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Cross-sectional and longitudinal association of the secretoglobin 1A1 gene A38G polymorphism with asthma phenotype in the Perth Infant Asthma Follow-up cohort

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Clinical and Experimental Allergy

Summary

Background Associations between Clara cell secretory protein gene variants (*SCGB1A1*, also known as CC16, CC10, CCSP and uteroglobin) and the asthma phenotype have been found in five out of eight studies world-wide. No study has investigated the contribution of *SCGB1A1* polymorphisms to the development and/or persistence of the asthma phenotype in a birth cohort followed over time.

Objective The aim of this study was to determine the role of the *SCGB1A1* gene in the development of the asthma phenotype.

Methods The Perth Infant Asthma Follow-up (PIAF) cohort ($n = 231$ unrelated infants, unselected for asthma and recruited at birth) were seen at 1 month, 6 and 11 years of age, and had a questionnaire, lung function, airway responsiveness (AR) and skin prick tests (SPTs) completed. Blood was taken at 6 and 11 years for total and specific immunoglobulin E (sIgE) and DNA extraction. SPT positivity had at least one positive SPT. sIgE > 4 kU/L had at least one sIgE above 4 kU/L. *SCGB1A1* A38G (rs3741240), that alters gene transcription, was genotyped using *Sau96I* restriction digestion of exon 1 PCR products.

Results At 6 and 11 years of age, 33.0% and 29.7% of those genotyped had doctor-diagnosed asthma, and 35.8% and 52.1% had SPT positivity. In cross-sectional analyses, children with 38G/38A or 38A/38A had increased AR at 1 month (1.72-fold, $P = 0.013$); sIgE > 4 kU/L [odds ratio (OR) = 6.95, 95% confidence interval (CI) = 1.35–35.91, $P = 0.021$]; house dust mite (HDM) SPT positivity (OR = 7.21, 95% CI = 1.09–47.78, $P = 0.041$) and sIgE (4.57-fold, $P = 0.045$) at 6 years; and doctor-diagnosed asthma (OR = 3.93, 95% CI = 1.24–12.47, $P = 0.02$) and cat SPT positivity (OR = 4.34, 95% CI = 1.01–18.77, $P = 0.049$) at 11 years. Longitudinal analyses of 6 and 11 years paired data showed that children with 38A/38A had increased persistent sIgE > 4 kU/L (OR = 11.87, 95% CI = 1.97–71.53, $P = 0.007$) and persistent HDM SPT positivity (OR = 7.84, 95% CI = 1.04–58.92, $P = 0.045$).

Conclusion *SCGB1A1* A38G may play a role in the development and persistence of the asthma phenotype in childhood.

Keywords airway responsiveness, asthma, atopy, children, longitudinal study, polymorphism, *SCGB1A1*

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Introduction

Few asthma genetic susceptibility studies have investigated the effect of age on the contribution of gene polymorphisms to asthma or asthma-associated phenotypes. This has been due to the limited number of

unselected cohorts with both sufficient longitudinal data and DNA. Thus, only a few studies have reported age-specific relationships between particular genotypes and asthma-related phenotypes [1–4]. The advantage of studying populations longitudinally lies in the repeat follow-up investigations of subjects at similar ages, using

the same methodology to assess disease phenotypes [5, 6]. This allows investigation of age-specific factors related to disease, enables subjects with a consistent homogenous phenotype to be delineated from those with intermittent variable phenotypes, and thus provides a greater likelihood of identifying genotype–phenotype associations that represent causal pathways to disease. The strong potential of this approach has been acknowledged [7].

Investigations on longitudinal cohorts ascertained in infancy have successfully identified a number of phenotypic features associated with a predisposition to the development of respiratory symptoms, asthma and/or atopy [8–11]. Ascertainment of genetic factors that predispose subjects to altered phenotypic variables, preceding the development of asthma or its diagnosis, may identify markers that could be used to target particular subjects for early intervention.

Compelling evidence suggests that the gene (*SCGB1A1*; also known as CC16, CC10, CCSP and uteroglobin) for Clara cell 16 kDa secretory protein could be important in age-related genetic influences on the development of asthma. Polymorphic markers on chromosome 11q12-13.1 showed significant evidence of association to serum specific immunoglobulin E (sIgE) levels in a population that included families from the Perth Infant Asthma Follow-up (PIAF) cohort [12]. This chromosomal region contains *SCGB1A1* which has been identified as a candidate in the aetiology of asthma both by its chromosomal location [13] and functionality because of its numerous anti-inflammatory activities and the significantly reduced *SCGB1A1* levels that have been reported in the airways of asthmatic patients [14, 15]. *SCGB1A1* inhibits phospholipase A2 [16, 17] and IFN- γ [18], and reduced levels are associated with numerous respiratory conditions in humans including bronchopulmonary dysplasia or acute respiratory distress in infants [19, 20], chronic obstructive pulmonary disease and lung cancer [21], asthma [14, 15, 22], bacterial pneumonia [23] and bronchiolitis obliterative syndrome in lung transplant recipients [24]. In knockout mice, *Scgb1a1* stimulates IFN- γ expression in naïve T cells and inhibits cytokine expression from Th2 differentiated cells [25].

