

Asthma Treatment Outcome in Children Is Associated with Vascular Endothelial Growth Factor A (*VEGFA*) Polymorphisms

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Abstract

Background: Asthma is a common chronic disease characterized by airway inflammation and structural remodeling. Vascular endothelial growth factor (VEGF), a major regulator of angiogenesis, is elevated in asthma patients. VEGF contributes to airway responsiveness and remodeling. It has been shown that treatment of asthma patients decreases VEGF levels, and inhibition of VEGF diminishes asthma symptoms in mice. Therefore, polymorphisms in the vascular endothelial growth factor A (*VEGFA*) gene might be associated with asthma treatment response.

Methods: This study enrolled 131 children with asthma treated with different therapies – specifically, the inhaled corticosteroid (ICS) fluticasone propionate or the leukotriene receptor antagonist (LTRA) montelukast. We performed an association analysis between improvement of lung function – assessed by measurement of the percentage of the predicted forced expiratory volume in 1 second (%predicted FEV₁), the ratio between the FEV₁ and the forced vital capacity (FEV₁/FVC) after 6 and 12 months of treatment, and asthma control after 12 months of treatment – and two polymorphisms, rs2146323 and rs833058, in the *VEGFA* gene.

Results: Polymorphism rs2146323 A>C in *VEGFA* was associated with response to ICS therapy. Asthma patients with the AA genotype had a greater improvement in the %predicted FEV₁ than those with the AC or CC genotype (p=0.018). Conversely, the AA genotype in rs2146323 was associated with uncontrolled asthma in patients regularly receiving LTRA therapy (p=0.020) and a worse FEV₁/FVC ratio in patients who episodically used LTRA therapy (p=0.044). Furthermore, polymorphism rs833058 C>T was associated with treatment response to episodically used LTRA therapy. A subgroup of patients with the TT genotype had an improvement in the %predicted FEV₁, compared with no improvement in patients with the CT or CC genotype (p=0.029).

Conclusions: Our results showed that treatment response to commonly used asthma therapies (ICS or LTRA) is associated with polymorphisms rs2146323 and rs833058 in *VEGFA*. With additional replication of this preliminary study, our findings could contribute to the development of individualized asthma therapy.

Background

Asthma is a chronic disease characterized by airway inflammation and structural remodeling.^[1] Recently, a role of angiogenesis in asthma pathology was recognized.^[2,3] Airway biopsies taken from adults and children with asthma have revealed an increase in the number of microvessels,^[4] which contribute to airway narrowing and airflow reduction, as well

as vasodilatation and increased microvascular permeability.^[5] Increased permeability causes plasma proteins to leak into extravascular space, leading to edema and alterations in the extracellular matrix.^[5] Cytokine vascular endothelial growth factor (VEGF) is a major regulator of angiogenesis and vascular permeability.^[6] VEGF promotes asthma pathologic features such as mucus production, collagen deposition, and smooth muscle hyperplasia, as shown in transgenic mouse models.^[7] VEGF

has been recognized as a mediator of vascular remodeling and inflammation; it enhances antigen sensitization and plays a role in T helper type 2-driven immunoresponse.^[8] Increased VEGF levels have been found in induced sputum^[9] and biopsy specimens of bronchial mucosa from asthma patients.^[10,11] VEGF expression has correlated inversely with the forced expiratory volume in 1 second (FEV₁) measurements and positively with airway hyperresponsiveness.^[11] Moreover, inhibition of VEGF diminishes asthma symptoms in mouse models.^[12] Correspondingly, it has been shown that commonly used asthma therapies inhibit VEGF, showing that treatment influences angiogenic remodeling in the airways of asthma patients.^[12]

One of the most effective types of therapies used for persistent asthma treatment are inhaled corticosteroids (ICS). They decrease airway vascularity, abnormal vascular permeability, basal membrane thickness, and airway hyperresponsiveness, and they increase the FEV₁.^[2,13] It has also been shown that ICS treatment inhibits VEGF expression in the airway wall, which might be the cause of ICS-dependent regression of hypervascularity.^[14] An alternative to ICS therapy for managing asthma is use of leukotriene receptor antagonists (LTRA). Evidence suggests leukotriene involvement in angiogenesis, through upregulation of VEGF expression.^[15] Treatment with the LTRA agents montelukast or pranlukast decreases vascular permeability, possibly by inhibiting upregulation of VEGF expression.^[7]

