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Airway smooth muscle thickness in asthma is related to severity but not duration of asthma

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ABSTRACT: **Asthma is characterised by an increased airway smooth muscle (ASM) area (ASM_{area}) within the airway wall. The present study examined the relationship of factors including severity and duration of asthma to ASM_{area}.**

The perimeter of the basement membrane (PBM) and ASM_{area} were measured on transverse sections of large and small airways from *post mortem* cases of fatal (n=107) and nonfatal asthma (n=37) and from control subjects (n=69). The thickness of ASM (ASM_{area}/PBM) was compared between asthma groups using multivariate linear regression.

When all airways were considered together, ASM_{area}/PBM (in millimetres) was increased in nonfatal (median 0.04; interquartile range 0.013–0.051; p=0.034) and fatal cases of asthma (0.048; 0.025–0.078; p<0.001) compared with controls (0.036; 0.024–0.042). Compared with cases of nonfatal asthma, ASM_{area}/PBM was greater in cases of fatal asthma in large (p<0.001) and medium (p<0.001), but not small, airways. ASM_{area}/PBM was not related to duration of asthma, age of onset of asthma, sex or smoking. No effect due to study centre, other than that due to sampling strategy, was found.

The thickness of the ASM layer is increased in asthma and is related to the severity of asthma but not its duration.

KEYWORDS: **Airway smooth muscle, asthma, severity**

The airway smooth muscle (ASM) layer area (ASM_{area}) seen on transverse sections is increased up to five-fold in cases of asthma [1]. The *post mortem* study of CARROLL *et al.* [2] showed that the thickness of the ASM layer in people with clinically mild or moderately severe asthma was less than that in patients who died of asthma and who had clinically severe disease. This suggests a relationship between asthma severity and ASM_{area}. In biopsy studies, the only structural change that distinguished severe from mild/moderate cases of asthma was the ASM_{area} [3, 4]. In addition to clinical severity, a number of other factors, such as sex, age, cigarette smoking, treatment, age at onset of asthma and its duration, might be related to the ASM_{area}. BAI *et al.* [5] examined the effects of age on ASM_{area} and the relative area fractions of smooth muscle and extracellular matrix by comparing younger (17–23 yrs) and older (40–49 yrs) adults with fatal asthma. The amount of ASM was greater in older *versus* younger subjects with fatal asthma after correction for the fraction of smooth muscle within the ASM layer.

The Melbourne longitudinal study of asthma followed patients with mild and severe asthma from childhood [6]. In general, individuals tended to show stable clinical severity from childhood through to middle age. Similarly, reductions in lung function have been shown to track with persistence of symptoms from childhood to adulthood [7]. These studies suggest that the severity of asthma may be determined early in life. Therefore, we hypothesised that the ASM_{area} would not be related to age or duration of asthma but to severity of asthma. This hypothesis was tested by examining the thickness of the ASM layer in a large number of cases of asthma and control subjects from six study centres.

MATERIAL AND METHODS

Further details regarding methods are available in the online supplementary material.

This article has supplementary material accessible from www.erj.ersjournals.com

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Subjects

Post mortem tissues from subjects with or without asthma contained in six tissue banks (table 1) were studied. These included a prospective study of fatal asthma [2]; a study of asthma management and mortality [8]; a study of airway development [9]; a study of fatal asthma [10]; a study from New Zealand of fatal asthma [11]; and a Canadian study of fatal asthma [12]. Subjects were classified as: control (death due to nonrespiratory causes; history of asthma excluded); nonfatal asthma (death due to nonrespiratory causes; history of asthma confirmed); or fatal asthma (cause of death consistent with a fatal attack of asthma; history of previous asthma confirmed). Approval for these studies and for the collaborative analysis was obtained from the Institutional Ethics Committees of the participating centres and from the Sir Charles Gairdner Hospital (Nedlands, Australia) Human Research Ethics Committee. Demographic data, including age at death, sex and, where available, smoking history, frequency of asthma symptoms, duration of asthma, age of onset of asthma and current treatment requirements were recorded. Smoking was categorised as: ever-smoker (current or ex-smoker); or never-smoker (at time of death).

