

ORIGINAL PAPER

***Alu*MICB dimorphism within the class I region of the major histocompatibility complex is associated with asthma and airflow obstruction in the Busselton population**

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Clinical and Experimental Allergy

Summary

Aim To examine the association between the *Alu* dimorphism within the first intron of the *MICB* gene and asthma and airflow obstruction.

Background The highly polymorphic non-classical MHC class I polypeptide-related (MIC) genes, *MICA* and *MICB*, encode stress inducible glycoproteins, which are expressed on a variety of epithelial cells, including those of the lungs.

Methods *Alu*MICB genotyping was performed on 1109 subjects from the Busselton Health Study. From a standard questionnaire, 359 individuals indicated that they had been diagnosed by a doctor with asthma. Lung function was assessed by the forced expired volume in 1 second (FEV₁) and expressed as a percent of the predicted value. Airflow obstruction was defined as FEV₁ < 80% predicted.

Results In men, a dominant relationship was found between the *Alu*MICB *DD* genotype and asthma ($P = 0.006$; $\chi^2_2 = 7.65$). Furthermore, multivariate analysis adjusted for age, height, weight and body mass index (BMI) showed a relationship between *DD* genotype and asthma in men in a dominant model (odds ratio (OR) = 1.97; 95% confidence interval (CI) = 1.11–3.51; $P = 0.021$). In women, an association was found between the *Alu*MICB *II* genotype and FEV₁ percent predicted as a continuous variable ($P = 0.001$). When adjusted for age and BMI, it showed a significant relationship between *Alu*MICB and airflow obstruction in a dominant model (OR = 14.11%, 95% CI 3.29–60.57, $P < 0.001$). However, no association was found between the *Alu*MICB *II* genotypes and airflow obstruction in men.

Conclusion These findings suggest the possible involvement of a MHC class I gene in abnormal airway structure in women and airway function in men.

Keywords airflow obstruction, *Alu*MICB, COPD, asthma, FEV₁

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Introduction

Asthma is a chronic inflammatory disease characterized by widespread obstruction of the airways, which includes airway hyper-responsiveness, pulmonary eosinophilic inflammation, and elevated serum IgE levels. Approximately two million Australians are affected by asthma and it is also one of the most common reasons for emergency department admissions.

In contrast to asthma where airflow obstruction is by definition reversible, chronic obstructive pulmonary disease (COPD) is usually defined as irreversible airflow

obstruction, most commonly associated with cigarette smoking. It is an imprecise term that lacks rigorous consensus definition. Airflow obstruction is most commonly measured by the forced expiratory volume in 1 second (FEV₁) as a ratio of the forced vital capacity (FVC). Irreversibility is demonstrated by absence of significant improvement in FEV₁ following administration of bronchodilator and/or spontaneous improvement as seen in asthma. As these two conditions may coexist, COPD may reasonably be diagnosed when, after the administration of a bronchodilator medication, the ratio of FEV₁ to FVC is < 70% and the FEV₁ is < 80% of the predicted

value [1, 2]. It is also associated with an abnormal response of the lungs to noxious particles and gases, particularly cigarette smoke and may occur in subjects with asthma [3].

Asthma and COPD share characteristic changes in lung function over the life course of an individual, in particular, accelerated decline in spirometric indices and the development of chronic airflow obstruction. Asthma and COPD are complex diseases that involve the interaction between multiple genetic and environmental factors. Compared with asthma, limited information regarding the role of inflammatory mediators and genetic factors in COPD have been studied [4]. COPD is now an increasing global health problem and is predicted by the World Health Organization to become the third most common cause of death and fifth most common cause of disability in the world by 2020 [5]. The roles of inflammatory mediators and genetic factors in COPD are not well understood but are now increasingly being studied [6, 7].

