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Chromosome 17q21 SNP rs8076131 risk allele associates with airway smooth muscle hypertrophy in fatal asthma

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To the Editor:

Airway remodeling in asthma is characterized by the presence of increased airway smooth muscle (ASM), increased mucus producing cells (submucosal mucous glands and epithelial goblet cells), and subepithelial fibrosis (deposition of collagen and extracellular matrix proteins) which all contribute to increased airway wall thickness¹. The increased thickness of the ASM layer in the remodeled airway in asthma is likely due to both hypertrophy and hyperplasia^{E1}. Increased thickness of the ASM layer is associated with inducible airway remodeling^{E2} and with functional changes including decline in FEV1². As the increased thickness of the ASM layer is related to asthma severity³, but not to duration of asthma or age³, in this study we have investigated whether the thickness of the lung ASM layer in asthmatics who have had a very severe exacerbation of asthma (i.e. a fatal episode of asthma) could be related to their genetic predisposition in harboring a chromosome 17q21 SNP risk allele. Chromosome 17q21 SNPs have been linked to the risk of acute severe asthma exacerbations in childhood⁴, rhinoviral wheezing illness (rhinovirus is a frequent trigger of asthma exacerbations)⁵, and to severe asthma⁶. However, none of these epidemiologic studies of chromosome 17q21 SNPs and severe asthma exacerbations have investigated whether a chromosome 17q21 SNP could influence either the risk of a fatal

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Conflict of Interest

The authors have no conflicts of interest to disclose related to this research. These include consultant arrangements, speakers' bureau participation, stock or other equity ownership, patent licensing arrangements, employment, or expert witness testimony.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

exacerbation of asthma, or ASM layer thickness and hypertrophy in asthmatics with fatal asthma exacerbations which is the focus of this study.

SNPs linked to chromosome 17q21 have been associated with increased levels of ORMDL3^{E3}. Studies in a transgenic mouse model which express increased levels of human ORMDL3 have demonstrated that in the absence of allergen challenge these mice spontaneously develop increased ASM and increased airway responsiveness without associated airway inflammation⁷. In addition, precision cut lung slices from ORMDL3 transgenic mice in the absence of allergen challenge exhibit increased methacholine induced ASM Ca²⁺ oscillation and increased ASM contractility compared to wild type mice⁸. While these studies in mice support a potential role for ORMDL3 expression in ASM contributing to increased ASM thickness in human subjects with asthma, they do not address whether asthmatics with SNPs linked to chromosome 17q21 have increased ASM. We also investigated this possibility in this study.

We genotyped post-mortem lung airway sections from 45 subjects (24 fatal asthma, 13 non-fatal asthma, 8 non-asthma) to determine whether they had either the chromosome 17q21 SNP rs8076131 risk allele AA, the intermediate risk allele AG, or the non-risk allele GG using a TaqMan SNP genotyping assay^{E4}. As not all SNPs are functional in regulating levels of gene expression, we focused on studying a SNP rs8076131 on chromosome 17q21 whose risk allele is known to regulate increased levels of ORMDL3 expression^{E5,E6}. The study subjects (Table 1A), airway tissue preparation, airway measurements (ASM, airway eosinophil, airway neutrophil, reticular basement membrane thickness, inner airway wall thickness, and mucus), and genotyping are all described in detail in the On-Line Supplementary Material^{E1–E20}. In brief, genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) lung blocks using QIAamp DNA FFPE tissue kit (Qiagen) according to the manufacturing instruction. Chromosome 17q21 ORMDL3 SNP rs8076131 genotyping was performed using the TaqMan SNP assay^{E4} (Thermo Fisher). The chromosome 17q21 SNP rs8076131 is located in an intron between exon 3 and exon 4 of ORMDL3 (Figure 1A). The SNP rs8076131 genotype was determined in anonymized airway sections, and the results of the SNP genotyping were correlated with previously quantitated inner airway wall thickness (which measures the ASM thickness combined with reticular basement membrane thickness), ASM layer thickness, ASM hypertrophy, and ASM hyperplasia using methods previously described in detail⁹.

