ERJ Express. Published on May 3, 2011 as doi: 10.1183/09031936.00194710

E-cadherin gene polymorphisms in asthma patients using inhaled corticosteroids

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Keywords: Asthma, airway remodeling, E-cadherin, epithelium, glucocorticosteroids, polymorphisms

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Abstract

E-cadherins form intercellular junctions that maintain epithelial integrity. Epithelial integrity is impaired in asthma and can be restored by inhaled corticosteroids (ICS).

Our aim was to investigate the association of *CDH1* gene polymorphisms (SNPs) with airway remodeling, inflammation and FEV_1 decline in asthma patients and assess whether ICS modulate these effects.

Bronchial biopsies of 138 asthmatics were available (population 1). Associations of 17 haplotype-tagging SNPs with epithelial E-cadherin expression, biopsy parameters and FEV_1/VC were tested. FEV_1 and VC data were collected in 281 asthmatics with 30-year follow-up (population 2). Linear Mixed Effect Models assessed associations of SNPs with FEV_1 decline.

Seven of the 17 SNPs were associated with airway remodeling, 3 with CD8+T-cell numbers, 2 with eosinophil counts and 7 with FEV_1 decline. All associations occurred only in patients using ICS. In general, alleles associated with less remodeling correlated with less FEV_1 decline and higher FEV_1/VC. Decreased epithelial E-cadherin expression was associated with 5 SNPs in no ICS users.

In conclusion our data show that *CDH1* polymorphisms are associated with epithelial E-cadherin expression and suggest that epithelial adhesion is an important contributor to airway remodeling and lung function in asthma. These effects are modified by the use of inhaled corticosteroids.

Introduction

Asthma is a chronic inflammatory disease of the airways characterized by bronchial hyperresponsiveness (BHR) and pathological changes in the epithelium and the submucosal area of the bronchi. Histological studies indicate that the epithelium is interrupted and fragile in bronchial biopsies of asthmatics [1,2]. Both genetic and environmental factors as well as their interactions are involved in asthma development and its course during lifetime. Recent advances in understanding the pathophysiology of asthma have pointed towards a prominent role of the airway epithelium in asthma development and severity [3,4].

Airway epithelium is the natural barrier between environment and the underlying tissue. Its integrity is important for protecting the airways against noxious inhalants like environmental tobacco smoke, particles and biological agents. Regulation of cell-cell junction stability and dynamics is crucial to maintain tissue integrity and allow tissue remodeling throughout development [5,6]. E-cadherin, the major cadherin expressed in epithelial cells, is an adhesion molecule able to establish and stabilize cellular junctions between adjacent cells in the presence of Ca^{2+} [7] and is coded by the *CDH1* gene on chromosome 16q22.1. Hirosako et al. suggested that human bronchial intraepithelial lymphocytes have roles distinct from subsets of other lymphocytes, and that CD8+ cells and CD103+ (ligand of E-cadherin) cells have potentially important functions in the bronchial epithelium [8].

Longitudinal studies have demonstrated that some asthma patients develop irreversible airway obstruction and progressive loss of lung function [9]. Inhaled corticosteroids (ICS) have been widely used for suppressing inflammation and asthma symptoms. Early use of ICS is found to be associated with less accelerated FEV_1 decline [10] and with improvement of BHR and airway remodeling [11]. Furthermore, corticosteroids are thought to modulate epithelial repair [12] and there is evidence that they up-regulate epithelial E-cadherin expression [13].

We hypothesized that loss of epithelial integrity resulting from loss of epithelial E-cadherin expression or disruption of E-cadherin-mediated cell-cell contacts predisposes airways to abnormal responses to inhaled substances. Subsequent remodeling processes and airway inflammation may lead to airway narrowing and accelerated lung function decline.

Therefore, we investigated the associations between *CDH1* polymorphisms and airway remodeling (basement membrane thickness and subepithelial vasculature), inflammation and annual FEV_1 decline in asthma patients and assessed whether ICS use modulates these associations.

Materials and Methods

Populations

Population 1: 138 asthmatics participated in cohort studies conducted by our research group in previous years. They were re-examined for lung function and

BHR and underwent bronchoscopy with biopsy collection [14,15]. Main exclusion criteria were: FEV_1 <1.2 L, bronchiectasis, upper respiratory tract infection (e.g. colds) and/or use of antibiotics or oral corticosteroids within the last 2 months before inclusion. DNA samples and biopsy data were available in 137 subjects.

