Haplotype in ABCC4 gene by PCR-SSCP technique in Iraqi Asthmatic patients

Ameera jasim Al-Aaraji¹, Suhayr Aesa Al- Qaysi² and Ali SalihBaay³

¹² College of nursing/Babylon University
³ College of Hammurabi medicine /Babylon University
Email : ameeraalaaraji@gmail.com

Abstract. The current study was carried out to discover the relation of ABCC4(rs1751034) haplotypes polymorphisms with asthma in Iraqi patients, this SNP was located on exon 19 and PCR-SSCP technique dealed in this study, whole blood was processed to DNA extraction .this study includes about 165 subjects (female and male) categorized in to two groups , the first group includes 86 patients suffered from asthma and the second group includes 50 apparently healthy subject as control group. the data show that there was strong association between ABCC4(rs1751034 ) and asthma in one haplotype from three (p<0.05). This study revealed that there was a link between ABCC4 polymorphisms with asthma , our finding required more work up to use this polymorphism as early suggestion of this disease.

Key words: ABCC4 , PCR-SSCP technique, asthma and polymorphisms.

1. Introduction :

Asthma is a chronic inflammatory disorder of the bronchial tree in which several cells play a role, especially eosinophils, mast cells, and T lymphocytes(1), and it is also recognized that asthma has a solid genetic component (2).In susceptible persons this inflammation causes repeated attacks of shortness of breath, wheezing, and cough especially in the early morning and/or at night. These clinical features are frequently accompanied with extensive but variable airflow obstruction which is partially or totally reversible either off hand or with management. This inflammation also causes increase in airway responsiveness to a range of triggers (3). Family and twin studies have indicated that genetics have a great role in the occurrence of asthma (4).

ABCC4 is a one of the family of ATP-binding cassette transporters ,it is named as a multidrug resistance protein, because of the fact that this gene has the ability to extrude with wide specificity. It is also considered as one of the large genes that is located on 13q32.1 chromosomes and has 1,325 amino acids encoded by the aforementioned gene (5)(6)(7).We studied the potential relation between ABCC4 gene polymorphisms and asthma. Few data on the role of ABCC4 variants are present in spite of the fact that it is a greatly polymorphic gene.ABCC4 variants are linked with diverse diseases; however, no report has involved a relationship between ABCC4 and atopic diseases in Iraqi population. Copsel et al. shown that an ABCC4 polymorphism regulate the cellular levels of cAMP and control the proliferation and differentiation of human leukemia cell, demonstrating its function in a cellular processes (8).Therefore, ABCC4 variants may also reveal an potential role in the inflammatory of asthma . There no data about the associations of ABCC4 polymorphisms with asthma in Iraqi patients.
Therefore, we investigated the important associations between asthma and ABCC4 polymorphisms in Iraqi population.

2. Materials and methods:

Study population: this is case control study includes about 165 subjects (female and male) categorized in to two groups, the first group includes 86 patients suffered from asthma and the second group includes 50 apparently healthy subject as control group. The age of all subjects were ranged between (8-60) years, mean ± SD for patients (36.34 ± 16.9) and for controls (37 ± 10.5), the mean of body mass index (BMI) km/m² for fist group (34.99 ± 11.68) and for second group (31.02 ± 8.96) as shown in table 1. Current study was carried out from out patients clinic (Respiratory unit in Merjan teaching hospital / Hilla province), and the samples under the super vision of pulmonologist.

| Table (1): demographic data of patient and control groups |
|-----------------------------------------------------------|
| **Age (Mean ± SD)** | **control** | **P value** |
| 36.34 ± 16.9 | 37 ± 10.5 | >0.05 |
| **BMI (Mean ± SD)** | 34.99 ± 11.68 | 31.02 ± 8.96 | <0.05 |

DNA extraction: from whole blood, we extracted DNA dependent on the instruction of faverogene manufacturing, in (biochemistry lab of clinical biochemistry department, College of medicine, university of Babylon).

After obtaining the DNA; concentration and purity of DNA were assessed using nanodrope. PCR conditions were implemented as a following Primers that used, forward, AGTTTTTCTCCTAGTTTTGCTGCAT, (NCBI primer blast).

PCR circumstances: PCR procedure implemented as a Follows; ABCC4(rs1751034) per-denaturation for five minutes at 94°C, then thirty five cycles (thirty sec at 94°C, thirty sec at 57°C, 30sec at 72°C, and finally 5 minutes at 72°C). products of PCR were assigned by electrophoresis pattern in gel (1.5% agarose, 100 V, 50 mA for 50 min) with red safe staining, the PCR size products were (370) bp. Statics, the results were analyze by using SPSS version 21, measurement of odd ratio.

