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ORIGINAL ARTICLE

Genetic variants of pulmonary SP-D predict disease outcome of COPD in a Chinese population

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ABSTRACT

Background and objective: **Although surfactant protein-D (SP-D) has been suggested as a biomarker for chronic obstructive pulmonary disease (COPD), the relationship between genetic variants of SP-D and disease outcome of COPD remains unknown. We hypothesized that genetic polymorphisms of SP-D are associated with COPD-related phenotypes and disease prognosis.**

Methods: **A hospital-based, case-controlled study was conducted prospectively. Six single nucleotide polymorphisms of the *SFTPD* gene were determined for genetic association analysis. Inflammatory cytokines and SP-D serum level were quantified. Frequency of exacerbation and change of lung function were assessed. All-cause 3-year mortality was registered.**

Results: **We studied 320 smokers (192 with COPD and 128 at-risk for COPD) who were prospectively monitored for at least 3 years. The serum levels of SP-D in COPD patients were significantly associated with the degree of airflow obstruction and frequency of exacerbation. Haplotype association analysis revealed that haplotype G-G-C-C-A was associated with lower risk of COPD ($P = 0.03$) in our study population. COPD patients with haplotype G-G-C-C-A had lower serum SP-D levels ($P < 0.001$), higher rates of positive response to bronchodilator treatment ($P = 0.01$), more improvement of forced expiratory volume in 1 s in yearly follow-up ($P = 0.03$) and better 3-year survival rate than COPD patients with non G-G-C-C-A haplotype ($P = 0.03$).**

Conclusions: **Genetic haplotype of SP-D may serve as a valuable prognostic indicator in Chinese patients with COPD.**

Key words: chronic obstructive pulmonary disease, haplotype, polymorphism, surfactant protein D.

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SUMMARY AT A GLANCE

We demonstrated for the first time in a Chinese population cohort that genetic polymorphisms of SP-D are not only associated with risk of COPD development, but also related to disease manifestation and that they predict outcomes.

Abbreviations: ATS, American Thoracic Society; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ELISA, enzyme-linked immunosorbent assay; FDR, false discovery rate; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; HR, hazard ratio; IL, interleukin; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphisms; SP-D, surfactant protein-D; TNF-α, tumour necrosis factor-α.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), encompassing chronic bronchitis with or without emphysema, is currently the seventh leading cause of death in Taiwan[1] and the third leading cause of death in the United States.[2] Worldwide COPD morbidity and mortality are expected to increase dramatically in the next 10 years.[2] Although cigarette smoking is the primary risk factor for COPD, not all smokers are equally likely to develop COPD in their lifetimes,[3] suggesting that genetics also plays an important role in the development of COPD.[4] Identifying genetic determinants and investigating their functions may lead to beneficial progress in understanding COPD pathobiology, diagnosis and treatment.[5]

Surfactant protein-D (SP-D), which lines the alveolar epithelium, is synthesized in type II pneumocytes and club cells as a large multimeric, calcium binding hydrophilic protein.[6] SP-D is thought to play a significant role in the pathogenesis of COPD by reducing oxidant production,[7] inflammatory responses in alveolar macrophages[8] and increasing apoptotic cell clearance.[9] In animal studies, increased oxidant production and reactive oxygen species are noted in the

lungs of SP-D$^{-/-}$ mice.[10] Furthermore, mice that lack SP-D develop chronic inflammation and emphysema, which can be prevented by administration of truncated recombinant human SP-D.[11] In fact, a higher level of serum SP-D is suggested as a biomarker associated with COPD risk.[12,13] Foreman and colleagues[14] demonstrated that certain genetic variants of SP-D are associated with changes in serum concentrations of SP-D and lung function, suggesting that SP-D is involved in the pathogenesis of COPD. Although genetic association studies highly suggest that the *SFTPD* gene contributes to the development of COPD,[14–16] little comprehensive work has been done regarding the relationship between polymorphisms of the *SFTPD* gene and COPD-related phenotypes and disease outcomes. We hypothesize that genetic variants of SP-D causing functional changes of SP-D protein are associated with the manifestation and disease outcome of COPD.

