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RESEARCH ARTICLE

Association between the length of the *MUC8*-minisatellite 5 region and susceptibility to chronic obstructive pulmonary disease (COPD)

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Abstract In asthma and chronic obstructive pulmonary disease (COPD), mucins display disease-related alterations caused by airway mucus obstruction. *MUC5AC, MUC5B* and *MUC8* are known as the major secretory mucins in human airway epithelial cells. Analysis of mucin genes has identified the presence of several features with a variable number of tandem repeats (VNTR; minisatellites) in the central region of each mucin. In our previous study, six minisatellites in the region of the *MUC8* gene were identified, and the *MUC8*-MS5 minisatellite showed the highest heterozygosity among them. In this study, we evaluated the relationship between *MUC8*-MS5 and susceptibility to asthma and COPD. A case-control study was performed with 229 controls, 123 COPD cases and 77 asthma cases. A significant association (OR 3.96) between short alleles (2/2 repeats) and the occurrence of COPD was observed [95% confidence interval (CI) 1.32–11.88; $p = 0.008$]. Hence, the increased frequency of 2/2 homo-short alleles were also found in asthma cases (3.11; CI 0.88–11.05; $p = 0.066$), though this association was not statistically significant. These results revealed a genetic association between *MUC8* and COPD, and that the specific short minisatellite alleles (2/2) of *MUC8*-MS5 may be a risk factor for COPD.

Keywords *MUC8* · COPD · Asthma · Risk factor · Minisatellite

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Introduction

Epithelial surfaces of human respiratory, gastrointestinal and reproductive tracts are protected by mucus secretions (Martínez-Antón et al. 2006). Mucus is secreted by the airway surface epithelium and glands, and it plays an important role in protecting the human airway from the external environment. In chronic inflammatory airway diseases, mucins display disease-related alterations in quantity, composition and glycosylation (Hollingsworth and Swanson 2004). There are seven secreted mucins, including five polymeric mucins (*MUC2, MUC5AC, MUC5B, MUC6*, and *MUC19*) and two nonpolymeric glycoproteins (*MUC7* and *MUC8*). *MUC7* and *MUC8* also encode mucin-like glycoproteins lacking a transmembrane domain (Byrd and Bresalier 2004). *MUC5AC, MUC5B* and *MUC8* are the airway mucin genes most prominently involved in chronic respiratory diseases (Yuta et al. 1997; Nadel 2001): *MUC5AC* and *MUC5B* are found in higher amounts in asthma and COPD patients. Furthermore, overexpressed *MUC8* was also identified in respiratory organs related to chronic inflammatory respiratory organs (Basbaum et al. 1999; Bernacki et al. 1999; Song et al. 2008).

In chronic inflammatory airway diseases, such as asthma and COPD, mucins display alterations in quantity, composition and glycosylation. Asthma and COPD affect more than 500 million people worldwide and are characterized by mucus hypersecretion as a common clinical feature (Ha and Rogers 2016). Despite recent advances and multiple available therapies and interventions, there remains a clinical need to determine the mechanisms that exacerbate airway diseases. The development of clinical biomarkers for use in prognosis will allow easier detection of conflict, and intervention can take place before the disease progresses.

Human minisatellites are located predominantly in the subtelomeric regions of chromosomes (Jeffreys et al. 1985), and *MUC8* is located at q24.3 of *MUC8* on chromosome 12 (Thornton et al. 2008). In many reports, the length of minisatellites of several genes (*HRAS1, hTERT, BORIS, MUC2* and *MUC6*) has been associated with susceptibility to certain human disorders (Krontiris et al. 1993; Leem et al. 2002; Jeong et al. 2007; Kwon et al. 2010; Yoon et al. 2010). We focused on the major components of mucus, involved in respiratory disorders, which are coded by mucin genes; these genes contain minisatellite regions (Vinall et al. 1998; Thornton et al. 2008). In a previous study, we characterized the genomic features of the *MUC8* region, and six novel minisatellites (*MUC8*-MS1, -MS2, -MS3, -MS4, -MS5, -MS6) were analyzed (Lee et al. 2009). Among those minisatellites, *MUC8*-MS5 showed the highest heterozygosity ($h = 0.662$) in the *MUC8* region. In several reports, abnormal expression of *MUC8* occurred in various diseases and in conditions associated with high risk to respiratory organs (Basbaum et al. 1999; Bernacki et al. 1999; Song et al. 2008). Therefore, we examined the association between *MUC8*-MS5 and asthma or COPD in this study. Here, we report the allelic variations of *MUC8*-MS5 and its association with asthma or COPD.

