No Association between Coding Polymorphism within Exon 4 of the Human Surfactant Protein B Gene and Pulmonary Function in Healthy Men

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Abstract: The coding polymorphism (rs1130866) within the surfactant protein B gene is known to associate with certain respiratory abnormalities. We investigated, using spirometry and fluorescence-based PCR, whether this variant influenced pulmonary function in healthy, nonsmoking men. We found no association of pulmonary function with genotype at the rs1130866 locus.

Key words: pulmonary function, genotype.

An important component of the respiratory system is the protein and phospholipid-based composition known as pulmonary surfactant. Pulmonary surfactant helps to lower the surface tension at the air–water boundary of terminal bronchioles and therefore aids alveolar gas exchange [1]. Several surfactant-associated proteins (SPs) are also known to play an active part in innate immunity by interacting with certain pathogenic organisms as well as inflammatory cells leading to cytokine production and phagocytosis [2].

One of the SPs, surfactant protein B (SFPB), is encoded by a 9.5 kb gene that resides on chromosome 2 in humans [3, 4]. The expression of SFPB is controlled by 5' flanking enhancer/transcription factor binding sequences [5], and transcription is sensitive to a range of modulating factors [5–7]. Newly translated SFPB protein undergoes complex cellular processing [8] and plays a central role in regulating surfactant composition and in the recycling of several surfactant-related molecules [1, 9]. A single nucleotide polymorphism (SNP) within exon 4 of SFPB (rs1130866) is characterised by a T/C substitution. Possession of the T allele causes isoleucine to replace threonine at position 131 in the immature SFPB protein [10], resulting in the removal of an N-linked glycosylation site at the Asp 129 residue [11, 12]. The polymorphism is known to associate with certain respiratory abnormalities [13, 14, 15], but its effect on pulmonary function as a quantifiable trait in disease-free individuals remains unclear. With this in mind, we specifically wished to assess whether the rs1130866 polymorphism could influence pulmonary function as a quantitative trait in healthy nonsmoking men.

Study participants (N = 55) were all healthy, nonsmoking males aged 18–42 years. The cohort consisted of 49 Caucasians, 2 Asian, 1 Black African and 3 of the participants were of mixed ancestry. Informed consent was obtained from each individual before the start of the investigation. The study was in accordance with the Declaration of Helsinki, and the research protocol was approved by the School of Health’s Research Ethics Committee.

Height (m) was measured whilst standing upright, using a stadiometer, and body mass (kg) was measured using a standard balance. Body mass index (BMI) was determined by dividing body mass (kg) by height (m²). Pulmonary function was assessed by the use of a handheld spirometer (Model No. 2120, Vitalograph, UK). Participants were shown how to use the device and asked to perform the manoeuvre three times with nose pinched. The mean of three manoeuvres was used for subsequent data analysis. Using this approach, we measured both forced vital capacity (FVC) and forced expiratory volume per second (FEV1).

DNA was extracted from whole blood using a Flexigene kit (Qiagen, UK) and following the manufacturer’s
instructions. DNA was quantified using a GeneQuant Pro spectrophotometer (Amersham Biosciences, UK). We employed 5' nuclease polymerase chain reaction technology (Taqman®) to amplify DNA (Applied Biosystems, Foster City, CA) using a MiniOpticon real-time thermocycler (BioRad, UK). Each reaction (25 µl) contained forward 5'-GCAGGAGGTGAGCTTGCA-3' and reverse 5'-CCCTGGTCATCGACTACTTCCA-3' primers (both at 1 µM), allele-specific fluorescently labelled reporter probes VIC-5'-CCCTCACAATCTGGT-3' (T allele probe), FAM-5'-CCCTCACAGTCTGGT-3' (C allele probe), both at 0.2 µM, and PCR universal master mix (Applied Biosystems, Foster City, CA) containing ampliTaq DNA polymerase Gold. The PCR protocol consisted of 95°C (10 min) followed by 40 cycles of 15 s at 92°C and 1 min at 60°C. Fluorescence for both FAM and VIC reporter signals were measured at the end of each cycle, and genotypes ascertained using MJOpticon Monitor analysis software (version 3.1). Allele calls were analysed for any deviation from Hardy-Weinberg equilibrium using a χ² test and mean, age, height, adjusted FVC and FEV1 values, expressed as percent of expected; for each genotype, were compared by analysis of variance (SPSS version 11.5) with Tukey’s post hoc test. The null hypothesis was rejected at P < 0.05.

