SP-D polymorphisms and the risk of COPD

Tania A. Shakoori[a,*], Don D. Sin[b], S. Nazim Hussain Bokhari[a], Farkhanda Ghafoor[c] and
A.R. Shakoori[d]
[a]*University of Health Science, Lahore, Pakistan*
[b]*Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada*
[c]*National Health Research Complex, SZMC, Lahore, Pakistan*
[d]*School of Biological Sciences, University of the Punjab, Lahore, Pakistan*

Abstract. *Introduction*: There are limited data linking serum levels of surfactant protein D, its genetic polymorphisms to the risk of Chronic Obstructive Pulmonary Disease (COPD).

Objectives: We sought to investigate these relationships using a case control study design.

Methods: Post bronchodilator values of FEV1/FVC <0.7 were used to diagnose COPD patients ($n = 115$). Controls were healthy subjects with normal spirometry ($n = 106$) Single nucleotide polymorphisms (rs721917, rs2243639, rs3088308) were genotyped using polymerase chain reaction (PCR) and restriction analysis. Serum SP-D levels were measured using a specific immunoassay.

Results: Allele 'A' at rs3088308 ($p < 0.00$, $B = -0.41$) and 'C' allele at rs721917 ($p = 0.03$; $B = -0.30$) were associated with reduced serum SP-D levels. Genotype 'T/T' at rs721917 was significantly associated with risk of COPD ($p = 0.01$). Patients with repeat exacerbations had significantly higher serum SP-D even after adjusting for genetic factors.

Conclusions: We report for the first time that rs3088308 is an important factor influencing systemic SP-D levels and confirm the previous association of rs721917 to the risk of COPD and serum SP-D levels.

Keywords: Surfactant protein D, SP-D SNPs, serum SP-D levels, COPD risk, SP-D haplotypes

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease with significant extra-pulmonary effects associated with progressive airflow limitation and abnormal inflammatory response [1]. The incomplete understanding of its pathophysiology is a major hurdle in the development of effective therapy. Many mechanisms have been proposed to explain the pathophysiology of COPD. These include protease/antiprotease imbalance [2], oxidant/antioxidant disturbances [3], persistent abnormal inflammatory response [4], accelerated unopposed apoptosis [5], autoimmunity [6,7], histone deacetylation leading to transcription of proinflammatory factors [8] and premature aging of the lung [9]. Surfactant protein D (SP-D) has immunomodulatory functions [10,11]. By keeping a potentially aggressive inflammatory reaction in check, SP-D diminishes production of proteases and oxidants [12]. In addition it causes effective efferocytosis and prevents autoantibody formation [13–15]. SP-D may be an important effector molecule in the pathogenesis of COPD. SNPs rs721917 rs2243639 and rs3088308 [16,17] were chosen to be genotyped in COPD patients and controls in order to determine their association with risk of COPD and serum SP-D levels. Each of these SNPs is from one of the three main functional domains of the protein and together they regulate the various components of SP-D.

Rs721917 is associated with increased susceptibility to tuberculosis [18] Influenza A virus [19] infection, allergic rhinitis [20], COPD [21], respiratory syncitial virus (RSV) infection in infants [17], atopy [22] and blood levels of SP-D [23]. Rs2243639, on the other hand, has been associated with ulcerative colitis [24]

*Corresponding author: Dr. Tania Ahmed Shakoori, Ph.D., University of Health Science, Lahore, Pakistan. E-mail: drtaniashakoori @yahoo.com.

Table 1
Primer sequences, restriction enzymes and restriction products for the three SNPs

SNP ID	Primer pairs	Annealing temperatures	PCR product	Enzyme	For major allele	For minor allele
rs3088308	5'ACGGAGGCACAGCTGCTG-3' 5'-GGAAAGCAGCCTCGTTCT-3'	54°C	115 bp	MnlI	54, 42 and 19 bp	96 and 19 bp
rs721917	5'CCCCATAGCAGAGGACAGAA3' 5'CCAGGGTGCAAGCACTGGAC3'	61°C	238 bp	HpyCH4 IV	Uncut (238 bp)	217 and 21 bp
rs2243639	5'-CCCCACTTCTCTCTCTGACC-3' 5'-CTGCTCACCTGCTGCCCCCG3'	56°C	238 bp	BstU1	165 and 73 bp	145, 73 and 20 bp

and risk of respiratory syncytial virus infection (RSV) in neonates [25]. No clinical associations have yet been reported for rs3088308.