Population 2: 281 asthma patients who have been referred to the asthma clinic at Beatrixoord Hospital in Haren, The Netherlands over the period 1966–1975. Patients who at first visit were < 45 years, had ≥ 20% fall in FEV_1 during a histamine challenge test (PC_{20} ≤32 mg/ml) and were symptomatic according to current American Thoracic Society criteria [16] were re-examined during the period 1991–1999 [9]. At both visits patients filled in a questionnaire on respiratory symptoms and underwent lung function, histamine challenge and skin prick tests. The clinical assessment was performed as previously described [10]. Before testing, participants had to be in stable condition without any exacerbation in the last 6 weeks. After their first visit subjects had annual routine check-ups for their asthma. Their medical records provided information on lung function and corticosteroid use during these check-ups. DNA samples have been collected from 253 subjects. Thirty two subjects of population 1 were derived from population 2. All participants originated from the northern region of The Netherlands. The study was approved by the medical ethics committee of the University Medical Center Groningen, and all participants signed an informed consent.

Biopsy collection and processing

Bronchoscopy, collection and processing of the bronchial biopsies were performed as previously described [14]. Immunohistochemistry was performed on 3µm formalin fixed, paraffin tissue specimens that were deparafinized with xylene, dehydrated in ethanol and after antigen retrieval, incubated with the primary antibodies: Anti-E-cadherin antibody (BD Bioscience #610181, Breda, The Netherlands) for epithelial E-cadherin expression, anti-eosinophilic peroxidase (EPX) antibody for eosinophils detection (laboratories of NA Lee JJ Lee, Mayo Clinic, Scottsdale, AZ), anti-CD8 (DAKO) antibody for T-lymphocytes and anti-CD31 monoclonal antibody for vessel's endothelial cells. Basement membrane (BM) thickness was calculated by dividing the BM surface area by BM length (µm). The number of positively stained inflammatory cells was counted in a total area of 0.1 mm^2 in the submucosa, 100µm under the BM. The number of CD31+ vessels in the submucosal area was counted in the whole section (excluding epithelium, muscle and mucus glands areas) therefore we measured the number of vessels per area (0.1mm^2). Finally E-cadherin expression was determined by the percentage of BM covered with E-cadherin-positive stained intact epithelium (figure 1). Further details on the used immunochemistry and quantification procedures are presented in the online depository.

Genotyping of Single Nucleotide Polymorphisms (SNPs)

We genotyped 17 haplotype tagging SNPs in *CDH1* according to HapMap CEU genotype data ($r^2 < 0.8$, Minor Allele Frequency >10%). Twelve of the SNPs were

genotyped by K-Bioscience Ltd (UK) using their competitive allele specific PCR system (KASar), five SNPs were derived from a genome wide association study on asthma using the 370Kb Illumina chip (see figure 1 for the genotyped SNPs).

Statistics

Normalization of the distribution of the variables was performed by natural logarithm transformation if necessary. Multiple linear regression analysis adjusted for gender, age and smoking was used to assess the effect of the SNPs on airway remodeling parameters (BM thickness and submucosal vessel numbers) and on inflammatory cells (eosinophils, CD8+T-cells) in population 1. Additionally, we used FEV_1/VC ratio post bronchodilation (bd) adjusted for gender, age, height and smoking as a marker of remodeling in both populations [17]. Since E-cadherin expression is known to be up-regulated by ICS [13,18,19] we tested interaction between ICS and genotypes by introducing interaction terms into the models. General (heterozygotes vs wild types and homozygotes mutant vs wild types) genetic models were used. When the number of subjects with the homozygote mutant genotype was < 5, only dominant (heterozygotes and homozygotes mutant pooled vs wild types) models were used. Association between epithelial E-cadherin expression (percentage of basement membrane covered with intact epithelium positive for E-cadherin) and genotypes was assessed with non-parametric tests using dominant genetic models. The association between denuded epithelium (percentage basement membrane covered with denuded epithelium) and epithelial E-cadherin expression was

tested with multiple linear regression adjusted for sex and age. In population 2, FEV_1 decline was analyzed with Linear Mixed Effect models (LME) as described before [10]. Only FEV_1 measurements after the age of 30 years were included since this is the age where the lung function is in a plateau phase or decline begins in normal subjects [20]. LME models were adjusted for gender, age, first FEV_1 after age 30, height, packyears of smoking and use of oral corticosteroids. Inclusion criteria required subjects having FEV_1 measurements before and after the introduction of ICS, with a minimum of three FEV_1 measurements over a period of at least 2 years. Subjects who never used ICS were excluded. FEV_1 levels and annual FEV_1 declines were estimated for the two periods (without and with ICS use) by introducing the variables: time, genotypes, ICS and their interactions. LME models were conducted in S-plus 7.0 (Insightful Corp., Seattle, Washington, USA). All other analyses were conducted with SPSS (version 16; SPSS Inc., Chicago, Illinois, USA). Two tailed p-values of <0.05 were considered statistically significant.