The frequencies of the C and T alleles were found to be 0.55 and 0.45, respectively, and there was no deviation from Hardy-Weinberg equilibrium (P = 0.88). The genotype distribution in our cohort was CC (N = 16), CT (N = 29) and TT (N = 10). Mean (±SD) BMI (27.9 ± 4.5), age (25.2 ± 6.0 years), body mass (92.3 ± 16.9 kg) and height (1.81 ± 0.09 m) were all independent of genotype. Figure 1 shows a genotyping plot obtained following PCR of participants’ DNA, using allele specific probes containing the VIC and FAM reporter dyes. Genotypes were identified by endpoint fluorescence. Figure 2 shows the FVC and FEV1 values obtained for each genotype expressed as a percent of expected value after adjustment for age and height. FVC and FEV1 were found to be unrelated to genotype.

The data obtained from this study infer that the common SNP (rs1130866) within the human SFPB gene does not seem to influence pulmonary function in healthy, non-smoking men. The findings are of particular interest as possession of the T allele leads to an exchange of polar threonine, for nonpolar isoleucine at residue 131 in the protein [10]. Although the biological consequences of this in humans are not fully understood, it has been shown, through the use of modified SFPB sequences cloned into an expression system that residue 131 is in a part of the propeptide necessary for intracellular transport [17]. Accordingly, our data would indirectly imply that transport of the propeptide from the endoplasmic reticulum to the cell exterior is unaffected by the presence of either threonine or isoleucine at position 131.
instead of a C nucleotide within codon 121 of the gene, is associated with fatal respiratory distress in neonates [18], and variation within intron 4 has been associated with susceptibility to lung cancer in chromate workers [19]. Indeed this latter point has led the authors to speculate that typing for intron 4 variants might be a useful screening procedure that could be employed before permitting individuals to work in the chromium industry [19]. Furthermore, using transcript analysis and ELISA, investigators have recently reported that the C allele of the C/A (−18) promoter polymorphism influences the amount of SFPB 4 detectable in bronchoalveolar lavage [20]. The amount of SFPB 4 protein produced may have an impact on pulmonary function, especially for individuals with lung diseases [20].

Although we found no association with genotype at the rs1130866 locus and FVC or FEV1, it is likely that other variants in SPs, or other genes important for pulmonary function, will have measurable effects on the trait in healthy individuals as pulmonary function is known to be a polygenic trait with high heritability [21–23]. Moreover, identifying how genomic variation affects pulmonary function in the absence of pathology may prove important for our future predictability of pulmonary disease. This is supported by studies showing that in young adults, poorer respiratory function is a factor that increases the risk of developing chronic obstructive pulmonary disease (COPD) in later life [24].

In terms of abnormal physiology, Wang and co-workers have previously speculated that the rs1130866 polymorphism may require other interacting factors to alter respiratory phenotype [12]. Indeed, this hypothesis now seems likely when we consider the following evidence. Firstly, our data suggest that pulmonary function in healthy nonsmoking males is not related to the rs1130866 SNP. The apparent lack of other “interacting” pathology in our cohort may account for our findings. Secondly, Guo et al. [13] have shown the importance of gene–gene and gene–environment interaction with the C allele of rs1130866 in a Mexican-based cohort. In this study, the C allele was specifically found to interact with the D25388 microsatellite marker and smoking in COPD individuals [13]. Further, using a family-based association study, Hersh et al. [16] have identified an association of the rs1130866 polymorphism with restricted pulmonary function in COPD. Quantitatively, FEV1 and FEV1/FVC did not associate with the polymorphism in this study, using mainly female probands. The same authors have also established a significant interaction of the rs1130866 polymorphism with smoking and COPD, using a mixed gender case-control design [16]. Interaction between surfactant protein A alleles and rs1130866 have been documented to modify the risk of respiratory distress syndrome [25, 26], and a recent study has reported that the C allele is associated with acute respiratory distress syndrome and need for assisted ventilation in patients with pneumonia [27].

In conclusion, we sought to establish whether association exists between the rs1130866 polymorphism and quantitative pulmonary function in a healthy, relatively young male-only cohort. Our initial findings infer that replacing threonine for isoleucine at position 131 of the SFPB propeptide does not translate into altered pulmonary performance in young men who are free of respiratory pathology. However, as pulmonary function declines in later life [28], it may be possible that the rs1130866 SNP exerts some influence over the phenotype in older populations. Finally, we suggest that confirmation of this experiment would be achieved by using a larger sample size with enhanced statistical power.

REFERENCES