Ministry of Higher Education and Scientific research

University of Babylon

College of Science for Women

Estimation of Angiotensin Converting Enzyme Insertion/Deletion Gene polymorphism in Iraqi Pateints with Asthma

A search Submitted by

Saja Abdullah Jumaa and Shahad Mohammed Abbas

For the year 2022/20223

Subervised by

Pro. Dr. ISRAA Adnan ALBaghdady

وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية العلوم للبنات/ قسم علوم الحياة

التغاير الشكلي من نوع حذف/اضافة للجين المحول للانجيوتنسين في مرضى الربو العراقيين

بحث مقدم من قبل الطالبتين

سجى عبد الله جمعة و شهد محمد عباس

لنيل شهادة البكلوريوس في قسم علوم الحياة/ كلية العلوم للبنات/ جامعة بابل

العام الدراسي2022/2023

باشراف

ا.د. اسراء عدنان ابراهيم

التغاير الشكلي من نوع حذف/اضافة للجين المحول للانجيوتنسين في مرضى الربو العراقيين

الخلاصة:

اجريت الدراسة الحالية على 50 مريض عراقي مصاب بالربو وعدد مساوي من الاشخاص الاصحاء الذين كعينة سيطرة لتحديد دور التغايرات الوراثية من نوع الحذف والاضافة للجين المحول للانجيوتنسين في الاصابة بالربو لاول مرة في العراق واظهر استخدام تقنية تضخيم الدنا المتسلسل وجود ارتباط بين التغايرات الوراثية للجين مع الاصابة بالربو في العينة المدروسة.

كلمات مفتاحية: الربو ، التغاير الوراثي، الاستعداد الوراثي

Estimation of Angiotensin Converting Enzyme Insertion/Deletion Gene polymorphism in Iraqi Pateints with Asthma

ABSTRACT:

The present study was conducted on 50 Iraqi patients with Asthma and similar number healthy subject to detect Angiotensin Converting Enzyme Insertion/Deletion gene polymorphism in Iraqi patients for the first time, the results of gene amplification using PCR technology reported that there is association between DD , ID and II of ACE gene polymorphism and Asthma in the studied patients.

Key words: asthma , genepolymorphism, genetic susceptability

INTRODUCTION:

Asthma is a chronic infammatory disease characterized by recurrent respiratory symptoms, reversible variable airway obstruction, airway infammation, and airway hyperreactivity [1]. It is one of the most common chronic diseases in developed countries, and estimations suggest that 300 million individuals are affected worldwide [2]. The phenotypic heterogeneity of asthma is well documented, and a number of clinical properties such as atopic phenotype or asthma severity have been applied to describe subtypes of asthma and even to manage clinical symptoms [3]. Susceptibility to asthma is related to the interaction between multiple genes and environmental factors. Whereas environmental factors are known to trigger or modulate asthma responses [4], the genetic components that underlie asthma susceptibility are not completely understood. Analysis of single-nucleotide polymorphisms (SNPs) has been widely used for the study of complex genetic disorders. The identification of variations in specific genes involved in the expression of asthma phenotypes could lead to a better understanding of the underlying pathways or even facilitate management tailored to the patient's genotype. Several studies have reported an association between asthma or atopy and genes coding for molecules involved in various pathways [5]. The study of cytokine genes is particularly important owing to the significant role of cytokines in pathophysiology. In the last decade, many authors have studied cytokine gene SNPs in different populations [6-12].

However, associations vary widely between different ethnic populations. In this sense, characterization of phenotypes following appropriate clinical criteria is a key component of genetic association studies. Other aspects underlying the

discrepancies observed in these studies include quality control measures, specif cally in laboratory procedures and statistical

analysis[13].

Angiotensin-converting enzyme (ACE) inactivates bradykinin, substance P and neurokinin A, which are believed to play important roles in the pathogenesis of asthma, especially in neurogenic inflammation[14], ACE is a key component of the renin-angiotensin system, the most important humoral pressure regulator. The formation of angiotensin II - the main vasoconstrictor and degradation of bradykinin - an important vasodilator takes place under the influence of this enzyme, which also enhances the proliferation and contractility of the smooth muscle of the respiratory tract, thereby contributing to the excessive bronchus. The angiotensin-converting enzyme gene - ACE is localized on the long arm of the 17th chromosome at the 17q23 locus, it consists of 26 exons and 25 introns, and its size is 45,000 bp. [15].

