Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2012;159:109–120
DOI: 10.1159/000335919

Received: August 10, 2011
Accepted after revision: December 19, 2011
Published online: May 30, 2012

Asthma Endophenotypes and Polymorphisms in the Histamine Receptor *HRH4* Gene

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Key Words
Single nucleotide polymorphism · Histamine receptor H4 · Infection-induced asthma

Abstract
Background: Histamine as an inflammatory mediator plays an important role in chronic allergic and asthmatic conditions. However, the role of genetic polymorphisms of the histamine receptor *HRH4* (histamine receptor H4) gene in asthma susceptibility and endophenotypes has not been studied yet. Our aim was to investigate the possible association between single nucleotide polymorphisms (SNPs) in the *HRH4* gene and asthma or some endophenotypes of asthma. ***Methods:*** Twenty-one SNPs of the *HRH4* gene were genotyped in 313 asthmatic patients and 360 controls using Sequenom® iPLEX® Gold Genotyping Technology. ***Results:*** Genotype distribution of three *HRH4* SNPs, namely rs17187619 [p = 0.002; odds ratio, OR (95% confidence interval, CI) = 2.4 (4.1–1.4)], rs527790 [p = 0.0002; OR (95% CI) = 3.3 (6.1–1.8)] and rs487202 [p = 0.00007; OR (95% CI) = 3.5 (6.6–1.9)] differed significantly between patients with or without infection-induced asthma. Haplotypes, which included the rs4800573–rs527790 CC allele combination, were found to be associated with infection-induced asthma [p = 0.0009, OR (95% CI) = 0.5 (0.4–0.8)]. The rs487202–rs574913 CA haplotype was more frequent among patients with infection-induced asthma [p = 0.0006, OR (95% CI) = 1.9 (1.3–2.6)]. None of the SNPs contributed directly to the risk of asthma. ***Conclusions:*** Our results suggest that genetic variation in the *HRH4* gene might influence the pathogenesis of infection-induced asthma.

Introduction

Asthma is a highly prevalent chronic inflammatory disease of the airways worldwide. This complex syndrome is characterized by variable and recurring symptoms including reversible airflow obstruction, bronchospasm, airway hyperresponsiveness and chronic inflammation of the airways. Clinically it is diagnosed on the

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basis of the symptoms of wheeze, dyspnea, cough, chest tightness and shortness of breath.

Asthma is a multifactorial disease with genetic, environmental, allergic, infectious, nutritional and emotional components contributing to its inception and evolution. The complexity of the disease originates from the abovementioned factors. Recent genetic studies suggest the role of an unknown number of genes with moderate effects in the pathomechanism of asthma [1].

Asthma can be manifested in different phenotypes. These phenotype categories can be defined by clinical and physiological criteria, and by environmental triggers and their pathobiology. The most common phenotype in children is allergic asthma, triggered by an allergic sensitization associated with the presence of a specific IgE [2]. Allergen-induced asthma can be categorized by certain features such as allergy of one or more environmental antigens (mono/poly) or based on the type of allergen: inhalative, indoor or outdoor. Non-allergic asthma can be caused by anything other than allergens. It may be triggered by inhalation of chemicals such as cigarette smoke or cleaning agents, taking aspirin, a chest infection, stress, laughter, cold air, food preservatives or a myriad of other factors. Exercise can also induce an asthma attack in people who have no other triggers and do not experience asthma under any other circumstances. Respiratory infections provoked by bacteria or viruses can also trigger asthma (called infection-induced asthma). Asthma can frequently occur together with other allergic diseases, such as allergic rhinitis or conjunctivitis, with confirmed links between these diseases [3].

Histamine is known to be an inflammatory mediator which contributes to the progression of allergic-inflammatory responses by enhancement of the secretion of proinflammatory cytokines and chemokines [4]. A variety of physiological and pathological functions of histamine are mediated by four different receptors: HRH1, HRH2, HRH3 and HRH4 (histamine receptor H1–4), among which types 1, 2 and 4 have been proven to affect inflammation and other immune processes and have been proposed as useful targets for the treatment of immune and inflammatory diseases [5, 6].

HRH4, similarly to all other histamine receptors, is a constitutively active, G protein-coupled molecule expressed mainly on immune cells, including mast cells, monocytes, eosinophils, basophils, dendritic cells, T cells and natural killer cells [7, 8]. However, it was recently demonstrated that HRH4 was functionally expressed on neurons in the mammalian central nervous system [9].

HRH4 is considered to have a role in a number of inflammatory disorders such as allergy, asthma, chronic pruritus and autoimmune diseases [10]. HRH4 ligands have shown efficacy in dampening airway inflammation and reducing asthma-like symptoms, although the exact mechanism of this function is unknown [11]. Administration of an HRH4 antagonist (JNJ7777120) to HRH4 genetically deficient animals has been demonstrated to diminish asthma-like parameters [12]. Interestingly, treatment with the HRH4 agonist 4-methylhistamine resulted in mitigation of airway hyperreactivity and inflammation [13]. Based on these studies HRH4 may mediate pro- or anti-inflammatory signals in asthmatic conditions and on the whole ligands for this histamine receptor may prove beneficial for the relief of asthma symptoms and could become effective therapeutic tools in the future.

Although genetic polymorphisms can alter gene expression or receptor functions, there have so far only been two studies about genetic variations in the *HRH4* gene [14, 15]. In our study we investigated whether *HRH4* gene polymorphisms might play a role in asthma progression or influence some phenotype categories of the disease.

Materials and Methods

Patients

The 313 children analyzed in this study were of Hungarian (Caucasian) origin and diagnosed with asthma bronchiale by specialist physicians based on the following characteristics: (1) recurrent breathlessness and expiratory dyspnea requiring treatment; (2) wheeze; (3) reversibility of wheezing and dyspnea by bronchodilator treatment measured as forced expiratory volume 1s (FEV_1) by a spirometer. The asthmatic cohort consisted of 203 male and 110 female patients with a mean age at diagnosis of 10.5 years. The characteristics of the study population are shown in table 1. Clinical data of the patients were collected using a questionnaire based on ISAAC (International Study of Asthma and Allergies in Childhood) questionnaires (http://isaac.auckland.ac.nz/) completed by the physician together with the parents of the patients. We analyzed the clinical data of the patients regarding the endophenotypes of asthma. Characterization of the patients was based on the decision of a specialist physician.