2. Methods

2.1. Subjects

We collected 106 controls from the general population of Lahore through advertising via posters at various public places in the city. One hundred and fifteen (115) cases were chosen from the inpatient wards and outpatient departments of Shaikh Zayed Medical Complex (SZMC) and Gulab Devi Chest Hospital, Lahore. The research project was approved by the ethical and scientific committees of both these hospitals prior to start of sampling. The inclusion criteria for cases were a clinical history suggestive of COPD (including breathlessness, chronic cough and sputum) along with airflow limitation as demonstrated by electronic spirometry (Spirolab 2, SDI Diagnostics, 10 Hampden Drive, Easton, MA 02375). Airflow limitation was diagnosed if FEV1/FVC ('Forced Expiratory Volume in 1 second' to 'Forced Vital Capacity ratio') was less than 0.7 after administration of salbutamol inhaler (200 μg, Salbo, Getz Pharma, KIA, Karachi, Pakistan). The disease was staged according to FEV1%predicted (Forced Expiratory Volume in 1 second percentage of predicted) as recommended by the GOLD committee [1]. The inclusion criteria for controls were the absence of respiratory symptoms and no airflow obstruction on spirometry. Exacerbations were diagnosed by a history of increase in dyspnoea, sputum volume or change of sputum colour as compared to the patients' routine [26]. All subjects (cases and controls) were Pakistani Punjabi men, 26 to 74 years old. Informed consent was obtained from each subject.

2.2. Sampling

Ten millilitres of venous blood was drawn from each subject. Five millilitre was allowed to clot and serum extracted, while the remaining 5 ml was collected in EDTA (Ethylene Diamine Tetraacetic Acid) vacutainer tubes and used to isolate DNA.

2.3. Genotyping

DNA was extracted from whole blood manually using a 'salting out' procedure [27]. Relevant fragments of DNA were amplified by polymerase chain reaction using sets of primers as shown in Table 1 [28]. PCR was performed in a thermocycler (Eppendorf Mastercycler® Personal, USA). The reaction mixtures for the three sets of primers are shown in Table 1. PCR cycle was optimized so that the three primer pairs amplified their respective PCR products at the same set of temperatures. Briefly, initial denaturation took place at 95°C for 3 minutes, followed by subsequent denaturation at 94°C for 45 seconds and annealing at 61°C for another 45 seconds. Finally renaturation occurred at 72°C for 45 seconds and the cycle repeated itself 35 times until final extension step at 74°C (5 minutes). Each fragment was restricted by a specific restriction enzyme as described previously by Kreuger et al. [28] (Table 1). Briefly, the 115 base pair PCR product for rs3088308 was cleaved by MnI1 into 3 fragments (54, 42 and 19 base pairs) for the major allele and 2 fragments (96 and 19 base pairs) for the minor allele (Fig. 1). The 238 base pair PCR product for rs721917 was cleaved by HpyCH4IV into 2 fragments in case of the minor allele and remained intact in the case of major allele (Fig. 2). Finally the 238 base pair PCR product for rs2243639 was restricted by BstU1 into 165 and 73 base pairs fragments for the major allele and 3 fragments (145, 73 and 20 base pairs) in case of minor allele (Fig. 3). Restriction samples were incubated for 6–12 hours as required to ensure proper digestion. Restriction fragments were visualized in 12% polyacrylamide gel in TBE buffer and stained with ethidium bromide or silver nitrate.

