ORIGINAL ARTICLE

MUC5AC and inflammatory mediators associated with respiratory outcomes in the British 1946 birth cohort

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ABSTRACT

Background and objective: Dysregulation of respiratory mucins, MUC5AC in particular, has been implicated in respiratory disease and MUC5AC expression is up-regulated in response to environmental challenges and inflammatory mediators. The aim of this study was to examine the effect of genetic variation on susceptibility to common respiratory conditions.

Methods: The association of MUC5AC and the closely linked genes MUC2 and MUC5B with respiratory outcomes was tested in the MRC National Survey of Health and Development, a longitudinal birth cohort of men and women born in 1946. Also examined were the functional variants of the genes encoding inflammatory mediators, IL13, IL1B, IL1RN, TNFA and ERBB1, for which there is a likely influence on MUC5AC expression and were explored potential gene–gene interactions with these inflammatory mediators.

Results: Statistically significant associations between the 3’ter MUC5AC simple nucleotide polymorphism (SNP) rs1132440 and various non-independent respiratory outcomes (bronchitis, wheeze, asthma, hay fever) were reported while the adjacent loci show slight (but largely non-statistically significant) differences, presumably reflective of linkage disequilibrium (allelic association) across the region. A novel association between bronchitis and a non-synonymous functional ERBB1 SNP, rs2227983 (aka epidermal growth factor receptor: R497K, R521K) is also reported and evidence presented of interaction between MUC5AC and ERBB1, and between MUC5AC and IL1RN with respect to bronchitis. The ERBB1 result suggests a clear mechanism for a biological interaction in which the allelic variants of epidermal growth factor receptor differentially affect mucin expression.

Conclusions: The MUC5AC association and the interactions with inflammatory mediators suggest that genetically determined differences in MUC5AC expression alter susceptibility to respiratory disease.

Key words: airway epithelium, asthma, genetics, inflammation, respiratory function test.

INTRODUCTION

Disturbances to the normal dynamics of the respiratory epithelial layer as a result of allergens, microorganisms and noxious agents that cause inflammation often leads to secretion of large quantities of airway mucus which allows the expulsion of the offending agent. In chronic airway disease, this mechanism is intensely active for lengthy periods, exacerbating disease symptoms, and in severe asthma, some airways become irreversibly occluded. Genetically determined differences in MUC5AC and MUC5B expressions are important factors in determining susceptibility to respiratory disease.

The major high molecular-weight glycoprotein components of respiratory mucus are the mucins MUC5AC and MUC5B. The quantities of MUC5AC and MUC5B are increased in a number of respiratory...
conditions and experimental models and expression of their genes, MUC5AC and MUC5B are up-regulated. Several mediators of inflammation such as the cytokines, interleukin 13 (encoded by the gene IL13), interleukin 1B (encoded by IL1B) and tumour necrosis factor α (encoded by TNFA), which are present at high levels within the asthmatic airways, are thought to orchestrate respiratory mucus hypersecretion and are known to up-regulate MUC5AC expression as well as secretion, and MUC5AC is regulated through an epidermal growth factor receptor (EGFR, encoded by the gene known as ERBB1) signalling pathway. Previous studies showed that the genetically variable MUC2 tandem repeat (TR) sequence of the main mucin domain had a different size distribution in atopic individuals with and without asthma. Although MUC2 expression shows some evidence of up-regulation in inflammatory disease, biologically, MUC2 appears a rather unlikely candidate for altering respiratory disease susceptibility because the protein is found at only very low levels in the airways. MUC5AC is located adjacent to MUC2 in a region of strong linkage disequilibrium (allelic association) on chromosome 11p15.5. Thus the association seen between MUC2 and asthma could in fact be a consequence of association between the MUC2 TR and a causative allele (variant) in MUC5AC, or possibly MUC5B. Here we explore the possible association between variants in these MUC genes (see Table S2 in the supporting information available online) and various respiratory- and allergy-related outcomes in the 1946 British birth cohort. We also test for gene–gene interactions with functional single nucleotide polymorphism (SNP) within the genes IL13, IL1B, TNFA and IL1RN which encodes the interleukin 1 receptor agonist and interacts with IL1B (see Table S1 in the supporting information available online), and ERBB1 with respect to disease outcome.