The control group was composed of 360 subjects (181 males, 179 females, mean age 21.7 years), and were healthy adult blood donors from the Hungarian National Blood Transfusion Service and minor outpatients from either the Orthopedic Department of the Budai Children's Hospital or the Urological Department of Heim Pal Hospital, Budapest. Clinical data of the controls were collected with the same questionnaire as used for the patient group. They were of the same ethnicity and geographical region as the patients of the asthmatic group and had no history of asthma or other allergic diseases.

Table 1. Characteristics of the study population

	Characteristics	Mean ± SD	Category 0	Category 1	Category 2	Category 3
Asthmatics (n = 313)	age	10.5 ± 4.8				
	gender [male (0)/female (1)]		110 (35)	203 (65)		
	GINA (no/1/2/3)		68 (22)	52 (17)	168 (54)	25 (8)
Allergen-induced asthma	allergy (no/yes)		117 (37)	196 (63)		
	mono- or poly-allergen [no (0)/ mono (1)/poly (2)]		128 (41)	23 (7)	162 (52)	
	inhalative allergen (no/yes)		128 (41)	185 (59)		
	outdoor allergen [no (0)/yes (1)]		166 (53)	147 (47)		
	indoor allergen [no (0)/yes (1)]		169 (54)	144 (46)		
Exercise-induced asthma	no (0)/yes (1)		249 (80)	64 (20)		
Infection-induced asthma	no (0)/yes (1)		224 (72)	89 (28)		
Nonallergic asthma	no (0)/yes (1)		253 (81)	60 (19)		
Other diseases	rhinitis [no (0)/yes (1)]		202 (65)	111 (35)		
	conjunctivitis [no (0)/yes (1)]		253 (81)	60 (19)		
Total IgE level	normal (0)/high (1)	281.0 ± 312.1[a]	46 (38)	76 (62)		
Absolute eosinophil cell count, g/l	normal (0)/high (1)	0.4 ± 0.3[b]	35 (31)	78 (69)		
Eosinophil	normal (0)/high (1)	5.4 ± 4.2[c]	70 (59)	49 (41)		
Controls (n = 360)	age	21.7 ± 13.9				
	gender [male (0)/female (1)]		179 (50)	181 (50)		

Values in each category are number of patients with percentages in parentheses.
[a] Measured as kU/l. [b] Measured as g/l. [c] Values are percentages.

The study was conducted according to the principles of the Declaration of Helsinki, and approved by the Ethics Committee of the Hungarian Medical Research Council. Written informed consent was obtained from all patients or the parents or guardians of the minors involved in the study.

In our study we analyzed some phenotypes of asthma based on the environmental trigger of asthma exacerbation. These were the allergen-induced, exercise-induced, infection-induced and nonallergic types of asthma. Allergen-induced asthma was defined by certain features such as allergy to one or more environmental antigens (mono/poly) or based on the type of allergen: inhalative, indoor or outdoor. Allergy was defined by a skin prick test positive for at least one allergen (with wheal diameter 3 mm greater than the saline control) and/or positive total or specific IgE levels. Serum IgE levels were classified as normal or high according to the following age-specific reference ranges (kU/l): 0–1 year: <15; 1–5 years: <60; 5–10 years: <90, and adult: <100. Exercise-induced asthma was defined on the basis of patients experiencing asthma exacerbations in a running test or during any physical activity. If the onset of asthma or asthma exacerbations were associated with an infection-linked acute respiratory illness, the asthma was classified as infection-induced asthma. Non-allergic asthma was diagnosed in cases where an asthmatic attack had no relation to allergen challenge or infection. We also studied the expression of other diseases such as allergic rhinitis and allergic conjunctivitis. Rhinitis was defined by troublesome sneezing or blocked or runny nose severely affecting the well-being of the patient during periods without a common cold or flu. Only those subjects whose rhinitis status was verified by specialists were involved in this dataset. Rhinoconjunctivitis-like symptoms were defined as an itchy, runny or stuffy nose, or sneezing and/or red, burning or weeping eyes occurring at random time points almost every day or from time to time, except when occurring during a respiratory infection.

The distinction between mild and moderate asthma and phenotypes of asthma was made according to the GINA classification (National Institute of Health: Global Strategy for Asthma Management and Prevention, 2010, http://www.ginasthma.org). Asthmatic patients could be diagnosed to belong to more than one endophenotype category as can be seen in table 1.

Methods

Genomic DNA was isolated from peripheral blood using the iPrep PureLink gDNA Blood Kit (Invitrogen, Carlsbad, Calif., USA) according to the manufacturer's instructions. Single nucleotide polymorphisms (SNPs) of the *HRH4* gene were selected with minor allele frequency of more than 9% from the allelic frequency data of the HapMap database using HapMap Genome Browser release No. 27 and the CEU population (CEPH: Utah residents with ancestry from northern and western Europe; http://hapmap.ncbi.nlm.nih.gov/). We covered the whole gene with these SNPs, the main features of which are summarized in table 2.

Table 2. Characteristics of the investigated *HRH4* SNPs, and genotype and allele frequencies in the asthmatic and control populations