The *SCGB1A1* gene contains few validated polymorphisms and the data for Caucasians from the HapMap (<http://www.hapmap.org>) shows that these are in linkage disequilibrium. A single nucleotide polymorphism, A38G, was identified in the 5' untranslated region of exon 1 (rs3741240) [13, 26] and the 38A allele has been associated with reduced gene transcription [27, 28]. The 38A/38A genotype has been associated with significantly lower levels of circulating *SCGB1A1* protein [29] in a paediatric asthma case control study [29] and also in children having an asthma attack [30]. Furthermore, the 38A allele has been consistently associated with an increased risk of asthma in a number of studies that used a variety of

cross-sectional designs, asthma measures and populations with different ethnicities [26, 29, 31–33]. Nevertheless, a few studies have not found *SCGB1A1* A38G to be associated with the asthma phenotype [34–36]. Overall, consistently reported associations of the 38A allele with the asthma phenotype strongly supported a functional role for the *SCGB1A1* A38G polymorphism in the development of asthma. None of the studies reported to date have investigated the role of this gene on the development of asthma over time using a number of asthma phenotypic measures in addition to the gold standard of doctor diagnosis.

To further elucidate the potential role of the *SCGB1A1* gene in the pathogenesis of asthma, this study analysed the relationship between the A38G polymorphism and asthma phenotypic features in the PIAF cohort investigated longitudinally over the first 11 years of life. Our primary hypothesis was that the *SCGB1A1* 38A/38A genotype would be persistently associated with increased odds of asthma diagnosis during childhood. Our secondary hypotheses were to determine whether the *SCGB1A1* 38A/38A genotype was associated with increased incidence/severity of several asthma-associated phenotypes.

Materials and methods

Population

The population has been described in detail previously [3, 11, 37, 38]. Briefly, 231 genetically unrelated children, unselected for parental history of asthma or atopy, were recruited before birth, and comprehensive respiratory phenotypic data were collected at several time points (1 month, 6 and 11 years of age). At recruitment, and each follow-up, the following were collected: respiratory history using a questionnaire administered to one or more parents, lung function [1 month, maximum expiratory flow at functional residual capacity (V_{maxFRC}); 6 and 11 years, forced expiratory volume in 1 s (FEV_1)] including airway responsiveness (AR) to histamine (maximum provocative concentration/dose at 1 month was 20 mg/mL, and 6 and 11 years was 7.8 μ mol) and skin prick tests (SPTs) to several common allergens [house dust mite (HDM); perennial rye grass pollen; cow's milk and egg white at 1 month, 6 and 11 years of age; as well as mixed grass, cat dander, dog dander, *Alternaria alternans* and *Aspergillus fumigatus* at 6 and 11 years of age]. SPTs' weals were measured after 15 min by taking the largest diameter and were only considered to be valid if the weal size was greater than that of the 0.9% saline negative control. At 6 and 11 years of age, blood was taken to measure total and sIgE levels, and to extract DNA from peripheral blood lymphocytes for genetic studies. Written parental consent was obtained at each follow-up, and assent was obtained from the study children of 11 years of

age. The Princess Margaret Hospital Human Ethics Committee approved this study.

Genotype

SCGB1A1 A38G was genotyped using PCR and restriction digestion as described previously [26]. Briefly, specific primers were used to amplify the region spanning the polymorphism from genomic DNA. PCR products were then digested with the *Sau96I* restriction enzyme that recognizes the 38G allele. Samples were run on 2% agarose gel electrophoresis, which contained ethidium bromide, and visualized with UV illumination. A random 10% of samples were DNA sequenced to confirm genotyping results, which matched the PCR-based results 100%.

Statistics

Hypotheses were investigated according to a prioritized set. Phenotypes, such as SPT positivity, that showed some evidence of association were further investigated. Binary variables were analysed by multiple logistic regression, and continuous outcomes were assessed by multiple linear regression using Minitab version 13. Subjects homozygous for the 38G allele were set as the baseline or reference group, because this was the most common genotype. Linear regression analyses to determine the genetically additive effect of SCGB1A1 genotype on each phenotype variable were performed by entering SCGB1A1 A38G genotype as a continuous covariate. Genotypes were coded as 38G/38G = 0, 38G/38A = 1 and 38A/38A = 2.

