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Original article

Association of polymorphisms of two histamine-metabolizing enzymes with allergic asthma in Egyptian children

Background: *Histamine released from mast cells and basophils plays a key role in the development of allergic diseases such as allergic asthma, rhinitis or anaphylaxis. Histamine-metabolizing enzymes: N-methyltransferase (HNMT) and amiloride binding protein 1(ABP) are involved in allergic inflammation.* ***Objective:*** *This study was undertaken to evaluate the relationship between polymorphisms of two genes encoding the histamine metabolizing enzymes HNMT and ABP1 with the development of allergic asthma in Egyptian children.* ***Methods:*** *This is a case control study performed on 100 atopic asthmatic and 94 healthy control children. Conventional method of PCR amplification was used for genotyping.* ***Results:*** *Distribution of HNMT -105 Thr → Ile (-314 C to T) single nucleotide polymorphism (SNP) genotypes and Thr and Ile (C and T) alleles among patients and controls revealed significant increase in the frequencies of Thr / Ile (CT) and Thr / Ile (CT) + Ile / Ile (TT) in atopic asthmatics than in controls (p= 0.04 and 0.002 respectively). There was also a significant increase in Ile (T) alleles in atopic asthmatic patients than controls (p= 0.002). The 2029 CG SNP polymorphism of ABP1gene was significantly associated with atopic asthma (p=0.0003).* ***Conclusion:*** *The results of this study suggest that genetic variations in the histamine-metabolizing enzyme (HNMT and ABP1) genes might contribute to the pathogenesis of asthma in the studied children.*

Keywords: Amiloride binding protein, asthma, atopy, children, N-methyltransferase.

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INTRODUCTION

Asthma is a highly prevalent disease that involves a complex interplay of environmental factors, airflow obstruction, bronchial hyperresponsiveness, and inflammation. The dominant feature that leads to clinical symptoms is smooth muscle contraction and inflammation, which results in narrowing of the airway and obstruction[1, 2].

Histamine is proved to be an important substance that controls body temperature and respiration in systemic anaphylaxis but its role in controlling blood pressure is minor. Histamine is mainly produced in mast cells and basophils in hematopoietic cells. In atopic bronchial asthma, histamine can control eosinophilia but not bronchial hypersensitivity [3].

Histamine-metabolizing enzymes including N-methyltransferase (HNMT) and amiloride binding protein 1(ABP1) are responsible for histamine degradation. Genetic variants of HNMT and ABP1 genes were found to be associated with altered enzyme activity. So the alleles which lead to decreased enzyme activity and, therefore, decreased inactivation of histamine may be responsible for altered susceptibility to asthma[4].

N-methylation catalyzed by cytosolic HNMT enzyme is the primary pathway for histamine bio-transformation in bronchial epithelium. HNMT might represent a common risk factor for development of asthma and allergic rhinitis and may be useful in identifying individuals who are candidates for early preventative pharmacotherapeutic intervention[5].

HNMT gene is located on the chromosome 2q22.1 and within the gene region, several polymorphisms have been identified. A common C314T polymorphism leading to Thr105Ile substitution was discovered[6] and it was found that the less common T allele (encoding Ile) was associated with decreased HNMT enzyme activity[7].

ABP1 enzyme is mainly expressed in kidney, colon, placenta, thymus and seminal vesicles and plays role in the inactivation of extracellular histamine[8].

The ABP1 gene has been located to chromosome 7q34-36, and within the gene region, several polymorphisms have been identified. Among these, His645Asp substitution (rs1049793) was found to be functional and was associated with significant decrease in the serum enzyme activity in vivo[9]. The importance of genetic variation of genes related to histamine (including histamine-metabolizing enzymes HNMT and ABP1) was widely discussed as regards these genes and their involvement in diverse diseases, including allergic diseases and asthma[10].

Genetic and genomic testing and analysis are already being incorporated into treatment decisions for patients with many diseases. There has been little integration of genomics and genetic testing for determination of best approaches to therapy for patients with asthma[11]. In asthma, understanding the impact of genetic variations on response to therapy has the potential to improve care, decrease side effects, and improve patient outcomes[12]. It is important to understand the therapeutic as well as the social and economic implications of the increased understanding of both the genetics of disease and responses to specific therapies[13].

