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## Genetic and histological evidence for autophagy in asthma pathogenesis

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asthma pathogenesis; autophagy; lung function; polymorphism; SNP; ATG5; autophagosome

To the Editor:

Asthma affects all age groups and presents itself as a spectrum of severity and symptoms. Reactive oxidative species (ROS) play a pivotal role in asthma pathogenesis. Exhaled levels of mediators associated with ROS positively correlate with asthma severity.(1) Autophagy, the process of cellular waste disposal through lysosomal -dependent pathways, is induced by ROS to remove oxidized proteins or organelles to minimize tissue damage.(2) Although autophagy is augmented in the lungs of COPD patients compared to healthy control subjects (3), evidence for autophagy in asthma, particularly moderate-to-severe asthma, has not been reported. We hypothesize that autophagy is associated with asthma pathogenesis, and sought to detect its presence using both genetic and histological approaches.

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We conducted a genetic association study to investigate whether single nucleotide polymorphisms (SNPs) in genes of the autophagy pathway are associated with asthma. We selected 5 genes of the autophagy pathway (unc-51-like kinase 1 (*ULK1*), sequestosome 1 (*SQSTM1*), microtubule-associated protein 1 light chain 3 beta (*MAP1LC3B*), beclin 1 (*BECN1*) and autophagy related 5 homolog (*ATG5*)). (2) We tested for genetic associations in an asthma family-based study from northeastern Quebec, Canada (The Saguenay-Lac-Saint-Jean (SLSJ) asthma Study) using the family based association test (FBAT) statistic and UNPHASED software for an odds ratio estimate.(4, 5) Patient recruitment has previously been described.(6) These SNPs have been genotyped previously in a genome-wide association study.(6) To reduce the likelihood of false positive findings, we reset the statistical significance threshold from  $p=0.05$  to  $p=0.001$  according to the Bonferroni method. To confirm our positive findings, we tested the association in a second family based population; the non-Hispanic Caucasian participants of the Childhood Asthma Management Program (CAMP), and patient recruitment has previously been described. (7) The SLSJ local ethics committee and the Institutional Review Board for CAMP approved the study, and all subjects gave informed consents.

In the SLSJ asthma study a total of 1338 individuals (483 nuclear families) with known asthma status were included in the analysis, and 336 individuals were either probands or affected siblings. Of this group, the male:female ratio was 0.83. The mean age was 16.45 (standard deviation (SD)  $\pm 9.43$ ) years. 77.1% were atopic. The mean (SD) forced expiratory volume in 1 second ( $FEV_1$ )% predicted was 94.1(20.1)%. A total of 39 SNPs were tested, and after Bonferroni correction, SNP rs12212740 G>A of *ATG5* remained statistically significant (Table 1). Allele G with allele frequency of 0.88 is over-transmitted to asthmatic offspring ( $p=0.0002$ ), (Odds ratio = 1.35, 95% confidence interval = 1.01 and 1.89). SNP rs12212740 was not associated with asthma in CAMP, however, it was associated with pre-bronchodilator  $FEV_1$  (pre- $FEV_1$ ) (adjusted for age, sex and height) ( $p=0.04$ ). In the SLSJ cohort, rs12212740 was associated with pre- $FEV_1$  ( $p=0.007$ ). In both populations, allele G was negatively associated with adjusted pre- $FEV_1$ . SNP rs12212740 is located in intron 3 of *ATG5*, 7kb downstream and 8kb upstream of exon 3 and 4, respectively. At present, the functional consequence of SNP rs12212740 is unknown, and it is probable that the association is due to the linkage disequilibrium between SNP rs12212740 and the true causative variant. Nevertheless, the association between a genetic variant of *ATG5* and pre- $FEV_1$  in both study populations suggest that autophagy is associated with reduced lung function in asthmatic subjects.

To determine if autophagy is present in the airways of asthmatic individuals, bronchial biopsy stored at the Tissue Bank of the Respiratory Health Network of the Fonds de la Recherche en Santé du Québec (McGill University Health Centre site) were obtained. Patient recruitment and bronchoscopy have previously been described.(8) Bronchial biopsy tissue from a moderately severe asthma patient and a healthy control were viewed by electron microscopy (EM) for double membrane autophagosomes.

