Journal of the Formosan Medical Association (2015) 114, 750–755

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfma-online.com

ORIGINAL ARTICLE

Association of Egr-1 and autophagy-related gene polymorphism in men with chronic obstructive pulmonary disease

Chiung-Zuei Chen [a], Chih-Ying Ou [a], Ru-Hsueh Wang [b], Cheng-Hung Lee [a], Chien-Chung Lin [a], Han-Yu Chang [a], Tzuen-Ren Hsiue [a,*]

[a] *Division of Chest Medicine, Department of Internal Medicine, National Cheng Kung University Hospital, Medical College, National Cheng Kung University, Tainan, Taiwan*
[b] *Department of Family Medicine, National Cheng Kung University Hospital, Medical College, National Cheng Kung University, Tainan, Taiwan*

Received 28 April 2013; received in revised form 30 June 2013; accepted 31 July 2013

KEYWORDS
autophagy;
chronic obstructive pulmonary disease;
polymorphism

Background/Purpose: : Autophagy is important in cellular homeostasis and control of inflammatory immune response. Increased autophagy has recently been associated with increased cell death and chronic obstructive pulmonary disease (COPD) pathogenesis. Two autophagy regulator genes have been identified: Egr-1 (early growth response), associated with different phenotype expressions in asthma; and, Atg16L1 (autophagy related 16-like 1), a candidate gene responsible for susceptibility to chronic inflammatory diseases. We will explore the role of the Egr-1 and Atg16L1 gene polymorphisms in COPD.
Methods: The genotypes of 151 male smoking patients with COPD and 100 male smoking controls were evaluated by polymerase chain reaction followed by restriction fragment length polymorphism analysis of the Egr-1 (−4071 A → G) rs7729723 and Atg16L-1 (T300A) rs2241880 variants.
Results: The G allele of the Egr-1 gene polymorphism was associated with an increased risk of developing COPD [odds ratio (OR), 2.05; 95% confidence interval (CI), 1.15–3.72], and participants with the G allele polymorphism (GG and GA genotypes) had a 2.56-fold higher risk (OR, 2.56; 95% CI, 1.31–5.16) of having COPD than those homozygous for the A allele [35.8% (54/151) vs. 24.0% (24/100); $p = 0.007$]. Participants with the A allele of the Atg16L1 gene polymorphism (AA and AG genotypes) had a 3.34-fold higher risk (OR, 3.34; 95% CI, 1.32–8.97) of

* Corresponding author. Department of Internal Medicine, National Cheng Kung University Hospital, 138 Sheng-Li Road, Tainan 704, Taiwan.
E-mail address: hsiue@mail.ncku.edu.tw (T.-R. Hsiue).

http://dx.doi.org/10.1016/j.jfma.2013.07.015

having COPD than those homozygous for the G allele [93.4% (141/151) vs. 81.0% (81/100); $p = 0.013$].
Conclusion: The Egr-1 and Atg16L1 genes' polymorphisms were significant risk factors for susceptibility to COPD. These results demonstrate that autophagy regulator genetic mutations are associated with COPD in male smokers.

Introduction

Chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of death worldwide, and the World Health Organization estimates it will be the third leading cause of death by 2020.[1] Surprisingly, there are no effective drug therapies for COPD able to significantly alter the disease's progression, and little is known of the underlying molecular mechanisms responsible for its occurrence.[1] Further knowledge of the genetics of COPD may lead to new treatments or to an increase in our understanding of the disease's pathogenesis.[2] However, current knowledge on the genetics of COPD is limited.

Autophagy (self-eating) is a lysosomal degradation pathway that is essential for survival, differentiation, development, and homeostasis. Autophagy has previously been implicated in several human diseases including cancer, neurodegeneration, aging, and inflammatory bowel disease.[3] Recently, Chen et al[4,5] demonstrated for the first time that increased autophagy was associated with increased cell death and contributes to COPD pathogenesis by promoting epithelial cell death. They also demonstrated a critical role for the early growth response-1 (Egr-1) gene in promoting autophagy and aptosis in response to cigarette smoke exposure *in vitro* and *in vivo*. Egr-1 is a zinc finger transcription factor, classified as an immediate-early response protein in human pulmonary epithelial cells. Exposure to cigarette smoke extract (CSE) resulted in rapidly induced autophagy by increasing the binding of Egr-1 to the autophagy gene light chain-3B (LC3B) promoter, increasing autophagic protein expression. In addition, knockdown of Egr-1 inhibited the expression of autophagic protein in Egr-$1^{-/-}$ mice, which displayed basal airspace enlargement and resisted cigarette-smoke induced autophagy and emphysema.[4] An Egr-1 gene polymorphism (−4071 A → G) had been associated with the serum IgE level and atopy in Chinese asthmatic children,[6] but its association with COPD has not been explored. In addition, previous studies demonstrated that loss of the autophagic protein Atg16L1 enhanced endotoxin-induced inflammatory cytokines.[7] Genome-wide association studies have also identified Atg16L1 (T300A) as a candidate gene responsible for susceptibility to chronic inflammatory bowel disease.[8,9]