SNP characteristics				Asthma				Control			
SNPs	alleles	function	position	genotype 11	genotype 12	genotype 22	MAF	genotype 11	genotype 12	genotype 22	MAF
rs17203314	A/G	promoter	20291455	221 (71)	76 (24)	8 (3)	0.15	262 (73)	72 (20)	4 (1)	0.12
rs524149	C/T	promoter	20292322	258 (82)	44 (14)	5 (2)	0.09	300 (83)	44 (12)	0 (0)	0.06
rs615283	G/A	promoter	20294493	217 (69)	73 (23)	13 (4)	0.16	227 (63)	98 (27)	10 (3)	0.18
rs880263	C/T	intron	20296661	224 (72)	76 (24)	6 (2)	0.14	265 (74)	76 (21)	3 (1)	0.12
rs1421119	T/C	intron	20297288	171 (55)	112 (36)	23 (7)	0.26	193 (54)	134 (37)	17 (5)	0.24
rs623590	C/T	intron	20297351	258 (82)	43 (14)	4 (1)	0.08	299 (83)	43 (12)	0 (0)	0.06
rs4483927	T/G	intron	20298398	171 (55)	112 (36)	23 (7)	0.26	193 (54)	133 (37)	18 (5)	0.25
rs17797945	C/T	intron	20300654	170 (54)	108 (35)	28 (9)	0.27	175 (49)	138 (38)	28 (8)	0.28
rs8088140	C/A	intron	20303138	175 (56)	116 (37)	16 (5)	0.24	208 (58)	120 (33)	15 (4)	0.22
rs17187619	T/C	intron	20303578	180 (58)	113 (36)	14 (4)	0.23	203 (56)	127 (35)	14 (4)	0.23
rs104000	A/G	intron	20305077	225 (72)	73 (23)	9 (3)	0.15	241 (67)	94 (26)	9 (3)	0.16
rs643552	T/C	intron	20306557	172 (55)	111 (35)	24 (8)	0.26	195 (54)	126 (35)	23 (6)	0.25
rs657132	G/A	intron	20307272	244 (78)	56 (18)	6 (2)	0.11	287 (80)	56 (16)	0 (0)	0.08
rs11665084	C/T	A138V	20310764	248 (79)	53 (17)	6 (2)	0.11	288 (80)	52 (14)	4 (1)	0.09
rs11662595	A/G	H206R	20310968	246 (79)	54 (17)	6 (2)	0.11	288 (80)	51 (14)	4 (1)	0.09
rs1421125	C/A	3′ UTR	20311909	116 (37)	144 (46)	47 (15)	0.39	129 (36)	154 (43)	58 (16)	0.4
rs17797975	T/C	3′ UTR	20313366	242 (77)	62 (20)	2 (1)	0.11	275 (76)	60 (17)	2 (1)	0.09
rs4800573	G/A	3′ UTR	20313668	236 (75)	61 (19)	9 (3)	0.13	240 (67)	93 (26)	9 (3)	0.16
rs527790	G/A	after HRH4	20314913	100 (32)	153 (49)	54 (17)	0.43	125 (35)	160 (44)	58 (16)	0.4
rs487202	C/G	after HRH4	20316814	99 (32)	150 (48)	53 (17)	0.42	120 (33)	155 (43)	56 (16)	0.4
rs574913	T/C	after HRH4	20317206	245 (78)	55 (18)	6 (2)	0.11	286 (79)	57 (16)	0 (0)	0.08

Asthma and control values are number of patients with percentages in parentheses. The indicated SNP characteristics are alleles (allele 1 and allele 2) with function and position according to the NCBI Genome Build 36.3. The genotype groups are indicated by: 11 = homozygote for the frequent allele; 12 = heterozygote; 22 = homozygote for the rare allele. MAF = Minor allele frequency.

Genotyping 21 of the SNPs was conducted using Sequenom® iPLEX® Gold Genotyping Technology (Sequenom, San Diego, Calif., USA) following the manufacturer's instructions at the McGill University and Génome Québec Innovation Centre (Montréal, Qué., Canada). This is a single-base primer extension followed by MALDI-TOF MS detection. Samples having SNP call rates <80% were excluded from the analysis. Genotyping quality control was evaluated through the inclusion of duplicate genotyping of one SNP for which >99% concordant results were obtained.

Total serum IgE levels and specific IgE levels of more than 100 allergens were determined by 3gAllergy blood tests in Immulite 2000 Immunoassay System (Siemens Healthcare Diagnostics, Deerfield, Ill., USA). The eosinophil cell counts were measured by Coulter MAXM Analyzer (Beckman Coulter, Krefeld, Germany).

Statistical Methods

The clinical and genotype data were analyzed as dichotomous variables. If the number of patients were sufficient ($n \geq 5$) in all genotype groups, the analyses were performed using additive (11 vs. 12 vs. 22), recessive (11/12 vs. 22) or dominant (11 vs. 12/22) models, with the common homozygotes signed as 11. Otherwise only the dominant model was used. Allele frequencies were calculated by allele counting and tested for deviation with the Hardy-Weinberg equilibrium by using the online software accessible at http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl. Multiple logistic regression analyses were completed, adjusted for age and gender, to assess the effect of the genetic background of asthma development and dichotomous clinical characteristics. Confidence intervals (CI) were calculated at the 95% level. General linear model procedures adjusted for age and gender were used to analyze the effect of the genetic background on dependent scale variables. Linkage disequilibrium (indicated with D' and r^2) and estimated haplotype frequency in cases and controls were calculated by Haploview 4.1 software: http://www.broad.mit.edu/mpg/haploview/. Haplotype blocks were generated for all the 21 studied SNPs of the *HRH4* gene and for 5 SNPs at the 3′ end of the studied region. Only those haplotypes were included where the difference between the haplotype frequencies in the two groups was more than 5%. The haplotype-specific odds ratio (OR) was estimated using logistic regression.

Bonferroni correction considering multiple testing for 21 SNPs was used. Alpha levels of $p < 0.0024$ were considered to be significant. Analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Ill., USA) and MedCalc 10.0.2.0 (MedCalc Software, Mariakerke, Belgium) programs. The power of the analyses was calculated using the Power and Sample Size Calculation 3.0 program.

Results

Study Population and Allele Frequencies

We investigated whether 21 SNPs in the *HRH4* gene showed association with asthma or some endophenotypes of asthma. The characteristics of the study population are shown in table 1 and the minor allelic and genotype frequencies are presented in table 2. Genotype distributions were in Hardy-Weinberg equilibrium for all SNPs in the population (table 2). The analyses performed on the whole population had adequate power (80% for $\alpha = 0.0024$) to detect loci with genotype ORs of 1.85 (MAF = 0.40) ranging to 2.87 (MAF = 0.06). Analyzing the subpopulations of asthma patients generated ORs of 3 (MAF = 0.50) ranging to 5 (MAF = 0.05), which were also detectable with adequate power (80% for $\alpha = 0.0024$).

