

## RESEARCH LETTER

WILEY

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

To the Editor,

Airway remodelling 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 thickness.<sup>1</sup> The increased thickness of the ASM layer in the remodelled airway in asthma is likely due to both hypertrophy and hyperplasia<sup>E1</sup>. Increased thickness of the ASM layer is associated with inducible airway remodelling<sup>E2</sup> and with functional changes including decline in FEV1.<sup>2</sup> As the increased thickness of the ASM layer is related to asthma severity,<sup>3</sup> but not to duration of asthma or age,<sup>3</sup> 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 (ie a fatal episode of asthma) could be related to their genetic predisposition in harbouring a chromosome 17q21 SNP risk allele. Chromosome 17q21 SNPs have been linked to the risk of acute severe asthma exacerbations in childhood,<sup>4</sup> rhinoviral wheezing illness (rhinovirus is a frequent trigger of asthma exacerbations)<sup>5</sup> and to severe asthma.<sup>6</sup> 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 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<sup>E3</sup>. 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 inflammation.<sup>7</sup> In addition, precision cut lung slices from ORMDL3 transgenic mice in the absence of allergen challenge exhibit increased methacholine induced ASM Ca<sup>2+</sup> oscillation and increased ASM contractility compared to wild type mice.<sup>8</sup> 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<sup>E4</sup>. 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<sup>E5,E6</sup>. 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<sup>E1-E20</sup>. 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<sup>E4</sup> (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 detail.<sup>9</sup>

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 ( $P = .006$  vs fatal asthma) and 23% of non-fatal asthma ( $p = \text{NS}$ ) (Table 1B). In addition, all of the fatal asthma samples were either homozygous or heterozygous for the rs8076131 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<sup>E7</sup> which

**TABLE 1A** Characteristics of Study Subjects

| Characteristic                                 | No asthma | Non-Fatal asthma | Fatal asthma |
|------------------------------------------------|-----------|------------------|--------------|
| Number of subjects                             | 8         | 13               | 24           |
| Age (mean) (y)                                 | 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%          |

Abbreviation: NA, Not Applicable.

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 remodelling 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 ( $P = .05$ ) (Figure 1B), as well as trends for increased ASM thickness ( $P = .08$ ) (Figure 1C), RBMt thickness ( $P = .12$ ) (Figure 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 ( $P < .02$ ) (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

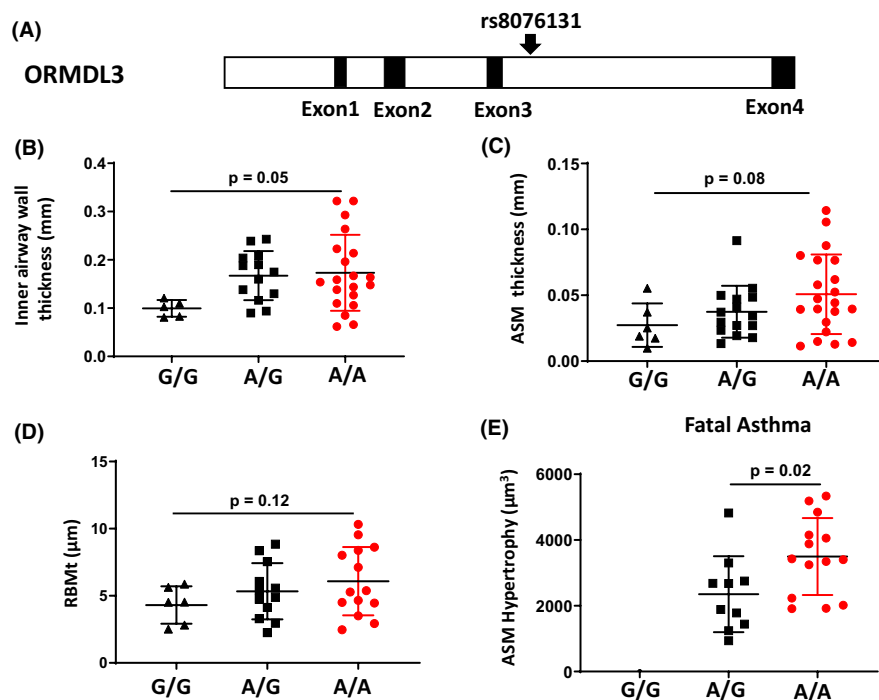


**FIGURE 1** Association of chromosome 17q21 SNP rs8076131 AA risk allele with inner airway wall thickness (in combined cohort of asthmatics and non-asthmatic controls) and with ASM hypertrophy in fatal asthmatics. A, SNP rs8076131 localizes to an intron of ORMDL3 in chromosome 17q21. B-D, chromosome 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 ( $\mu\text{m}^3$ )

**TABLE 1B** Chromosome 17q21 SNPRs8076131 genotype

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

Note: Results depict % of study subjects in No Asthma, Non-Fatal Asthma and Fatal

Asthma groups with a particular genotype.

