GENETIC VARIATION ALONG THE HISTAMINE PATHWAY IN CHILDREN WITH ALLERGIC VS NON-ALLERGIC ASTHMA

BY

BRIDGETTE L. JONES
MD, UNIVERSITY OF ARKANSAS SCHOOL OF MEDICINE, 2002

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Brooke L. Fridley, PhD
Chairperson

J. Steven Leeder, PharmD PhD
Committee Member

Lanny J. Rosenwasser, MD
Committee Member

Date Defended: November 18, 2014

The Thesis Committee for BRIDGETTE L. JONES certifies that this is the approved version of the following thesis:

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Brooke L. Fridley, PhD
Chairperson,

Date approved: December 2, 2014

ABSTRACT:

Rationale: Previous studies have suggested that antihistamines may be therapeutic in some patients with asthma. Variation in genes along the histamine production, response, and degradation pathway may be important in predicting response to antihistamines. We hypothesize that genetic variation in genes of the histamine pathway differs between children with allergic versus non-allergic asthma.

Methods: Children 7-18 years of age (n=118) with asthma participated in this IRB-approved protocol and were classified as allergic (N = 68) or non-allergic (N = 50) based on allergy skin testing. DNA isolation and genotyping were performed for 10 SNPs within 4 genes (*HDC, HNMT, ABP1, HRH1, HRH4*) within the histamine pathway. Chi Square tests were used to test for associations between genotypes and allergic or non-allergic asthma among participants. Significance was determined by $p < 0.05$.

Results: We observed differences in genotype frequency between participants with allergic versus non-allergic asthma for 2 SNPs: HNMT-1639(rs6430764) (31% allergic with TT vs. 14% non-allergic with TT, p=0.04) and HNMT -464 (rs2071048) genotype (33% allergic with TT vs. 12% non-allergic with TT, p=0.03) after controlling for race. Differences in genotype frequency were also observed between allergic and non-allergic phenotypes in stratified analyses among African Americans.

Conclusion: Genetic variants within the histamine pathway appear to be associated with an allergic versus non-allergic asthma phenotype. Further studies are needed to validate our findings in a larger cohort. There is also the need to determine the functional significance of identified SNPs and their impact on antihistamine response in patients with asthma and allergic disease.

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INTRODUCTION

Asthma is a chronic inflammatory disease characterized by airway hyper-responsiveness, airflow obstruction and variable reversibility in response to various exposures. According to the CDC's National Asthma Surveillance Report, since 2001 the prevalence of asthma has increased by 2.9% each year from 20.3 million persons in 2001 to 25.7 million persons in 2010 [1]. Asthma is also one of the most common chronic childhood diseases in developed countries; and according to the 2012 National Health Interview Survey, more than 7.1 million children had an asthma diagnosis in 2011 [1].

Asthma is a complex disease whereby the underlying pathophysiology is not completely understood. Several phenotypes of asthma have been identified based on underlying inflammatory mediators or triggers [2]. Allergic and non-allergic asthma are common phenotypic classifications among patients with asthma. Allergic asthma is defined as asthma with allergen hypersensitivity while non-allergic asthma is defined as asthma without allergen hypersensitivity. It is reported that up to 80% of asthma patients classify as allergic asthma[3]. Non-allergic asthma includes a diverse group of described phenotypes such as infection induced asthma, pre-asthma wheezing, eosinophilic asthma, neutrophilic asthma, paucigranulocytic asthma, aspirin sensitive asthma, exercise induced bronchospasm, severe asthma, and flare prone asthma [4]. Better understanding of the underlying pathophysiology of differing asthma phenotypes is important in improving disease evaluation and management.

Histamine (2-[4-imidazole] ethylamine) is a biogenic amine and a known mediator in the pathogenesis of allergic rhinitis and asthma [5, 6]. In the lungs, histamine receptor activation results in bronchospasm and airway obstruction. Plasma histamine levels have been found to correlate with asthma severity [7, 8] and histamine receptor activation results in increased

vascular permeability, mucus production, and contraction of airway smooth muscle cell [9-12]. Histamine also has an important immunoregulatory role as it affects T cell-helper type I and II responses. The amine also directly participates in the functions and activity of dendritic cells [13].

Targeting histamine as a therapeutic treatment has also shown benefit among some patients with asthma. The use of type 1-receptor antihistamines (H1 antihistamines) has been shown to reduce respiratory symptoms and need for rescue medications in children with allergic asthma [14]. Furthermore, the use of antihistamines in atopic children and children considered high-risk for atopy appeared to prevent the onset of asthma when compared with placebo [15, 16]. These data suggest that histamine plays an important role in disease pathogenesis of asthma and the therapeutic response to asthma treatments, especially among targeted asthma phenotypes such as allergic asthma. It is important for us to learn more about the role of histamine in the body in relation to inflammatory diseases such as asthma so as to use this knowledge to improve therapeutic outcomes in diseases that involve histamine.