By investigating asthma patients we found significant correlations between the genotypes and some endophenotypes of asthma. Analyses of *HRH4* SNPs among patients with or without infection-induced asthma showed that genotype distribution of three *HRH4* SNPs differed in these two groups of patients. The *HRH4* rs487202 GG genotype occurred more frequently among patients with infection-induced asthma than those without in an additive model [28 (33%) vs. 25 (12%), p = 0.0002, OR (95% CI) = 3.6 (6.9–1.8)] (table 3). This difference was even more pronounced when we compared patients with GG genotype to those with at least one C allele (recessive model) [28 (33%) vs. 25 (12%), p = 0.00007, OR (95% CI) = 3.5 (6.6–1.9)] (table 3).

rs527790 also associated with infection-induced asthma. Patients with infection-induced asthma had more rs527790 AA genotype compared to patients without this endophenotype of asthma [additive model: 28 (32%) vs. 26 (12%), p = 0.0004, OR (95% CI) = 3.3 (6.3–1.7); recessive model: 28 (32%) vs. 26 (12%), p = 0.0002, OR (95% CI) = 3.3 (6.1–1.8)] (table 3).

We could analyze the rs17187619 only using the dominant model because of the low number of patients with the rs17187619 CC genotype. There were fewer patients with at least one C allele among the infection-induced asthma group compared to patients without infection-induced asthma [25 (28%) vs. 102 (47%), p = 0.002, OR (95% CI) = 2.4 (4.1–1.4)].

We observed different genotype distributions in three *HRH4* SNPs when we compared asthmatic patients with or without allergic rhinitis (table 4). Patients with the rs1421125 AA genotype suffered more often from allergic rhinitis since this genotype was more frequent among patients with allergic rhinitis than the others in the study population [25 (23%) vs. 22 (11%), p = 0.005, OR (95% CI) = 2.7 (5.6–1.4)]. When we analyzed this SNP under the dominant model we could also observe this association [CC/CA 25 (23%) vs. AA 22 (11%), p = 0.004, OR (95% CI) = 2.5 (4.8–1.3)].

Genotype distribution was very similar for rs527790 and rs487202. Among allergic rhinitis patients, 27 (25%) carried the rare homozygote genotype rs527790 AA or rs487202 GG. Only 27 (14%) or 26 (13%) of asthmatic patients without allergic rhinitis had the rare homozygote genotype [p = 0.006, OR (95% CI) = 2.3 (4.3–1.3) for rs527790 and p = 0.006, OR (95% CI) = 2.4 (4.4–1.3) for rs487202, respectively].

Patients with conjunctivitis had less rs615283 GG genotype than patients without this coexisting disease [6 (10%) vs. 7 (3%), p = 0.008, OR (95% CI) = 4.8 (15.5–1.5)] (table 4).

When we used Bonferroni correction for the tests, associations with p values >0.0024 were only nominally significant. Therefore, only the association between infection-induced asthma and rs487202, rs527790 and rs17187619 can be considered as a true correlation.

Regarding the similarity of the genotype frequencies between rs487202 and rs527790, we calculated the linkage disequilibrium coefficients for rs487202 and rs527790 from the genotypes determined in our population. We found that these SNPs had a strong linkage disequilibrium ($D' = 1.0$, $r^2 = 0.9$; fig. 1).

The allelic and genotype distributions of asthmatics and controls were similar (table 2). There was no difference between patients with and those without allergy-induced asthma, even when we considered the type or number of allergens the individuals were sensitive to: mono- or poly-allergen, inhalative allergen, outdoor or indoor allergen. Patients with exercise-induced or non-allergic asthma, allergic rhinitis or allergic conjunctivitis did not have different allelic or genotype distributions compared with patients without this special endophenotype of asthma. The distributions of the SNPs did not correlate with the severity of asthma (GINA categories), IgE level, eosinophil count or eosinophil percentage (data not shown).

Haplotype Analysis

To ascertain whether haplotype blocks rather than single SNPs were responsible for the observed associations between this region and infection-induced asthma, we analyzed the haplotype block of 5 SNPs surrounding rs487202 and rs527790. We found that certain haplotypes were associated with infection-induced asthma (table 5).

Table 3. Genotype distribution of *HRH4* SNPs in patients with or without infection-induced asthma

SNP	Model	Alleles (1/2)	Infection-induced asthma	Genotype 11 (%)	Genotype 12 (%)	Genotype 22 (%)	n	p value	OR	95% CI
rs17203314	dominant	A/G	yes	69 (78)	19 (22)		88	0.2	0.6	1.2–0.4
			no	152 (70)	65 (30)		217			
rs524149	dominant	C/T	yes	77 (88)	11 (13)		88	0.3	0.7	1.5–0.3
			no	181 (83)	38 (17)		219			
rs615283	dominant	G/A	yes	58 (67)	29 (33)		87	0.1	1.5	2.6–0.9
			no	159 (74)	57 (26)		216			
rs880263	dominant	C/T	yes	71 (81)	17 (19)		88	0.1	0.6	1.0–0.3
			no	153 (70)	65 (30)		218			
rs1421119	dominant	T/C	yes	48 (55)	39 (45)		87	0.6	1.1	1.9–0.7
			no	123 (56)	96 (44)		219			
rs623590	dominant	C/T	yes	77 (88)	11 (13)		88	0.4	0.7	1.5–0.3
			no	181 (83)	36 (17)		217			
rs4483927	dominant	T/G	yes	48 (55)	39 (45)		87	0.6	1.1	1.9–0.7
			no	123 (56)	96 (44)		219			
rs17797945	additive	C/T	yes	48 (55)	28 (32)	12 (14)	88	0.2	1.8	4.1–0.8
			no	122 (56)	80 (37)	16 (7)	218			
rs17797945	dominant	C/T	yes	48 (55)	40 (45)		88	1.0	1.0	1.7–0.6
			no	122 (56)	96 (44)		218			
rs8088140	additive	C/A	yes	53 (60)	28 (32)	7 (8)	88	0.2	1.9	5.5–0.7
			no	122 (56)	88 (40)	9 (4)	219			
rs8088140	dominant	C/A	yes	53 (60)	35 (40)		88	0.5	0.8	1.4–0.5
			no	122 (56)	97 (44)		219			
rs17187619	dominant	T/C	yes	63 (72)	25 (28)		88	0.002	2.4	4.1–1.4
			no	117 (53)	102 (47)		219			
rs104000	dominant	A/G	yes	67 (76)	21 (24)		88	0.5	0.8	1.5–0.5
			no	158 (72)	61 (28)		219			
rs643552	additive	T/C	yes	54 (61)	29 (33)	5 (6)	88	0.3	0.6	1.7–0.2
			no	118 (54)	82 (37)	19 (9)	219			
rs643552	dominant	T/C	yes	54 (61)	34 (39)		88	0.3	0.7	1.2–0.4
			no	118 (54)	101 (46)		219			
rs657132	dominant	G/A	yes	72 (83)	15 (17)		87	0.5	0.8	1.6–0.4
			no	172 (79)	47 (21)		219			
rs11665084	dominant	C/T	yes	77 (88)	11 (13)		88	0.1	0.5	1.0–0.2
			no	171 (78)	48 (22)		219			
rs11662595	dominant	A/G	yes	75 (85)	13 (15)		88	0.2	0.6	1.2–0.3
			no	171 (78)	47 (22)		218			
rs1421125	additive	C/A	yes	27 (31)	41 (47)	20 (23)	88	0.0	2.4	5.0–1.1
			no	89 (41)	103 (47)	27 (12)	219			
rs1421125	dominant	C/A	yes	27 (31)	61 (69)		88	0.2	1.5	2.6–0.9
			no	89 (41)	130 (59)		219			
rs1421125	recessive	C/A	yes	68 (77)		20 (23)	88	0.0	2.1	4.0–1.1
			no	192 (88)		27 (12)	219			