Materials and methods

Study population

We conducted a case-control study for the minisatellites of *MUC8*-MS5 using the genomic DNA from 229 unrelated controls, 123 COPD cases and 77 asthma cases. Controls were selected from the Department of Preventive Medicine and Internal Medicine of Dong-A University hospitals from 2000 to 2004 (Busan, Korea). A total of 229 controls with no personal history of asthma or COPD completed an interview (Kwon et al. 2010). The control set for analysis included 109 females and 120 males with a median age of 59.5 years. Individuals with COPD demonstrated a post-bronchodilator FEV1/FVC < 0.7, while controls had normal spirometry (Han et al. 2016). The diagnosis of asthma was made using the asthma-specific International Classification of Diseases, Tenth Revision (ICD-10) codes (J459, J450, J460, J461, J469) by Internal Medicine doctors (Kwon et al. 2016). The controls and cases with asthma or COPD were matched according to sex and age, and all participants provided written, informed consent. All samples were obtained from two different hospitals in Korea: Dong-A University Hospital (#IRB-06-10-02 & IRB-07-10-7) and Kangwon National University School of Medicine (#IRB-2012, 06-007).

Genotyping of *MUC8*-MS5

For genotyping, genomic DNA was isolated from peripheral leukocytes of 400 µL whole blood using a Blood and Cell Culture DNA Mini Kit (Qiagen, CA). We analyzed *MUC8*-MS5 VNTR polymorphisms by PCR, using the following primers: Forward (19 bp) 5′-ATGTGGGCCGGCAGGAGAC-3′; Reverse (20 bp) 5′-GGCTCCGTTTTGGGGCTGTT-3′ (Lee et al. 2009). PCR reactions (40 µL) were performed in reaction mixes containing 100 ng genomic DNA, 10 µM primers, 2.5 U Go Taq Flexi DNA polymerase (Promega, WI), 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 5.0 mM $MgCl_2$, 0.2 mM dTTP, dCTP, dGTP and dATP. PCR was performed in a 9700 Thermocycler (Perkin-Elmer, CT), and the general thermocycling conditions were as follows: 2 min initial denaturation at 94 °C, followed by 30 cycles of 45 s at 94 °C and 45 s at 68 °C, then a final 7 min extension at 72 °C. PCR products were separated by gel electrophoresis (1 V cm^{-1}) using 2.5% MetaPhor agarose (Lonza, USA) in 1× TAE buffer.

Statistical analyses

For the association of genetic variants with asthma or COPD, we estimated odds ratios (ORs) and their 95% confidence intervals (95% CIs) using logistic regression analysis. ORs were estimated using the natural logarithm and its standard error. All tests were two-sided, with $p < 0.05$ considered statistically significant. Statistical analysis was performed using MS Excel with CHITEST and R statistical software (v2.5.2, http://www.r-project.org) (Kwon et al. 2010).

Results

Allelic frequency of *MUC8*-MS5 among the controls, asthma and COPD cases

The *MUC8* gene is located on chromosome 12q24.3, and we previously analyzed six VNTRs within the *MUC8* region (Lee et al. 2009). It has been reported in several studies that differential expression of *MUC8* was related to chronic inflammation of respiratory organs (Basbaum et al. 1999;

Bernacki et al. 1999; Song et al. 2008). In a previous study, *MUC8*-MS5 was identified between intron 3 and exon 4, and four alleles were detected (2, 5, 9 and 29 repeats), with eight haplotype-patterns among 200 individuals (Lee et al. 2009). This region showed the highest heterozygosity among the *MUC8* VNTR regions in 200 controls.