METHODS

Study design and populations

A prospective, case-controlled, hospital-based study was conducted from January 2003 to January 2010. All subjects older than 40 years old who visited the outpatient department of the pulmonary clinic in National Cheng Kung University Hospital were screened, and pulmonary function test was arranged for every participant. The experimental group was defined as patients diagnosed with COPD, on the basis of their medical history, airway symptoms and signs, chest radiographical findings, and spirometric results, according to the diagnostic guidelines and criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD).[17] Chronic airway obstruction was defined as the ratio of post-bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) less than 0.7 of predicted from pulmonary function testing, according to the current standard protocols of the American Thoracic Society (ATS).[18] The control group consisted of healthy smokers with normal spirometry. All participants were prospectively monitored for at least 3 years, and all patients were in clinically stable condition and received appropriate COPD therapy recommended by GOLD guidelines.[17] The clinical stability was defined as neither exacerbated airway symptoms in need of antibiotics or corticosteroids, nor change of maintained inhalational drugs for at least 4 weeks prior to enrolment. The research protocol was approved by the ethics committee of National Cheng Kung University Hospital (BR-100-100), and an informed consent was obtained from each patient.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the QIAmp DNA Blood Midi Kit (Qiagen, Crawley, UK). We included two functional single nucleotide polymorphisms (SNP) (rs2243639, Thr160Ala or 538A > G and rs721917, Met11Thr or 92T > C) with known functional effect on *SFTPD* gene expression[19] and then explored the tag SNP on the HapMap website (http://hapmap.ncbi.nlm.nih.gov). A total of four tag SNP for Han Chinese in Beijing (CHB) were selected to encompass the entire *SFTPD* gene using the Tagger-pairwise Tagging algorithm (filter: minor allele frequency (MAF) ≥ 0.10 and $r^2 \geq 0.8$). The SNP was further genotyped using TaqMan chemistry as designed by Applied Biosystems (Foster City, CA, USA). Haplotype block was then determined using the Haploview software, version 4.2 (Daly Lab at the Broad Institute, Cambridge, MA, USA) and on the basis of the same genotyping data with an MAF ≥ 0.10.

Serum SP-D concentration and measurement of biomarkers

Serum SP-D concentrations were determined with an enzyme-linked immunosorbent assay (ELISA) kit (Biovendor, Inc., Heidelberg, Germany). Additionally, tumour necrosis factor-α, interleukin (IL)-6 and IL-8 were also measured in plasma of subjects on enrolment using ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA).

Parameters for COPD-related phenotypes

Post-bronchodilator FEV_1 was measured according to the ATS guidelines,[18] using equipment for lung function testing (Chestec 65, Chest Company, Tokyo, Japan). The severity of airflow obstruction was classified using FEV_1 according to the GOLD criteria.[17] The classification criteria were as follows: GOLD1 = FEV_1/FVC < 0.7 and $FEV_1 \geq 80\%$ predicted; GOLD2 = FEV_1/FVC < 0.7 and $50\% \leq FEV_1 < 80\%$ predicted; GOLD3 = FEV_1/FVC < 0.7 and $30\% \leq FEV_1 < 50\%$ predicted; GOLD4 = FEV_1/FVC < 0.7 and $FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted, plus chronic respiratory failure. Post-bronchodilator spirometry was measured in the pulmonary function laboratory 20 min after the administration of inhaled fenoterol 400 μg. Additionally, patients were asked to eliminate short-acting bronchodilators for 8 h and long-acting bronchodilators for 12–24 h before testing. The frequency of a positive post-bronchodilator response, defined as the FEV_1/FVC remains <0.7 with FEV_1 > 12% and >200 mL, was calculated. Pulmonary function test was measured annually for every patient. The changes of FEV_1 and frequency of medical visits were defined as the average of each in 1 year. Both hospitalization and emergency room visits for pulmonary symptoms and signs were regarded as medical visits due to exacerbated disease. We also categorized exacerbation risk as high, which was ≥2 medical visits per year in contrast to low, defined as <2 medical visits per year.

