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Polymorphisms of histamine-metabolizing enzymes and clinical manifestations of asthma and allergic rhinitis

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Clinical and Experimental Allergy

Summary

Background Polymorphisms of enzymes involved in histamine biodisposition may affect clinical symptoms in diseases related to histamine, such as asthma or allergic rhinitis (AR).

Objective This study aims to analyse two common polymorphisms in genes coding for histamine-metabolizing enzymes in patients with allergic diseases.

Methods Five-hundred and sixty-five individuals participated in the study, including 270 unrelated patients with asthma and/or AR recruited from a single centre and 295 healthy volunteers. Participants were analysed for the presence of Thr105Ile and His645Asp amino acid substitutions at histamine *N*-methyltransferase (HNMT) and diamine oxidase (amiloride binding protein 1) enzymes, respectively, by amplification-restriction procedures.

Results The variant *HNMT* allele frequencies were slightly higher among patients with asthma [16.0%, 95% confidence interval (CI) 12.0–20.0] and among patients with rhinitis (13.2, 95% CI 10.3–16.1) as compared with healthy subjects (11.5 95% CI 8.9–14.1). The variant *ABP1* allele frequencies were similar among patients with asthma (30.8%, 95% CI 25.7–35.9), rhinitis (28.7, 95% CI 24.8–32.6) and healthy subjects (26.8 95% CI 23.2–30.3). Individuals carrying mutated *ABP1* alleles presented allergy symptoms with significantly lower IgE levels as compared with individuals without mutated genes, with a significant gene-dose effect ($P < 0.001$). In addition, the percentage of individuals presenting symptoms without eosinophilia was significantly higher among homozygous carriers of *ABP1* variant alleles ($P < 0.020$) as compared with the rest of the atopic patients.

Conclusion There is a lack of association between the allelic variants studied and the risk of developing allergic asthma and rhinitis. However, patients carrying the His645Asp polymorphism of *ABP1* are more prone to developing symptoms with lower IgE levels.

Keywords allergic rhinitis, asthma, diamine oxidase, histamine *N*-methyltransferase

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Introduction

Histamine released from mast cells and basophils plays a key role in the development of allergic diseases such as allergic asthma, rhinitis or anaphylaxis [1, 2].

Released histamine is degraded through two enzymes: histamine *N*-methyltransferase (HNMT, EC 2.1.1.8) and diamine oxidase [amiloride binding protein 1 (ABP1); EC 1.4.3.6]. HNMT is a cytosolic enzyme involved in the inactivation of intracellular histamine [3–5]. The ABP1 enzyme is secreted and seems to play a role in the inactivation and scavenging of extracellular histamine [6–8].

The genes coding for *HNMT* and *ABP1* are polymorphic. The *HNMT* gene, located in chromosome 2q22.1, shows eight single-nucleotide polymorphisms (SNPs), but only one of these is non-synonymous. This SNP is located in exon 4 and causes the amino acid substitution Thr105Ile [9]. The variant allele is unambiguously related to decreased enzyme activity [5, 10].

Three non-synonymous SNPs have been mapped to the *ABP1* gene, located in chromosome 7q34–36. We have shown functional impairment on serum ABP1 activity only for the SNP with the refSNP ID: rs1049793 (NCBI SNP database), which codes for an altered protein with the amino acid substitution His645Asp [11]. The other amino

acid changes, namely Thr16Met with the refSNP ID: rs10156191 and Ser332Phe with the refSNP ID: rs1049742, do not cause major changes on ABP1 enzyme activity [11].

Because histamine plays a prominent role in the pathogenesis of allergic diseases, it has been hypothesized that individuals carrying variant alleles leading to a decreased inactivation of histamine may show altered susceptibility or a different evolution of such diseases. In this regard, promising findings indicating the association of the HNMT Thr105Ile polymorphism with asthma were initially reported [12], although further independent studies failed to identify such an association for patients with asthma or bronchial hyperresponsiveness [13, 14] or other pathologies related to histamine such as ASA-induced urticaria/angio-oedema [15], ulcerative colitis [16] or gastric ulcer [17].