Asthma severity score

Those with a history of asthma and with available information were assigned a category for asthma severity, unrelated to the cause of death, based on guidelines from the Global Initiative for Asthma (GINA) [13]. Subjects were classified as having severe asthma if they were using oral corticosteroids, reported hospitalisations for asthma (ever) or had daily symptoms. Subjects were classified as having moderate asthma if they had none of the above, but had symptoms on most days or nights (>3 days·week^{-1}), used regular inhaled corticosteroids or used reliever medications on most days or nights. The remaining cases were, therefore, classified as mild.

Tissue preparation and measurement

Post mortem tissues (ranging from whole lungs to dissected airways or parenchyma) were fixed by inflation or immersion. Airways had been sampled systematically in some centres [2, 9, 12], and at the discretion of the pathologist, for diagnostic purposes, at others [8, 10, 11]. Cases were excluded if there was macroscopic or microscopic evidence of lung injury or disease (such as pneumonia). Sections of large and small airways were cut at a thickness of either 4 or 5 μm and stained with haematoxylin and eosin or using either Masson's or Gomori's trichrome technique. Planimetry was perfomed on each transverse section of airway in order to measure the length of the basement membrane (PBM), and either planimetry or point counts were used to measure the ASM_{area} [14].

Analysis

Analyses were conducted using SPSS version 13.0 (SPSS, Chicago IL, USA), primarily for ASM_{area} corrected for PBM as a marker of airway size. As ASM_{area}/PBM was not normally distributed, univariate differences in $\log_{10}ASM_{area}$/PBM between asthma categories were tested using ANOVA and Tukey's test *post hoc*. Correlations were tested using Pearson's correlation. Associations between categories were tested using the Chi-squared test. In multivariate analyses, general linear models were used to examine the effects of study centre, age, duration of asthma, age of onset of asthma, sex, asthma group and PBM on ASM_{area}/PBM (logarithmically transformed). Analyses were also repeated for airways categorised as small (PBM<4 mm), medium (PBM 4–10 mm) or large (PBM>10 mm) airways. Probabilities of <5% were considered significant and adjusted r^2 are presented.

RESULTS

The baseline characteristics of the cases are shown in table 2. There were 213 subjects with airways available for analysis. Clinical data were not available for all cases (table 2). The age distribution was similar for the three case groups, but there were relatively more females in the asthma groups ($p=0.013$). In the 57 cases for whom data were available, there was a strong relationship between nonfatal asthma and clinically mild or moderate asthma, and fatal asthma and clinically severe asthma (Chi-squared=15.3; $p<0.001$). There were no significant differences between groups with regard to smoking (information available in 99 cases), age, age at onset or duration of asthma.

When all airways were considered together (fig. 1a), the thickness (ASM_{area}/PBM) of ASM was increased in the nonfatal (median 0.040 mm; interquartile range 0.013–0.051 mm; $p=0.034$) and fatal cases of asthma (0.048 mm; 0.025–0.078 mm; $p<0.001$) compared with controls (0.036 mm; 0.024–0.042 mm). Fatal and nonfatal cases of asthma were not significantly different ($p=0.105$). ASM_{area}/PBM increased with airway size and the

TABLE 1 Sources and preparation of tissue for study

Centre	Subjects n	Groups	Fixation	Airways samples	Sections	
					Thickness μm	Stain
Perth (AU)	65	C, NFA, FA	Inflation; formaldehyde	Large and small airways	5	H&E
Melbourne (AU)	17	FA	Immersion; formaldehyde	Sample of random blocks	5	H&E
Sydney (AU)	24	C, NFA, FA	Immersion; formaldehyde	Large and small airways	4	Gomori's
São Paulo (BR)	23	C, FA	Immersion; formaldehyde	Sample of blocks	4	Masson
Auckland (NZ)	41	FA	Immersion; formaldehyde	Sample of blocks	4	Gomori's
Calgary (CA)	48	C, NFA, FA	Inflation; glutaraldehyde	Large and small airways	5	Masson

AU: Australia; BR: Brazil; NZ: New Zealand; CA: Canada; C: control; NFA: nonfatal asthma; FA: fatal asthma; H&E: haematoxylin–eosin.