**METHODS**

**Study participants**

The MRC National Survey of Health and Development is a socially stratified sample of 5362 of all British births during 1 week of March 1946. The data collections from which we have collated information were carried out at age 43 (1989) and 53 years (1999) when research nurses visited study members in their own homes and asked a series of health and lifestyle questions. At age 53, 2989 of the cohort members were interviewed. Contact was not attempted for the 1979 individuals who had previously refused to take part, were living abroad, were untraced since the previous contact at 43 years or had already died. The responding sample at age 53 is in most respects representative of the national population of a similar age and considered to be representative of a European population since the study began before mass immigration into the United Kingdom. Blood and buccal samples were collected from consenting participants at age 53 (ethical approval reference MREC no. 98/2/121).

**Outcome variables**

Table 1 shows a description of all outcome variables and measures. The outcome variables indicating whether individuals had ever had asthma or hay fever were as described previously. Forced expiratory volume in 1 s and forced vital capacity, were recorded at each visit using a Micromedical turbine electric spirometer (Cardinal Health UK 232 Ltd, Basingstoke, UK).

**Confounders**

Potential confounders were chosen because they were previously reported to be significantly associated with one or more of the outcome variables, or considered to be of direct biological relevance. These were smoking status, childhood social class, own social class, gender and region of birth, as well as height for lung function measurements (see Table 2 for demographic details of the key outcome and confounder variables).

**Genotyping**

Details of DNA extraction, genotyping and validation as well as choice of SNP are given in the online supporting information (Text and Tables S1, S2 in the supporting information available online).

**Data analyses**

LDmax (http://www.sph.umich.edu/csg/abecasis/GOLD/docs/ldmax.html) was used to calculate pairwise measures of linkage disequilibrium. All further statistical analyses were performed using SPSS or STATA software. For each categorical outcome, contingency tables were constructed to compare the distribution of genotypes or alleles between the ‘affected’ and ‘unaffected’ groups with respect to disease variables. Multiple logistic regression models were then used to adjust the important associations for potential confounders. For these analyses, each of the SNP markers was coded by genotype (co-dominant model, with alleles grouped where necessary—see supplementary information). MUC5ACTR has two common length alleles and several rare ones which were considered as genotypes made up of three alleles L (long) S (short) and R (rare). For binary (yes/no) outcomes, because the MUC2 TR data are recorded as a continuous variable, we compared the MUC2 TR allele size distributions (using a Mann–Whitney test) between the two groups, as done for our previously published study in which allele length was associated with asthma. Regression analysis was carried out to relate MUC2 allele length to lung function, using the combined MUC2 allele lengths for each individual and categorized into four gender-specific quartiles. Finally, potential interactions between each of the inflammatory loci and MUC5AC rs1132440 were explored and assessed using the likelihood ratio test.
To display these differences in distribution in a simple manner graphically, we combined heterozygotes and homozygotes for the minor allele in each case.

**RESULTS**

11p15.5 mucin gene variants typed in the National Survey of Health and Development cohort

Details and allele frequencies for polymorphisms within MUC2, MUC5AC and MUC5B are shown in Table S2 in the supporting information available online. No significant deviation from Hardy–Weinberg equilibrium was observed for any polymorphism. The MUC2 TR allele lengths ranged from 3.21 to 11.64 kb. As previously reported,23,26 there was a major mode between 7 and 8 kb with a minor mode of around 4 to 4.5 kb.

Linkage disequilibrium within the 11p15.5 MUC gene complex

As reported previously for other markers in this region,26 all of the MUC5AC and MUC5B markers are significantly associated with one or more of the others (see Table S3 in the supporting information available online).

Statistically significant association was also found between the MUC2 TR allele length distribution and the MUC5AC TR genotypes, LL and LS being associated with shorter MUC2 alleles, \((P < 0.001,\) Kruskal–Wallis test) as found previously using family inferred haplotypes.26 The MUC2 TR and MUC5AC rs1132440 showed a similar trend, although this was not statistically significant \((P = 0.084).\) Thus a general pattern of association can be seen to extend from MUC2 to MUC5AC.