The aim of this study was to evaluate the relationship between polymorphisms of two genes encoding the histamine metabolizing enzymes HNMT and ABP1 and atopic asthma among a group of Egyptian children.

METHODS

Patient selection

This case control (retrospective) study was performed on194 children: 100 atopic asthmatics and 94 sex and age-matched healthy children free from any diseases or allergic symptoms as a control group. The asthmatic children had their ages ranging from 7 to 15 years (mean= 9.97; SD = 2.5 years) and they were 68 males and 32 females.

They were treated for asthma in the Pediatric Pulmonology, Allergy and Clinical Immunology unit of Mansoura University Children Hospital. Asthma diagnosis was made according to the Global Initiative for Asthma (GINA) recommendation[14], based on clinical asthma symptoms and spirometry to assess bronchodilator responsiveness. This was measured 20 minutes after administration of 200 mcg of salbutamol via nebulizer; a ≥12% increase in forced expiratory flow in one second (FEV1) was diagnostic. Clinical diagnosis of atopy depended on current or past symptoms of atopic dermatitis, allergic rhino-conjunctivitis (seasonal or perennial) or food allergy[15].

Atopy was confirmed when children fulfilled one of the following criteria: total IgE level higher than the upper normal limits for age (32 IU/ml); blood eosinophilia (more than 5%) in differential count or absolute count (more than 450/μl); skin prick testing that is 3 mm greater than control or detection of specific IgE to one or more of common allergens as *Dermatophagoides Pteronyssinus*, grass, trees, cat, dog, Aspergillus fumigates ,mixed mites, mixed pollens, house dust and mixed molds. Patients must stop antihistamine use (2 days for first generation and 7 for second generation) before skin prick testing. Exclusion criteria included patients with chronic illness as TB or diabetes. All participants as well as their parents gave written informed consent. Local ethics committee accepted the project. The study was performed according to the Code of Ethics of Declaration of Helsinki.

DNA extraction

Two milliliters of venous blood were collected from all subjects and genomic DNA was extracted from EDTA–anticoagulated peripheral blood leucocytes using QIA amp DNA Blood Mini Kit supplied by Qiagen GmbH (Cat, No.51104, Hiden, Germany)[16]. The average DNA concentration (0.17±0.014μg/μl) was determined from absorbance at 260 nm (Jenway, Genova Model, UK). All samples had a 260/280 nm absorbance ratio between 1.52 and 1.71. The integrity of the DNA was checked by electrophoresis on 2 % agarose gel stained with ethidium bromide.

Genotyping of HNMT -105 Thr → Ile (-314 C to T) SNP (rs1801105) and ABP1 -645 His→ Asp (T to A) SNP (rs1049793):

Conventional method of PCR amplification was used for genotyping of HNMT -105 Thr → Ile (-314 C to T) SNP (rs1801105) in exon 4 of HNMT gene by the method described by Garcia-Martin et al.,[16].

A 394 bp exon 4 region containing the polymorphism site was amplified by using the following primer sequences (based on the published human HNMT sequence Gene Bank Accesion No. U44109): a 32-mer HNMT- forward primer 5′-GAA AAA CGT TCT TTC TAT CTG TTT GTA TAT AA-3′ and a 25-mer reverse primer 5′- ATT TGG GCA GAT CAT GGT CAC TTG T-3′ (Fermentas Life Science, Ontario, Canada) in which a deliberated primer mismatch designed to introduce an artificial EcoRV restriction site when the T allele is present at position -314.

Genotyping of ABP1 -645 His → Asp (- 2029 C to G) SNP (rs1049793) was done also by the method described by Garcia-Martin et al[17]. A 110

bp exon 2 region containing the polymorphism site was amplified by using the following primer sequences (based on the diamine oxidase gene sequence Gene Bank Accession No. X78212): a 21-mer forward primer 5′- GGT CAC CTG AAC CCG GTT AAC -3′ and a 21-mer reverse primer 5′- TTG TGA CCT CTG AAC TTG CCG -3′ (Fermentas Life Science, Ontario, Canada) in which a deliberated primer mismatch designed to introduce an artificial AvaII restriction site when the G allele is present at position -2029.