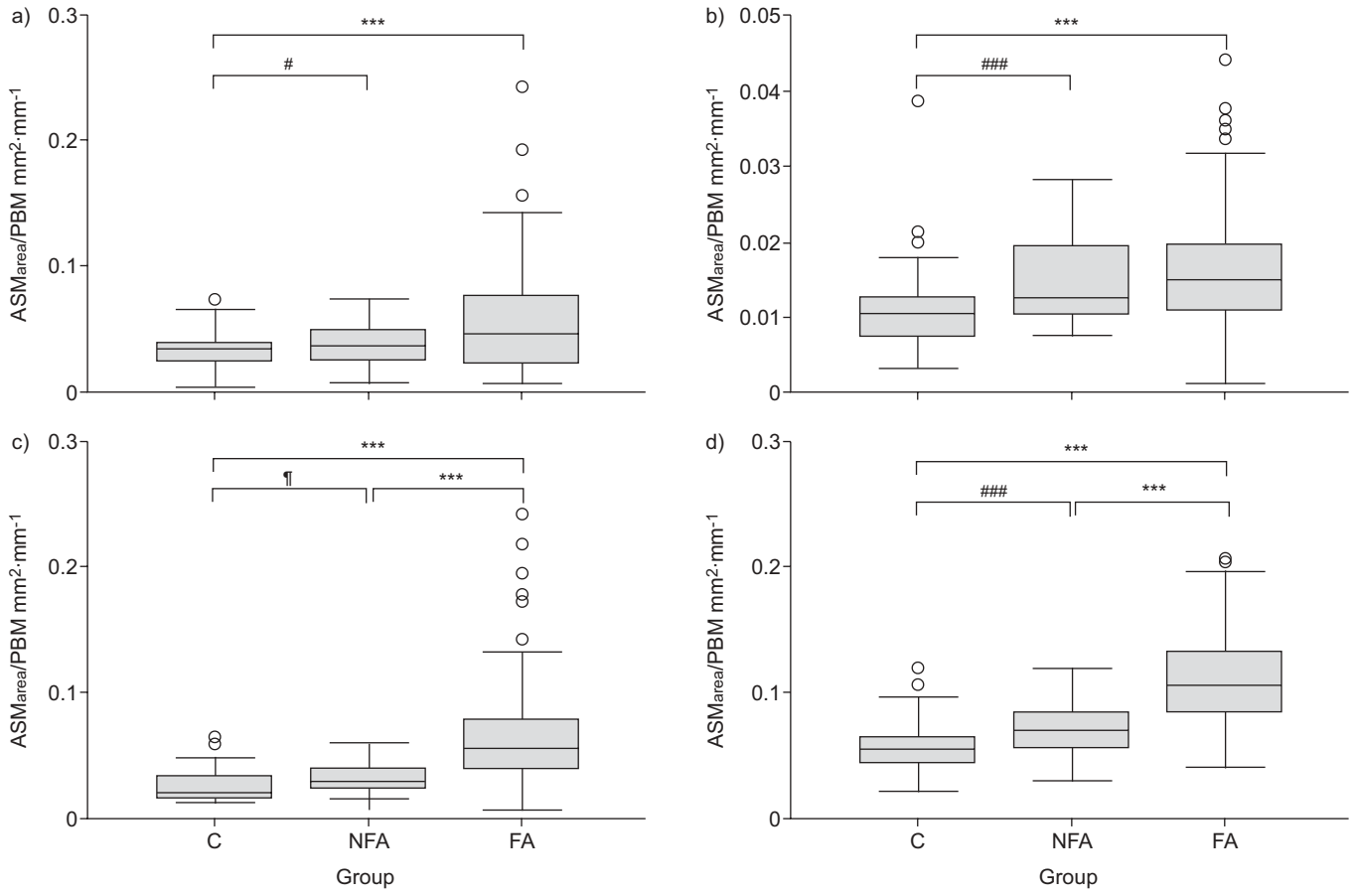

FIGURE 1. Area of the airway smooth muscle (ASM_{area}) relative to basement membrane perimeter (PBM) in controls (C) and cases of nonfatal asthma (NFA) and fatal asthma (FA) in: a) all airways combined; b) small airways (PBM <4 mm); c) medium airways (PBM 4–10 mm); and d) large airways (PBM >10 mm). Boxes represent median and interquartile range and whiskers represent 95% confidence interval. ○: outliers. ***: $p<0.001$; ###: $p=0.001$; #: $p=0.034$; ¶: $p=0.003$.