Here, we demonstrated by EM in a tissue sample from a moderately severe asthma subject evidence of autophagy in asthma pathogenesis. Using EM, double membrane autophagosomes were detected in fibroblasts and epithelial cells. A representative fibroblast from a bronchial biopsy tissue of a moderate asthma subject is depicted in Figures 1A-C, and epithelial cells in figures 2A-C. Corresponding fibroblast and epithelial cells from a healthy control are depicted in figures 1D-F and figures 2D-F, respectively, where fewer or no autophagosome was detected.

This is the first report to present genetic and histological evidence of autophagy in asthma pathogenesis. *ATG5* is involved in the elongation step of the autophagosome formation.

ATG5 forms a complex with ATG12 and ATG16L1 and the complexes are found on the outer membrane of the forming autophagosome.(2) We speculate that the positive association of allele G with asthma and the negative association with pre-FEV<sub>1</sub> in asthmatics may be due to the inverse relationship between pre-FEV<sub>1</sub> and asthma severity(9). If allele G is a risk factor for low pre-FEV<sub>1</sub>, given that pre-FEV<sub>1</sub> tends to be lower in those with more severe forms of asthma, the allele would also increase the risk of developing moderate-to-severe asthma. The genetic association of *ATG* with pre-FEV<sub>1</sub> in asthmatics and the detection of autophagosomes in fibroblasts and epithelial cells in tissues from a moderately severe asthma patient suggest an association between autophagy and reduced lung function in asthmatic subjects. At present the mechanistic pathway of autophagy in asthma is unclear, but it opens up a new avenue to explore the mechanism of the chronic nature of asthma pathogenesis.

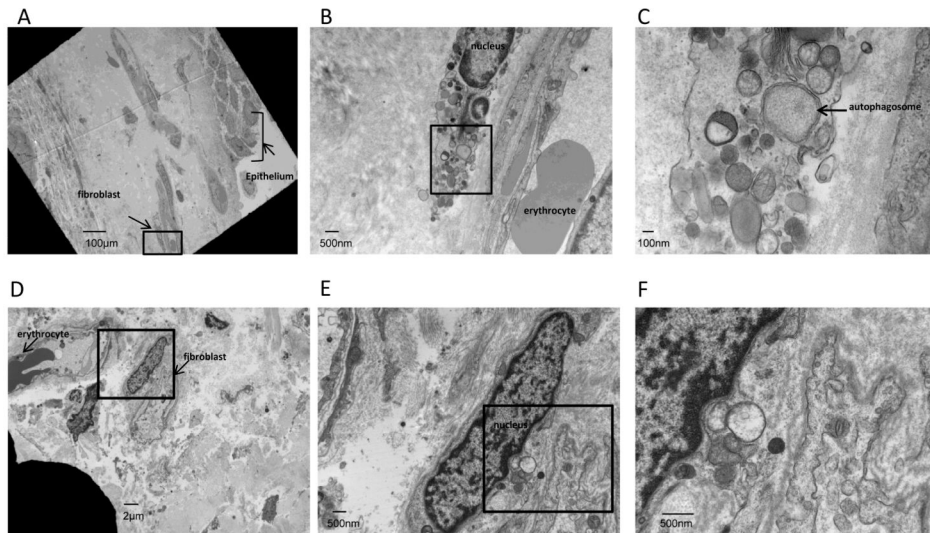
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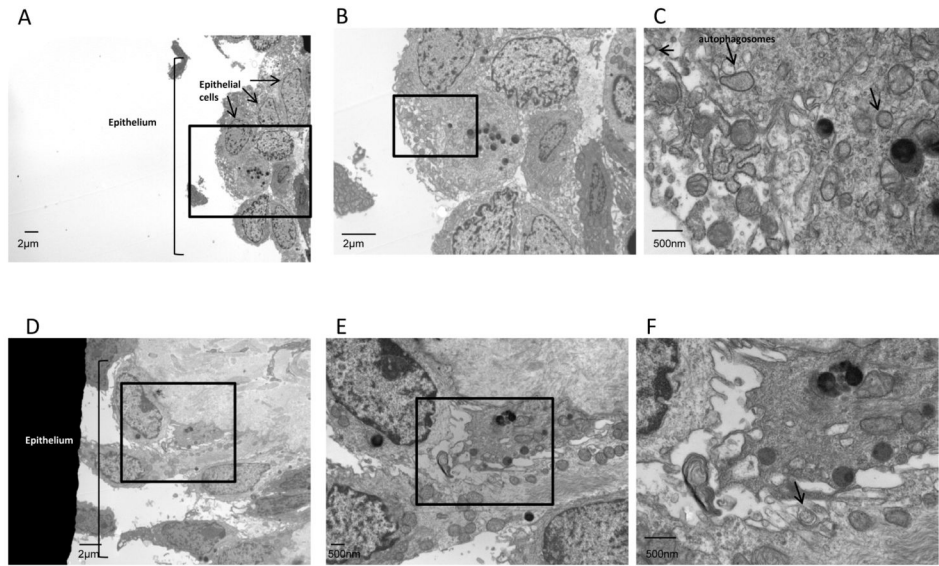
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**Fig 1.**