Although no studies have addressed the role of *ABP1* polymorphisms in patients with allergic diseases, it can be hypothesized that *ABP1* polymorphisms may modify the pathogenesis of asthma or allergic rhinitis (AR) because of the involvement of ABP1 in the metabolism of circulating histamine [6–8].

The present study was undertaken to answer several open questions: first, to obtain additional data to confirm or discount the putative association of the HNMT Thr105Ile polymorphism with asthma. Second, the present study is the first to address a putative role of *ABP1* gene polymorphisms in asthma. Third, the present study is the first to address a putative association of *HNMT* or *ABP1* gene polymorphisms in AR. Fourth, this study provides useful data on allele frequencies for the polymorphisms analysed because the information available on such allele frequencies in healthy subjects is scarce. Finally, this study analyses whether detrimental mutations in one enzyme may induce a functional pressure that modifies allele frequencies in the other enzyme.

Materials and methods

Study population

We studied a cohort of 270 Caucasian unrelated consecutive patients with allergic asthma, and/or AR recruited in the Allergy Department, Hospital Infanta Cristina, (Badajoz, Spain).

The clinical characterization included questionnaires on allergic diseases and pharmacological therapy, familial antecedents (defined as the presence of disease in a first- or second-degree relative), age, gender, determination of total and specific IgE, haemogram with determination of eosinophils, skin tests to common food or environmental allergens, pulmonary function tests, smoking and drinking habits. All patients were diagnosed by an allergy specialist and had a positive skin prick test (defined as a

weal diameter 3 mm greater than the negative control) [18] for one or more inhalant allergens. All patients diagnosed with allergic asthma had clinical symptoms and physical examinations compatible with asthma and they showed airway reversibility as documented by an inhalant bronchodilator-induced improvement of >12% of forced expiratory volume in 1 s (FEV₁) and/or an airway hyper-reactivity methacholine-positive test. The equations used for lung function were those described for Spanish individuals [19]. AR was defined by clinical features and a classical history of rhinitis (nasal obstruction, sneezing, itching and watery discharge) with positive skin tests to one or more inhalant allergens and correlation with other positive diagnostic tests as described elsewhere [20].

A total of 295 healthy unrelated controls ethnically matched with patients and from the same geographic area were recruited. All individuals were Caucasian. Control subjects were selected among medical students and the staff of the University and Hospital participating in the study. Medical examination and history were obtained from each individual to exclude preexisting disorders. Subjects with personal or familial (up to second-degree relatives) antecedents of autoimmune or allergic diseases, positive prick tests or high IgE levels (over 100 IU/mL) were excluded. The protocol was approved by the Ethics Committee of the Hospital Infanta Cristina (Badajoz, Spain), and all patients and controls were included in the study after informed consent was obtained. All patients and over 95% of the healthy subjects agreed to participate in the present study. Possible sources of bias related to differences in age or allergen exposure are unlikely because patients and control subjects has similar mean ages and geographic origin. Regarding genetic polymorphisms, it has been shown that the frequencies for genetic variation for several genes are not modified by age [21]. Still, a putative source of bias related to occupational exposure cannot be ruled out. Table 1 summarizes the characteristics of the individuals included in the study.

Genotyping

Genomic DNA was obtained from peripheral leucocytes and purified according to standard procedures. The presence of SPNs was investigated by amplification restriction and electrophoresis in agarose gels. The analysis of the *HNMT* mutation leading to the Thr105Ile substitution was carried out after the amplification of a gene fragment comprising exon 4 using the following primers: GAA AAA CGT TCT TTC TAT CTG TTT GTA TAT AA and ATT TGG GCA GAT CAT GGT CAC TTG T. After an initial step of 2 min at 94 °C, polymerase chain reaction (PCR) amplification was carried out for 40 cycles of 25 s at 94 °C, 1 min at 52 °C and 1 min at 72 °C, and a final extension of 5 min at 72 °C. The 394 base pairs (bp) PCR product was