TABLE 2 Subject characteristics

	Controls	Asthma cases	
		Nonfatal	Fatal
Subjects n	69	37	107
Age yrs	35 (13–90)	27 (12–69)	35 (10–76)
Sex#			
Male	46 (67)	20 (54)	47 (44)
Female	23 (33)	17 (46)	60 (56)
Smoking status			
Ever	12 (33)	14 (54)	15 (40)
Never	24 (67)	12 (46)	22 (60)
Asthma severity* **			
Mild/moderate		18 (64)	4 (14)
Severe		10 (36)	25 (86)
Asthma duration yrs		16 (10–26)¶	18 (7–27.5)+
Age at asthma onset yrs		9 (2–20)¶	12.5 (5–26.8)+

Data are presented as median (interquartile range) or n (%), unless otherwise stated. Data for age are presented as median (range). ***: $p<0.001$ for group effect; #: $p=0.013$ (Chi-squared test). ¶: n=25; +: n=44.

relationships of ASM_{area}/PBM and asthma group for different airway sizes are shown in figure 1b–d. In nonfatal cases of asthma, compared with controls, ASM_{area}/PBM was significantly increased in large ($p=0.001$), medium ($p=0.003$) and small airways ($p=0.001$). In cases of fatal asthma, ASM_{area}/PBM was greater compared with controls for each airway size group ($p<0.001$ for all comparisons) and compared with nonfatal cases of asthma for large and medium airways ($p<0.001$), but not for small airways ($p=0.399$). Univariate analysis showed an effect of centre, but not sex or cigarette smoking. ASM_{area}/PBM was associated weakly with age ($r^2=0.166$; $p=0.041$) and with duration of asthma ($r^2=0.226$; $p=0.032$), but not with age at onset of asthma ($r^2=0.081$; $p=0.78$). These relationships and differences between groups were similar when airways were grouped by size. Analysis by asthma group showed an effect of age (fig. 2) in the nonfatal cases of asthma ($r^2=0.387$; $p<0.001$). However no effect of age was apparent for controls or fatal asthma cases.

The results of the general linear models are summarised in table 3. The primary model explained 50% of the variance in ASM_{area}/PBM. Only subject group (control or nonfatal or fatal asthma) showed a significant effect on ASM_{area}/PBM. There was no significant effect of age, sex or smoking, and there were no significant interactions between age, asthma group or

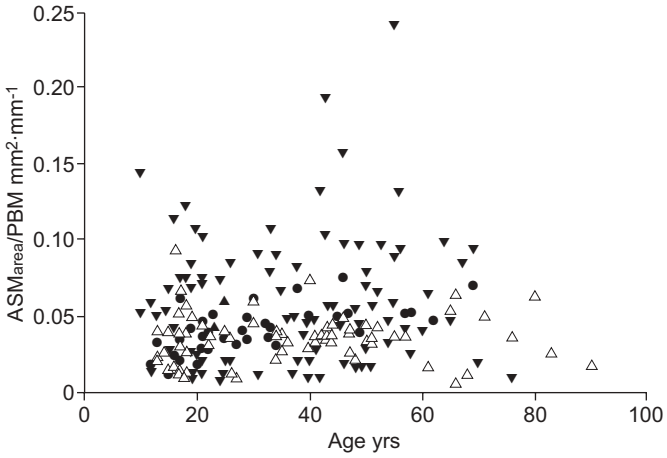

FIGURE 2. Area of the airway smooth muscle (ASM_{area}) relative to basement membrane perimeter (PBM) by age for asthma cases categorised as control (△), nonfatal asthma (●) or fatal asthma (▼).

smoking. In separate models, no effects of duration of asthma or age at onset of asthma were observed on ASM_{area}/PBM. If PBM was included in the model, it had a strong independent effect on ASM_{area}/PBM ($p<0.001$), but did not significantly change the effects of other variables. When cases of nonfatal asthma were analysed separately, there was a significant but weak effect of age on ASM_{area}/PBM for all airways ($r^2=0.06$; $p=0.044$) and a stronger effect if the analysis was confined to adults aged 21–60 yrs ($r^2=0.255$; $p=0.046$). There was no effect of age in the fatal asthma or control groups.

