The Role of SLC22A4 Gene Polymorphisms in the Response to Salbutamol in Asthmatic Patients

Alshymaa A. Ahmed, Nora M. Said, Alia A. El Shahaway and Nagwan A. Ismail

Introduction

Bronchial asthma is one of the most prevalent chronic respiratory disorders affecting around 358 million people worldwide (Soriano et al., 2017). Despite the growing understanding of its pathogenesis and the advances of the management guidelines, inadequate control of asthma still presents a serious problem (Pauwels, 1996), as patients with inadequately controlled asthma often have limited therapeutic options and remain at high risk of serious morbidity and mortality (Chung et al., 1996). The aim of asthma pharmacogenetics is to reduce the asthma burden via utilizing genetic information to personalize asthma pharmacotherapy. Novel gene variants have been identified to influence the response to asthma drugs. Studies to identify novel polymorphisms that associate with response to asthma drugs are still required (Lima et al., 2009).

The milestone drug in relieving asthma symptoms are the inhaled bronchodilators including the adrenergic β2 receptor agonists and the anticholinergic drugs. Many factors underlying the large standard deviation of Bronchodilator Response (BDR), these factors include personal, environmental as well as inherited genetic factors (Tarnoki et al., 2015). Effective and rapid absorption of inhaled bronchodilators across the airway epithelium is a prerequisite to reach their targets in the smooth muscle layer. The majority of the currently used bronchodilators are cationic in the physiologic pH, they cannot diffuse freely through the airway epithelial cell membrane lipid bilayer and so, transporters are required to facilitate the transmission of these organic cations through the cell membrane barriers (Horvath et al., 2006).

A number of polyspecific transporters that facilitate the passage of drugs through the membrane barriers have been identified. These include the Solute Carrier (SLC) transporter family (Horvath et al., 2006). Solute Carrier (SLC) is a superfamily of transporters including over 300 members divided into 52 families. Solute carriers mediate the influx of a diverse range of compounds including both endobiotic and exogenous chemicals.
(Zhou and You, 2007; Koepsell and Endou, 2003). Two main subfamilies of human SLC transporters have been identified, Organic Cation Transporters (OCT, encoded by SLC22A1-22A5 genes) and organic anion transporters (OAT, encoded by SLC22A6-22A11 genes) (Okuda et al., 1996; Kekuda et al., 1998; Gründemann et al., 1997).

Organic Cation Transporters include electrogenic transporters (i.e., OCT1/SLC22A1, OCT2/SLC22A2 and OCT3/SLC22A3) and electroneutral pH dependent transporters named (OCTN1/SLC22A4 and OCTN2/SLC22A5) (Ayrton and Morgan, 2008). Organic cation transporters share a common topological structure that contains twelve transmembrane domains, an intracellular N- and C-terminus with a large extracellular loop between the first and second transmembrane domains (Koepsell and Endou, 2004).

The Organic Cation Transporter (OCTN1) is expressed in different tissues like kidney, muscles and bone marrow cells (Tamai et al., 1997). Initially, it was known as a carnitine transporter, but recently, L-ergothioneine "the xenobiotic amino acid" is considered to be its main physiological substrate (Gründemann et al., 2005). It can transport zwitterions, as well as several organic cationic drugs at both directions (Tamai et al., 1997). Sakamoto et al. (2013) found that OCTN1 was highly expressed in the lung (Sakamoto et al., 2013).

The SLC22A4 gene encodes for the OCTN1 and is located on chromosome 5q31. This chromosome region contains multiple genes involved in immune response (Santiago et al., 2006). SLC22A4 gene polymorphisms have been implicated to be associated with increased incidences of autoimmune and inflammatory disorders such as; rheumatoid arthritis, type 1 diabetes mellitus, inflammatory bowel diseases and bronchial asthma (Santiago et al., 2006; Tekuhiro et al., 2003; Peltekova et al., 2004; Li et al., 2012). In addition; Single Nucleotide Polymorphisms (SNPs) of SLC22A4 have received a great attention in clinical fields as they can modulate the transporter gene expression and functions, causing individual variations in response to drugs (Nakahara et al., 2008; Hou et al., 2015), such as the association of rs460089 and rs1050152 with Imatinib therapy outcome in chronic myeloid leukemia patients (Jaruskova et al., 2017; Angelini et al., 2013).