The synthesis of histamine begins with the alpha-decarboxylation of L-histidine by the enzyme histadine decarboxylase (HDC) [6, 17]. Histamine exerts its effects by activating histamine receptors on various cells throughout the body. There are four known subtypes of histamine receptors (HR): HR1, HR2, HR3, and HR4. HR1 is expressed on several cell types including airway and vascular smooth muscle cells, hepatocytes, chrondrocytes, nerve cells, endothelial cells, dendritic cells, monocytes, lymphocytes, eosinophils, neutrophils. Activation of the HR1 receptor leads to vasodilatation, erythema, increased vascular permeability, edema, and bronchoconstriction [9, 18, 19]. The receptor is responsible for allergic responses such as sneezing, itching, edema, and mucus production. The HR2 receptor is expressed on various cells

types such as gastric parietal cells, smooth muscle cells, neurons, neutrophils, monocytes, macrophages, dendritic cells, lymphocytes, endothelial cells, and epithelial cells [10]. Activation of HR2 is commonly known to increase gastric acid secretion. However, it also is involved in smooth muscle relaxation, airway mucus production, and inhibition of histamine release from basophils and mast cells [19-21]. HR3 is expressed in the central and peripheral nervous system as a presynaptic receptor controlling the release of histamine and neurotransmitters in the human brain [10, 19, 22-24]. The HR3 receptor has been linked to satiety, sleep, and cognition [10, 25, 26]. HR4 is expressed in the bone marrow, and peripheral blood hematopoietic cells such as neutrophils, eosinophils, monocytes, and T cells [27]. It is also expressed on both basophils and mast cells [28]. HR4 has a role in autoimmune and allergic conditions and is involved in cellular chemotaxis and inflammatory mediator release [10, 19]. Recent evidence also suggests that the receptor may play a significant role in histamine induced pruritus [29, 30]. HR4 inverse agonists have been shown to attenuate itch both in animal and human studies [31, 32].

Histamine is degraded by two major enzymes, Histamine N-methyltransferase (HNMT) and Diamine Oxidase (DAO), for subsequent removal from the body [6, 10]. HNMT is responsible for the majority of histamine degradation and is widely expressed in various tissues, including the bronchial epithelium, skin, ileum, and stomach [6, 10, 33, 34]. DAO is mainly involved in degradation of extracellular histamine and is expressed mainly in the colon and kidney [6, 10, 35].

The enzymes responsible for histamine production and degradation and receptors which mediate the effects of histamine may be important in diseases that involve the amine such as asthma. Variation has been observed among the genes coding for these proteins (*HDC, H1R, H2R, H3R, H4R, HNMT, ABP1*) [6]. Although the functional significance of many of these SNPs is

unknown, several studies have been conducted to explore potential associations between histamine related genes and asthma and allergic disease [6, 17, 33-43]. We have previously reported that the *HRH1* gene was more highly expressed in buccal tissue from those with asthma compared to those without asthma [44]. It is plausible that variation among genes involved in histamine production, response, and/or degradation may influence the disposition and effect of the amine within the body. Identification of genetic variants related to the pharmacology of histamine may be important to better understanding of the underlying pathophysiology of asthma sub-types in addition to predicting how asthma patients may respond to treatments that affect histamine (e.g. antihistamines). Therefore, we conducted a pilot investigation to identify differences in genetic variants along the histamine production, response and degradation pathway between children with allergic and non-allergic asthma.

MATERIALS AND METHODS

Study population

All study participants were enrolled in an IRB-approved protocol after obtaining parental permission and, when appropriate (i.e. age ≥ 7), child assent. Convenience sampling was utilized for this clinic based case –control study. Children with asthma were enrolled from Allergy, Asthma and Immunology outpatient clinics at Children's Mercy in Kansas City, MO. Children were then classified as non-allergic vs. allergic asthma. Asthma was defined by ≥ 12% post-bronchodilator reversibility in forced expiratory volume in 1 second (FEV1) or by an Allergy/Asthma specialist diagnosis based on clinical symptoms in children unable to perform spirometry. The diagnosis of allergic rhinitis was determined by at least one positive skin prick test (mean wheal/flare diameter ≥ mean diameter of the positive histamine control) to at least one

seasonal or perennial allergen. Subjects with non-allergic asthma were defined as children with negative skin prick test to regional seasonal and perennial allergens in the past year.