Table 1. Characteristics of the individuals included in the study

	Patients with asthma	Patients with rhinitis	Healthy subjects
Men	73 (45.9)	122 (47.3)	135 (45.8)
Women	86 (54.1)	136 (52.7)	160 (54.2)
Age $\pm$ SD (range)	30.2 $\pm$ 13.6 (14–79)	29.3 $\pm$ 12.8 (2–79)	27.5 $\pm$ 14.6 (19–84)
Smokers (%)	56 (35.2)	103 (40.0)	98 (33.2)
Familial antecedents (%)	74 (46.5)	118 (45.7)	0

The sum of patients with asthma and rhinitis exceeds 270 because 149 patients had asthma and rhinitis. The category smokers include ex-smokers.

Table 2. HNMT and ABP1 polymorphism in patients with allergic diseases and healthy controls

	No. (%; 95% CI; <i>P</i>)		
Genetic polymorphism	Asthma (<i>n</i> = 159)	Rhinitis (<i>n</i> = 258)	Healthy subjects (<i>n</i> = 295)
HNMT Thr/Thr	115 (72.3; 65.4–79.3; <i>P</i> = 0.130)	199 (77.1; 72.0–82.2; <i>P</i> = 0.668)	232 (78.6; 74.0–83.3)
HNMT Thr/Ile	37 (23.3; 16.7–29.8; <i>P</i> = 0.367)	50 (19.4; 14.6–24.2; <i>P</i> = 0.934)	58 (19.7; 15.1–24.2)
HNMT Ile/Ile	7 (4.4; 1.2–7.6; <i>P</i> = 0.082)	9 (3.4; 1.3–5.7; <i>P</i> = 0.180)	5 (1.7; 0.2–3.2)
ABP1 His/His	76 (47.8; 40.0–55.6; <i>P</i> = 0.335)	132 (51.2; 45.1–57.3; <i>P</i> = 0.746)	155 (52.5; 46.8–58.2)
ABP1 His/Asp	68 (42.8; 35.1–50.5; <i>P</i> = 0.771)	104 (40.3; 34.3–46.3; <i>P</i> = 0.803)	122 (41.3; 35.7–47.0)
ABP1 Asp/Asp	15 (9.4; 4.9–14.0; <i>P</i> = 0.192)	22 (8.5; 5.1–11.9; <i>P</i> = 0.272)	18 (6.1; 3.4–8.8)

The sum of patients with asthma and rhinitis exceeds 270 because 149 patients had asthma and rhinitis. *P*-values correspond to the comparison with healthy subjects.

HNMT, histamine *N*-methyltransferase; ABP1, amiloride binding protein 1; CI, confidence interval.

incubated with the endonuclease *EcoRV*, which digests the mutated allele in two fragments of 179 and 215 bp, leaving intact the wild-type sequence [16].

Regarding the SNP within the *ABP1* gene causing the His645Asp substitution, the following primers were used: GGT CAC CTG AAC CCG GTT AAC and TTG TGA CCT CTG AAC TTG CCG. After an initial step of 2 min at 94 °C, PCR amplification was carried out for 40 cycles of 25 s at 94 °C, 1 min at 61 °C and 1 min at 72 °C and a final extension period of 5 min at 72 °C. The amplified 476 bp fragment contains a constitutive restriction site for the endonuclease *AvaII*, and a variant-allele specific restriction site. After endonuclease digestion, the wild-type gene yields fragments of 369 and 107 bp whereas the mutated gene is digested to fragments of 252, 117 and 107 bp [16].

