

Gene expression profiling of laser microdissected airway smooth muscle tissue in asthma and atopy

C. Y. Yick¹, A. H. Zwinderman², P. W. Kunst³, K. Grünberg⁴, T. Mauad⁵, S. Chowdhury⁶, E. H. Bel¹, F. Baas⁷, R. Lutter^{1,6} & P. J. Sterk¹

¹Department of Respiratory Medicine, Academic Medical Center; ²Department of Epidemiology and Bioinformatics, Academic Medical Center; ³Department of Respiratory Medicine, Admiraal De Ruyter Hospital, Goes; ⁴Department of Pathology, VU Medical Center, Amsterdam, the Netherlands; ⁵Department of Pathology, São Paulo University Medical School (USP), São Paulo, Brazil; ⁶Department of Experimental Immunology, Academic Medical Center; ⁷Department of Genome Analysis, Academic Medical Center, Amsterdam, the Netherlands

To cite this article: Yick CY, Zwinderman AH, Kunst PW, Grünberg K, Mauad T, Chowdhury S, Bel EH, Baas F, Lutter R, Sterk PJ. Gene expression profiling of laser microdissected airway smooth muscle tissue in asthma and atopy. *Allergy* 2014; **69**: 1233–1240.

Keywords

airway smooth muscle; asthma; gene expression analysis; RNA-Seq; transcriptomics.

Correspondence

Ching Yong Yick, Department of Respiratory Medicine (F5-260), Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands.
Tel.: +31-20-5664359
Fax: +31-20-5669001
E-mail: c.y.yick@amc.uva.nl

Accepted for publication 26 May 2014

DOI:10.1111/all.12452

Edited by: Marek Sanak

Abstract

Background: Asthma and atopy share common characteristics including type 2 helper-T-cell-mediated inflammation. However, only asthma is associated with variable airways obstruction. The complex cellular and molecular pathways distinguishing asthma and atopy can now be captured by transcriptomic analysis (RNA-Seq). We hypothesized that the transcriptomic profile of airway smooth muscle (ASM) distinguishes atopic asthma from atopic healthy controls. First, we compared the ASM transcriptomic profiles of endobronchial biopsies between glucocorticoid-free, atopic asthma patients, and atopic and nonatopic healthy controls. Second, we investigated the association between ASM transcriptomic profiles and airway function.

Methods: Twelve asthma patients and 12 control subjects (six atopic, six nonatopic) underwent bronchoscopy. RNA of laser-dissected ASM from 96 bronchial biopsy specimens was sequenced with Roche GS FLX. Gene networks were identified using Ingenuity Pathway Analysis. RNA-Seq reads were assumed to follow a negative binomial distribution. With the current sample size, the estimated false discovery rate was approximately 1%.

Results: One hundred and seventy four ASM genes were differentially expressed between asthma patients and atopic controls, 108 between asthma patients and nonatopic controls, and 135 between atopic and nonatopic controls. A set of eight genes discriminated asthma patients from nonasthmatic controls, irrespective of atopy. Four of these genes (*RPTOR*, *VANGLI*, *FAM129A*, *LEPREL1*) were associated with airway hyper-responsiveness ($P < 0.05$).

Conclusion: Airway smooth muscle from asthma patients can be distinguished from that of atopic and nonatopic control subjects by a specific gene expression profile, which is associated with airway hyper-responsiveness.

Abbreviations

ASM, airway smooth muscle; ECM, extracellular matrix; ERS, European Respiratory Society; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; GINA, Global Initiative for Asthma; IPA, Ingenuity Pathway Analysis; PC₂₀, provocative concentration of methacholine causing a 20% drop in forced expiratory volume in 1 s.

Asthma is characterized by episodic airway narrowing and structural changes of the airways called airway remodeling. The latter includes an increase in airway smooth muscle (ASM) mass and altered deposition of extracellular matrix (ECM) inside and outside the ASM layer (1, 2). Increasing evidence show that epithelial to mesenchymal transition may play an important role in this increase in ASM mass (3). The main functional abnormality in asthma is altered airway

dynamics, as shown by spontaneous variability of airways obstruction (4) and airway hyper-responsiveness (5). The extent of airway hyper-responsiveness seems to be determined by the interplay of various cells and mediators in the airways. However, the clinical expression of asthma is reflected by the extent of airway narrowing, which is mediated mechanically by the ASM layer. Previous studies have supported the importance of ASM in asthma. The severity of asthma has shown to be associated with the thickness of the ASM layer, suggesting that ASM not only plays a key role in the pathophysiology of this disease, but also represents a potential target for new asthma therapies (6–8).

Apart from its contractile properties, ASM also exhibits synthetic and proliferative activity in close interaction with its ECM and intramuscular inflammatory cells (9). Notably, each of these elements is associated with altered dynamics of airway function in asthma. This holds true for ASM protein expression (10), the composition of ECM inside the ASM layer (11), and mast cell counts within ASM (12, 13).

Atopy shares common features with asthma. It has been shown that atopy as such, similar to asthma, is characterized by a Th2-type response resulting in allergic inflammation that includes eosinophils and mast cells (14). Although being associated with certain features of airway remodeling, it appears that atopy is not related to altered dynamics of airway function. This suggests that the difference between asthma and atopy as such may be determined by the activity of ASM.

Gene expression profiling can provide insight into the phenotypic characteristics of ASM in asthma and atopy. One of the most powerful recent techniques for gene expression profiling is transcriptome sequencing (RNA-Seq), which allows an unbiased and detailed characterization of the RNA expression and thus can improve our understanding of the cellular and molecular pathways involved in disease (15–17).

We hypothesized that the transcriptomic profile of the ASM layer in endobronchial biopsies distinguishes atopic asthma patients from atopic healthy controls. Therefore, we compared the ASM transcriptomic profiles of endobronchial biopsies between glucocorticoid-free, atopic asthmatics, and atopic and nonatopic healthy controls. Second, we investigated the association between the ASM transcriptomic profile and airway function.

Methods

Additional information regarding the study methods is included in the Supporting Information.

Design

The study consisted of two visits. At visit 1, asthma patients and controls were screened for eligibility to participate according to the inclusion and exclusion criteria of this study followed by lung function measurements including standardized spirometry and methacholine bronchoprovocation test [expressed as dose–response slope (18)]. At visit 2, bronchoscopy was performed and four endobronchial biopsies per subject were collected. This study was approved by the local

medical ethics committee and was part of a larger study registered at the Netherlands Trial Register (The role of the airway smooth muscle layer in asthma; NTR1306; www.trialregister.nl).

Subjects

Glucocorticoid-free, atopic asthma patients ($n = 12$), atopic ($n = 6$), and nonatopic ($n = 6$) healthy controls were recruited by the Department of Respiratory Medicine of the Academic Medical Center, Amsterdam, the Netherlands. The sample size was based on *a priori* power and false discovery rate calculation (see below). Asthma patients had controlled disease according to Global Strategy for Asthma Management and Prevention (GINA) guidelines (19) and no exacerbations within 6 weeks prior to participation. Control subjects had no history or symptoms of pulmonary diseases. All subjects gave written informed consent prior to enrollment.

RNA-Seq and data analysis

Study procedures were performed as described in our recent RNA-Seq studies (8, 17). In short, the biopsies were collected at B 7–9 of the right lung, incubated overnight in RNAlater (Qiagen, Venlo, the Netherlands), frozen, and cut up entirely into 10- μ m cryosections. After staining with hematoxylin and eosin, ASM was captured with laser capture microdissection using Leica AS LMD (Leica, Wetzlar, Germany). TRIzol (Invitrogen Carlsbad, CA, USA) was used to isolate RNA from the captured ASM.

Amplified cDNA was prepared from the isolated RNA with Ovation RNA-Seq System (NuGEN, San Carlos, CA, USA) and used to construct cDNA libraries of >350 bp in size with SPRIworks Fragment Library System II (Beckman-Coulter, Brea, CA, USA). Addition of multiplex identifier adaptors (Roche, Penzberg, Germany) allowed multiplexing of samples.

Two million enriched DNA library beads, which were obtained by emulsion-based clonal amplification using the GS FLX Titanium emPCR Kit (Lib-L, Roche) (20), were loaded into each region of a two-region PicoTiterPlate device. RNA-Seq was performed on the Genome Sequencer FLX system (454/Roche) with the number of nucleotide cycles set at 200 (20). The sequence reads were mapped against the human genome (hg19) (21) using Genome Sequencer Reference Mapper v2.6 (Roche). Gene network analysis by the Ingenuity Pathway Analysis (IPA) software application generated an IPA network score for each gene network found. Networks with a score ≥ 2 have at least 99% confidence that it is not generated by chance. RNA-Seq data were uploaded to the Gene Expression Omnibus (GSE38003). Quantitative PCR (qPCR) was performed on selected genes to validate the sequencing data.

Statistical analysis

Unpaired *t*-tests or Mann–Whitney *U*-tests were used to compare subject characteristics between the study groups.

RNA-Seq yielded read numbers per gene per study participant, which were assumed to follow a negative binomial distribution, also known as the Poisson-gamma mixture. Comparison of these numbers of reads per gene between study groups was carried out with the R package DESeq, which is dedicated to the analysis of RNA sequencing data and includes normalization of RNA-Seq libraries based on size factors (22). Correlations between genes and airway function parameters were examined using Spearman's correlation coefficient. For evaluating the association between airway function and gene expression, a *P*-value of <0.05 was considered statistically significant. Statistical analyses were performed using SPSS 19 (IBM Corporation, Armonk, NY, USA) and R statistical program v2.15.0 (The R Foundation for Statistical Computing, Vienna, Austria).

Sample size calculation

The sample size was based on limiting the false discovery rate. We conservatively assumed that all pathways that are associated with asthma consist of $\geq 10\,000$ genes (23). This number represents about a third of the 31 227 genes of the human genome (hg19) tested. The average fold change of the gene expression of asthma vs controls was presumed to be about 1.2, which coincides with a Cohen's effect size of 0.5. A significant level of 0.0001 was used to correct for multiple testing. Accordingly, we expected to find 310 true and two false significant genes (24, 25), resulting in a false discovery rate of approximately 1%.

Results

Subject characteristics

Subject characteristics are summarized in Table 1. Lung function parameters were similar between the study groups. As expected, airway hyper-responsiveness expressed as the dose-response slope was significantly greater in patients with asthma as compared to atopic and nonatopic controls.

RNA-Seq

Airway smooth muscle could be isolated from all biopsy specimens with laser capture microdissection (Fig. S1). Sample characteristics including the RNA-Seq read numbers of the three study groups are shown in Table S1.

The transcriptomes found by RNA-Seq were mapped to 14 583 unique genes for asthma, 12 835 for atopic, and 12 001 for nonatopic controls. A total of 174 genes were differentially expressed between atopic asthma and atopic controls (Table S2, Fig. 1A), 108 between atopic asthma and nonatopic controls (Table S3, Fig. 1B), and 135 between atopic and nonatopic controls (Table S4, Fig. 1C). There were no significant differences in read numbers of reference genes [glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), β -actin (*ACTB*), β -2-microglobulin (*B2M*), and lactate dehydrogenase A (*LDHA*)] between asthma and control subjects.

Table 1 Subject characteristics of asthma, atopic, and nonatopic healthy controls

	Asthma	Healthy, atopic	Healthy, nonatopic
Subjects (<i>n</i> ; male/female)	12 (3/9)	6 (2/4)	6 (2/4)
Age (years)†	24 (20–27)	26 (23–36)	27 (23–34)
FEV ₁ postbronchodilator (%pred.)‡	107 (10)	108 (6)	114 (17)
FEV ₁ /FVC postbronchodilator (%pred.)‡	100 (9)	108 (7)	107 (6)
PC ₂₀ (mg/ml)§	2.19 (1.30–3.67)	>8	>8
Dose-response slope (% decline FEV ₁ /μmol methacholine)¶*	5.50 (1.76–13.64)	0.31 (0.15–0.41)	0.12 (0.09–0.17)

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; PC₂₀, provocative concentration of methacholine causing a 20% drop in FEV₁; %pred., % predicted.