Random samples were genotyped twice for quality assurance. For rs721917 the major allele remains undigested. Comparison with restriction samples pos-
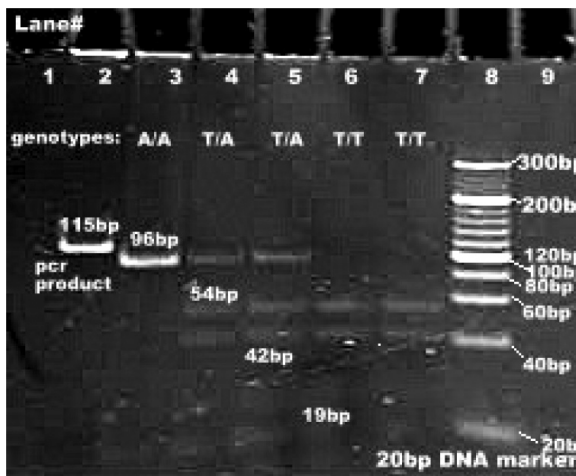
Fig. 1. 12% polyacrylamide gel (ethidium bromide stained) showing restriction analysis of PCR product for SNP, rs3088308 (115bp) by the enzyme MnlI. A/A genotype shows two bands of 96bp and 19bp in lane 3, T/T genotype shows three bands of 54, 42 and 19 base pairs (lanes 6 and 7), whereas all 4 bands (96, 54, 42 and 19 base pairs) are visible in case of T/A (lanes 4 and 5). Lane 2 shows the unrestricted PCR product and lane 1 is empty. Lane 8 shows 20bp DNA ladder. Due to prolonged running of the gels the 19 base pair restriction bands and the 20 base pair band of the DNA ladder are only faintly visible.
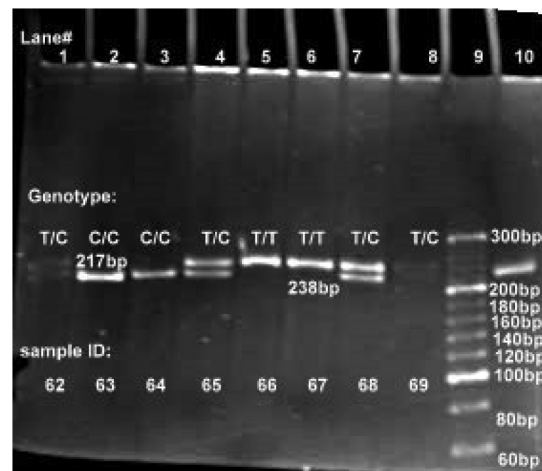

Fig. 2. 12% polyacrylamide gel (stained with ethidium bromide) showing restriction analysis of PCR product for rs721917 (238bp) by the enzyme HpyCH4IV. T/T genotype shows the uncut PCR product of 238 base pairs (lanes 5 and 6), C/C genotype shows two restriction bands of 217 and 21 base pairs (lanes 2 and 3), whereas all 3 bands (238, 217 and 21 base pairs) are visible in case of heterozygous genotype T/C (lanes 1, 4, 7 and 8). Lane 10 shows the unrestricted PCR product of 238 base pairs. Lane 9 shows the 20bp DNA ladder. The 21 base pair restriction bands and the 20 and 40 base pair bands of the DNA ladder have run out of the gel due to protracted electrophoresis.

sessing the minor allele which were digested using the same mastermix of the enzyme and buffer as the samples with major allele, was used to ensure that lack of digestion was indeed due to the presence of major allele and not because of failure of the enzyme.

2.4. Elisa

The SP-D assay was performed in Immunoassay Lab. of National Health Research Complex, SZMC, Lahore. Commercial enzyme linked immunosorbent assay kits (BioVendor – Laboratorní medicína a.s. CT Park Modrice, Evropska 873 664 42 Modrice, Czech Republic) were used. Standards, quality controls and patient samples were run in duplicates. All procedures were carried out observing temperature conditions detailed in assay procedure. In brief, diluted samples were incubated in microplate wells pre-coated with monoclonal anti-human surfactant protein D antibody. After overnight incubation and washing, HRP conjugate was applied and another two hour incubation with adhered surfactant protein D was carried out. After washing the wells, the remaining HRP conjugate was allowed to react with the substrate solution (TMB). An acidic 'stop solution' was added to thwart the reaction and absorbance of the yellow product was recorded. The absorbance rep-

resented the concentration of SP-D. The OD (optical density) values were plotted against concentrations of standards to generate a standard curve. Concentrations of unknown samples were determined by means of this standard curve. Throughout the assay procedure, assay precision was maintained by using calibrated micro pipettes and multi channel pipettes from Gilson and washing was done with automatic plate washer.

2.5. Statistical analysis

Hardy Weinberg equilibrium, genotype, allele and haplotype frequencies were calculated using SHesis software [30]. Chi square fisher exact test and odds ratio were calculated to test association of the genotypes alleles with COPD and to check for deviations from HWE. SPSS 17 was used for the rest of the analysis. Continuous variables were expressed as mean $\pm$ SD. Binary logistic regression was applied to explore the relationship of age, smoking history and the genotypes of SNP rs721917 with risk of COPD. Serum levels of SP-D across different genotypes were compared using ANOVA. Multiple linear regression analysis was performed to analyse the effect of exacerbation status, steroid intake, smoking status, 'A' allele at rs3088308, C allele at rs721917, BMI, age, and FEV1% of predicted on serum SP-D levels. For the purposes of re-