A variable response to asthma treatment is well documented.^[16] Large interindividual variation exists in the treatment response to ICS because 5–10% of patients do not respond to treatment.^[17] Because of the highly repeatable individual treatment response, it is assumed that genetic variations contribute to this phenomenon.^[18] Identifying polymorphisms contributing to asthma treatment response would make it possible to select the most suitable asthma therapy for individual patients on the basis of genotype information.^[18] Given the clear role of VEGF in asthma and asthma treatment, we hypothesized that single nucleotide polymorphisms (SNPs) in the vascular endothelial growth factor A (*VEGFA*) gene, located on 6p12 (chr6:43737921–43754224), might contribute to asthma treatment response. *VEGFA* is highly polymorphic, with at least 50 known SNPs.^[19] For our study, SNPs rs2146323 and rs833058 in *VEGFA* were selected on the basis of the study performed by Sharma et al.,^[20] who showed an association between rs2146323 and lung function improvement when children were treated with ICS therapy. Despite promising results, their study was never replicated.

The aim of this study was to determine whether polymorphisms rs2146323 and rs833058 in *VEGFA* were associated with response to treatment – specifically, with ICS or LTRA therapy – in Slovenian children with asthma.

Methods

Patients

We carried out a prospective study, which enrolled 131 Slovenian children who attended pediatric care at the University Clinical Centre Maribor (Maribor, Slovenia). Children with physician-diagnosed asthma, who first received asthma treatment or were put on a therapy-free period, were included in the study. Their mean age was 12 ± 3 years, and 56% of them were male. They were observed during 12 months of asthma therapy. All children underwent spirometry tests, a broncho-provocation test with methacholine, and measurement of exhaled nitric oxide in expired air. The patients' clinical and laboratory parameters are listed in table I. Patients were divided into three groups, and different therapeutic approaches with two different therapeutic agents (the ICS fluticasone propionate and the LTRA montelukast) were used. The first group comprised 40 children with asthma who received ICS therapy. Children under 7 years received 250 µg/day of ICS therapy, and children older than 7 years received 400 µg/day. The second group comprised 47 children with asthma who received LTRA therapy on a regularly scheduled regimen. Children under 12 years received 5 mg/day, and older ones received 10 mg/day. The third group comprised 44 children who received LTRA therapy episodically. The LTRA was given at the onset of asthma symptoms. Therapy was given at bedtime for a minimum of 7 days or until symptoms had resolved for 48 hours up to a maximum of 20 days.^[21] To assess the improvement of pulmonary function, we determined the %predicted FEV₁ and the ratio between the FEV₁ and the forced vital capacity (FVC). First, we assessed these variables at the beginning of the trial, when patients had gone 1 month without any asthma therapy, to determine baseline pulmonary function. Afterward, to reveal the effect of treatment, we subtracted the %predicted FEV₁ and the FEV₁/FVC ratio after 6 and 12 months from the corresponding values at the onset of the treatment. Furthermore, asthma control was determined after 12 months of therapy. Asthma was uncontrolled when asthma control test (ACT) scores were under 20 at least once in 12 months of follow-up, and asthma was controlled when ACT scores were constantly higher than 20. The study was approved by the national medical ethics committee.