Results

Clinical characteristics and genotype distribution

Clinical characteristics of both populations are presented in Table 1. All subjects were atopic and hyperresponsive at first clinical assessment. Population 1 had a higher mean lung function and 57 (41.3%) subjects used ICS, while 146 (52%) subjects were on ICS treatment in population 2. For the analysis on FEV_1 decline 125 subjects met the inclusion criteria. Table 2 presents details on corticosteroid

use in populations 1 & 2. Genotype distributions in the two asthmatic populations are presented in Table E1 in the online depository. All SNPs were in Hardy-Weinberg equilibrium ($p>0.05$). The linkage disequilibrium plot of the SNPs in population 2 is shown in figure 2.

Epithelial E-cadherin expression

Five *CDH1* SNPs were significantly associated with epithelial E-cadherin expression (rs8056633, rs16958383, rs2276330, rs3785078, rs7203904). The minor alleles of these SNPs were significantly associated with lower expression of E-cadherin in individuals without ICS and generally with similar expression as the wild types in those with ICS use (table 3 and figure 3; table E2 online depository). There were no significant differences in epithelial E-cadherin expression between never and ever (ex and current pooled) smokers nor among the 3 smoking categories (data not shown). The association between denuded epithelium with epithelial E-cadherin expression was very close to significance ($b=-0.003$; $p=0.05$; natural logarithm scale).

Airway remodeling: BM thickness and subepithelial vasculature

In population 1, 7 out of 17 SNPs were associated with BM thickness and subepithelial vasculature in the presence of ICS (rs7188750, rs8056633, rs16958383, rs2276630, rs3785078, rs7203904 and rs17690554). The minor alleles of these polymorphisms were all associated with less BM thickness and/or lower number of vessels in the submucosal area (table 3 and figure 3). Without

ICS use these effects of the minor alleles were less prominent or even reversed, but not significantly so. Interactions between these SNPs and ICS with respect to airway remodeling were significant for rs2276330 and rs3785078 (tables E3.1 and E3.2 online depository).

Airway Inflammation: submucosal CD8+Tcell and eosinophil counts

With ICS use, rs11075699, rs2276630 and rs1125557 were significantly associated with CD8+T-cell counts in the submucosal area. CD8+T-cell counts were higher in subjects heterozygous for rs11075699 and in carriers of the minor C-allele of rs2276330, while subjects with a homozygous mutant genotype for rs1125557 had lower CD8+T-cell counts compared to wild types. Minor alleles of rs2902185 and rs10431924 were associated with higher eosinophil counts. These associations were absent in asthmatics without ICS. Interactions between SNPs and ICS on inflammatory cell counts (CD8+T-cells and eosinophils respectively) were statistically significant for 3 out of 5 SNPs (rs1125557, rs10431924 and rs2276330) and additionally for rs7188750 (figure 3; tables E4.1 and E4.2 online depository).

FEV_1 decline and FEV_1/VC

Seven out of 17 SNPs were significantly associated with FEV_1 decline in the presence of ICS. The minor alleles for rs8056633, rs16958383, rs7203904 and rs17690554 were associated with less FEV_1 decline. For example, during ICS use FEV_1 decline was 20.4 ml/year for subjects with the wild type genotype of

rs16958383, while it was 36.6 ml/year less in homozygote carriers of the minor allele (p=0.004, figure 3). The minor alleles for rs1125557, rs7199991 and rs7186053 were associated with accelerated FEV_1 decline. A significant interaction of ICS with *CDH1* SNPs on FEV_1 decline was present only for rs7199991 and rs3785078. There were no associations with lung function decline during no ICS use (table 3; table E5 online depository).

In population 2, SNPs that were associated with less FEV_1 decline were also associated with higher FEV_1/VC post bd with ICS use. Additionally, rs4783573, rs7188750 and rs3785078 were associated with higher FEV_1/VC post bd (table 3 and figure 3). Interactions of these SNPs with ICS on FEV_1/VC post bd were significant. In population 1, these SNPs had the same direction of association with FEV_1/VC post bd, but only the interaction with rs7203904 was significant (tables E6.1 and E6.2 online depository).