The ACE gene contains a polymorphism based on the presence (or insertion [I]), or absence (or deletion [D]) of a 287-base pair element in intron 16 on chromosome 17q23, According to the presence of this element, three different genotypes may occur: DD and II homozygotes, and ID heterozygotes. [14].

Increased serum ACE levels may affect asthma severity, and studies have shown increased levels of angiotensin II in patients with severe asthma that’s the reason behind conducting this study which is originally planed to determine the presence or absence of the angiotensin converting enzyme genepolymorphism in Iraqi asthma patients.

MATERIALS AND METHODS:

The study carried out on 50 blood samples of asthma patients and 50 saples of healthy subjects having no previous detection of any autoimmune disease, samples obtained from msc student in our collegue. The student collected the samples d from both patients and the healthy subjects by vein puncture then 2.5 ml of blood were put in EDTA anticoagulant tubes and kept in -20 °C until the DNA was extracted. DNA from whole the blood was extracted using Favrogene Genomic DNA extracting Kit (Favrogene, Taiwan) according to manufacturer instruction and kept refugerated until use, The DNA quality and integrity detected by using electrophoresis on a agarose gel containing red sfe stain (Intron, Korea) with the concentration 0.7% by mixing 10 µl of DNA together with 2µl of loading dyejuice (Genedirex, Korea) in 0.2 ml PCR tube. Then the samples were loaded individually into the gel wells, and subjected to electrical power at 100 volt for 10 min using AgaroPower™ device (Bioneer, Korea), later the DNA bands were visualized using UV transiluminator at 350 nm and documented by digital camera.

Detecting of ACE insertion deletion polymorphism was carried out using PCR using the forward primer 5′ -CTG GAG ACCACT CCC ATC CTT TCT-3 ′ and Reverse primer : 5′ -GAT GTG GCC ATC ACA TTC GTC AGA T-3′ used by Deepika *et al*[16].

Preperation of the primers were done by adding nuclease free distal water (Promega, USA) according to the instruction of the manufacturing company (Promega, USA) to obtain primers solution with the concentration of 100 Pico mole/ µl as stock solution which used to prepare working solution with 10 Pico mole/ µl concentration and then soluble primers stored at -20 C until used. PCR reactions for the healthy subjects and patients was carried out using GO Taq Green mastermix (Promega, USA), with a final reaction volume 25 µl containing: 1 µl

forward primer, 1 µl reverse primer,5 µl DNA, 2X GO Tag Green Mastermix 12.5 µl and Nuclease free distil water 4.5 µl. PCR amplification PCR was performed in a thermocycler Verti96 Thermo cycler (Applied biosystem, USA) with the same condition of Deepika *et al*. (2013) [16]. Represented by an initial denaturation step for 5 min at 95 °C, then 30 cycles consisting of 30 s of denaturation at 94 ° C, 45 s of annealing at 59 °C and a final extension for 5 min at 72 °C. The products were run on 2% agarose gel containing red safe dye with the use of 100bp DNA ladder H3 (Genedirex, Korea) as a size marker under electrical power (100 volt) for 10 minets and then (50 volt) for 1 hour using AgaroPower™ device (Bioneer, Korea) and photographed using digital camera.

A product of 490 bp indicates a genotype homozygous for insertion (II), 190 bp homozygous for DD and the presence of 490 and 190 bp products indicate heterozygous genotype[16].

RESULTS AND DISCUSSION:

The DNA extracted the blood samples quality and integrity were estimated through electrophoresis on agarose 0.7% for 10 min. The quality, estimated by noticing the DNA bands which appeared as single not diffused bands and without having any smear which may result from DNA degradation.

The PCR amplification results in different patterns of DD, ID, and II gene polymorphism in both patients and the healthy subject these pattern represented in table (1).