Outcome evaluation

Survival status was obtained by research assistants who were blinded to patients' baseline disease severity. After at least 3 years of follow-up, all patients' medical charts were reviewed, or patients were contacted by telephone to check for any mortality. Exact dates of death if noted were obtained. All-cause mortality was registered.

Table 1 Demographic characteristics of patients with COPD and healthy smoking controls (a), and multivariate regression analysis of the seven significant parameters in the univariate analyses (b)

(a)

Univariate analysis	COPD (n = 192)	Control (n = 128)	P
Demographic parameters			
Age (years)	68.6 ± 11.4	58.3 ± 12.8	<0.001
Male (%)	100	100	—
Body mass index (kg/m^2)	23.2 ± 3.8	25.3 ± 3.9	<0.001
Smoking history (pack-years)	44.8 ± 32.7	37.3 ± 25.6	0.02
Current/ex-smoker (%)	23/77	20/80	0.56
Spirometry			
FEV_1/FVC (%)	53.3 ± 11.7	80.2 ± 6.3	<0.001
FEV_1 % of predicted	57.9 ± 21.8	89.3 ± 5.2	<0.001
FVC % of predicted	84.2 ± 18.5	97.5 ± 7.4	0.54
Bronchodilator response (%)	20	1	<0.001
Medical history			
Family history of COPD (%)	34	30	0.41
History of asthma (%)	0	0	—
Inflammatory cytokines, SP-D			
TNF-α (pg/mL)	10.8 ± 9.0	9.4 ± 7.3	0.30
IL-8 (pg/mL)	4.4 ± 6.0	2.2 ± 2.5	0.002
IL-6 (pg/mL)	8.7 ± 26.5	4.1 ± 3.1	0.24
SP-D (ng/mL)	63.7 ± 43.8	69.3 ± 52.2	0.53

(b)

Multivariate analysis	COPD (n = 192)	Control (n = 128)	P
Demographic parameters			
Age (years)	68.6 ± 11.4	58.3 ± 12.8	<0.001
Body mass index	23.2 ± 3.8	25.3 ± 3.9	0.07
Smoking history (pack-years)	44.8 ± 32.7	37.3 ± 25.6	0.04
Spirometry			
FEV_1/FVC (%)	53.3 ± 11.7	80.2 ± 6.3	<0.001
FEV_1 % of predicted	57.9 ± 21.8	89.3 ± 5.2	<0.001
Bronchodilator response (%)	20	1	<0.001
Inflammatory cytokines, SP-D			
IL-8 (pg/mL)	4.4 ± 6.0	2.2 ± 2.5	0.20

—, inconclusive results; COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; IL, interleukin; SP-D, surfactant protein D; TNF-α, tumour necrosis factor-α.

Statistical analysis

Data for continuous variables are presented as means ± standard deviations or number (%). For comparison of clinical information, the baseline differences between two independent groups (patients and controls, or patients with G-G-C-C-A/non G-G-C-C-A haplotype) were analysed using the independent t-test and chi-squared test for comparison of continuous and nominal variables, respectively. Logistic regression modelling was used for further judgement of confounding factors, and odds ratios (OR) with 95% confidence intervals (CI) were estimated. Kaplan–Meier survival analysis was used for outcome evaluations, which were compared with the log–rank test. Tests for comparison of significant alleles or genotypes between patients and controls were performed using the chi-squared test or Fisher's exact test. We used the false discovery rate[20] to adjust for multiple testing. Hardy–Weinberg equilibrium analysis was calculated in accordance with standard procedures, also using the chi-squared test. Tests for haplotype association with susceptibility to COPD were performed using the Haploview software, version 4.2. Statistically significant P-values were corrected for multiple testing using permutation testing (1000 iterations randomly). A P-value of <0.05 was considered significant. Statistical analyses were performed using SPSS Statistics 18 for Windows 7 (IBM, Armonk, NY, USA).