Single strand conformational polymorphism (SSCP) method: PCR products were denaturation by SSCP dye (0.25 gm bromophynol blue, 0.025 xylene cyanol, 9.5 formamid and 100 μl (2gm in 50 ml NaOH) sodium hydroxide). In SSCP procedure, the products were electrophoresis. Four μl of DNA + 20μl dye were poured into wells of eight percent acrylamide/bis gel comprising 29% acrylamide, and 1% Bis acrylamide. In more details; 3.2 ml of 30% acrylamide/bis (stoke solution 29:1) mingle with 2.4 ml of 5x TBE, then 10μl TEMED and 200 μl of ten percent ammonium per sulfate (APS) were added to 6.4 ml of dH2O. The gel was brood in 37°C for 30 minutes to polymerize. After the polymerizer, the tank of electrophoresis apparatus was packed in 0.5 TBE buffer, and the comb was taken away, then the wells were washed of polyacrylamide gel by the buffer. A pre-run was carried out for 30 minutes under a constant current 50 mA and 100 V. After the pre-run the a 4μl of the sample and 20μl dye were put in water bath at 90°C for 10 minute, and the tube directly was placed in to ice, then the sample loaded in the well by mechanical pipet. The electrophoresis run was carried out until the bromophenol blue dye reach to the two third of the gel, under a constant current 50mA and 100 V for 24 hours. The gel then stained by stain solution [10% ethanol, 0.5% acetic acid and 0.2% silver nitrate], other solution [3% sodium hydroxide, 0.1% formaldehyde], the substances were complete to 50 ml of water and then placed in water bath at 60°C and stop solution [10%...
ethanol, 0.5 \% acetic acid. The gel then imaged and analyzed by UV trans illuminator (9). Haplotype frequency were determination by variety of bands among studied groups.

3. Results:

The data of current study involved gene polymorphism of ABCC4 in asthma comparison with control group. The products of PCR for ABCC4 was 370 bp as show in fig (1) The data of haplotype polymorphism in ABCC4 gene reveal three pattern of haplotype written as (A, B and C) (Table 2, figure 2).

![Figure (1) Electrophoresis pattern of PCR product (370) of ABCC4 gene on red safe stained agarose gel (1.5\%), 100 V for 50 min, lanes (1-15) PCR products and lane L DNA marker (100bp).](image)

![Figure (2) Electrophoresis Pattern of PCR-SSCP of ABCC4 gene polymorphism for patients and control on 30\% acrylamide](image)

The data of ABCC4 polymorphism which display in table (2) and fig (2) explained the difference of haplotypes in studied groups, there were three pattern (A,B and C), In current study one (A) haplotype association with asthma in Iraqi population. While other patterns (B and C) did not show association with asthma.

| haplotype | Control\% | Patients\% | Odd ratio | CI (95\%)              | P value |
|-----------|-----------|------------|-----------|------------------------|---------|
| A         | 20\%      | 36\%       | 2.2545    | 0.9920-5.1238          | 0.0523  |
| B         | 30\%      | 18.6\%     | 0.5333    | 0.2366-1.2024          | 0.1296  |
| C         | 50\%      | 45.35\%    | 0.8298    | 0.4128-1.6681          | 0.1225  |

Table (1): showed that most abundant haplotype were A and C among patient groups (36\%, 45\%) and in control groups (20\%, 50\%) respectively.
On other hand there was A pattern polymorphism show highly significant differences between studied groups (p= 0.0523). While other patterns (B and C) did not show any significant (P =0.1296 and p=0.1225) respectively. between patient and control.

4. Discussion:

Asthma is a complex, chronic pulmonary disorder characterized by a extensive clinical spectrum, with contributions from several environmental and genetic factors(10)(11). Recent studies have shown that various gene polymorphisms influence the onset and progression of asthma(12)(13). Genetic studies of intermediate phenotypes may ultimately facilitate the find of susceptibility genes for asthma (14)(15). We first identified a significant association between the ABCC4 (rs1751034) and asthma. PCR-SSCP technique was used to detected Gene polymorphisms for the reason that it can be give features about haplotype 2 structure of the specific fragments of the gene, The PCR-SSCP distinguish stereo state of single strand of DNA which reliant on chemical shape of nucleotide, furthermore it identified a deletion, insertion, and duplication of DNA chain in one strand which it need in elevation fee in alternative technique. In the present study, we selected rs1751034 SNP within the ABCC4 gene to examine this potential role in asthma pathogenesis. Till now, the roles of this gene in asthma have not been determined.

