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Role of *THRB*, *ARG1*, and *ADRB2* Genetic Variants on Bronchodilators Response in Asthmatic Children

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Abstract

Background: An interindividual variability in response to short-acting bronchodilator drugs (short-acting inhaled β2-agonists, SABA) exists and this is linked in part to genetic factors. The aim of this study was to verify the influence of single nucleotide polymorphisms (SNPs) of a previously studied gene (*ADRB2*) and of new candidate genes (*THRB* and *ARG1*) on the acute response to SABA in children with asthma.
Methods: One hundred asthmatic children (mean age 9.6 ± 3.0 years, 77 boys) underwent allergological and lung function evaluations. Spirometry was performed before and after bronchodilation test (BD test). The *ADRB2* region containing the Arg16Gly (rs1042713) and Gln27Glu (rs1042714) variants were amplified by polymerase chain reaction, whereas *ARG1* rs2781659 (A>G) and *THRB* rs892940 (G>A) SNPs were genotyped by high-resolution melting (HRM) analysis.
Results: Seventy-seven percent of children developed asthma in the first 6 years of life. Allergic sensitization was observed in 92% (total immunoglobulin G: $529.8 \pm 477.$ kU/L). All patients exhibited respiratory allergy: 43% has multiple respiratory, 22% to single respiratory, and 27% multiple respiratory and food allergies. Fifty four percent children showed positive BD response (forced expiratory volume in 1 second [FEV1] > 12%). Presence of Arg/Gly or Gly/Gly genotypes in position 16 of *ADRB2* was significantly associated to a worse BD response (post-BD FEV1: $108.68\% \pm 15.62\%$ in Arg/Arg vs. $101.86\% \pm 14.03\%$ in Arg/Gly or Gly/Gly patients, $p = 0.02$). No significant association was found between spirometric parameters before and after BD for the other three examined SNPs.
Conclusion: The influence of genetic variability on responsiveness to drugs could become a key parameter to optimize a tailored therapy for young patients with asthma, especially if drug-resistance occurs.

Keywords: asthma, bronchodilator response, children, genetic variants, pharmacogenetics

Introduction

ASTHMA IS THE MOST COMMON CHRONIC DISEASE OF CHILDHOOD, with onset occurring in ~50% of patients in early childhood.[1,2] It is a heterogeneous disease characterized by chronic airway inflammation and respiratory symptoms, such as wheezing, shortness of breath, chest tightness, and cough, associated with variable expiratory airflow limitation.[1]

Despite the availability of several classes of drugs for the treatment of asthma, β2-agonists still represent the first-line therapy for acute bronchoconstriction and for prevention of exercise-induced bronchospasm. Inhaled selective β2-agonists are the most extensively used drugs for the acute relief of asthma symptoms.[3] Short-acting inhaled β2-agonists (SABA) are used by all asthmatic patients such as rescue bronchodilator medications to treat acute bronchoconstrictive symptoms, whereas long-acting β2 agonists

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(LABA) are administered in combination with inhaled corticosteroids (ICS) to provide prolonged bronchodilation and control of asthma symptoms.[4]

However, many patients with asthma do not respond to this class of drugs; in addition, a wide interindividual variability in pharmacological response exists, likely because of the interaction between clinical, environmental, and genetic factors.[1]

Recently, pharmacogenetic studies have identified several single nucleotide polymorphisms (SNPs) that seem to play an important role in treatment response in patients with asthma. In particular, some investigators have reported a significant association between two genetic variants within the β2-adrenergic receptor (*ADRB2*) gene (Arg16Gly and Gln27Glu) and bronchodilators (BD) response in children and adults with asthma,[5–11] although this association has not been confirmed in all studies.[12–19]

ADRB2 is a small intronless gene located on chromosome 5q31–q32,[20] a region genetically linked to asthma and its phenotypes.[21,22] Originally, nine polymorphisms within the coding region of *ADRB2* were described, four of which (Gly16Arg, Gln27Glu, Val34Met, and Thr164Ile) lead to nonsynonymous changes in the amino acid sequence.[23] *In vitro* studies have shown that *ADRB2* gene variants involving amino acid substitutions at positions 16 and 27 of the receptor sequence result in conformational changes leading to downregulation and desensitization in response to agonist stimulation.[6,24,25]