Table 3 (continued)

SNP	Model	Alleles (1/2)	Infection-induced asthma	Genotype 11 (%)	Genotype 12 (%)	Genotype 22 (%)	n	p value	OR	95% CI
rs17797975	dominant	T/C	yes no	64 (73) 178 (82)	24 (27) 40 (18)		88 218	0.1	1.7	3.1–1.0
rs4800573	dominant	G/A	yes no	68 (77) 168 (77)	20 (23) 50 (23)		88 218	0.9	1.0	1.9–0.6
rs527790	additive	G/A	yes no	23 (26) 77 (35)	37 (42) 116 (53)	28 (32) 26 (12)	88 219	0.0004	3.3	6.3–1.7
rs527790	dominant	G/A	yes no	23 (26) 77 (35)	65 (74) 142 (65)		88 219	0.2	1.4	2.5–0.8
rs527790	recessive	G/A	yes no	60 (68) 193 (88)		28 (32) 26 (12)	88 219	0.0002	3.3	6.1–1.8
rs487202	additive	C/G	yes no	22 (26) 77 (35)	35 (41) 115 (53)	28 (33) 25 (12)	85 217	0.0002	3.6	6.9–1.8
rs487202	dominant	C/G	yes no	22 (26) 77 (35)	63 (74) 140 (65)		85 217	0.2	1.4	2.6–0.8
rs487202	recessive	C/G	yes no	57 (67) 192 (88)		28 (33) 25 (12)	85 217	0.00007	3.5	6.6–1.9
rs574913	dominant	T/C	yes no	75 (85) 170 (78)	13 (15) 48 (22)		88 218	0.2	0.7	1.3–0.3

The genotype distribution of SNPs in the studied populations were compared with logistic regression and the p value, OR and 95% CI are indicated. In cases with sufficient patient numbers in all genotype groups (n ≥ 5) the analyses were performed using additive (11 vs. 12 vs. 22), recessive (11/12 vs. 22) or dominant (11 vs. 12/22) models, the common homozygote is signed as 11, heterozygote as 12 and rare homozygote as 22. Otherwise only the dominant model was used.

Table 4. Genotype distribution of *HRH4* SNPs in patients with or without allergic rhinitis or conjunctivitis

SNP	Model	Alleles (1/2)	Rhinitis	Genotype 11	Genotype 12	Genotype 22	n	p value	OR	95% CI
rs1421125	additive	C/A	yes no	37 (34) 79 (40)	47 (43) 97 (49)	25 (23) 22 (11)	109 198	0.005	2.7	5.6–1.4
rs1421125	dominant	C/A	yes no	84 (77) 176 (89)		25 (23) 22 (11)	109 198	0.004	2.5	4.8–1.3
rs527790	dominant	G/A	yes no	82 (75) 171 (86)		27 (25) 27 (14)	109 198	0.006	2.3	4.3–1.3
rs487202	dominant	C/G	yes no	82 (75) 167 (87)		27 (25) 26 (13)	109 193	0.006	2.4	4.4–1.3
SNP	Model	Alleles (1/2)	Conjunctivitis	Genotype 11	Genotype 12	Genotype 22	n	p value	OR	95% CI
rs615283	additive	G/A	yes no	36 (62) 181 (74)	16 (28) 57 (23)	6 (10) 7 (3)	58 245	0.008	4.8	15.5–1.5

Values for each genotype are number of patients with percentages in parentheses. The genotype distribution of SNPs in the studied populations were compared with logistic regression, the p value, OR and 95% CI are indicated. In cases with sufficient patient numbers in all genotype groups (n ≥ 5) the analyses were performed using additive (11 vs. 12 vs. 22), recessive (11/12 vs. 22) or dominant (11 vs. 12/22) models, the common homozygote is signed as 11, heterozygote as 12, rare homozygote as 22. Otherwise only the dominant model was used.