DNA Isolation and Single Nucleotide Polymorphism (SNP) Genotyping

Genomic DNA was extracted from EDTA-containing whole-blood samples, using a QIAamp DNA Blood Mini Kit (Qiagen,

Table 1. Clinical characteristics of patients with asthma

Characteristic	All patients	ICS recipients	LTRA recipients		p-Value ^a
			Regular	Episodic	
Subjects (n)	131	40	47	44	
Age (y; mean $\pm$ SD)	12 $\pm$ 3	12 $\pm$ 3	11 $\pm$ 3	12 $\pm$ 3	
Male sex (n [%])	74 [56]	20 [50]	25 [53]	29 [66]	
PC20 (mg/mL; median [IQR])	0.26 [0.73]	0.14 [0.21]	0.26 [0.62]	0.83 [1.11]	<0.0001
FeNO (ppb; median [IQR])	34 [11]	51 [62]	35 [37]	27 [28]	0.005
%Predicted FEV ₁ (median [IQR])					
At baseline	97 [14]	97 [16]	97 [15]	97 [14]	0.612
Change after 6 months of treatment	1 [10]	3 [11]	0 [11]	-1 [12]	0.459
Change after 12 months of treatment ^b	3 [12]	5 [9]*	0 [15]**	1 [14]***	0.212
FEV ₁ /FVC ratio (median [IQR])					
At baseline	89 [8]	88 [9]	89 [5]	90 [9]	0.173
Change after 6 months of treatment	1 [6]	2 [8]	1 [6]	0 [5]	0.235
Change after 12 months of treatment	1 [7]	0 [10]	1 [8]	0 [7]	0.832
Controlled asthma (n [%])	104 [79]	28 [70]	39 [85]	37 [82]	0.203

a Different treatment approaches (ICS, regular use and episodic use of LTRA) were statistically compared using the Kruskal-Wallis test.

b The change from baseline in the %predicted FEV₁ after 12 months of treatment with each therapy was statistically compared using the paired Wilcoxon test.

FeNO=fractional exhaled nitric oxide; **FEV₁**=forced expiratory volume in 1 second; **FVC**=forced vital capacity; **ICS**=inhaled corticosteroid; **IQR**=interquartile range; **LTRA**=leukotriene receptor antagonist; **PC20**=concentration of methacholine causing a 20% fall in FEV₁; **ppb**=parts per billion; **SD**=standard deviation; *p=0.0002, **p=0.376, ***p=0.290.

Hilden, Germany) according to the manufacturer's instructions. The genotypes of the analyzed SNPs were determined using a 5'-nuclease allelic discrimination assay in a 96-well format. Primers and probes were purchased from Applied Biosystems (Foster City, CA, USA) for SNP genotyping assays for rs2146323 and rs833058 in *VEGFA*. Allelic discrimination assays were performed in duplicate in 10 μ L reaction volumes, using approximately 10 ng of DNA as a template, 2 $\times$ TaqMan Universal PCR Master Mix, and the pre-designed SNP genotyping assays provided by Applied Biosystems. The temperature conditions for the PCR were set at 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and at 60°C for 1 minute. Genotyping of the amplified PCR products was determined on the basis of the differences in VIC and FAM fluorescent levels, using the ABI Prism 7500 sequence detection system (Applied Biosystems) and SDS 1.2 software.

Statistical Analysis

The Hardy-Weinberg equilibrium was tested using the chi-squared test for the goodness-of-fit (one degree of freedom) model. Data distribution was evaluated by the D'Agostino & Pearson test. Parametric statistics (paired and unpaired t-tests)

were used on normally distributed data, and non-parametric statistics (the Mann-Whitney, Wilcoxon, and Kruskal-Wallis tests) were used if the distribution deviated from normal. To determine the treatment success of each therapy, changes from baseline in the %predicted FEV₁ after 12 months of therapy were compared using a paired Wilcoxon test. In addition, changes in the %predicted FEV₁ and the FEV₁/FVC ratio were compared in patients with different genotypes. When two groups were compared, statistical significance was determined using a Mann-Whitney test, and when three groups were compared, the Kruskal-Wallis test was used. Genotypic distribution and allelic frequencies were compared by Fisher's exact test calculated on contingency tables. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated using the same test. We used GraphPad Prism software (version 5.0 for Windows; GraphPad Software, San Diego, CA, USA). A p-value of less than 0.050 was accepted as statistically significant.