Statistical analysis

Case-control analyses were performed with the χ^2 statistics or Fisher's exact test, when appropriate. The association between *HNMT* and *ABP1* mutations and the risk of developing allergic diseases was estimated by an odds ratio (OR) with a 95% confidence interval (CI) by dividing individuals into two groups: Carriers of variant alleles either in heterozygosity or homozygosity, and non-carriers of variant alleles. The χ^2 values and the statistical significance of the differences of clinical or analytical parameters among individuals with different genotypes and the gene-dose effects were calculated

using the non-parametric Kruskal–Wallis (K–W) test. Potential confounders considered were gender, age, smoking habits or familial antecedents of the disease. Differences were considered to be significant when *P*-values were under 0.05. Statistical analysis was performed using the statistical package for the social sciences (SPSS) version 10.07 for Windows (SPSS Inc., Chicago, IL, USA). The statistical power was evaluated as described elsewhere [22] with a genetic model analysing the frequency for carriers of the disease gene with a RR value = 2, (α = 0.05), as recommended for pharmacogenomic studies [23, 24]. The power calculated for bilateral associations is as follows: association with the *HNMT* polymorphism, 88% and 95% for asthma and rhinitis, respectively, and association with the *ABP1* polymorphism, 94% and 98% for asthma and rhinitis, respectively.

Results

Association of variant alleles with the risk of developing allergic diseases

No association between *HNMT* and *ABP1* allelic variants was observed in any of the groups of patients or in the control subjects, and all genotypes were in Hardy–Weinberg equilibrium. The frequency analyses for the non-synonymous *HNMT* and *ABP1* polymorphisms are summarized in Tables 2 and 3. The *HNMT* amino acid change (Table 2) was distributed equally between patients

Table 3. IgE plasma levels according HNMT and ABP1 polymorphisms

Nonsynonymous polymorphism	IgE/mL: median; mean, SD (95% CI; <i>P</i>)	
	Asthma	Rhinitis
HNMT Thr/Thr	219; 328.6, 257.5 (252.7–404.5)	144; 265.4, 249.5 (219.5–311.2)
HNMT Thr/Ile	292; 545.9, 464.3 (351.7–740.1; <i>P</i> = 0.130)	286; 512.3, 565.7 (364.1–660.5; <i>P</i> = 0.032)
HNMT Ile/Ile	177; 218.6, 152.7 (94.8–342.3; <i>P</i> = 0.483)	177; 178.8, 29.1 (156.4–201.2; <i>P</i> = 0.430)
ABP1 His/His	207; 486.7, 439.2 (363.3–610.1)	197; 398.4, 249.7 (320.9–475.8)
ABP1 His/Asp	169; 238.2, 345.6 (170.1–306.4; <i>P</i> = 0.001)	147; 200.6, 204.4 (154.0–247.1; <i>P</i> = 0.001)
ABP1 Asp/Asp	96; 142.3, 144.6 (62.2–222.4; <i>P</i> = 0.017)	144; 175.2, 152.8 (107.4–243.0 = <i>P</i> = 0.023)

IgE values are expressed as IU/mL. *P*-values correspond to the comparison with individuals lacking mutations.

HNMT, histamine *N*-methyltransferase; ABP1, amiloride binding protein 1; SD, standard deviation; CI, confidence interval.

and control subjects, and no statistically significant differences were observed either for patients with asthma or rhinitis, as compared with healthy individuals. The variant *HNMT* allele frequencies were slightly higher among patients with asthma (16.0%, 95% CI 12.0–20.0, *P* = 0.054) and among patients with rhinitis (13.2%, 95% CI 10.3–16.1, *P* = 0.403) as compared with healthy subjects (11.5%, 95% CI 8.9–14.1). The OR (95% CI) for carriers of variant *HNMT* alleles is 1.4 (0.9–2.2) for patients with asthma and 1.1 (0.7–1.6) for patients with rhinitis. Among rhinitis patients, the OR were 1.0 for patients with rhinitis only and 1.1 for patients with asthma and rhinitis.

Regarding the *ABP1* polymorphism (Table 2), no statistically significant differences were observed between patients with asthma or rhinitis, as compared with healthy subjects. The variant *ABP1* allele frequencies were similar among patients with asthma (30.8%, 95% CI 25.7–35.9, *P* = 0.197) and among patients with rhinitis (28.7%, 95% CI 24.8–32.6, *P* = 0.484) and among healthy subjects (26.8%, 95% CI 23.2–30.3). The OR (95% CI) for carriers of variant *ABP1* alleles is 1.2 (0.8–1.8) for patients with asthma and 1.1 (0.8–1.5) for patients with rhinitis. Among rhinitis patients, the OR were 1.0 (0.7–1.3) for patients with rhinitis only and 1.1 (0.7–1.5) for patients with asthma and rhinitis.