Discussion

Our results indicate that *E-cadherin* (*CDH1*) gene polymorphisms are associated with airway remodeling, inflammation and lung function decline in individuals with asthma. In summary, with ICS use 7 of the 17 SNPs were associated with airway remodeling, 3 SNPs with number of CD8+T-cells and 2 with eosinophil counts in the submucosa, and 7 SNPs with FEV_1 decline. Consistently, alleles associated with less airway remodeling correlated also with less FEV_1 decline and higher FEV_1/VC post bd. While all associations occurred during ICS use, associations

with epithelial E-cadherin expression were significant for 5 SNPs in the absence of ICS. These results may indicate that ICS may influence the way these polymorphisms express their effect and are consistent with *in vitro* results showing that administration of glucocorticosteroids can up-regulate cadherins expression, improve cell-cell contact and strengthen the epithelial barrier [19,21,22].

Altered expression of epithelial junctional proteins on bronchial epithelium may contribute to a defective epithelial barrier [1,23] with subsequent functional and clinical manifestations. In our longitudinal study (population 2), the minor alleles of 3 SNPs (rs1125557, rs7199991 and rs7186053) were associated with accelerated FEV_1 decline during ICS use. Rs7199991 is in complete LD (R^2=1 according to www.hapmap.org; version 4.0) with a promoter region SNP (rs16260) suggesting this may have functional significance. So far E-cadherin gene polymorphisms have not been studied in asthma, but recent findings have shown associations of *CDH1* SNPs with Crohn's disease [24], another impaired epithelial-barrier disorder which may share the same pathways with asthma [25,26]. Moreover, in genome wide association studies *CDH1* has recently been associated with susceptibility to ulcerative colitis [27] and colorectal cancer [28]. The new associations suggest that changes in the integrity of the intestinal epithelial barrier may contribute to the pathogenesis of these diseases hence *CDH1* polymorphisms are important for understanding the pathogenesis of impaired epithelial barrier diseases.

Additionally our study showed that the minor alleles of 4 other SNPs (rs8056633, rs16958383, rs7203904 and rs17690554) were associated with less FEV_1 decline. The latter 4 alleles were also associated with less airway remodeling in biopsies of asthmatics using ICS in population 1. The fact that we observe a significant decreased BM thickness in the presence of ICS is in line with findings of Broekema et al. who previously showed that symptomatic asthmatics using ICS in our population 1 had significantly thinner BM than asymptomatic asthmatics who did not use ICS (5.7 and 6.5 μm respectively, $p<0.05$) [15]. However, our results highlight that this decrease in BM thickness is likely not present in all asthmatics using ICS, but only in those with a specific *E-cadherin* genotype.

Rasmussen et al. used FEV_1/VC ratio after bonchodilatation as an indirect measure of airway remodeling [17] and our results point in the same direction i.e alleles associated with less airway remodeling (rs7188750, rs8056633, rs16958383, rs3785078, rs7203904 and rs17690554) were also associated with higher FEV_1/VC post bd and less FEV_1 decline in population 2. In population 1, only rs7203904 showed a significant association with FEV_1/VC ratio which is likely due to a lower power: lower number of ICS users (57 subjects (41%) vs 146 subjects (52%)) and less FEV_1/VC post bd variance (standard deviation 6.5 vs 13.5) compared to population 2. In contrast, Kosciuch et al. concluded that there

is no relationship between BM thickness and lung function tests like FEV_1, FEV_1%VC, RV%pred, TLC%pred, but they included only asthmatic patients who never used ICS or who were withdrawn from ICS for at least 3 months [29]. Our study suggests that discrepant findings may, at least partially, be due to a modulating effect of both ICS and *CDH1* SNPs on airway remodeling and lung function. As previous studies have shown, treatment with ICS may reduce or control the intensity of airway remodeling, improve FEV_1 and reduce loss of lung function [30-32]. Yet it has to be elucidated how ICS prevent these adverse effects. Based on our findings and on evidence that corticosteroids up-regulate cadherin expression [18,33] we speculate that ICS interaction with *CDH1* SNPs might be one possible explanation.

The fact that we do not observe significant differences in epithelial E-cadherin expression between the mutant alleles and wild types during ICS use could be attributed to the fact that the mutant alleles interact with ICS and promote E-cadherin up-regulation to a similar level as the wild types do. However in the absence of ICS the mutant alleles are not capable of producing the same amount of protein compared to wild types. The nearly significant, inverse association we found between damaged/denuded epithelium and epithelial E-cadherin expression indicates that E-cadherin is involved in epithelial integrity and it is likely that epithelial damage is increased in subjects with lower E-cadherin expression.