Table 2
Characteristics of study subjects

Characteristic	Controls	COPD
Subjects (n)	115	106
Age-yrs (mean $\pm$ SD)	37 ± 11	61 ± 13
Smoking status	15 never-smokers, 8 ex-smokers, 92 current smokers	4 never smokers, 58 ex-smokers, 44 current smokers
Mean pack-years*	18 ± 17	48 ± 31
BMI kg/m^2	25 ± 5	21 ± 4
FEV$_1$/FVC %	84 ± 5	53 ± 14
FEV$_1$%predicted	97 ± 15	44 ± 19
SP-D levels (ng/mL)	121.5 ± 61	150.4 ± 97

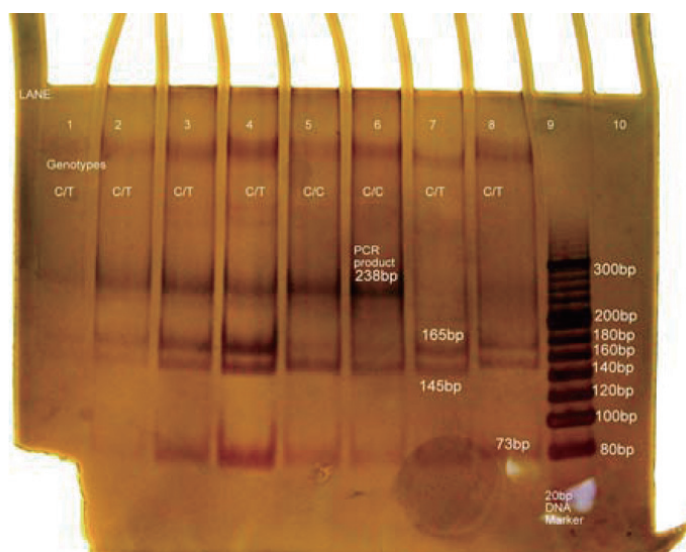*Mean pack years include never smokers.

Fig. 3. 12% polyacrylamide gel (after silver staining) showing restriction of PCR product for SNP rs2243639 by the enzyme BstU1. 145 and 73 bp bands are visible in lanes 5 and 6 showing the C/C genotype. T/T genotype is not seen in this gel. Heterozygous genotype is seen in lanes 1, 2, 3, 4, 7 and 8 as is indicated by bands of 165 and 145 base pairs. (20 bp bands and DNA ladder bands less than 80bp have 'run out' of the gel during the course of the electrophoresis).

gression, SP-D levels, age and BMI were transformed to their natural log to mitigate the effect of outliers. A 'p' value of less than 0.05 was considered statistically significant.

3. Results

3.1. Subjects

All subjects were Pakistani Punjabi men. Their characteristics are shown in Table 2.

3.1.1. Genotypes and allele frequencies
Results of PCR and restriction analysis revealed genotypes and allele frequencies (Table 3, Fig. 4). For the SNP rs721917, allele T was more frequent amongst cases (57.41 versus 48.8%) whereas allele C (51.19% versus 42.59%) and genotype C/C (21.4% versus 7.4%) was more common in the controls group. The genotype distribution in controls conformed to HWE ($p = 0.08$) but deviated significantly from it in cases ($p < 0.001$). Chi square test showed the difference in allele frequencies to be significant at $p = 0.038$. There was no significant difference in the case of rs2243639 and rs3088308 the genotype or allele distribution between cases and controls did not differ significantly when tested with Chi square.

3.1.2. Haplotypes
Haplotype T T A (alleles in rs3088308, rs721917 and rs2243639 respectively) was significantly less frequent in COPD cases (8.8%) as compared to controls (29%) at $p = 0.03$ with an odds ratio of 0.236 (95% CI 0.057 to 0.975).

Table 3
Genotypes and allele frequencies in cases and control

		Controls (n = 106)	COPD (n = 115)	'p' for difference in allele/genotype frequencies	'p' for HWE test (controls/cases)
rs3088308					
Genotypes (%):	T/T	58.4	65.1	0.31	0.04/0.35
	A/T	29.4	30.3		
	A/A	11.2	5.5		
Allele frequencies (%)	T	73.6	79.6	0.14	
	A	26.4	20.2		
*rs721917**					
Genotypes (%):	T/T	19.0	22.2	0.038	0.08/< 0.001
	T/C	59.5	70.4		
	C/C	21.4	7.4		
Alelle frequencies (%)	T	48.8	57.4	0.12	
	C	51.2	42.6		
rs2243639					
Genotypes (%):	C/C	14.3	14.3	0.50	0.66/0.50
	C/T	57.1	50.8		
	T/T	11.9	11.9		
Allele frequencies (%)	C	42.9	52.7	0.15	
	T	57.1	37.3		