*Asthma vs Healthy, atopic *P* < 0.01; Asthma vs Healthy, nonatopic *P* < 0.01.

†Mean (min-max).

‡Mean (SD).

§Geometric mean (95% CI).

¶Median (P25–P75).

Gene network identification

Each set of differentially expressed ASM genes observed between the study groups was uploaded to the IPA software application for gene network identification. The highest scoring network for asthma vs atopic controls was associated with the network functions carbohydrate metabolism, small molecule biochemistry, and cell-to-cell signaling and interaction (Fig. S2A). The highest scoring network for asthma vs nonatopic controls was associated with the network function cellular movement (Fig. S2B). For atopic vs nonatopic controls, the highest scoring network was associated with network functions including cellular development, growth, and proliferation (Fig. S2C).

A substantial overlap in network components existed between the highest scoring networks of asthma vs atopic controls, and asthma vs nonatopic controls. Key components in the gene network of atopic and nonatopic controls combined vs asthma included ERK1/2, also known as mitogen-activated protein kinase 1 (MAPK 1), p38 mitogen-activated protein kinase (p38 MAPK), and nuclear factor kappa-B (NFκB) (Fig. S3).

Gene expression and airway function

Eight genes were differentially expressed in asthma vs both atopic and nonatopic controls, but not between atopic and nonatopic controls (Table 2). Read numbers of four of these genes, including *RPTOR* (regulatory associated protein of

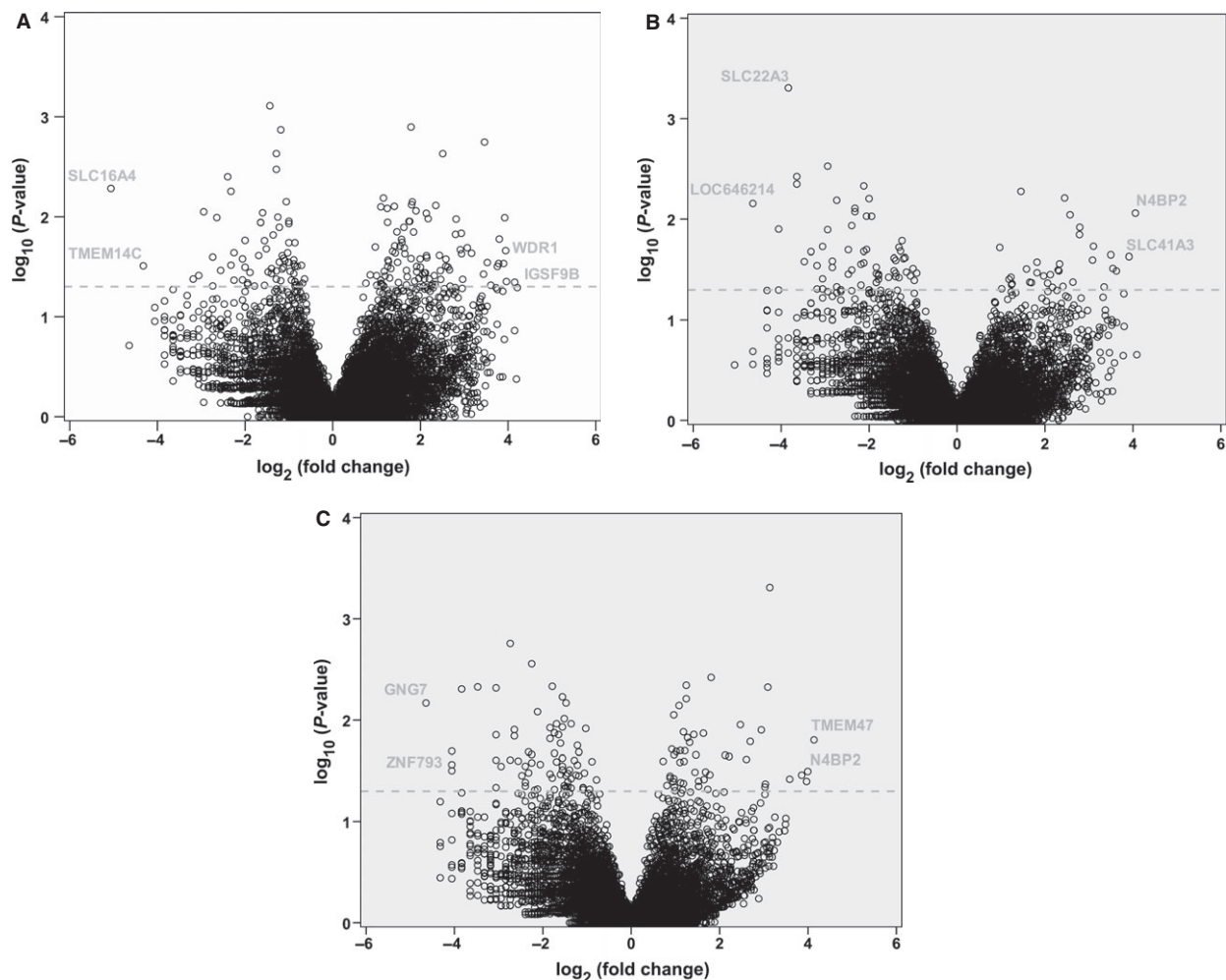


Figure 1 Volcano-plots of ASM genes. ASM genes from asthma and healthy atopic controls (A), asthma and healthy nonatopic controls (B), and healthy atopic and nonatopic controls (C) are shown. Positive log₂ (fold change) values represent up-regulation in asthma

compared to healthy controls, whereas negative values represent down-regulation. Dots above the dotted line represent differentially expressed genes between asthma and healthy controls.

mTOR, complex 1), *VANGLI* (vang-like 1), *FAM129A* (family with sequence similarity 129, member A), and *LEPRELI* (leprecan-like 1), are shown in Fig. 2. qPCR results confirmed the RNA-Seq data (Table S5). These four genes correlated significantly with the methacholine dose–response slope. An increase in *RPTOR* and *VANGLI* was associated with a steeper dose–response slope, indicating greater responsiveness to methacholine ($r = 0.421$, $P = 0.04$; $r = 0.746$, $P < 0.01$). Conversely, an increase in *FAM129A* and *LEPRELI* was associated with a less steep methacholine dose–response slope and hence lesser responsiveness ($r = -0.460$, $P = 0.02$; $r = -0.465$, $P = 0.02$).

Discussion

The present study shows that the ASM gene expression profile of patients with atopic asthma is different from that

of atopic and nonatopic healthy controls. Pathway analysis yielded multiple gene networks that were associated with cell signaling, development, and proliferation. The networks describing the distinction between asthma and both control groups were largely similar and included various well-known inflammatory mediators and protein complexes. Eight genes were found to be differentially expressed in asthma as compared to atopic and nonatopic healthy controls, and four of these genes were correlated with airway hyper-responsiveness. These results suggest that muscle function and inflammatory pathways are differentially regulated in the airways of patients with asthma.

The novelty of our study is that we were able to apply next-generation high-throughput sequencing on *in vivo* collected human bronchial ASM cells for transcriptome analysis. In contrast to microarray chips, RNA-Seq allows an unbiased analysis of the transcriptome as it is not dependent on

Table 2 Differentially expressed genes between asthma and both atopic and nonatopic controls

	Asthma vs Atopic control*		Asthma vs Nonatopic control*		Atopic vs Nonatopic control	
	Log fold change	P-value	Log fold change	P-value	Log fold change	P-value
<i>AK8</i>	3.46	<0.01	2.17	0.05	−1.32	0.71
<i>VANGL1</i>	2.11	0.04	3.50	0.02	1.34	0.86
<i>GPATCH4</i>	1.91	0.04	2.30	0.03	0.33	0.93
<i>RPTOR</i>	1.79	<0.01	1.45	<0.01	−0.36	0.94
<i>HFM1</i>	1.58	0.02	1.23	0.04	−0.38	0.59
<i>MTRNR2L8</i>	1.10	<0.01	0.97	0.02	−0.14	0.86
<i>FAM129A</i>	−1.12	0.03	−1.06	0.04	0.07	0.87
<i>LEPREL1</i>	−1.43	<0.01	−0.97	0.04	0.45	0.37

*Fold change >1 signifies up-regulated in asthma, whereas <1 down-regulated.

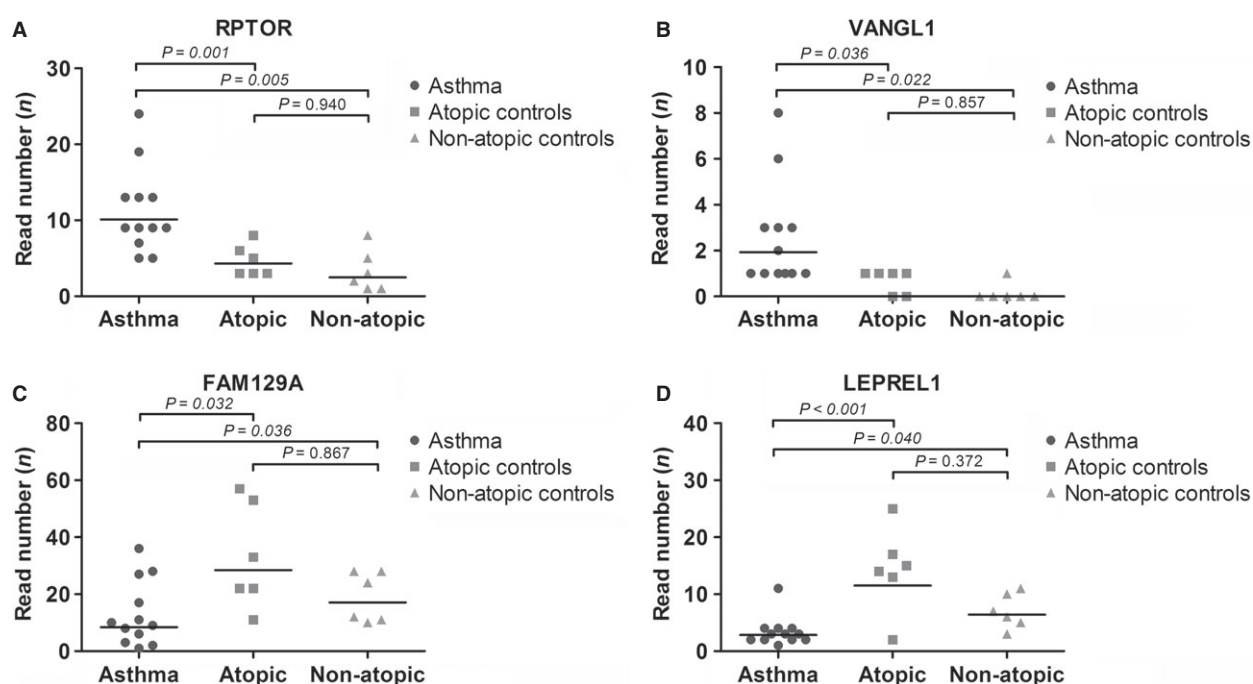


Figure 2 Gene expression levels of airway function associated genes. *RPTOR* (A), *VANGL1* (B), *FAM129A* (C), and *LEPREL1* (D) are differentially expressed between asthma vs healthy atopic and nonatopic controls. No significant difference was found in read

numbers between healthy atopic vs nonatopic controls for all four genes. Read numbers per subject and the geometric mean per study group is shown.

predefined probe sets and therefore not limited to a selection of known genes or nucleotide sequences (16). Consequently, RNA-Seq enables the discovery and characterization of novel, disease-related genes, thereby providing a step change in disentangling the pathogenesis of comorbid disease entities such as asthma and atopy.