Because both *HNMT* and *ABP1* enzymes are involved in histamine metabolism, we analysed the frequency of the combination of both amino acid polymorphisms. For this, individuals were divided into four groups according to whether they were carriers of mutations in the *HNMT* or *ABP1* genes. Although the percentage of individuals carrying *HNMT* or *ABP1* allelic variants, either isolated or combined, was higher among patients with asthma than among healthy subjects, the differences were not statistically significant. A percentage of carriers of *HNMT*-only (12.6% and 12.0%) or *ABP1*-only variant alleles (37.1% and 38.0%) or in the frequency for carriers of *HNMT* plus *ABP1* variant alleles (15.7% and 10.9%) was observed for patients with asthma and patients with rhinitis, respectively. The percentage of carriers of variant alleles among healthy subjects was *HNMT* only (9.8%),

ABP1 only (35.2%) and *HNMT* plus *ABP1* (11.9%). Among patients with rhinitis, no differences in the percentage of carriers of *HNMT* (11.6% and 12.2%) or *ABP1* variant alleles (36.8% and 38.9%) or in the frequency for carriers of *HNMT* plus *ABP1* variant alleles (10.6% and 11.12%) were observed for patients with rhinitis only and patients with asthma and rhinitis, respectively.

Association of variant alleles with clinical and analytical parameters

Because the data shown in Table 2 indicated a higher frequency of variant alleles among patients, we determined whether factors like gender, age and clinical data such as eosinophilia or IgE levels might be related to the association between polymorphisms and asthma or rhinitis. There was no evidence of gender, age, smoking habits or familial antecedent-dependent differences for allele frequencies in the asthma and rhinitis groups of patients (not shown). Patients with asthma who were carriers of mutations either to the *HNMT* or to the *ABP1* gene variants tended to have similar scores in pulmonary function tests, with mean volume of air expired in the first second during maximal expiratory effort (FEV₁), expressed as percentage of the actual value compared with standard values for Spanish individuals [19] as follows: 101.4%, 98.9% and 99.2% for patients with the *HNMT* Thr/Thr, Thr/Ile and Ile/Ile genotypes, respectively, and 104.4%, 90.6% and 99.2% for patients with the *ABP1* genotype His/His, His/Asp and Asp/Asp, respectively. The percentage of the vital capacity that expired in the first second of maximal expiration (FEV₁/FVC) values were 88.6%, 92.5% and 86.2% for patients with the *HNMT* Thr/Thr, Thr/Ile and Ile/Ile genotypes, respectively, and 88.8%, 87.1% and 91.2% for patients with the *ABP1* genotype His/His, His/Asp and Asp/Asp, respectively. None of the lung function values observed among patients with mutations indicated statistically significant differences when compared with values from patients carrying no mutations.

Table 4. Frequency of presentation of eosinophilia according HNMT and ABP1 polymorphisms

Non-synonymous polymorphism	Percentage of eosinophilia patients (95% CI; <i>P</i>)	
	Asthma	Rhinitis
HNMT Thr/Thr	22.4 (15.9–28.8)	15.2 (11.0–19.8)
HNMT Thr/Ile	25.8 (19.0–32.6; <i>P</i> = 0.701)	23.0 (17.8–28.1; <i>P</i> = 0.179)
HNMT Ile/Ile	33.0 (25.7–40.3; <i>P</i> = 0.533)	20.2 (15.3–25.1; <i>P</i> = 0.688)
ABP1 His/His	22.5 (16.0–29.0)	14.0 (9.7–18.2)
ABP1 His/Asp	30.2 (23.1–37.3; <i>P</i> = 0.344)	25.0 (19.7–30.2; <i>P</i> = 0.032)
ABP1 Asp/Asp	0 (0–0.1; <i>P</i> = 0.025)	0 (0–0.1; <i>P</i> = 0.020)

Eosinophilia was defined as an eosinophils count over 400/μL or over 6% of white cells.