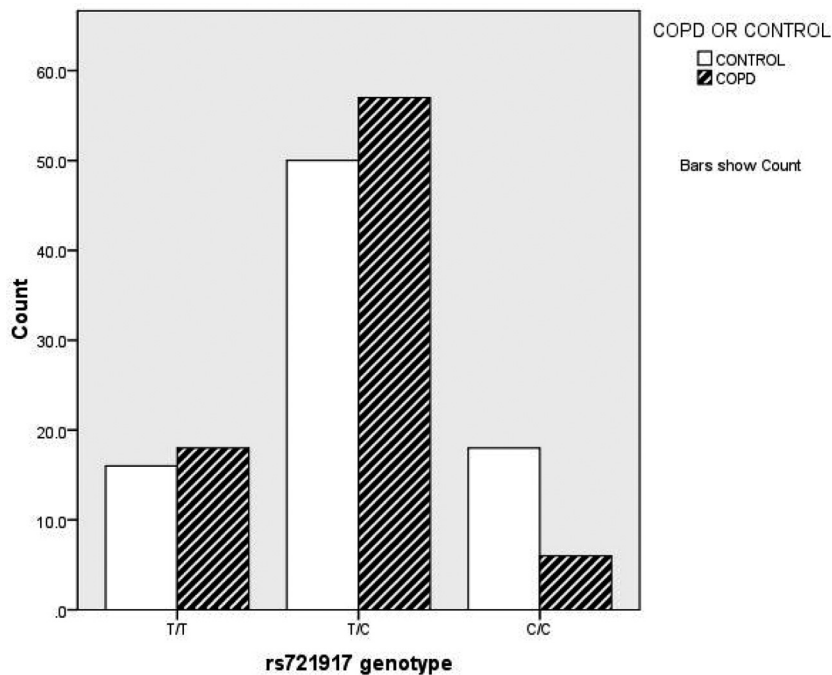*Genotyping results were available for 80 cases and 85 controls for rs721917.

Fig. 4. Distribution of genotypes of SNP rs721917 ($p = 0.038$). The dots represent outliers.

3.1.3. Logistic regression for SNP alleles as risk factors for COPD

Binary logistic regression verified genotype T/T of rs721917 as a significant risk factor for COPD ($B = +2.4$, $p = 0.01$, odds ratio $= 11.17$) along with advanced age ($B = +0.15$, $p = 0.00$, odds ratio $= 1.16$) as shown in Table 4. The logistic regression model fitted the data adequately as is mirrored by the Hosmer and Lemeshow Test.

3.1.4. Serum SP-D levels and SNPs

Mean SP-D serum levels differed significantly between the different genotypes of rs3088308 and rs721917 (Table 5, Fig. 5). For rs3088308 the serum

Table 4
Genetic and environmental risk factors for development of COPD

Risk factors for COPD	B	S.E.	'P'	Odds ratio	95% CI
Age (years)	0.15	0.03	< 0.001	1.16	1.10–1.23
Smoking (Pack years)	0.02	0.01	0.11	1.02	0.99–1.05
rs721917 (C/C)	–	–	0.04	–	–
rs721917 (T/T)	2.41	0.95	0.01*	11.17	1.75–71.18
rs721917 (C/T)	1.34	0.80	0.10	3.80	0.79–18.39

Hosmer and Lemeshow Test Chi-square $= 10.93$; $p = 0.21$;
Omnibus Tests of Model Coefficients Chi-square $= 191.31$ ($p < 0.001$).

Table 5
Mean serum SP-D values in ng/ml in different genotypes

rs3088308			
T/T	A/T	A/A	(ANOVA) P
149.75 ± 87.17	111.16 ± 62.51	99.51 ± 47.78	0.01
rs721917			
T/T	C/T	C/C	(ANOVA) p
175.94 ± 86.00	127.24 ± 81.31	112.12 ± 43.23	0.02
rs2243639			
C/C	C/T	T/T	(ANOVA) P
183.31 ± 89.88	129.08.1 ± 102.49	148.35 ± 27.36	0.40