Tests of association between mucin genetic variants and respiratory variables

Each mucin genetic variable (see Table S2 in the supporting information available online) was analysed for association with each of the respiratory outcomes detailed in Table 1. MUC5AC rs1132440 genotype counts showed statistically significant association with hay fever, bronchitis and wheeze at 43 years \((3 \times 2\) contingency tables chi-square \(P\)-values 0.001 to 0.02) and were marginally associated with asthma \((P = 0.06,\) Table S4).
in the supporting information available online). Curiously, for all outcomes, there was an increase in heterozygote frequency and a decrease in the rarer GG homozygote in affected individuals (Fig. S1 in the supporting information available online). The change in heterozygote frequency had the effect of causing a statistically significant deviation from Hardy–Weinberg equilibrium in both the yes and no groups for hay fever \( (P = 0.01) \). Allele count differences \( (2 \times 2 \) contingency tables) were only statistically significant for bronchitis and wheeze \( (P = 0.026 \) and 0.027 respectively).

The **MUC5AC** TR genotype variable showed marginally significant association with hay fever \( (P = 0.044) \) but was not significant with any other outcomes. Because the **MUC5AC** TR dataset is somewhat smaller than the SNP dataset (Table S2 in the supporting information available online) because of the requirement for high-quality blood DNA for the Southern blot analysis, tests for association of **MUC5AC** rs1132440 with all outcomes were also performed on the smaller dataset. Significance remained for bronchitis, wheeze and hay fever, suggesting that associations with **MUC5AC** rs1132440 are stronger than those with **MUC5AC** TR. For the **MUC5B** SNP data, a significant association was observed between the exon 2 SNP (rs2672785) and wheeze at 43 years \( (P = 0.022) \). There was a trend towards longer **MUC2 TR** alleles in the asthma and wheeze groups, but this was not statistically significant.

The measures of lung function (forced expiratory volume in 1 s, forced vital capacity, \( \Delta \) forced expiratory volume and forced expiratory volume in 1 s/forced vital capacity), adjusted for gender and height, showed just one significant association, namely heterozygotes for **MUC5B** rs2672785 showed slightly \( (1\%) \) but significantly reduced forced expiratory volume in 1 s/forced vital capacity in 1989 \( (P = 0.028) \). This remained significant after full adjustment for the other confounders.

Because **MUC5AC** rs1132440 showed both stronger association and association with more respiratory outcomes than any of the other loci, all further analyses were conducted using only this locus.

### Adjusting for confounders and identifying risk genotypes

In an adjusted model, all previously identified associations remain significant (Table 3).

The rare homozygote genotypes of rs1132440 appeared to confer protection against bronchitis \( \) (odds ratio \( 95\% \) confidence interval) \( = 0.689 \) \( (0.50–0.94) \), wheeze \( (0.382 \) \( (0.20–0.74) \)) and asthma \( (\text{odds ratio} = 0.614 \) \( (0.40–0.94) \)) as the odds ratios are significantly less than 1 \( (P-\text{values} \leq 0.025 \) in all cases) (Table 3). For hay fever, heterozygosity appeared to confer risk \( \) (adjusted odds ratio \( 95\% \) confidence interval) \( = 1.24 \) \( (1.00–1.54) \); \( P = 0.049 \).

### The inflammatory markers

Details of the inflammatory response markers tested are given in Table S1.

Significantly different allelic distributions between the affected and unaffected groups were found for: the **IL13** promoter SNP \( (\text{rs1800925}) \) in asthma \( (P = 0.038) \); the **IL13**exonic SNP \( (\text{rs20541}) \) in asthma \( (P = 0.0007) \)*; the **ERBB1** SNP \( (\text{rs2227983}) \) in bronchitis \( (P = 0.007) \). For both **IL13** SNPs, the rare allele confers risk and is overrepresented in the asthmatic affected group. In contrast, the rare **ERBB1** rs2227983 allele is significantly underrepresented in the affected bronchitis group. Logistic regression analysis showed significant associations between genotype and outcome in each of these cases, which remained significant after adjustment for the potential confounders and the association between **IL1B** and asthma became significant (Table 4).