The association between *ADRB2* variants and response to inhaled β2-agonists has been controversial, and discordant findings have been reported so far.[26] Some authors have found that the Arg/Arg genotype at position 16 is associated with a favorable response to SABA, whereas Arg/Gly and Gly/Gly genotypes are associated to a poor response.[5–11] However, other studies reported opposite results or were unable to show any association.[12–19] Similar results were obtained when studying polymorphisms at position 27 of the *ADRB2* or combinations of the two sites (haplotypes).[26]

Because the response to inhaled β2-agonists in patients with asthma is a complex phenotype, more genes are likely involved.[27] Among them, the arginase 1 gene (*ARG1*) seems to be a potential novel bronchodilator response gene, although only a few studies examined the possible association between this gene and BD response in patients with asthma.[27] *ARG1* maps to chromosome 6q23 and encodes one isoform of the enzyme arginase that metabolizes L-arginine. L-Arginine homeostasis is involved in the regulation of airway function, because the availability of this amino acid to nitric oxide synthase (NOS) determines the production of the endogenous bronchodilator nitric oxide (NO).[28]

Another possible candidate is represented by the *THRB* gene, located on chromosome 3p24.2, encoding for the β subunit of the thyroid hormone receptor, which is one of two genes (α and β) encoding for several isoforms.[29] The thyroid hormone receptor is located in the nucleus and upon binding to the thyroid hormone regulates (both repressing and activating) transcription through binding to T3 response elements either as a homodimer or heterodimer with retinoid X receptor β. Thyroid hormones have been implicated in the growth and development of the lung and other organs in pre- and postnatal stages.[30,31] Genetic variants in *THRB* may affect the expression of this receptor and have widespread downstream effects on transcription regulation that may contribute to inflammation, constriction of the bronchial smooth muscle, and obstruction of the airways.

Emerging data on new SNPs of *ARG1* and *THRB* genes need to be confirmed by further studies.

The aim of this study was to assess a potential effect of polymorphisms of the two novel candidate genes *ARG1* and *THRB* and of the most studied *ADRB2* gene on acute BD response and their association with clinical parameters, such as asthma severity and comorbidities in children with mild-to-moderate persistent asthma.

Materials and Methods

Study population

This is a preliminary observational prospective study including 100 asthmatic children, with a mean age (±SD) of 9.6 ± 3.0 years (77 boys and 23 girls).

Patients were recruited from the Allergy and Respiratory Unit, Pediatric Department, Department of Medicine and Aging Sciences of ''G. d'Annunzio'' University of Chieti-Pescara, Italy, between February and April 2017. Genetic analysis was conducted at the Laboratory of Molecular Genetics, Department of Psychological, Health and Territorial Sciences of the ''G. d'Annunzio'' University of Chieti-Pescara.

Inclusion criteria were as follows: age between 6 and 17 years; Caucasian ethnicity; diagnosis of asthma according to most recent Global Initiative for Asthma (GINA) guidelines[1]; being diagnosed with asthma for at least 1 year and available follow-up clinical data and collection of at least three spirometries, performed at separate visits; ICS therapy with fluticasone dipropionate.

Exclusion criteria were as follows: congenital abnormalities and/or malformations; bronchopulmonary dysplasia and/or chronic lung diseases different from asthma; chronic systemic and inflammatory diseases; neoplastic disorders (past or current); chemotherapy or radiotherapy; HIV, and B and C hepatitis.

Preterm infants born at a gestational age <36 weeks and children with a weight at birth <2.5 kg were excluded to remove the potential effect of early factors that could affect future lung function, such as bronchopulmonary dysplasia.[32]

The ethical committee of the University of Chieti approved the study (N° 03/2017) that was performed in accordance with the Declaration of Helsinki (1964); written informed consent was obtained from all parents and all children.

Lung function evaluation

Spirometry was performed in accordance with the European Respiratory Society (ERS) recommendations for the standardization of spirometry.

The main parameters measured were as follows[33]: forced expiratory volume in 1 second (FEV1; normal value >80%); forced vital capacity (FVC; normal value >80%); peak expiratory flow (normal value >80%); forced expiratory flow at 25%–75% (FEF25–75; normal value >70%); FEV1/FVC% ratio (normal value >83%–85%).