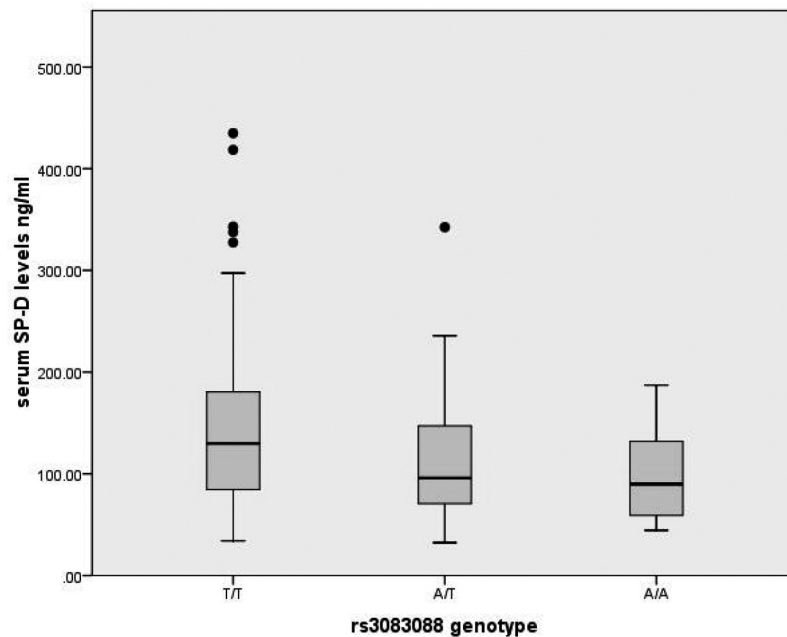

Fig. 5. Serum SP-D levels in genotypes of rs3088308 ($p = 0.01$). Note: The dots represent outliers within 1.5 inter-quartile ranges(IQR).

levels of SP-D were highest in T/T genotype (149.75 ± 87.17 ng/ml), lower in genotype A/T (111.16 ± 62.51 ng/ml) and lowest in genotype A/A (99.51 ± 47.78 ng/ml). This pattern was significant at $p = 0.01$. Similarly with rs721917, the highest SP-D values were recorded in genotype T/T (175.95 ± 86.00 ng/ml), followed by C/T (127.24 ± 81.31 ng/ml) and the lowest in C/C (112.12 ± 43.23 ng/ml). These differences were

also statistically significant at $p = 0.02$. For rs2243639 there were no statistically significant differences between the three genotypes ($p = 0.40$).

3.1.5. Determinants of serum SP-D levels

Multiple linear regression analysis revealed the contribution of various predictor variables (including age, BMI, smoking status, exacerbation status, allele A at

Table 6
The effect of different demographic, clinical and genetic factors on SP-D blood levels

	Predictor variables	Unstandardized coefficients		Standardized coefficients	'P'
		B	Std. Error	Beta	
	(Constant)	4.81	0.99	NA	< 0.001
1	Exacerbation presence	0.39	0.16	0.28	0.01^*
2	Steroid use[a]	-0.63	0.19	-0.36	$< 0.001^*$
3	Smoking status	0.06	0.19	0.04	0.75
4	'A' allele at rs3088308	-0.41	0.11	-0.33	$< 0.001^*$
5	'C' allele at rs721917	-0.30	0.13	-0.21	0.03^*
6	Natural log of BMI	-0.69	0.26	-0.26	0.01^*
7	Natural log of age	0.58	0.19	0.36	$< 0.001^*$
8	FEV1% predicted	0.003	0.002	0.18	0.16

Dependent Variable: Natural log of Surfactant protein D serum levels;
Regression Model's Adjusted R Square $= 0.377$, ANOVA: $p < 0.001$;
BMI: Body mass Index, FEV1% predicted: Forced expiratory volume in 1st second in litres percentage predicted for the age gender and ethnic background, NA: not applicable.
[a] Steroid intake at the time of sampling.

rs3088308, allele C at rs721917 and FEV1) to serum SP-D (Table 6). We found that 'C' allele at rs721917 ($p = 0.03$; $B = -0.30$) and 'A' at rs3088308 ($p < 0.001$, $B = -0.41$) were significantly associated with decreased SP-D levels. Other significant contributors to serum SP-D levels included age ($p < 0.001$) body mass index ($p = 0.01$), exacerbation status ($p = 0.01$) and steroid intake ($p < 0.001$).

4. Discussion

The most important findings in this study are the correlation of SNPs rs721917 and rs3088308 with serum SP-D levels and of rs721917 with the risk of COPD. Single nucleotide polymorphism rs3088308 causes the substitution of nucleotide adenine (A) in place of a thymidine (T) in the seventh exon of the SFTPD gene leading to the replacement of serine (Ser) by threonine (Thr) at position number 270 in the carbohydrate recognition domain (CRD) of SP-D protein molecule. We found serum levels of SP-D in genotype A/A to be 11% lower as compared to heterozygotes (A/T) and 34% lower than genotype T/T at $p = 0.01$. We are reporting this association for the first time. Our study also confirmed the previously reported association of rs721917 with systemic SP-D levels [21,29]. In the current study, SP-D levels subjects with genotype C/C (of rs721917) were 12% lower than heterozygotes (C/T) and 36.% lower as compared to genotype T/T. The percentage difference between genotypes appears to be more in case of rs3088308 as compared to rs721917. A multiple regression model was developed to understand and quantify the effect of various genetic and environmen-