Almost all the linear and logistic regression models were adjusted for gender and age, as they are known to influence the outcomes measured. Several phenotypic variables were adjusted for tobacco smoke exposure by including the 'number of cigarettes smoked by the child's mother per week' in the analyses of asthma ever and AR to histamine. Analyses of raw lung function values (V_{maxFRC} at 1 month or FEV_1 at 6 and 11 years of age) were adjusted using a subject's gender (0, 1) and the number of cigarettes smoked by their mother per week, as well as variables for age, age² and height (all mean corrected). In addition, each subject's FEV_1 , measured by spirometry, was expressed as a percentage of their predicted FEV_1 , which was calculated using the age, gender and height of each subject and therefore represented an estimate of adjusted lung function [39].

Binary outcome variables were coded as 0 for a negative response and 1 for a positive response to the following:

- *Asthma ever*: 'Has your child ever had asthma?' and 'Who diagnosed the asthma? – doctor' (6 and 11 years of age)

- *SPT positivity*: at least one positive SPT (of at least 2 mm in diameter at 1 month and of at least 3 mm in diameter at 6 and 11 years of age)
- *sIgE > 4 kU/L*: levels of sIgE to mixed grasses or HDM greater than 4 kU/L (6 and 11 years of age)

This cut-off was selected as for HDM it had greater than 95% confidence of detecting IgE antibodies to the major HDM allergens, in a predominantly paediatric population with asthma or rhinitis [40]. Furthermore, in a group of non-allergic children with current ascaris infection, and hence high total IgE levels, none of the children had sIgE to the major HDM allergens above the 4 kU/L cut-off. SPT positivity to mixed grasses, HDM and cat were assessed individually as they were the most common reactions in this cohort (6 and 11 years of age).

AR to histamine was calculated as a dose-response slope (DRS) (1 month, 6 and 11 years of age). sIgE to HDM and mixed grasses and total IgE levels were skewed, and were thus log transformed before analysis (6 and 11 years of age) to achieve normal distributions.

Longitudinal phenotype classification

In order to be classified into a longitudinal category of disease, an individual had to have a response at both visits. Therefore, only children with paired data were included. For each individual, responses to 'has your child ever been diagnosed with asthma by a doctor?' and 'at what age?' were analysed at both the 6 and 11 year follow-up. Data on SPT positivity, sIgE > 4 kU/L and any provocative dose of histamine that resulted in a 20% reduction in FEV_1 (PD₂₀) were coded as binary variables (1 = present, 0 = absent) at each visit at age 6 and 11 years. For PD₂₀, the maximum dose at completion of the histamine challenge (7.8 µmol) was arbitrarily used as the cut-off [41]. Although we have previously cautioned against comparing AR results between different aged cohorts, due to unresolvable difficulties in correcting the dose for size [42, 43], in the current study, we have simply used the data to dichotomize results, and avoided quantitative comparisons between the age groups. Responses were classified into one of the four categories as follows: never present – phenotype not present at both visits; early remittent – phenotype present at age 6 and absent at age 11; persistent – phenotype present at both visits and late onset – phenotype absent at age 6 and present at age 11.

Longitudinal statistical analyses

Longitudinal categories were coded as binary variables and entered into logistic regression analyses. (1) Never present and early remittent phenotypes = 0 with late onset and persistent = 1 or (2) never present = 0 and

persistent = 1. Statistical models of *SCGB1A1* A38G for longitudinal phenotype were adjusted for gender.

Results

Population characteristics

The male:female ratio for each of the three follow-ups was consistent (Table 1). Levels of maternal tobacco smoking were similar when the children were followed-up at 6 and 11 years of age and slightly lower than during pregnancy. The frequency of SPT positivity rose from 1 month to 11 years of age and asthma was most common at 6 years of age (Table 1). The mean, standard deviation (SD) and range of the children's height, lung function and IgE levels are described in Table 1. There were no significant differences between the frequencies of phenotypic outcomes at 1 month of age in children followed-up at 6 years of age compared with those not followed-up [37]. There were also no significant differences in phenotypic outcomes assessed at 6 and 11 years of age between those that could be genotyped at 11 years of age vs. those assessed but not genotyped in this cohort [3].