PCR was carried out in 50 microliters final reaction volume using Ready Mix (RED. Taq-PCR Reaction Mix) (purchased from Sigma Aldrich, Saint Louis, USA).The following mixture was prepared for each sample: 25 µl RED-Taq PCR reaction Mix (1X), 2µl (20 pmol) of forward primer, 2µl (20 pmol) of reverse primer, 2µl (200 ng) of genomic DNA and 19 µl of deionized water. This mix was put in a thin wall PCR microcentrifuge tube and gently centrifuged to collect all components to the bottom of the tube. Thermal cycling was carried out using Techne TC-312 thermal cycler (Model FTC312D, Barlworld Scientific Ltd, UK). The following program was used: initial denaturation at 94 ºC for 5 min followed by 35 cycles of denaturation at 94 ºC for 30 sec, annealing at 58 ºC (HNMT) or 62 ºC (ABP1) for 30 sec, and an extension step at 72 ºC for 30 sec, followed by a final linear extension step at 72 ºC for 5 min. Amplification products of 394 bp for HNMT and 476 bp for ABP1 were obtained when electrophoresed in a 2.5% (wt/vol) agarose gel stained with ethidium bromide, and bands were visualized under ultraviolet (UV) via Light UV Transilluminator (Model TUV-20, OWI Scientific,Inc.800 242-5560).

The PCR products of HNMT (394 bp) were digested using EcoRV enzyme (Fermentas Life Science, Conventional restriction enzymes, Catalog number ER0031), 12µl of each PCR product was digested overnight with 3 µl (30 U) of the enzyme at 37 °C. The enzyme recognition site is 5'-GTCTC (N)1-3' and 3'-CAGAG(N)5-5'. Homozygous wild genotype (Thr/Thr; CC) was presented as a single band at 394 bp when electrophoresed in a 2.5% (wt/vol) agarose gel against DNA molecular marker. Two bands at 215 bp and 179 bp identified Ile/Ile (TT) homozygous genotype, and three bands at 394, 215 and 179 bp indicated heterozygous Thr/ Ile (CT) genotype.

To determine the His (C) allele of ABP1 at position -645, PCR products of 476 bp were digested by AvaII enzyme (Fermentas Life Science, Conventional restriction enzymes, Catalog number ER0151), 12µl of each PCR product was digested overnight with 3 µl (30 U) of the enzyme at 37 °C. The enzyme recognition site is 5'-GGCC-3' and 3'-CCGG-5'.

Two bands (369 bp and 107 bp) indicated the His/His (CC) wild genotype, while three bands at 252 bp, 117 bp and 107 bp identified the mutated Asp/Asp (GG) genotype. The presence of all bands indicated the His/Asp (CG) genotype.

Statistical Methods

Statistical package of social science (SPSS) version 16 was used for statistical analysis. The qualitative data were presented in the form of number and percentage. Chi-square test or fisher's exact were used as a test of significance for qualitative data. Odds ratio and 95% confidence intervals were calculated for determination of disease association and expected risk for the disease. The quantitative data were expressed as mean and standard deviation. Significance was considered at p value less than 0.05.

RESULTS

The demographic characteristics of the studied children were summarized in Table 1. Among the studied patients cough was present in 90% followed by wheezes and difficulty of breathing (86% and 72% respectively). Allergic rhinitis was present in 72%, atopic dermatitis in 32% and food allergy in 52%. Family history of allergic disorders was present in 76%. Assessment of asthma severity, revealed that 70% of cases had moderate pulmonary affection (FEV1% =60-80% predicted), 20% had mild asthma affection (FEV1%≥ 80%) and 10 cases had severe affection (FEV1% less than 60%).