DISCUSSION

In the present study, the thickness of the ASM layer (ASM_{area}/PBM) was increased in cases of asthma compared with controls, and increased in cases of fatal asthma (clinically severe) compared with nonfatal cases of asthma (mild or moderate clinical severity). In multivariate analyses, there was no relation to duration of asthma, age at onset of asthma, smoking or sex. These findings suggest that the increased thickness of the ASM layer is present at an early stage in the natural history of asthma and may determine clinical severity.

We found a strong relationship between clinical severity and asthma group (nonfatal or fatal asthma). The underlying severity of asthma is generally constant over long periods [6, 7], and the data from the present study suggest that ASM thickness and asthma severity are associated. Most previous studies have examined ASM in cases categorised as fatal or nonfatal asthma, but have not reported grades of clinical severity [1]. In mild-to-moderate cases studied during life, only small biopsy specimens can be obtained [3, 15]. These studies have consistently shown that the relative ASM_{area} is increased in asthma, and at least three studies have shown that the amount of ASM distinguished mild/moderate from severe cases of asthma [3, 4, 16]. The clinical severity of asthma in the present study was based upon GINA guidelines [13] and used available data on these *post mortem* cases. Data on lung function were not evaluable for enough subjects to be included in the model. Therefore, severity may have been generally underestimated since cases were categorised by their most severe criteria. The presence of lung function abnormalities would have placed more subjects into more severe categories. However, even in the absence of lung function data, the nonfatal cases that were scored as severe showed similar amounts of ASM to those that were mild to moderate (fig. E1 of online supplementary material).

TABLE 3 Parameter estimates for general linear model for airway smooth muscle area/basement membrane perimeter

	B coefficient ± SEM (95% CI)	p-value
Sex (*versus* male)	0.001 ± 0.014 (-0.027–0.028)	0.947
Age	0.001 ± 0.000 (-0.0003–0.0004)	0.790
Duration of asthma#	0.001 ± 0.000 (-0.0004–0.0013)	0.259
Age at onset of asthma#	0.001 ± 0.000 (-0.0012–0.0004)	0.285
Asthma group (*versus* fatal)		
Control	-0.040 ± 0.016 (-0.064– -0.015)	0.002
Nonfatal	-0.040 ± 0.016 (-0.072– -0.009)	0.013

#: assessed in separate models with asthma cases alone.

The thickness of the ASM layer was found to be independent of age at onset and duration of asthma. This raises the possibility that the thickness of the ASM layer is determined early in the course of asthma. Although asthma severity is related to lung function [6, 7], the relationships between lung function, severity of asthma and ASM thickness could not be assessed in the present study. Others have found a relation between duration of asthma and lung function [17], and between age and airway wall thickness, with a similar trend for ASM [5]. In the present study, a relationship between ASM thickness and age was found on univariate analysis in the nonfatal asthma group. There was a small overall effect of age in the same group in the general linear model. It is possible that the effects of age are small relative to the effect of severity and are, therefore, only evident in cases of mild-to-moderate severity.

The effects of age were not observed if PBM (the marker of airway size) was included in the model. Although we found that the results were similar if analyses were confined to specific airway size groups, there is potential for confounding by airway size since small airways have a thinner layer of ASM than do larger airways. Children, having smaller airways, have a thinner ASM layer in airways at the same anatomical site as adults [9]. Although the present study included cases over a wide age range (10–>80 yrs), there were only 13 children aged <15 yrs, too few for separate analysis. Analyses confined to adults showed a stronger effect of age in the nonfatal asthma cases. Therefore, a small effect of ageing on the thickness of the ASM layer cannot be excluded. There was a small increase in ASM_{area} with age in the nonfatal asthma group. The lack of change with age in the control group suggests that ageing *per se* does not increase the thickness of the ASM. The effect in the nonfatal asthma group may have been apparent because subtle changes, possibly related to matrix deposition or ASM cell enlargement or proliferation over time, are discernible against a background of mild remodelling, whereas, in the fatal cases

of asthma, these subtle changes may be masked by the marked remodelling seen with such cases.