The genotyping of the chromosome 17q21 SNP rs8076131 demonstrated that the non-risk GG allele was present in none of the 24 fatal asthmatics (0% of fatal asthmatics had the GG non-risk allele) whereas the GG non-risk allele was present in 50% of control non-asthmatics ($p=0.006$ vs fatal asthma) and 23% of non-fatal asthma ($p=NS$) (Table 1B). In addition, all of the fatal asthma samples were either homozygous or heterozygous for the rs807613 A risk allele. The absence of the non-risk GG allele in the fatal asthma group may be due to the potential low prevalence of the non-risk GG allele in fatal asthmatics, or to the number of fatal asthmatics studied ($n=24$). It should be noted that we identified the non-risk GG allele in 50% of control non-asthmatics (4/8 control subjects), 24% of non-fatal asthmatics (3/13 asthma subjects), and 0% of fatal asthmatics despite studying many more fatal asthmatics ($n=24$) compared to non-fatal asthmatics ($n=13$), or non-asthmatics ($n=8$). If

future studies do not identify the non-risk GG allele in fatal asthmatics a blood screening test for the risk and non-risk rs8076131 allele in asthmatics could be evaluated to determine whether the presence of the risk allele could predict asthmatics at risk for fatal episodes of asthma. The prevalence of the non-risk GG allele is approximately 21% in Europeans of northern, western, or Italian heritage^{E7} which is similar to the 23% prevalence we have noted in the non-fatal asthma lungs which were all obtained from Caucasians in Australia and Canada.

As the genotyping of the lungs (fatal asthma, non-fatal asthma, control) suggested a relationship between the rs8076131 A risk allele and fatal asthma, we next examined whether the airways from these lungs exhibited changes in airway remodeling and/or airway inflammation that could predispose asthmatics with the rs8076131 A allele to fatal episodes of asthma. We initially examined the relationship of the AA SNP rs8076131 risk allele to the inner airway wall thickness (which measures the ASM thickness combined with reticular basement membrane thickness), RBMt thickness, ASM thickness, ASM hypertrophy, and ASM hyperplasia in all the 45 lungs studied (asthma n=37, and non-asthma=8). Subjects with the SNP rs8076131 AA risk allele had significantly increased inner airway wall thickness compared to subjects with the GG non-risk allele ($p=0.05$) (Fig 1B), as well as trends for increased ASM thickness ($p=0.08$) (Fig 1C), RBMt thickness ($p=0.12$) (Fig 1D), but no difference in ASM hypertrophy, ASM hyperplasia, airway eosinophils, airway neutrophils, or mucus compared to subjects with the GG non-risk allele (data not shown).

As we observed a relationship between the SNP rs8076131 AA risk allele and inner airway wall thickness and ASM thickness in the cohort as a whole, we next examined whether these relationships were present in either the subset of fatal asthmatics, non-fatal asthmatics, and/or non-asthmatics which made up the cohort. Fatal asthmatics with the chromosome 17q21 SNP rs8076131 AA risk allele had significantly increased ASM hypertrophy in airway sections compared to fatal asthmatics with the intermediate risk AG allele ($P<0.02$) (Figure 1E), but no increase in inner airway wall thickness, ASM thickness, or RBMt thickness (data not shown). None of the fatal asthmatics had the non-risk GG allele in this group of 24 fatal asthmatics who were randomly selected. Thus, having the non-risk GG allele in the cohort as a whole is associated with lower inner airway wall thickness compared to the AA allele. This may also be the case in fatal asthma, but this requires further study and the identification of fatal asthmatics with the non-risk GG allele. In the cohort as a whole, subjects with the non-risk GG allele ($n=7$) had the lowest levels of ASM thickness (Figure 1C) and inner airway wall thickness (Fig 1B).

The fatal asthma group with the AA risk allele had no difference in ASM hyperplasia, airway eosinophils, airway neutrophils, or mucus, compared to fatal asthmatics with the intermediate risk AG allele (data not shown). In the non-fatal asthma subjects ($n=13$), the non-asthma subjects ($n=8$), or all asthma subjects combined (fatal and non-fatal)($n=37$) there was no difference in inner airway wall thickness, ASM thickness, airway eosinophils, airway neutrophils or mucus, comparing the AA and GG group, or the AA and AG groups (data not shown). However, it is possible that the low numbers of subjects in the non-fatal asthma group ($n=13$) and the non-asthma group ($n=8$) may have reduced power to detect differences between these groups.

The use of corticosteroids, age of asthma onset, and gender are confounding variables that could potentially influence levels of ASM hypertrophy in the two genotype groups of fatal asthmatics (AA and AG) in Fig 1E. We therefore performed additional analysis of the data in the two genotype groups of fatal asthmatics (14 AA; 10 AG) in Figure 1E to determine whether there were any differences between the two groups in the use of corticosteroids, age of onset of asthma, and gender. This analysis demonstrated that there was a non-significant trend ($p=0.20$) for a difference between the fatal asthma AA group and the fatal asthma AG group in the use of corticosteroids, and no difference in age of onset of asthma, or gender (Supplementary Table 1).