DNA Extraction and Genotyping

Five ml of blood was collected into a glass tube containing ACD or calcium EDTA anticoagulant, mixed by repeated inversion and either stored for up to 7 days at 4ºC or immediately frozen at -70ºC. Genomic DNA was extracted from blood using the Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare, Piscataway, NJ). Genotyping assays were performed on genomic DNA (12-16 ng) using commercially available TaqMan assays to detect the following SNPs of interest: rs10156191 (*ABP1* 47C/T), rs10156191 (*ABP1* 4107 C/G), rs1049742 (*ABP1* 995C/T), rs17740607 (*HDC* 92C/T), rs901865 (*HRH1* -17C/T), rs11665084 (*HRH4* 413C/T), rs6430764 (*HNMT* -1639C/T), rs2071048 (*HNMT* 464C/T), rs1050900 (*HNMT* 3'UTRA/T), and rs11558538 (*HNMT* 314C/T) (Applied Biosystems, Foster City, CA) and KAPA Probe Fast qPCR master mix (Kapa Biosystems, Boston, MA). SNPs were chosen based on their potential functional significance and previously investigated variants (rs11558538, rs10156191, rs10156191, rs901865), as well as those with an expected minor allele frequency (MAF) within our participant population (≥2%) among expected racial groups of African Americans and Caucasians [6, 33, 45, 46]. Genotyping was performed on an Eco ^{TM} Real-Time PCR System (Illumina, San Diego, CA) with a thermal profile of 95ºC for 3 minutes followed by 40-50 cycles of 95ºC for 3 seconds and 64ºC for 20 seconds. All samples were performed in duplicates to rule out random error.

Statistical Analysis

Concordance with Hardy-Weinberg equilibrium (HWE) was confirmed according to Chi Square distribution for each SNP using available online software (Hardy-Weinberg equilibrium

calculator including analysis for ascertainment bias) [47]. HWE tests were completed by racial group Statistical analyses were performed using SAS 9.2 (Cary, NC). Chi-Square test and Student T-test was used to compare demographics between the allergic asthma and non-allergic asthma groups. Fisher's Exact tests (general genotypic model 2 degree of freedom test or co-dominant genetic model) and Cochran-Armitage Trend tests (additive genetic model) were used to compare the genotype frequency for each SNPs between asthmatic subjects with and without allergic sensitization. All analyses were completed by race followed by Cochran-Mantel-Haenszel (CMH) tests to compare the frequencies of genotype between allergic and non-allergic subjects, adjusted by the confounding race effect. Significance was determined by the nominal $p<0.05$ (not adjusting for testing of multiple SNPs). Adjustment for multiple testing with a Bonferroni correction would result in significance determined for $p < 0.005$. As exploratory analyses, we further evaluated genotype frequency according to the subgroup stratification of race.

RESULTS

A total of 118 children between the ages of 7 through 18 years were included in this preliminary study. Participant demographics are shown in **Table 1.** Allergic asthma was more common among males and African Americans than non-allergic asthma ($p<0.01$ and 0.04 respectively). Among African Americans allergic asthma was more frequent than non-allergic asthma ($p<0.01$). There were no differences in age between the two asthma phenotypes.

Table 1: Demographic characteristics of all participants and among those classified as allergic and non-allergic.

Demographics	Total Subjects (N=118)	Subjects with Allergic Asthma (N=68)	Subjects with Non-Allergic Asthma (N=50)	P-value
Age in years: Mean±SD	12.5 ± 3.3	12.7 ± 3.3	12.06 ± 3.2	0.80
**Gender % (N):				0.008
Males	54% (64)	69% (44)	31% (20)	
Females	46% (54)	44 %(24)	56 %(30)	
***Race % (N):				0.04
†African American	52% (61)	69% (42)	31% (19)	
Caucasian	38% (45)	44 %(20)	56% (25)	
Other	10% (12)	50% (6)	50% (6)	

** Increased frequency of those with allergic asthma compared to those with non-allergic asthma among males

***Increased frequency of allergic asthma among African Americans

†Allergic asthma was more frequent than non-allergic asthma among African Americans

We observed differences in genotype frequency for 2 of the 10 SNPs evaluated between subjects with allergic and non-allergic asthma after correcting for the confounder of race. The homozygous variant (TT) genotype for *HNMT* -464 (rs2071048) was more common among those with allergic asthma than those with non-allergic asthma (**Table 2**, TT=0.48 allergic asthma vs. TT=0.24 non-allergic asthma, p=0.03; Bonferroni corrected p value 0.3) (see Table 3 for genotypes stratified by race). The homozygous variant *HNMT*-1639 (rs6430764) genotype (TT) was also more common among allergic asthma participants than non-allergic asthma (**Figure 2**, TT=0.31 allergic vs. TT=0.14 non-allergic, p=0.04; Bonferroni corrected p-value 0.4) (see Table 3 for genotypes stratified by race). There were no significant differences observed for genotype for the other SNPs evaluated after correcting for race (**Table 2**).