Frequency of *SCGB1A1* A38G

The genotype frequencies of this polymorphism were not significantly different between follow-up groups and were similar to those previously reported for unselected Caucasian populations [26, 31, 33]. The frequencies of the 38A/38A and 38G/38A genotypes expressed as the percentage (*n*) of the population at each follow-up were 9.7% (15) and 40.3% (62) at 1 month, 9.9% (10) and 39.6% (40) at 6 years and 10.6% (16) and 40.4% (61) at 11 years of age. The 38A allele frequencies of 1 month, 6 and 11 year follow-up cohorts were 29.85%, 29.7% and 30.8%, respectively. Genotype frequencies in each follow-up cohort were in Hardy-Weinberg equilibrium (1 month, $P = 0.998$; 6 years, $P = 0.998$; 11 years, $P = 0.998$).

Cross-sectional genotype/phenotype analyses

Data were available on more children at each follow-up than those children seen at both 6 and 11 years of age (i.e. children with paired data). Therefore, cross-sectional analyses were firstly completed using the prioritized set of hypotheses. Several outcome measures showed evidence of a significant association with the *SCGB1A1* A38G genotype at different ages including 1 month – AR

Table 1. Description of genotyped subjects from Perth Infant Asthma Follow-up (PIAF) cohort

	Pregnancy/birth <i>n</i> = 160	1 month <i>n</i> = 154	6 years <i>n</i> = 102	11 years <i>n</i> = 151
Males: <i>n</i> (%)	92 (57.5)	89 (57.8)	55 (54.5)	85 (56.3)
Age: mean, SD (range)		4.72, 1.67 (2.00–9.00) weeks	6.27, 0.49 (4.24–8.68) years	11.13, 0.92 (9.25–13.47) years
Length/height in cm: mean, SD (range)		54.87, 2.70 (47.20–61.00)	117.52, 5.77 (107.00–133.00)	146.27, 8.78 (123.00–170.00)
Mothers that currently smoke: <i>n</i> (%)	37 (23.1)		19 (19.4)	29 (20.1)
SPT positivity: <i>n</i> (%)		7 (4.8)	34 (35.8)	75 (52.1)
sIgE > 4 kU/L: <i>n</i> (%)			20 (23.0)	42 (31.8)
Doctor-diagnosed asthma: <i>n</i> (%)			32 (33.0)	43 (29.7)
Lung function: mean, SD (range)		V _{max} FRC 97.14, 49.55 (17.20–321.88) mL	FEV ₁ 1.22, 0.23 (0.74–1.78) L	FEV ₁ 2.16, 0.40 (1.32–3.68) L
Predicted lung function (%): mean, SD (range)			123.98, 21.45 (70.19–183.48)	99.07, 11.60 (69.22, 135.17)
Dose-response slope to histamine: mean, SD (range)		%V _{max} FRC per mg/mL 70.74, 79.26 (–2.22–389.48)	%FEV ₁ per µmol 15.62, 38.40 (–3.07–258.06)	%FEV ₁ per µmol 3.61, 6.79 (–0.92–50.81)
Airway responsive to histamine: <i>n</i> (%)		PC40 ≤ 20 mg/mL 118 (94.4)	PD20 ≤ 7.8 µmol 51 (60.7)	PD20 ≤ 7.8 µmol 47 (34.3)
Total IgE in kU/L: mean, SD (range)			162.35, 263.33 (2.00–2000.00)	206.22, 620.94 (1.20–6642.00)
Specific IgE to HDM in kU/L: mean, SD (range)			13.84, 35.40 (0.35–148.01)	8.51, 24.53 (0.10–194.49)
Specific IgE to mixed grasses in kU/L: mean, SD (range)			2.68, 11.36 (0.35–100.00)	7.27, 24.79 (0.20–150.00)

SPT, skin prick test; sIgE, specific immunoglobulin E; FEV₁, forced expiratory volume in 1 s; V_{max}FRC, maximum expiratory flow at functional residual capacity; HDM, house dust mite.

to histamine; 6 years – sIgE > 4 kU/L, SPT positivity to HDM and sIgE levels to HDM and 11 years – doctor diagnosis of asthma ever, sIgE > 4 kU/L and SPT positivity to cat. These results are presented in Table 2. Trends towards significance were observed between *SCGB1A1* A38G genotype and doctor diagnosis of asthma ever, SPT positivity, SPT positivity to mixed grasses, total IgE levels and percentage predicted FEV₁ at 6 years of age as well as SPT positivity, sIgE > 4 kU/L, SPT positivity to HDM and sIgE levels to HDM at 11 years of age. Using multiple regression analyses, no associations were found between *SCGB1A1* A38G and V'maxFRC or atopic status at 1 month; AR to histamine, FEV₁, SPT positivity to cat or sIgE levels to mixed grasses at 6 years of age; or AR to histamine, FEV₁, SPT positivity to mixed grasses, total IgE levels or sIgE to mixed grasses at 11 years of age (Table S1).