Our hypothesis is that genetic polymorphism in autophagy regulator genes such as Egr-1 and Atg16L1 may be linked to susceptibility to COPD. The aim of this study is to explore the role of Egr-1 (−4071 A → G) and the Atg16L1 (T300A) gene in COPD. The genotypes of Egr-1 and Atg16L1 for patients with COPD and control individuals will be evaluated by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis. If we prove our hypothesis that autophagy regulator genetic variation is associated with airflow obstruction in smokers, we may be able to support the evidence of the role of autophagy in the pathogenesis of COPD. This may present an opportunity for the development of novel target therapies for autophagy in COPD patients.

Patients and methods

Study population

This was a hospital-based, case-control study consisting of 251 participants. The case group included 151 men aged ≥40 years with smoking-related COPD (smoking history ≥10 pack−years) recruited from National Cheng-Kung University Hospital. They were diagnosed as having COPD based on their medical histories, chest radiographic findings, and spirometric results, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2008 guidelines.[1] Inclusion criteria for COPD included the following: chronic airway symptoms and signs such as coughing, dyspnea, and wheezing, and chronic airway obstruction defined as a ratio of the postbronchodilator forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) of <70%. Patients were excluded if they had a history of asthma. The severity classification according to the GOLD stages[1] for these 151 patients were mild (37/151; 24.5%), moderate (65/151; 43.1%), severe (34/151; 22.5%), and very severe (15/151; 9.9%).

The control group included 100 asymptomatic men ≥40 years of age (smoking history ≥10 pack−years) without clinical symptoms of COPD and with normal pulmonary function (FEV_1/FVC ≥70% and FEV_1 ≥80% predicted). Most of the control participants visited our hospital for health checkups. We enrolled only men in this study because there are very few women who smoke or have COPD in our population.

The study was approved by the Ethics Committee of the National Cheng-Kung University Medical College and Hospital, and informed consents were obtained.

DNA extraction and polymerase chain reaction restriction fragment length polymorphism

Genomic DNA was isolated using Puregene commercial DNA isolation kits according to standard procedures (Gentra Systems, Inc., Minneapolis, MN, USA). The total volume of the PCR amplification mixture was 50 μL and contained 100 ng of genomic DNA, 0.5 mM of a dNTP Mix, 0.5 U *Taq* DNA polymerase, 0.2 mM of each nucleotide, and 1.5 mM

$MgCl_2$. The amplification protocol was done using the Perkin Elmer DNA Thermal Cycler 9600 (Perkin Elmer, Waltham, MA, USA).

Two polymorphisms in the autophagy regulator genes: an A to G polymorphism in Egr-1 (−4071 A → G; rs7729723) and the polymorphism Atg16L1 (T300A) (rs2241880), an A to G polymorphism resulting in the amino acid exchange at position 300 of the protein (Thr300Ala), were genotyped using PCR and restriction fragment length polymorphism analysis according to the method of Chan et al[6] and Csöngei et al.[10] For the A to G polymorphism in Egr-1 (−4071 A → G), the primer sequences were 5′-GTG ATT CTC ATT GGC CTG GT-3′ (sense) and 5′-TAC TAT TCC CCA GCC AGC AG-3′ (antisense). For the Atg16L1 T300A, the primer sequences were 5′-CTC TGT CAC CAT ATC AAG CGT GG-3′ (sense) and 5′-TCT AGA AGG ACA GGC TAT CA CAG ATG-3′ (antisense). The fragments were separated by electrophoresis through 3% agarose gel, stained with ethidium bromide and transilluminated with ultraviolet light.