Table 2: Comparison of genotype frequencies in subjects with allergic and non-allergic asthma

Gene (locus)	SNP (*major/minor allele)	Genotype	Allergic Asthma Subjects % (N)	Non-Allergic Asthma Subjects % (N)	**p value
HDC (15q21-q2)	rs17740607 (C/T)	CC	1 (1)	4 (2)	0.62
		CT	12 (8)	14 (7)	
		TT	87 (59)	82 (41)	
HRH1 (3p25)	rs901865 (C/T)	CC	57 (39)	72 (36)	0.09
		CT	35 (24)	28 (14)	
		TT	8 (5)	0 (0)	
HRH4 (18q11.2)	rs116650859 (C/T)	CC	93 (63)	92 (46)	0.84
		CT	7 (5)	8 (4)	
		TT	0 (0)	0 (0)	
HNMT (2q.22)	rs6430764 (C/T)	CC	19 (13)	18 (9)	**0.04**
		CT	50 (34)	68 (34)	
		TT	31 (21)	14 (7)	
	rs2071048 (C/T)	CC	9 (6)	12 (6)	**0.03**
		CT	42 (29)	64 (32)	
		TT	49 (33)	24 (12)	
	rs11558538 (C/T)	CC	90 (61)	78 (39)	0.63
		CT	9 (6)	20 (10)	
		TT	1 (1)	2 (1)	
	rs1050900 (A/T)	AA	66 (45)	52 (26)	0.69
		AT	26 (18)	46 (23)	
		TT	8 (5)	2 (1)	
ABP1 (7q34-36)	rs10156191 (C/T)	CC	26 (18)	38 (19)	0.16
		CT	54 (37)	52 (26)	
		TT	19 (13)	10 (5)	
	rs1049742 (C/T)	CC	79 (54)	86 (43)	0.56
		CT	21 (14)	12 (6)	
		TT	0 (0)	2 (1)	
	rs1049793 (C/G)	CC	27 (18)	36 (18)	0.55
		CG	51 (35)	44 (22)	
		GG	22 (15)	20 (10)	

*****Major allele represents the most frequently-occurring allele; serves as reference for modeling.
** P-values represented from Co-dominant genotype model from Cochran-Mantel-Haenszel test corrected for race confounder

Exploratory analysis among racial groups

Genetic differences relative to race were observed**.** The *HDC*92 (rs17740607) TT genotype was more common among African Americans than Caucasians (TT=0.98 African American vs. 0.71 Caucasian, $p<0.0001$; Cochran-Armitage Trend $p=0.0005$). There were no other differences in genotype frequencies observed between the racial groups.

Recognizing that disease phenotype and pathophysiology differ between different races and due to findings of differences in genotype between racial groups we performed further exploratory analyses to evaluate allele and genotype frequencies among racial subgroups with allergic and non-allergic asthma (**Table 3**). Similar to the findings among the entire cohort, the *HNMT*-1639 (rs6430764) TT genotype was more frequent among African Americans with allergic asthma than non-allergic asthma (TT=0.24 allergic asthma vs.TT=0 non-allergic asthma, $p=0.04$).

Table 3: Comparison of genotype frequencies among allergic and non-allergic asthma within stratified Caucasian and African American cohorts

		African American			Caucasian		
SNP	**Genotype**	**Allergic Asthma Subjects % (N)**	**Non-Allergic Asthma Subjects % (N)**	***p value**	**Allergic Asthma Subjects % (N)**	**Non-Allergic Asthma Subjects % (N)**	***p value**
HDC 92 rs17740607	CC	2(1)	0	**	0	8(2)	0.64
	CT	0	0		25(5)	24(6)	
	TT	97(41)	100(19)		75(5)	68(17)	
HRH1 -17 rs901865	CC	48(20)	74(14)	0.12	75(15)	72(18)	1.0
	CT	40(17)	26(5)		25(5)	28(7)	
	TT	12(5)	0		0	0	
HRH4 413 rs11665085	CC	95(40)	95(18)	1.0	85(17)	88(22)	1.0
	CT	4(2)	5(1)		15(3)	12(3)	
	TT	0	0		0	0	
HNMT -1639 rs 6430764	CC	26(11)	26(5)	**0.04**	10(2)	16(4)	0.21
	CT	50(21)	74(14)		45(9)	64(16)	
	TT	24(10)	0		45(9)	20(5)	
HNMT -464 rs2071048	CC	10(4)	11(2)	0.13	10(2)	16(4)	0.21
	CT	38(16)	63(12)		45(9)	64(16)	
	TT	52(22)	26(5)		45(9)	20(5)	
HNMT 314 rs11558538	CC	97(41)	100(19)	**	80(16)	68(17)	0.73
	CT	2(1)	0		15(3)	28(7)	
	TT	0	0		5(1)	4(1)	
HNMT 3'UTR rs1050900	AA	67(28)	58(11)	0.44	65(13)	48(12)	0.11
	AT	28(12)	42(8)		20(4)	48(12)	
	TT	5(2)	0		15(3)	4(1)	
ABP1 47 rs10156191	CC	12(5)	37(7)	0.08	50(10)	36(9)	0.19
	CT	67(28)	53(10)		30(6)	56(14)	
	TT	21(9)	10(2)		20(4)	8(2)	
ABP1 995 rs1049742	CC	81(34)	84(16)	0.30	75(15)	88(22)	0.43
	CT	19(8)	11(2)		25(5)	12(3)	
	TT	0	5(1)		0	0	
ABP1 4107 rs1049793	CC	24(10)	26(5)	1.0	30(6)	52(13)	0.17
	CG	50(21)	47(9)		60(12)	32(8)	
	GG	26(11)	26(5)		10(2)	16(4)	

*P-values represent Fishers Exact p-value for comparison of genotype frequency among Allergic and non-allergic asthma for African Americans and Caucasians respectively using a co-dominant genotype model

**P-value not provided due to minor allele frequency < 0.05

DISCUSSION

Histamine related genes may be important in the pathogenesis of asthma. We observed an association between genetic variation within *HNMT* and the allergic asthma phenotype in our cohort of children whereby the *HNMT* TT genotype was associated with allergic asthma. The same genotype was also associated with allergic asthma among African American children. We further identified an HNMT haplotype, which includes the -1639 SNP, that was associated with asthma phenotype. Our results warrant further investigation into the role of histamine related genes among those with allergic asthma.