Apart from COPD, the prevalence and severity of asthma has also been increasing throughout the world [8], including Australia [9]. However, the increasing prevalence may be reaching a plateau [10]. The precise aetiology of asthma is unknown and many studies have shown that both genetic and environmental factors are involved in the disease and its associated phenotypes [11, 12]. One of the most consistent primary genetic associations for asthma and related respiratory diseases is with markers on chromosome 6p21–24. This association is highly reproducible with multiple independent studies to date implicating the region [13–18]. The human major histocompatibility complex (MHC) is located within this chromosomal region and investigations of candidate genes within this region have mainly focused on the class II region (*HLA-DR* and *HLA-DQ*) and the TNF gene cluster [19–24]. However, none of the positive association studies for these loci exclude other regions within the MHC. For these reasons, we have investigated other parts of the MHC in order to determine possible associations with asthma and COPD.

MIC gene family was discovered in 1994 by Bahram [25] and Leelayuwat [26]. This gene family consists of two *MIC* encoding gene (*MICA* and *MICB*) and five pseudogenes (*MICC–MICG*) which are located within the class I region of the MHC. The *MICA* and *MICB* genes are highly polymorphic and are located telomeric to the TNF gene, between the B-associated transcript (BAT1) and 46.4 kb and 141.2 kb centromeric from *HLA-B*, respectively [25, 27]. The genomic structure of the *MIC* gene family is similar to that of the MHC class I genes with distinct functional domains encoded by separate exons. *MICA* and *MICB* transcripts share 83% amino acid homology. To date, 59 *MICA* alleles and 20 *MICB* alleles have been identified [28–32].

MIC genes have been associated with several autoimmune disorders such as diabetes, celiac disease, psoriasis, and arthritis [33–37]. *MIC* molecules do not present peptide nor bind β -2 microglobulin. However, the two encoding genes, *MICA* and *MICB*, are expressed in epithelial cells and a wide variety of other human tissues [38]. They are stress-induced molecules recognized by $\gamma\delta$ T cell receptors of $\gamma\delta$ T cells, natural killer (NK) cells and cytotoxic CD8⁺ $\alpha\beta$ T cells through the killer cell lectin-like receptor subfamily K member 1 (KLRK1/NKG2D) receptor [39]. NK cells are normally present in considerable number in human lung interstitium [40–42] and patients with asthma have increased NK cell activity when compared with normal healthy blood donors [43–45]. *MIC* expression has been suggested as analogous to a flag raised by cells under stress (either infection, cell damage or cellular transformation) to recruit lymphocytes such as $\gamma\delta$ T cells and NK cells [39]. It has also been suggested that the bronchial epithelium is selectively damaged in asthma with weakening of the junctional adhesion structure and subsequent increased permeability and susceptibility to injury [46]. Therefore *MIC* could be involved in the injury of asthmatic bronchial epithelium as the binding of *MIC* on target cells to KLRK1 on NK and $\gamma\delta$ T cells could stimulate effector responses from these cells [39], thereby increasing NK cell activity. Furthermore, a previous study has shown that the increase in NK cell activity in asthma reflects a predisposition of individuals with high NK cell activity to develop exaggerated T cell responses to inhaled antigens and hence to be at risk of developing asthma [47]. As *MIC* molecules bind to KLRK1 on NK and $\gamma\delta$ T cells, this further supports a possible biological role of *MIC* in the initiation of immunological diseases [48, 49].

In this report we describe a study of the *Alu* dimorphism within the first intron of the *MICB* gene and its association with asthma and abnormal airway function in 1109 European–Australian from the Busselton population of Western Australia.

Materials

Subjects

The Busselton Health Study comprises a series of cross-sectional surveys of all adults and children residing in the Shire of Busselton from 1966 to 1981 and a follow-up study of all previous participants (residing within and outside this Shire) in 1994/1995. Busselton is a coastal town in the South West region of Western Australia. It has a population that is predominantly of European origin. In the present case/control study, all subjects ($n = 1109$) who attended both the 1981 and the 1994 survey and who had complete demographic, lung function and skin prick test (SPT) data were included. Subjects were considered to have 'asthma' if they answered yes to the question 'Has

your doctor ever told you that you had asthma/bronchial asthma?' at either survey. All other subjects were considered as 'controls'. The study protocol was approved by the Institutional Ethics Committee of the University of Western Australia and written informed consent was obtained from all study participants.