Several of the eight differentially expressed genes between the study groups were also identified in our previous RNA-Seq study on whole biopsy specimens (17). Additionally, gene expression data in the current study and our previously published transcriptomics studies have been successfully validated by qPCR. Taken together, this confirms that the implemented study methods and the current results are valid. Four

genes, *RPTOR*, *VANGL1* (also known as *KITENIN*), *FAM129A*, and *LEPREL1*, were significantly correlated with the methacholine dose–response slope. Notably, in a recent study, we observed that *FAM129A* is related to airway function (8). More specifically glucocorticoids increase the read number of *FAM129A*, which is correlated with reduced airway responsiveness. This is consistent with the results of the current study, showing that both atopic and nonatopic healthy controls have higher read numbers of *FAM129A* and that an increase in *FAM129A* was associated with a less steep methacholine dose–response slope and hence lesser responsiveness. Although none of these genes have been directly associated with asthma previously, they have been related

with muscle development and function, and with components known for their roles in inflammatory and cell regulatory processes, including *MAPK* and *mTOR* (26–29). Presumably, the difference in ASM transcriptomic profile in asthma patients as compared to atopic and nonatopic controls leads to a different regulation of ASM function and inflammatory pathways, resulting in altered airway function. In addition, targeting these genes may even lead to an improvement in airway function in asthma patients. Consequently, asthma as opposed to atopy might be explained by the expression of these genes.

Although not statistically different, the ASM transcriptomic profile between atopic and nonatopic controls was also different. The gene networks found with IPA comprised NF κ B, alpha-catenin, cadherins, 14-3-3, and ABL1 as important constituents. E-cadherin and alpha-catenin are important in cell–cell adhesion in the epithelium (30), which is impaired in asthma (31). However, their role in ASM is still unknown. Overexpression of 14-3-3 may enhance phosphorylation of myosin II, which is well-known for its role in muscle contraction (32), while ABL1 has been shown to be a critical mediator in the clustering of acetylcholine receptors (33). Taken together, atopy appears to affect gene expression in the ASM layer, but this is unrelated to airway function.

In addition, although not differentially expressed between the study groups, our gene expression analyses (GEO GSE38003) also identified several genes that have been associated to epithelial cells in previous studies, such as *CDC20B*, *POF1B*, and *AQP3* (34–36). Increasing evidence in the literature shows that airway remodeling in asthma may be contributed by epithelial to mesenchymal transition (37). Although speculative, the identification of these epithelial-associated genes in ASM supports this hypothesis.

The subjects in the present study were carefully phenotyped. Asthma patients were glucocorticoid-free and had controlled disease, which remained stable with minimal clinical symptoms during the study period. Even though they were allowed to use inhaled short-acting β 2-agonists as rescue therapy, none of the asthma patients used any during the study period. Consequently, the transcriptomic profiles of the ASM layer could not be affected by medication usage.

The handling and workup of the biopsies required a strict standard operating procedure. The advantage of the Ovation RNA-Seq System is that a low amount of 500 pg RNA is required as minimal input. Therefore, the yield of RNA from the endobronchial biopsies (ranging 30–821 ng) was sufficient. Furthermore, this RNA-Seq kit uses isothermal, linear amplification, which is initiated both at the 3' end and randomly throughout the target transcriptome, thereby maximizing the coverage as reads are distributed throughout the whole transcriptome (38, 39). Combined with the sequencing technology of the GS FLX system, any detrimental effects of partially degraded RNA on gene expression analysis could be largely resolved.

Biopsies were taken from prespecified locations of the right lung to allow accurate comparison of the gene expression profile across the study groups. Nevertheless, some bias and confounding cannot be excluded. Firstly, previous studies

have shown that the extent of airway wall remodeling and inflammation may depend on the location in the bronchial tree (40). Secondly, the composition of the ASM layer may be different between the study groups. ECM proteins and inflammatory cells are often found inside the ASM layer (10). This could have confounded the currently observed transcriptomic profiles. However, the differentially expressed genes between the study groups did not include specific transcripts of inflammatory cells, suggesting that the transcriptomic profile was not affected by a difference in inflammatory cells. Apart from inflammation, we observed that the ECM proteins collagen and laminin were differentially expressed. Previous studies have indicated that there is a close interaction between ASM cells and ASM microenvironment, including ECM expression, in determining airway function (11, 41). This suggests that ECM synthesis by ASM cells can be one among the critical mechanisms that distinguishes asthma from allergic inflammation.

The differentially expressed genes between the study groups and the gene networks found in this study are identifying novel protein complexes and signaling pathways that have not been associated with asthma previously. These can be considered potential targets for therapeutic intervention. The next step toward selecting those targets will be an external validation in other asthma populations with the implementation of a systems biology approach (15). In addition, next-generation sequencing technologies also have the prospect of elucidating the mode of action of existing asthma therapies (*e.g.* glucocorticoids), the mechanisms that are driving asthma exacerbations, and those that are predisposing to asthma onset and progression (8).

Acknowledgment

This study was supported by a research grant from the Lung Foundation Netherlands (project number 3.2.09.065). Thais Mauad is funded by CNPQ (Brazilian Research Council).

Author contributions

CYY carried out the study procedures, participated in the design of the study, wrote the manuscript, and carried out the statistical analyses with AHZ. AHZ as a biostatistician assisted in designing the study, the analysis plan, power calculations, and statistical analysis and participated in drafting the manuscript. PWK performed all bronchoscopic procedures. KG and TM participated in the design of the study. SH designed the primers for the qPCR validation of the gene sequencing data. EHB, RL, and FB participated in the design of the study and its coordination. PJS conceived the study, participated in the design of the study and its coordination. Additionally, all co-authors helped to draft the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Study methods.

Table S1. Sample characteristics.

Table S2. List of differentially expressed genes in airway smooth muscle of asthma vs healthy atopic controls.

Table S3. List of differentially expressed genes in airway smooth muscle of asthma vs healthy nonatopic controls.

Table S4. List of differentially expressed genes in airway smooth muscle of healthy atopic vs nonatopic controls.

Table S5. Read numbers and Cq-ratios.

Figure S1. Laser capture microdissection of ASM.

Figure S2. Gene networks by IPA.

Figure S3. Gene networks by IPA combined.

References

- Fixman ED, Stewart A, Martin JG. Basic mechanisms of development of airway structural changes in asthma. *Eur Respir J* 2007;**29**:379–389.
- James AL, Elliot JG, Jones RL, Carroll ML, Mauad T, Bai TR et al. Airway smooth muscle hypertrophy and hyperplasia in asthma. *Am J Respir Crit Care Med* 2012;**185**:1058–1064.
- Hackett TL. Epithelial-mesenchymal transition in the pathophysiology of airway remodelling in asthma. *Curr Opin Allergy Clin Immunol* 2012;**12**:53–59.
- Reddel H, Jenkins C, Woolcock A. Diurnal variability – time to change asthma guidelines? *BMJ* 1999;**319**:45–47.
- Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;**16**:53–83.
- James AL, Bai TR, Mauad T, Abramson MJ, Dolnikoff M, McKay KO et al. Airway smooth muscle thickness in asthma is related to severity but not duration of asthma. *Eur Respir J* 2009;**34**:1040–1045.
- Dowell ML, Lavoie TL, Solway J, Krishnan R. Airway smooth muscle: a potential target for asthma therapy. *Curr Opin Pulm Med* 2014;**20**:66–72.
- Yick CY, Zwiderman AH, Kunst PW, Grünberg K, Mauad T, Fluiter K et al. Glucocorticoid-induced changes in gene expression of airway smooth muscle in patients with asthma. *Am J Respir Crit Care Med* 2013;**187**:1076–1084.
- Koziol-White CJ, Panettieri RA Jr. Airway smooth muscle and immunomodulation in acute exacerbations of airway disease. *Immunol Rev* 2011;**242**:178–185.
- Slats AM, Janssen K, van Schadewijk A, van der Plas DT, Schot R, van den Aardweg JG et al. Expression of smooth muscle and extracellular matrix proteins in relation to airway function in asthma. *J Allergy Clin Immunol* 2008;**121**:1196–1202.
- Yick CY, Ferreira DS, Annoni R, von der Thüsen JH, Kunst PW, Bel EH et al. Extracellular matrix in airway smooth muscle is associated with dynamics of airway function in asthma. *Allergy* 2012;**67**:552–559.
- Slats AM, Janssen K, van Schadewijk A, van der Plas DT, Schot R, van den Aardweg JG et al. Bronchial inflammation and airway responses to deep inspiration in asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007;**176**:121–128.
- Siddiqui S, Hollins F, Saha S, Brightling CE. Inflammatory cell microlocalisation and airway dysfunction: cause and effect? *Eur Respir J* 2007;**30**:1043–1056.
- Djukanović R, Lai CK, Wilson JW, Britten KM, Wilson SJ, Roche WR et al. Bronchial mucosal manifestations of atopy: a comparison of markers of inflammation between atopic asthmatics, atopic nonasthmatics and healthy controls. *Eur Respir J* 1992;**5**:538–544.
- Auffray C, Adcock IM, Chung KF, Djukanović R, Pison C, Sterk PJ. An integrative systems biology approach to understanding pulmonary diseases. *Chest* 2010;**137**:1410–1416.
- Metzker ML. Sequencing technologies – the next generation. *Nat Rev Genet* 2010;**11**:31–46.
- Yick CY, Zwiderman AH, Kunst PW, Grünberg K, Mauad T, Dijkhuis A et al. Transcriptome sequencing (RNA-Seq) of human endobronchial biopsies: asthma versus controls. *Eur Respir J* 2013;**42**:662–670.
- O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. *Am Rev Respir Dis* 1987;**136**:1412–1417.
- GINA report, Global strategy for asthma management and prevention (update December 2009). Available at: <http://www.ginasthma.com>. Accessed November 23, 2012.
- Weterman MA, Sorrentino V, Kashner PR, Jakobs ME, van Engelen BG, Fluiter K et al. A frameshift mutation in LRSAM1 is responsible for a dominant hereditary polyneuropathy. *Hum Mol Genet* 2012;**21**:358–370.
- UCSC Genome Bioinformatics. Available at: <http://genome.ucsc.edu>. Last Accessed November 23, 2012.
- Anders S, Huber W. Differential expression analysis for sequence count data. *Genome Biol* 2010;**11**:R106.
- Loza MJ, McCall CE, Li L, Isaacs WB, Xu J, Chang BL. Assembly of inflammation-related genes for pathway-focused genetic analysis. *PLoS ONE* 2007;**2**:e1035.
- Ferreira JA, Zwiderman AH. On the Benjamini-Hochberg method. *Ann Statist* 2006;**34**:1827–1849.
- Ferreira JA, Zwiderman AH. Approximate sample size calculations with microarray data: an illustration. *Stat Appl Genet Mol Biol* 2006;**5**:Article 25.
- Lee S, Song YA, Park YL, Cho SB, Lee WS, Lee JH et al. Expression of KITTENIN in human colorectal cancer and its relation to tumor behavior and progression. *Pathol Int* 2011;**61**:210–220.
- Bentzinger CF, Romanino K, Cloëtta D, Lin S, Mascarenhas JB, Oliveri F et al. Skeletal muscle-specific ablation of raptor, but not of rictor, causes metabolic changes and results in muscle dystrophy. *Cell Metab* 2008;**8**:411–424.
- Ohanna M, Sobering AK, Lapointe T, Lorenzo L, Praud C, Petroulakis E et al. Atrophy of S6K1(-/-) skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. *Nat Cell Biol* 2005;**7**:286–294.
- Tiainen P, Pasanen A, Sormunen R, Myllyharju J. Characterization of recombinant human prolyl 3-hydroxylase isoenzyme 2, an enzyme modifying the basement membrane collagen IV. *J Biol Chem* 2008;**283**:19432–19439.
- Nawijn MC, Hackett TL, Postma DS, van Oosterhout AJ, Heijink IH. E-cadherin: gatekeeper of airway mucosa and allergic sensitization. *Trends Immunol* 2011;**32**:248–255.
- Carayol N, Campbell A, Vachier I, Mainprize B, Bousquet J, Godard P et al. Modulation of cadherin and catenins expression by tumor necrosis factor-alpha and dexamethasone in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2002;**26**:341–347.