Previous investigations among varying sample sizes have yielded mixed results regarding the relationship between histamine related genes and asthma [34-38, 40-43, 48]**.** We believe that our study underscores the importance of conducting asthma related genetic studies within cohorts of well-defined asthma phenotypes and among diverse racial and ethnic groups. African Americans, who bear significant morbidity and mortality associated with asthma, are a particularly under studied racial group among genetic studies of asthma [49]. Previous studies of histamine pathway genes have included very low numbers of African Americans or none at all [34-38, 40-43, 48]**.** It is essential that relationships between genetics and disease are identified among diverse populations so as to provide medical knowledge which will lead to improved diagnostic and therapeutic treatments among various racial and ethnic groups.

HNMT, located on chromosome 2q22, has been the most widely investigated gene, in relation to asthma and allergic disease, among the histamine related genes. One particular SNP, *HNMT* 314 C/T, resulting in a change in amino acid (Ala138Val) and protein configuration, has been extensively studied. This particular SNP has been shown to result in decreased HNMT activity [33]. Many have postulated that reduced HNMT activity may lead to histamine accumulation and

therefore, affect pathophysiology among diseases that involve histamine. Several studies have been conducted to determine the potential clinical impact of this variant among those with allergic disease, asthma, and atopic dermatitis. A study conducted by MJ Kennedy et al., revealed that the *HNMT* 314 T variant allele was associated with atopic dermatitis among children [39]. Another study conducted among a cohort of predominately Caucasian adults observed an association between *HNMT* 314T variant allele and asthma [34]. Furthermore, a study performed by Szczepankiewicz, et al, found that the homozygous variant genotype (TT) for *HNMT* 314 occurred more frequently among Caucasian asthmatic children when compared to healthy controls[43]. Conflicting reports exist among various ethnic groups and asthma phenotypes in relation to the *HNMT* 314 SNP and asthma [36, 37, 40, 41]. Sasaki, et al. also did not find a relationship between the SNP and allergic asthma in the Japanese cohort[40]. We also did not observe and association between the HNMT 314 variant and asthma among our cohort which is likely due to differences in linkage disequilibrium associated with differing racial groups.

We observed two novel associations between *HNMT* SNPs, *HNMT*-464 and *HNMT*-1639, and allergic asthma. The *HNMT*-1639 SNP was also specifically relevant within the African American cohort in relation to asthma phenotype. Although the functional relevance of these non-coding SNPs is currently unclear, our findings suggest that further investigations should include these variants as they may be in linkage with SNPs that result in protein changes or, given their position relative to the *HNMT* gene, may affect levels of expression. Furthermore, we observed an association between an *HNMT* haplotype that consists of wild type alleles and non-allergic asthma which supports our findings related to individual gene variants and the allergic phenotype. Investigation of single SNPs may not adequately predict disease association, which

may lead to conflicting results among studies. Future studies should also include haplotype investigations among the histamine related genes to more clearly elucidate the role of these genes in disease.

ABP1, located on chromosome 7q 34-36, encodes for DAO. Potentially functionally relevant SNPs have been identified for this gene. Two *ABP1* SNPs, *ABP1* 4107C/G and *ABP1* 47C/T have been previously associated with altered DAO enzyme activity [5, 35]. In addition, Maintz, et al. observed that the *ABP1* 47 T allele was associated with "histamine intolerance", defined as headache and flushing when consuming histamine-rich substances. These findings suggest that variants within *ABP1* may be related to the function of diamine oxidase activity which may be clinically important in disease phenotypes that involve histamine (e.g. allergic asthma). Previous literature has not reported an association between *ABP1* genetic variation and asthma [20,21]. Garcia-Martin, et al. reported an association between *ABP1* 4107C/G (His645Asp) and allergic asthma and/or allergic rhinitis among 270 Caucasians and 295 unrelated controls [37]. The allergic phenotype in their study was well-defined by clinical history and environmental allergy skin prick testing. Garcia-Martin did not identify an association between *ABP1* 4107C/G and asthma or allergic disease.