Fatal Asthma vs No Asthma: \* $P = .02$ , \*\* $P = .006$ , \*\*\* $P = .0006$ .

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

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

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 (Figure 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 Figure 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 ( $P = .20$ ) 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 (Table S1).

The increased thickness of the ASM layer in asthma has previously been shown to be due to both ASM hyperplasia (increased numbers of ASM)<sup>E10,E11</sup> and ASM hypertrophy (increased size of ASM)<sup>E10,E11</sup>. The increased thickness of the ASM layer in asthma may theoretically be due to either pathways extrinsic to ASM (ie airway inflammation) and/or to pathways intrinsic to ASM<sup>1,E11</sup>. In extrinsic pathways, a cytokine or mediator needs to be released from a non-ASM cell type (eg T cell, eosinophil, epithelium, mast cell)<sup>E5,E12,E13</sup> 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 hypertrophy<sup>8</sup> 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 $\alpha$  unfolded protein response pathway<sup>E14</sup>, SERCA2b<sup>E14</sup>, sphingolipids<sup>E15,E16</sup>, as well as chemokines and cytokines<sup>E14</sup>. In vitro studies have shown that siRNA knock-down of ATF6 $\alpha$  inhibits ASM proliferation<sup>E17</sup>. 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.

## 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.

## FUNDING INFORMATION

This study was supported by NIH Grants AI 070535, AI 124236 and AI 107779 (DHB). AP and NW were supported by NIH T32 AI 007469.

Marina Miller<sup>1</sup>

Peter Rosenthal<sup>1</sup>

Ning Weng<sup>1</sup>

Alex Pham<sup>1</sup>

Gyu-Young Hur<sup>1</sup>

John Elliot<sup>2</sup>

Francis H.Y. Green<sup>3</sup>

Alan James<sup>2</sup>

David H. Broide<sup>1</sup> 

<sup>1</sup>Department of Medicine, University of California San Diego, La Jolla, CA, USA

<sup>2</sup>Department of Pulmonary Physiology and Sleep Medicine, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia

<sup>3</sup>Department of Pathology & Laboratory Medicine, University of  
Calgary, Alberta, Canada

#### Correspondence

David Broide, University of California San Diego, 9500  
Gilman Drive, MC 0635, La Jolla, CA 92093-0635, Australia  
Email: dbroide@health.ucsd.edu

#### ORCID

David H. Broide  <https://orcid.org/0000-0001-8405-9090>

#### REFERENCES

1. Hur GY, Broide DH. Genes and pathways regulating decline in lung function and airway remodeling in asthma. *Allergy Asthma Immunol Res.* 2019;11:604-621.
2. Pepe C, Foley S, Shannon J, et al. Differences in airway remodeling between subjects with severe and moderate asthma. *J Allergy Clin Immunol.* 2005;116:544-549.
3. James AL, Bai TR, Mauad T, et al. Airway smooth muscle thickness in asthma is related to severity but not duration of asthma. *Eur Respir J.* 2009;34:1040-1045.
4. Bisgaard H, Bønnelykke K, Sleiman PM, et al. Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. *Am J Respir Crit Care Med.* 2009;179:179-185.
5. Çalışkan M, Bochkov YA, Kreiner-Møller E, et al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med.* 2013;368:1398-1407.
6. Binia A, Khorasani N, Bhavsar PK, et al. Chromosome 17q21 SNP and severe asthma. *J Hum Genet.* 2011;56:97-98.
7. Miller M, Rosenthal P, Beppu A, et al. ORMDL3 transgenic mice have increased airway remodeling and airway responsiveness characteristic of asthma. *J Immunol.* 2014;192:3475-3487.
8. Chen J, Miller M, Unno H, et al. Orosomucoid-like 3 (ORMDL3) up-regulates airway smooth muscle proliferation, contraction, and  $Ca_{2+}$  oscillations in asthma. *J Allergy Clin Immunol.* 2018;142:207-218.
9. Elliot JG, Noble PB, Mauad T, et al. Inflammation-dependent and independent airway remodelling in asthma. *Respirology.* 2018;23:1138-1145.

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

## **SUPPLEMENTARY ONLINE REPOSITORY**

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

Marina Miller MD PhD<sup>1</sup>, Peter Rosenthal BS<sup>1</sup>, Ning Weng PhD<sup>1</sup>, Alex Pham PhD<sup>1</sup>, Gyu-Young Hur MD<sup>1</sup>, John Elliot MSc<sup>2</sup>, Francis H.Y. Green MB ChB MD<sup>3</sup>, Alan James MD PhD<sup>2</sup>, David H. Broide MB ChB<sup>1</sup>.