Intron 1 point mutation rs3792876 T/C (slc2F2) in SLC22A4 gene affects binding of runt-related transcription factor (RUNX1), regulating the transcription of SLC22A4 (Hou et al., 2015). The recessive genotype of slc2F2 showed a positive association with autoimmune diseases including Rheumatoid arthritis, autoimmune thyroiditis and Crohn's disease (Hou et al., 2015; Jaruskova et al., 2017; Angelini et al., 2013; De Ridder et al., 2007). Another polymorphism; rs2073838 A/G (slc2F1) is on intron 2 of SLC22A4 gene (Barton et al., 2005), which also showed a positive association with Rheumatoid arthritis (Hou et al., 2015).

**Research Hypothesis**

Based on the molecular function and the frequent associations of these SNPs with autoimmune diseases we hypothesized that rs3792876 and rs2073838 can affect the absorption of inhaled bronchodilators such as the short-acting β2 Adrenergic receptors agonist (Salbutamol) leading to an impaired response to these drugs.

**Objectives of this Work**

To evaluate the influence of SLC22A4 gene polymorphisms on the response to inhaled bronchodilators as the main asthma reliever.

**The Aim of this Work**

This may participate in improving asthma therapy outcome in non-responding patients due to genetic causes by rapid shifting to other lines of drugs that relieve asthma symptoms and to encourage the invention of structurally different inhaled bronchodilators.

**Methods**

This cross-sectional study was conducted at Clinical Pathology and Chest Departments, Faculty of Medicine, Zagazig University Hospitals in Zagazig city, Al Sharqia Governorate, Egypt, from July 2017 to September 2018. Eligible patients for inclusion were constitutive patients, from both genders, at any age above 7 years old "to be able to participate in pulmonary function tests" and from the same Egyptian ethnicity. Patients were diagnosed to have mild to moderate persistent bronchial asthma and admitted to the chest clinic for follow up and reevaluation. The initial diagnosis of patients was confirmed by revising the medical history of patients including clinical manifestations, examination findings, X-ray films if present and spirometry results including Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV1) and FEV1/FVC ratio. For the diagnosis of asthma and assessing asthma severity; the GINA 2016 criteria was used (http://ginasthma.org/wp-content/uploads/2016/04/GINA-2016-main-report_tracked.pdf). Skin prick test was performed at Allergy and Immunology Unit, Zagazig University, under the supervision of the third author. Positive and negative controls were included to validate the prick test.

Patients not on regular treatment, having other complications like bronchiectasis or chest infections and patients who refused or unable to give informed written consent for participation were excluded from the study. Patients with intermittent and severe asthma were excluded as the former usually seek medical advice at the primary care units during attacks and are lost from follow up for several months (Shahidi and Fitzgerald,
2010), also, it is difficult to assess BDR in stable asthmatic patients whom there is no resting bronchoconstriction (Koskela et al., 2006), the later were excluded as they are usually resistant to inhaled bronchodilators and already require high doses of inhaled and systemic corticosteroids (Poon et al., 2012).

**Data Collection**

Personal, family, past, medical histories and history of atopy were taken from each patient after giving written informed consent. In addition, data from patients' records were collected.

**Bronchodilator Reversibility Testing**

Each patient was assessed by measuring the changes of FEV1 in response to inhaled Salbutamol, patients were asked to stop long and short acting β2 agonists 12 and 6 hours, respectively and to stop smoking at least one hour, before the assessment. A nose clip was used and the spirometric maneuvers were performed in the standing position. A minimum of 3 maneuvers was done, with a minimum exhalation time of 6 seconds per maneuver, unless there was an obvious plateau (i.e., no volume change) for at least 1 second, or the subject could not exhale further. The paper printout of the volume-time and flow-volume displays were subjected to the acceptability criteria of the American Thoracic Society (ATS); FEV1, FEV1/FVC ratio, post-bronchodilator FEV1 were recorded for each patient. A response was considered positive when a change in FEV1 ≥ 12% and 200 mL after administration of one to two buffs (200-400μg) of salbutamol (Larocca et al., 2013). The test was performed by qualified staff members in the chest clinic under the supervision of the fourth author. Patients were classified as Responders (positive BDR) and Non-responders (negative BDR).