Longitudinal genotype/phenotype analyses

Paired data at 6 and 11 years of age ($n = 76$ – 151) were used to identify those children with never present, early remit-

tent, persistent or late onset asthma phenotypes of doctor-diagnosed asthma ($n = 151$), AR to histamine ($n = 81$), SPT positivity ($n = 96$), sIgE > 4 kU/L ($n = 76$) and SPT positivity to HDM ($n = 90$). No evidence of association was identified for the *SCGB1A1* A38G genotype and longitudinal doctor-diagnosed asthma [38A/38A vs. 38G/38G: odds ratio (OR) = 1.68, 95% confidence interval (CI) = 0.46–6.18, $n = 151$, $P = 0.433$] or SPT positivity (38A/38A vs. 38G/38G: OR = 1.72, 95% CI = 0.37–7.97, $n = 96$, $P = 0.485$). Overall, the *SCGB1A1* A38G genotype appears to be associated with persistent and late onset AR to histamine and persistent atopy defined using SPT responses and sIgE to aeroallergens (Table 3).

Compared with subjects with 38G/38G, those with 38A/38A had an odds of persistent or late AR of 5.22 (95% CI = 0.82–33.26, $P = 0.08$) and 38G/38A subjects had an intermediate odds of 2.19 (95% CI = 0.83–5.78, $P = 0.115$). This association exhibits a linear trend with an increase in odds of 2.24 (95% CI = 1.06–4.74, $P = 0.035$) for each additional 38A allele. Subjects with persistent and late onset AR have 3.70-fold significantly increased odds (95%

Table 2. Summary of cross-sectional *SCGB1A1* A38G genotype/phenotype associations in the Perth Infant Asthma Follow-up (PIAF) cohort

Phenotype	<i>SCGB1A1</i> genotype			Linear trend
	38G/38G	38G/38A	38A/38A	
1 month	$n = 77$	$n = 62$	$n = 15$	
DRS (%FEV ₁ per mg/mL histamine) $n = 128$				
Adjusted mean	33.52	57.76	55.81	1.44#
95% CI	22.66–49.59	38.23–87.26	29.06–107.19	1.06–1.95#
P -value		0.013	0.159	0.022
6 years	$n = 51$	$n = 41$	$n = 10$	
sIgE > 4kU/L $n = 87$				
Odds ratio	1	3.14	6.95	2.73
95% CI		0.97–10.13	1.35–35.91	1.25–5.99
P -value		0.056	0.021	0.012
HDM SPT positivity $n = 97$				
Odds ratio	1	2.83	7.21	2.73
95% CI		0.84–9.51	1.09–47.78	1.13–6.57
P -value		0.093	0.041	0.025
HDM sIgE (kU/L) $n = 87$				
Adjusted mean	0.49	1.05	2.24	2.13#
95% CI	0.22–1.10	0.47–2.34	0.64–7.90	1.12–4.04#
P -value		0.11	0.045	0.023
11 years	$n = 74$	$n = 61$	$n = 16$	
Asthma diagnosis ever $n = 149$				
Odds ratio	1	1.29	3.93	1.76
95% CI		0.58–2.83	1.24–12.47	1.03–3.01
P -value		0.532	0.020	0.038
Cat SPT positivity $n = 149$				
Odds ratio	1	2.24	4.34	2.12
95% CI		0.79–6.34	1.01–18.77	1.05–4.25
P -value		0.128	0.049	0.035

$P < 0.05$ grey; all models adjusted for age and gender; linear trend is the change (added or multiplied #) in odds ratio or adjusted mean and 95% CI for each additional A allele.

Please refer to the text for a summary of associations that did not reach statistical significance and to the online supplementary table for detail.

SPT, skin prick test; sIgE, specific immunoglobulin E; FEV₁, forced expiratory volume in 1 s; HDM, house dust mite; CI, confidence interval; DRS, dose-response slope.

Table 3. Results summary for the *SCGB1A1* A38G genotype/longitudinal phenotype analyses in the Perth Infant Asthma Follow-up (PIAF) cohort

	Never present	Early remittent	Persistent	Late onset
6 years	absent	present	present	absent
11 years	absent	absent	present	present
<i>SCGB1A1</i> 38A/38A vs 38G/38G Asthma, OR=1.68, 95%CI=0.46-6.18, <i>P</i>=0.433 AR, OR=5.22, 95%CI=0.82-33.26, <i>P</i>=0.08 SPT positivity, OR=1.72, 95%CI=0.37-7.97, <i>P</i>=0.485				
<i>SCGB1A1</i> 38A/38A vs 38G/38G sIgE > 4kU/L, OR=11.87, 95%CI=1.97-71.53, <i>P</i>=0.007 SPT positivity to HDM, OR=7.84, 95%CI=1.04-58.92, <i>P</i>=0.045				

Dark vs. light cross-hatch represents groups compared in the longitudinal analyses.