32. Koga Y, Ikebe M. A novel regulatory mechanism of myosin light chain phosphorylation via binding of 14-3-3 to myosin phosphatase. *Mol Biol Cell* 2008;**19**:1062–1071.
33. Finn AJ, Feng G, Pendergast AM. Postsynaptic requirement for Abl kinases in assembly of the neuromuscular junction. *Nat Neurosci* 2003;**6**:717–723.
34. Lizé M, Herr C, Klimke A, Bals R, Dobbels M. MicroRNA-449a levels increase by several orders of magnitude during mucociliary differentiation of airway epithelia. *Cell Cycle* 2010;**9**:4579–4583.
35. Rizzolio F, Bione S, Villa A, Berti E, Cassetti A, Bulfone A et al. Spatial and temporal expression of POF1B, a gene expressed in epithelia. *Gene Expr Patterns* 2007;**7**:529–534.
36. Schreiber R, Nitschke R, Greger R, Kunzelmann K. The cystic fibrosis transmembrane conductance regulator activates aquaporin 3 in airway epithelial cells. *J Biol Chem* 1999;**274**:11811–11816.
37. Berair R, Saunders R, Brightling CE. Origins of increased airway smooth muscle mass in asthma. *BMC Med* 2013;**11**:145.
38. Kurn N, Chen P, Heath JD, Kopf-Sill A, Stephens KM, Wang S. Novel isothermal, linear nucleic acid amplification systems for highly multiplexed applications. *Clin Chem* 2005;**51**:1973–1981.
39. Adiconis X, Borges-Rivera D, Satija R, DeLuca DS, Busby MA, Berlin AM et al. Comparative analysis of RNA sequencing methods for degraded or low-input samples. *Nat Methods* 2013;**10**:623–629.
40. Araujo BB, Dolhnikoff M, Silva LF, Elliot J, Lindeman JH, Ferreira DS et al. Extracellular matrix components and regulators in the airway smooth muscle in asthma. *Eur Respir J* 2008;**32**:61–69.
41. Burgess JK, Ceresa C, Johnson SR, Kanabar V, Moir LM, Nguyen TT et al. Tissue and matrix influences on airway smooth muscle function. *Pulm Pharmacol Ther* 2009;**22**:379–387.

Online Supplement

Gene expression profiling of laser microdissected airway smooth muscle tissue in asthma and atopy

Ching Yong Yick¹, Aeilko H Zwinderman², Peter W Kunst³, Katrien Grünberg⁴, Thais Mauad⁵, Saheli Chowdhury⁶, Elisabeth H Bel¹, Frank Baas⁷, Rene Lutter^{1, 6}, Peter J Sterk¹.

¹ Department of Respiratory Medicine, Academic Medical Center, Amsterdam, The Netherlands

² Department of Epidemiology and Bioinformatics, Academic Medical Center, Amsterdam, The Netherlands

³ Department of Respiratory Medicine, Admiraal De Ruyter Hospital, Goes, The Netherlands

⁴ Department of Pathology, VU Medical Center, Amsterdam, The Netherlands

⁵ Department of Pathology, São Paulo University Medical School (USP), São Paulo, Brazil

⁶ Department of Experimental Immunology, Academic Medical Center, Amsterdam, The Netherlands

⁷ Department of Genome Analysis, Academic Medical Center, Amsterdam, The Netherlands

Study Methods

Subjects

The inclusion criteria for asthma patients were: controlled disease according to GINA guidelines (1), aged 18-50 years; non-smoking or stopped >12 months with ≤ 5 pack years; no exacerbations within 6 weeks prior to participation; glucocorticoid-naïve or stopped using glucocorticoids by any dosing route ≥ 8 weeks prior to participation; no pulmonary diseases other than asthma; no pulmonary medication except inhaled short-acting β_2 -agonists as rescue therapy; airway hyperresponsiveness defined by a methacholine provocative concentration of methacholine causing a 20% drop in forced expiratory volume in 1 second (FEV_1) ≤ 8 mg/mL; post-bronchodilator FEV_1 >70% of predicted; atopy defined by a positive skin prick test. The inclusion criteria for healthy atopic and non-atopic controls were similar, except that they had no pulmonary diseases and no airway hyperresponsiveness. The atopic status of healthy controls was determined by skin prick test.

Lung function

Spirometry was performed according to European Respiratory Society recommendations (2). Airway responsiveness was measured by methacholine bronchoprovocation test using the standardized tidal volume method with a maximum methacholine dose of 16 mg/mL (3). To enable comparison between asthma patients and healthy controls, we used the validated dose response slope (4). FEV_1 reversibility was determined by measuring FEV_1 pre- and post-inhalation of 400 μ g Salbutamol. The atopy skin prick test included 12 common aeroallergen extracts (ALK, Hørsholm, Denmark).

Bronchoscopy and biopsy specimen processing

Fiberoptic bronchoscopy was performed as described previously (5, 6). Local anaesthesia was provided by Lignocaine spray in the nose and throat prior to the bronchoscopy. During the procedure, additional Lignocaine solution was instilled into lung segments to dampen the cough reflex. A cup forceps (KW2411S; Pentax, Breda, The Netherlands) was used to collect

49 4 endobronchial biopsies per participant at B 7-9 of the right lung. Special care was taken in
50 positioning the forceps laterally to the bronchial carina in order to minimize the amount of
51 connective tissue and at the same time maximize the yield of ASM in the biopsies.

53 *Isolation of RNA from ASM*

54 Biopsy specimens were put into RNAlater (Qiagen, Venlo, The Netherlands) for overnight
55 incubation, embedded in Tissue-Tek (Sakura Finetek, Alphen aan den Rijn, The Netherlands)
56 and frozen in liquid nitrogen-cooled isopentane. 10µm sections of the frozen biopsies were
57 mounted on PEN-membrane covered microscope slides (Carl Zeiss, Sliedrecht, The
58 Netherlands), and stained with haematoxylin and eosin. ASM sections from the 4 frozen
59 biopsies per subject were collected by laser capture microdissection (AS LMD, Leica,
60 Wetzlar, Germany) and pooled into the same microcentrifuge tube containing TRIzol
61 (Invitrogen Carlsbad, CA, US).

63 Total RNA was isolated according to the TRIzol protocol provided by the manufacturer.
64 RNase-free glycogen (UltraPure Glycogen, Invitrogen) was added to this protocol as a carrier
65 to maximize the yield of RNA. Amplified cDNA was prepared from the isolated RNA with
66 Ovation RNA-Seq System (NuGEN, San Carlos, CA, US) and purified with Agencourt
67 RNAClean XP (Beckman-Coulter, Brea, CA, US). GS FLX Titanium Rapid Library multiplex
68 identifier (MID) adaptors (Roche, Penzberg, Germany) were added to the cDNA libraries to
69 allow multiplexing of samples. The concentration of cDNA was measured on the Qubit
70 fluorometer (Invitrogen) using the dsDNA BR Assay Kit (Invitrogen). Amplified cDNA and
71 cDNA libraries were quality-checked using the Agilent 2100 Bioanalyzer and the amount of
72 cDNA molecules was measured on the FLUOstar OPTIMA (BMG Labtech, Ortenberg,
73 Germany).

RNA-Seq and data analysis

A 2-region PicoTiterPlate device was used for the sequencing run. Each region was loaded with 2 million enriched DNA Library Beads. The GS FLX system (454/Roche) was used for RNA-Seq with the number of nucleotide cycles set at 200 (7). The GS Reference Mapper v2.6 (Roche) was used to map the sequence reads against the human genome (hg19) (8) with the following settings. Multiplex Identifiers (MIDs) were specified using the RLMIDs scheme for Rapid Library preparation kit MID adaptors with the Number of Errors allowed set to 1. The expected depth was set at 0 and the minimum read length at 20bp. Duplicate reads were excluded from computation of the consensus for a contig and frequency statistics. A minimum overlap length of 40bp and minimum overlap identity of 90% were used for alignment. Mapping of the reads by GS Reference Mapper follows a sequence census approach (9), which is in its concept similar to the serial analysis of gene expression (SAGE) method. In our current study, the median read length of the contigs is 261bp. With on average 92% of the sequence reads with a median read length of 250 nucleotides mapped to the reference genome based on contigs with a median length of 260bp, the coverage is approximately 31x in the current study (10).

Genes that were significantly up- or down-regulated were uploaded to the web-based Ingenuity Pathway Analysis (IPA) software application (11) for gene network analysis. The IPA network score for each gene network displayed as the negative log of the p-value of that specific network, gives the likelihood that the set of genes in this network could be explained by chance alone. Therefore, networks with a score ≥ 2 have at least 99% confidence that it is not generated by chance.

qPCR validation of RNA-Seq data

Quantitative PCR (qPCR) was performed on selected genes to validate the sequencing data. PCR conditions were optimized in rt-PCR and subsequently in the qPCR (StepOnePlus; Applied Biosystems, Foster City, CA, USA) using mRNA from NCI-H292 cells (ATCC: CRL-

1848) either exposed or not to IFN- γ (100pg/mL for 24h) and mRNA from umbilical cord endothelial cells. Samples were run in duplicate and the product was analyzed by melting temperature and on gel. A high amount of cDNA corresponds with a low Cq-value and vice versa. qPCR data is expressed as the ratio of quantification cycles (Cq) of the genes compared to those of GAPDH (12). To enable a comprehensive comparison between RNA-Seq and qPCR data, geometric means of the read numbers per gene per study group were used.

For *LEPREL1* a primer set was developed flanking intron-exon borders to rule out genomic contamination. We used: forward: 5'-gcctacatcaagcttaaccag-3' and reverse: 5'-gctgtcgccctgtaattctc-3' (116bp product) (Invitrogen). *RPTOR* (106bp) Qiagen primers were used successfully (Cat. # PPH05799A). GAPDH was quantified for normalization: forward: 5'-tcattctctgccccctctgc-3'; reverse: 5'-gagtccttcaggataccaa-3'. The qPCR-program was as follows: hot start (95°C), 10'' (95°C), cycle (40x): 15'' (95°C), 60'' (60 °C).

Gene network identification

Each set of differentially expressed ASM genes observed between the study groups was uploaded to the IPA software application for gene network identification. Sixteen gene networks with an IPA score ≥ 2 were identified when the 174 differentially expressed genes between *asthma and atopic controls* were used as input. The highest scoring network with a score of 38 was associated with the network functions carbohydrate metabolism, small molecule biochemistry, and cell-to-cell signaling and interaction (Figure S2A). With the 108 differentially expressed genes between *asthma and non-atopic controls*, 13 gene networks with IPA scores ≥ 2 were found. The highest network scored 43 and was associated with the network function cellular movement (Figure S2B). IPA analysis of the 135 differentially expressed genes between *atopic and non-atopic controls* resulted in 17 gene networks with

129 IPA scores ≥ 2 with the highest scoring network receiving 53 (Figure S2C). The associated
130 network functions included cellular development, growth, and proliferation.