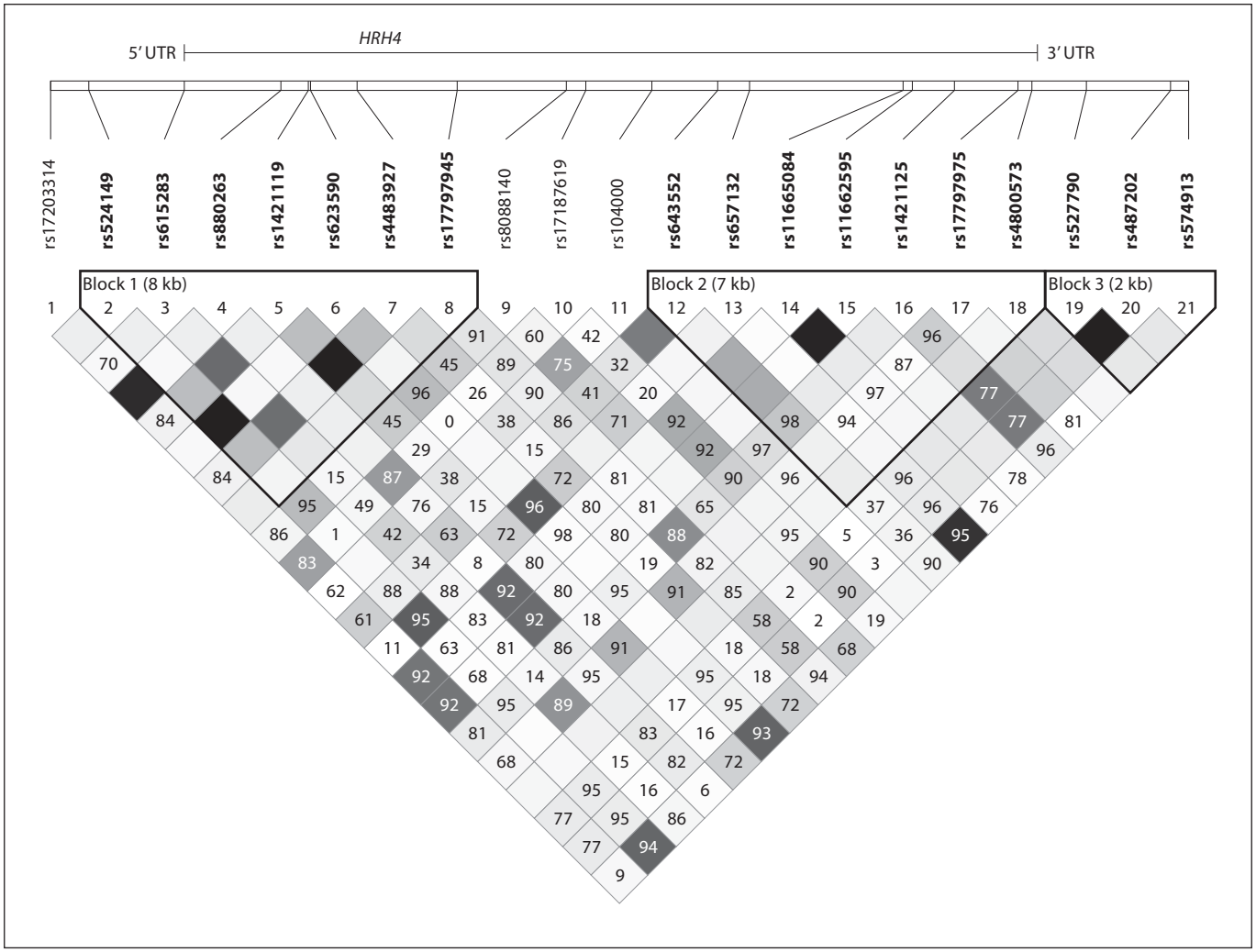

Fig. 1. Linkage disequilibrium analysis for the 21 *HRH4* SNPs in a Hungarian population. Pairwise linkage disequilibrium is expressed as r^2 and D' (both from 0 to 1). The value of r^2 is indicated by the shade of the boxes whereby the more dense shade represents the higher linkage ($r^2 = 0$ is white, $0 < r^2 < 1$ are shades of grey and $r^2 = 1$ is black). D'·100 is indicated in the boxes as numbers when $D' < 1$.

Some haplotypes, which included the rs4800573–rs527790 CC allele combination, were less frequent in infection-induced asthma patients than in other patients [chromosomes, n = 60 (34%) vs. n = 214 (49%), p = 0.0009, OR (95% CI) = 0.5 (0.4–0.8)]. Interestingly, rs4800573 alone was not associated with infection-induced asthma. This was only the case in haplotype with rs527790, or in 3–4 SNP-defined haplotype blocks with rs17797975, rs527790 and rs487202 (table 5). The rs487202–rs574913 CA haplotype was more frequent among patients with infection-induced asthma [94 (53%) vs. 167 (38%), p = 0.0006, OR (95% CI) = 1.9 (1.3–2.6)].

Next, haplotype analysis was performed for all the 21 studied SNPs of the *HRH4* gene (table 6). We found no difference between the asthmatic and control groups. However, the frequency of the TGTGCCGGCTAACGATCCTCA haplotype was significantly higher in the exercise-induced asthma group than in asthmatic patients [n = 22 (18%) vs. n = 38 (8%), p = 0.0015, OR (95% CI) = 2.5 (1.4–4.4)], indicating that patients with this haplotype might be more susceptible to exercise-induced asthma. The other haplotypes did not show a correlation with the formation of asthma-associated diseases if we considered Bonferroni correction.

Table 5. Haplotype analysis of 2–5 SNPs located in the 3′ UTR region or downstream region of *HRH4* gene