Statistical analysis

Descriptive statistics values are given as mean ± standard error and relative frequencies or absolute numbers. Deviations from Hardy−Weinberg equilibrium analysis were performed using χ^2 tests with one degree of freedom. Associations between specific genotypes and phenotypes were analyzed for significance using logistic regression model. Interaction was estimated by calculating odds ratios (ORs) adjusted for age and cumulative cigarette consumption in pack−years between the COPD and control groups. Multivariate logistic regression analysis was used to assess the contributions of the genetic factors to the risk of developing COPD and progressive airflow limitations in all smokers. Because only three (2%) patients and three (3%) controls with the GG genotypes of Egr-1(−4071 A → G) were noted, GG and GA genotypes were combined into a single category for the statistical comparisons. We used the JMP software program for multivariate analysis (SAS Institute, Inc. Cary, NC, USA). Power analysis was calculated using Quanto version 1.2.4 (hydra.usc.edu/GxE/; 2009). The genetic effects of the Egr-1 (−4071 A → G) polymorphisms were assumed to be G allele dominant and the effects of the Atg16L1 (T300A) polymorphisms were assumed to be A allele dominant. The population risk was assumed to be 10%. Adjustment for multiple corrections was made using the Bonferroni approach. In each analysis, a difference with $p < 0.05$ was accepted as significant.

Results

The characteristics of the control and COPD subjects are summarized in Table 1. The mean age and smoking history were lower in the control group than the COPD group; we adjusted for this incomplete matching in the analysis of the data. The Hardy−Weinberg equilibrium was used to test the genotypes for Egr-1 (−4071 A → G) and Atg16L1 (T300A) in both the COPD and control groups, and no obvious deviations were found ($\chi^2 < 3.84$); the test results that presented as χ^2 were 1.60 and 0.98 for Egr-1 genotypes in COPD and control groups, 1.01 and 3.75 for ATG16L1 genotypes in COPD and control groups, respectively.

Table 1 Patient characteristics of the COPD and control group smokers.

	COPD (n = 151)	Controls (n = 100)
Age (y)	69.0 ± 0.8	57.3 ± 1.0
Smoking/pack−years	49.1 ± 2.3	34.7 ± 2.8
Current smokers[a]	63/144 (43.8)	65/97 (67.0)
FEV_1 % predicted	55.1 ± 1.5	98.6 ± 1.9
FEV_1/FVC ratio	50.6 ± 0.8	78.0 ± 0.9

Data are presented as mean ± SD or n (%).
*All p values are <0.001.
COPD = chronic obstructive pulmonary disease.
[a] Smoking status for seven patients and three controls was unknown.

Participants with the G allele (GG and GA genotypes) of the Egr-1(−4071 A → G) polymorphism had an increased risk of developing COPD than those homozygous for the A allele (OR, 2.56; 95% CI, 1.31−5.16). The frequency of the G allele as a combined GG and GA genotype was significantly higher in the COPD group than the control group [35.8% (54/151) vs. 24.0% (24/100); $p = 0.007$; Table 2]. The power to detect genetic risks of 2.56 (OR) was 91.9% if the inheritance mode was G allele dominant. No significant difference in the Egr-1 genotypes between the COPD and control group was noted if the inheritance mode was G allele recessive.

Subjects with the G allele (GG and GA genotypes) of the Egr-1(−4071 A → G) polymorphism also had lower lung function data (FEV_1 % of prediction and ratio of FEV_1/FVC) than those homozygous for the A allele, but the difference was not significant (69.4 ± 3.0 % vs. 73.7 ± 2.0 %, $p = 0.24$ and 59.3 ± 1.7 vs. 62.5 ± 1.1; $p = 0.11$, respectively).

Participants with the AA genotypes and AG genotypes of Atg16L1 (T300A), both had an increased risk of developing COPD than those homozygous for the G allele (OR, 3.64; 95% CI, 1.35−10.50; $p = 0.013$ and OR, 3.23; 95% CI, 1.18−9.48; $p = 0.026$ respectively, but the difference for AG vs. GG

Table 2 Association of the Egr-1 polymorphism (rs 7729723) with COPD.