**Genotyping**

SLC22A4 gene polymorphisms were carried out as follows; Two milliliters of whole blood was withdrawn on EDTA containing BD Vacutainer tubes. DNA extraction using (Thermo Fisher Scientific, USA) kit "according to the manufacturer's guidelines" was carried out by author 1 and 3 in Zagazig University Hospital research lab. The real-time PCR for the studied SNPs were performed in the Central Research Lab of the hospital by a qualified staff member and a well-trained technician under the supervision of the first three authors, on (StepOnePlus™ Real-Time PCR, Thermo Fisher Scientific, USA). The reaction mixture included; 10 μL from the TaqMan® Universal PCR Master Mix (Thermo Fisher Scientific, USA), 0.5 μL from the readymade primer-probe mixture of one SNP (Custom TaqMan® SNP Genotyping Assays, Thermo Fisher Scientific, USA), 3 μL from the extracted DNA and 6.5 μL nuclease-free water, annealing temperature was set at 60°C.

**Confirmation by DNA Sequencing**

Genotyping of ambiguous samples and confirmation of 10% of samples was carried in Zagazig University Hospital lab using direct Sanger sequencing: - (1) PCR amplification using the following primers; for rs2073838 (forward 5'-ACGTTGGATGGAAAAAGTCTGCAGAGCC-3', reverse 5'-ACGTTGGATGTGGCCAAGGTGTTGCAAG-3') and for rs3792876 (forward 5'-ACGTTGGATGACGTATCCACCAAC3', reverse 5'-ACGTTGGATGACGAAGTTTCCCCGTTA-3') (Newman et al., 2005). PCR amplification reaction mixture contains, 12.5 μL of the ready master mix (Top Taq Master Mix Kit Qiagen, Germany), 1μL of 10 pmol from each primer, 6 ul of genomic DNA, 4.5 μL nuclease free water, using thermal cycler (Biometra TProfessional PCR, Germany) with cycling condition of initial denaturation at 95°C for 5 min, 35 cycles of (denaturation at 95°C for 1min, annealing at 61°C for rs2073838 and 54°C for rs3792876 for 40 sec and extension at 72°C for 1min, followed by a final extension at 72°C for 10 min (2) first purification of amplified PCR products with QIAquick PCR Purification Kit (50) (Qiagen GmbH, Hilden, Germany). (3) Cycle sequencing using Bigdye Terminator V3.1cycle sequencing kit (Thermo Fisher Scientific Inc., Ontario, Canada). (4) the second purification of the products using BigDye X Terminator Purification Kit (Thermo Fisher Scientific Inc., Ontario, Canada). (5) Sequencing using the Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific Inc., Ontario, Canada). All were performed according to manufacturer's guidelines by two qualified staff members under the supervision of the first two authors (6) Nucleotide Blast online program (https://blast.ncbi.nlm.nih.gov) was used for results interpretation.

**Statistical Analysis**

SPSS program version 21 (IBM Corp., Chicago, IL, USA) statistical software was used for data analysis. Shapiro–Wilk test was used to test the normality of quantitative results. Ages, BMI and duration of disease are expressed as mean and standard deviation. FEV1, FEV1/FVC and changes in FEV1are expressed as median and range. T-test and Mann Whitney test were used to compare means and medians among the studied groups respectively. Genotypes, haplotype and allele frequencies were expressed as numbers and percentages. The differences in SLC22A4 genotypes as well as haplotypes frequencies among the investigated groups were analyzed using the Chi-square test and the Fisher's exact test for results ≤5. Odds Ratios (ORs) and 95% confidence intervals were calculated. P≤0.05 was considered to indicate statistically significant differences.
Ethical Approvals

The Institutional Review Board (IRB) and the ethical committee of Zagazig University Hospitals approved this study. All subjects gave written informed consent before enrollment in this work. Approval number (IRB#4725/26-6-2017).

Sample Size Calculation

The sample size of this study was calculated using this formula (Charan and Biswas, 2013):

\[ n = \frac{Z_{1-\alpha^2}^2 \times p(1-p)}{d^2} \]

As \( Z_{1-\alpha^2} \) is the standard normal variate at 1% type one error = 2.58, \( p \) is the prevalence of these mutations in normal population = 0.07 (Newman et al., 2005) and \( d \) is the absolute error or precision = 0.05. Ten percent of the calculated sample size was added to the final sample size to compensate for lost samples or data.