CI = 1.41–9.69, *P* = 0.008) of doctor-diagnosed asthma by the age of 11 years, than those with never or early remittent AR.

Subjects with 38A/38A had 11.87-fold odds (95% CI = 1.97–71.53, *P* = 0.007) and 38G/38A had 2.69-fold odds (95% CI = 0.64–11.30, *P* = 0.176) of persistent sIgE > 4 kU/L than never sIgE > 4 kU/L, compared with those with 38G/38G. There was an increase in the odds of sIgE > 4 kU/L for each additional A allele of 3.36 (95% CI = 1.37–8.24, *P* = 0.008). This definition was created to identify subjects most likely to have IgE antibodies to HDM and was found to be associated with doctor diagnosis of hayfever (6 years: $\chi^2_1 = 3.38$, *P* = 0.066 and 11 years: $\chi^2_1 = 22.84$, *P* < 0.001).

Compared with subjects with 38G/38G, those with 38A/38A had a 7.84-fold increased odds (95% CI = 1.04–58.92, *P* = 0.045) of persistent SPT positivity to HDM than never present SPT positivity to HDM, and 38G/38A had an odds of 2.25 (95% CI = 0.65–7.81, *P* = 0.202). This association exhibited a linear trend with odds of 2.60 (95% CI = 1.05–6.42, *P* = 0.039) for each additional 38A allele of early persistent HDM SPT positivity.

Discussion

Longitudinal studies have identified associations of family history of asthma, tobacco smoke and allergen exposure with impaired lung function and increased AR in infancy [44, 45] and with symptoms in early life [46–48]. These findings have indicated that both genetic and environmental exposures can alter early lung development. These altered lung function parameters in early

life were also found to be predictive of poor asthma-related outcomes in later childhood [11, 37, 49]. The genetic factors that predispose infants to developing asthma and/or a particular asthma phenotype [50–53] and that influence the natural course of their condition [2, 3, 54] are complex and likely to be heterogeneous.

In this study, we show that the *SCGB1A1* A38G polymorphism is associated with AR in infancy, and further that during childhood, this genetic variant is increasingly associated with asthma and AR, and persistently associated with atopic outcomes. These data suggest that *SCGB1A1* may have a continuing important influence on the airway from infancy to mid-childhood, as these phenotypes are perhaps the most dominant asthma-related phenotypes that characterize each phase of this period. These findings are in line with *SCGB1A1* having a broad range of roles within the airway and in immune function.

It is possible that the *SCGB1A1* 38A allele is not responsible for the observed associations and is simply linked to another causative allele. However, data for Caucasians from the HapMap (<http://www.hapmap.org>) shows that *SCGB1A1* is not in linkage disequilibrium with the other well-known candidate genes for asthma in the 11q12–13 region, including the β chain of the high affinity IgE receptor (*FCER1B*), glutathione S-transferase P 1 (*GSTP1*), chemoattractant receptor expressed on TH2 cells (*CRTH2*) or interleukin 18 (*IL18*). *SCGB1A1* does lie within a region that exhibits linkage disequilibrium with eight other genes. Five of these genes are also members of the secretoglobulin family (*SCGB2A1*, *SCGB2A2*, *SCGB1D1*, *SCGB1D2* and *SCGB1D4*). However, the *SCGB2A1*, *SCGB2A2* and *SCGB1D2* genes are breast cancer markers

and have only been found to co-express with each other [55, 56], while *SCGB1D1* has only been found in tears [57]. *SCGB1D4* is known to induce IFN- γ [58], but has only one polymorphism (rs1528732) in linkage disequilibrium with areas outside the gene, and the frequency of the polymorphism is too low to account for the associations in this study. The other three genes in linkage disequilibrium with *SCGB1A1* are asparaginase-like 1 protein (*ASRGL1*), AHNK nucleoprotein isoform 2 (*AHNK*) and inner centromere protein antigens 135/155 kDa (*INCEP*). Their function and/or cell specific expression make them unlikely candidates for an aetiological role in asthma or atopy [59–61].

SCGB1A1 is the only gene in the linkage disequilibrium block for which the protein is normally expressed in the lung [62–65] and is one of the most common proteins secreted into the airway. Although the primary function of *SCGB1A1* in the respiratory system has yet to be fully elucidated, it has been shown to have multiple anti-inflammatory activities [21, 66], and has been found to co-express with several other genes thought to be involved in the aetiology of asthma and atopy [56]. Therefore, it is likely that a reduction in *SCGB1A1* protein levels as a result of having the 38A allele could impact on respiratory health and may be the reason why infants with the 38A/38A genotype have increased AR to histamine, a non-specific inflammatory stimulus.