To examine the association between *MUC8*-MS5 and respiratory diseases, we analyzed the allelic variations of *MUC8*-MS5 with 229 controls, 77 asthma and 123 COPD cases (Table 1). Table 1 summarizes the frequency of allelic lengths among controls and cases. There were no significant differences for allelic frequencies between controls and the two cases. The heterozygosity of this region was approximately 0.78 in controls and cases. Compared to our previous study, we detected three additional alleles (10, 28 and 30 repeats) in this region. For further analysis, each allele was grouped into two sets (common and rare alleles) according to their frequency in controls. The expected frequency for rare alleles was set as < 1% (Kwon et al. 2010). We detected seven alleles in this study, which included five common alleles for 2, 5, 9, 29 and 30 repeats and two rare alleles for 10 and 28 repeat copies for *MUC8*-MS5 in controls (Table 1). The rare allele with 28 repeats was detected only in controls; this seems to depend on the sample size. There are also no significant differences between the frequency of rare alleles of controls and cases. Rare *MUC8*-MS5 alleles appeared at a frequency of less than 1% (0.87% in controls, 0% in COPD and 0.65% in asthma). Additionally, for each allelic frequency, similar frequency was represented between controls and cases (Table 1).

Genetic susceptibility of the short allelic length of *MUC8*-MS5 to COPD

Because some minisatellite alleles have been reported to be in association with human diseases (Bailly et al. 1996; Kwon et al. 2010), we analyzed the effects of allelic length of minisatellites on disease. As shown in Table 1, the length of the minisatellites in this region can be divided into short alleles (2, 5, 9 and 10 repeats) and long alleles (28 ~ 30 repeats). According to length, minisatellites can be divided into three categories: L/L, L/S and S/S (Table 2). In the analysis of allelic length, a similar frequency was shown across the three groups (controls vs. COPD, $p = 0.4887$; controls vs. asthma, $p = 0.4699$).

For further analysis, we analyzed the frequency of genotype patterns for *MUC8*-MS5 among control, COPD and asthma groups (Table 3). Interestingly, we found that the frequency of homozygous genotypes with the shortest rare allele (2/2 repeats) showed a significant difference between

Table 1 Frequency of *MUC8*-MS5 alleles in controls and cases

	Allele		Control		COPD			Asthma		
	Repeat	Size (bp)	N = 458	Frequency	N = 246	Frequency	*p* value	N = 154	Frequency	*p* value
Short allele	2	493	68	0.1485	49	0.1992	0.0850	20	0.1299	0.5693
	5	616	65	0.1419	34	0.1382	0.8926	30	0.1948	0.1169
	9	780	91	0.1987	47	0.1911	0.8078	27	0.1753	0.5249
	10	821	1	0.0022	0	0	–	1	0.0065	0.4175
	Total		225	0.4913	130	0.5284	0.3467	78	0.5065	0.7437
Long allele	28	1556	3	0.0066	0	0	–	0	0	–
	29	1600	89	0.1943	41	0.1667	0.3672	30	0.1948	0.9896
	30	1641	141	0.3079	75	0.3049	0.9348	46	0.2987	0.8310
	Total		233	0.5087	116	0.4715	0.3467	76	0.4935	0.7437
H*	0.7857				0.7839			0.7872		

Short alleles (2, 5, 9 and 10 repeats); Long alleles (28 ~ 30 repeats)

*H** heterozygosity

Table 2 Analysis of allelic length of minisatellites in *MUC8*-MS5 region

Allelic length	Control		COPD			Asthma		
	N = 229	Frequency	N = 123	Frequency	*p* value	N = 77	Frequency	*p* value
L/L	63	0.2751	32	0.2602	0.7633	23	0.2987	0.6903
L/S	107	0.4672	52	0.4228	0.4239	30	0.3896	0.2359
S/S	59	0.2576	39	0.3171	0.2356	24	0.3117	0.3561
L/S + S/S	166	0.7249	91	0.7398	0.7633	54	0.7013	0.6903

L long alleles (28 ~ 30 repeats), *S* short alleles (2, 5, 9 and 10 repeats)