Table (1): Results of PCR amplification of ACE gene

DD genotype	ID genotype	II genotype
Healthy subject no. 22	Healthy subject no.21	Healthy subject no. 7
Patient no. 32	Patient no. 17	Patient no. 1

ACE gene polymorphism of the genetically analyzed 50 control healthy subjects and 50 patients are shown in Table 1. shows difference between the patient and control groups, these results refer that there is an association between ACE gene polymorphism in Iraqi patients, but this results require further investigation on larger number of both patients and healthy subject to confer this results.

Angiotensin Converting Enzyme Insertion/Deletion Gene polymorphism have been previously approved to be associated with differences in plasma ACE levels. The insertion appears to reduce ACE expression. Thus, the DD genotype is associated with the highest plasma levels of ACE, while the II genotype is associated with the lowest levels[17].

Iskandar et al. 2020 showed that ACE D/D genotype had significantly higher occurrence in atopic asthmatic patients, compared to healthy control subjects; patients with D/D genotype had 6.8-fold higher risk for atopic asthma development than those with non- D/D genotype [18]. Pasiyeshvili and Zheleznyakova showed that the relative risk of developing BA was 2.67 for patients with the ACE D/D genotype; 0.46 for the ACE D/I genotype; and 0.69 for the ACE I/I genotype, suggesting a possible protective role of the I allele [19]. The authors point out that individuals carrying ACE D/D genotype predominate (54.2%) among BA patients. This is also supported by a study indicating that children with the D/D genotype are in the group of high risk for BA development [20].

On the other hand, some studies failed to establish any associations of ACE gene polymorphisms with BA. For instance, ACE gene polymorphism is not significantly associated with BA or with its severity among Egyptian adults [21]. Similarly, the ACE genotype frequencies also do not significantly differ between the patients with BA and healthy controls in study conducted in Turkey [22], Iran [413], and Japan [24].

REFFERENCES:

1.Patel , S.J., and Teach, S, Asthma. Pediatr Rev. 2019 ;40(11):549-567. doi: 10.1542/pir.2018-0282.

3. Hansbro PM, Scott GV, Essilfi e AT, Kim RY, Starkey MR, Nguyen DH, Allen PD, Kaiko GE, Yang M, Horvat JC, Foster PS. Th2 cytokine antagonists: potential treatments for severe asthma. ExpertOpinInvestigDrugs.2013;22(1):49-69.

4. Sokol K, Sur S, Ameredes BT. Inhaled environmental allergens and toxicants as determinants of the asthma phenotype. Adv Exp Med Biol. 2014;795:43-73. doi: 10.1007/978-1-4614- 8603-9_4.

5. Ober C,Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. Genes Immun. 2006;7(2):95-100.

6. Christensen U, Haagerup A, Binderup HG, Vestbo J, Kruse TA, Borglum AD. Family based association analysis of the IL2 and IL15 genes in allergic disorders. Eur J Hum Genet. 2006;14(2):227-35.

7. Movahedi M, Mahdaviani SA, Rezaei N, Moradi B, Dorkhosh S, Amirzargar AA. IL-10, TGF-B, IL-2, IL-12, and IFN-gamma Cytokine Gene Polymorphisms in Asthma. Journal of Asthma. 2008;45(9):790-4.

8. Krynytska, I., Marushchak, M., Mykolenko, A., Smachylo, I., Sopel, O., Kucher.S. Bronchial Asthma: Genetic Factors Contributing to its Pathogenesis. Macedonian Journal of Medical Sciences. 2021. 05; 9:590-594. doi.org/10.3889/oamjms.2021.6788

9. Hui Q, Hao Y, Ye F, Pang B, Niu W and Zhang Q (2022) Genetically high angiotensin-converting enzyme concentrations causally increase asthma risk: A meta-analysis using Mendelian randomization. Front. Med. 9:941944. doi: 10.3389/fmed.2022.941944

10. Hammad , H., and Lambrecht, B.N. The basic immunology of asthma. Cell. 2021 Mar 18;184(6):1469-1485. DOI: 10.1016/j.cell.2021.02.016

11. Randolph AG, Lange C, Silverman EK, Lazarus R, Silverman ES, Raby B, Brown A, Ozonoff A, Richter B, Weiss ST. The IL12B gene is associated with asthma. Am J Hum Genet. 2004;75(4):709-15.