tal factors on serum SP-D levels. As shown in Table 6, age (Beta $= 0.36$, $p < 0.001$), and steroid intake (Beta $= -0.36$, $p < 0.001$) and rs3088308 influence serum SP-D levels (Beta $= -0.33$, $p < 0.001$) along with exacerbation status (Beta $= 0.28$, $p = 0.01$), body mass index (Beta $= -0.26$, $p = 0.01$) and SNP rs721917 (Beta $= -0.21$, $p = 0.03$). Our study supports the previous studies correlating age [29], body mass index [31, 32], corticosteroid intake [33] and rs721917 [29,31,39] with systemic SP-D levels. We have previously reported on significantly raised serum SP-D levels in COPD exacerbations [34]. In the current study we confirm those findings using a larger sample size ($n = 153$) and a more comprehensive regression model incorporating genetic markers (rs3088308 and rs721917) as covariates. Thus we show that even when genetic factors are considered, exacerbation remains a significant determinant of systemic SP-D levels. These data highlight the potential utility of serum SP-D as a biomarker for exacerbations as previously reported [34]. A study by Sims et al. [35] reports on decreased SP-D levels in broncho-alveolar lavage fluid (BALF) of COPD patients and that these levels increased after corticosteroid therapy. This is consistent with our findings since alveolar damage in COPD causes leakage of SP-D from the pulmonary compartment into the systemic circulation resulting in lowered BALF but high serum SP-D levels. Similarly steroid therapy stimulates the production of SP-D, leading to inmcreased levels in BALF. Steroids also control the inflammatory reaction of COPD and restore the integrity of alveolar membrane which explains lower serum SP-D levels in patients on corticosteroid therapy [35].

Why amino acid substitutions would affect serum levels of SP-D is unknown. It is widely believed that
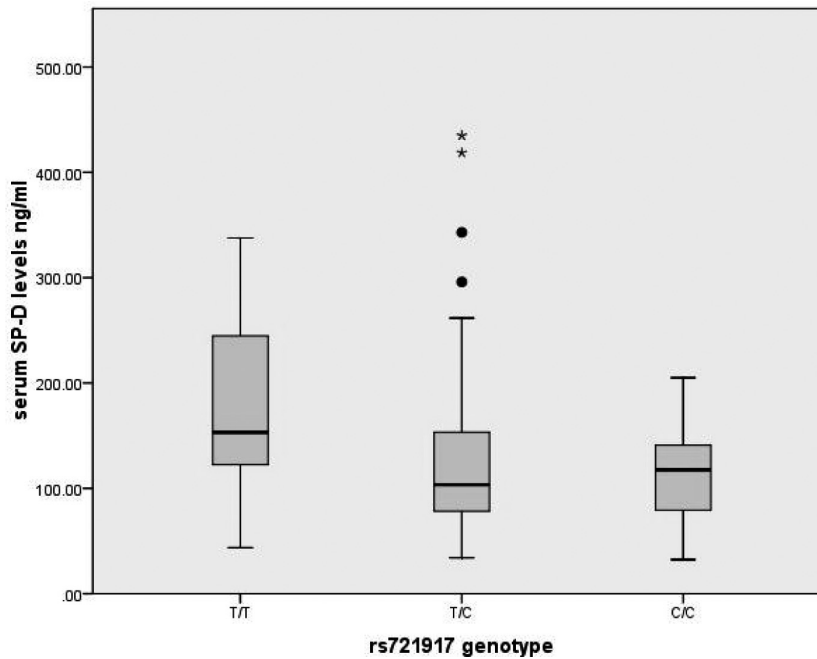
Fig. 6. Serum SP-D levels in genotypes of rs721917 ($p = 0.02$). Note: The dots represent outliers within 1.5 IQR while * represents outliers within 3 IQR.

the predominant source of systemic SP-D is via leakage from the alveolar compartment [36,37]. It is possible that even slight alterations in the molecular structure of SP-D may modify the function of SP-D, perhaps disturbing balance between its immuno-stimulatory and immuno-modulatory roles. Altered defence capabilities of this key innate immune molecule may lead to an abnormal inflammatory response to environmental insults which may in turn compromise the integrity of the alveolar membrane, increasing the amount of SP-D that leaks into the circulation. More research is however required to investigate the exact mechanisms.