Results

This study analyzed improvement in lung function and asthma control in children with asthma who were treated for 12 months. Three different therapeutic approaches were used, and treatment response was observed according to genotypes of

two SNPs, rs2146323 and rs833058, in the *VEGFA* gene. The genotype distributions for both polymorphisms were in Hardy-Weinberg equilibrium. When patients were not stratified according to treatment, genotype frequencies of patients with asthma were AA 16%, AC 44%, and CC 40% in rs2146323; and TT 11%, CT 47%, and CC 41% in rs833058. When changes in lung function after 12 months were analyzed, only patients receiving ICS therapy had a significant improvement in the %predicted FEV₁ ($p=0.0002$). Patients receiving LTRA therapy regularly ($p=0.376$) or episodically ($p=0.290$) did not show improvement in lung function (table I). There were no differences in asthma control after 12 months of treatment between the three therapeutic approaches ($p=0.203$) [table I].

However, further pharmacogenetic analysis of *VEGFA* polymorphisms revealed genotype-dependent variation in lung function improvement and asthma control after treatment with different therapies (tables II–IV). Comparison of lung function after ICS therapy showed an rs2146323 genotype-dependent treatment response. Comparison of the dynamics of the %predicted FEV₁ after 6 and 12 months of ICS therapy revealed that patients with the AA genotype responded to treatment better than patients with the AC or CC genotype (table II and figure 1a). After 6 months of treatment, patients with the AA genotype had a 6% increase in the %predicted FEV₁, whereas patients with the AC or CC genotype had only a 1% improvement in the %predicted FEV₁ ($p=0.045$). After 12 months, the increase in the %predicted FEV₁ was 9% in patients with the AA genotype and 5% in the others ($p=0.018$). Polymorphism rs2146323 was associated with improvement in the %predicted FEV₁ after ICS therapy but not after LTRA therapy, whether patients took it regularly or episodically

(figure 1b and 1c). However, there was an association between polymorphism rs2146323 and asthma control in patients regularly receiving LTRA therapy (table III). Sixty percent of patients with the AA genotype had uncontrolled asthma despite receiving LTRA therapy regularly for 12 months, whereas only 10% of patients with the AC or CC genotype had uncontrolled asthma when receiving the same treatment ($p=0.020$; OR 0.07; 95% CI 0.009, 0.57). No association was found between rs2146323 and asthma control after 12 months of ICS therapy or episodically used LTRA therapy (table III). Furthermore, there was an association between rs833058 in *VEGFA* and patients' response to LTRA therapy (table II). The subgroup of patients with the TT genotype had improvement in the %predicted FEV₁ after 12 months of receiving episodic LTRA therapy, compared with no improvement in patients with the CC or CT genotype (9% vs 1%; $p=0.029$). In addition, there was a weak association between the change in the FEV₁/FVC ratio after 6 months of episodically used LTRA therapy and rs2146323 (table IV), since patients with the AA genotype had a worse FEV₁/FVC ratio, whereas it remained unchanged in patients with the AC or CC genotype ($p=0.044$). This difference did not remain significant after 12 months of treatment ($p=0.459$). There were no other genotype-dependent differences in the FEV₁/FVC ratio (table IV). SNP rs833058 was not associated with asthma control (table III).

Discussion

It is well documented that VEGF is increased in asthma patients and inversely correlates with airway function and hyperreactivity.^[22] It has been shown that VEGF levels are de-

Table II. Changes in the percentage of the predicted forced expiratory volume in 1 second (%predicted FEV₁) after 6 and 12 months of treatment, according to vascular endothelial growth factor A (*VEGFA*) polymorphism genotypes

Treatment	Treatment duration (months)	Change in the %predicted FEV ₁					
		rs2146323 polymorphism			rs833058 polymorphism		
		AA	AC+CC	p-Value ^a	TT	CT+CC	p-Value ^a
ICS (fluticasone propionate)	6	6	1	0.045	-2	5	0.058
	12	9	5	0.018	4	6	0.459
LTRA (montelukast)							
	Regular use						
	6	5	0	0.142	0	0	0.931
	12	-1	0	0.667	6	-1	0.407
	Episodic use						
	6	-6	0	0.222	6	-2	0.131
	12	-6	1	0.460	9	1	0.029

a Changes in the %predicted FEV₁ from baseline were statistically compared between genotypes (AA and AC+CC in rs2146323; TT and CT+CC in rs833058) after 6 and 12 months of treatment, using the Mann-Whitney test.