Methods

Lung function

Lung function was measured using a pneumotachograph spirometer (Welch Allyn Model No: 6100; Skaneateles Falls, NY, USA). The best FEV₁ and FVC were recorded from three reproducible and acceptable maximum expiratory manoeuvres, according to American Thoracic Society Criteria [50]. The measured levels were expressed as a percentage of the predicted normal level using prediction equation from the Busselton Population [32]. Airway responsiveness was recorded as the dose-response slope [51] of the relationship between the fall in FEV₁ from the post-saline measurements in response to increasing doses of an inhaled bronchoconstrictor, methacholine, to a maximum dose of 4 µM.

Skin prick test

Skin prick testing (SPT) was performed using eight standardized allergens, including house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), mould mix, dog and cat dander (*Canis familiaris* and *Felis domesticus*), grass and rye pollens, and both negative (saline) and positive (histamine) control solutions. An SPT result was considered positive if the largest diameter of the weal size was 3 mm larger than that of the negative saline control. Subjects in whom the weal size of the positive histamine controls was less than 3 mm were excluded.

Genotyping

The sense and antisense primers used for PCR of the *Alu*_{MICB} locus were as previously published (Kulski et al., 2002). Briefly, the PCR reaction contained 20 ng of template DNA primers at 10 µM each, dNTPs at 2 mM each, 0.5 U of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA, USA) and 3 mM MgCl₂ in 10 mM Tris-HCl buffer pH 8.3. PCR was performed using a MJ Research Dyad machine under the same cycling conditions as previously described. The PCR products were analysed in 2% TBE agarose by electrophoresis. PCR products with *Alu* (I allele) are 665 bp in length and 503 bp (D allele) without *Alu*.

Statistical analysis

Statistical analysis was performed using the software package Minitab for Windows v13 (Minitab Inc., PA,

USA). Statistical significance was defined at the standard 5% level. The primary outcome variable of the association analyses was case-control status. The secondary outcome within the case group was FEV₁. Two levels of airflow obstruction were defined (a) A01: FEV₁ < 80% predicted and FEV₁/FVC < 90% predicted, and [52] A02: FEV₁ < 70% predicted and FEV₁/FVC < 90% predicted. The principal explanatory variable was the *Alu*_{MICB} sequence variant. The genotypes were coded into three classes and analysed categorically with the most common homozygous genotype (DD) as the reference category. Genotype-phenotype analysis was also conducted under both a dominant (i.e. DD = 0, ID, and II = 1) and recessive (i.e. DD and ID = 0, II = 1) model. Bonferroni's correction was carried out by multiplying the number of hypothesis to the *P*-value obtained.

Hardy-Weinberg equilibrium was tested on a contingency table of observed-vs-predicted genotypic frequencies using a modified Markov-chain random walk algorithm [53].

ANOVA was used to compare continuous outcomes across the levels of each genotype, and χ^2 tests or a hybrid approximation to Fisher's exact test [54] on contingency tables for comparisons of the distributions of categorical variables across genotypes.

Generalized linear models (linear and logistic regression; GLMs) [55] were used to model the effects of multiple covariates on the continuous and dichotomous outcomes, including an investigation of the need for interaction or polynomial terms. Sex, age, height, weight, and body mass index (BMI) were included as potential covariates in multivariate analyses.

Results

Four hundred and seventy-five men (42.8%) and 634 women (57.2%) were genotyped (Table 1). No significant deviation from the Hardy-Weinberg equilibrium was observed in the population for the *Alu* dimorphism within

Table 1. Characteristics of study population

	Asthma patients	Healthy controls
Number (%)	358 (32.28)	751 (67.72)
Age (years)	49.75 ± 17.21	58.71 ± 14.26
M/F	136/222	339/426
Height (cm)*	1.68 ± 0.09	1.68 ± 0.09
Weight (kg)*	73.19 ± 13.53	73.63 ± 13.92
BMI*	25.95 ± 4.08	26.08 ± 3.97
Ever smoke (N, %)	173 (48.19)	341 (45.41)
Never smoke (N, %)	186 (51.81)	410 (54.60)
FEV ₁ *	2.74 ± 1.05	2.82 ± 0.89
FVC*	3.67 ± 1.21	3.69 ± 1.10
FEV ₁ /FVC*	0.73 ± 0.11	0.76 ± 0.69

* ± Standard deviation. FVC, forced vital capacity; FEV₁, forced expired volume in 1 s; BMI, body mass index; M, male; F, female.