The cross-sectional and longitudinal results from this birth cohort are the first to suggest a role for *SCGB1A1* genotype in the development and persistence of atopy. Previous studies have predominantly used a case control study design, comparing asthmatics with and/or without atopy with healthy controls. In an adult Indian population, the *SCGB1A1* gene was associated with asthma and total IgE levels [67], although British, Japanese, Icelandic and German populations found no association of *SCGB1A1* with atopic asthma [33–35]. One other study of an unselected cohort of adult Danes found atopy was not involved in the association of *SCGB1A1* A38G with asthma [31]. Yet, our results are supported by the previous studies of *SCGB1A1* gene and protein function. *Scgb1a1* knockout mice have a magnified allergic response to OVA immunization, with increased Th2 cytokine expression found in lung homogenates [68]. One mechanism of action elucidated was that *SCGB1A1* was found to specifically block PGD₂ receptor-mediated nuclear factor (NF)- κ B activation and suppress cyclo-oxygenase (COX)-2 gene expression [69]. Furthermore, *SCGB1A1* was found to induce IFN- γ production from naïve CD4⁺ T cells and to suppress Th2 cytokine expression of polarized CD4⁺ Th2 cells [70]. Together, these data suggest a biologically plausible role for the *SCGB1A1* 38A allele and associated lower *SCGB1A1* protein levels in the development of atopy.

The study of this cohort involved collecting phenotypic data on 253 children over a period of 12 years. As three different cohorts of children at each age group are represented by the cross-sectional phenotype/genotype results, there is the potential for introduced bias. The method of classifying longitudinal phenotypes using binary outcome measures would have resulted in the loss of valuable information on the changing severity of disease. These factors limited the power of this study, perhaps contributing to a number of the relationships that approached but did not reach statistical significance. CIs were included in the results presented to give an indication of the power of the study – as can be seen with the association between 38A/38A genotype and sIgE > 4 kU/L, the CIs were quite wide (OR = 6.95, 95% CI = 1.35–35.91, *P* = 0.021). We did not correct for multiple testing using Bonferroni, as we believe that it is an unrealistically conservative correction of *P*-values [71, 72]. Instead, we chose to investigate a set of prioritized hypotheses and report the analyses completed, to allow for appropriate interpretation of results [73]. The results from the longitudinal and cross-sectional analyses in this study were similar, and most importantly the 38A/38A genotype was consistently associated with deleterious outcomes, suggesting that these associations may represent an aetiological pathway to disease.

Few studies to date have investigated the contribution of genetic factors to longitudinally assessed phenotypes. This is partly due to the limited number of well-characterized longitudinal populations with comprehensive respiratory phenotype including lung function [5, 74]. One study analysed the CD14 C-159T polymorphism vs. cross-sectional and longitudinal phenotypes in an Australian population recruited at 8 years of age and followed-up seven times to the age of 25 years [2]. This study identified an age-specific association between CD14 –159CC and increased odds of early onset atopy and early onset AR to histamine, compared with the heterozygous and homozygous TT genotypes. This study used very similar methods of longitudinal classification, but was limited by the lack of data from early life, as recruitment began when the children were already in mid-childhood.

In *SCGB1A1*, the 38A allele is the allele consistently associated with increased prevalence and severity of a number of cross-sectional and longitudinal asthma-associated phenotypes in an unselected cohort recruited before birth and followed longitudinally. In conclusion, the *SCGB1A1* 38A/38A genotype may be increasingly involved in the development of asthma and AR with age and persistently involved in the development of IgE-mediated symptomatic atopy in childhood. Further assessment of this and other longitudinal cohorts should establish whether the *SCGB1A1* A38G polymorphism continues to play a role in the aetiology of asthma in early adulthood.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Results of cross-sectional analyses that did not reach statistical significance.

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ONLINE SUPPLEMENTARY TABLE:

Results of cross-sectional analyses that did not reach statistical significance.

