ORIGINAL ARTICLE

SNP rs3088308 is a risk factor for poor lung function in healthy smokers

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Abstract

Objective: To see if single nucleotide polymorphisms of pulmonary innate immune molecule surfactant protein D were associated with poor lung function in smokers.

Methods: The study was conducted at Shaikh Zayed Hospital, Lahore, Pakistan, from April 2008 to August 2010, and comprised relatives and attendants of patients, as well as college and university students. Self-reported healthy smokers who demonstrated no airflow obstruction on spirometry were included. Deoxyribonucleic acid was extracted from their blood sample and genotyped for single nucleotide polymorphisms rs721917 and rs3088308 by polymerase chain reaction and restriction analysis. Serum was separated for measurement of surfactant protein D levels by a commercially available enzyme-linked immunosorbent assay based kit. Lung functions were compared between subjects possessing major and minor alleles using two-tailed Student's t-test. Multiple linear regression analysis was conducted to analyse the effect of age, smoking and the two single nucleotide polymorphisms on forced expiratory volume in 1 second.

Results: Of the 122 participants, all of whom were men, 98(80.33%) were smokers while 24(19.67%) had never smoked. Of the former, 90(91.84%) were current smokers and 8(8.16%) were ex-smokers. The overall mean age was 35.8±10.9 years. The mean surfactant protein D level was 121.4±61.6ng/ml. In case of rs3088308, all lung function variables were reduced in patients with a minor allele and the results for forced expiratory volume in 1 second ($p=0.016$), forced expiratory volume in 1 second (%) predicted ($p=0.009$), forced vital capacity ($p=0.048$) and forced vital capacity (%) predicted ($p=0.048$) were statistically significant. Age had the highest influence on lung function ($p<0.001$) followed by smoking status ($p=0.04$) and single nucleotide polymorphisms rs3088308 minor allele ($p=0.04$).

Conclusion: Single nucleotide polymorphisms rs3088308 was found to modulate serum surfactant protein D levels and may be a risk factor for development of chronic obstructive pulmonary disease among smokers.

Keywords: Smoking, Genetic polymorphism, Surfactant protein D, Risk of COPD. (JPMA 66: 1137; 2016)

Introduction

Smoking is associated with accelerated decline in lung function. However, smoking and lung function decline do not have a typical linear relationship as suggested by the fact that only 15-20% of all smokers develop chronic obstructive pulmonary disease (COPD). Thus there may be genetic factors that modulate the expression of smoking on lung function decline. One interesting but understudied molecule in the pathogenesis of smoking-related lung disease is surfactant protein D (SFTPD). SFTPD is a complex pneumoprotein that is involved primarily in innate immune response of the lung.[1] It modulates macrophage function to carefully orchestrate the inflammatory response against aero-pathogens and irritants. It also plays an important role in turning off the inflammatory response once the pathogens have been neutralised, thus limiting collateral damage from inflammatory and oxidative stress.[2-5]

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The human SFTPD gene is associated with multiple single nucleotide polymorphisms (SNPs) in protein coding as well as non-coding regions. For instance, SNP rs721917 causes amino acid substitution in N terminal domain of the mature protein whereas rs3088308 substitutes amino acids in the carbohydrate recognition domain. Both of these polymorphisms affect serum SFTPD levels.[6-8] However, the effects of these polymorphisms on lung function are not well documented. This study was planned to determine whether polymorphisms in SFTPD are associated with lung function in a group of healthy young men.

Subjects and Methods

The study was conducted at the Shaikh Zayed Hospital, Lahore, Pakistan, from April 2008 to August 2010, and comprised relatives and attendants of patients, as well as college and university students. The study was approved by the institutional review board. Informed consent was obtained from all the participants. The subjects were self-reported healthy men, and did not demonstrate clinically significant signs or symptoms indicative of respiratory disease. They were not on any medications. Their lung