12. Ferreira MA, Matheson MC, Duffy DL, Marks GB, Hui J, Le Souef P, Danoy P, Baltic S, Nyholt DR, Jenkins M, Hayden C, Willemsen G, Ang W, Kuokkanen M, Beilby J, Cheah F, de Geus EJ, Ramasamy A, Vedantam S, Salomaa V, Madden PA, Heath AC, Hopper JL, Visscher PM, Musk B, Leeder SR, Jarvelin MR, Pennell C, Boomsma DI, Hirschhorn JN, Walters H, Martin NG, James A, Jones G, Abramson MJ, Robertson CF, Dharmage SC, Brown MA, Montgomery GW, Thompson PJ, Australian Asthma Genetics Consortium. Identifi cation of IL6R and chromosome 11q13.5 as risk loci for asthma. Lancet. 2011;378(9795):1006-14.

13. Tomita H, Sato S, Matsuda R, Ogisu N, Mori T, Niimi T, et al. Genetic polymorphism of the angiotensin-converting enzyme (ACE) in asthmatic patients. Respir Med. 1998;92:1305---10.

15.Olena Rechkina, Nataliia Gorovenko, Vira Stryzh, Zoia Rossokha, Svitlana Kyriachenko, Serhii Rudenko. Models of Gen-gene Interaction in Determining the Severity of Bronchial Asthma in Children. American Journal of Internal Medicine. Special Issue: New Approaches to Manage Difficult-to-Control, Severe Asthma. Vol. 8, No. 4, 2020, pp. 182-191. doi: 10.11648/j.ajim.20200804.17

15. Crisan D., Carr J. (2000). Angiotensin I-Converting Enzyme. Genotype and Disease Associations. J. Mol. Diagn. 2 (3): 105–115. doi: 10.1016/S1525-1578(10)60624-1.

16.Deepika, NLN. ; Reddy, KR.; Rani, VU.; Balakrishna, N. ; Prasanna Latha, KP. and Parveen J. Do ACE I/D gene polymorphism serve as a predictive marker for age at onset in PCOS? J Assist Reprod Genet .2013. 30:125–130.

7. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin Iconverting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest. 1990;86:1343-1346.

18. Iskandar H, Bakri S, Mannulusi B, Patellongi IJ. DD genotype of the I/D angiotensin-converting enzyme gene polymorphism is a higher risk for atopic asthma. Int J Med Rev Case Rep. 2020;4(5):11-4.

19. Pasiyeshvili T, Zheleznyakova N. Clinical and prognostic value of ACE gene polymorphism in patients with asthma and obesity. Crimean Ther J. 2015;1:65-8.

20. Rechkina O, Gorovenko N, Stryzh V, Rossokha Z, Kyriachenko S, Rudenko S. Models of gen-gene interaction in determining the severity of bronchial asthma in children. Am J Intern Med. 2020;8(4):182-91.

21. El-Shafei MS, Farres MN, Shahin RY. Evaluation of angiotensin converting enzyme gene polymorphism and susceptibility to bronchial asthma among Egyptians. Allergol Immunopathol (Madr). 2012;40(5):275-80. https://doi.org/10.1016/j. aller.2011.05.010
PMid:21889830
22. Bora E, Soylar R, Arıkan-Ayyıldız Z, Uzuner N, Giray-Bozkaya Ö, Erçal D, et al. Plasminogen activator inhibitor-1 and angiotensin converting enzyme gene polymorphisms in Turkish asthmatic children. Allergol Immunopathol (Madr). 2013;41(1):11-6. https://doi.org/10.1016/j.aller.2011.12.003
PMid:22361338
23. Alizadeh-Navaei R, Rafiei A, Hedayatizadeh-Omran A, Mohammadzadeh I, Arabi M. Gene susceptibility in Iranian asthmatic patients: A narrative review. Ann Med Health Sci Res. 2014;4(6):837-40. https://doi.org/10.4103/2141-9248.144871
PMid:25506473
24. Nakahama H, Obata K, Nakajima T, Nakamura H, Kitada O, Sugita M, et al. Renin-angiotensin system component gene polymorphism in Japanese bronchial asthma patients. J Asthma. 1999;36(2):187-93. https://doi. org/10.3109/02770909909056316 .