The BD test is essential in the evaluation of bronchoreversibility: four separate doses of 100 mg of Fenoterol were

given by metered dose inhaler using a spacer; spirometry was repeated after a 15-minute delay.[34] An increase in FEV1 > 12% from baseline was considered a positive BD response, whereas increases <12% were considered a negative BD response.[1]

For all children at least three spirometries before and after BD test were collected.

Allergological evaluation

Allergological evaluation included the following[35,36]:

- SPT (Allergopharma) for the most important outdoor and indoor inhalants (grasses, parietaria, olive, ragweed, poplar, cypress, alternaria, cat, dog, Aspergillus, Dermatophagoides pteronyssinus) and food (cow's milk, wheat, egg, tomato, cod, peanut, cocoa, hazelnut, soy); a mean wheal diameter >3 mm larger than the negative control was considered as a positive reaction.
- Total immunoglobulin E (IgE) were considered high based on the age-specific reference value of ImmunoCAP Total IgE (Phadia).
- Specific IgE (ImmunoCAP; Phadia, AB, Uppsala, Sweden) against the most important outdoor and indoor inhalants (grasses, parietaria, birch, olive, ragweed, poplar, cypress, alternaria, cat, dog, Aspergillus, Dermatophagoides pteronyssinus and Dermatophagoides farinae) and foods (cow's milk, wheat, egg, tomato, cod, peanut, cocoa). Specific IgE were scored according to the RadioAllergoSorbent Test (RAST) rating: RAST rating 1 (0.35–0.69 kUA/L); RAST rating 2 (0.70–3.49 kUA/L); RAST rating 3 (3.50–17.49 kUA/L); RAST rating 4 (17.50–49.99 kUA/L); RAST rating 5 (50.0–100.00 kUA/L); RAST rating 6 (>100 kUA/L). IgE were considered positive at a level of 0.35 kUA/L (class I or RAST rating 1) or higher.

Genotyping

Genomic DNA was isolated from peripheral blood lymphocytes using standard methods, and quantified by measuring ultraviolet absorption using a spectrophotometer.

Specific regions of the ADRB2 gene containing the Arg16Gly (rs1042713) and Gln27Glu (rs1042714) variants were amplified by polymerase chain reaction (PCR) in 25 μL reaction volume containing 50 ng of genomic DNA in AB Applied Biosystems 2720 thermal cycler (Applied Biosystems, Foster City, CA), using the KAPA Taq DNA polymerase (Resnova, Genzano, Italy). Primers were designed using Primer3 software. PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 35 cycles of 95°C for 30 seconds, 62°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. The amplification products were submitted to direct sequencing procedure using BigDye Term v3.1 CycleSeq Kit (Life Technologies, Monza, Italy) followed by automatic sequencing analysis (ABI PRISM 3130XL).

The SNPs rs2781659 (A>G) in *ARG1* gene and rs892940 (G>A) in *THRB* gene were genotyped by high-resolution melting (HRM) analysis. HRM was performed on 96-well PikoReal Real-Time PCR System (Thermo Scientific™) using the Luminaris Color HRM Master Mix (Thermo Scientific) according to the manufacturer's instructions, as previously described.[37] Each sample was run in triplicate. Genotypes of the samples were assigned by comparing the melting patterns with that of reference genotypes. To evaluate the sensitivity of HRM, some of the results were randomly confirmed by direct sequencing.

Statistical analysis

Data are expressed as mean ± SD unless otherwise stated. Categorical variables are reported as percentages and tested by the χ^2 test. Linear regression analyses was performed to assess, under a dominant genetic model, the association of each SNP with respiratory parameters. Adjustments for age, sex, and body mass index were made. Student's *t*-test and analysis of covariance were used to compare between group differences. For each investigated locus, Hardy–Weinberg equilibrium was calculated.

Statistical analyses were performed with SPSS, version 22.0 (SPSS, Inc., Chicago, IL). Values of $p < 0.05$ were considered statistically significant.

Results

The study population included 100 patients with a mean age of 9.6 ± 3.0 years (77% of the study population were boys, reflecting the known higher prevalence of asthma in male subjects aged 2–13 years).[38]

All children were born at gestational age >36 weeks (38.7 ± 2.1 weeks), with a mean birth weight of 3.21 ± 0.56 kg.