SNPs involved in the haplotype analysis	Haplotype	Infection-induced asthma (n = 88)	No infection-induced asthma (n = 219)	p value	OR	95% CI
rs17797975 – rs4800573 – rs527790 – rs487202 – rs574913	CCTCA	14%	9%	0.08	1.6	0.9–2.7
rs17797975 – rs4800573 – rs527790 – rs487202 – rs574913	TCCGA	26%	37%	0.01	0.6	0.4–0.9
rs17797975 – rs4800573 – rs527790 – rs487202 – rs574913	TCCGG	8%	12%	0.2	0.6	0.3–1.2
rs17797975 – rs4800573 – rs527790 – rs487202 – rs574913	TCTCA	39%	29%	0.02	1.5	1.1–2.3
rs17797975 – rs4800573 – rs527790 – rs487202 – rs574913	TTCGA	13%	13%	0.9	1	0.6–1.8
rs17797975 – rs4800573 – rs527790 – rs487202	CCTC	14%	9%	0.08	1.6	0.9–2.7
rs17797975 – rs4800573 – rs527790 – rs487202	TCCG	34%	49%	0.0009	0.5	0.4–0.8
rs17797975 – rs4800573 – rs527790 – rs487202	TCTC	39%	29%	0.02	1.5	1.1–2.2
rs17797975 – rs4800573 – rs527790 – rs487202	TTCG	13%	13%	0.9	1	0.6–1.8
rs4800573 – rs527790 – rs487202 – rs574913	CCGA	26%	37%	0.01	0.6	0.4–0.9
rs4800573 – rs527790 – rs487202 – rs574913	CCGG	8%	12%	0.1	0.6	0.3–1.2
rs4800573 – rs527790 – rs487202 – rs574913	CTCA	53%	38%	0.0011	1.8	1.3–2.6
rs4800573 – rs527790 – rs487202 – rs574913	TCGA	13%	13%	0.9	1	0.6–1.8
rs17797975 – rs4800573 – rs527790	CCT	14%	9%	0.08	1.6	0.9–2.7
rs17797975 – rs4800573 – rs527790	TCC	34%	49%	0.0009	0.5	0.4–0.8
rs17797975 – rs4800573 – rs527790	TCT	39%	29%	0.02	1.5	1.1–2.2
rs17797975 – rs4800573 – rs527790	TTC	13%	13%	0.9	1	0.6–1.8
rs4800573 – rs527790 – rs487202	CCG	34%	49%	0.0009	0.5	0.4–0.8
rs4800573 – rs527790 – rs487202	CTC	53%	38%	0.0011	1.8	1.3–2.6
rs4800573 – rs527790 – rs487202	TCG	13%	13%	0.9	1	0.6–1.8
rs527790 – rs487202 – rs574913	CGA	39%	50%	0.02		
rs527790 – rs487202 – rs574913	CGG	8%	12%	0.2	0.6	0.3–1.2
rs527790 – rs487202 – rs574913	TCA	53%	38%	0.0011	1.8	1.3–2.6
rs17797975 – rs4800573	TC	73%	78%	0.2	0.8	0.5–1.1
rs17797975 – rs4800573	CC	14%	9%	0.08	1.6	0.9–2.7
rs17797975 – rs4800573	TT	13%	13%	0.9	1	0.6–1.8
rs4800573 – rs527790	CT	53%	38%	0.0011	1.8	1.3–2.6
rs4800573 – rs527790	CC	34%	49%	0.0009	0.5	0.4–0.8
rs4800573 – rs527790	TC	13%	13%	0.9	1	0.6–1.8
rs527790 – rs487202	CG	47%	62%	0.0011	1.8	1.3–2.6
rs527790 – rs487202	TC	53%	38%	0.0011	1.8	1.3–2.6
rs487202 – rs574913	CA	53%	38%	0.0006	1.9	1.3–2.6
rs487202 – rs574913	GA	39%	50%	0.01	0.6	0.4–0.9
rs487202 – rs574913	GG	8%	12%	0.2	0.6	0.3–1.2

Haplotype frequencies determined by rs17797975, rs4800573, rs527790, rs487202 and rs574913 SNPs were compared in patients with or without infection-induced asthma. The percentages of the haplotypes were determined by the Haploview 4.1 program. The haplotype frequencies were compared with logistic regression and the p value, OR and 95% CI are indicated. Only those haplotypes were included where the difference between haplotype frequencies in the two groups was more than 6%.

Table 6. Haplotype analysis of the 21 *HRH4* SNPs

Haplotype sequence	Group 1 allele frequency	Group 2 allele frequency	p value	OR	95% CI
TGTGCCGGCTAACGATCCTCA			0.0048	2.6	1.3–4.9
allergic asthma	12%				
nonallergic asthma		5%			
TGTGCCGGCTAACGATCCTCA			0.0056	2.4	1.3–4.5
inhalative allergen-induced asthma	12%				
noninhalative allergen-induced asthma		6%			
CGCATCTGCCAGCAGGTCCGA			0.0076	0.5	0.3–0.8
inhalative allergen-induced asthma	7%				
noninhalative allergen-induced asthma		13%			
TGTGCCGGCTAACGATCCTCA			0.009	2.2	1.2–3.9
conjunctivitis	16%				
nonconjunctivitis		8%			
TGTGCCGGCTAACGATCCTCA			0.0015	2.5	1.4–4.4
exercise-induced asthma	18%				
non-exercise-induced asthma		8%			

Haplotype frequencies determined by all studied SNPs in *HRH4* gene were compared in the patient populations. The percentages of the haplotypes were determined by the Haploview 4.1 program. The haplotype frequencies were compared with logistic regression and the p value, OR and 95% CI are indicated. Only those haplotypes are included where the difference between haplotype frequencies in the two groups was more than 5%.

Discussion

Histamine is known to have an important role in chronic allergic and asthmatic conditions. However, little is known about the influence of genetic polymorphisms in histamine-associated genes on the development of asthma or its endophenotypes.

In our research we investigated the associations between 21 SNPs in the *HRH4* gene and asthma or some endophenotypes of asthma. We observed that genotype distributions were remarkably different in various endophenotypes of asthma. Genotype distributions of three *HRH4* SNPs (rs17187619, rs527790 and rs487202) were found to differ significantly between patients with or without infection-induced asthma. Comparing asthmatic patients with or without allergic rhinitis we observed distinct genotype distributions in rs1421125, rs527790 and rs487202. rs615283 was also associated with conjunctivitis in asthmatics. Additionally, we found that certain haplotypes containing rs487202 and rs527790 were associated with infection-induced asthma. Haplotype analysis for the 21 SNPs of *HRH4* revealed that TGTGCCGGCTAACGATCCTCA was associated with higher susceptibility to exercise-induced asthma. None of the SNPs contributed directly to the risk of asthma.