\*The associations between both **IL13** SNP and the asthma outcome on this dataset have been reported previously.\textsuperscript{28}
Tests for gene–gene interactions

Significant interactions with respect to bronchitis were identified between MUC5AC rs1132440 and ERBB1 rs2227983 (P = 0.019), IL1RN VNTR (P = 0.009) and TNFA rs1800629 (P = 0.046). The ERBB1 and IL1RN interactions are illustrated graphically in Figure S2 in the supporting information available online. The association of MUC5AC with bronchitis is only significant in individuals who lack the IL1RN*2 (risk) allele (Fig. S2A in the supporting information available online) and only in individuals homozygous for the ERBB1 common rs2227983 (r) allele—that is non-carriers of the rarer K allele (Fig. S2B in the supporting information available online).

DISCUSSION

Abnormal expression of mucins is a central feature of airway pathology. Here we report significant associations between a MUC5AC SNP rs1132440 and occurrence of asthma, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. Although this particular SNP, wheeze, hay fever and bronchitis. 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Table 4  Association between inflammatory mediator genotypes and respiratory outcomes

| Variable    | ERBB1 (L and S)* | ERBB1 (rs2227983) | IL13 (rs180925) | IL13 (rs20541) | IL1B (rs16944) | IL1RN (TR) 2 and X | TNF (rs1800629) |
|-------------|------------------|-------------------|-----------------|----------------|----------------|-------------------|-----------------|
|             | 1          | 2          | 1          | 2          | 1          | 2          | 1          | 2          | 1          | 2          | 1          | 2          | 1          | 2          |
| Ever bronchitis | 0.96     | 1.01     | 0.90    | 0.53     | 1.22     | 1.02     | 1.03     | 1.03     | 0.93     | 1.02     | 1.08     | 0.93     | 0.92     | 0.99     |
| 1989        | (0.75–     | (0.76–   | (0.72–   | (0.32–   | (0.98–   | (0.57–   | (0.82–   | (0.55–   | (0.75–   | (0.72–   | (0.87–   | (0.64–   | (0.73–   | (0.57–   |
|             | 1.23)    | 1.34)    | 1.11)   | 0.88)    | 1.52)    | 1.82)    | 1.29)    | 1.91)    | 1.15)    | 1.44)    | 1.33)    | 1.37)    | 1.14)    | 1.72)    |
| wheeze 1989 | 1.20     | 1.03     | 0.69    | 1.20     | 0.82     | 0.85     | 0.65     | 0.65     | 1.11     | 1.32     | 0.99     | 1.75     | 0.76     | 1.81     |
|             | (0.77–   | (0.60–   | (0.46–   | (0.62–   | (0.54–   | (0.30–   | (0.41–   | (0.20–   | (0.76–   | (0.74–   | (0.67–   | (0.99–   | (0.50–   | (0.83–   |
|             | 1.87)   | 1.77)   | 1.02)   | 2.34)   | 1.25)   | 2.41)   | 1.02)   | 2.12)   | 1.64)   | 2.38)   | 1.47)   | 3.08)   | 1.16)   | 3.92)   |
| Ever asthma | 1.04     | 1.01     | 1.13    | 1.13     | 1.45     | 1.02     | 1.47     | 1.46     | 1.20     | 1.54     | 1.22     | 1.54     | 0.87     | 1.39     |
| 1999        | (0.75–   | (0.69–   | (0.92–   | (0.76–   | (1.08–   | (0.46–   | (1.11–   | (0.68–   | (0.90–   | (1.00–   | (0.92–   | (0.98–   | (0.64–   | (0.74–   |
|             | 1.45)   | 1.49)   | 1.38)   | 1.66)   | 1.93)   | 2.26)   | 1.96)   | 3.14)   | 1.60)   | 2.37)   | 1.63)   | 2.41)   | 1.17)   | 2.63)   |
| Ever hay fever | 1.10    | 1.02     | 0.90    | 1.15     | 1.24     | 0.96     | 1.13     | 1.22     | 1.12     | 0.98     | 1.04     | 1.14     | 0.84     | 1.19     |
| 1999        | (0.87–   | (0.78–   | (0.67–   | (0.68–   | (1.00–   | (0.55–   | (0.91–   | (0.69–   | (0.92–   | (0.70–   | (0.85–   | (0.91–   | (0.68–   | (0.73–   |
|             | 1.39)   | 1.34)   | 1.19)   | 1.95)   | 1.53)   | 1.67)   | 1.39)   | 2.17)   | 1.37)   | 1.38)   | 1.27)   | 1.61)   | 1.04)   | 1.94)   |