P-values correspond to the comparison with individuals lacking mutations.

HNMT, histamine *N*-methyltransferase; ABP1, amiloride binding protein 1.

When patients were stratified on the basis of analytical data, statistically significant differences were observed, as summarized in Table 3. Individuals carrying the ABP1 Asp polymorphism tended to have significantly lower IgE plasma levels. The median and mean values are shown in Table 3. The mean IgE values indicate statistically significant differences related to the ABP1 polymorphisms. The K–W test yielded a χ^2 value = 17.32 (*P* < 0.001) and 20.33 (*P* < 0.001) for a gene–dose effect in patients with asthma and rhinitis, respectively. This effect was not observed in healthy subjects. We observed a statistically significant difference in the IgE levels in patients with rhinitis carrying the HNMT Thr/Ile genotype, which was attributable to patients with rhinitis alone and was not present among patients with asthma and rhinitis. Such a difference is marginal, and it is in contradiction with previous reports [14], and therefore it may be due to chance. For the rest of the patients, no association of IgE levels with the HNMT polymorphism was observed.

Eosinophilia, defined as an eosinophil count over 400/μL or over 6% of white cells, was analysed in relation to the HNMT and ABP1 polymorphisms. No relation between the HNMT polymorphism was observed either for patients with asthma or rhinitis, and among rhinitis patients, the lack of association occurred regardless of whether patients also had asthma or not. Among homozygous subjects for the variant *ABP1* gene, statistically significant differences were observed (Table 4). Whereas 23.7% of patients with asthma had eosinophilia, none of the 14 patients with a homozygous *ABP1* genotype had eosinophilia. With regard to patients with rhinitis, 20.6% of them had eosinophilia, and none of the 22 patients with a homozygous *ABP1* genotype had eosinophilia. Among these 22 patients, 14 had asthma plus rhinitis and eight had rhinitis alone.

Discussion

The results obtained in the study indicate that the polymorphism of the *ABP1* enzyme, involved in the degrada-

tion of circulating histamine, may influence the clinical presentation of asthma or AR. Individuals who are carriers of *ABP1* alleles tend to develop clinical symptoms even though analytical parameters are not severely altered. These findings are consistently observed in patients with asthma or rhinitis, and agree with our findings indicating that the same *ABP1* polymorphism is related to a severe clinical course of ulcerative colitis, another disorder highly related to immunity [16], and suggest that the clinical course of allergic and other diseases related to high histamine levels [25] may be altered in patients with impaired histamine metabolism.

This study provides additional support for a key role of histamine in the development of symptoms of allergic asthma and rhinitis. However, it cannot be ruled out that the association of SNPs with some clinical aspects of allergic diseases, or the controversial association of the HNMT polymorphism with asthma may be due to other genes linked to *HNMT* or *ABP1* in these diseases. To obtain data on such a hypothesis, we performed an analysis of the SNP associations in the areas where *HNMT* and *ABP1* genes are located using the Marker application, <http://www.gmap.net/marker> using data from a population consisting of Utah residents with ancestry from northern and western Europe [26]. Figure 1a shows that the *HNMT* gene displays several linked SNPs, as has been described elsewhere [9], and that there is little association of these SNPs with other SNPs in close genes. The SNP analysed in the present study (Fig. 1a) does not show a relevant SNP association beyond the *HNMT* gene. Figure 1b shows that in the area where *ABP1* and close genes are located, the SNP association is negligible, thus suggesting that the findings obtained in the present study are directly related to *ABP1*, and not to an association with close genes. The lack of association of the polymorphisms analysed and pulmonary function tests among patients suffering from asthma is expected, because it has been shown that plasma histamine is not related to the severity of airways' obstruction [27, 28].