function results showed no airway obstruction, with forced expiratory volume in 1 second/forced vital capacity (FEV1/FVC) > 0.70. The mean FEV1 was 3.22±0.59 litres and mean FEV1/FVC was 84.23±5.34 %. Spirometry was performed according to a standard protocol using electronic spirometer (Spirolab 2, SDI Diagnostics). Manoeuvres were repeated until technically acceptable curves were obtained. Best of three readings were considered.[9] Smoking history was measured in pack-years. Blood was sampled for deoxyribonucleic acid (DNA) extraction and serum separation. DNA was isolated from blood manually as previously described.[10] For genotyping, SFTPD gene was amplified for SNPs rs721917 and rs3088308 and restricted with HpyCH4 IV and Mn1I, respectively, as previously reported.[8,11] Random samples were genotyped twice for quality assurance. Serum SFTPD levels were measured using a commercially available kit (BioVendor - Laboratornimedicinaa.s. CT Park Modrice, Evropska 873 664 42 Modrice, Czech Republic) following the manufacturer's instructions. SPSS 17 for statistical analysis. Mean values of lung function variables were compared between minor and major allele groups using a 2-tailed, independent sample t-test. Multiple linear regression (MLR) analysis was conducted to analyse the effect of various predictor variables on lung function variable FEV1. The variables included in the regression model were age, smoking history, smoking status, 'A' allele at rs3088308 and 'C' rs7219197. Continuous variables (age, FEV1, and pack years) which were not normally distributed were transformed to their natural log. P<0.05 was considered significant.

Results

Of the 122 participants, all of whom were men, 98(80.33%) were smokers while 24(19.67%) had never smoked. Of the former, 90(91.84%) were current smokers and 8(8.16%) were ex-smokers. The overall mean age was 35.8±10.9 years. The mean SFTPD level was 121.4±61.6ng/ml. (Table-1). In terms of frequencies of genotypes, there were 84(69%) rs721917 and 89(73%) rs3088308 (Table-2).

Table-1: Subject characteristics.

Characteristic	N	Mean	Std. Deviation
Subjects age (years)	118	35.75	10.87
Body mass Index (kg/m2)			
◆ Underweight (14.91 - 18.40 kg/m2)-11 (9%)			
◆ Normal (18.41 - 22.90 kg/m2) - 34 (27.9%)			
◆ Overweight (22.91 - 27.50 kg/m2) 36 (29.5%)			
◆ Obese (27.51 - 40.00 kg/m2)- 30 (24.6%)	111	24.23	5.01
Pack years of smoking*			
◆ Never smoked- 24 (19.7%)			
◆ Smokers (current or ex-smoker): 98 (80.3%)	116	13.41	15.41
Serum SFTPD levels (ng/ml)	91	121.41	61.58

*Pack years: number of cigarettes smoked per day/20 x number of years of smoking.
SFTPD: Surfactant Protein D.

Table-2: Frequencies of genotypes in the subjects.

SNP (n)	Genotype	Frequency	Percentage
rs721917 (n=84)	M/M (C/C)	18	14.8
	M/m (T/C)	50	41.0
	m/m (T/T)	16	13.1
rs3088308 (n=89)	M/M (A/A)	50	41.0
	M/m (A/T)	25	20.5
	m/m (T/T)	14	11.5

SNP: Single nucleotide polymorphisms
M/M: homozygous for major allele;
M/m: heterozygous with major and minor allele;
m/m: homozygous for minor allele.

Mean FEV1 was 3.22±0.59 litres and mean FEV1/FVC was 84.23±5.34%.In case of rs3088308,all lung function variables were reduced in patients with a minor allele (T) and the results for FEV1(p=0.016), FEV1% predicted (p=0.009), FVC (p=0.048) and FVC% predicted (p=0.048) were statistically significant (Table-3,4). MLR (Table-5) revealed that age had the highest influence on lung function (p<0.001) followed by smoking status (p=0.04) and SNP rs3088308minor allele (p=0.04).T allele ofrs721917 was also linked to low FEV1 but the association did not reach statistical significance (p=0.08).

Serum surfactant protein D levels (sSFTPD) differed significantly between genotypes of both rs721917 and rs3088308 (Figure). In the case of rs721917, serum SFTPD levels were reduced with the 'C' allele (110.53 ± 49.04 ng/m) and raised (160.38±78.14ng/ml) with the 'T' allele (p=0.003). In the case of rs3088308, the minor allele (T)

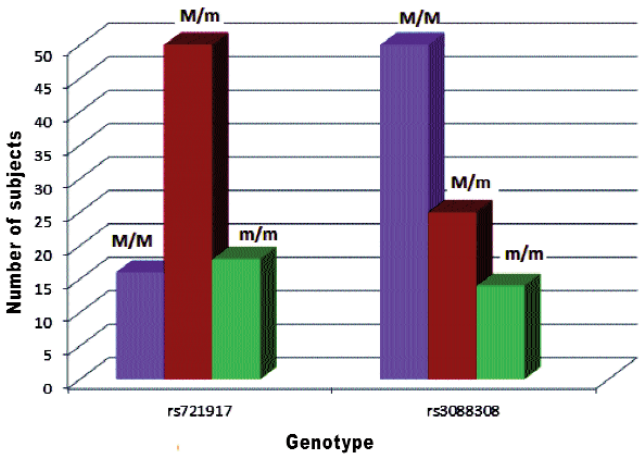

Figure: Genotype frequencies in the subjects.