It must be noted that this study might have some limitations. Our study population is biased as the patient and control groups differ significantly in their age and gender. We think this does not influence our results significantly as the adult controls had no history of childhood respiratory disease and certainly will not develop childhood asthma. Gender may play a role in asthma risk, but in this multifactorial disease it could only have a slight effect. In addition, whilst this possibility cannot be totally excluded, it seems very implausible that populations with the same ethnicity and from the same environment would differ significantly in polymorphisms of the *HRH4* gene just because of their age and gender. Furthermore, in multiple logistic regression analyses we adjusted for age and gender, and with this we corrected the possible differences caused by these factors. In addition, we carried out corrections for multiple testing in respect of the number of SNPs tested. We also carried out the evaluation in multiple subgroups, which could exaggerate the significance of our results. But, as detailed previously, there is independent evidence in the literature suggesting that the *HRH4* gene might play a role in asthma, which could be regarded as independent a priori hypotheses that strengthen the statistical power of our findings [16].

The expression pattern of HRH4 suggests its role in autoimmune and inflammatory diseases as it is expressed on the surface of several cell types playing central roles in these disorders, including eosinophils, mast cells, dendritic cells or Th2 cells. Indeed, HRH4 is implicated in the pathogenesis of colitis, allergy, asthma, pruritus, or autoimmune diseases and, due to its proven role in inflammatory functions, HRH4 appears to be a promising therapeutic target for the treatment of a variety of immune disorders [10].

In vitro and animal studies have shown that signal transduction through HRH4 could influence migration, cytokine response, mediator release or differentiation of these cells [7]. According to these results it is plausible to hypothesize that genetic variations, which might alter the expression and/or functions of this receptor, can influence the pathogenesis of and susceptibility to these diseases.

To date there has only been two studies in the literature about SNPs in the *HRH4* gene. Yu et al. [15] found three novel *HRH4* SNPs significantly associated with atopic dermatitis. These were rs77485247 (ss142022671), rs74604924 (ss142022677) and rs77041280 (ss142022679). We did not analyze these SNPs because they were novel and therefore not validated by the HapMap project. The nearest SNPs studied by us were rs615283 and rs880263 (close to rs77485247), rs11662595 and rs1421125 (close to rs74604924 and rs77041280, which are at adjacent nucleotide positions). The genotype distribution of these SNPs did not differ between the patient and control populations. However, we found some associations regarding this genomic region, as the rs615283 GG genotype associated with coexisting conjunctivitis and patients with the rs1421125 AA genotype suffered more often from allergic rhinitis. The same working group investigated the role of these SNPs also in systemic lupus erythematosus, but found no association with the development of the disease [14]. They also investigated copy number variations of *HRH4*, which they found to increase the risk of systemic lupus erythematosus.

Some observations also indicate the significance of the relationship between the development of asthma or asthma attacks and the presence of histamine. For example, ingestion of histamine-rich food may provoke, among others, the symptoms of asthma through histamine intolerance [17]. SNPs in enzymes involved in the metabolism of histamine such as HDC (histidine decarboxylase), HNMT (histamine N-methyltransferase), ABP1 (amiloride binding protein 1, also termed DAO) and SLC22A3 solute carrier family 22 (extraneuronal monoamine transporter), member 3, also termed OCT3, are also described in association with asthma; however, the results are contradictory.

SNP in the *HNMT* gene, but not in *ABP1*, significantly influenced the risk of asthma in a Polish population [18]. Polymorphisms in the *HDC* and *SLC22A3* genes influenced the risk of both rhinitis and asthma [19]. The association of asthma with *HNMT* SNP could not be observed in two Caucasian (Spanish and German) and an Indian population [20–22]. *HNMT* gene polymorphism did not influence the endophenotypes of asthma in the German group either [21]. Our results supported these findings as we also found no correlation between the risk of asthma and the endophenotypes they studied.

Regarding our findings, it was proven that histamine was a key mediator not only in general asthmatic conditions but also in exercise-induced bronchoconstriction or in infection-induced asthma. Altogether 5 SNPs in the *HRH4* gene (rs615283, rs17187619, rs1421125, rs527790 and rs487202) were found to influence asthma endophenotypes. There is no information about the potential function of these SNPs in either HRH4 isoforms. It is known that the HRH4 protein has three splice variants which code the 390, 302- and 67-amino acid isoforms. Among the two exonic SNPs (rs11665084 and rs11662595) the rs11665084 is present both in the 390- and 302-amino acid isoforms, but rs11662595 is present only in the 390-amino-acid-long isoform. None of these SNPs are present in the 67-amino-acid-long isoform. Nevertheless, it is notable that our most important finding was with two SNPs (rs527790 and rs487202) downstream of the *HRH4* gene. This is an intergenic region, the nearest flanking genes of *HRH4* are *IMPACT* (upstream) and *LOC390843* pseudogene (downstream), however, they do not overlap with *HRH4* and are not in linkage disequilibrium with it. The localization of these SNPs suggests the existence of some possible functional regulatory elements in this region.

This study is the first examining the association of *HRH4* SNPs and asthma, although histamine has been known to be a relevant molecule for chronic allergic and asthmatic conditions for a long time. *HRH4* represents an interesting candidate gene for inflammatory immunological diseases such as asthma due to its predominant expression on immune cells and its important role in inflammatory and immunological processes. According to our results polymorphisms in the *HRH4* gene influence some aspects of asthma, further supporting the belief that this receptor might play an important role in the disease.

In conclusion, the results gained in this study suggest that genetic variation in the *HRH4* gene contributes to the pathogenesis of some endophenotypes of asthma. In the regulation of allergic diseases such as asthma, targeting of HRH4 has already been suggested as a promising new therapeutic tool. Further studies are needed to elucidate how the results gained in this study fit in the pathomechanisms of asthma and asthma endophenotypes. The precise correlation between nucleotide polymorphisms in the genes of histamine receptors or in histamine-metabolizing enzymes remains a black hole in our present knowledge. Future research on the function of this receptor and information regarding the SNPs of *HRH4* may help to identify new candidates for therapeutic targeting and enlighten our understanding of the pathogenesis of asthma endophenotypes.

Acknowledgements

We thank all the patients and control subjects for participating in the study. This study was supported by OTKA (Hungarian Scientific Research Fund): K81941 (C. Szalai); ETT (Ministry of Health, Hungary): 415/2009 (C. Szalai) and NKTH (National Research and Technology): TECH_08-A1/2-2008-0120 (A. Falus, C. Szalai).

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