The results revealed significantly higher Thr / Ile (CT) and Thr / Ile (CT) + Ile / Ile (TT) frequencies in atopic asthmatic patients than controls (p= 0.04 and 0.002 respectively). Also a significant increase in Ile (T) alleles in atopic asthmatic patients was observed (p= 0.0003). The same result was found in cases with positive family history of allergic disorders (p=0.004 and 0.0001 respectively). Asthmatic patients with coexisting allergic rhinitis showed significant increase in Thr / Ile (CT) genotype and in Ile (T) alleles when compared to controls (p=0.002 and 0.0003 respectively), however asthmatic patients with AD did not show these significant difference (table 2). There was no significant difference between cases with mild asthma and those with moderate to severe asthma in relation to Thr genotype and Ile (C and T) alleles (table 3).

Distribution of ABP1 -645 His→ Asp (-2029 C to G) SNP genotypes and His and Asp alleles (C and G) among patients and controls revealed that -2029 CG SNP polymorphism of ABP1gene is significantly associated with asthma in the diseased children group (total CG and GG genotypes). CG genotype and G allele are highly distributed in asthmatic children when compared to controls (p=0.003 and 0.0004 respectively). Patients with allergic rhinitis, atopic dermatitis and those with positive family history of atopic diseases showed the same significant difference in genotype and alleles when compared to controls (table 4). These similar results can be explained by strict selection of atopic patients and high percentage of patients with positive family history of allergy and allergic rhinitis.

There was no significant difference between patients with mild asthma and cases with moderate to severe asthma regarding ABP1 -645 His→ Asp (-2029 C to G) SNP genotypes and His and Asp alleles (C and G) (table 5).

Table 1. Demographic data of the studied atopic asthmatic patients.

Sex	68 (68%) male 32 (32%) females
Age Range Mean(SD)	 7-15 years 9.97 years (±2.5)
Cough	90 (90%)
Wheezes	86 (86%)
Difficulty of breathing	72 (72%)
Allergic rhinitis	72 (72%)
Atopic dermatitis	32 (32%)
Food allergy	52 (52%)
Mean duration of illness(SD)	5.5 years(± 3.6)
Family history of atopy	76%
Asthma severity Mild Moderate Severe	 20% 70% 10%

Table 2. Distribution of HNMT -105 Thr → Ile (-314 C to T) SNP genotypes and Thr and Ile (C and T) alleles among patients and controls.

	Thr / Thr (CC)	**Thr / Ile (CT)**	**Ile / Ile (TT)**	**Thr / Ile (CT) + Ile / Ile (TT)**	**Thr (C)**	**Ile (T)**
Asthma only (100) **Control (94)** **OR** **(95%CI)**	58 (58%) 77 (81.9%)* 0.3 (0.16-0.58)	35 (35%) 15 (16%) 2.1933 (1.42-5.6)	7 (7%) 2 (2.1%) 3.4 (0.7 -17.1)	42 (42%) 17 (18.1%) 2.7 (1.45 - 5.36)	151 (75.5%) 169 (89.9%) 0.3 (0.19-0.61)	49 (24.5%) 19 (10.1%) 2.8 (1.62-5.1)
P	0.0005*	0.004*	0.1	0.002*	0.0003	0.0003
Asthma and AR (72) **Control (94)** **OR** **(95%CI)**	40 (55.5%) 77(81.9%)* 0.27 (0.13-0.55)	27 (37.5%) 15 (16%) 3.16 (1.52-6.55)	5 (6.9%) 2 (2.1%) 3.4 (0.6- 18.05)	32 (44.4%) 17 (18.1%) 3.6 (1.7 -7.3)	107 (74.3%) 169 (89.9%) 0.32 (0.17-0.59)	37 (25.7%) 19 (10.1%) 3.07 (1.6 – 5.6)
P	0.0004	0.002	0.24	0.0004	0.0003	0.0003
Asthma and AD (32) **Control (94)** **OR** **(95%CI)**	21 (65.6.%) 77 (81.9%)* 0.4 (0.17-1.03)	10 (31.3%) 15 (16%) 2.3 (0.94-6.06)	1 (3.1%) 2 (2.1%) 1.4 (0.12-16.9)	11 (34.4%) 17 (18.1%) 2.2 (0.9-5.5)	52 (81.3%) 169 (89.9%) 0.4 (0.22-1.07)	12 (18.7%) 19 (10.1%) 2.05 (0.93-4.5)
P	0.09	0.07	1	0.08	0.07	0.07
Family history **Control (94)** **OR** **(95%CI)**	31 (40.8%) 77 (81.9%)* 0.15 (0.07-0.3)	29 (38.1%) 15 (16%) 2.8 (1.3-5.8)	16 (22.4%) 2 (2.1%) 12.2 (2.7-55.3)	45 (59.2%) 17 (18.1%) 6.5 (3.2-13.1)	91 (59.8%) 169 (89.9%) 0.16 (0.09-0.29)	61 (40.2%) 19 (10.1%) 5.9 (3.3-10.5)
P	0.0001	0.004	0.0001	0.0001	0.0001	0.0001