All 100 patients included in the study were affected by mild-to-moderate persistent asthma at the time of the recruitment, with a mean number of asthma episodes of 5.3 ± 3.6 per year. Seventy-seven percent of children developed asthma symptoms in the first 6 years of life, with a mean age of 3.41 ± 2.51 years at the first episode. Forty-one percent of patients showed a positive family history in a first-degree relative for asthma. Family history for allergy was found in 66% of children.

The allergological evaluation showed allergic sensitization in most of the study population (92%), with eosinophil counts in peripheral blood ≥4% as high total IgE levels (529.8 ± 477.5 kU/L). In particular, all patients exhibited respiratory allergy, of which 43% has multiple respiratory allergies; 22% single respiratory allergy and 27% multiple respiratory and food allergies. The most relevant respiratory allergens were Dermatophagoides pteronyssinus (89%), grasses, and olive (47%); less frequently, sensitization against dog (34%), cat (30%), parietaria (29%), cypress (17%), alternaria (8%), and Aspergillus (7%). The most common food allergies were peanuts (85%), hazelnut (41%), tomato (66%), and soy (41%), whereas allergy against wheat and egg white (30%), cow's milk (18%), yolk (11%), and cod (4%) were less common.

The most common comorbidities shown by patients included rhinitis (95%), mainly persistent rhinitis (51%); atopic dermatitis (54%), which was still active in 31% of the study population; urticaria, and/or anaphylaxis (31%).

The genotypes distribution of the SNPs for the three genes are reported in Table 1.

Positive BD response (FEV1 > 12%) was observed in 54% children of children.

Table 1. Genotype Distribution of the Single Nucleotide Polymorphisms in *ADRB2*, *ARG1*, and *THRB* Genes

	Genetic model				
	Codominant model			*Dominant model*	
ADRB2 (Arg16Gly)	GG	GA	AA	GA+AA	GG
	45	47	8	55	45
ADRB2 (Gln27Glu)	CC	CG	GG	CG+GG	CC
	47	33	20	53	47
ARG1	AA	AG	GG	AG+GG	AA
rs2781659 (A>G)	43	40	17	57	43
THRB	GG	GA	AA	GA+AA	GG
rs892940 (G>A)	43	42	15	57	43

Lung function spirometric parameters before and after BD test are summarized in Table 2.

Linear regression analyses (Table 3) and Student's *t*-test (Table 4) were performed to assess, under a dominant genetic model, the association of each SNP with respiratory parameters.

Presence of Arg/Gly or Gly/Gly genotypes in position 16 of *ADRB2* gene was significantly associated to lower basal FEV1 ($91.22\% \pm 14.34\%$ vs. $98.34\% \pm 12.42\%$, $p = 0.01$) and to worse post-BD FEV1 ($101.86\% \pm 14.03\%$ vs. 108.68 ± 15.62, $p = 0.02$), respectively, in Arg/Gly or Gly/Gly patients versus Arg/Arg children (Tables 3, 4 and Fig. 1).

No significant association was found between spirometric parameters before and after BD for the other three examined SNPs or between any of the four SNPs and clinical parameters examined (comorbidities, family history for asthma and allergy, and age of the first wheezing episode).

All the investigated genotype frequencies were within the Hardy–Weinberg equilibrium (χ^2 test $p > 0.05$), except for *ADRB2* rs1042714 (C>G).

Discussion

This study aimed to investigate the potential genetic influence on a poor response to SABA medication in children with mild-to-moderate persistent asthma.

Recently, pharmacogenetic studies have identified several SNPs that may influence the poor BD response (acute and chronic) observed in a relevant percentage of asthmatic patients. In this study, we evaluated the influence of four SNPs, two within the most studied gene *ADRB2* and one each of the two novel candidate genes *ARG1* and *THRB*, on poor bronchial responsiveness in a group of asthmatic children. Some of these SNPs, such as *ADRB2* SNPs, were already previously explored, whereas only a few data are available on others, such as *THRB* and *ARG1*. Thus, we performed a preliminary study to evaluate on a small sample the usefulness of the investigations on these latter genes in BD response, to be further analyzed in larger samples.