131

132 A substantial overlap in network components existed between the highest scoring networks
133 of asthma *versus* atopic controls, and asthma *versus* non-atopic controls. Key components in
134 the gene network of atopic and non-atopic controls combined *versus* asthma included
135 ERK1/2, also known as mitogen-activated protein kinase 1 (MAPK 1), p38 mitogen-activated
136 protein kinase (p38 MAPK), and nuclear factor kappa-B (NFkB) (Figure S3).

137

References

1. GINA report, Global strategy for asthma management and prevention (update December 2009). Available at: <http://www.ginasthma.com>. Accessed November 23, 2012.
2. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J* 2005 August;**26**:319-338.
3. Sterk PJ, Fabbri LM, Quanjer PhH, Cockcroft DW, O'Byrne PM, Anderson SD, et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;**16**:53-83.
4. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. *Am Rev Respir Dis* 1987;**136**:1412-1417.
5. Yick CY, Zwinderman AH, Kunst PW, Grünberg K, Mauad T, Fluiters K, et al. Glucocorticoid-induced changes in gene expression of airway smooth muscle in patients with asthma. *Am J Respir Crit Care Med* 2103;**187**:1076-1084.
6. Busse WW, Wanner A, Adams K, Reynolds HY, Castro M, Chowdhury B, et al. Investigative bronchoprovocation and bronchoscopy in airway diseases. *Am J Respir Crit Care Med* 2005;**172**:807-816.
7. Weterman MA, Sorrentino V, Kasher PR, Jakobs ME, van Engelen BG, Fluiters K, et al. A frameshift mutation in LRSAM1 is responsible for a dominant hereditary polyneuropathy. *Hum Mol Genet* 2012;**21**:358-370.
8. UCSC Genome Bioinformatics. Available at: <http://genome.ucsc.edu>. Accessed November 23, 2012.
9. Morozova O, Marra MA. Applications of next-generation sequencing technologies in functional genomics. *Genomics* 2008;**5**:255-264.

- 165 10. Lander ES, Waterman MS. Genomic mapping by fingerprinting random clones: a
166 mathematical analysis. *Genomics* 1988;**2**:231-239.
- 167 11. Ingenuity Systems. Available at: <http://www.ingenuity.com>. Accessed November 23,
168 2012.
- 169 12. Real-time PCR Data Markup Language, MIQE guidelines. Available at:
170 <http://www.rdml.org/miqe.php>. Accessed November 23, 2012.
171

172 **Table S1 – Sample characteristics**

	Asthma	Healthy, atopic	Healthy, non-atopic
RNA yield (ng; 4 biopsies per subject)^a	170 (80 – 422)	109 (64 – 194)	199 (34 – 263)
cDNA yield (ng; 50ng RNA input)^a	1790 (433 – 5040)	1829 (908 – 5040)	1566 (798 – 4395)
cDNA library concentration (x10⁹ mol/μL)^a	1.14 (0.57 – 1.83)	1.06 (0.92 – 1.25)	0.94 (0.75 – 1.29)
RNA-Seq read number (n)	746,820	383,740	269,900
Read number mapped to hg19 (%)	92	93	92

^a Data are shown as Mean (range)

Table S2 – List of differentially expressed genes in airway smooth muscle of asthma vs. healthy atopic controls

Gene symbol	Gene name	NCBI Gene ID	Log2 fold change ^a	p-value
<i>IGSF9B</i>	immunoglobulin superfamily, member 9B	22997	4.15	0.05
<i>RASGEF1C</i>	RasGEF domain family, member 1C	225426	3.96	0.04
<i>WDR1</i>	WD repeat domain 1	9948	3.95	0.02
<i>ANKRD45</i>	ankyrin repeat domain 45	339416	3.92	0.01
<i>HOMER2</i>	homer homolog 2 (Drosophila)	9455	3.90	0.03
<i>PGS1</i>	phosphatidylglycerophosphate synthase 1	9489	3.80	0.02
<i>CDC20B</i>	cell division cycle 20 homolog B (S. cerevisiae)	166979	3.79	0.03
<i>POF1B</i>	premature ovarian failure, 1B	79983	3.76	0.03
<i>RNF114</i>	ring finger protein 114	55905	3.61	0.05
<i>PLD3</i>	phospholipase D family, member 3	23646	3.53	0.03
<i>AK8</i>	adenylate kinase 8	158067	3.46	<0.01
<i>LOC145820</i>	Uncharacterized	145820	3.44	0.04
<i>RGS9</i>	regulator of G-protein signaling 9	8787	3.17	0.02
<i>HORMAD2</i>	HORMA domain containing 2	150280	3.07	0.02
<i>DLGAP2</i>	discs, large (Drosophila) homolog-associated protein 2	9228	2.97	0.04
<i>POU2AF1</i>	POU class 2 associating factor 1	5450	2.94	0.02
<i>KLHL5</i>	kelch-like 5 (Drosophila)	51088	2.93	0.01
<i>PI4KB</i>	phosphatidylinositol 4-kinase, catalytic, beta	5298	2.91	0.02
<i>KCTD16</i>	potassium channel tetramerisation domain containing 16	57528	2.80	0.01
<i>FAM69A</i>	family with sequence similarity 69, member A	388650	2.80	0.02
<i>ADCY1</i>	adenylate cyclase 1 (brain)	107	2.68	0.05
<i>PKHD1L1</i>	polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1	93035	2.66	0.03
<i>MED15</i>	mediator complex subunit 15	51586	2.63	0.03
<i>RGPD1</i>	RANBP2-like and GRIP domain containing 1	400966	2.58	0.03
<i>C9orf171</i>	chromosome 9 open reading frame 171	389799	2.58	0.03

<i>PPP1R13B</i>	protein phosphatase 1, regulatory subunit 13B	23368	2.51	<0.01
<i>CA8</i>	carbonic anhydrase VIII	767	2.46	0.04
<i>C5orf15</i>	chromosome 5 open reading frame 15	56951	2.46	0.03
<i>PCDHA1</i>	protocadherin alpha 1	56147	2.44	0.02
<i>SPSB1</i>	splA/ryanodine receptor domain and SOCS box containing 1	80176	2.35	<0.01
<i>IL18R1</i>	interleukin 18 receptor 1	8809	2.33	0.05
<i>SFI1</i>	Sfi1 homolog, spindle assembly associated (yeast)	9814	2.32	0.04
<i>POLN</i>	polymerase (DNA directed) nu	353497	2.27	0.05
<i>TBC1D14</i>	TBC1 domain family, member 14	57533	2.25	0.05
<i>COL28A1</i>	collagen, type XXVIII, alpha 1	340267	2.20	0.04
<i>ANO3</i>	anoctamin 3	63982	2.18	0.01
<i>HSF2BP</i>	heat shock transcription factor 2 binding protein	11077	2.14	0.03
<i>SLC38A9</i>	solute carrier family 38, member 9	153129	2.14	0.02
<i>ANO4</i>	anoctamin 4	121601	2.13	<0.01
<i>VANGL1</i>	vang-like 1 (van gogh, Drosophila)	81839	2.11	0.04
<i>CRMP1</i>	collapsin response mediator protein 1	1400	2.09	0.04
<i>PPFIBP2</i>	PTPRF interacting protein, binding protein 2 (liprin beta 2)	8495	2.09	0.04
<i>KCND2</i>	potassium voltage-gated channel, Shal-related subfamily, member 2	3751	2.07	0.05
<i>MB21D2</i>	Mab-21 domain containing 2	151963	2.05	0.04
<i>RPGRIP1L</i>	RPGRIP1-like	23322	1.99	0.03
<i>ANKLE2</i>	ankyrin repeat and LEM domain containing 2	23141	1.96	0.04
<i>BAGE3</i>	B melanoma antigen family, member 3	85318	1.91	0.03
<i>GPATCH4</i>	G patch domain containing 4	54865	1.91	0.04
<i>DEC1</i>	deleted in esophageal cancer 1	50514	1.90	<0.01
<i>HEXA</i>	hexosaminidase A (alpha polypeptide)	3073	1.87	0.04
<i>LRRC7</i>	leucine rich repeat containing 7	57554	1.84	0.01
<i>CAPN11</i>	calpain 11	11131	1.82	<0.01
<i>CLPTM1L</i>	CLPTM1-like	81037	1.80	<0.01
<i>RPTOR</i>	regulatory associated protein of MTOR, complex 1	57521	1.79	<0.01

<i>PSMF1</i>	proteasome (prosome, macropain) inhibitor subunit 1 (PI31)	9491	1.79	0.03
<i>FGF12</i>	fibroblast growth factor 12	2257	1.76	0.01
<i>SLC2A13</i>	solute carrier family 2 (facilitated glucose transporter), member 13	114134	1.74	0.01
<i>FMN1</i>	formin 1	342184	1.70	0.04
<i>FKBP5</i>	FK506 binding protein 5	2289	1.68	0.03
<i>OR8U8</i>	olfactory receptor, family 8, subfamily U, member 8	504189	1.68	0.05
<i>PLEKHA4</i>	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 4	57664	1.65	0.02
<i>B4GALT5</i>	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 5	9334	1.63	0.02
<i>EFCAB11</i>	EF-hand calcium binding domain 11	90141	1.62	0.01
<i>HFM1</i>	HFM1, ATP-dependent DNA helicase homolog (S. cerevisiae)	164045	1.58	0.02
<i>SHANK2</i>	SH3 and multiple ankyrin repeat domains 2	22941	1.57	0.02
<i>PIAS4</i>	protein inhibitor of activated STAT, 4	51588	1.54	0.02
<i>HIP1</i>	huntingtin interacting protein 1	3092	1.50	0.05
<i>GPC5</i>	glypican 5	2262	1.48	0.03
<i>RBFOX1</i>	RNA binding protein, fox-1 homolog (C. elegans) 1	54715	1.46	0.01
<i>ERBB4</i>	v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	2066	1.43	0.02
<i>RGS7</i>	regulator of G-protein signaling 7	6000	1.42	0.02
<i>MGAT4C</i>	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme C (putative)	25834	1.41	<0.01
<i>FGGY</i>	FGGY carbohydrate kinase domain containing	55277	1.37	0.02
<i>TRRAP</i>	transformation/transcription domain-associated protein	8295	1.33	0.03
<i>ATP8B4</i>	ATPase, class I, type 8B, member 4	79895	1.30	0.04
<i>TAF3</i>	TAF3 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 140kDa	83860	1.28	0.03
<i>NCOR2</i>	nuclear receptor corepressor 2	9612	1.26	0.03
<i>CSMD3</i>	CUB and Sushi multiple domains 3	114788	1.26	0.03
<i>CMIP</i>	c-Maf inducing protein	80790	1.25	0.01
<i>SLC9A8</i>	solute carrier family 9, subfamily A (NHE8, cation proton antiporter 8), member 8	23315	1.24	<0.01
<i>SLC24A3</i>	solute carrier family 24 (sodium/potassium/calcium exchanger), member 3	57419	1.20	0.04
<i>PLEKHG3</i>	pleckstrin homology domain containing, family G (with RhoGef domain) member 3	26030	1.18	0.04