Table-3: Lung function in major allele (A) and minor allele (T) group of rs3088308.

	rs3088308 genotype	N	Mean	Std. Deviation	Std. Error Mean	't' test p value
Forced Expiratory volume in 1st second in litres	M/M	44	3.34	0.64	0.09	0.016*
	M/m or m/m	25	2.97	0.51	0.10	
FEV1 % predicted	M/M	44	99.25	14.57	2.20	0.009*
	M/m or m/m	25	89.52	14.03	2.81	
Forced vital capacity in litres	M/M	44	3.96	0.75	0.11	0.048*
	M/m or m/m	25	3.61		0.55	0.11
FVC % predicted	M/M	44	98.05	14.38	2.17	0.048*
	M/m or m/m	25	90.80	14.28	2.86	
FEV1/FVC	M/M	44	84.23	4.53	0.68	0.086*
	M/m or m/m	25	82.16	5.32	1.06	
FEV1/FVC% predicted	M/M	44	104.16	5.99	0.90	0.334
	M/m or m/m	25	102.72	5.77	1.15	
Peak expiratory flow (PEF)	M/M	44	6.57	1.57	0.24	0.269
	M/m or m/m	25	6.12	1.70	0.34	
PEF % predicted	M/M	44	73.23	17.63	2.66	0.355
	M/m or m/m	25	68.96	19.45	3.89	
FEF2575	M/M	44	3.75	1.15	0.17	0.089
	M/m or m/m	25	3.27	1.01	0.20	
FEF2575 %predicted	M/M	44	84.07	19.43	2.93	0.076
	M/m or m/m	25	75.16	20.24	4.05	

M/M: homozygous for major allele;
M/m: heterozygous with major and minor allele;
m/m: homozygous for minor allele
FEV1: Forced expiratory volume in 1 second
FVC: Forced vital capacity. FEF: Forced expiratory flow. PEF: Peak expiratory flow

Table-4: Lung function in Major allele (C) and minor allele (T) group of rs721917.

	T allele in rs721917	N	Mean	Std. Deviation	Std. Error Mean	't' Test p value
Forced Expiratory volume in 1st second in litres	M/M	15	3.39	0.73	0.19	0.21
	M/m or m/m	51	3.17	0.58	0.08	
FEV1 % predicted	M/M	15	99.87	16.75	4.33	0.38
	M/m or m/m	51	96.00	14.43	2.02	
Forced vital capacity in litres	M/M	15	4.02	0.84	0.22	0.29
	M/m or m/m	51	3.80	0.65	0.09	
FVC % predicted	M/M	15	98.53	17.03	4.40	0.55
	M/m or m/m	51	95.98	13.78	1.93	
FEV1/FVC	M/M	15	84.66	5.33	1.38	0.312
	M/m or m/m	51	83.19	4.78	0.67	
FEV1/FVC %predicted	M/M	15	105.13	7.12	1.84	0.272
	M/m or m/m	51	103.24	5.42	0.76	
Peak expiratory flow	M/M	15	6.3267	1.33	0.34	0.913
	M/m or m/m	51	6.3790	1.69	0.24	
PEF % predicted	M/M	15	70.47	16.95	4.38	0.781
	M/m or m/m	51	71.98	18.84	2.64	
FEF2575	M/M	15	4.0147	1.34	0.35	0.106
	M/m or m/m	51	3.4786	1.04	0.15	
FEF2575 %predicted	M/M	15	86.80	23.04	5.95	0.253
	M/m or m/m	51	80.08	18.86	2.64	

M/M: homozygous for major allele;
M/m: heterozygous with major and minor allele;
m/m: homozygous for minor allele

Table-5: Determinants of FEV1 in healthy adults.