Table 2. *Aluymicb* dimorphism in asthmatic patients and control subjects

		Genotypes					
		<i>DD</i>		<i>ID</i>		<i>II</i>	
Groups (n = 1109)		N	Frequency	N	Frequency	N	Frequency
Asthma patients	Male	116	0.85	16	0.12	4	0.03
	Female	182	0.82	36	0.16	4	0.02
	Total	298	0.83	52	0.15	8	0.02
Healthy control	Male	249	0.73	76	0.22	14	0.04
	Female	328	0.80	76	0.18	8	0.02
	Total	577	0.77	152	0.20	22	0.03

Bivariate analysis: asthma vs controls (total population: $\chi^2 = 6.00$; $P = 0.05$).

Bivariate analysis: asthma vs controls (men: $\chi^2 = 7.82$ $P = 0.02$; women: $\chi^2 = 0.52$; $P = 0.77$).

Multivariate analysis: asthma vs controls (men $P < 0.001$, odds ratio 1.97; 95% confidence interval (1.11–3.51)).

Adjusted for age, height, weight and body mass index.

the first intron of the *MICB* gene. Individuals in the study cohort were assigned as having genotype *DD*, *ID* or *II* for those homozygous for 'no *Aluymicb*', 'heterozygous', or 'homozygous for *Aluymicb* insertion', respectively (Table 2).

Associations with asthma

Bivariate analysis suggested a difference in genotypic frequencies between the asthma and control subjects that was marginally significant ($\chi^2 = 6.00$, $P = 0.05$) (Table 2). After stratification by gender, a stronger association was detected in men ($\chi^2 = 7.82$, $P = 0.02$) (Table 2). This association in men was also found to be dominant ($\chi^2 = 7.65$, $P = 0.006$). Multivariate analysis adjusted for age, height, weight, BMI and smoking history showed that men with *DD* genotype are approximately twice as likely to have asthma than those with the other genotypes.

Associations with forced expired volume in 1 second (FEV₁)

Analysis of variance indicated an association between the *Aluymicb II* genotype and reduced percentage predicted FEV₁ in women ($F_{2,631} = 7.62$; $P = 0.001$) but not in men ($F_{2,472} = 0.26$; $P = 0.77$) (Table 3). The frequencies of men and women with 'airflow obstruction' (AO1 and AO2) as determined by FEV₁ and FEV₁/FVC ratio are shown in Table 4. When binary logistic regression analysis was performed between genotypes and AO1, an odds ratio (OR) of 7.47 (95% confidence interval (CI) 1.88–29.73; $P = 0.02$) was detected in women with age, BMI and smoke as covariates. Further analysis performed on the more stringent AO2 criteria confirmed this finding (OR = 14.11; 95% CI = 3.29–60.57; $P = 0.002$) (Table 4). There were no associations with genotype and airway responsiveness (data not shown).

Discussion

In this study, the frequency of the two *Aluymicb* alleles and their association with asthma and lung function were

Table 3. Genotype frequencies of mean FEV₁% and adjusted FEV₁ in women

		Genotypes		
		<i>DD</i> (N = 510)	<i>ID</i> (N = 112)	<i>II</i> (N = 12)
Women	N	510	112	12
	Mean FEV ₁ % [^]	109.61	108.48	84.74
	FEV ₁ (L)*	2.33	2.275	1.778

[#] $P = 0.001$; [^] $P < 0.0001$.