Previous studies have also investigated the potential relationship between SNPs within *HDC,* which is located on located on chromosome 15q21-q22, and asthma/allergic disease. *HDC* mRNA expression was increased in the nasal mucosa of Japanese patients with allergic rhinitis compared to those without the disease [17]. A study by Gervasini, et a.l, which included *HDC* nonsynonymous SNPs, (*HDC* 1932 A/C (Glu644Asp, rs2073440) and *HDC* 92C/T (Thr31Met, rs17740607)) and newly identified synonymous SNPs (rs854163, rs35047996) was conducted in

a well-defined allergic phenotype population of Caucasian subjects from Spain. They identified an association between *HDC* 1932 A/C polymorphism and allergic rhinitis alone and allergic rhinitis with asthma. Similar to our findings, they did not find an association between asthma and/or allergic disease and the *HDC* 92 C/T SNP. In our study, we conducted a limited investigation of the gene in relation to asthma phenotype as we were limited by our smaller sample size. Future studies should include additional *HDC* SNPs so as to more clearly elucidate the role of the histamine related genes in asthma.

The four genes encoding the histamine receptors, *HRH1, HRH2, HRH3,* and *HRH4*, are located on 3p25, 5q35.2, 20q13.33, and 18q11.2, respectively, and their relationship to asthma and allergic disease have been previously investigated [6]. Similar to our findings Sasaki did not find an association between SNPs within the histamine receptor genes and allergic asthma among Japanese participants. Others have also investigated *HRH4* and its potential association with asthma and allergic disease. In our study we did not observe differences in allele or genotype frequency between the allergic and non-allergic asthmatics for *HRH4* 413C/T. In a previous study, conducted in Caucasian children of Hungarian decent, investigators looked at 21 SNPs, including *HRH4* 413 C/T, in relation to various asthma phenotypes (e.g. allergic asthma, exercise induced asthma). In their study, where allergic asthma was defined by environmental allergy skin prick testing, they also did not find an association between the *HRH4* 413C/T SNP and the disease [42]. However, they did identify an association between other variants, which were not included in our study (*HRH4* rs527790, rs487202, rs17187619) and "infection-induced asthma". Of the *HRH4* nonsynonymous SNPs currently identified, none are reported to be observed consistently at high frequency among African Americans [6]. Therefore, this gene may be of less importance among a cohort like ours. As such, we conducted a limited investigation of *HRH4*

within our group. Yu, et al. also investigated *HRH4* genetic variants and found an association with 3 different SNPs (rs77485247, rs74604924, rs77041280) and atopic dermatitis. More recent data from the same group also suggests that amplification of the *HRH4* copy number is also associated with atopic dermatitis [50]. As the HR4 receptor has been most extensively associated with pruritus, their findings are biologically plausible and deserve more investigation [51, 52]. Perhaps *HRH4* is most important in the pathogenesis of atopic dermatitis and may not be as influential in the pathogenesis of asthma.

The most significant limitation of our study was the small sample size and further limited sample size for analyses conducted among racial groups. We may not have been able to detect small differences in gene frequency between the allergic and non-allergic groups. However, the differences that we did observe are not likely due to chance given the large difference required for detection in a small sample size. Racial heterogeneity among our cohort is a limitation and further studies should focus on larger racially homogeneous populations given known differences in inheritance and linkage disequilibrium between racial groups. We believe that our findings among the African American participants deserve more investigation given the large differences in genotype frequency observed between the allergic and non-allergic groups in this racial group. It is likely that the differences in genotype observed among the entire cohort for *HNMT*1639 were most likely driven by the African American racial group. We also recognize that SNPs identified to be associated with allergic asthma are in non-coding regions and therefore may only be "tagging" SNPs. Further work is needed to determine the potential functional significance of these SNPs and to identify other more functionally relevant SNPs that may be in linkage.

We believe that our findings are important in further understanding the pathophysiology of asthma among different patient populations. With validation, this information may be useful in utilizing genotype in predicting therapeutic response to antihistamines for the treatment of asthma. As those with altered histamine production or degradation or receptor function may respond differently to antihistamines. Future studies are needed to confirm our results in a larger cohort of participants with allergic asthma, specifically focusing on potentially relevant and understudied subgroups (e.g. African American). In addition, further studies are required to determine the functional significance of identified SNPs and their impact on disease phenotype as well as inclusion of other potentially important histamine related genes (*HRH2*, *HRH3*) and haplotypes. Our study supports re-visiting the role of histamine in asthma and the role of antihistamine treatment for those with well-defined asthma phenotypes.

REFERENCES

1. *National Heart, Lung, and Blood Institute Expert Panel Report 3 (EPR3): Guidelines for the Diagnosis and Management of Asthma 2007*. 2007 [cited; Available from: http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm.

2. Barnes, P.J., *Pathophysiology of allergic inflammation.* Immunol Rev, 2011. **242**(1): p. 31-50.

3. Bourdin, A., et al., *Upper airway x 1: allergic rhinitis and asthma: united disease through epithelial cells.* Thorax, 2009. **64**(11): p. 999-1004.