Allele frequency and polymorphism	COPD (n = 151)	Controls (n = 100)	OR (95% CI)	p
Egr-1−4071				
A	81.1	86.5	1	0.017
G	18.9	13.5	2.05 (1.15−3.72)	
AA	97 (64.2)	76 (76.0)	1	0.007
AG and GG[a]	54 (35.8)	24 (24)	2.56 (1.31−5.16)	

Data are presented as %, n (%), or n (range).
CI = confidence interval; COPD = chronic obstructive pulmonary disease; OR = odds ratio.
[a] Only three (2%) patients and three (3%) controls with the GG genotypes, AG and GG genotypes were combined into a single category for the statistical comparisons.

genotypes was not significant after the Bonferroni correction for multiple tests; Table 3). The A allele (combined AA and AG genotypes) of the Atg16L1 (T300A) polymorphism had an increased risk of developing COPD than those homozygous for the G allele [93.4% (141/151) vs. 81.0% (81/100); OR, 3.34; 95% CI, 1.32–8.97; $p = 0.013$]. The power to detect genetic risks of 3.34 (OR) was 77.5% if the inheritance mode was A allele dominant. No significant difference in the Atg16L1 genotypes between the COPD and control group was noted if the inheritance mode was A allele recessive. Accordingly, individuals with the A allele (combined AA and AG genotypes) of the Atg16L1 (T300A) polymorphism had lower lung function than those homozygous for the G allele (FEV_1 % of prediction: 60.8 ± 1.0 % vs. 67.4 ± 2.7 %, $p = 0.023$ and ratio of FEV_1/FVC: 70.9 ± 1.8 % vs. 84.3 ± 4.9 %, $p = 0.011$). No significant difference in the lung function data between the Atg16L1 G allele (combined GG and GA genotypes) and homozygous for the A allele was noted if the inheritance mode was A allele recessive.

There was no significant difference between the severity of airway obstruction (FEV_1 % of prediction and ratio of FEV_1/FVC) and genotypes (Egr-1 of AA vs. AG + GG and Atg16L1 of AA + AG vs. GG) in the COPD patients.

In the genetic models for the allele of Egr-1 (−4071 A → G) polymorphism; the frequency of the G allele for the Egr-1 polymorphism was higher in the COPD group than the control group (57/302, 18.9% vs. 27/200, 13.5%; OR, 2.05; 95% CI, 1.15–3.72; $p = 0.017$). In the genetic models for the allele of the Atg16L1 (T300A) polymorphism, the frequency of the A allele for the Atg16L1 polymorphism was higher in the COPD group than the control group (215/302, 71.2% vs. 125/200, 62.5%; OR, 1.58; 95% CI, 1.02–2.47; $p = 0.047$), but the difference was not significant after the Bonferroni correction for multiple tests.

The prevalence of the minor allele (G allele) of Egr-1 (rs 7729723) polymorphism in the single nucleotide polymorphism database (dbSNP) has been reported to range from 15.9% to 25.6% in the Han Chinese populations (Asian). The frequency of the G allele in the present study was 13.5% (27/200) within the control group, close to that reported in the dbSNP. The prevalence of the minor allele (G allele) of Atg16L1 (rs 2241880) polymorphism in the dbSNP has been reported to range from 23.8% to 39.0% in the Han Chinese populations. The frequency of the G allele in the present study was 38.0% (76/200) within the control group, consistent with that reported in the dbSNP.

Table 3 Association of the Atg16L1 polymorphism (rs 2241880) with COPD.

Allele frequency and polymorphism	COPD ($n = 151$)	Controls ($n = 100$)	OR (95% CI)	p
Atg16L1				
A	71.2	62.5	1.58 (1.02–2.47)	0.047
G	28.8	37.5	1	
AA	74 (49.0)	43 (43.0)	3.64 (1.35–10.50)	0.013
AG	67 (44.4)	38 (38.0)	3.23 (1.18–9.48)	0.026
GG	10 (6.6)	19 (19.0)	1	

Data are presented as %, *n* (%), or *n* (range).
CI = confidence interval; COPD = chronic obstructive pulmonary disease; OR = odds ratio.