<i>GALNT13</i>	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 13 (GalNAc-T13)	114805	1.16	0.05
<i>PCBD2</i>	pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 2	84105	1.16	<0.01
<i>RGS6</i>	regulator of G-protein signaling 6	9628	1.13	0.05
<i>MARCH1</i>	membrane-associated ring finger (C3HC4) 1, E3 ubiquitin protein ligase	55016	1.10	0.05
<i>MTRNR2L8</i>	MT-RNR2-like 8	100463486	1.10	<0.01
<i>PTPRD</i>	protein tyrosine phosphatase, receptor type, D	5789	1.10	0.04
<i>EYA2</i>	eyes absent homolog 2 (Drosophila)	2139	1.10	0.05
<i>PACRG</i>	PARK2 co-regulated	135138	1.00	0.04
<i>MYO1D</i>	myosin ID	4642	1.00	0.05
<i>TCF7L2</i>	transcription factor 7-like 2 (T-cell specific, HMG-box)	6934	0.97	0.02
<i>BTBD9</i>	BTB (POZ) domain containing 9	114781	0.86	0.04
<i>NEAT1</i>	nuclear paraspeckle assembly transcript 1 (non-protein coding)	283131	0.76	0.05
<i>NT5E</i>	5'-nucleotidase, ecto (CD73)	4907	0.38	0.05
<i>RAD51AP1</i>	RAD51 associated protein 1	10635	0.31	0.03
<i>HSPBP1</i>	HSPA (heat shock 70kDa) binding protein, cytoplasmic cochaperone 1	23640	0.23	0.03
<i>CCDC150</i>	coiled-coil domain containing 150	284992	0.23	0.05
<i>LACTB</i>	lactamase, beta	114294	0.20	0.04
<i>KCNC2</i>	potassium voltage-gated channel, Shaw-related subfamily, member 2	3747	0.18	0.05
<i>CDH10</i>	cadherin 10, type 2 (T2-cadherin)	1008	0.15	0.03
<i>ABCD2</i>	ATP-binding cassette, sub-family D (ALD), member 2	225	0.06	0.04
<i>SFT2D2</i>	SFT2 domain containing 2	375035	0.06	0.04
<i>BCAS4</i>	breast carcinoma amplified sequence 4	55653	0.06	0.04
<i>PCDH20</i>	protocadherin 20	64881	-0.06	0.02
<i>LPP</i>	LIM domain containing preferred translocation partner in lipoma	4026	-0.64	0.04
<i>THSD4</i>	thrombospondin, type I, domain containing 4	79875	-0.69	0.05
<i>NRXN3</i>	neurexin 3	9369	-0.71	0.03
<i>KCNQ5</i>	potassium voltage-gated channel, KQT-like subfamily, member 5	56479	-0.76	0.05

<i>XIST</i>	X (inactive)-specific transcript (non-protein coding)	7503	-0.81	0.03
<i>ITPR1</i>	inositol 1,4,5-trisphosphate receptor, type 1	3708	-0.84	0.05
<i>PLCB4</i>	phospholipase C, beta 4	5332	-0.86	0.02
<i>SPARCL1</i>	SPARC-like 1 (hevin)	8404	-0.86	0.03
<i>PRDM6</i>	PR domain containing 6	93166	-0.86	0.04
<i>MYLK</i>	myosin light chain kinase	4638	-0.86	0.04
<i>PRUNE2</i>	prune homolog 2 (Drosophila)	158471	-0.86	0.05
<i>LAMA4</i>	laminin, alpha 4	3910	-0.89	0.03
<i>SLIT2</i>	slit homolog 2 (Drosophila)	9353	-0.89	0.03
<i>COL4A2</i>	collagen, type IV, alpha 2	1284	-0.89	0.04
<i>PTPN14</i>	protein tyrosine phosphatase, non-receptor type 14	5784	-0.94	0.04
<i>GAB1</i>	GRB2-associated binding protein 1	2549	-0.97	0.04
<i>ZSWIM6</i>	zinc finger, SWIM-type containing 6	57688	-1.00	0.01
<i>ROR1</i>	receptor tyrosine kinase-like orphan receptor 1	4919	-1.00	0.01
<i>BACH2</i>	BTB and CNC homology 1, basic leucine zipper transcription factor 2	60468	-1.00	0.02
<i>FAM5C</i>	family with sequence similarity 5, member C	339479	-1.00	0.03
<i>LOC92249</i>	Uncharacterized	92249	-1.03	0.02
<i>COL4A5</i>	collagen, type IV, alpha 5	1287	-1.06	<0.01
<i>SEN7</i>	SUMO1/sentrin specific peptidase 7	57337	-1.09	0.03
<i>PAM</i>	peptidylglycine alpha-amidating monooxygenase	5066	-1.12	0.03
<i>FAM129A</i>	family with sequence similarity 129, member A	116496	-1.12	0.03
<i>APOOL</i>	apolipoprotein O-like	139322	-1.15	0.02
<i>SMAD2</i>	SMAD family member 2	4087	-1.15	0.03
<i>INPP4B</i>	inositol polyphosphate-4-phosphatase, type II, 105kDa	8821	-1.18	<0.01
<i>SBNO1</i>	strawberry notch homolog 1 (Drosophila)	55206	-1.18	0.04
<i>USP47</i>	ubiquitin specific peptidase 47	55031	-1.22	0.01
<i>DSTN</i>	destrin (actin depolymerizing factor)	11034	-1.22	0.03
<i>FAT1</i>	FAT tumor suppressor homolog 1 (Drosophila)	2195	-1.25	0.01
<i>CHRM3</i>	cholinergic receptor, muscarinic 3	1131	-1.29	<0.01

<i>MEIS1</i>	Meis homeobox 1	4211	-1.29	<0.01
<i>GALNT11</i>	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11 (GalNAc-T11)	63917	-1.29	0.03
<i>ABCD3</i>	ATP-binding cassette, sub-family D (ALD), member 3	5825	-1.29	0.04
<i>ACTA2</i>	actin, alpha 2, smooth muscle, aorta	59	-1.36	0.02
<i>PLOD2</i>	procollagen-lysine, 2-oxoglutarate 5-dioxygenase	5352	-1.40	0.03
<i>FAM188A</i>	family with sequence similarity 188, member A	80013	-1.40	0.04
<i>LEPREL1</i>	leprecan-like 1	55214	-1.43	<0.01
<i>HSPA4</i>	heat shock 70kDa protein 4	3308	-1.47	0.04
<i>PRKAA2</i>	protein kinase, AMP-activated, alpha 2 catalytic subunit	5563	-1.51	0.02
<i>GJA1</i>	gap junction protein, alpha 1, 43kDa	2697	-1.56	0.02
<i>SLC35A1</i>	solute carrier family 35 (CMP-sialic acid transporter), member A1	10559	-1.56	0.05
<i>SEMA6A</i>	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	57556	-1.60	<0.01
<i>VPS45</i>	vacuolar protein sorting 45 homolog (S. cerevisiae)	11311	-1.64	0.01
<i>FHL1</i>	four and a half LIM domains 1	2273	-1.64	0.01
<i>NUAK1</i>	NUAK family, SNF1-like kinase, 1	9891	-1.69	0.02
<i>API5</i>	apoptosis inhibitor 5	8539	-1.79	0.05
<i>XPA</i>	xeroderma pigmentosum, complementation group A	7507	-1.89	0.04
<i>ACTR10</i>	actin-related protein 10 homolog (S. cerevisiae)	55860	-1.94	0.05
<i>CRY2</i>	cryptochrome 2 (photolyase-like)	1408	-1.94	0.05
<i>ZFYVE20</i>	zinc finger, FYVE domain containing 20	64145	-2.00	0.02
<i>PAPD5</i>	PAP associated domain containing 5	64282	-2.06	0.03
<i>KREMEN1</i>	kringle containing transmembrane protein 1	83999	-2.06	0.04
<i>ENSA</i>	endosulfine alpha	2029	-2.25	0.02
<i>CCT6B</i>	chaperonin containing TCP1, subunit 6B (zeta 2)	10693	-2.25	0.04
<i>LMO4</i>	LIM domain only 4	8543	-2.32	<0.01
<i>SP140</i>	SP140 nuclear body protein	11262	-2.32	0.03
<i>RPE</i>	ribulose-5-phosphate-3-epimerase	6120	-2.40	<0.01
<i>LDHB</i>	lactate dehydrogenase B	3945	-2.56	0.03

<i>CHMP2A</i>	charged multivesicular body protein 2A	27243	-2.64	0.01
<i>FBXL3</i>	F-box and leucine-rich repeat protein 3	26224	-2.74	0.03
<i>FAM76A</i>	family with sequence similarity 76, member A	199870	-2.74	0.05
<i>PHF10</i>	PHD finger protein 10	55274	-2.94	<0.01
<i>COA5</i>	cytochrome C oxidase assembly factor 5	493753	-3.06	0.04
<i>LIPC</i>	lipase, hepatic	3990	-3.18	0.04
<i>TMEM14C</i>	transmembrane protein 14C	51522	-4.32	0.03
<i>SLC16A4</i>	solute carrier family 16, member 4 (monocarboxylic acid transporter 5)	9122	-5.06	<0.01

^a Log2 fold change asthma vs. healthy atopic controls: positive number signifies up-regulated in asthma, negative number signifies down-regulated in asthma

174

175

Table S3 – List of differentially expressed genes in airway smooth muscle of asthma vs. healthy non-atopic controls

Gene symbol	Gene name	NCBI Gene ID	Log2 fold change ^a	p-value
<i>N4BP2</i>	NEDD4 binding protein 2	55728	4.06	<0.01
<i>SLC41A3</i>	solute carrier family 41, member 3	54946	3.91	0.02
<i>STX2</i>	syntaxin 2	2054	3.62	0.03
<i>PRDM15</i>	PR domain containing 15	63977	3.54	0.03
<i>VANGL1</i>	vang-like 1 (van gogh, Drosophila)	81839	3.50	0.02
<i>KLHL23</i>	kelch-like 23 (Drosophila)	151230	3.35	0.05
<i>INPP5B</i>	inositol polyphosphate-5-phosphatase, 75kDa	3633	3.10	0.02
<i>C22orf46</i>	chromosome 22 open reading frame 46	79640	3.08	0.03
<i>C3orf64</i>	chromosome 3 open reading frame 64	285203	2.79	0.01
<i>PTCH1</i>	patched 1	5727	2.78	0.01
<i>RGS3</i>	regulator of G-protein signaling 3	5998	2.63	0.04
<i>FLJ34690</i>	uncharacterized protein	284034	2.57	<0.01
<i>COL6A2</i>	collagen, type VI, alpha 2	1292	2.45	<0.01
<i>CIR1</i>	corepressor interacting with RBPJ, 1	9541	2.41	0.05
<i>RPAP3</i>	RNA polymerase II associated protein 3	79657	2.33	0.03
<i>CDC42BPB</i>	CDC42 binding protein kinase beta (DMPK-like)	9578	2.32	0.03
<i>GPATCH4</i>	G patch domain containing 4	54865	2.30	0.03
<i>AK8</i>	adenylate kinase 8	158067	2.17	0.05
<i>PI4KA</i>	phosphatidylinositol 4-kinase, catalytic, alpha	5297	2.12	0.04
<i>OCA2</i>	oculocutaneous albinism II	4948	2.06	0.04
<i>IKBKAP</i>	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	8518	1.97	0.04
<i>PHF17</i>	PHD finger protein 17	79960	1.82	0.03
<i>NUP214</i>	nucleoporin 214kDa	8021	1.82	0.03
<i>PHACTR4</i>	phosphatase and actin regulator 4	65979	1.69	0.04