*Model adjusted for age, height, weight, body mass index and smoke.

reported. The overall frequencies of the less common genotypes in the current cohort were similar to a previously reported Caucasoid population (18.4% and 2.8% for *ID* and *II* genotypes, respectively) [56]. An association was found between *Aluymicb* genotypes and asthma ($\chi^2 = 6.00$, $P = 0.05$) but after stratification by gender, *Aluymicb* was found to be significantly associated with asthma in men only ($\chi^2 = 7.82$, $P = 0.02$), independent of lung function level. We cannot discount any environmental interactions that affect males more than females. For instance, occupational exposure in men [57] has been shown to be a factor associated with increased IgE levels in men, a possible mechanism which could differentially affect interactions between genotype and asthmatic phenotypes in men and women. On the other hand, female with homozygous *Aluymicb* insertion, the *II* genotype, appear to be 14 times more susceptible to airflow obstruction, independent of smoking history. This observation may be due to gender-specific differences in exposure to a wide range of environmental factors such as hormonal status, relative difference in airway and parenchymal size, or asthma management. It could also be due to a linkage disequilibrium effect from a nearby gene in linkage with *Aluymicb*, such as *HLA-B* as previously described [56]. This warrants further investigation especially in the class I

Table 4. Genotype frequencies in men and women with chronic airflow obstruction

		Genotypes								
		<i>DD</i>		<i>ID</i>		<i>II</i>				95% confidence interval
Lung function*	Sex	Frequency	<i>N</i>	Frequency	<i>N</i>	Frequency	<i>N</i>	<i>P</i> value	Odds ratio	
AO1	Men	0.75	33	0.23	10	0.02	1			
Non-AO1		0.77	332	0.19	82	0.04	17	0.74	–	–
AO1	Women	0.70	21	0.20	6	0.10	3			
non-AO1		0.81	489	0.18	106	0.01	9	0.02	7.47	1.88–29.73
AO2	Men	0.77	24	0.19	6	0.03	1			
Non-AO2		0.77	341	0.19	86	0.04	17	0.98	–	–
AO2	Women	0.60	9	0.20	3	0.20	3			
Non-AO2		0.81	501	0.18	109	0.01	9	0.002	14.11	3.29–60.57

*FEV₁ expressed as percent of predicted value. All FEV₁/FVC < 0.9 of the predicted value

AO1-FEV₁ < 80%; FEV₁/FVC < 0.9 of the predicted value.

AO2 = FEV₁ < 70%; FEV₁/FVC < 0.9 of the predicted value.

Non-AO1 = FEV₁ > 80%; FEV₁/FVC < 0.9 of the predicted value.

Non-AO2 = FEV₁ > 70%; FEV₁/FVC < 0.9 of the predicted value.

FVC, forced vital capacity; FEV₁, forced expired volume in 1 s.

MHC region. The association observed could also be due to a gene–gene interaction between *Alu*MICB and a yet to be identified sex-linked locus which is expressed only in women. Furthermore, an estrogen responsive element (ERE) half-palindrome site for ‘AGGTCA’ is present within the *Alu* sequence 55 bp upstream of the *Alu*MICB insertion. Estrogen has been shown to have a pro-inflammatory effect, which may be relevant to the inflammatory components of asthma and airflow obstruction [58]. Furthermore, it has been previously suggested that *Alu* retroposons may play an important role in evolution of regulation of the primate gene expression by nuclear hormone receptors [59]. The ERE found within the *Alu* sequence, together with the insertion of an *Alu*MICB sequence, may exert a negative effect on the level of pulmonary function in women where estrogen levels are higher. Furthermore, it has also been shown that HLA class I expression can be down-regulated by hormones in human breast carcinoma cells [60]. The *Alu* insertion could also have an influence on the regulation and function of NK cells or it could interact with infectious agents such as respiratory syncytial virus, seen in up to 85% of asthma exacerbations in children and almost 50% in adults [61, 62].

Based on our analysis, the present study suggests that *Alu*MICB may play an important role in the pathogenesis of asthma and impairment of lung function. Further studies in different populations are warranted to confirm this finding. Although it is not known whether *Alu*MICB or another gene/genes closely linked to *Alu*MICB is responsible for the association found in this study, we can conclude that a gene/genes located in the Class I MHC region is associated with reduced airway function.

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