<sup>1</sup>Department of Medicine, University of California San Diego, La Jolla, California

<sup>2</sup>Department of Pulmonary Physiology and Sleep Medicine, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia

<sup>3</sup>Department of Pathology & Laboratory Medicine, University of Calgary, Alberta, Canada

## **SUPPLEMENTARY METHODS**

The methods are all described below and in detail in the references<sup>E1-E20</sup>.

### **Study Subjects**

Lung tissue from study subjects (n=45) included in this study were obtained from the Airway Disease Biobank at the Sir Charles Gairdner Hospital in Perth, Australia as previously described<sup>E1,E18</sup>. Approval for this study was obtained from the University of California San Diego Human Subjects committee. Ethical approval was obtained from the University of Calgary, University of Edmonton, University of Saskatchewan, University of Manitoba, and Sir Charles Gairdner Hospital. Subjects were categorized as fatal asthma (n=24) if they died from asthma with a confirmed history, non-fatal asthma (n=13) if they died suddenly of non-respiratory causes but with a confirmed history of asthma, and non-asthma (n=8) if they had no history of asthma, wheeze, or lung disease as previously described<sup>E1,E18</sup>. Information regarding the diagnosis of asthma (symptoms, use of asthma medications, hospitalizations, lung function) was obtained from next-of-kin, hospital and coroner files, and the subjects medical practitioner<sup>E1,E18</sup>.

### **Airway tissue preparation**

Post-mortem lung tissue samples were fixed in inflation using formaldehyde or glutaraldehyde and embedded in paraffin wax. Airway sections were cut at 0.5, 4 and 30- $\mu$ m thickness and stained with hematoxylin and eosin for airway measurements of ASM, reticular basement membrane thickness, eosinophils, neutrophils, and mucus as previously described<sup>E1,E18</sup>.

### **Airway ASM measurement**

ASM parameters were estimated using stereological software (newCAST version 4.2.1, Visiopharm, Hoersholm, Denmark) on airways cut in cross-section as previously described<sup>E1,E18</sup>. On 30- $\mu$ m sections, the area of the ASM layer was measured by planimetry, the volume density of ASM nuclei estimated using the optical disector approach<sup>E1,E18</sup>, and the total number of ASM cells per mm of airway length calculated<sup>E1,E18</sup>. Average ASM cell volume was estimated as the inverse of the ASM cell volume density (i.e. volume of the ASM layer/number of cells), correcting for the volume fraction of smooth muscle (versus extracellular matrix, cells, blood vessels), as previously described<sup>E1,E18</sup>.

### **Airway eosinophil and neutrophil measurement**

Airway eosinophil and airway neutrophil cell numbers were counted within the inner airway wall (between the basement membrane and the inner border of the ASM layer) around the entire airway section based on hematoxylin and eosin staining criteria and morphology as previously described<sup>E1,E18</sup>. Results are expressed as the number of eosinophils or neutrophils per area within the inner airway wall.

### **Reticular Basement Membrane Thickness**

Reticular basement membrane thickness (RBMt) was measured on 5- $\mu$ m sections and results expressed in  $\mu$ m as previously described<sup>E1,E18</sup>.

### **Inner Airway Wall Thickness**

Inner airway wall thickness was defined as the area between the inside of the basement membrane and the outer border of the ASM divided by the perimeter of the basement membrane and measured on 5  $\mu$ m sections with results expressed in mm as previously described<sup>E19</sup>.

### **Airway mucus measurement**

Mucus gland thickness (mm) was measured as previously described<sup>E20</sup>.

### **Statistical analysis**

We used the Graphpad Stat program to determine if the data was normally distributed. As the data was normally distributed all comparisons were analyzed using a Student's t-test. Results are reported as the mean and standard error of the mean (SEM). Statistical analysis was performed using Prism software (Graphpad, La Jolla, CA) and a two-sided P value of 0.05 was considered statistically significant.

## SUPPLEMENTARY REFERENCES

- E1. James AL, Elliot JG, Jones RL, Carroll ML, Mauad T, et al. Airway smooth muscle hypertrophy and hyperplasia in asthma. *Am J Respir Crit Care Med* 2012; 185:1058-1064.
  
- E2. Noble PB, Jones RL, Cairncross A, Elliot JG, Mitchell HW, et al. Airway Narrowing and Bronchodilation to Deep Inspiration in Bronchial Segments from Subjects with and without Reported Asthma. *J Appl Physiol*; 2013 114: 1460-71.
  
- E3. Verlaan DJ, Berlivet S, Hunninghake GM, Madore AM, Larivière M, et al. Allele-specific chromatin remodeling in the ZPBP2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease. *Am J Hum Genet* 2009; 85:377-393.
  
