

Letter to the Editor

Genetic and histologic evidence for autophagy in asthma pathogenesis

To the Editor:

Asthma affects all age groups and presents itself as a spectrum of severity and symptoms. Reactive oxygen 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 lysosome-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 patients with chronic obstructive pulmonary disease compared with healthy control subjects,³ evidence for autophagy in asthmatic patients, particularly those with 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 histologic approaches.

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 β [*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), by using the family-based association test 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 a *P* value of .05 to a *P* value of .001, according to the Bonferroni method. To confirm our positive findings, we tested the association in a second family-based population, the non-Hispanic white 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 provided informed consent.

In the SLSJ asthma study a total of 1338 subjects (483 nuclear families) with known asthma status were included in the analysis, and 336 subjects were either probands or affected siblings. Of this group, the male/female ratio was 0.83. The mean age was 16.45 years (SD, ± 9.43 years), and 77.1% were atopic. The mean FEV₁ percent predicted value was 94.1% (SD, 20.1%). A total of 39 SNPs were tested, and after Bonferroni correction, SNP rs12212740 G>A of *ATG5* remained statistically significant (Table I). Allele G, with an allele frequency of 0.88, is overtransmitted to asthmatic offspring (*P* = .0002; odds ratio, 1.35; 95% CI, 1.01-1.89). SNP rs12212740 was not associated with asthma in CAMP; however, it was associated with prebronchodilator FEV₁ (adjusted for age, sex, and height; *P* = .04). In the SLSJ cohort rs12212740 was associated with prebronchodilator FEV₁ (percent predicted; *P* = .007). In both populations allele G was negatively associated with adjusted prebronchodilator FEV₁. SNP rs12212740 is located in intron 3 of *ATG5*, which is located 7 kb downstream and 8 kb

TABLE I. Association of SNPs and asthma in the SLSJ asthma study

Gene	SNP	Allele	Allele frequency	No. of informative families	<i>P</i> value
<i>BECN1</i>	rs10512488	A	0.82	180	.1019
<i>MAP1LC3B</i>	rs7204722	T	0.82	167	.3834
	rs8051218	T	0.95	64	.141
	rs2873702	A	0.99	19	-.9351
	rs2241617	T	0.87	118	.8613
<i>SQSTM1</i>	rs3748400	T	0.75	196	.8694
	rs166624	G	0.86	131	-.5718
	rs10516140	C	0.8	168	.9025
	rs565280	G	0.59	224	.9447
	rs155788	T	0.91	97	-.1876
	rs10277	C	0.6	221	.8631
	rs1065154	T	0.6	222	.8886
	rs248244	C	0.95	60	-.7241
	rs248247	G	0.62	232	-.7656
	rs248248	C	0.78	198	.4782
<i>ULK1</i>	rs2303677	C	0.78	180	-.2451
	rs155793	C	0.91	91	-.6668
	rs748197	G	0.55	232	.3494
	rs11246867	G	0.95	68	.0187
	rs9652059	C	0.85	148	.0126
<i>ATG5</i>	rs10902472	C	0.95	64	.0387
	rs7487166	G	0.87	132	.036
	rs9398071	T	0.77	176	-.0694
	rs6920944	C	0.91	77	.9309
	rs10484575	C	0.92	87	-.8106
	rs9386514	T	0.8	164	-.1043
	rs9486306	T	0.87	117	-.2731
	rs1769972	T	0.95	61	.739
	rs3804332	T	0.96	65	-.4294
	rs3851210	C	0.94	82	.2755
	rs3804333	C	0.78	174	-.1332
	rs633724	C	0.66	222	.0145
	rs9486314	C	0.82	143	-.1427
	rs12529626	A	0.9	107	.0627
	rs10485352	A	0.96	65	-.4297
	rs12212740*	G	0.88	130	.0002
	rs1766208	C	0.88	132	-.5169
	rs3761796	A	0.99	15	.3656
	rs2355380	T	0.7	220	.2373

Thirty-nine SNPs were tested for association with asthma in the SLSJ asthma study. The gene symbols, rs numbers for each SNP, major alleles of each SNP, numbers of informative families that contributed to the test statistic, and unadjusted *P* values are listed. Positive *P* values represent overtransmission of the major allele to affected offspring, and negative *P* values represent undertransmission.