P is significant if < 0.05, AR: allergic rhinitis; AD: atopic dermatitis

Table 3. Distribution of ABP1 -645 His→ Asp alleles n(C and G) among patients and controls.

ABP1 -645 His→ Asp genotypes	His / His (CC)	His / Asp (CG)	Asp / Asp (GG)	His / Asp (CG) + Asp / Asp (GG)	His (C)	Asp (G)
Asthma cases (100) **Control (94)** **OR** **(95%CI)**	43 (43%) 66 (70.2%) 0.45 (0.42 - 0.84)	47 (47%) 24 (25.5%) 2.5 (1.4 – 4.7)	10 (10%) 4 (4.3%) 2.3 (0.71 -7.74)	57 (57%) 28 (29.8%) 2.97 (1.63 - 5.42)	133(66.5%) 156 (83%) 0.40 (0.25 – 0.65)	67 (33.5%) 32 (17%) 1.96 (1.23 - 3.13)
P	0.01	0.003	0.16	0.0004	0.0003	0.004
Allergic rhinitis (72) **OR** **(95%CI)**	30 (41.7%) 0.3 (0.15-0.5)	37 (51.4%) 3.08 (1.6-5.93)	5 (6.9%) 1.6 (0.43- 6.49)	42 (58.3%) 3.3 (1.73- 6.28)	97 (67.4%) 0.306 (0.18 -0.5)	47 (32%) 2.3 (1.41-3.95)
P	0.0004	0.001	0.5	0.0004	0.0001	0.001
Atopic dermatitis (32) **OR** **(95%CI)**	10 (31.3%) 0.19 (0.08-0.45)	18 (56.2%) 3.7 (1.6-8.6)	4 (12.5%) 3.2 (0.75-13.6)	22 (68.7%) 5.1 (2.17-12.3)	38 (59.3%) 0.29 (0.16-0.56)	26 (40.6%) 3.3 (1.7-6.2)
P	0.0001	0.002	0.11	0.0001	0.0002	0.0002
Positive Family history (76) **OR** **(95%CI)**	37 (48.6%) 0.4 (0.2-0.7)	33 (43.4%) 2.3 (1.1-4.2)	6 (8%) 1.9 (0.5-7.1)	39 (51.3) 2.4 (1.3-4.6)	107 (70.4%) 0.4 (0.29-81)	45 (29.6%) 2 (1.2-3.4)
P	0.004	0.01	0.3	0.004	0.06	0.06

Table 4. Distribution of HNMT -105 Thr → Ile (-314 C to T) SNP genotypes and Thr and Ile (C and T) alleles in relation to asthma severity.

HNMT -105 Thr → Ile SNP	Mild asthma (n=20) No (%)	Moderate to Severe asthma (n=80)	OR (95%CI)	P value
Thr / Thr (CC)	13 (65%)	45(56.2%)	0.6	0.6
Thr / Ile (CT)	6 (30%)	29(36.3%)	(0.2-1.9)	0.4
Ile / Ile (TT)	1 (5%)	6(7.5%)	1.6	1
Thr / Ile (CT) + Ile / Ile (TT)	7(35%)	35(43.7%)	(0.5-4.8)	0.6
Thr (C)	32 (80%)	119(74.4%)	1.5	0.5
Ile (T)	8 (20%)	41(25.6%)	1.3 (0.5– 3.2)	0.5

P is significant if < 0.05

Table 5. Distribution of ABP1 -645 His→ Asp alleles n(C and G) in relation to asthma severity.