RESULTS

Characteristics of COPD patients and healthy smokers

From January 2003 to January 2010, there were 320 subjects consisting of 192 COPD patients and 128 healthy smokers who were enrolled in the study. The median duration of follow-up was 35 ± 22 months

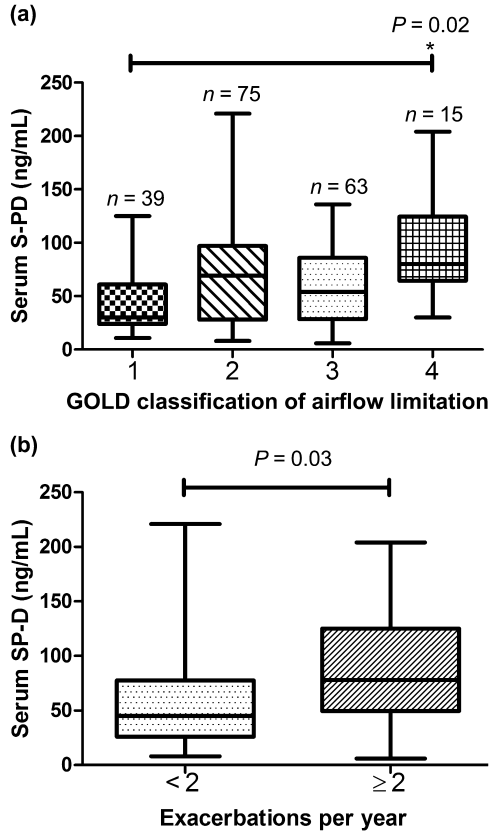

Figure 1 (a) SP-D serum levels in association with the degree of airflow obstruction, as classified by GOLD guidelines (GOLD 4 vs GOLD 1: $P = 0.02$). GOLD 1, 2, 3 and 4 represent $FEV_1 \geq 80\%$ of predicted, $50\% \leq FEV_1 < 80\%$ of predicted, $30\% \leq FEV_1 < 50\%$ of predicted, and $FEV_1 < 30\%$ of the predicted value. (b) SP-D serum levels in association with exacerbation risk defined as the frequency of medical visits for chronic obstructive pulmonary disease exacerbations per year (≥2 vs <2 exacerbations per year, $P = 0.03$). FEV_1: forced expiratory volume in 1 s; GOLD, Global Initiative for Chronic Obstructive Lung Disease; SP-D, surfactant protein D.

(range: 1–84 months). The baseline characteristics of the two groups are listed in Table 1a. Older age (68.6 ± 11.4 years vs 58.3 ± 12.8 years, $P < 0.001$), lower body mass index (23.2 ± 3.8 vs 25.3 ± 3.9, $P < 0.001$), heavier smoking history (44.8 ± 32.7 vs 37.3 ± 25.6 pack-years, $P = 0.02$) and higher IL-8 level (4.4 ± 6.0 vs 2.2 ± 2.5, $P = 0.002$) were found in COPD patient group. Multivariate analysis using logistic regression modelling revealed that only age and smoking history were significant factors for susceptibility to COPD (Table 1b).

Association of SP-D serum level with COPD severity

Disease severity was assessed by degree of airflow obstruction (GOLD 1, 2, 3 or 4) and exacerbation risk (low or high). As illustrated in Figure 1a, SP-D serum levels were significantly higher in the patient group with the most severe airflow obstruction (GOLD 4) compared with patients with the least severe obstruction (GOLD 1) (93.3 ± 45.8 vs 43.7 ± 32.9 ng/mL, $P = 0.02$). High risk of exacerbation (≥2 medical visits per year, 83.2 ± 51.1 ng/mL SP-D) was significantly associated with higher SP-D serum levels than group with a low risk of exacerbation (<2 medical visits per year, 55.8 ± 41.4 ng/mL SP-D) ($P = 0.03$) (Fig. 1b).

