A Pilot Study of Polymorphism of Adrenergic Beta-2 Receptor and Mild Asthma: A Clinical and Pharmacogenetic Study

Parisa Adimi Naghan\textsuperscript{a}, Fanak Fahimi\textsuperscript{b,c,*}, Seyed Alireza Nadji\textsuperscript{d}, Nima Naderi\textsuperscript{e,f}, Fatemeh Soleimani\textsuperscript{b} and Mohammad Reza Masjedi\textsuperscript{g}

\textsuperscript{a}Chronic Respiratory Disease Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. \textsuperscript{b}Clinical Pharmacy Department, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. \textsuperscript{c}Pharmaceutical Care Department, Chronic Respiratory Disease Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. \textsuperscript{d}Virology Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. \textsuperscript{e}Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. \textsuperscript{f}Toxicology Department, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. \textsuperscript{g}Chronic Respiratory Disease Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract

Glycine allele at codon 16 has previously been associated with the increase in asthma severity, bronchial hyperresponsiveness and also the increase in inhaled corticosteroid dependence. This study was designed to evaluate the genetic alleles in mild asthma.

Thirty-four patients with diagnosis of mild asthma (FEV\textsubscript{1} ≥ 80%, positive methacholine test) and body mass index (BMI ≤ 30 Kg/m\textsuperscript{2}) were included in the study. They could only use short acting beta-2 agonists for asthma control. Smoking, infection, occupational sensitizers’ exposure, gastroesophageal reflux, diabetes mellitus and heart failure were also considered as exclusion criteria. All patients were genotyped at 16\textsuperscript{th} and 27\textsuperscript{th} codons.

Among all, 20 (58.8%) Arg/Gly, 14 (41.2%) Arg/Arg and no Gly/Gly genotype were detected at codon 16. Genotyping at codon 27 revealed 2 (5.9%) Glu/Glu, 13 (38.2%) Glu/Gln and 18 Gln/Gln (52.9%).

Based on the obtained results, Arg/Gly mutation had a higher rate among the studied subjects compared to Arg/Arg polymorphism. This is a pilot study which shows a probable usefulness of genotyping for predicting of asthma severity.

Keywords: Beta-adrenoceptor; Polymorphism; Asthma; Clinical study.

Introduction

Asthma is a chronic polygenic disease. The total cost per patient is estimated around 1,000 Euros yearly for patients with mild persistent asthma (1).
of long term use of β2 adrenergic agonists (5, 6). Based on the above-mentioned facts, we, along with a few other researchers, believe that the severity of asthma might be related to β2 adrenergic receptor genotype (7). In addition, a study by Drysdale on 13 polymorphisms revealed a huge diversion in distribution of some haplotypes between Caucasian, African-American, Asian and Hispanic-Latino (8). Such a difference was observed in some other studies which described ethnic-specific pharmacogenetic differences that could change the response of individuals to β2 adrenergic agonists (9-10).

In addition, our study would give us a basic view of Iranian mild asthmatic patients’ polymorphisms in β2 adrenoceptor gene. This pilot could benefit future studies as the first of its kind. The gene encoding this receptor is located on the short arm of chromosome 5 (11) and encodes one of the seven-transmembrane families of receptors that is coupled to the G protein and is expressed in various cell types like smooth muscle cells, neutrophils, eosinophils, macrophages and epithelial cells (12). Expression of β2 adrenergic receptors and their coupling are mediated through a dynamic process with a negative feedback cycle regulated in a way that in case of prolonged exposure to agonists or pre-inflammatory cytokines, down-regulation of receptors and a subsequent decreased response occur (13). In case of exposure to glucocorticoids, up-regulation of receptors occurs (14-15). There are 9 points in this gene that may undergo mutation (16). So far, 6 different types of polymorphisms have been detected (17), out of which, the arginine-to-glycine substitution at codon 16 and substitution of glutamic acid for glutamine at codon 27 are more common among the Caucasian population (16). The two above-mentioned substitutions along with the substitution of Threonine for Isoleucine at codon 164 have been shown to affect the function of receptor in in-vitro studies (2). β2 adrenoceptor agonists cause the dilation of airways and therefore, are indicated for the treatment of asthma (18). Several studies have discussed possible drug-related changes in β2 adrenergic receptors or signal transduction in cells that can control the disease. For example, in a study, polymorphism of β2 adrenergic receptors resulted in down regulation of them (19). The expression of Gln27 has been associated with hyperresponsiveness of airways in another study (20).