Predictor variables	Unstandardised Coefficients		Standardised Coefficients		
	B	Std. Error	Beta	t	Sig.
(Constant)	2.93	0.33		9.05	0.00
Natural Log of age in years	-0.40	0.08	-0.62	-5.14	0.00*
Natural Log of pack years	0.03	0.02	0.14	1.17	0.25
Smoker or Non smoker	-0.32	0.15	-0.22	-2.06	0.04*
A allele at rs3088308	-0.08	0.04	-0.22	-2.07	0.04*
T allele in rs721917	-0.08	0.04	-0.18	-1.76	0.08

Dependent Variable: Natural Log of FEV1 in liters, Adjusted R squared 0.381, ANOVA: 0.000
FEV1: Forced expiratory volume in 1 second
ANOVA: Analysis of variance.

was associated with significantly lower sSFTPD levels (99.61±46.04ng/ml) compared to the major allele, 'A', (134.15±64.66ng/ml) (p=0.013).

Discussion

SFTPD is an immune regulator of the lung. It is a lung specific biomarker, which may be useful for diagnosing and tracking progression of various lung diseases and its genetic polymorphisms have been associated with various lung pathologies.[12-20] The SNP rs721917 replaces methionine (nucleotide sequence ATG) with threonine (ACG) at residue number 11 in the amino terminal domain of the mature protein. This SNP modulates sSFTPD (serum levels), multimerises SFTPD[21] and may influence the pathogenesis of various diseases including tuberculosis (TB),[22] Influenza A virus,[23] respiratory syncytial virus (RSV) infection in infants[24] atopy,[25] allergic rhinitis[24] COPD,[12,14] interstitial pneumonia,[26] lung cancer[20] and Sjogren's syndrome.[27] Consistent with previous literature, the current study found that rs721917 associated with serum SFTPD levels.

The SNP rs3088308 causes a substitution at amino acid residue number 270 where threonine (Thr) replaces serine (Ser). This residue corresponds to the carbohydrate recognition domain of the mature protein which binds various pathogens as well as apoptotic cells. Although no clinical associations have yet been reported for rs3088308, it causes an amino acid substitution in the functional ligand binding site of this pattern recognition molecule,[28] and hence merited exploration. We have previously shown for the first time that rs3088308 is associated sSFTPD levels in COPD patients.[8] We found a similar association in the current study in healthy smokers. Our results are in agreement with a recent study by Johansson et al.,[29] who investigated the SFTPD polymorphism in smokers. They also reported a significant correlation of rs3088308 with sSFTPD levels. Their results however differed from ours with respect to the association of lung function with rs3088308. They did not find significant correlation of lung function with this SNP. In contrast, we found in the present study that most of the lung function variables were significantly higher in subjects carrying the major allele. Rs3088308 causes amino acid substitution in the carbohydrate recognition domain of the mature protein. This may affect the functionality of this molecule, which in turn may predispose certain individuals to accelerated lung function decline in response to environmental insults such as cigarette smoke. As far as rs721917 is concerned, Johansson et al. reported significant correlation with lung function. Our results are more in line with Foreman et al.'s findings, which reported a lack of significant association of lung function with rs721917.[6] Differences between our findings and Johansson et al.'s study may be explained by differences in genetic and ethnic backgrounds of the study subjects.

There were several limitations to our study. First, the sample size was relatively small. However, unlike genome-wide association studies (GWAS), our study interrogated targeted sites for genotyping, enabling us to have sufficient power to assess their relationship with lung function. Second, the cohort was composed only of men. While it is a weakness of the study, it is also a strength as it reduced the variability of the cohort and largely removed the effects of biomass on lung function. Third, we studied young men, who did not have airways disease such as COPD. As such, we could not evaluate the relationship of the SFTPD polymorphisms with COPD. However, an important strength was that because these young men were healthy, the relationship of SFTPD polymorphisms with lung function was not confounded by co-morbidities or medications. Further, because it is well known that reduced lung function in young adulthood is a significant risk factor for future development of COPD, we were able to evaluate the effects of these polymorphisms in young men, who are predisposed to COPD.[30]

Conclusion

SNP rs3088308 was found to modulate serum SFTPD levels and therefore is a risk factor for accelerated lung function decline in healthy smokers, and may lead to COPD.

Disclaimer: None.

Conflict of Interest: None.

Funding: The study was funded by the Higher Education Commission (HEC) Pakistan.

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