Saileshet al using the TaqMan Allelic Discrimination technique with Taq Manprobes which has repeatedly been used to describe single nucleotide polymorphisms (SNPs) and to define the genotypes of gross deletion mutations. They found that a functional polymorphism of the ABCC4 gene (−1508A>G) may affect its promoter activity, thereby affecting release of periostin, from innate immune cells in asthma (16). van de Ven et al. reported that ABCC4 plays an important role in dendritic cell migration in humans and that inhibition of ABCC4 activity decreases dendritic cell migration in the skin (17). However, no association study to date has examined the relationships between this gene and asthma.

5. References:

[1] Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J. 2008;31(1):143–78.
[2] Shimoda T, Obase Y, Kishikawa R, Iwanaga T. Association of matrix metalloproteinase 8 genetic polymorphisms with bronchial asthma in a Japanese population. Allergy Rhinol. 2013;4(3):ar-2013.
[3] Maier A, Vincent MJ, Gadagbui B, Patterson J, Beckett W, Dalton P, et al. Integrating asthma hazard characterization methods for consumer products. Regul Toxicol Pharmacol. 2014;70(1):37–45.
[4] Willemsen G, Van Beijsterveldt TCEM, Van Baal CGCM, Postma D, Boomsma DI. Heritability of self-reported asthma and allergy: a study in adult Dutch twins, siblings and parents. Twin Res Hum Genet. 2008;11(2):132–42.
[5] Reid G, Wielinga P, Zelcer N, van der Heijden I, Kuil A, de Haas M, et al. The human multidrug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidal antiinflammatory drugs. Proc Natl Acad Sci. 2003;100(16):9244–9.
[6] Dermauw W, Van Leeuwen T. The ABC gene family in arthropods: comparative genomics and role in insecticide transport and resistance. Insect Biochem Mol Biol. 2014;45:89–110.
[7] Huynh T, Norris MD, Haber M, Henderson MJ. ABCC4/MRP4: a MYCN-regulated transporter and potential therapeutic target in neuroblastoma. Front Oncol. 2012;2:178.
[8] Copsel S, Garcia CI, Diez F, Vermeulen M, Baldi A, Bianciotti LG, et al. Multidrug resistance protein 4 (MRP4/ABCC4) regulates cAMP cellular levels and controls human
leukemia cell proliferation and differentiation. J Biol Chem. 2011;jbc-M110.

[9] Sambrook J, Russell DW, Russell DW. Molecular cloning: a laboratory manual (3-volume set). Immunol. 2001;49:895–909.

[10] Baos S, Calzada D, Cremades L, Sastre J, Quiralte J, Florido F, et al. Biomarkers associated with disease severity in allergic and nonallergic asthma. Mol Immunol. 2017;82:34–45.

[11] Kauffmann F, Demenais F. Gene-environment interactions in asthma and allergic diseases: challenges and perspectives. J Allergy Clin Immunol. 2012;130(6):1229–40.

[12] Tizaoui K, Kaabachi W, Hamzaoui K, Hamzaoui A. Association of single nucleotide polymorphisms in toll-like receptor genes with asthma risk: a systematic review and meta-analysis. Allergy Asthma Immunol Res. 2015;7(2):130–40.

[13] Vercelli D. Genetic polymorphism in allergy and asthma. Curr Opin Immunol. 2003;15(6):609–13.

[14] Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonasson GM, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet. 2009;41(3):342.

[15] Weidinger S, Gieger C, Rodriguez E, Baurecht H, Mempel M, Klopp N, et al. Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. PLoS Genet. 2008;4(8):e1000166.

[16] Palikhe S, Uuganbayar U, Trinh HKT, Ban G-Y, Yang E-M, Park H-S, et al. A Role of the ABCB4 Gene Polymorphism in Airway Inflammation of Asthmatics. Mediators Inflamm. 2017;2017.

[17] van de Ven R, Scheffer GL, Reurs AW, Lindenbergh JJ, Oerlemans R, Jansen G, et al. A role for multidrug resistance protein 4 (MRP4; ABCC4) in human dendritic cell migration. Blood. 2008;112(6):2353–9.