This study aimed at evaluating β2 adrenergic receptor polymorphism and its correlation with mild asthma in Iranian patients.

**Experimental**

The study was conducted according to the ethical guidelines of Shahid Beheshti University of Medical Sciences for human studies and approved by the ethics committee of the university.

Patients with diagnosis of mild asthma (FEV1 ≥ 80% of predicted value, and positive methacholine test) who referred to the pulmonary clinic of Masih Daneshvari Hospital with the following inclusion criteria were entered into the study.

- Cases with a history of gastroesophageal reflux, diabetes mellitus, heart failure, chronic obstructive pulmonary disease COPD, Churg-Strauss syndrome, bronchitis obesity (BMI > 30 Kg/m2), smoking or infections within one month prior to the study procedure or exposure to occupational sensitizers were excluded from the study. In addition, those who took medications which causes cough or exacerbate asthma condition, i.e. angiotensin converting enzyme inhibitors (ACEIs), nonsteroidal anti-inflammatory drugs (NSAIDs) and β-blockers, were not included in the study either. They could only use short acting beta2 agonists for asthma control.

- Respiratory parameters of FEV1, FEV1/FVC were measured. Blood samples were collected and stored in -20°C for genotyping analysis.

**Molecular procedure**

**Genome extraction and PCR method**

Genomic DNA was extracted from peripheral blood obtained using phenol chloroform method (21). The 2AR genotypes were determined by primer-induced restriction site assay. The primers were selected according to a previous study by Martinez et al. (22) and were 5’-GCCTTCTTGTGCTGGCACCCCAT-3’ and
Polymorphism of Adrenergic Beta-2 receptor

5'-CAGACGCTCGAACTTGCCATG-3'. Then, a PCR product which included the regions of \( \beta_2 \)AR-16 and \( \beta_2 \)AR-27 polymorphisms was generated. PCR reactions were carried out in a volume of 50 µL containing 50 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl\(_2\), 1.5 U of Taq polymerase, 0.2 mM of each deoxynucleotide triphosphate and 30 pm of each primer. Temperature cycling was 94°C for 60 s, 60°C for 60 s and 72°C for 60 sec for 44 cycles and then a final extension for 7 min at 72°C. The size of generated PCR product was 168 bp (Figure 1).

Polymorphism detection

For detection of \( \beta_2 \)AR polymorphism, 15 µL of PCR product was digested with 1 U of Nco. (New England Biolabs), 2 µL PCR water, 2 µL of 50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl\(_2\) and 1 mM dithiothreitol (pH 7.9) at 37°C for 12 h. Nco. cuts 22 bp from the 3'-end of both alleles and 18 bp from the Gly-16 allele. The restriction digests were electrophoresed on 3% agarose gels and visualized with ethidium bromide staining and gel documentation. The Gln27Glu polymorphism was identified in the second restriction digest using another aliquot of the same PCR product. Twelve µL of PCR product was digested with 2 U of Bbv. (New England Biolabs), 5 µL PCR water, in 2 µL of 50 mM NaCl, 10 mM of Tris-HCl, 10 mM of MgCl\(_2\) and 1 mM of dithiothreitol (pH = 7.9) at 37°C for 12 h. Bbv digests the Gln-27 allele to produce 105 and 63-bp fragments which are separated from uncut Glu-27 alleles on 3% agarose gels (Figure 2).

Results

Genotypes of 34 patients with mild asthma were evaluated in terms of polymorphisms at codons 16 and 27. Table 1 shows the demographics data and genotypic characteristics of patients. The mean ± SD age of patients was 29.97 ± 8.23 years (range = 16–52 years). Male to female ratio was 14/20.

Among all, 20 (58.8%) Arg/Gly, 14 (41.2%) Arg/Arg, and no Gly/Gly genotypes were detected at codon 16. Genotyping at codon 27 revealed 2 (5.9%) Glu/Glu, 13 (38.2%) Glu/Gln, and 18 Gln/Gln (52.9%). Frequency of Arg and Glu allele were estimated 70.6% and 25.75% respectively. Data of patients’ haplotypes in the study is shown in Table 2.