ADRB2 Arg16Gly and Gln27Glu were present in 55% and 53% of our study population, respectively. Similar frequencies have been previously reported in asthmatic patients and healthy subjects,[39] suggesting they are unlikely directly involved in the pathogenesis of asthma. Nevertheless, a possible

Table 2. Spirometry Parameters Before and After Bronchodilator Test

	FEV1 (%)	*FVC (%)*	*FEV1/FVC*	*PEF (%)*	*FEF 75 (%)*	*FEF 50 (%)*	*FEF 25–75 (%)*	*Δ FEV1 (%)*	*↑FEV1 (mL)*	*Δ FVC (%)*	*Δ FEF 75 (%)*
Baseline spirometry parameters											
Mean	92.89	93.91	92.75	81.22	77.05	79.93	81.69				78.89
SD	15.84	14.91	81.9	16.95	31.27	23.56	19.726				22.80
Post-BD spirometry parameters											
	FEV1 (%)	*FVC (%)*	*FEV1/FVC*	*PEF (%)*	*FEF 75 (%)*	*FEF 50 (%)*	*FEF 25 (%)*	*FEF 25–75 (%)*	*Δ FEV1 (%)*	*↑FEV1 (mL)*	*Δ FVC (%)*	*Δ FEF 75 (%)*
Mean	104.93	98.21	90.24	91.57	106.84	101.46	96.36	100.93	13.05	216.36	5.89	47.61
SD	15.08	13.32	6.38	19.27	36.21	25.27	20.07	24.97	9.89	170.96	9.45	40.62

FEV1, forced expiratory volume in 1 second; PEF, peak expiratory flow; FVC, forced vital capacity; FEF25–75, forced expiratory flow at 25%–75%; Δ, delta; ↑, increase.

Table 3. Differences in Spirometry Parameters Before and After BD Test in Relation to ADRB2, THRB, and ARG1 SNPs (Linear Regression, Adjusted for Age, Sex, and BMI)

SNPs	*Dependent variable*	*Regression coefficient β*	p *value*
ADRB2 Arg16Gly	Baseline FEV1 (%)	–0.203	0.05
	Baseline FEV1/FVC	–0.090	0.39
	Baseline FEF 25–75 (%)	–0.029	0.78
	FEV1 post-BD (%)	**–0.244**	**0.02**
	FEV1/FVC post-BD (%)	–0.049	0.63
	Δ FEV1 post-TBD (%)	–0.064	0.53
	↑FEV1 (mL) post-TBD	–0.028	0.79
	FEV1% [1]	**–0.229**	**0.03**
	FEV1% [2]	**–0.232**	**0.03**
ADRB2 Gln27Glu	Baseline FEV1 (%)	0.051	0.62
	Baseline FEV1/FVC	–0.104	0.30
	Baseline FEF 25–75 (%)	0.063	0.53
	FEV1 post-BD (%)	0.122	0.23
	FEV1/FVC post-BD (%)	0.009	0.93
	Δ FEV1 post-TBD (%)	–0.040	0.69
	↑FEV1 (mL) post-TBD	0.003	0.97
	FEV1% [1]	0.008	0.94
	FEV1% [2]	0.076	0.47
THRB	Baseline FEV1 (%)	–0.003	0.98
	Baseline FEV1/FVC	0.080	0.43
	Baseline FEF 25–75 (%)	–0.131	0.19
	FEV1 post-BD (%)	–0.021	0.83
	FEV1/FVC post-BD (%)	–0.025	0.8
	Δ FEV1 post-TBD (%)	0.046	0.64
	↑FEV1 (mL) post-TBD	0.107	0.29
	FEV1% [1]	0.003	0.97
	FEV1% [2]	–0.007	0.95
ARG1	Baseline FEV1 (%)	–0.17	0.09
	Baseline FEV1/FVC	0.079	0.43
	Baseline FEF 25–75 (%)	–0.111	0.26
	FEV1 post-BD (%)	–0.153	0.13
	FEV1/FVC post-BD (%)	0.042	0.67
	Δ FEV1 post-TBD (%)	0.004	0.96
	↑FEV1 (mL) post-TBD	0.053	0.6
	FEV1% [1]	–0.036	0.73
	FEV1% [2]	–0.128	0.23

FEV1 [1] and [2] are patients' value of basal FEV1 at two other different visits.
BD, bronchodilator; SNP, single nucleotide polymorphism.
Underscored boldface represents statistically significant results. Δ, delta; ↑, increase.

role of these variants in affecting severity of symptoms and treatment response has been suggested.[40]