A double membrane autophagosome was detected in a fibroblast from a bronchial biopsy tissue sample of a moderate asthma subject. Tissue was viewed at magnifications of (A) 1480x, (B) 16100x and (C) 62200x. Boxed areas were viewed under higher magnification in subsequent figures. An arrow indicated an autophagosome. Corresponding fibroblast from a healthy subject was viewed at magnifications of (D) 4030x, (E) 16100x and (F) 37000x, respectively.



**Fig 2.** Autophagosomes were detected in bronchial epithelial cells from a moderate asthma subject. Tissue was viewed at magnifications of (A) 4030x, (B) 9760x and (C) 37000x, respectively. Corresponding epithelial cells of a healthy subject was viewed at magnifications of (D) 6390x, (E) 16100x and (F) 37000x, respectively. Arrows pointed at double membrane autophagosomes.

Table 1

## Association of SNPs and asthma in the SLSJ asthma study

39 SNPs were tested for association with asthma in the SLSJ asthma study. The gene symbols, rs number for each SNP, the major allele of each SNP, the number of informative families that contributed to the test statistic, and the unadjusted p-values are listed. Positive p-values represent over-transmission of the major allele to affected offspring, and negative p-values represent under-transmission. After Bonferroni correction, SNPs 12212740 of *ATG5* remained statistically significant (**bolded\***).

Gene	SNP	Allele	Allele frequency	# of informative families	p-value
<i>BECN1</i>	rs10512488	A	0.82	180	0.1019
	rs7204722	T	0.82	167	0.3834
	rs8051218	T	0.95	64	0.141
<i>MAP1LC3B</i>	rs2873702	A	0.99	19	-0.9351
	rs2241617	T	0.87	118	0.8613
	rs3748400	T	0.75	196	0.8694
<i>SQSTM1</i>	rs166624	G	0.86	131	-0.5718
	rs10516140	C	0.8	168	0.9025
	rs565280	G	0.59	224	0.9447
	rs155788	T	0.91	97	-0.1876
	rs10277	C	0.6	221	0.8631
	rs1065154	T	0.6	222	0.8886
	rs248244	C	0.95	60	-0.7241
	rs248247	G	0.62	232	-0.7656
	rs248248	C	0.78	198	0.4782
	rs2303677	C	0.78	180	-0.2451
	rs155793	C	0.91	91	-0.6668
	rs748197	G	0.55	232	0.3494
<i>ULK1</i>	rs11246867	G	0.95	68	0.0187
	rs9652059	C	0.85	148	0.0126
	rs10902472	C	0.95	64	0.0387
<i>ATG5</i>	rs7487166	G	0.87	132	0.036
	rs9398071	T	0.77	176	-0.0694
	rs6920944	C	0.91	77	0.9309

Gene	SNP	Allele	Allele frequency	# of informative families	p-value
	rs10484575	C	0.92	87	-0.8106
	rs9386514	T	0.8	164	-0.1043
	rs9486306	T	0.87	117	-0.2731
	rs1769972	T	0.95	61	0.739
	rs3804332	T	0.96	65	-0.4294
	rs3851210	C	0.94	82	0.2755
	rs3804333	C	0.78	174	-0.1332
	rs633724	C	0.66	222	0.0145
	rs9486314	C	0.82	143	-0.1427
	rs12529626	A	0.9	107	0.0627
	rs10485352	A	0.96	65	-0.4297
	rs12212740	G	0.88	130	0.0002
	rs1766208	C	0.88	132	-0.5169
	rs3761796	A	0.99	15	0.3656
	rs2355380	T	0.7	220	0.2373