Results

A total of 190 patients were eligible for inclusion in this study, four patients with failed spirometry testing and six with failed genotyping were excluded from analysis. The remaining 180 patients included 97(53.9%) males and 83(46.1%) females, with mean age \( \pm SD \) (29.9\pm13.3); range (8-51) years and mean body mass index \( \pm SD \) (27.5\pm4.6); range (18.1-37.3) Kg/m². Duration since the first diagnosis in these patients ranged from 1 to 36 years with mean \( \pm SD \) 15.3\pm8.5. Forty-eight patients (26.7%) were current smokers. Eighty-three (46.1%) of the included patients had a positive family history of bronchial asthma. 55(33.5%) patients had associated allergic rhinitis, 20(11.1%) had allergic rhinitis with conjunctivitis, 8(4.4%) had allergic rhinitis with dermatitis and one patient (0.5%) had allergic rhinitis with conjunctivitis and dermatitis. 89(49.4%) of patients had positive skin tests for respiratory allergens like pollen, house dust, molds, animal fur and dust mites.

Based on the bronchodilator reversibility test; 105(58.3%) patients were responders and 75(41.7%) were not, with median FEV1 absolute and percent changes (0.30 L and 15%) in responders versus (0.13 L and 8%) in non-responders \( P<0.01 \) as shown in Table 1. The severity of disease, baseline pulmonary function tests and medication history of patients in the two groups were also illustrated in Table 1. There were no significant differences regarding the severity of the disease and baseline pulmonary functions. By taking the history of medications in the past few months, patients with poor response to inhaled bronchodilators required the administration of oral steroids to relieve asthma symptoms during the past 12 months and needed to take maintenance medications and increased rescue medications in the past four months more frequently \( P<0.01 \).

A comparison was made between responders and non-responders regarding age, gender, BMI, duration of the diseases, family history, associated allergic diseases and results of skin test, to exclude the possible effect of these factors on the responsiveness to inhaled bronchodilators. Results are summarized in Table 2. No significant differences between the two groups were detected regarding these features.

Table 1: Pulmonary function tests and medical history of patients

|                     | Responders n=105 | Non responders n=75 | P value |
|---------------------|------------------|---------------------|---------|
| Asthma severity n(%)| Mild 64 (60.9)    | 36 (48.0)           | 0.08    |
|                     | Mod 41 (39.1)    | 39 (52.0)           |         |
| Baseline FEV1/FVC%  | Median 64        | 62                  | 0.06    |
|                     | Range 40-75      | 36-75               |         |
| Baseline FEV1 (litre)| Median 2        | 1.85                | 0.06    |
|                     | Range 1.5-2.5    | 1.2-2.4             |         |
| Post BD FEV1 (litre)| Median 2.24     | 1.99                | <0.01   |
|                     | Range 1.69-2.95  | 1.87-2.54           |         |
| FEV1 Change (litre) | Median 0.30     | 0.13                | <0.01   |
|                     | Range 0.19-0.46  | 0.04-0.24           |         |
| FEV1 change (%)     | Median 15        | 8                   | <0.01   |
| (Reversibility %)   | Range 12-19      | 2-11                | <0.01   |
| Need for oral steroids| Yes 5          | 27                  | <0.01   |
| during acute asthma attack in the past 12 months | No 100 | 48 |
| Need to increase rescue medications in the past 4 weeks | Yes 11 | 65 |
| Need for maintenance | No 94          | 10                  | <0.01   |
| medications in the past 4 weeks | Yes 11 | 63 |
| FEV1: Forced Expiratory Volume in one second. FVC: Forced Vital Capacity. Significant P values are written in bold lines. |
Table 2: Demographic features, family history, history of allergy and prick test results of responders in comparison to non-responders

|                          | Responders n=105 | Non responders n=75 | P value |
|--------------------------|------------------|---------------------|---------|
| **Age (year) n(%)**      |                  |                     |         |
| <18                      | 23(21.9)         | 13(17.3)            | 0.20    |
| ≥18                      | 82(78.1)         | 62(82.7)            |         |
| **Duration of the disease (year)** |                  |                     |         |
| Mean                     | 15               | 15.8                | 0.30    |
| SD                       | 8.4              | 8.7                 |         |
| **BMI (Kg/m2)**          |                  |                     |         |
| Mean                     | 27.5             | 27.4                | 0.50    |
| SD                       | 4.5              | 4.8                 |         |
| **Gender n(%)**          |                  |                     |         |
| Males                    | 56 (53.3)        | 41(54.6)            | 0.80    |
| Females                  | 49 (46.7)        | 34(45.4)            |         |
| **Smoking status n(%)**  |                  |                     |         |
| Smokers                  | 25               | 23                  | 0.30    |
| Non smokers              | 80               | 52                  |         |
| **Family history of asthma n(%)** |            |                     |         |
| Positive                 | 50(47.6)         | 33(44.0)            | 0.60    |
| Negative                 | 55(52.4)         | 42(56.0)            |         |
| **Associated allergic diseases n(%)** |        |                     |         |
| Yes                      | 43(40.9)         | 41(54.6)            | 0.06    |
| No                       | 62(59.1)         | 34(45.4)            |         |
| **Skin test n(%)**       |                  |                     |         |
| Positive                 | 48(45.7)         | 41(54.6)            | 0.20    |
| Negative                 | 57(54.3)         | 34(45.4)            |         |