ICS=inhaled corticosteroid; LTRA=leukotriene receptor antagonist.

Table III. Asthma control after 12 months of treatment, according to vascular endothelial growth factor A (*VEGFA*) polymorphism genotypes

<i>VEGFA</i> polymorphism	Asthma status	Patients (n [%])					
		rs2146323 polymorphism			rs833058 polymorphism		
		AA	AC + CC	p-Value ^a	TT	CT + CC	p-Value ^a
ICS (fluticasone propionate)	Controlled	8 [80]	20 [67]	0.693	2 [33]	25 [76]	0.060
	Uncontrolled	2 [20]	10 [33]		4 [67]	8 [24]	
LTRA (montelukast)	Regular use	2 [40]	37 [90]	0.020 ^b	5 [100]	34 [83]	1.000
		3 [60]	4 [10]		0 [0]	7 [17]	
	Episodic use	5 [83]	32 [82]	1.000	2 [50]	35 [85]	0.140
		1 [17]	7 [18]		2 [50]	6 [15]	

a Asthma control after 12 months of treatment was defined as asthma being controlled (ACT scores >20) or uncontrolled (ACT scores <20). Asthma control was statistically compared between genotypes (AA and AC + CC in rs2146323; TT and CT + CC in rs833058), using Fisher's exact test.

b OR 0.07; 95% CI 0.009, 0.57.

ACT = asthma control test; **CI** = confidence interval; **ICS** = inhaled corticosteroid; **LTRA** = leukotriene receptor antagonist; **OR** = odds ratio.

creased by different asthma therapies, such as ICS^[23] and LTRA.^[7] ICS treatment has an effect on distinct components of airway remodeling: vasodilatation, microvascular permeability, and angiogenesis, all regulated by VEGF.^[24] The role of leukotrienes in VEGF expression has also been reported. When cell cultures were treated with leukotriene LTD₄ or LTE₄, *VEGF* messenger RNA (mRNA) expression increased markedly. In the same experiment, when cell culture was pretreated with an LTRA, *VEGF* expression remained unchanged,^[15] providing evidence of LTRA involvement in angiogenesis. Taken together, clear evidence exists of the involvement of commonly used asthma therapeutics on the VEGF pathway, which makes it a potential target for pharmacogenetic research. One of the goals of pharmacogenetic studies is to identify the most effective therapy on the basis of the patient genotype, which would enable physicians to select the most appropriate therapy for individual patients.^[25] An association between genetic polymorphisms in *VEGFA* and response to ICS or LTRA treatment could be helpful for selecting therapy tailored to each individual. This study assessed the effect of two *VEGFA* polymorphisms on 6- and 12-month asthma therapy outcomes in a Slovenian pediatric population receiving ICS or LTRA therapy. The most evident result of our study was an association between polymorphism rs2146323 in *VEGFA* and the change in the %predicted FEV₁ after 6 and 12 months of ICS treatment in children with asthma. We used the %predicted FEV₁ to measure the treatment response because it has been shown that VEGF levels inversely correlate with FEV₁.^[26] For polymorphisms rs2146323 and rs833058, a recessive genetic model was used because it showed the best fit in our study and it had