Discussion

\( \beta_2 \)AR (beta-2 adrenergic receptor) polymorphism has been studied and discussed by several studies in terms of asthma susceptibility, severity and responsiveness (2, 4, 7, 20, 22-25).

In our mild asthmatic patients, allele frequency of Arg allele (70.6%) was dominant compared to the frequency of Gly allele (29.4%). This finding is consistent with results of Liggette...
that the Gly16 polymorphism may play a role in the pathogenesis of asthma severity, but no significant relationship was found between the polymorphism at amino acid 27 and asthma or the asthma severity (28). Though, one of the reasons that our patients’ asthma severity was rated as mild, could be related to the fact that no patients with Gly/Gly polymorphism was among them.

Ramsey et al. could not find any association between 27Glu/Gln or Arg/Gln polymorphism and asthma (29).

On the other hand, in a study by Weir et al. which evaluated the polymorphisms at codones 16, 27, and 164 in 86 patients, B2AR polymorphism was not recognized a causative strong factor for asthma exacerbation. No significant difference between Gly allele in our patient population, might explain their mild symptoms. At 16th codon, the presence of Arg allele seems to be protective in asthma severity in comparison with the other alleles resulted in milder asthma occurrence (27). In Liggette study, two groups of patients with and without nocturnal asthma were compared. The allele frequency of Gly16 was found to be higher in subjects with nocturnal asthma (80%) as compared with non-nocturnal asthmatic patients (52%). In addition, we could not find any patient with Gly/Gly similar to a study by Turki et al. (17). The study resulted in a small number of Gly homozygotes in non-nocturnal asthmatic patients, supporting the theory that Gly allele could be related to nocturnal asthma. In another study by Holloway et al, it was shown that the Gly16 polymorphism may play a role in the pathogenesis of asthma severity, but no significant relationship was found between the polymorphism at amino acid 27 and asthma or the asthma severity (28). Though, one of the reasons that our patients’ asthma severity was rated as mild, could be related to the fact that no patients with Gly/Gly polymorphism was among them.

Ramsey et al. could not find any association between 27Glu/Gln or Arg/Gln polymorphism and asthma (29).

On the other hand, in a study by Weir et al. which evaluated the polymorphisms at codones 16, 27, and 164 in 86 patients, B2AR polymorphism was not recognized a causative strong factor for asthma exacerbation. No significant difference between Gly allele

### Table 1. Polymorphisms in codon 16 and 27 of mild asthmatic patients.

| Codon   | Genotype | Number (%) |
|---------|----------|------------|
| Codon 16 | Arg/Arg  | 14 (41.2)  |
|         | Arg/Gly  | 20 (58.8)  |
|         | Gly/Gly  | 0 (0)      |
|         | Glu/Glu  | 2 (5.9)    |
| Codon 27| Glu/Gln  | 13 (38.2)  |
|         | Gln/Gln  | 18 (52.9)  |

### Table 2. The rate of β2AR haplotypes in our study.

| Codon/haplotype | Study results |
|----------------|--------------|
| Gly 16 - Glu 27 | 9 (29.0)     |
| Gly 16 - Gln 27 | 9 (29.0)     |
| Arg 16 - Glu 27 | 9 (29.0)     |
| Arg 16 - Gln 27 | 4 (12.9)     |
| Total           | 31 (100)     |
frequencies was detected between patients with mild and moderate asthma. Gly16/Glu27 haplotype was more prevalent in mild asthmatic patients (2).

In general, it is clear that various host and environmental factors other than polymorphism can affect the severity of asthma; factors such as poor economic condition, lower level of education, concomitant diseases such as diabetes, heart failure, etc (30). For example, allergy was found as another significant predictor in a model derived from a retrospective logistic regression analysis, which proves that the positive history of allergy is a risk factor of asthma severity (31). But the dominant role of genetics should not be ignored.

This study had some limitations as well. Owing to the extensively limited inclusion criteria, we were not able to enroll more patients in the study. So, small sample size limits the power of the study to represent this data as a perfect model of Iranian asthmatic patients. Studies with a bigger sample size should be conducted to give more reliable data for general population of Iranian patients. As a first time conducted to give more reliable data for general population study, it would be better to include a group of non-asthmatic patients as control group. However, this was the first study in this realm conducted on asthmatic patients in Iran and paved the way for further investigations on such patients.