Allele and genotype frequencies of SP-D

The information for each SNP of the *SFTPD* gene tested in our sample population is summarized in Supplementary Table S1, along with Hardy–Weinberg equilibrium data. Comparisons of allele and genotype frequencies between COPD and control groups are given in Supplementary Table S2 and Table 2. Before correction for the false discovery rate for multiple testing, dominant allele model of rs721917 increased the risk for COPD susceptibility ($P = 0.05$, OR = 1.2, 95% CI: 1.00–1.43). However, there was no statistical significance after correction for multiple testing.

Haplotype analysis of SP-D between COPD patients and healthy smokers

Haplotype analysis revealed that the frequency of the G-G-C-C-A haplotype was significantly higher in the control group compared with the COPD group (frequency in COPD group: 0.17 vs 0.23 in the control group, $P = 0.03$), suggesting that this haplotype played a potentially protective role in the development of COPD (Fig. 2 and Table 3).

Association of SP-D haplotype with COPD-related phenotypes

We analysed the effect of SP-D G-G-C-C-A haplotype on COPD-related phenotypes, which included baseline demographic, pulmonary function and systemic biomarkers. As listed in Table 4, patients with haplotype G-G-C-C-A had better improvement of pulmonary function ($P = 0.03$, OR = 1.53) in the disease follow-up and were more sensitive to bronchodilator response ($P = 0.01$, OR = 1.72). The SP-D serum level was significantly lower in those patients with the G-G-C-C-A haplotype ($P < 0.001$, OR = 0.57). There were no other differences in demographic variables or inflammatory cytokines. In concordance with the results, we also found that the SP-D serum level positively correlated with the degree of airflow limitation and exacerbation risk in both groups of patients encompassing G-G-C-C-A and non-G-G-C-C-A haplotypes (Supplementary Table S3).

Effect of SP-D haplotype on COPD survival

Figure 3 shows the significant difference in the 3-year survival between COPD patients with and without the G-G-C-C-A haplotype. The 3-year survival rate for patients with the haplotype G-G-C-C-A was 90%, while the rate among patients with the non-G-G-C-C-A haplotype was only 77% ($P = 0.03$, log–rank test). Cox proportional hazard regression modelling (Table 5) revealed that worse airflow obstruction

Table 2 Genotype frequencies of *SFTPD* for patients with COPD and healthy smokers

SNP		Genotype frequency			*P*	OR (95% CI)	FDR
		COPD, *n* (%)	Control, *n* (%)				
rs911887	*G/G*	75 (39.3)	55 (43.0)	Dominant	0.51	0.94 (0.78–1.13)	0.64
	A/G	93 (48.7)	57 (44.5)				
	A/A	23 (12.0)	16 (12.5)	Recessive	0.90	1.00 (0.57–1.89)	0.82
rs2243639	*A/A*	133 (69.3)	76 (59.4)	Dominant	0.07	1.32 (0.98–1.78)	0.35
	A/G	53 (27.6)	45 (35.2)				
	G/G	6 (3.1)	7 (5.5)	Recessive	0.30	0.98 (0.93–1.03)	0.50
rs10887199	*G/G*	65 (33.9)	44 (34.4)	Dominant	0.92	0.99 (0.84–1.17)	0.77
	A/G	81 (42.2)	63 (49.2)				
	A/A	46 (24.0)	21 (16.4)	Recessive	0.10	1.10(0.98–1.23)	0.25
rs2255601	*T/T*	85 (44.5)	44 (34.4)	Dominant	0.07	1.18 (0.99–1.41)	0.35
	C/T	80 (41.9)	62 (48.4)				
	C/C	26 (13.6)	22 (17.2)	Recessive	0.38	0.96 (0.87–1.06)	0.54
rs721917	*T/T*	86 (44.8)	43 (33.9)	Dominant	0.05	1.20 (1.00–1.43)	0.50
	C/T	81 (42.2)	62 (48.8)				
	C/C	25 (13.0)	22 (17.3)	Recessive	0.29	0.95 (0.86–1.05)	0.58
rs726288	*A/A*	112 (58.3)	73 (57.0)	Dominant	0.82	1.03 (0.80–1.34)	0.82
	A/G	67 (34.9)	48 (37.5)				
	G/G	13 (6.8)	7 (5.5)	Recessive	0.64	1.01 (0.96–1.07)	0.71

The effect of dominant/recessive models of each SNP on the susceptibility to COPD was analysed and corrected for the FDR of multiple testing.