*After Bonferroni correction, SNP rs12212740 of *ATG5* remained statistically significant.

upstream of exons 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 prebronchodilator FEV₁ in both study populations suggests that autophagy is associated with reduced lung function in asthmatic patients.

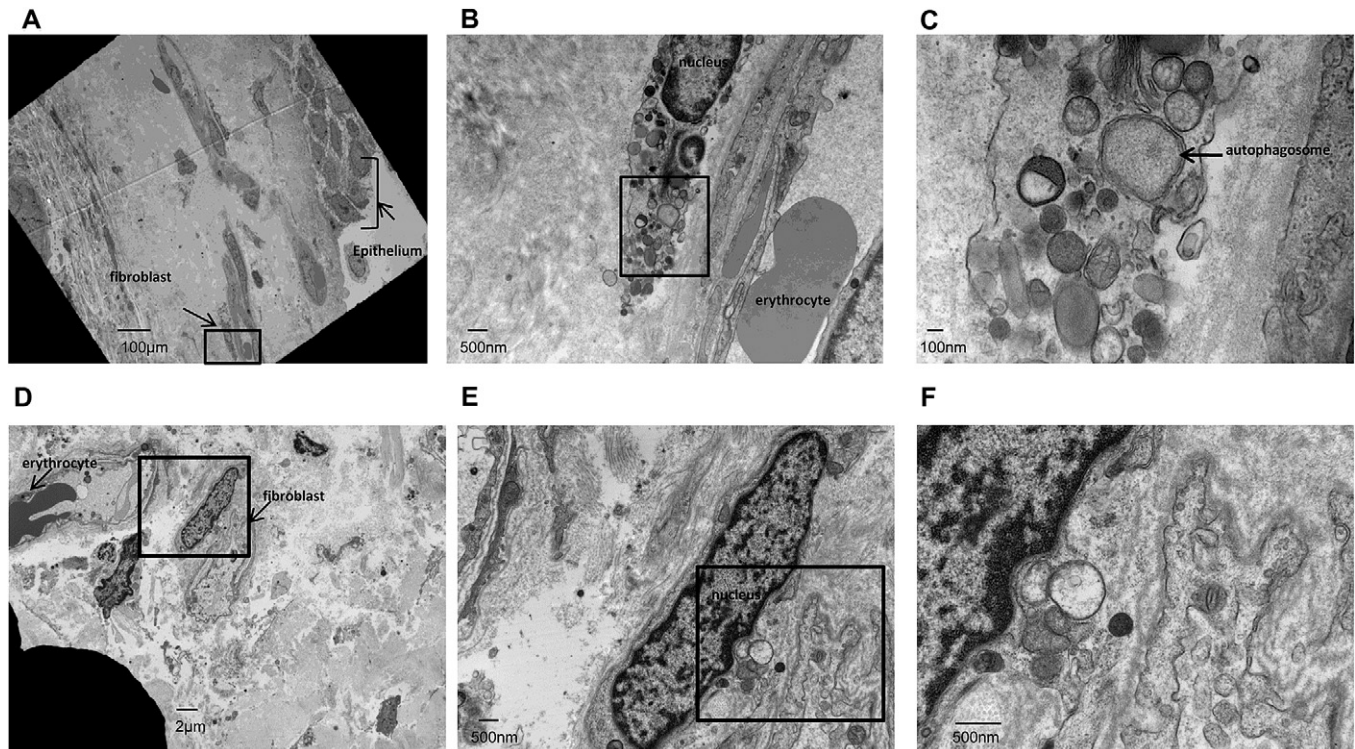


FIG 1. A double-membrane autophagosome was detected in a fibroblast from a bronchial biopsy tissue sample of a patient with moderate asthma. Tissue was viewed at magnifications of $\times 1,480$ (A), $\times 16,100$ (B), and $\times 62,200$ (C). Boxed areas were viewed at higher magnification in subsequent illustrations. An arrow indicates an autophagosome. A corresponding fibroblast from a healthy subject was viewed at magnifications of $\times 4,030$ (D), $\times 16,100$ (E), and $\times 37,000$ (F), respectively.

Bronchial biopsy specimens 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 to determine whether autophagy is present in the airways of asthmatic patients. Patient recruitment and bronchoscopy have previously been described.⁸ Bronchial biopsy tissue from a patient with moderately severe asthma and a healthy control subject were viewed by means of electron microscopy (EM) for double-membrane autophagosomes.