Acknowledgment

The authors would like to thank the molecular laboratory of Baghiyatollah Medical University staff, especially Dr. Morovvati for his help.

The study is performed as part of a Pharmacy student (Fatemeh Soleimani) Pharm. D. thesis. The study was supported financially by Neuroscience Research Center of Shahid Beheshti University of Medical Sciences.

References

(1) Antonicelli L, Bucca C, Neri M, De Benedetto F, Sabbatani P, Bonifazi F, Eichler HG, Zhang Q and Yin DD. Asthma severity and medical resource utilisation. *Eur. Respir. J.* (2004) 23: 723-729.
(2) 2-Weir TD, Mallek N, Sandford AJ, Bai TR, Awadh N, Fitzgerald JM, Cockcroft D, James A, Liggett SB and Paré PD. Beta2-Adrenergic receptor haplotypes in mild, moderate and fatal/near fatal asthma. *Am. J. Respir. Crit. Care Med.* (1998)158:787-791.
(3) Bai TR, Mak JCW and Barnes PJ. A comparison of beta-adrenergic receptors and *in-vitro* relaxant responses to isoproterenol in asthmatic airway smooth muscle. *Am. J. Respir. Crit. Care Med.* (1992) 6:647-651.
(4) Reihsaus E, Innis M, MacIntyre N and Liggett SB. Mutations in the gene encoding for the ß2 adrenergic receptor in normal and asthmatic subjects. *Am. J. Respir. Cell Mol. Biol.* (1993) 8:334-339.
(5) Crane J, Pearce N, Flatt A, Burgess C, Jackson R, Kwong T, Ball M and Beasley R. Prescribed fenoterol and death from asthma in New Zealand, 1981-83: case-control study. *Lancet* (1983) 1: 917-920.
(6) Spitzer WO, Suisssa S, Ernst P, Horwitz RL, Habbick B, Cockcroft D, Boivin JF, McNutt M, Buist AS and Reubck AS. The use of beta-agonists and the risk of death and near death from asthma. *N. Eng. J. Med.* (1992) 326: 501-506.
(7) Hall IP, Wheatley A, Wilding P and Liggett SB. Association of Glu 27 beta 2-adrenerceptor polymorphism with lower airway reactivity in asthmatic subjects. *Lancet* (1995) 345:1213-1214.
(8) Drysdale CM, McGraw DW, Stack CB, Stephens JC; Judson RS, Nandabalan K, Arnold K, Ruano G and Liggett SB. Complex promoter and coding region beta2 adrenergic receptor haplotypes alter receptor expression and predict *in-vivo* responsiveness. *Proc. Natl. Acad. Sci. U S A.* (2000) 12: 97(19):10483-10488.
(9) Haselkorn T, Lee J and Mink D. Racial disparities in asthma related health outcomes in severe or difficult-to-treat asthma. *Ann. Allerg. Asthma Im.* (2008) 101: 256-263.
(10) Choudhry, S, Ung N, Avila P, Ziv E, Nazario S, Casal J, Torres A, Gorman J, Salari K and Rodriguez-Santana J. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. *Am. J. Resp. Crit. Care. Care.* (2005) 171: 563-570.
(11) Kobila BK, Dixon RA, Frielle T, Dohtman HG, Bolanowski MA, Sigal IS, Yang-Feng TL, Francke U, Caron MG and Leffkowitz RJ. cDNA for the human beta 2-adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA* (1987) 84: 46-50.
(12) Liggett S. Update on current concepts of the molecular basis of [beta] 2-adrenergic receptor signaling. *Journal of Allergy and Clinical Immunology* (2002)110:S223-S228.
(13) Shore SA, Laporte J, Hall IP, Hardy E and Panettieri RA Jr. Effect of IL-1 beta on responses of cultured human airway smooth muscle cells to bronchodilator agonists. *Am. J. Respir. Cell Mol. Biol.* (1997) 16: 702-712.
(14) Taylor DR and Hancox RJ. Interactions between corticosteroids and beta agonists. *Thorax.* (2000) 55: 595-602.
(15) Mak J, Nishikawa M and Barnes P. Glucocorticosteroids increase beta 2-adrenergic receptor transcription in human lung. American Journal of Respiratory and Critical Care Medicine (1995) 152: 1675-1681.