CI, confidence interval; COPD, chronic obstructive pulmonary disease; FDR, false discovery rate; OR, odds ratio; SNP, single nucleotide polymorphisms.

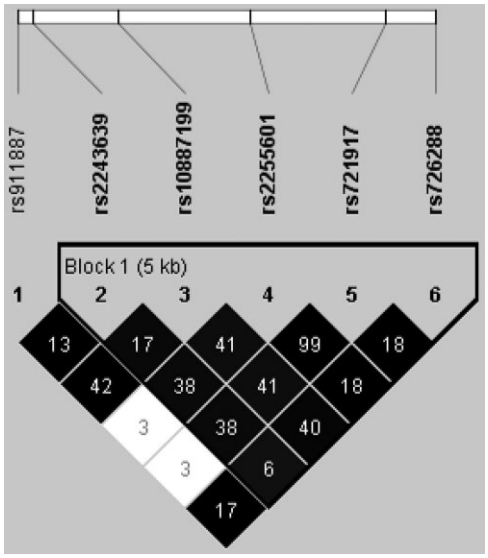

Figure 2 Haplotype block (delineated by bold lines) and tag SNP of SP-D as determined with Haploview software, version 4.2. The strength of the linkage disequilibrium between the two SNP was measured by r^2 (r: correlation coefficient) (black: $r^2 = 100$ and is the strongest; white: $r^2 = 0$ is the weakest). SNP, single nucleotide polymorphisms; SP-D, surfactant protein D.

(hazard ratio (HR): 0.92, 95% CI: 0.85–0.97), higher exacerbation risk (HR: 5.35, 95% CI: 2.34–8.44) and protective haplotype G-G-C-C-A (HR: 0.79, 95% CI: 0.33–0.89) were independently associated with the prognosis after adjustment for all the other confounding factors.

Table 3 Haplotype analysis of *SFTPD* gene between patients and healthy smokers

SNP markers	Haplotype	Frequency		*P*
		COPD (*n* = 192)	Control (*n* = 128)	
rs2243639	A-A-T-T-G	0.24	0.24	0.91
rs10887199	A-G-T-T-A	0.21	0.19	0.43
rs2255601	G-G-C-C-A	0.17	0.23	0.03*
rs721917	A-A-T-T-A	0.20	0.16	0.16
rs726288	A-G-C-C-A	0.17	0.17	0.85

*Permutation *P*-value.

COPD, chronic obstructive pulmonary disease; SNP, single nucleotide polymorphisms.

DISCUSSION

SP-D, as an innate immunity molecule, plays an important role in host defence and regulation of inflammation, which are essential factors in the pathogenesis of asthma,[21] lung injury[22] and COPD.[23] In our study population, we demonstrated the association of SP-D serum levels, after verifying smoking history and drug history in our patients to avoid potential confounding effects, with exacerbations and baseline airflow obstruction of COPD, which is consistent with other reports.[24,25] In fact, several studies also found that genetic variants of the *SFTPD* gene were associated with serum concentrations of SP-D; Kim *et al.* from the genome-wide association

Table 4 Association of the G-G-C-C-A haplotype of SP-D with COPD-related phenotypes