In our study we additionally observed that specific *CDH1* polymorphisms in the presence of ICS tend to be associated with recruitment of CD8+T-cells in the submucosa. Previously, Sont et al. found that despite ICS treatment CD8+T-cell infiltration might persist in asthma patients and that CD8+ but not CD4+ cells are associated with BHR [34] and lung function decline [35], and recently Hirosako et al. showed that CD8 and CD103 (ligand for E-cadherin) are highly expressed in asthmatic bronchial intraepithelial lymphocytes. In this study the percentage of CD8+ cells was higher than the percentage of CD4+ cells in intraepithelial lymphocytes in asthma. The expression of CD103 was significantly higher in CD8+ cells compared with CD4+ cells, suggesting that the interaction between E-cadherin (CD103) and CD8+ cells is the reason for the higher percentage of CD8+ cells in asthmatic intraepithelial lymphocytes [8]. Furthermore, Cepek et al. demonstrated that T-lymphocytes express a member of cadherins superfamily and this may contribute to T-cell-mediated immune surveillance via heterotypic adhesions with mucosal epithelial cells [36]. Combining these findings we could speculate that there might be a link between specific *CDH1* polymorphisms and expression or binding of E-cadherin on T-cells, a finding that needs further investigation.

One could discuss that the lack of multiple testing correction is responsible for the current results. We decided not to apply a sequential (classical) Bonferroni correction for a number of reasons. Firstly, our choice for the current study was explicitly driven by previous observations, suggesting E-cadherin expression is

related to epithelial integrity, airway remodeling and disease progression. Secondly, the independent variables in our analyses (e.g. airway remodeling, FEV_1 decline) are mutually related, indicating that a rigid statistical procedure like a Bonferroni correction for multiple testing would not do justice to their biologically linked nature. Finally, although adjustment for multiple testing will decrease the chance of a type I error, it will also increase the chance of a type II error, so that a true association is not found. This is especially possible in a relatively small study like ours. We thus follow the advice given by T.V. Perneger: "Simply describing what was done and why, and discussing the possible interpretations of each result should enable the reader to reach a reasonable conclusion without the help of Bonferroni adjustments" [37].

In our study, the associations between the SNPs and the biopsy parameters in population 1 were confirmed in population 2, where the same risk alleles were associated with FEV_1 decline. This is called a loose replication [38]. The observed interaction of *CDH1* SNPs with ICS use makes it difficult to find another study that is suitable for replication of our results. Firstly, no other study on adult asthma patients exists with longitudinal data on lung function both with and without ICS use during such a long follow-up. Secondly, bronchial biopsy studies in asthmatics usually investigate fewer patients than our study which decreases the power to find associations.

In conclusion our data show that *CDH1* single nucleotide polymorphisms are associated with epithelial E-cadherin expression and suggest that epithelial adhesion is an important contributor to airway remodeling and lung function in asthma. These effects are modified by the use of inhaled corticosteroids.

Acknowledgments

We thank F Volbeda and A Dijkstra (Dep. Pulmonology) for collecting the biopsies, M Broekema and M Lodewijk (Dep. Pathology and Medical Biology) for processing and staining of the biopsies and M Olivier (Dep. Genetics) for her help with genotyping. University Medical Center Groningen (UMCG).

Grant support

This study was in part supported by a grant from the Netherlands Asthma Foundation (AF 3.2.07.15)

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Tables:

Table 1: Characteristics of population 1 and 2

	Population 1	Population 2
Number of asthmatics	n=138	n=281
Year of assessment	2002-2006	1991-1999
Males (%)	50.7	60.5
Age (years)	47.2 (12.7)	51.3 (9.1)
ICS use	57 (41.3)	146 (52.0)
LABA use	37 (26.8)	NA
non/ex/current smokers (%)	44.9/34.1/21.0	40.6/32.0/27.4
FEV_1% predicted pre bd (ml)	91.7 (18.0)	70.1 (23.6)
FEV_1% predicted post bd (ml)	99.9 (17.0)	82.5 (22.4)
FEV_1/VC pre bd	71.2 (8.8)	60.2 (14.2)
FEV_1/VC post bd	75.6 (6.5)	64.8 (13.5)
CD31+Vessel numbers*	18.0 (8.2)	NA
BM Thickness (µm)	6.0 (1.6)	NA
CD8+Cells*	23.0 (1-204)	NA
EPX+Cells*	2.0 (0-40)	NA
Epithelial E-cadherin expression#	88.4 (0-100)	NA

All values are presented in mean (standard deviation) or median (range).
FEV_1=forced expiratory volume in one second, VC=vital capacity, bd= bronchodilator, ICS= inhaled corticosteroids, LABA=long acting beta-agonists, EPX= eosinophilic peroxidase, BM=basement membrane, * per 0.1mm^2 of submucosal area, #% of BM covered with E-cadherin-positive stained intact epithelium, NA=not applicable