BMI: Body Mass Index. SD: Standard Deviation

Table 3: Current treatment regimen of patients

|                                          | Responders n=105 | Non responders n=75 | P value |
|------------------------------------------|------------------|---------------------|---------|
| **BD with low dose ICS**                 | 42               | 26                  | 0.40    |
| **BD with medium dose ICS**              | 23               | 25                  | 0.09    |
| **BD with medium dose ICS and Oral corticosteroids** | 3            | 1                   | 0.40    |
| **BD with medium dose ICS and Leukotriene antagonists** | 11          | 13                  | 0.10    |
| **BD and Leukotriene antagonists**       | 26               | 10                  | 0.06    |

BD: Bronchodilators
ICS: Inhaled Corticosteroids

Table 4: The distribution of SLC22A4 (rs2073838 and rs3792876) SNPs among responders to bronchodilators and non-responders

| SNP              | Responders (105) n (%) | Non responders (75) n (%) | P value | Odds ratio (95% CI) |
|------------------|------------------------|---------------------------|---------|---------------------|
| rs2073838        |                        |                           |         |                     |
| AA               | 10(9.5)                | 2(2.6)                    | 0.060   | (0.05-1.2)          |
| AG and GG        | 95(90.5)               | 73(97.4)                  | 0.060   | 3.8(0.8-18.1)       |
| rs3792876        |                        |                           |         |                     |
| TT               | 6(5.7)                 | 13(17.3)                  | **0.013** | (1.2-9.5)         |
| CT and CC        | 99(94.3)               | 62(82.7)                  | **0.010** | 0.2(0.1-0.8)       |

CI: Confidence Interval
Significant P values are written in bold lines

Table 5: SLC22A4 (rs2073838 and rs3792876) haplotypes distribution among responders to bronchodilators and non-responders

| Haplotype | Responders (157) n (%) | Non responders (197) n (%) | P value | Odds ratio (95% CI) |
|-----------|------------------------|---------------------------|---------|---------------------|
| AC        | 54                     | 25                        | **<0.01** | 0.5(0.29-0.8)       |
| AT        | 17                     | 23                        | 0.07    | 1.8(0.9-3.5)        |
| GC        | 90                     | 60                        | 0.10    | 0.7(0.4-1.1)        |
| GT        | 36                     | 49                        | **<0.01** | 2.0(1.2-3.3)       |

CI: Confidence Interval
Significant P values are written in bold lines
The responding status to salbutamol was assessed in relation to the current therapeutic regimen of patients; results are illustrated in Table 3. There was no significant effect of the therapeutic regimen on the passage of Salbutamol through the BDR receptors in smoker asthmatics.

The genotype and allele frequencies of SLC22A4 rs3792876 and rs2073838 among cases are illustrated in the supplementary Table 1. The distributions of the examined SNPs among cases are within Hardy-Weinberg Equilibrium. The frequency of rs2073838 genotypes showed no significant difference when compared among responders and non-responders. On the other hand, the recessive genotype of rs3792876 occurred more frequently among the non-responding patients P=0.01. The differences in the frequencies of both SNPs among the two groups are illustrated in Table 4. Haplotype analysis of the two SNPs showed that the AC haplotype was more frequent in responders and the GT haplotype was more frequent in non-responders P=0.01, as shown in Table 5.

Based on the gender, age of onset, family history, severity of asthma and the presence of atopy, patients were categorized into groups and the distribution of SLC22A4 genotypes within these groups were compared. No significant association of these factors with the distribution of genotypes was detected. Results are illustrated in Table 6.

**Discussion**

This study included 180 bronchial asthma patients admitted to the Chest clinic of Zagazig university hospital for follow up. Bronchodilator reversibility test was performed to categorize patients into responders and non-responders to salbutamol. 105(58.3%) patients were responders and 75(41.7%) were non-responders.