The main finding of this study was a significant association between Arg/Gly or Gly/Gly genotypes on position 16 of *ADRB2* and a worse BD response identified by lower value of FEV1 after BD test versus Arg/Arg patients ($p = 0.02$). These results are in accordance with many previous reports,[5–11] although not confirmed in all performed studies.[12–19]

A meta-analysis published in 2009 was conducted to examine the association between *ADRB2* polymorphisms and the response to inhaled β2-adrenergic agonists in children with asthma. Three case–control or family-based studies were included, involving 960 asthmatic children (692 children with negative BD response, defined as <15% improvement in FEV1 and 268 children with positive BD response). The authors observed a significant association between favorable therapeutic response to inhaled β2-adrenergic agonists in asthmatic children and the Arg/Arg phenotype at position 16 of the *ADRB2*, as compared with the Arg/Gly or Gly/Gly phenotypes. In line with our study results, no association was observed between clinical response to β2-agonists and polymorphism at position 27 of the *ADRB2* (odds ratio = 1.04; 95% confidence interval = 0.76–1.42).[26]

In a small study on 16 clinically stable patients with moderate asthma, Lima et al., observed that albuterol-evoked FEV1 was higher and the response was more rapid in Arg16 homozygotes compared with the carriers of the Gly16 variant.[5]

The results of this study on the association between *ADRB2* Gly16 variant and a worse respiratory function in asthmatic children suggest that this SNP may be associated to a worse lung function, leading to worse asthma control and consequently a greater asthma severity.

Up to now, few studies on the potential association between *ADRB2* SNPs and asthma severity or poor asthma control are available and the results are often discordant. For example, in contrast with our data, recently Scichilone et al. demonstrated that also the Arg/Arg genotype is associated

Table 4. Differences in Spirometry Parameters Before and After BD Test in Relation to *ADRB2* Arg16Gly SNPs (T Test)

ADRB2 Arg16Gly	*Number*	*Mean*	*SD*	t *Test* p *Value*	*Adjusted* p *for age, sex, and BMI*
Age (years)					
WT	45	8.75	2.79	***0.009***	
SNPs	55	10.31	2.98		
Birth weight (kg)					
WT	45	3.18	0.56	0.661	
SNPs	55	3.23	0.56		
Gestational age (weeks)					
WT	45	38.62	2.34	0.690	
SNPs	55	38.8	1.99		
BMI (kg/m^2)					
WT	45	18.52	3.46	***0.046***	
SNPs	55	20.2	4.59		
Baseline FEV1 (%)					
WT	45	95.83	15.74	0.093	0.054
SNPs	55	90.49	15.66		
Baseline FEV1/FVC					
WT	45	103.33	121.54	0.244	0.386
SNPs	55	84.09	9.54		
Baseline FEF 25–75 (%)					
WT	45	78.99	22.89	0.966	0.778
SNPs	55	78.80	22.94		
FEV1 post-BD (%)					
WT	45	108.68	15.62	***0.024***	***0.019***
SNPs	55	101.86	14.03		
FEV1/FVC post-BD (%)					
WT	45	90.90	5.37	0.353	0.631
SNPs	55	89.70	7.11		
Δ FEV1 post-TBD (%)					
WT	45	14.25	9.99	0.274	0.535
SNPs	55	12.07	9.79		
↑FEV1 (mL) post-TBD					
WT	45	208.44	127.28	0.676	0.787
SNPs	55	222.96	201.24		
FEV1 [1] (%)					
WT	45	98.34	12.42	**0.015**	**0.035**
SNPs	55	91.22	14.34		
FEV1 [2] (%)					
WT	45	112.50	14.46	**0.019**	**0.034**
SNPs	55	105.59	13.10		

WT = wild type or Arg/Arg; SNPs (Arg/Gly + Gly/Gly). FEV1 [1] and [2] are patients' value of basal FEV1 at two other different visits. Underscored boldface represents statistically significant results. Δ, delta; ↑, increase.

with the occurrence of severe asthma.[41] Rebordosa et al. previously reported that the Arg allele was associated with poorer asthma control, a steeper lung function decline, and airway hyperresponsiveness.[19] However, this study used only clinical criteria of asthma severity (Asthma Control Test[41]), whereas in our study we assessed this allele in relation to lung function over time.