ABP1 -645 His→ Asp genotypes	Mild asthma (20)	Moderate to Severe asthma(80)	OR (95%CI)	P value
His / His (CC)	6(30%)	30(37.5%)	1.4 (0.48-4.03)	0.6
His / Asp (CG)	10(50%)	37(46.3%)	0.86 (0.32-2.3)	0.81
Asp / Asp (GG)	4(20%)	13(16.3%)	0.8 (0.22-2.78)	0.74
His / Asp (CG) + Asp / Asp (GG)	14(70%)	50(62.2%)	0.71 (0.24-2.05)	0.61
His (C)	22(55%)	97(60.6%)	1.2 (0.62-2.5)	0.58
Asp (G)	18(45%)	63(39.4%)	0.79 (0.39-1.59)	0.58

P is significant if < 0.05

DISCUSSION

Asthma is considered to be multifactorial disorder with immunological, environmental, and genetic factors. Genetic variations, such as single nucleotide polymorphisms (SNPs), in histamine related genes involved in complex inflammatory reactions of asthma have gained much attention recently. HNMT activity was measured in human trachea and bronchi by Yamauchi et al.,[18] and the contractile response of isolated human bronchi to histamine was potentiated in the presence of an HNMT inhibitor suggesting that HNMT plays an important role in degrading histamine and in regulating the airway response to histamine.

In this study, there was a significant association of -314CT polymorphism of HNMT gene with asthma (total CT and TT genotypes). CT genotype and T allele were found to be significantly more frequent among asthmatic children when compared with their control counterparts. These findings are in accordance with other investigators [4, 19] However; other studies did not find any link between T allele and developing asthma.[17, 20, 21]

Our asthmatic patients with coexisting AD did not show significant difference in CT genotype and T allele in relation to controls. On the other hand Kennedy et al.,[5] found that the frequencies of the T314 variant allele (0.12, p=0.04) and combined CT/TT genotype (0.24, p=0.02) were significantly higher in children with AD compared to controls (allele and genotype frequencies = 0.06 and 0.12).

This common polymorphism leads to Thr105Ile substitution. It was discovered by Preuss et al.,[10] as a functional polymorphism associated with decreased HNMT enzyme activity. Other disorders associated with altered histamine metabolism were neuronal degeneration, inflammatory bowel disease and alcoholism.

In our study, we found that -2029 CG SNP polymorphism of ABP1gene was significantly associated with asthma in the diseased children group (total CG and GG genotypes). CG genotype and G allele were significantly distributed in asthmatic children when compared with their corresponding control groups. Moreover, there was a significant association between -2029 CG SNP polymorphism of ABP1gene and the high risk to develop asthma. Also this studied polymorphism of ABP1gene leads to His645Asp substitution and it was found to be a functional polymorphism as it was associated with marked reduction in activity of the serum enzyme in vivo.[9] Szczepankiewicz et al.,[4] did not observe any association of this polymorphism with asthma. However, he stated that involvement of His645Asp SNP in histamine metabolism cannot be ignored and may be related to precise clinical phenotype rather than asthma per se that needs further research.[4]Also this contrast in results may be due to race difference.

Another study by Garcia-Martin et al[17] revealed that patients carrying mutated ABP1 genes tended to have more manifestations of asthma although they show lower IgE levels. This apparent controversy may be explained if these patients tend to develop clinical symptoms even with a lower IgE stimulation[17]. However assessment of specific IgE is more sensitive than total IgE.

In our study there was no significant association of specific genotype in both studied polymorphisms with asthma severity, on the other hand Garcia-Martin et al[17]found that none of the lung function values observed among patients with mutations differed significantly from values obtained for patients carrying no mutations. These findings can be explained by previous studies showed that plasma histamine is not related to the severity of airways' obstruction[22, 23].

In conclusion, the results of this study suggest that genetic variations in the histamine-metabolizing enzymes (HNMT and ABP1) genes contribute to the pathogenesis of asthma in studied children. Further studies with large number of cases are needed to elucidate the precise correlation between nucleotide polymorphisms in these genes and the patho-mechanisms of asthma and asthma endo-phenotypes. Future research on SNPs of HNMT and ABP1may help to identify new therapeutic candidates targeting asthma.

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