Discussion

In the present study, a significant positive association between autophagy regulator gene mutation Egr-1 (−4071 A → G) and COPD was noted. Egr-1 has the capacity to regulate genes relevant to the pathogenesis of COPD, such as those related to matrix metalloproteinase expression and remodeling, including the active airway obstruction and fibrosis characteristic of the earlier course of emphysema.[11,12] Egr-1 has been characterized as an immediate early response gene that is induced rapidly following various cellular stresses including oxidative stress, an important causative factor in COPD,[13–15] and was sustained induced in late-stage emphysema patients.[11] Comprehensive gene expression analyses have identified Egr-1 as a gene whose expression changed significantly in COPD.[12] Currently, there is no evidence that the Egr-1 genetic polymorphism, which causes variability in the regulation of autophagy, may determine individual susceptibility to COPD in individuals carrying such polymorphisms. However, it is possible considering the results of our study.

A significant association between autophagy-related gene mutation Atg16L1 (T300A) and COPD was noted. The Atg16L1 (T300A) polymorphism, which leads to the amino acid exchange at position 300 of the protein (Thr300Ala) is suggested to be functionally effective and is associated with Crohn's disease.[8–10] Crohn's disease is a chronic inflammatory bowel disease. Environmental risk factors such as cigarette smoke and genetic factors such as α1-antitrypsin deficiency affect the pathogenesis of both COPD and Crohn's disease.[16] These studies suggest that COPD and Crohn's disease may have common inflammatory pathways, including genetic variants in regulating autophagy. This hypothesis was consistent with our findings. The only difference was that in patients with Crohn's disease, the G allele was the risk allele of susceptibility for diseases,[8–10] whereas the A allele was the risk allele of susceptibility for COPD in the present study. Individuals with the A allele carriers (AA and AG genotypes) of Atg16L1 (T300A) polymorphism have an increased risk of developing COPD than those homozygous for the G allele (OR 3.34, 95% CI: 1.32–8.97). The described result may be due to the fact that autophagy can promote both cell survival and cell death, depending on the different specific stimuli, environmental conditions, and cell type.[17] For example, psoriasis is a chronic inflammatory disease of the skin, inflammatory cytokines such as C-reactive protein, tumor necrosis factor-α, interleukin-6, and interleukin-8 have been found to be etiologically involved in both psoriasis and COPD,[18] an effect similar to the disease susceptibility in the present study was noted in a previous study for psoriasis vulgaris. The frequency of homozygous for the A allele for Atg16L1 (T300A) was higher in the psoriasis group as compared to the controls (36.5% vs. 27.8%),[19] but the frequency of homozygous for the A allele was significantly

decreased in Crohn's disease as compared to the controls (12.5% vs. 27.3%).[20]

The genetic polymorphisms for autophagy in the present study were associated with the development of COPD, but not with the spirometric severity of COPD. Inconsistent results for the genetic predisposition of smokers to acquired COPD and genetics bases of COPD severity with rapid decline of FEV1 was also found in the matrix metalloproteinase; matrix metalloproteinase 9 polymorphisms are associated with the development of smoking-induced pulmonary emphysema, but not associated with a rapid decline of lung function. This result may be due to COPD susceptibility and progressive airflow limitations are different clinical phenotypes associated with different genes.[2] Another possible reason was that spirometry is not a suitable severity assessment tool in the present study. Autophagy play a role in cigarette smoke induced emphysema.[8] COPD patients with emphysema were documented by high-resolution computed tomography scan (HRCT) in clinical practice; severity assessment by HRCT scan would be a more informative evaluation rather than conventional spirometry. Further studies for COPD patients using HRCT to assess the association between autophagy gene polymorphism and COPD severity would provide stronger evidence of the role of autophagy genes in disease progression.

One limitation of the present study was that only men were enrolled. The majority of patients with smoking-related COPD in our population are men (smoking prevalence for women is $<5\%$).[21] In contrast to industrialized countries in the West, COPD morbidity remains male predominant in Asian countries. [22]The second limitation was that this study was not replicated in an independent cohort. The third limitation was that a relatively small number of participants were recruited and the findings were limited to the Chinese population. A large group of study participants including various demographics should be considered for future studies to clearly demonstrate a significant contribution of these polymorphisms.

In conclusion, in our population, there were two genes involved in autophagy. The Egr-1 and the Atg16L1 genes' polymorphisms were significant risk factors for susceptibility to smoking-related COPD. Autophagy can be pharmacologically induced by inhibiting negative regulators such as the target of rapamycin with rapamycin or inhibited by targeting the class III P13K involved in autophagosome formation.[23] If further studies can confirm that loss of function autophagy genetic mutations play a major role in airflow obstruction in patients with COPD, possible therapies can be developed to treat these patients. Further, scientists can attempt some mechanism-based studies to verify the functional significance of these polymorphisms in context with various diseases in the future.