(16) Litonjua AA, Silverman EK, Tantisira KG, Sparrow D, Sylvia JS and Weiss ST. Beta 2-adrenergic receptor polymorphisms and haplotypes are associated with airways hyperresponsiveness among nonsmoking men. Chest. (2004) 126: 66-74.

(17) Turki J, Pac J, Green SA, Martin RJ and Liggett SB. Genetic polymorphisms of the beta 2-adrenergic receptor in nocturnal and nonnocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype. J. Clin. Invest. (1995) 95: 1635-1641.

(18) Johnson M. The beta-adrenoceptor. Am. J. Respir. Crit. Care Med. (1998) 158: S146-S153.

(19) Joos L and Sandford AJ. Genotype predictors of response to asthma medications. Curr. Opin. Pulm. Med. (2002) 8: 9-15.

(20) D’Amato M, Vitiani LR and Petrelli G. Association of persistent bronchial hyperresponsiveness with b2-Adrenoceptor (ADRB2) Haplotypes: a population study. Am J Respir Crit Care Med. (1998) 158:1968-1973.

(21) Sambrook J and Russell DW. Preparation and analysis of eukaryotic genomic DNA. In: Sambrook J, Russell DW (eds), Molecular Cloning: a Laboratory Manual 3rd ed, chapter 6. Cold Spring Harbor Laboratory Press, New York (2001)6.4-6.12.

(22) Martinez FD, Graves PE, Baldini M, Solomon S and Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. J. Clin. Invest. (1997) 100:3184-3188.

(23) Turner SW, Khoo SK, Laing IA, Palmer LJ, Gibson NA, Rye P, Landau LI, Goldblatt J and Le Souef PN. B2 adrenoceptor Arg16Gly polymorphism, airway responsiveness, lung function and asthma in infants and children. Clin. Exp. Allergy. (2004) 34: 1043-1048.

(24) Bleeker ER, Nelson HS, Kraft M, Corren J, Meyers DA, Yancey SW, Anderson WH, Emmett AH and Ortega HG. Beta2-receptor polymorphisms in patients receiving salmeterol with or without fluticasone propionate. Am. J. Respir. Crit. Care Med. (2010) 181: 676-687.

(25) Israel E, Chinchilli VM, Ford JG, Boushey HA, Cherniack R, Craig TJ, Deykin A, Fagan JK, Fahy JV, Fish J, Kraft M, Kunselman SJ, Lazarus SC, Lemanske RF Jr, Liggett SB, Martin RJ, Mitra N, Peters SP, Silverman E, Sorkness CA, Szefler SJ, Wechsler ME, Weiss ST and Drazen JM; National Heart, Lung, and Blood Institute’s Asthma Clinical Research Network. Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial. Lancet (2004) 364: 1505-1512.

(26) Liggett SB. The pharmacogenetics of beta2-adrenergic receptors: Relevance to asthma. J. Allerg. Clin. Immun. (2000) 105:847-849.

(27) Tan S, Hall IP, Dewar J and Dow E. Association between beta 2-adrenergic receptor polymorphism and susceptibility to bronchodilator desensitization in moderately severe stable asthmatics. Lancet. (1997) 350:995-999.

(28) Holloway JW, Dunbar PR, Riley GA, Sawyer GM, Fitzharris PF, Pearce N, Le Gros GS and Beasley R. Association of beta2-adrenergic receptor polymorphisms with severe asthma. Clin. Exp. Allergy. (2000) 30: 1097-1103.

(29) Ramsay CE, Hayden C, MTiller KJ and Burton PR. Polymorphisms in the beta2-adrenoreceptor gene is associated with decreased airway responsiveness. Clin Exp Allergy. (1999) 29: 1.

(30) Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O’Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE and Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. Eur. Respir. J. (2008) 31: 78-143.

(31) Salamzadeh J, Wong ICK, Hosker HSR and Chrystyn H. A logistic regression analysis of predictors for asthma hospital re-admissions. Iranian J. Pharm. Res. (2003) 5-9.

This article is available online at http://www.ijpr.ir