Table 2: Characteristics of corticosteroid use in populations 1 and 2

	Population 1 (n=138)	Population 2 (n=281)
Subjects using ICS at final survey, n (%)	57 (41.3)	146 (52.0)
Daily ICS dose (µg/day)*	800 (100-2000)	800 (50-6000)
Subjects using oral CS at final survey, n (%)	0	14 (5.0)
Subject using LABA among ICS users, n (%)	31 (54.4)	NA
Subjects included in analysis on FEV_1 decline, only ever ICS-users (n=125)		
Subjects who ever used ICS, n (%)		125 (100.0)
Subjects who ever used oral CS, n (%)		69 (55.2)
Age at start of ICS use (years)		42 (21-70)
Duration of ICS use (years)		13.5 (2.2-25.7)
Daily ICS dose (µg/day)*		694 (179-2400)
Duration of oral CS use (years)		9.8 (0.1-34.8)
Daily oral CS dose (µg/day)		7.6 (2.1-15.0)

Data are presented as median values (min-max) unless stated otherwise.
CS: corticosteroids, ICS: inhaled corticosteroids, LABA: long acting beta-agonists, NA: not applicable
* All doses were recalculated to equivalents of Beclomethasone: 100 µg/day of Beclomethasone is equivalent to 100 µg/day of Budesonide (not by Turbuhaler), to 50 µg/day of Budesonide by Turbuhaler and to 50 µg/day of Fluticasone.

Table 3: Summary table of *CDH1* SNPs significantly associated with airway remodeling and lung function decline in the presence of ICS and their associations with FEV_1/VC post bd and epithelial E-cadherin expression

CDH1 gene SNPs		ICS use					No ICS use
		BM thickness (µm)	Number of CD31+vessels /0.1mm^2	FEV_1/VC post bd (%)	Annual change in FEV_1 (ml/year)	% epithelial E-cadherin expression[#]	% epithelial E-cadherin expression[#]
		B				Median Difference [$]	Median Difference[$]
rs1125557	AG GG	0.5 0.2	3.9 2.9	1.7 -4.2	**-19.7*** **-17.6***	-2	-9
rs7199991	AC CC	-0.3	3.2	0.9	**-13.5*** -5.1	-4	-11
rs7186053	GA AA	0.2	2.0	-2.2 -4.6	**-16.7*** -13.7	-3	-9
rs7188750	GA/AA	-0.9	**-6.9***	**5.2***	5.6	2	-21
rs8056633	TG GG	-0.7	**-4.7***	**7.1***	8.6 **27.2***	-4	**-17***
rs16958383	GA AA	**-1.0***	**-6.1***	**5.5***	10.5 **36.6***	-3	**-48***
rs2276330	TC/CC	**-1.1***	**-6.9***	4.5	6.6	-6	**-33***
rs3785078	AC/CC	**-1.1***	**-6.6***	**6.1***	10.5	-4	**-46***
rs7203904	GC CC	-0.6	**-4.7***	**5.1*** **9.8***	1.7 **27.4***	-5	**-18***
rs17690554	CG GG	**-1.0***	-2.8	**7.5***	**15.3*** **26.9***	-3	-14

*p value<0.05, B= regression coefficient, Median Difference = Median of heterozygotes and homozygotes mutant pooled- Median of wild types, $= significance tested with Mann–Whitney *U* test, BM=basement membrane, 0.1mm^2 of submucosal ares, [#] percentage of BM covered with E-cadherin-positive stained intact epithelium, FEV_1= forced expiratory volume in one second, VC= vital capacity, bd= bronchodilator

Figure Legends:

Figure 1: Immunostaining of airway wall biopsies of asthma patients

Representative pictures of $CD31^+$ vessels: A= strong; B= weak staining; and epithelial E-cadherin expression: C=strong;
D= moderate; E= weak staining. Magnification: objective 20x