1 month	38G/38G		38G/38A	38A/38A	Linear trend
	n=77		n=62	n=15	
<i>V'maxFRC</i> n=153	Adjusted mean	88.46	79.58	87.48	0.95#
	95%CI	74.62-104.86	66.42-95.36	64.60-118.47	0.83-2.95
	p value		0.254	0.943	0.457
<i>SPT positivity</i> n=147	Odds ratio	1	0.71	2.75	1.88
	95%CI		0.11-4.52	0.36-20.92	0.95-3.72
	p value		0.327	0.712	0.07
6 years	38G/38G		38G/38A	38A/38A	Linear trend
	n=51		n=41	n=10	
<i>Asthma Ever</i> n=101	Odds ratio	1	1.56	3.32	1.72
	95%CI		0.63-3.88	0.74-14.79	0.89-3.33
	p value		0.335	0.116	0.104
<i>SPT positivity</i> n=99	Odds ratio	1	2.37	2.5	1.88
	95%CI		0.97-5.8	0.49-12.66	0.95-3.72
	p value		0.059	0.268	0.07
<i>Mixed grasses SPT positivity</i> n=98	Odds ratio	1	3.44	1.23	1.75
	95%CI		0.99-11.94	0.11-14.29	0.72-4.27
	p value		0.051	0.869	0.220
<i>Cat SPT positivity</i> n=98	Odds ratio	1	1.27	2.24	1.42
	95%CI		0.24-6.75	0.18-28.42	0.43-4.69
	p value		0.782	0.533	0.562
<i>Total IgE (kU/l)</i> n=87	Adjusted mean	61.60	95.20	85.28	1.29#
	95%CI	35.73-106.20	55.08-164.55	36.02-201.89	0.83-2.00#
	p value		0.177	0.523	0.258
<i>Mixed grasses sIgE (kU/l)</i> n=87	Adjusted mean	0.549	0.782	0.653	1.19#
	95%CI	0.329-0.918	0.433-1.41	0.255-1.67	0.966-1.48#
	p value		0.244	0.720	0.404
<i>FEV₁</i> n=94	Adjusted mean	1.25	1.21	1.30	-0.005
	95%CI	1.18-1.33	1.13-1.29	1.15-1.44	-0.0002-0.005
	p value		0.316	0.568	0.867
<i>% predicted FEV₁</i> n=94	Adjusted mean	125.4	124.47	137.98	3.44
	95%CI	119.41-131.40	117.49-132.00	123.54-152.43	-3.12-10.00
	p value		0.886	0.118	0.306
<i>DRS (%FEV₁ per μmol histamine)</i> n=87	Adjusted mean	10.18	9.72	9.9	0.973#
	95%CI	6.41-16.17	5.86-16.10	4.06-24.13	0.673-1.41#
	p value		0.857	0.952	0.885

11 years				
	38G/38G	38G/38A	38A/38A	Linear trend
	n=74	n=61	n=16	
<i>SPT positivity</i> n=149	Odds ratio 1	1.04	2.25	1.31
	95%CI	0.52-2.07	0.70-7.27	0.80-2.14
	p value	0.913	0.176	0.288
<i>sIgE > 4kU/l</i> n=137	Odds ratio 1	2.02	2.61	1.72
	95%CI	0.90-4.49	0.81-8.47	1.00-2.95
	p value	0.086	0.110	0.051
<i>HDM SPT positivity</i> n=148	Odds ratio 1	1.34	2.46	1.49
	95%CI	0.64-2.78	0.80-7.62	0.89-2.49
	p value	0.437	0.118	0.125
<i>Mixed grasses SPT positivity</i> n=149	Odds ratio 1	1.09	1.31	1.13
	95%CI	0.50-2.39	0.39-4.38	0.65-1.94
	p value	0.835	0.659	0.669
<i>Total IgE (kU/l)</i> n=137	Adjusted mean 56.85	66.23	96.67	1.25#
	95%CI 33.26-97.20	37.46-117.12	40.05-233.34	0.848-1.86
	p value	0.600	0.240	0.256
<i>HDM sIgE (kU/l)</i> n=137	Adjusted mean 0.514	0.89	1.27	1.62#
	95%CI 0.24-1.1	0.41-1.91	0.38-4.18	0.93-2.82#
	p value	0.187	0.158	0.092
<i>Mixed grasses sIgE (kU/l)</i> n=138	Adjusted mean 0.640	0.684	0.873	1.14#
	95%CI 0.35-1.17	0.36-1.30	0.32-2.36	0.728-1.77#
	p value	0.839	0.541	0.577
<i>FEV₁</i> n=147	Adjusted mean 2.08	2.07	2.02	-0.021
	95%CI 2.00-2.15	1.99-2.15	1.89-2.15	-0.063-0.020
	p value	0.843	0.405	0.474
<i>DRS (%FEV1 per μmol histamine)</i> n=144	Adjusted mean 5.9	5.7	7.17	1.03#
	95%CI 4.76-7.31	4.54-7.15	5.00-10.27	0.902-1.17#
	p value	0.765	0.291	0.666

Footnote: $p < 0.1$ light grey, all models adjusted for age and gender, linear trend is the change (added or multiplied#) in odds ratio or adjusted mean and 95%CI for each additional A allele.