Acknowledgments

We are grateful to Jia-Ling Wu for providing statistical consulting services from the Biostatistics Consulting Center at National Cheng Kung University Hospital. This study was supported by grants NSC 95-2314-B-006-026 and NSC 101-2314-B-006-073 from the National Science Council.

References

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). *Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease, updated*. Bethesda, MD: National Heart, Lung and Blood Institute/World Health Organization; 2008 [accessed 17.05.08]. http://www.goldcopd.org.
2. Molfino NA. Genetics of COPD. *Chest* 2004;**125**:1929—40.
3. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008;**132**:27—42.
4. Chen ZH, Kim HP, Sciurba FC, Lee SJ, Feghali-Bostwick C, Stolz DB, et al. Egr-1 regulates autophagy in cigarette smoke-induced chronic obstructive pulmonary disease. *PLoS One* 2008;**3**:e3316.
5. Ryter SW, Chen ZH, Kim HP, Choi AM. Autophagy in chronic obstructive pulmonary disease: homeostatic or pathogenic mechanism? *Autophagy* 2009;**5**:235—7.
6. Chan IHS, Tang NLS, Leung TF, Huang W, Lam YYO, Wong GWK, et al. Association of early growth response-1 gene polymorphisms with total IgE and atopy in asthmatic children. *Pediatr Allergy Immunol* 2009;**20**:142—50.
7. Saitoh T, Fujita N, Jang MMH, Uematsu S, Yang BG, Satoh T, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production. *Nature* 2008;**456**:264—8.
8. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007;**39**:596—604.
9. Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007;**39**:207—11.
10. Csöngei V, Járomi L, Sáfrany E, Sipeki C, Magyari L, Faragó B, et al. Interaction of the major inflammatory bowel disease susceptibility allele in Crohn's disease patients. *World J Gastroenterol* 2010;**16**:176—83.
11. Zhang W, Yan SD, Zhu A, Zou YS, Williams M, Godman GC, et al. Expression of Egr-1 in late stage emphysema. *Am J Pathol* 2000;**157**:1311—20.
12. Ning W, Li CJ, Kaminiski N, Feghali-Bostwick CA, Alber SM, Di YP, et al. Comprehensive gene expression profiles reveal pathways related to the pathogenesis of chronic obstructive pulmonary disease. *Proc Natl Acad Sci U S A* 2004;**101**:14895—900.
13. Kiffin R, Bandyopadhyay U, Cuervo AM. Oxidative stress and autophagy. *Antioxid Redox Signal* 2006;**8**:152—62.
14. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. *EMBO J* 2007;**26**:1749—60.
15. Chen Y, McMillan-Ward E, Kong J, Israels SJ, Gibson SB. Mitochondrial electron-transport-chain inhibitors of complexes I and II induce autophagic cell death mediated by reactive oxygen species. *J Cell Sci* 2007;**120**:4155—66.
16. Yang P, Tremanine WJ, Meyer RL, Prakash UB. Alpha 1-antitrypsin deficiency and inflammatory bowel diseases. *Mayo Clin Proc* 2000;**75**:450—5.
17. Levine B. Cell biology: autophagy and cancer. *Nature* 2007;**446**:745—7.
18. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med* 2009;**361**:496—509.
19. Douroudis K, Kingo K, Traks T, Reimann E, Raud K, Rätsep R, et al. Polymorphisms in the ATG16L1 gene are associated with psoriasis vulgaris. *Acta Derm Venereol* 2012;**92**:85—7.
20. Lacher M, Schroepf S, Ballauff A, Lohse P, von Schweinitz D, Kappler R, et al. Autophagy16-like 1 rs2241880 G allele is

associated with Crohn's disease in German children. *Acta Paediatr* 2009;**98**:1835—40.
21. Regional COPD Working Group. COPD prevalence in 12 Asia-Pacific countries and regions: projections based on the COPD prevalence estimation model. *Respirology* 2003;**8**:192—8.
22. Tan WC, Ng TP. COPD in Asia: where East meets West. *Chest* 2008;**133**:517—27.
23. Rubinsztein DC, Gestwicki JE, Murphy LO, Klionsky DJ. Potential therapeutic applications of autophagy. *Nat Rev Drug Discov* 2007;**6**:304—12.