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

This is the first report to present genetic and histologic 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 prebronchodilator FEV₁ in asthmatic patients might be due to the inverse relationship between prebronchodilator FEV₁ and asthma severity.⁹ If allele G is a risk factor for low prebronchodilator FEV₁, given that

prebronchodilator FEV₁ tends to be less in those with more severe forms of asthma, the allele would also increase the risk of moderate-to-severe asthma. The genetic association of ATG5 with prebronchodilator FEV₁ in asthmatic patients and the detection of autophagosomes in fibroblasts and epithelial cells in tissues from a patient with moderately severe asthma suggest an association between autophagy and reduced lung function in asthmatic patients. 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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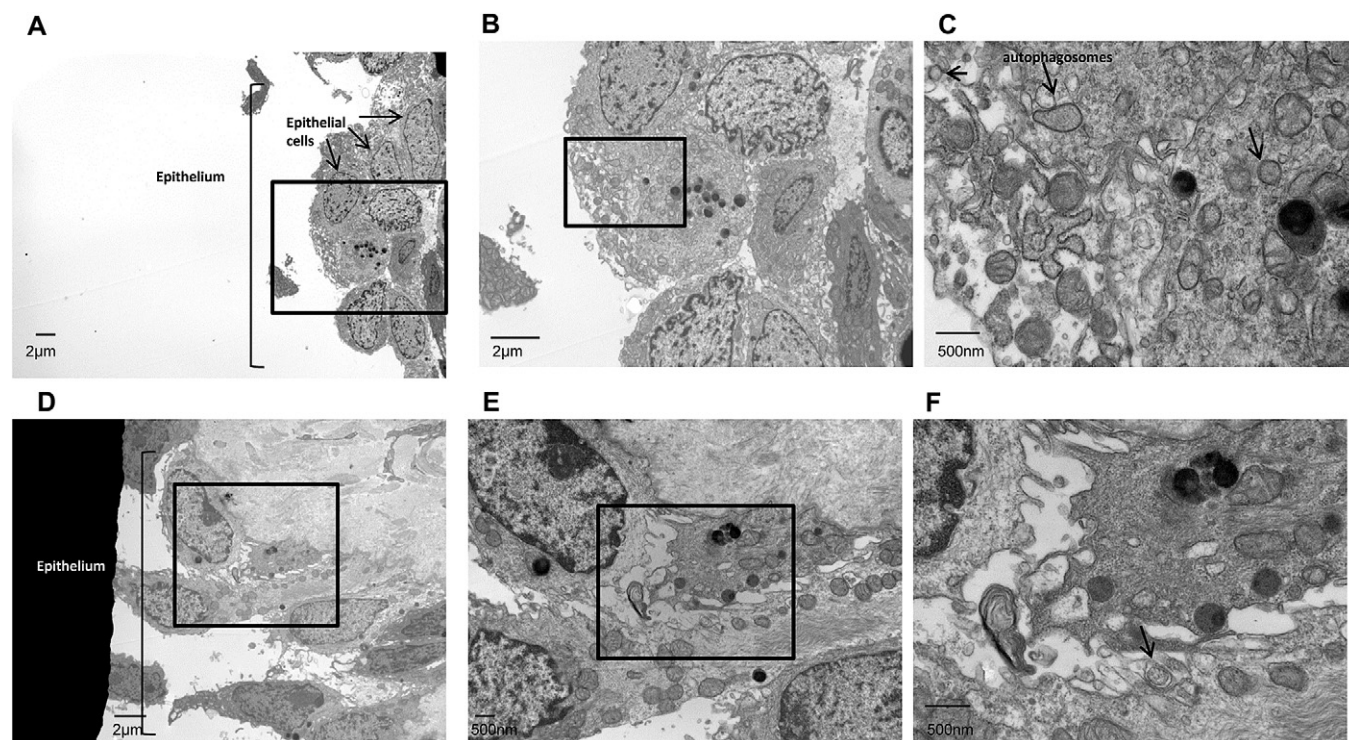


FIG 2. Autophagosomes were detected in bronchial epithelial cells from a patient with moderate asthma. Tissue was viewed at magnifications of $\times 4030$ (A), $\times 9760$ (B), and $\times 37,000$ (C), respectively. Corresponding epithelial cells of a healthy subject were viewed at magnifications of $\times 6,390$ (D), $\times 16,100$ (E), and $\times 37,000$ (F), respectively. Arrows indicate double-membrane autophagosomes.

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