4. Handoyo, S. and L.J. Rosenwasser, *Asthma phenotypes.* Curr Allergy Asthma Rep, 2009. **9**(6): p. 439-45.

5. Maintz, L. and N. Novak, *Histamine and histamine intolerance.* Am J Clin Nutr, 2007. **85**(5): p. 1185-96.

6. Garcia-Martin, E., et al., *Histamine pharmacogenomics.* Pharmacogenomics, 2009. **10**(5): p. 867-83.

7. Busse, W.W. and C.A. Swenson, *The relationship between plasma histamine concentrations and bronchial obstruction to antigen challenge in allergic rhinitis.* J Allergy Clin Immunol, 1989. **84**(5 Pt 1): p. 658-66.

8. Simon, R.A., et al., *The relationship of plasma histamine to the activity of bronchial asthma.* J Allergy Clin Immunol, 1977. **60**(5): p. 312-6.

9. O'Byrne, P.M. and M.D. Inman, *Airway hyperresponsiveness.* Chest, 2003. **123**(3 Suppl): p. 411S-6S.

10. Jones, B.L. and G.L. Kearns, *Histamine: new thoughts about a familiar mediator.* Clin Pharmacol Ther. **89**(2): p. 189-97.

11. Schmelz, M., et al., *Specific C-receptors for itch in human skin.* J Neurosci, 1997. **17**(20): p. 8003-8.

12. Clough, G.F., A.R. Bennett, and M.K. Church, *Effects of H1 antagonists on the cutaneous vascular response to histamine and bradykinin: a study using scanning laser Doppler imaging.* Br J Dermatol, 1998. **138**(5): p. 806-14.

13. Akdis, C.A., M. Jutel, and M. Akdis, *Regulatory effects of histamine and histamine receptor expression in human allergic immune responses.* Chem Immunol Allergy, 2008. **94**: p. 67-82.

14. Grant, J.A., et al., *Cetirizine in patients with seasonal rhinitis and concomitant asthma: prospective, randomized, placebo-controlled trial.* J Allergy Clin Immunol, 1995. **95**(5 Pt 1): p. 923-32.

15. Bustos, G.J., D. Bustos, and O. Romero, *Prevention of asthma with ketotifen in preasthmatic children: a three-year follow-up study.* Clin Exp Allergy, 1995. **25**(6): p. 568-73.

16. Warner, J.O., *A double-blinded, randomized, placebo-controlled trial of cetirizine in preventing the onset of asthma in children with atopic dermatitis: 18 months' treatment and 18 months' posttreatment follow-up.* J Allergy Clin Immunol, 2001. **108**(6): p. 929-37.

17. Hirata, N., et al., *Expression of histidine decarboxylase messenger RNA and histamine N-methyltransferase messenger RNA in nasal allergy.* Clin Exp Allergy, 1999. **29**(1): p. 76-83.

18. Togias, A., *H1-receptors: localization and role in airway physiology and in immune functions.* J Allergy Clin Immunol, 2003. **112**(4 Suppl): p. S60-8.

19. Zhang, M., R.L. Thurmond, and P.J. Dunford, *The histamine H(4) receptor: a novel modulator of inflammatory and immune disorders.* Pharmacol Ther, 2007. **113**(3): p. 594-606.

20. White, M.V., *The role of histamine in allergic diseases.* J Allergy Clin Immunol, 1990. **86**(4 Pt 2): p. 599-605.

21. Jutel, M., K. Blaser, and C.A. Akdis, *Histamine in allergic inflammation and immune modulation.* Int Arch Allergy Immunol, 2005. **137**(1): p. 82-92.

22. Lovenberg, T.W., et al., *Cloning and functional expression of the human histamine H3 receptor.* Mol Pharmacol, 1999. **55**(6): p. 1101-7.

23. Hill, S.J., et al., *International Union of Pharmacology. XIII. Classification of histamine receptors.* Pharmacol Rev, 1997. **49**(3): p. 253-78.

24. Arrang, J.M., M. Garbarg, and J.C. Schwartz, *Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor.* Nature, 1983. **302**(5911): p. 832-7.

25. Hancock, A.A., *H3 receptor antagonists/inverse agonists as anti-obesity agents.* Curr Opin Investig Drugs, 2003. **4**(10): p. 1190-7.

26. Fox, G.B., et al., *Two novel and selective nonimidazole H3 receptor antagonists A-304121 and A-317920: II. In vivo behavioral and neurophysiological characterization.* J Pharmacol Exp Ther, 2003. **305**(3): p. 897-908.

27. Nakamura, T., et al., *Molecular cloning and characterization of a new human histamine receptor, HH4R.* Biochem Biophys Res Commun, 2000. **279**(2): p. 615-20.

28. Zhu, Y., et al., *Cloning, expression, and pharmacological characterization of a novel human histamine receptor.* Mol Pharmacol, 2001. **59**(3): p. 434-41.

29. Dunford, P.J., et al., *The histamine H4 receptor mediates allergic airway inflammation by regulating the activation of CD4+ T cells.* J Immunol, 2006. **176**(11): p. 7062-70.