Haplotype analysis	G-G-C-C-A (*n* = 57)	non-G-G-C-C-A (*n* = 135)	Crude *P*	Adjusted *P*	OR (95% CI)
Demographic parameters					
Age (years)	67.2 ± 12.5	69.1 ± 10.9	0.42	—	—
Body mass index	23.8 ± 3.6	23.0 ± 3.9	0.13	—	—
Smoking history (pack-years)	46.1 ± 35.0	44.3 ± 32.0	0.77	—	—
Current smoker (%)	19	21	0.57	—	—
History of inhaled LAMA (%)	32	35	0.45	—	—
History of inhaled LABA + ICS (%)	29	25	0.22	—	—
History of inhaled LAMA + LABA + ICS (%)	39	40	0.19	—	—
Clinical parameters					
FEV_1 % predicted	56.4 ± 20.9	58.3 ± 22.7	0.59	—	—
Medical visit (times/year)	1.0 ± 1.6	1.4 ± 2.4	0.36	—	—
Change of FEV_1 (mL/year)	128.6 ± 197.4	31.0 ± 161.8	0.01	0.03	1.53 (1.30–1.77)
Positive bronchodilator response (*n*/%)	18/31.6	21/15.7	0.005	0.01	1.72 (1.49–1.91)
Inflammatory cytokines, SP-D					
TNF-α (pg/mL)	10.2 ± 7.1	11.1 ± 9.6	0.92	—	—
IL-8 (pg/mL)	4.4 ± 6.2	4.3 ± 5.9	0.95	—	—
IL-6 (pg/mL)	6.7 ± 10.1	9.4 ± 30.5	0.57	—	—
SP-D (ng/mL)	52.6 ± 38.9	94.8 ± 42.2	<0.001	<0.001	0.57 (0.31–0.84)

—, inconclusive results; CI, confidence interval; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; ICS, inhaled corticosteroid; IL-6, interleukin-6; IL-8, interleukin-8; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; OR, odds ratio; SP-D: surfactant protein-D; TNF-α, tumour necrosis factor-α.

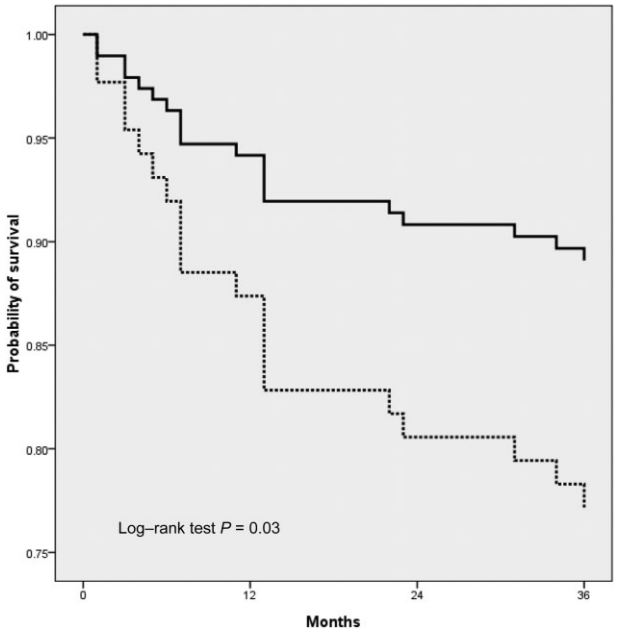

Figure 3 Kaplan–Meier survival analysis of patients with the G-G-C-C-A versus non G-G-C-C-A haplotype. ( ) Haplotype non-GGCCA, ( ) Haplotype GGCCA.

Table 5 Cox proportional hazard regression analysis for 3-year mortality rate between patients with haplotype G-G-C-C-A and non-G-G-C-C-A

Survival analysis	Crude *P*	Adjusted *P*	HR (95% CI)
Demographic parameters			
Age	0.10	—	—
Body mass index	0.38	—	—
Smoking history (pack-years)	0.18	—	—
Clinical parameters			
FEV_1 % of predicted	0.01	0.02	0.92 (0.85–0.97)
Medical visit (times/year)	0.02	0.03	5.35 (2.34–8.44)
Change of FEV_1 (mL/year)	0.08	—	—
Positive bronchodilator response (*n*/%)	0.10	—	—
SFTPD level and haplotype			
Haplotype G-G-C-C-A	0.02	0.03	0.79 (0.33–0.89)
SFTPD (ng/mL)	0.31	—	—

—, inconclusive results; CI, confidence interval; FEV_1, forced expiratory volume in 1 s; HR, hazard ratio.

analysis suggested SP-D as a serum biomarker as well as a genetic susceptibility marker of COPD.[16]