The findings of the present study and the stability of asthma severity from early childhood to middle age seen in longitudinal studies suggest that the increased thickness of the ASM layer in asthma is an early event that changes little with the duration of asthma and possibly very little with age. The increase in the thickness of the ASM layer might occur at the time of, or prior to, the appearance of the first symptoms of asthma. One study has suggested that some of the inflammatory and remodelling changes (increased thickness of the basement membrane) in childhood asthma develop at an age of 2–5 yrs [18], and a recent study [19] has shown increased area fraction of ASM within the airway wall and increased numbers of ASM cells in children aged 8–14 yrs with asthma, compared with controls. Prospective studies are required to determine the origins of increased ASM thickness in asthma.

The data examined in the present study came from six centres, each of which collected, fixed, embedded and sectioned the tissue samples, and measured the airway dimensions in the sections (table 1). Therefore, it is possible that the effect of the centre itself might account for differences between groups, and, on univariate analysis, there was a significant effect of centre on ASM_{area}/PBM. This effect was found to be due to differences in the sampling strategies used at each centre. Some centres opportunistically sampled predominantly small airways [8, 10, 11], whereas others systematically sampled large and small airways [2, 9, 12]. The Perth (Australia) centre sampled all available small airways on tissue blocks, whereas the Calgary (Canada) centre sampled only small airways along three axial paths, resulting in relatively fewer small airways than large airways compared with the other centres. Differences between centres were present only in the small airways, which were affected by these sampling strategies [20]. However, the relationship between ASM_{area} and PBM was almost identical for the Perth and Calgary centres, both of which sampled all available large and small airways [20]. There were also differences in fixation, measurement and staining techniques between the centres. Some lungs were fixed by inflation and others without inflation. This has been shown to have no significant effect on airway wall area or PBM [20, 21]. The use of point counts or planimetry gives the same result provided appropriate calibration is carried out and the same area of interest is measured. Centres that participated in the present study routinely calibrate measurement devices. The area of interest that is included in measurements of point counts or planimetry is affected by tissue thickness (due to overlap), magnification and tissue staining. Preliminary experiments determined that the ASM layer is equally well defined by different stains, and by both planimetry and point counts in tissue sections of 3–4 μm in thickness at a magnification of 40×. In all cases, the ASM layer was defined by the inner and outer edges of the muscle, rather than connective tissue.

Another potential limitation to the present study is the lack of clinical information on all subjects, as shown in table 2. The general linear model, which included all subjects, examined the effects of age (but not duration or age of onset of asthma) and case group (control or nonfatal or fatal asthma) on the thickness of the ASM layer (ASM_{area}/PBM). Like the models limited to smaller numbers by the availability of data on duration of asthma, age of onset of asthma and smoking, no effect of time on ASM thickness was observed.

Smoking has been shown to adversely affect asthma in a number of ways. Subjects with asthma who smoke show reduced responses to corticosteroids [22], increased symptoms [23], reduced lung function and a greater decline in lung function [24, 25]. However, we did not detect any effect of smoking on ASM thickness.

We also found no effect of age of onset or duration of asthma on the thickness of the ASM layer. Previous studies have shown an effect of duration of asthma on lung function [17]. It has also been suggested that the length of time that asthma goes untreated is related inversely to lung function [26]. Therefore, to the extent that fixed airflow obstruction reflects ASM thickness [16], these studies raise the possibility that ASM remodelling may increase with time, especially time without treatment. However, there are no prospective studies of ASM dimensions to support this view. In children with asthma, treatment with inhaled corticosteroids did not improve lung function decline [27]. It has been shown that treatment with high-dose inhaled corticosteroids can reduce the thickness of the reticular basement membrane [27, 28]. The degree to which treatment can reverse remodelling of the ASM is unknown. The presence of inflammation and fibrosis in asthma and the association of persistent inflammation with the development of fibrosis have led to the view that inflammation is the primary event in asthma, and results eventually in airway remodelling, including subepithelial deposition of extracellular matrix proteins, such as collagen, and increased ASM. The present study suggests the view that the increased ASM may be independent of the inflammation, or, at least, that persistent inflammation does not result in gradually increasing thickness of ASM in asthma.

In conclusion, although the thickness of the ASM layer is related to the clinical severity of asthma, it does not seem to be related to duration of asthma. This suggests that remodelling of ASM is an early event in the natural history of asthma and may be a factor that determines asthma severity.

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STATEMENT OF INTEREST

A statement of interest for M.J. Abramson can be found at www.erj.ersjournals.com/misc/statements.dtl

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