed-Hussein et al., 2018). The association between the SNP rs1042714 and BDR to salbutamol was not found in studies in adults from southern India (Shah et al., 2015) and a recent meta-analysis (Hikino et al., 2021). Also, no association of the SNP rs1042714 and BDR was found in studies that used other β2A drugs, such as fenoterol (Scaparrotta et al., 2019) and salmeterol (Bleecker et al., 2006), or even when bronchoprovocation was performed with a bronchoconstrictor drug like methacholine (Toraih et al., 2019).

The hypotheses proposed to explain the contradictory results between the present study and some previous studies are differences in study design (phenotypic classification and selection), ethnic compositions, number of study participants, population-specific gene-environment, and gene-gene interactions. It is important to emphasize that the analyses of our study involved a cohort composed only of women with a heterogeneous age distribution. However, the patients are from the same socioeconomic background and have similar access to healthcare services.

Regarding asthma's complex genetic basis, researchers have explored the impact of various genetic polymorphisms, including those in the *ADRB2* gene, in developing polygenic risk scores (PRS). PRS are tools used to predict an individual's genetic susceptibility to a particular disease or prognosis based on the cumulative effect of multiple genetic variants (Sordillo et al., 2021). Previous studies have developed PRSs for asthma, but as far as we know, there is no clinically validated PRS.

Despite being one of the main chronic lung diseases, there are currently no specific laboratory tests for the diagnosis or prognosis of asthma. Association studies using genetic variants

functionally characterized as rs1042714 can lead to biotechnological innovations that will allow the implementation of new prognostic and therapeutic guidelines to propose personalized treatments. Therefore, the adverse effects of treatments on patients can be reduced, and more efficient and targeted use of resources can be enabled.

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DISCLOSURE

The authors report no conflicts of interest.

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