The increased thickness of the ASM layer in asthma has previously been shown to be due to both ASM hyperplasia (increased numbers of ASM)^{E10,E11} and ASM hypertrophy (increased size of ASM)^{E10,E11}. The increased thickness of the ASM layer in asthma may theoretically be due to either pathways extrinsic to ASM (i.e. airway inflammation) and/or to pathways intrinsic to ASM^{1,E11}. In extrinsic pathways, a cytokine or mediator needs to be released from a non-ASM cell type (e.g. T cell, eosinophil, epithelium, mast cell, etc.)^{E5,E12,E13} to act on ASM to induce hypertrophy or hyperplasia. In contrast, in an intrinsic pathway the stimulus to induce ASM hypertrophy or hyperplasia arises from within the ASM cell. For example, ORMDL3 expressed at increased levels in the endoplasmic reticulum in ASM activates an intrinsic pathway in ASM to induce hypertrophy⁸ independent of any mediator or cytokine released from a non-ASM cell type. ORMDL3 is an endoplasmic reticulum localized transmembrane protein which regulates several downstream pathways including the ATF6 α unfolded protein response pathway^{E14}, SERCA2b^{E14}, sphingolipids^{E15,E16}, as well as chemokines and cytokines^{E14}. In vitro studies have shown that siRNA knockdown of ATF6 α inhibits ASM proliferation^{E17}. However, at present it is not known whether this or other pathways regulated by ORMDL3 contribute to increase ASM hypertrophy mediated by SNPs on chromosome 17q21 such as rs8076131.

In summary, we demonstrated that all of the 24 fatal asthmatics were either homozygous or heterozygous for the chromosome 17q21 risk A rs8076131 allele, and that none of the fatal asthmatics had the non-risk GG allele. The AA risk allele of SNP rs8076131 on chromosome 17q21 (compared to the non-risk GG allele) was significantly associated with inner airway wall thickness and a trend for ASM thickness in a cohort of lungs from asthmatics and controls. In addition, fatal asthmatics with the chromosome 17q21 SNP rs8076131 AA risk allele had a significantly increased ASM hypertrophy in airway sections compared to fatal asthmatics with the intermediate risk AG allele. Further study is needed to determine whether fatal asthmatics with the rs8076131 AA risk allele also have significantly thicker ASM layer in airway sections compared to fatal asthmatics with the GG non-risk allele.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ASM	Airway smooth muscle
FEV1	Forced expiratory volume in 1 second
ORMDL3	ORM1 like 3
SNP	Single nucleotide polymorphisms
RBMt	Reticular basement membrane thickness

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Figure 1: Association of chromosome 17q21 SNP rs8076131 AA risk allele with inner airway wall thickness (in combined cohort of asthmatics and non-asthmatic controls), and with ASM hypertrophy in fatal asthmatics

A). SNP rs8076131 localizes to an intron of ORMDL3 in chromosome 17q21. **(B-D)**, Chromosome 17q21 ORMDL3 SNP rs8076131 genotyping was performed using a TaqMan SNP assay in a combined cohort of asthmatics and non-asthmatic controls (n=45). AA risk allele, GG non-risk allele. The results of the SNP genotyping were correlated with **(B)**, inner airway wall thickness (mm), **(C)**, ASM thickness (mm), **(D)**, and reticular basement membrane thickness (mm). **(E)** Chromosome 17q21 ORMDL3 SNP rs8076131 genotyping was also performed in a subset of fatal asthmatics (n=24) and correlated with ASM hypertrophy (μm^3).

Tablec 1A.

Characteristics of Study Subjects

Characteristic	No Asthma	Non-Fatal Asthma	Fatal Asthma
Number of subjects	8	13	24
Age (mean) (years)	47 ±7	36 ±3	36±4
Gender (male/female) (number)	5/3	9/4	14/10
Age of onset of asthma (median, range) (years)	NA	17 (2-55}	7 (1-61)
Duration of asthma (median, range) (years)	NA	13 (2-31)	16 (2-60)
Corticosteroid use (oral or inhaled)(%)	NA	32%	65%

NA = Not Applicable

Table 1B.

Chromosome 17q21 SNPRs8076131 genotype

	GG (non-risk)	AG	AA (risk)
No Asthma	50%	25%	25%
Non-Fatal Asthma	24%	38%	38%
Fatal Asthma	0% ***	42% **	58% *

Results depict % of study subjects in No Asthma, Non-Fatal Asthma, and Fatal Asthma groups with a particular genotype

Fatal Asthma vs No Asthma:

*
P= NS

**
P=0.02

P=0.006

Fatal Asthma vs Non-Fatal Asthma: P=NS for all genotypes

Non-Fatal Asthma vs No Asthma: P=NS for all genotypes