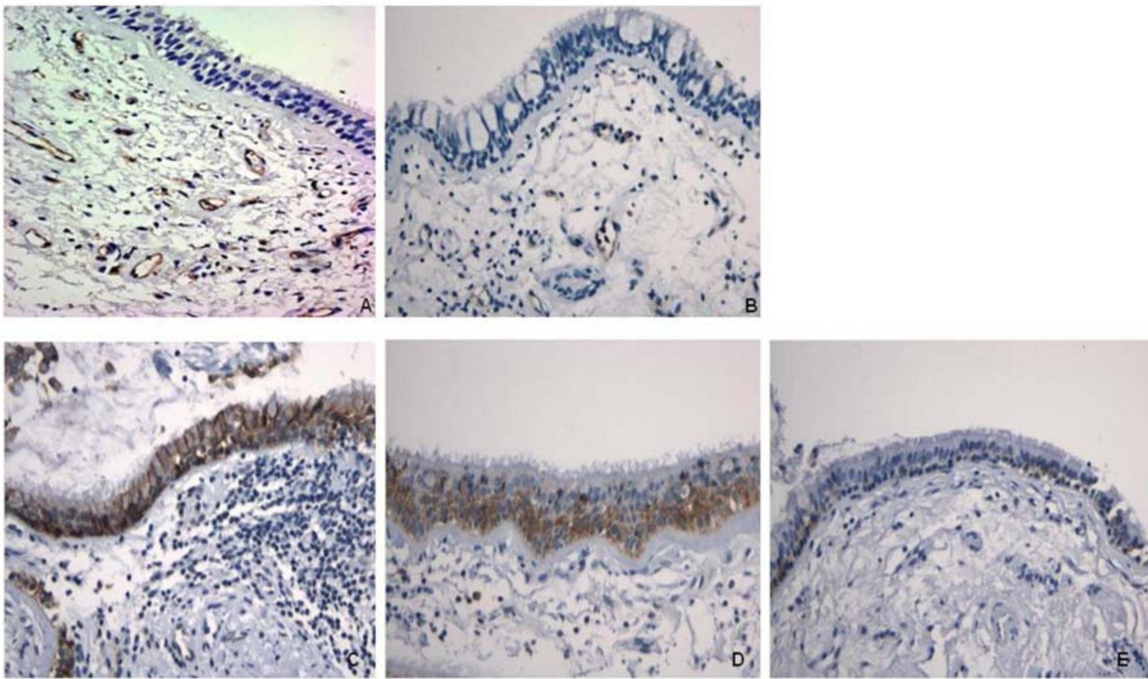


Figure 2: Linkage disequilibrium plot and correlation coefficients ($100 \cdot r^2$) of 17 genotyped *CDH1* gene single nucleotide polymorphisms in population 2 (n=281)

The location of single nucleotide polymorphisms is given for the HapMap, Release November 2008 (www.hapmap.org).