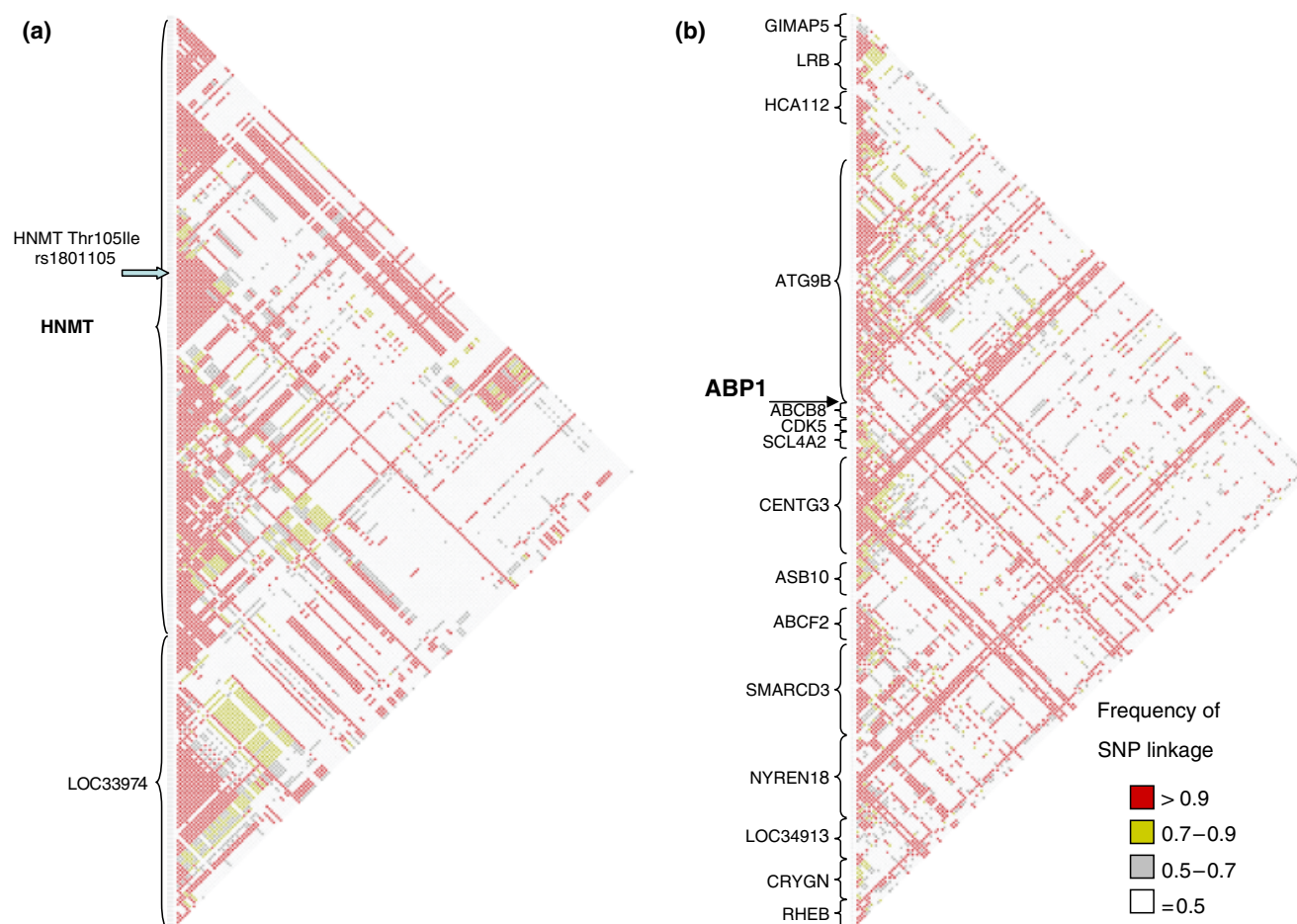


Fig. 1. Measure of linkage disequilibrium of single-nucleotide polymorphisms (SNPs) within genes in the area where histamine *N*-methyltransferase (*HNMT*; a) and amiloride binding protein 1 (*ABP1*; b) genes are located. Linkage disequilibrium was calculated for pairs of biallelic loci as described elsewhere [41]. The analysis was carried out on a population consisting of Utah residents with ancestry from northern and western Europe [26]. The figure was elaborated with the Marker application, <http://www.gmap.net/marker>.