To our knowledge, only Zhang et al. examined a cohort of children with longitudinal respiratory data and reported associations between *ADRB2* SNP haplotypes and lung function, airway hyperresponsiveness and asthma susceptibility. The authors observed an association between the Gly16Gln27 haplotype and higher FEV1 at age 6 and both higher FEV1 and FVC at age 11, and an association between Arg16Gln27 with both lower FEV1 and FVC at age 11.[42] However, this was a prospective study on a general population of children unselected for asthma.

Data on the possible influence of the other *ADRB2* SNP (Gln27Glu) are limited and inconclusive, but the majority of previous investigations[7,11,15,26] did not support its association with bronchoreversibility. Hall et al. even observed lower airway reactivity in asthmatic with Glu 27.[43] On the contrary, it has been suggested that contrasting results about possible influence of *ADRB2* SNPs on BD response may be related to linkage disequilibrium,[15,25,44–46] when considered in isolation polymorphisms that are commonly inherited together as *ADRB2* haplotypes.[26] However, another study based on haplotype rather than SNP analysis gave different

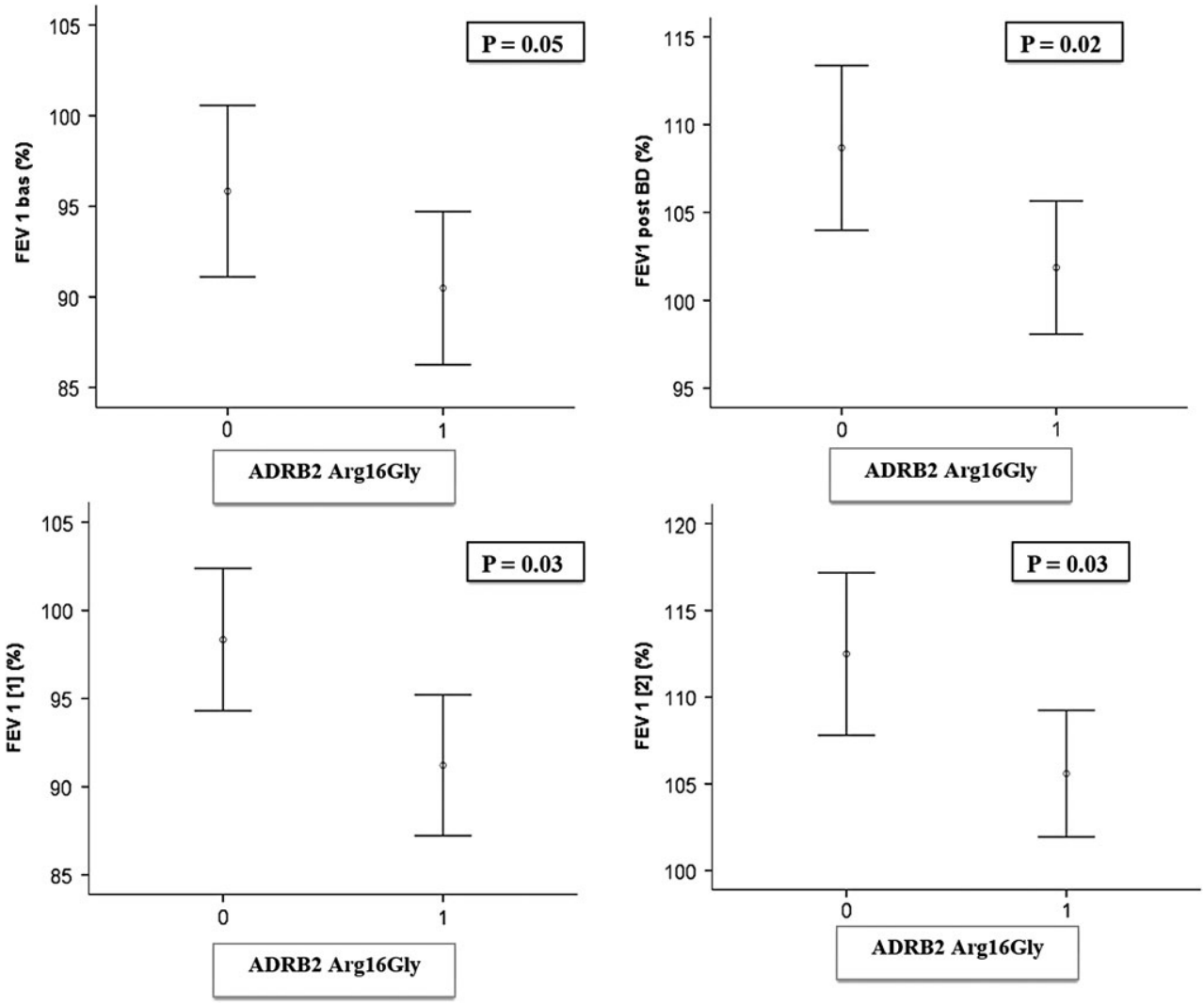

FIG. 1. Differences in FEV1 in relation to presence of *ADRB2* Arg16Gly (0 = wild type; 1 = SNPs). (a) FEV1 before BD test; (b) FEV1 post-BD test; (c, d) FEV1 [1] and [2] are patients' value of basal FEV1 at two other different visits. BD, bronchodilator; FEV1, forced expiratory volume in 1 second; SNP, single nucleotide polymorphism.

results and some other reports did not support the role of these SNPs on BD response in asthmatic patients.[39]

Therefore, contrasting data may also depend on various factors mainly related to different patient populations that include the sample size, the enrollment rates, the age and the disease severity, especially the different ethnic groups studied by various investigators.

On the contrary, factors such as different definition of ''treatment-resistant asthma,'' different treatment regimens and different β2-agonists administered, different routes of administration (inhalations vs. intravenous) and lengths of time (acute vs. chronic use), and different outcome measures to assess drug responsiveness (e.g., FEV1 increase by 15% from baseline, vs. increase by 12%) could explain discordant results across different studies.[26]

In contrast, we did not find any association between spirometric parameters before and after BD and the other two SNPs examined in *ARG1* and *THRB* genes.

SNPs related to these genes were identified after genotyping a great number of SNPs in many candidate genes in children and parents of the CAMP study.[27,47] The association between these variants and acute BD response was screened using a novel algorithm implemented in a family-based association test that ranked SNPs in order of statistical power. Genes that had SNPs with median power in the highest quartile were then taken for replication analyses in three other asthma cohorts.[27]