At first, comparison was made between the two groups regarding the baseline pulmonary function tests, severity of asthma, demographic criteria, family history and history allergy to exclude the presence of significant differences between responders and non-responders regarding these factors, as these factors may influence the responsiveness to bronchodilators rather than the examined SNPs. As indicated from previous studies such as; Heffler et al. (2012) concluded that increased risk of treatment failure is associated with older age, and associated with atopy, and Ahmad and Singh (2010) concluded that there is a down-regulation of adrenergic receptors in smoker asthmatics. To exclude the possible effect of the current treatment on the passage of Salbutamol through the
organic cation transporters, we evaluated the responding status of patients in relation to the therapy and there were no significant effects. Chiappori et al. (2012) found out that corticosteroids increase Salbutamol transport through the OCT3 in human bronchial smooth muscle cell lines, although this increase was not significant but this point requires further in vivo investigations.

The difference of frequencies of both SNPs was examined among responders and non-responders, a significant association between (rs3792876) and bronchodilators resistance was detected, as the recessive phenotype (TT) was more frequent in non-responders group rather than the responders. While for (rs2073838) a significant association was not detected. In addition, the GT haplotype was more frequent in non-responders and AC was more frequent in responders. The genotype distribution of rs2073838 in both groups did not differ significantly from the distribution in normal populations studied in previous literatures such as Plenge et al., (2005) study on north American and Sweden populations (Plenge et al., 2005). For rs3792876, the distribution in responders goes with that in previous studies while in non-responders the distribution differs significantly from normal populations (Hou et al., 201; Jaruskova et al., 2017; Angelini et al., 2013; De Ridder et al., 2007; Barton et al., 2005; Shahidi and Fitzgerald, 2010; Koskela et al., 2006; Poon et la., 2012; Larocca et al., 2013; Newman et al., 2005; Charan and Biswas, 2013; Heffler et al., 2016; Hegewald et al., 2012; Dunn et al., 2015; Ahmad and Singh, 2010; Chiappori et al., 2012; Plenge et al., 2005; Santiago et al., 2006). Finally, none of the studied genotypes was associated with gender, age of onset, positive family history, history of atopy or asthma severity.

Up to our knowledge, no previous literature studied the association between SLC22A4 gene polymorphisms and response to bronchodilators in asthmatic patients. Horvath et al found that mRNA of OCTN1, as well as OCTN2, were highly expressed in airway epithelia of the lung tissue and the OCTN1 activity was largely associated with the apical border of the epithelial cell in the trachea, also, they found that Albuterol and fomoterol inhibited the in vitro uptake of a cationic fluorophore, suggesting the involvement of OCTN1/2 of the airway epithelial cells in the delivery of inhaled cationic bronchodilators to the underlying tissues (Horvath et al., 2006).

Although previous studies confirmed the expression of these transporters in lung tissues and its role in transporting cationic drugs through the epithelial layer of trachea and bronchioles via cell line based transporting methods, clinical evidence on these data are still inadequate (Ehrhardt et al., 2017). Drake et al. (2014) found a significant increase in bronchodilator response in association with two rare SNPs in the SLC22A15 gene rs1281748 and rs1281743 Drake et al. (2014), this finding indicates the possible influence of Organic Cation Transporter polymorphisms on the response to inhaled bronchodilator.

**Limitations and Replication Studies**

The calculated sample size was the minimum required to achieve an 80% statistical power of the study. Because of the deficiency of previous results concerning the association of SLC22A4 gene polymorphism “particularly the studied SNPs” and the bronchodilator responses, as well as the relatively small sample size and the lack of funding sources, replication studies with larger sample size and more statistical power including both asthmatic and non-asthmatic normal populations are recommended to confirm or deny these results.

**Conclusion**

The homozygous form of (rs3792876) in the RUNX1 binding site in SLC22A4 gene can interfere with the absorption of inhaled bronchodilator (Salbutamol), although, further investigations on this point are required.

**Recommendations**

We recommend expanding this study on other Organic Cation Transporters polymorphisms, in relation to other drugs in the Bronchial Asthma therapy.

**Conflict of Interest**

Authors have no conflict of interest to declare.

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**Author Contributions**

The requirements for authorship have been met. The original idea was the first authors, who also performed the data analysis, manuscript writing and submission for publication. The second author contributed to sample processing, results collection and participated in manuscript writing. The third author contributed to allergy skin prick test performance, clinical diagnosis of patients, history taking and manuscript revision. The fourth author contributed to the clinical diagnosis of patients, history taking, spirometry, blood samples withdrawal and manuscript revision. Authors certify
that we have personally written at least 90 percent of the manuscript. Finally, the manuscript has been read and approved by all the authors.

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