30. Yamaura, K., et al., *Expression of histamine H4 receptor in human epidermal tissues and attenuation of experimental pruritus using H4 receptor antagonist.* J Toxicol Sci, 2009. **34**(4): p. 427-31.

31. Kollmeier A, F.K., Chen B, Dunford PJ, Greenspan AJ, Xia Y, Xu XL, Zhou B, Thurmond R., *The H4 receptor antagonist, JNJ 39758979, is effective in reducing histamine-induced pruritus in a randomized clinical study in healthy subjects.* J Pharmacol Exp Ther, 2014.

32. Dunford, P., et al., *Histamine H4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus* J Aller Clin Immunol, 2007. **119**(1): p. 176-183.

33. Preuss, C.V., et al., *Human histamine N-methyltransferase pharmacogenetics: common genetic polymorphisms that alter activity.* Mol Pharmacol, 1998. **53**(4): p. 708-17.

34. Yan, L., et al., *Histamine N-methyltransferase pharmacogenetics: association of a common functional polymorphism with asthma.* Pharmacogenetics, 2000. **10**(3): p. 261-6.

35. Ayuso, P., et al., *Genetic variability of human diamine oxidase: occurrence of three nonsynonymous polymorphisms and study of their effect on serum enzyme activity.* Pharmacogenet Genomics, 2007. **17**(9): p. 687-93.

36. Deindl, P., et al., *No association of histamine- N-methyltransferase polymorphism with asthma or bronchial hyperresponsiveness in two German pediatric populations.* Pediatr Allergy Immunol, 2005. **16**(1): p. 40-2.

37. Garcia-Martin, E., et al., *Polymorphisms of histamine-metabolizing enzymes and clinical manifestations of asthma and allergic rhinitis.* Clin Exp Allergy, 2007. **37**(8): p. 1175-82.

38. Gervasini, G., et al., *Variability of the L-Histidine decarboxylase gene in allergic rhinitis.* Allergy. **65**(12): p. 1576-84.

39. Kennedy, M.J., et al., *Association of the histamine N-methyltransferase C314T (Thr105Ile) polymorphism with atopic dermatitis in Caucasian children.* Pharmacotherapy, 2008. **28**(12): p. 1495-501.

40. Sasaki, Y., et al., *Lack of association between atopic asthma and polymorphisms of the histamine H1 receptor, histamine H2 receptor, and histamine N-methyltransferase genes.* Immunogenetics, 2000. **51**(3): p. 238-40.

41. Sharma, S., et al., *Lack of association of histamine-N-methyltransferase (HNMT) polymorphisms with asthma in the Indian population.* J Hum Genet, 2005. **50**(12): p. 611-7.

42. Simon, T., et al., *Asthma endophenotypes and polymorphisms in the histamine receptor HRH4 gene.* Int Arch Allergy Immunol. **159**(2): p. 109-20.

43. Szczepankiewicz, A., et al., *Polymorphisms of two histamine-metabolizing enzymes genes and childhood allergic asthma: a case control study.* Clin Mol Allergy. **8**: p. 14.

44. Vyhlidal, C.A., et al., *Detecting gene expression in buccal mucosa in subjects with asthma versus subjects without asthma.* Pediatr Allergy Immunol, 2013. **24**(2): p. 138-43.

45. Hon, Y., et al., *Endogenous histamine and cortisol levels in subjects with different histamine N-methyltransferase C314T genotypes: a pilot study.* Molecular Diagnosis and Therapuetics, 2006. **10**(2): p. 109-14.

46. Sasaki, Y., et al., *Lack of association between atopic asthma and polymorphisms of the histamine H1 receptor, histamine H2 receptos, and histamine N-methyltransferase.* Immunogenetics, 2000. **51**: p. 238-240.

47. Santiago Rodriguez, T.R.G.a.I.N.M.D. *Hardy-Weinberg Equilibrium Testing of Biological Ascertainment for Mendelian Randomization Studies*. American Journal of Epidemiology 2008 [cited; Available from: http://www.oege.org/software/hwe-mr-calc.shtml.

48. Maintz, L., et al., *Association of single nucleotide polymorphisms in the diamine oxidase gene with diamine oxidase serum activities.* Allergy. **66**(7): p. 893-902.

49. Scirica, C.V. and J.C. Celedon, *Genetics of asthma: potential implications for reducing asthma disparities.* Chest, 2007. **132**(5 Suppl): p. 770S-781S.

50. Chen, B., et al., *Association between copy-number variations of the human histamine H4 receptor gene and atopic dermatitis in a Chinese population.* Clin Exp Dermatol, 2013. **38**(3): p. 295-300; quiz 300-1.

51. Bell, J.K., D.S. McQueen, and J.L. Rees, *Involvement of histamine H4 and H1 receptors in scratching induced by histamine receptor agonists in Balb C mice.* Br J Pharmacol, 2004. **142**(2): p. 374-80.

52. Dunford, P.J., et al., *Histamine H4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus.* J Allergy Clin Immunol, 2007. **119**(1): p. 176-83.