already been used for rs2146323 in a previous study.^[19] Patients who were minor allele homozygotes showed a bigger improvement in the %predicted FEV₁, in contrast with combined heterozygotes and major allele homozygotes. Patients with the AA genotype in rs2146323 showed greater improvement in the %predicted FEV₁ compared with the carriers of a C allele (AC or CC genotype) at both time points. On the other hand, 60% of the patients with the AA genotype in rs2146323 had uncontrolled asthma despite regularly receiving LTRA therapy, whereas only 10% of patients with the AC or CC genotype had uncontrolled asthma when receiving the same therapy. Furthermore, patients with the AA genotype had a worse FEV₁/FVC ratio than those with the AC or CC genotype after 6 months of treatment. These data show that ICS treatment of patients with the AA genotype in rs2146323 would be beneficial, whereas LTRA treatment might not be the most appropriate therapy selection. Therapy selection according to genotype could improve treatment success in asthma. Sharma et al.^[20] showed an association of rs2146323 with the post-bronchodilator FEV₁/FVC ratio because, after 4 years of ICS therapy, non-carriers of the A allele had a mean FEV₁/FVC ratio similar to that of untreated patients. These data are consistent with our results confirming a positive correlation between the AA genotype in rs2146323 and ICS treatment success. Furthermore, we found an association between polymorphism rs833058 and the change in the %predicted FEV₁ in patients after episodically receiving LTRA therapy for 12 months. A subgroup of patients with the TT genotype in rs833058 had an improvement in the %predicted FEV₁ when receiving episodic LTRA therapy. The role of polymorphisms analyzed in

the present study is not known. Furthermore, currently available SNP effect-predicting programs, like PolyPhen and SNPeffect, do not provide information about their possible functional role, probably because rs2146323 is located in an intron and rs833058 is in the intergenic region, according to the National Center for Biotechnology Information's dbSNP database. Linkage dis-

equilibrium (LD) analysis, generated using the SNAP web-based tool,^[27] revealed weak LD between analyzed polymorphisms ($r^2=0.214$; $D'=0.897$), which may partly explain the differential association with treatment response.

So far, most of the pharmacogenetic studies on ICS and asthma have focused on analyzing corticosteroid receptors or known targets in the corticosteroid pathway.^[28,29] The fact that VEGF levels are influenced by ICS therapy is supported by numerous data, such as a dexamethasone dosage-dependent decrease in *VEGFA* mRNA expression in human vascular smooth cells^[30] and ICS-decreased VEGF levels in induced sputum.^[11]

Although therapies commonly used for treating asthma symptoms do not target VEGF directly, they reduce its levels and have a positive effect on patients.^[14] However, direct anti-VEGF therapy might be a promising approach for diminishing asthma symptoms.^[31] Our results support the idea that genetic variation significantly contributes to interpatient variability in response to ICS or LTRA therapy. A pharmacogenetic approach is important for identifying therapy non-responders, especially for chronic and common diseases such as asthma.^[18] This study of small groups of patients has preliminarily demonstrated the existence of a *VEGFA* polymorphism-dependent asthma treatment response. To confirm these results, it would be reasonable to replicate the study in larger patient groups, especially with patients who receive ICS therapy.

This preliminary study possesses several limitations that should be avoided in further studies. The number of study subjects was limited and therapy selection was not randomized, although this did not influence the pharmacogenetic part of the research. The %predicted FEV₁ and the FEV₁/FVC ratio were used as primary outcomes, but secondary outcomes such as peak expiratory flow and others should be incorporated into future studies. Asthma patients had %predicted FEV₁ values of nearly 100%, which is why we could not expect major improvements, and a methacholine test should be performed after 1 year of treatment. In any case, a placebo group should be included in the study design. It would also be appropriate to select therapy on the basis of patients' rs2146323 genotype data and to compare the results of treatment with those in a group of patients for whom therapy was not chosen according to genotype.

Conclusions

Response to two commonly used types of asthma therapy (ICS or LTRA) was associated with polymorphisms rs2146323 and rs833058 in *VEGFA*. Genotype AA in rs2146323 was