<i>BDNF-AS1</i>	BDNF antisense RNA 1 (non-protein coding)	497258	1.66	0.04
<i>TNC</i>	tenascin C	3371	1.58	0.03
<i>RPTOR</i>	regulatory associated protein of MTOR, complex 1	57521	1.45	<0.01
<i>SEC31A</i>	SEC31 homolog A (<i>S. cerevisiae</i>)	22872	1.25	0.04
<i>ESRRG</i>	estrogen-related receptor gamma	2104	1.24	0.04
<i>HFM1</i>	HFM1, ATP-dependent DNA helicase homolog (<i>S. cerevisiae</i>)	164045	1.23	0.04
<i>ANKRD30BL</i>	ankyrin repeat domain 30B-like	554226	1.18	0.04
<i>JAK2</i>	Janus kinase 2	3717	1.01	0.05
<i>MTRNR2L8</i>	MT-RNR2-like 8	100463486	0.97	0.02
<i>DDX26B</i>	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26B	203522	0.90	<0.01
<i>ARV1</i>	ARV1 homolog (<i>S. cerevisiae</i>)	64801	0.54	0.03
<i>SLC20A1</i>	solute carrier family 20 (phosphate transporter), member 1	6574	0.48	0.02
<i>ABO</i>	ABO blood group (transferase A, alpha 1-3-N-acetylgalactosaminyltransferase; transferase B, alpha 1-3-galactosyltransferase)	28	0.45	0.03
<i>SNX5</i>	sorting nexin 5	27131	0.40	0.03
<i>SEC14L3</i>	SEC14-like 3 (<i>S. cerevisiae</i>)	266629	0.37	0.03
<i>SCUBE3</i>	signal peptide, CUB domain, EGF-like 3	222663	0.30	0.03
<i>AATK</i>	apoptosis-associated tyrosine kinase	9625	0.29	0.04
<i>TEAD4</i>	TEA domain family member 4	7004	0.23	0.05
<i>PI16</i>	peptidase inhibitor 16	221476	0.18	0.03
<i>SERPINB5</i>	serpin peptidase inhibitor, clade B (ovalbumin), member 5	5268	0.15	0.05
<i>LOC649395</i>	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide pseudogene	649395	0.11	0.04
<i>IL26</i>	interleukin 26	55801	-0.04	0.05
<i>ENO2</i>	enolase 2 (gamma, neuronal)	2026	-0.12	0.01
<i>FLYWCH1</i>	FLYWCH-type zinc finger 1	84256	-0.27	0.05
<i>CAPN14</i>	calpain 14	440854	-0.40	<0.01
<i>FOXJ3</i>	forkhead box J3	22887	-0.92	0.03
<i>LEPREL1</i>	leprecan-like 1	55214	-0.97	0.04

<i>ZNF804B</i>	zinc finger protein 804B	219578	-1.00	0.03
<i>FAM129A</i>	family with sequence similarity 129, member A	116496	-1.06	0.04
<i>LIFR</i>	leukemia inhibitory factor receptor alpha	3977	-1.15	0.03
<i>KIF16B</i>	kinesin family member 16B	55614	-1.15	0.03
<i>TBX5</i>	T-box 5	6910	-1.18	0.02
<i>ADAMTS17</i>	ADAM metalloproteinase with thrombospondin type 1 motif, 17	170691	-1.22	0.02
<i>C1orf21</i>	chromosome 1 open reading frame 21	81563	-1.25	0.02
<i>ABCC4</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	10257	-1.29	0.02
<i>NRF1</i>	nuclear respiratory factor 1	4899	-1.32	0.02
<i>RAD50</i>	RAD50 homolog (S. cerevisiae)	10111	-1.32	0.04
<i>TMEM108</i>	transmembrane protein 108	66000	-1.40	0.03
<i>SLC23A2</i>	solute carrier family 23 (nucleobase transporters), member 2	9962	-1.40	0.03
<i>SCN7A</i>	sodium channel, voltage-gated, type VII, alpha subunit	6332	-1.40	0.04
<i>ATP2B1</i>	ATPase, Ca ⁺⁺ transporting, plasma membrane 1	490	-1.43	0.02
<i>SYNRG</i>	synergin, gamma	11276	-1.43	0.03
<i>CCDC45</i>	centrosomal protein 95kDa	90799	-1.56	0.04
<i>RBM4</i>	RNA binding motif protein 4	5936	-1.79	0.04
<i>CDC5L</i>	CDC5 cell division cycle 5-like (S. pombe)	988	-1.79	0.04
<i>MMADHC</i>	methylmalonic aciduria (cobalamin deficiency) cblD type, with homocystinuria	27249	-1.79	0.04
<i>RBM41</i>	RNA binding motif protein 41	55285	-1.84	0.03
<i>COPS4</i>	COP9 constitutive photomorphogenic homolog subunit 4 (Arabidopsis)	51138	-1.84	0.03
<i>AFAP1L2</i>	actin filament associated protein 1-like 2	84632	-1.84	0.04
<i>RAB22A</i>	RAB22A, member RAS oncogene family	57403	-1.84	0.04
<i>PDHX</i>	pyruvate dehydrogenase complex, component X	8050	-1.94	<0.01
<i>ETS2</i>	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	2114	-2.00	<0.01
<i>EIF2AK2</i>	eukaryotic translation initiation factor 2-alpha kinase 2	5610	-2.06	<0.01
<i>ZNF41</i>	zinc finger protein 41	7592	-2.06	0.02
<i>ZNF37BP</i>	zinc finger protein 37B, pseudogene	100129482	-2.12	<0.01
<i>ADH1B</i>	alcohol dehydrogenase 1B (class I), beta polypeptide	125	-2.12	0.02

<i>TMEM98</i>	transmembrane protein 98	26022	-2.12	0.03
<i>UNC50</i>	unc-50 homolog (C. elegans)	25972	-2.18	0.03
<i>ADC</i>	arginine decarboxylase	113451	-2.18	0.04
<i>TMEM71</i>	transmembrane protein 71	137835	-2.32	<0.01
<i>AQP3</i>	aquaporin 3 (Gill blood group)	360	-2.32	<0.01
<i>ITGB6</i>	integrin, beta 6	3694	-2.40	0.01
<i>SPTBN4</i>	spectrin, beta, non-erythrocytic 4	57731	-2.40	0.03
<i>HERC2P2</i>	hect domain and RLD 2 pseudogene 2	400322	-2.47	0.02
<i>FAM46C</i>	family with sequence similarity 46, member C	54855	-2.47	0.04
<i>RNF170</i>	ring finger protein 170	81790	-2.64	0.05
<i>SCML2</i>	sex comb on midleg-like 2 (Drosophila)	10389	-2.74	<0.01
<i>ZNF227</i>	zinc finger protein 227	7770	-2.74	0.05
<i>MRPL22</i>	mitochondrial ribosomal protein L22	29093	-2.84	0.03
<i>PPP2R2D</i>	protein phosphatase 2, regulatory subunit B, delta	55844	-2.84	0.03
<i>HELB</i>	helicase (DNA) B	92797	-2.94	<0.01
<i>SEC61A1</i>	Sec61 alpha 1 subunit (S. cerevisiae)	29927	-2.94	0.01
<i>WDR63</i>	WD repeat domain 63	126820	-3.06	0.02
<i>KCNK12</i>	potassium channel, subfamily K, member 12	56660	-3.06	0.04
<i>ZNF577</i>	zinc finger protein 577	84765	-3.06	0.05
<i>ZNF793</i>	zinc finger protein 793	390927	-3.18	0.03
<i>MFAP3</i>	microfibrillar-associated protein 3	4238	-3.18	0.05
<i>CYP4X1</i>	cytochrome P450, family 4, subfamily X, polypeptide 1	260293	-3.32	0.02
<i>E2F6</i>	E2F transcription factor 6	1876	-3.47	0.03
<i>PERP</i>	PERP, TP53 apoptosis effector	64065	-3.64	<0.01
<i>TPTE2</i>	transmembrane phosphoinositide 3-phosphatase and tensin homolog 2	93492	-3.64	<0.01
<i>SLC22A3</i>	solute carrier family 22 (extraneuronal monoamine transporter), member 3	6581	-3.84	<0.01
<i>SCGB1A1</i>	secretoglobin, family 1A, member 1 (uteroglobin)	7356	-4.06	0.01
<i>LOC646214</i>	p21 protein (Cdc42/Rac)-activated kinase 2 pseudogene	646214	-4.64	<0.01

^a Log2 fold change asthma vs. healthy non-atopic controls: positive number signifies up-regulated in asthma, negative number signifies down-regulated in asthma

Table S4 – List of differentially expressed genes in airway smooth muscle of healthy atopic vs. non-atopic controls

Gene symbol	Gene name	NCBI Gene ID	Log2 fold change ^a	p-value
<i>TMEM47</i>	transmembrane protein 47	83604	4.14	0.02
<i>N4BP2</i>	NEDD4 binding protein 2	55728	4.00	0.03
<i>CRY2</i>	cryptochrome 2 (photolyase-like)	1408	3.97	0.04
<i>PGM5P2</i>	phosphoglucomutase 5 pseudogene 2	595135	3.86	0.03
<i>BTF3L4</i>	basic transcription factor 3-like 4	91408	3.58	0.04
<i>COL6A2</i>	collagen, type VI, alpha 2	1292	3.14	<0.01
<i>FLJ34690</i>	uncharacterized protein	284034	3.09	<0.01
<i>GEM</i>	GTP binding protein overexpressed in skeletal muscle	2669	3.04	0.04
<i>CHMP2A</i>	charged multivesicular body protein 2A	27243	3.03	0.05
<i>C3orf64</i>	chromosome 3 open reading frame 64	285203	2.94	0.01
<i>CEBPZ</i>	CCAAT/enhancer binding protein (C/EBP), zeta	10153	2.69	0.02
<i>CDC42BPB</i>	CDC42 binding protein kinase beta (DMPK-like)	9578	2.61	0.02
<i>ARMCX4</i>	armadillo repeat containing, X-linked 4	100131755	2.47	0.01
<i>IKBKAP</i>	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	8518	2.21	0.02
<i>MAP2</i>	microtubule-associated protein 2	4133	2.13	0.02
<i>DCLK2</i>	doublecortin-like kinase 2	166614	1.81	<0.01
<i>RCAN1</i>	regulator of calcineurin 1	1827	1.78	0.03
<i>CSRP1</i>	cysteine and glycine-rich protein 1	1465	1.73	0.05
<i>SH3RF1</i>	SH3 domain containing ring finger 1	57630	1.68	0.03
<i>ABL1</i>	c-abl oncogene 1, non-receptor tyrosine kinase	25	1.65	0.04
<i>HIF1A</i>	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	3091	1.63	0.01
<i>KIAA0947</i>	uncharacterized protein	23379	1.53	0.04
<i>BICD1</i>	bicaudal D homolog 1 (Drosophila)	636	1.53	0.04
<i>PGM5</i>	phosphoglucomutase 5	5239	1.46	0.02