From this particular strategy, combining evidence for association from the four asthma cohort, SNPs from *ARG1* were significantly associated to BD response.[27] However, in our cohort of asthmatic children with a specific phenotype such as persistent allergic asthma, the potential role of rs2781659 in *ARG1* and rs892940 in *THRB* were not confirmed, so further investigations are needed to clarify their possible influence on BD responsiveness.

It needs to be acknowledged that a main limitation of our study is the sample size. However, this is one of the few

studies performed in a pediatric population, confirming previous results mainly related to adult populations. In addition, our study presented other strengths:

(1) a complete assessment of the patients (clinical, allergological, and lung function evaluation); (2) search of possible influence of four SNPs of three different genes on acute BD response; (3) strict selection of patients used to limit bias; (4) homogeneity of the study population as for other comorbidities such as allergy and rhinitis as well as for therapy, limiting their influence on BD responsiveness; and (5) search of correlation with patients' lung function over time and presence of the examined allele.

In particular, the choice of a pediatric population excluded chronic factors involved in asthma pathogenesis in adult patients such as chronic obstructive pulmonary disease, industrial and workplace exposures, and long-term cigarette smoking that may significantly compromise the response to SABA in acute asthmatic attacks, regardless of the patient's genotype.[26] Moreover, the strict selection of patients implicates that all were affected by mild-to-moderate asthma, that in according with GINA guidelines,[1] require therapy with ICS.

On the contrary, results obtained with regard to the potential role of *ADRB2* are in agreement with previous studies, so we can suppose that our population, although small, is representative of asthmatic pediatric population also for the results obtained about the other SNPs. However, further larger study are now required to confirm our findings.

Conclusions

This study confirms the association between Arg16Gly in *ADRB2* gene and a worse BD response to SABA in children with persistent asthma.

In line with previous studies, our data do not support a role of Gln27Glu in relation to a poor BD response in these patients.

Furthermore, we were unable to confirm a possible influence of *ARG1* and *THRB* on BD response to SABA in our population. Therefore, further investigations are needed to clarify their correlation with a poor bronchoreversibility in asthmatic children.

The influence of genetic variability on drug responsiveness can be a key factor for a better therapeutic management of drug-resistant children, to develop a tailored therapy for each little asthmatic patient.

Author Disclosure Statement

The authors declare there are no competing financial interests.

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