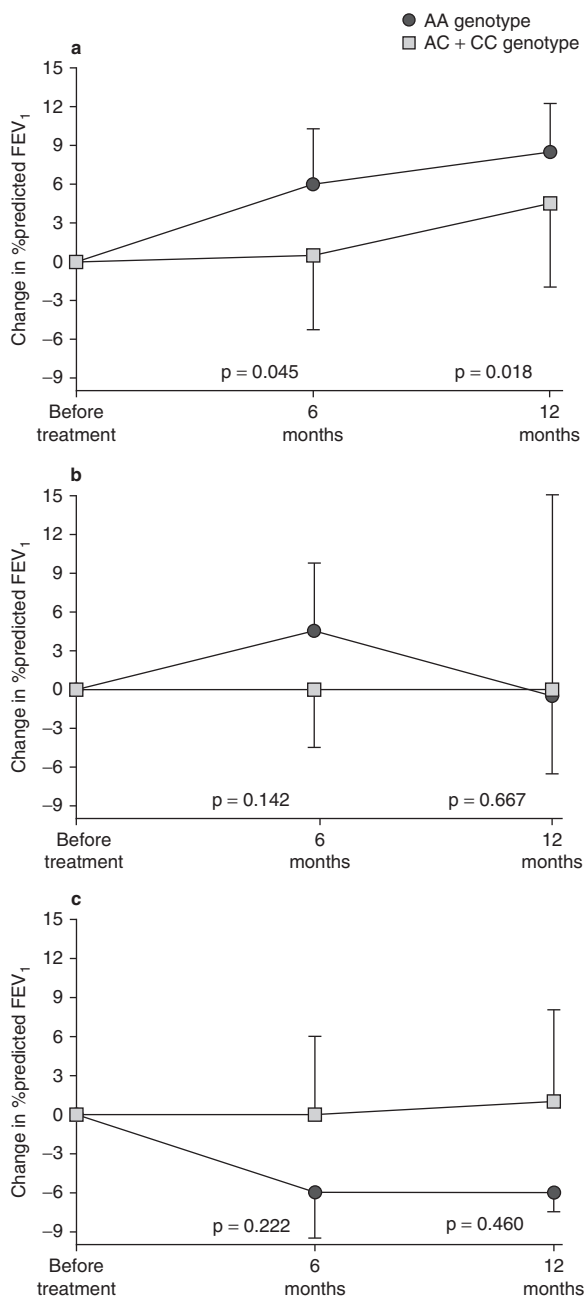


Fig. 1. Changes in the percentage of the predicted forced expiratory volume in 1 second (%predicted FEV₁) after 6 and 12 months of (a) treatment with the inhaled corticosteroid fluticasone propionate, (b) regular use of the leukotriene receptor antagonist montelukast, and (c) episodic use of montelukast, according to the rs2146323 genotype.

Table IV. Changes in the forced expiratory volume in 1 second/forced vital capacity (FEV₁/FVC) ratio after 6 and 12 months of therapy, according to vascular endothelial growth factor A (*VEGFA*) polymorphism genotypes

Treatment	Treatment duration (months)	Change in the FEV ₁ /FVC ratio					
		rs2146323 polymorphism			rs833058 polymorphism		
		AA	AC + CC	p-Value ^a	TT	CT + CC	p-Value ^a
ICS (fluticasone propionate)	6	3	2	0.673	0	2	0.609
	12	1	0	0.766	-3	1	0.544
LTRA (montelukast)							
	Regular use						
	6	3	1	0.713	0	2	0.782
	12	1	1	0.949	3	1	0.557
	Episodic use						
	6	-2	0	0.044	-4	0	0.261
	12	-1	0	0.459	1	0	0.967

a Changes in the FEV₁/FVC ratio from baseline were statistically compared between genotypes (AA and AC + CC in rs2146323; TT and CT + CC in rs833058) after 6 and 12 months of treatment, using the Mann-Whitney test.

ICS = inhaled corticosteroid; LTRA = leukotriene receptor antagonist.

associated with greater improvement in the %predicted FEV₁ when treated with ICS and, conversely, it was associated with a higher degree of uncontrolled asthma and a worse FEV₁/FVC ratio when treated with LTRA. The subgroup of patients with the TT genotype in rs833058 had improvement in the %predicted FEV₁ after 12 months of receiving episodic LTRA therapy, compared with no improvement in patients with the CC or CT genotype. These data show that the choice of therapy could be optimized on the basis of patients' *VEGFA* genotype data. This study needs to be confirmed in a larger sample of patients to validate our results.

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