<i>MTHFD1L</i>	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	25902	1.44	0.03
<i>COL4A1</i>	collagen, type IV, alpha 1	1282	1.42	0.01
<i>STOX2</i>	storkhead box 2	56977	1.32	0.02
<i>MYLK</i>	myosin light chain kinase	4638	1.28	0.01
<i>CHRM3</i>	cholinergic receptor, muscarinic 3	1131	1.24	<0.01
<i>TRIO</i>	triple functional domain (PTPRF interacting)	7204	1.24	<0.01
<i>FSTL1</i>	folliculin-like 1	11167	1.21	0.02
<i>RAI14</i>	retinoic acid induced 14	26064	1.20	0.03
<i>MAST4</i>	microtubule associated serine/threonine kinase family member 4	375449	1.18	0.04
<i>APBB2</i>	amyloid beta (A4) precursor protein-binding, family B, member 2	323	1.18	0.01
<i>YWHAB</i>	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	7529	1.17	0.01
<i>GNG12</i>	guanine nucleotide binding protein (G protein), gamma 12	55970	1.16	0.05
<i>PDE5A</i>	phosphodiesterase 5A, cGMP-specific	8654	1.12	0.04
<i>SAMD4A</i>	sterile alpha motif domain containing 4A	23034	1.11	0.02
<i>TBL1X</i>	transducin (beta)-like 1X-linked	6907	1.10	0.03
<i>INPP4B</i>	inositol polyphosphate-4-phosphatase, type II, 105kDa	8821	1.08	<0.01
<i>MYH10</i>	myosin, heavy chain 10, non-muscle	4628	1.02	0.02
<i>SLIT2</i>	slit homolog 2 (Drosophila)	9353	0.99	0.05
<i>FHOD3</i>	formin homology 2 domain containing 3	80206	0.98	0.05
<i>ESYT2</i>	extended synaptotagmin-like protein 2	57488	0.97	0.02
<i>CREB5</i>	cAMP responsive element binding protein 5	9586	0.96	0.04
<i>MACF1</i>	microtubule-actin crosslinking factor 1	23499	0.96	<0.01
<i>SYNPO2</i>	synaptopodin 2	171024	0.95	0.04
<i>SPARCL1</i>	SPARC-like 1 (hevin)	8404	0.92	0.02
<i>NRXN3</i>	neurexin 3	9369	0.88	0.04
<i>MSRB3</i>	methionine sulfoxide reductase B3	253827	0.88	0.04
<i>DDX26B</i>	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26B	203522	0.84	0.03
<i>LPP</i>	LIM domain containing preferred translocation partner in lipoma	4026	0.81	0.04

<i>LAMA4</i>	laminin, alpha 4	3910	0.72	0.03
<i>SCUBE3</i>	signal peptide, CUB domain, EGF-like 3	222663	0.72	0.04
<i>USP35</i>	ubiquitin specific peptidase 35	57558	-0.34	0.05
<i>KCNC2</i>	potassium voltage-gated channel, Shaw-related subfamily, member 2	3747	-0.51	0.02
<i>TRIM29</i>	tripartite motif containing 29	23650	-0.54	0.03
<i>LIPA</i>	lipase A, lysosomal acid, cholesterol esterase	3988	-0.74	0.02
<i>EHBP1</i>	EH domain binding protein 1	23301	-0.79	00.04
<i>LRP1B</i>	low density lipoprotein receptor-related protein 1B	53353	-0.89	0.03
<i>C1orf21</i>	chromosome 1 open reading frame 21	81563	-0.94	0.05
<i>CDH10</i>	cadherin 10, type 2 (T2-cadherin)	1008	-1.00	0.01
<i>PPFIA1</i>	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 1	8500	-1.03	0.01
<i>LSAMP</i>	limbic system-associated membrane protein	4045	-1.06	0.03
<i>IPCEF1</i>	interaction protein for cytohesin exchange factors 1	26034	-1.09	<0.01
<i>DSCAM</i>	Down syndrome cell adhesion molecule	1826	-1.09	0.05
<i>ERBB4</i>	v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	2066	-1.09	0.05
<i>ZNF804B</i>	zinc finger protein 804B	219578	-1.09	0.05
<i>CDH13</i>	cadherin 13, H-cadherin (heart)	1012	-1.18	0.04
<i>CMIP</i>	c-Maf inducing protein	80790	-1.18	0.02
<i>PCDH9</i>	protocadherin 9	5101	-1.22	0.03
<i>L3MBTL4</i>	l(3)mbt-like 4 (Drosophila)	91133	-1.22	0.02
<i>SYTL2</i>	synaptotagmin-like 2	54843	-1.25	0.03
<i>CTNNA2</i>	catenin (cadherin-associated protein), alpha 2	1496	-1.29	0.02
<i>TAF3</i>	TAF3 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 140kDa	83860	-1.36	0.05
<i>ACER3</i>	alkaline ceramidase 3	55331	-1.36	0.04
<i>PTPRN2</i>	protein tyrosine phosphatase, receptor type, N polypeptide 2	5799	-1.36	0.01
<i>FSTL5</i>	folliculin-like 5	56884	-1.40	0.03
<i>RNF130</i>	ring finger protein 130	55819	-1.43	0.05
<i>WDR37</i>	WD repeat domain 37	22884	-1.43	0.04

<i>FGF12</i>	fibroblast growth factor 12	2257	-1.43	0.03
<i>NRG3</i>	neuregulin 3	10718	-1.47	0.05
<i>SLC2A13</i>	solute carrier family 2 (facilitated glucose transporter), member 13	114134	-1.47	0.04
<i>NRG1</i>	neuregulin 1	3084	-1.47	<0.01
<i>LPHN3</i>	latrophilin 3	23284	-1.51	0.05
<i>SLC35F3</i>	solute carrier family 35, member F3	148641	-1.51	0.04
<i>MGAT4C</i>	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme C (putative)	25834	-1.51	<0.01
<i>ITGBL1</i>	integrin, beta-like 1 (with EGF-like repeat domains)	9358	-1.56	0.03
<i>SKAP1</i>	src kinase associated phosphoprotein 1	8631	-1.56	0.03
<i>RGS7</i>	regulator of G-protein signaling 7	6000	-1.56	0.02
<i>CDH4</i>	cadherin 4, type 1, R-cadherin (retinal)	1002	-1.56	0.01
<i>OLFM3</i>	olfactomedin 3	118427	-1.56	<0.01
<i>ETS2</i>	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	2114	-1.60	0.03
<i>DOCK2</i>	dedicator of cytokinesis 2	1794	-1.60	0.02
<i>GRID1</i>	glutamate receptor, ionotropic, delta 1	2894	-1.64	0.01
<i>KCNH8</i>	potassium voltage-gated channel, subfamily H (eag-related), member 8	131096	-1.69	0.02
<i>NEDD9</i>	neural precursor cell expressed, developmentally down-regulated 9	4739	-1.69	0.01
<i>RBM4</i>	RNA binding motif protein 4	5936	-1.74	0.05
<i>PIK3C2G</i>	phosphoinositide-3-kinase, class 2, gamma polypeptide	5288	-1.74	0.02
<i>FAM168A</i>	family with sequence similarity 168, member A	23201	-1.74	0.01
<i>ITGB6</i>	integrin, beta 6	3694	-1.79	0.04
<i>CLPTM1L</i>	CLPTM1-like	81037	-1.79	<0.01
<i>KCND2</i>	potassium voltage-gated channel, Shal-related subfamily, member 2	3751	-1.84	0.03
<i>ABCC4</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	10257	-1.84	0.02
<i>TRRAP</i>	transformation/transcription domain-associated protein	8295	-1.84	0.01
<i>CCDC45</i>	centrosomal protein 95kDa	90799	-2.06	0.03
<i>TOX3</i>	TOX high mobility group box family member 3	27324	-2.12	0.05
<i>ZNF385B</i>	zinc finger protein 385B	151126	-2.12	<0.01

<i>DMBT1</i>	deleted in malignant brain tumors 1	1755	-2.18	0.05
<i>NXPH1</i>	neurexophilin 1	30010	-2.25	0.03
<i>PPP1R13B</i>	protein phosphatase 1, regulatory subunit 13B	23368	-2.25	0.02
<i>FKBP5</i>	FK506 binding protein 5	2289	-2.25	<0.01
<i>GPR158</i>	G protein-coupled receptor 158	57512	-2.32	0.03
<i>SLCO1A2</i>	solute carrier organic anion transporter family, member 1A2	6579	-2.32	0.02
<i>ZFAT</i>	zinc finger and AT hook domain containing	57623	-2.40	0.04
<i>ERN1</i>	endoplasmic reticulum to nucleus signaling 1	2081	-2.40	0.04
<i>TSNARE1</i>	t-SNARE domain containing 1	203062	-2.40	0.04
<i>FAM134B</i>	family with sequence similarity 134, member B	54463	-2.40	0.03
<i>FREM2</i>	FRAS1 related extracellular matrix protein 2	341640	-2.47	0.04
<i>PTPRS</i>	protein tyrosine phosphatase, receptor type, S	5802	-2.56	0.03
<i>ZNF41</i>	zinc finger protein 41	7592	-2.64	0.01
<i>GABRA2</i>	gamma-aminobutyric acid (GABA) A receptor, alpha 2	2555	-2.64	0.01
<i>CDH19</i>	cadherin 19, type 2	28513	-2.74	0.02
<i>ELK3</i>	ELK3, ETS-domain protein (SRF accessory protein 2)	2004	-2.74	<0.01
<i>UNC50</i>	unc-50 homolog (C. elegans)	25972	-2.94	0.03
<i>IPO13</i>	importin 13	9670	-3.06	0.05
<i>C9orf171</i>	chromosome 9 open reading frame 171	389799	-3.06	0.02
<i>ARSF</i>	arylsulfatase F	416	-3.06	0.01
<i>TMEM71</i>	transmembrane protein 71	137835	-3.07	<0.01
<i>LGR5</i>	leucine-rich repeat containing G protein-coupled receptor 5	8549	-3.47	<0.01
<i>VWC2</i>	von Willebrand factor C domain containing 2	375567	-3.84	<0.01
<i>ZNF793</i>	zinc finger protein 793	390927	-4.06	0.03
<i>HERC2P2</i>	hect domain and RLD 2 pseudogene 2	400322	-4.06	0.03
<i>ANKRD45</i>	ankyrin repeat domain 45	339416	-4.06	0.02
<i>GNG7</i>	guanine nucleotide binding protein (G protein), gamma 7	2788	-4.64	<0.01

^a Log2 fold change healthy atopic vs. non-atopic controls: positive number signifies up-regulated in healthy atopic controls, negative number signifies down-regulated.

179 **Table S5 – Read numbers and Cq-ratios**

	<i>LEPREL1</i>		<i>RPTOR</i>	
	Read number (n) ^a	Cq-ratio	Read number (n) ^a	Cq-ratio
Asthma	3	1.33	10	1.20
Atopic controls	12	1.28	4	1.25
Non-atopic controls	6	1.19	2	1.30

^a Geometric mean values

181

Figure S1 – Laser capture microdissection of ASM

This figure shows a cryosection from a biopsy specimen of an asthma patient prior (a) and after (b) collection of ASM by laser capture microdissection at 10x magnification.

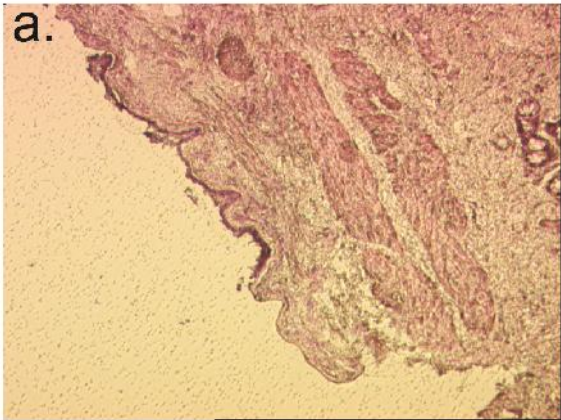
Figure S2 – Gene networks by IPA

The highest scoring gene networks are shown with the differentially expressed genes between asthma and healthy atopic controls (a; IPA score 38), asthma and healthy non-atopic controls (b; IPA score 43), and healthy atopic and non-atopic controls (c; IPA score 53) as input.

Figure S3 – Gene networks by IPA combined

This figure shows the highest scoring networks of asthma *versus* healthy atopic, and asthma *versus* healthy non-atopic controls combined, with ERK1/2, p38 MAPK, and NFκB as key components.

a.



b.