- E4. De la Vega FM, Lazaruk KD, Rhodes MD, Wenz MH. Assessment of two flexible and compatible SNP genotyping platforms: TaqMan SNP Genotyping Assays and the SNPLex Genotyping System. *Mutat Res* 2005; 573:111-135.
  
- E5. Schedel M, Michel S, Gaertner VD, Toncheva AA, Depner M, et al. Polymorphisms related to ORMDL3 are associated with asthma susceptibility, alterations in transcriptional regulation of ORMDL3, and changes in Th2 cytokine levels. *J Allergy Clin Immunol* 2015;136:893-903.

- E6. Andiappan AK, Sio YY, Lee B, Surri B, Matta S, et al. Functional variants of 17q12-21 are associated with allergic asthma but not allergic rhinitis. *J Allergy Clin Immunol* 2016;137:758-66.
- E7. SNPedia. <https://www.snpedia.com/index.php/Rs8076131>
- E8. Heard BE, Hossein S. Hyperplasia of bronchial muscle in asthma. *J Pathol* 1973; 110:339-331.
- E9. Woodruff PG, Dolganov GM, Ferrando RE, Donnelly S, Hays SR, et al. Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. *Am J Respir Crit Care Med* 2004; 169:1001-1006.
- E10. Ebina M, Yaegashi H, Chiba R, Takahashi T, Motomiya M, et al. Hyperreactive site in the airway tree of asthmatic patients revealed by thickening of bronchial muscles. A morphometric study. *Am Rev Respir Dis* 1990; 141:1327-1332.
- E11. Regamey N, Ochs M, Hilliard TN, Mühlfeld C, Cornish N, et al. Increased airway smooth muscle mass in children with asthma, cystic fibrosis, and non-cystic fibrosis bronchiectasis. *Am J Respir Crit Care Med* 2008; 177:837-843.

- E12. Schmiedel BJ, Seumois G, Samaniego-Castruita D, Cayford J, Schulten V, et al. 17q21 asthma-risk variants switch CTCF binding and regulate IL-2 production by T cells. *Nat Commun* 2016; 7:13426.
- E13. Ha SG, Ge XN, Bahaie NS, Kang BN, Rao A, et al. ORMDL3 promotes eosinophil trafficking and activation via regulation of integrins and CD48. *Nat Commun* 2013; 4:2479.
- E14. Miller M, Tam AB, Cho JY, Doherty TA, Pham A, et al. ORMDL3 is an inducible lung epithelial gene regulating metalloproteases, chemokines, OAS, and ATF6. *Proc Natl Acad Sci U S A*. 2012; 109:16648-16653.
- E15. Breslow DK, Collins SR, Bodenmiller B, Aebersold R, Simons K, et al. Orm family proteins mediate sphingolipid homeostasis. *Nature* 2010;463:1048-1053.
- E16. Miller M, Rosenthal P, Beppu A, Gordillo R, Broide DH. Oroscomucoid like protein 3 (ORMDL3) transgenic mice have reduced levels of sphingolipids including sphingosine-1-phosphate and ceramide. *J Allergy Clin Immunol* 2017; 139:1373-1376.
- E17. Unno H, Miller M, Rosenthal P, Beppu A, Das S, et al. Activating transcription factor 6 $\alpha$  (ATF6 $\alpha$ ) regulates airway hyperreactivity, smooth muscle proliferation, and contractility. *J Allergy Clin Immunol* 2018; 141:439-442.

- E18. James AL, Noble PB, Drew SA, Mauad T, Bai TR, et al. Airway smooth muscle proliferation and inflammation in asthma. *J Appl Physiol* 2018; 125:1090-1096.
- E19. James AL, Maxwell PS, Pearce-Pinto G, Elliot JG, Carroll NG. The relationship of reticular basement membrane thickness to airway wall remodeling in asthma. *Am J Respir Crit Care Med*. 2002;166:1590-1595.
- E20. Elliot JG, Noble PB, Mauad T, Bai TR, Abramson MJ, et al. Inflammation-dependent and independent airway remodelling in asthma. *Respirology* 2018; 23:1138-1145.

**Supplementary Table 1**

|                                                 | <b>Fatal Asthma</b>    |                         |
|-------------------------------------------------|------------------------|-------------------------|
| Genotype                                        | AG                     | AA                      |
| Total number of subjects                        | 10                     | 14                      |
| Gender (male/female)<br>(number)                | 6/4                    | 8/6                     |
| Age of onset of asthma<br>(median, range) years | 7.5<br>(1-45)          | 6.0<br>(1-61)           |
| Corticosteroid use<br>(oral or inhaled) (%)     | 50%<br>(5/10 subjects) | 78%<br>(11/14 subjects) |