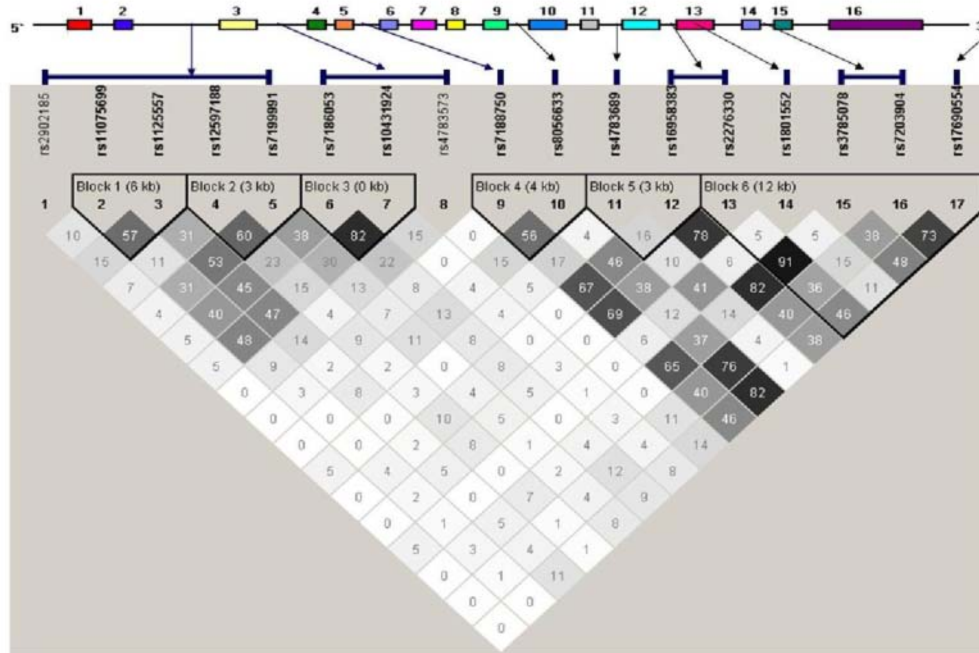
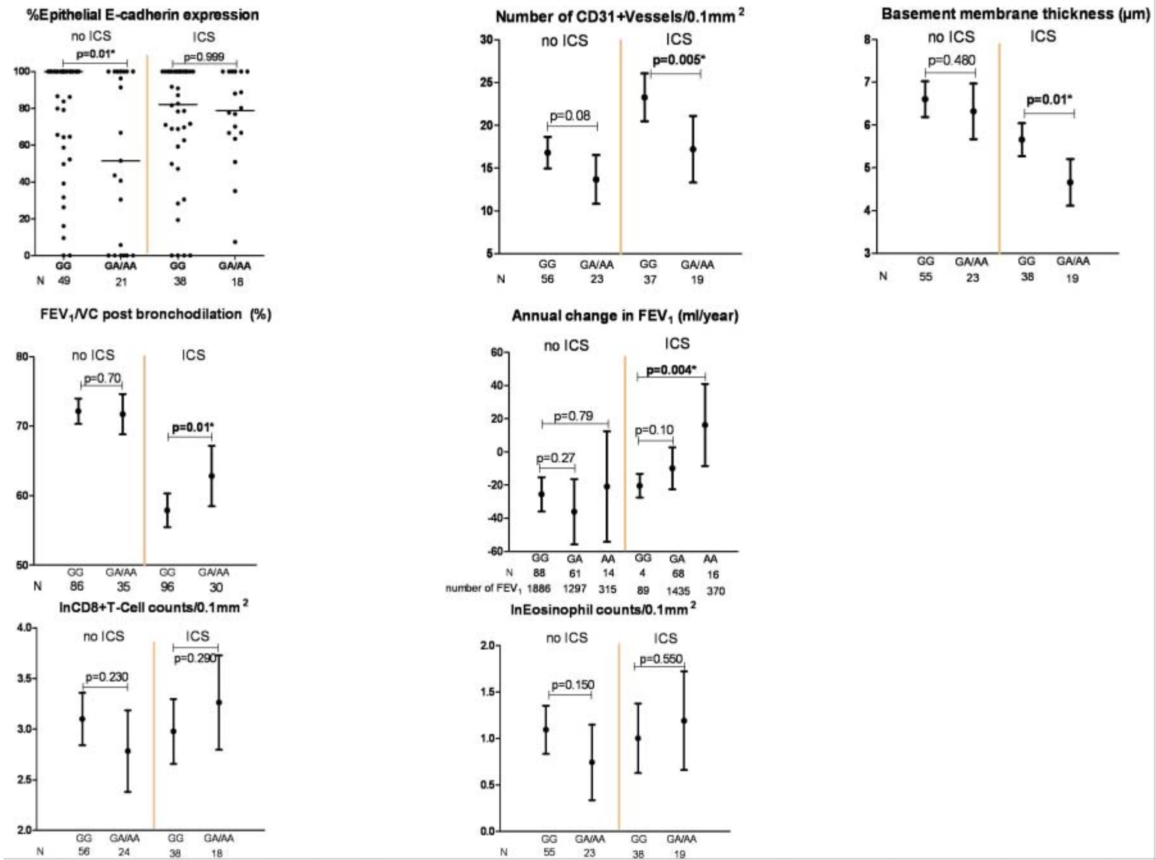


Figure 3: *CDH1* rs16958383 associations with epithelial E-cadherin expression, airway remodeling, inflammation and lung function decline in adult asthma patients.

CDH1 rs16958383 represents SNPs associated with airway remodeling (rs7188750, rs8056633, rs16958383, rs2276330, rs3785878, rs7203904 and rs17690554). General Linear Models were used to estimate the means of each dependent variable adjusted for gender, age, and smoking.

SNPs= single nucleotide polymorphisms, N= number of subjects, FEV_1= forced expiratory volume in one second, VC= vital capacity, BM= basement membrane, ln= natural logarithm of cell counts per $0.1mm^2$ of submucosal area, % = percentage of BM covered with E-cadherin-positive stained intact epithelium, ICS= inhaled corticosteroids


%Epithelial E-cadherin expression
no ICS
ICS
p=0.01*
p=0.999
GG
GA/AA
N 49 21 38 18
Number of CD31+Vessels/0.1mm 2
p=0.08
p=0.005*
N 56 23 37 19
Basement membrane thickness (µm)
p=0.480
p=0.01*
N 55 23 38 19
FEV1/VC post bronchodilation (%)
p=0.70
p=0.01*
N 86 35 96 30
Annual change in FEV1 (ml/year)
p=0.79
p=0.27
p=0.004*
p=0.10
GG GA AA GG GA AA
N 88 61 14 4 68 16
number of FEV1 1886 1297 315 89 1435 370
lnCD8+T-Cell counts/0.1mm 2
p=0.230
p=0.290
N 56 24 38 18
lnEosinophil counts/0.1mm 2
p=0.150
p=0.550
N 55 23 38 19