In previous studies, controversial findings on a putative role of the *HNMT* Thr105Ile polymorphism were reported. The present study is the first to address the role of non-synonymous *ABP1* polymorphism in patients with asthma or AR, thus permitting the simultaneous analysis of both histamine-metabolizing enzymes. In addition, the present study addresses an as yet unexplored topic: the role of *HNMT* polymorphism in AR. The findings obtained in the present study indicate a non-significant increase in the frequency for variant *HNMT* and *ABP1* alleles among patients with asthma or rhinitis, thus indicating that these parameters are not major susceptibility factors for the development of any of these disorders. This is consistent with the findings reported by other authors for patients with allergic asthma [13]. In addition, in agreement with the findings reported in the present study, it has been shown that *HNMT* polymorphisms are not related to IgE concentration in patients with asthma [14].

The aetiology of immune diseases seems to be related to gene–environmental interaction. Multiple environmental

factors are known to cause or modulate allergic disorders [29], and the involvement of a common set of susceptibility genes has been proposed [30, 31]. However, little is known about the genetic components related to the susceptibility to react to environmental allergens. Several studies have analysed candidate atopy and asthma genes, with little success in the identification of genetic factors clearly involved in such diseases. Clustering studies indicated several regions related to asthma or inflammatory diseases [30, 32]. A total of 16 clustered and three non-clustered asthma loci have been reported in chromosomes 4, 7, 11, 13 and 16 [30], thus suggesting that there may be a genetic background in inflammatory diseases. However, the genes have not been identified. In a study analysing microsatellite markers and SNPs in 24 candidate genes, including cytokines, growth factors, hormones and enzymes that have been implicated in asthma [31, 33, 34], none of the gene variations analysed displayed an association with atopic asthma [31]. The influence of genetic variability in the development of inflammatory diseases

can be explored from another perspective. Because histamine is metabolized by two polymorphic enzymes, it may be hypothesized that individuals with impaired ability to metabolize histamine may be more prone to developing histamine-related symptoms and therefore to showing inflammatory diseases. Such an effect may be valid both in tissues where histamine is released and in the rest of the body in the event that histamine plasma levels are increased in patients with atopic diseases. Although histamine plasma levels are highly variable due to circadian rhythm and interindividual variability in patients with allergic diseases [35–37], several independent studies indicate an increase of histamine and a decrease of the histamine metabolite *N*-methylhistamine in plasma among patients with asthma, as compared with healthy subjects [27, 36, 38, 39]. Histamine plasma levels significantly increase during the early asthmatic response to allergens [39]. In addition, histamine causes nasal airway exudation in subjects with seasonal rhinitis [40]. Thus, it may be speculated that individuals with altered histamine biodisposition may display atopic symptoms even though the amount of histamine released is not high. It is noteworthy that patients carrying mutated *ABP1* genes tended to have more manifestations of asthma although they show lower IgE levels in Table 3. This apparent controversy may be explained if these patients tend to develop clinical symptoms with a lower IgE stimulation. The occurrence of genetic factors that may modulate clinical response may be of great importance to prevent clinical symptoms in patients with histamine-related diseases. The identification of such genetic factors may lead to the use of simple genetic tests capable of identifying individuals with a genetic predisposition to develop severe symptoms.

The findings obtained in the present study open up the possibility for the use of *ABP1* polymorphism as a factor related to the clinical presentation of histamine-related diseases. Nevertheless, because the present study is the first to analyse the influence of the *ABP1* polymorphism on allergic diseases, our findings require independent confirmation in patients with other immune diseases. Further studies should focus on the role of *HNMT* and *ABP1* polymorphisms in other histamine-related diseases, with special focus on those causing life-threatening symptoms such as serious anaphylactic reactions.

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