Logistic regression odds ratios (OR) after adjusting for the confounders listed in Table 3; 1 is heterozygote and 2 is homozygous for the rarer or risk allele. Significant associations are shown in bold and OR 95% confidence intervals are in parentheses. ERBB1 microsatellite and IL1RN VNTR are multiallelic so to simplify analysis; the allelic data were binned into two appropriate categories, defined by reviewing the literature for allelic functional relevance. For the ERBB1/EGFR microsatellite, repeat numbers were defined as either short (S) or long (L). S being 8–18 repeats of 20 or greater denoted as L. The IL1RN tandem repeat lengths were categorized as 2 or X; 2 referring to the IL1RN*2 allele (previously described risk allele) and X includes all other alleles (IL1RN*1, 3, 4 and 5). N values range from 2194–2361.
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Supporting Information
Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Figure S1 Bar charts showing as percentages the MUC5AC rs1132440 genotype frequency distribution in the affected and unaffected groups for outcomes bronchitis 1989, wheeze most days and nights 1989, ever asthma (1999) and ever hay fever (1999). See Table S1 for n values. Note that in each case the heterozygotes show elevated frequencies in the yes group while the rarer homozygotes show lower frequency.

Figure S2 Bar charts showing the distributions as percentages of the MUC5AC rs1132440 genotypes in carriers and non-carriers of minor/risk alleles of ERBB1 and IL1RN with respect to Ever Bronchitis. A IL1RN *2 a) carriers of the IL1RN risk allele (22, 24, 25, 26) (NS). b) non-carriers of the IL1RN risk allele (34, 44, 45, 46, 55) P = 0.004. B ERBB1 rs2227983. (a) carriers of the rare ERBB1 allele (AA and AG) (NS). (b) non-carriers (GG, and homozygous for the ancestral allele) P < 0.001. P-value from Pearson chi-square; NS, not significant.

Table S1 Details of variants within the genes encoding the inflammatory mediators and functional evidence for these. Note that the marker names refer to physical positions within the gene or its regulatory sequence. t rs2227983 was previously listed as rs11543848 (NDBC dbSNP). Minor allele frequencies (MAF) range from 0.17 to 0.49 and genotype distributions were in accordance with Hardy–Weinberg equilibrium, with the exception of IL1B rs16944 (P = 0.01).

Table S2 MUC5AC and MUC5B polymorphisms typed on the 1946 cohort. Note that for simplicity the MUC5AC TR alleles have been categorized into a tri-allelic model where r refers to all rare alleles.

Table S3 Pairwise linkage disequilibrium (LD) measures for MUC5AC and MUC5B markers. (a) Significance of association is shown as chi-square P-values. (b) D’ measure of LD. Loci are shown in chromosomal order from MUC5AC TR through to rs2075859 in exon 9 of MUC5B (see Supporting Table S1) Note that all adjacent SNP are highly associated with each other. Although there is breakdown of LD in between exons 2 and 9 of MUC5B, with no significant association between rs2672785 and rs2075859, LD is still detectable across the two MUC5 genes since MUC5AC rs1132440 and MUC5ACTR are significantly associated with one of the MUC5B SNP even though the D’ values are small. Significant values are shown in bold.

Table S4 Chi-square P-values from contingency tables of MUC5AC and MUC5B genotypes and the categorical respiratory outcomes; Mann–Whitney P-value for MUC5ACTR. N values given are for MUC5AC rs1132440; those for other loci, in particular MUC2 TR and MUC5AC TR are a little lower (see manuscript text), y/n signifies yes or no as indicated on Table 1. Similar empirical P-values were obtained by permutation analysis.