Genetic associations of SP-D with COPD were first investigated early in 2001 and focused mainly on a Mexican population.[26] Van Diemen *et al*. showed that the rs2243639 (Thr160Ala) *SFTPD* SNP was associated with FEV_1/inspiratory vital capacity.[15] However, they did not encompass the whole block of the *SFTPD* gene and only picked up two loci, rs721917 (Met11Thr) and rs2243639 (Thr160Ala), as risk targets. Although we were unable to detect an association between alleles and/or genotypes of *SFTPD*

and COPD, we identified a potential trend in the risk for dominant rs721917 model ($P = 0.05$ before false discovery rate correction) towards COPD susceptibility. Further analysing the entire *SFTPD* gene with four additional tag SNPs of *SFTPD*, we demonstrated a protective G-G-C-C-A haplotype (rs2243639, rs10887199, rs2255601, rs721917 and rs726288) for the development of COPD. Our findings reinforce the genetic influence of SP-D on COPD susceptibility in a Chinese population in Taiwan. Moreover, the conclusion that SP-D is involved in the predisposition to COPD and emphysema was also recently validated in the Japanese population.[27]

We found that the G-G-C-C-A haplotype of SP-D was not only associated with lower risk of COPD, but also COPD patients with the G-G-C-C-A haplotype had lower SP-D serum levels, higher rates of positive response to bronchodilator therapy and better improvement of FEV_1 in yearly follow-ups. Importantly, when we compared survival between subgroups of COPD patients for all-cause 3-year mortality, there was a significant difference between COPD patients with and without the G-G-C-C-A haplotype. In our study population, the risk for 3-year mortality in patients with the G-G-C-C-A haplotype was 21% lower than in patients with the non-G-G-C-C-A haplotype. Eleven patients died within 1 year after enrolment, and the effect of G-G-C-C-A haplotype on survival among patients completing the 12-month follow-up period remained significant (data not shown). Presently, there is little, if any, data regarding genetic variants of SP-D haplotypes as predictors of disease outcome in COPD.

It is difficult to explain why smokers with the G-G-C-C-A haplotype of SP-D had lower risk for COPD. We speculate that smokers with the G-G-C-C-A haplotype of SP-D resist the pathogenesis of COPD through biological changes of SP-D function. Previous studies confirmed that oxygen radicals resulting from smoke lead to disruption of the quaternary structure of SP-D protein *in vivo* and *in vitro*.[25,28] Under nitric oxide stimulation, SP-D initiates a pro-inflammatory response through modification of cysteine residues in the hydrophobic tail domain of SP-D, resulting in the dissociation of multimers into oligomers that reduce its anti-inflammatory activity.[29,30] Oxidative and nitrosative stresses are hallmarks of COPD development and perpetuate ongoing pulmonary damage. Whether the G-G-C-C-A haplotype of SP-D has more resistance to structural modification caused by these stresses, thus maintaining its native anti-inflammatory function, or the non-G-G-C-C-A haplotype of SP-D is more susceptible to stresses, leading to dysfunction of SP-D and development of COPD, remains to be identified.

There are some limitations to our study. First, lack of dynamic records of all the inflammatory biomarkers could have limited their significance. Second, we did not collect bronchoalveolar fluid from the study participants, which could have resulted in inconclusive findings in the local levels and functions of SP-D. Third, a large population-based study should be designed for replication and further validation of our results.

In conclusion, we reported that serum levels of SP-D positively correlated with pulmonary function impairment at baseline and frequency of disease exacerbation in the follow-up period. From subgroup analysis, the protective G-G-C-C-A haplotype of the *SFTPD* gene was an important biomarker for predictive and therapeutic modalities of COPD.

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Supplementary Information

Additional Supplementary Information can be accessed via the *html* version of this article at the publisher's web-site:

Supplementary Table S1 Summary of SNPs tested in our study.

Supplementary Table S2 Allele frequencies of *SFTPD* between patients and healthy smokers. MAF: minor allele frequency.

Supplementary Table S3 Correlation of *SFTPD* serum level with degree of airflow obstruction and exacerbation risk.