Table 3 Frequency of *MUC8*-MS5 haplotypes and risk of COPD

	Haplotypes	Controls		COPD			Asthma		
		N = 229	Frequency	N = 123	Frequency	OR (95% CI) *p* value	N = 77	Frequency	OR (95% CI) *p* value
L/L	30/30	24	0.1048	10	0.0813	ND	8	0.1039	ND
	30/29	29	0.1266	18	0.1463	ND	13	0.1688	ND
	30/28	1	0.0044	0	0.0000	ND	0	0.0000	ND
	29/29	8	0.0349	4	0.0325	ND	2	0.0260	ND
	29/28	1	0.0044	0	0.0000	ND	0	0.0000	ND
L/S	30/9	29	0.1266	17	0.1382	ND	10	0.1299	ND
	30/5	14	0.0611	9	0.0732	ND	6	0.0779	ND
	30/2	20	0.0873	11	0.0894	ND	1	0.0130	ND
	29/10	1	0.0044	0	0.0000	ND	0	0.0000	ND
	29/9	18	0.0786	6	0.0488	ND	5	0.0649	ND
	29/5	14	0.0611	4	0.0325	ND	4	0.0519	ND
	29/2	10	0.0437	5	0.0407	ND	4	0.0519	ND
	28/9	1	0.0044	0	0.0000	ND	0	0.0000	ND
S/S	10/9	0	0	0	0.0000	ND	1	0.0130	ND
	9/9	7	0.0306	2	0.0163	ND	1	0.0130	ND
	9/5	14	0.0611	10	0.0813	ND	7	0.0909	ND
	9/2	15	0.0655	10	0.0813	ND	2	0.0260	ND
	5/5	5	0.0218	4	0.0325	ND	5	0.0649	ND
	5/2	13	0.0568	3	0.0244	ND	3	0.0390	ND
	2/2	5	0.0218	10	0.0813	3.96 (1.32–11.88) 0.0084*	5	0.0649	3.11 (0.88–11.05) 0.0657

L long alleles (28 ~ 30 repeats), *S* short alleles (2, 5, 9 and 10 repeats), *ND* no significant difference

*Statistically significant ($p < 0.05$)

controls, COPD and asthma: these were 2.18 vs. 8.13 vs. 6.49%, respectively (Table 3). Specifically, the association between COPD cases and the homozygous 2/2 repeats genotype was represented with statistically significant higher frequency [OR 3.96; CI 1.32–11.88; $p = 0.0084$)]. In the case of asthma, the homozygous 2/2 repeat genotype increased 2.98-fold compared with control (OR 3.11; CI 0.88–11.05; $p = 0.0657$), but it could not be considered a statistically significant association. The current results show a tendency towards risk, but the sample population appears to be somewhat small for statistical analysis.

Discussion

Excessive airway mucus in asthma and COPD is a powerful inducer of chronic airway inflammation and associated lung disease, even when there is no bacterial infection (Ha and Rogers 2016). Abnormal expression of mucin genes occurs in conditions associated with various diseases and high risk of respiratory organs. Mucus was secreted by the airway surface epithelium and glands, and *MUC8*, one of the secretory mucins, was found to be overexpressed in diseases associated with chronic inflammatory respiratory infections (Basbaum et al. 1999; Song et al. 2008). Despite recent advances in various therapies for asthma and COPD, the treatment of advanced disease is not easy. If genetic clinical biomarkers are available, treatment interventions can be promoted before disease progression. Therefore, since polymorphic minisatelites are known within the *MUC8* region, epidemiological studies are needed to determine whether these polymorphisms are associated with susceptibility to diseases such as asthma or COPD.

In this study, polymorphisms of *MUC8* minisatellites were examined to investigate the genetic minisatellite allele, which represents a higher risk for respiratory disease development. As a result, in the case of the homozygous 2/2 repeat genotype, the association of COPD occurrence was significantly increased about four-fold. The number of patients examined in this study seems to be somewhat small, but this results show a link between minisatellites polymorphism and susceptibility to COPD or asthma, suggesting the need for further investigation, such as large-scale epidemiological studies based on this. Such a study could provide a helpful reference for understanding the function and complex genomic properties of mucins.

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Compliance with ethical standards

Conflict of interest Se-Ra Lee declares that she does not have conflict of interest. Won-Tae Kim declares that he does not have conflict of interest. Tae Nam Kim declares that he does not have conflict of interest. Jong Kil Nam declares that he does not have conflict of interest. Woo Jin Kim declares that he does not have conflict of interest. Sun-Hee Leem declares that she does not have conflict of interest.

Ethical approval Dong-A University Hospital (#IRB-06-10-02 & IRB-07-10-7) and Kangwon National University School of Medicine (#IRB-2012, 06–007) approved this study.

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