The other important finding of our study is the association of genotype 'T/T' of rs721917 or the 'Met variant' with the risk of COPD. Genotype distributions in cases for rs721917 deviated significantly from Hardy Weinberg Equilibrium, giving an early hint of possible association of these SNPs with COPD ($p < 0.001$). Chi square test showed that genotype distributions between cases and controls indeed did differ significantly for rs721917 at $p = 0.038$ respectively. When the risk of COPD was assessed in the presence of age, smoking history and body mass index using a multiple linear regression model, rs721917 remained significantly associated with the risk of COPD (odds ratio = 11.17, $p = 0.01$). These data raise the possibility of SNP analysis in early detection of 'at risk smokers' for this progressive disease with no cure.

Our finding is in contrast to a previous study by Foreman et al. [21] who found the C allele to be associated with COPD. There are several possible reasons for this discrepancy. Although Foreman's study was larger than our study, they used the NETT (National Emphysema Treatment Trial) subjects who had bilateral emphysema and with FEV1 of $< 45\%$. Thus these subjects represented one extreme phenotype of COPD. This may be one of the reasons why Foreman's findings were not replicated in the 'Bergen cohort' or by the "ECLIPSE" Study Group. Another reason for the differences in results could be variation in the genetic backgrounds of subjects. This phenomenon has been well described by Brandt and coworkers [22]. They used the same protocol on black and white subjects but found the 'Met allele' to be associated with atopy and asthma in only black subjects. Genetic and phenotypic difference would also explain the contrasting findings by Ishii et al. [41]. It is also notable that serum SP-D is increased in COPD as compared to controls [33]. As higher SP-D levels are linked with 'T' allele [22,29], our finding of the association of COPD with 'T' allele of rs721917 is consistent with this observation.

Several studies have previously linked rs721917 with various pathologies. Allele C (threonine variant) has been related with susceptibility to tuberculosis [18] and allergic rhinitis [20] whereas allele 'T' (methion-

ine variant) has been connected with respiratory syncitial virus (RSV) infection in infants [17] and atopy in asthma [22].

There are several limitations to our study. First, due to the modest sample size, we cannot entirely rule out the associations of the other two polymorphisms with COPD. Second, all our subjects were men. Although this limits the general application of our findings, on the positive side, this removed confounding by gender to our findings [38]. It should be also noted that we recruited patients with the same ethnicity (Pakistani Punjabi), which reduced the genetic heterogeneity in our study. Third the control population is not in Hardy Weinberg equilibrium for rs3088308. This has the potential of false positive associations in genetic association studies. However we did not find any association of SNP rs3088308 with COPD risk to begin with. The SNP which did show association with COPD did not depart from Hardy Weinberg equilibrium. Fourth, we evaluated only three SNPs, one from each functional domain of the molecule. The SNP rs721917 affects the N terminal domain (potentially disturbing multimerization of the molecules), rs2243639 affects collagen domain (disrupting collagen domain mediated immunological interactions) and rs3088308 affects the carbohydrate recognition domain (the main ligand binding domain). Five other coding SNPs have been described in public databases such as NCBI. A larger study including all the eight coding SNPs may provide additional insight on the role of SP-D in COPD. Finally we did not show significant correlations of smoking with either serum SP-D levels [40] or risk of COPD [35] in our study group. This may be due to the modest sample size or the fact that many of the patients were ex smokers with significant smoking history who had quit smoking but their disease progressed as is the general pattern. Still another reason for lack of finding an association between smoking and serum SP-D or COPD risk could be that our control group was predominantly composed of smokers with normal lung functions. This group could well be resistant to harmful effects of cigarette smoke.

5. Conclusions

The single nucleotide polymorphisms of SFTPD, rs721917 and rs3088308 are major determinants of serum SP-D levels and genotype T/T of SNP rs721917 is a risk factor for COPD.

Venues of research

Sampling: Pulmonology Department, Shaikh Zayed Medical Complex and Gulab Devi Chest Hospital, Lahore, Pakistan.

Spirometry: Heart Lung Lab, SZMC and Gulab Devi Chest Hospital Lahore, Pakistan.

Genetic Analysis: School of Biological Sciences, University of the Punjab, Lahore, Pakistan.

ELISA: Immunoassay lab, National Health Research Complex, SZMC, Lahore, Pakistan.

Funding

COI Disclosures: None of the authors have any conflicts of interests to declare

Informed consent was taken from all participants and the study conforms to the Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964).

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