Letter to the Editor

TGF-β1 polymorphisms and asthma severity, airway inflammation, and remodeling

To the Editor:

The development and progression of asthma are determined by multiple genetic and environmental factors. A relatively new hypothesis is that chronic injury by environmental irritants and/or defective repair of the airway epithelium leads to the release of cytokines and growth factors.[1] These factors drive chronic airway inflammation and remodeling seen in asthma. Airway inflammation and remodeling are 2 important pathologic features associated with airway hyperresponsiveness (AHR) and fixed airflow obstruction.[2]

TGF-β1 is a cytokine whose level increases in asthma.[3] It is involved in airway pathology.[4] The release of TGF-β1 in the submucosa, mainly by eosinophils, is stimulated by environmental factors such as allergens and cigarette smoke. Experimental studies show that exposure to cigarette smoke increases *TGFB1* gene expression[5] and decreases eosinophil migration in the airway.[6]

Single nucleotide polymorphisms (SNPs) in the *TGFB1* gene have been associated with asthma development, yet only a few studies have investigated associations with asthma severity and the underlying airway inflammation[7] and none with FEV_1 decline and airway remodeling in asthma. Our aim was to investigate associations between *TGFB1* SNPs and asthma severity (ie, AHR, FEV_1 level, asthma remission, FEV_1 decline) and features of airway wall inflammation and remodeling in 2 asthma populations. In addition, we assessed interactions between *TGFB1* SNPs and smoking.

Details on the clinical assessment, outcome definitions, immunochemistry and quantification procedures, genotypic information, and statistical analysis are presented in this article's Online Repository at www.jacionline.org. The study was approved by the medical ethics committee of the University Medical Center Groningen, and participants signed informed consent.

The clinical characteristics of the 2 populations and genotype distributions are shown in Tables E1 and E2, A and B, in this article's Online Repository at www.jacionline.org. All SNPs were in Hardy-Weinberg equilibrium ($P > .05$). Fig E1 in this article's Online Repository at www.jacionline.org shows the linkage disequilibrium plots of the SNPs.

Table I presents a summary of the main results. Rs6957-G associated with more severe AHR and airflow obstruction, increased subepithelial eosinophil and macrophage counts, and increased basement membrane thickness. These associations were more pronounced in ever-smokers. Rs10417924-A associated with less AHR and a trend for higher FEV_1 (ever-smokers), more asthma remission, and higher subepithelial $CD8^+$ T cells. Rs1800469-T associated with less FEV_1 decline and lower FEV_1 (ever-smokers) and increased goblet cells. Rs1800470-C associated with less FEV_1 decline and lower FEV_1. Rs4803455-A associated with accelerated FEV_1 decline.

TGFB1 by smoking interaction: Significant effects on severity phenotypes were observed mainly in ever-smokers (see Fig 1; also see Fig E2 in this article's Online Repository at www.jacionline.org). Additional significant associations were found in ever-smokers compared with the total group (see Table I).

TABLE I. Main associations of *TGFB1* SNPs with markers of airway pathology and severity of asthma

		Total group		Ever-smokers	
***TGFB1* SNPs**	**Outcome**	**B or OR**	***P* value**	**B or OR**	***P* value**
rs6957-G 3′UTR	FEV_1 decline[1]	−0.2	.97	7.5	.16*
	FEV_1% predicted pre-BD[1,2]	−7.2	**<.001**	−9.6	**.001**
	FEV_1% predicted post-BD[1,2]	−6.9	**.001**	−8.0	**.006**
	AHR severity[1,2]	1.6	**.04**	3.6	**<.001**
	Asthma remission[1,2]	0.5	**.09**	0.5	.20
	Eosinophils in biopsies (ln)[2]	0.45	**.02**	0.78	**.003**†
	Macrophages in biopsies (ln)[2]	0.26	**.045**	0.48	**.01**†
	Basement membrane thickness[2]	0.39	.17	0.076	**.04**
rs10417924-T 3′UTR	FEV_1 decline[1]	−0.3	.94	2.3	.64
	FEV_1% predicted pre-BD[1,2]	3.7	.40	5.4	**.08**
	FEV_1% predicted post-BD[1,2]	3.5	.40	4.3	.16
	AHR severity[1,2]	0.7	.13	0.5	**.04**
	Asthma remission[1,2]	2.2	**.03**	3.0	**.01**
	$CD8^+$ T cells in biopsies (ln)[2]	0.37	**.02**	0.46	**.03**
rs1800469-T Promoter	FEV_1 decline[1]	6.3	**.06**	8.9	**.03**
	FEV_1% predicted pre-BD[1,2]	−2.7	.16	−6.4	**.02***
	FEV_1% predicted post-BD[1,2]	−2.5	.19	−5.4	**.05**†
	Severe asthma[1,2]	1.7	.40	1.3	.74
	Goblet cells in biopsies (ln)[2]	0.23	**.03**	0.27	**.08**
rs1800470-C Exon 1	FEV_1 decline[1]	10.7	**.002**	11.00	**.01**
	FEV_1% predicted pre-BD[1,2]	−4.1	**.04**	−6.8	**.01**†
	FEV_1% predicted post-BD[1,2]	−4.7	**.02**	−7.0	**.01**
	Severe asthma[1,2]	1.5	.60	1.4	.74
rs4803455-A Intron 2	FEV_1 decline[1]	−11.4	**.003**	−15.7	**<.001**
	FEV_1% predicted pre-BD[1,2]	3.2	.16	3.8	.22
	FEV_1% predicted post-BD[1,2]	2.1	.33	3.0	.32

Associations of *TGFB1* SNPs with FEV_1 decline were investigated in population 1. Associations with cross-sectional lung function level and clinical outcomes were assessed in populations 1 (n = 243) and 2 (n = 137) and meta-analyzed. Associations with pathologic outcomes were investigated in population 2. Populations are shown as superscript numerals.

Complete asthma remission: FEV_1% predicted post-BD > 90% and absence of AHR and symptoms and medication; severe asthma: FEV_1% predicted pre-BD < 80% and ICS use. The number of positively stained inflammatory cells was counted in a total area of 0.1 mm^2 in the submucosa, 100 μm under the basement membrane. Goblet cell numbers were counted on periodic acid–Schiff–stained biopsy sections and expressed per 1000 μm of basement membrane.

Dominant genetic models were used in multiple linear and logistic regression adjusted for sex, age, ICS use, and smoking status (total group).

Two tailed *P* values of < .05 were considered statistically significant and *P* values between .05 and .09 of borderline significance (boldface text).

AHR, Airway hyperresponsiveness PC_{20} histamine < 16 mg/mL (population 1) and PC_{20} AMP < 320 mg/mL (population 2); *AMP*, adenosine 5-monophosphate; *B*, regression coefficient; *BD*, bronchodilation; *ICS*, inhaled corticosteroids; *OR*, odds ratio; *3′UTR*, 3 prime untranslated region; *VC*, vital capacity.

*Significant interaction ($P < .05$).

†Interaction of borderline significance ($.05 \le P \le .09$).

We found a limited number of significant interactions, but several of the significant associations seen in ever-smokers had an interaction of borderline significance ($.05 \le P \le .09$). Tables E3 to E14

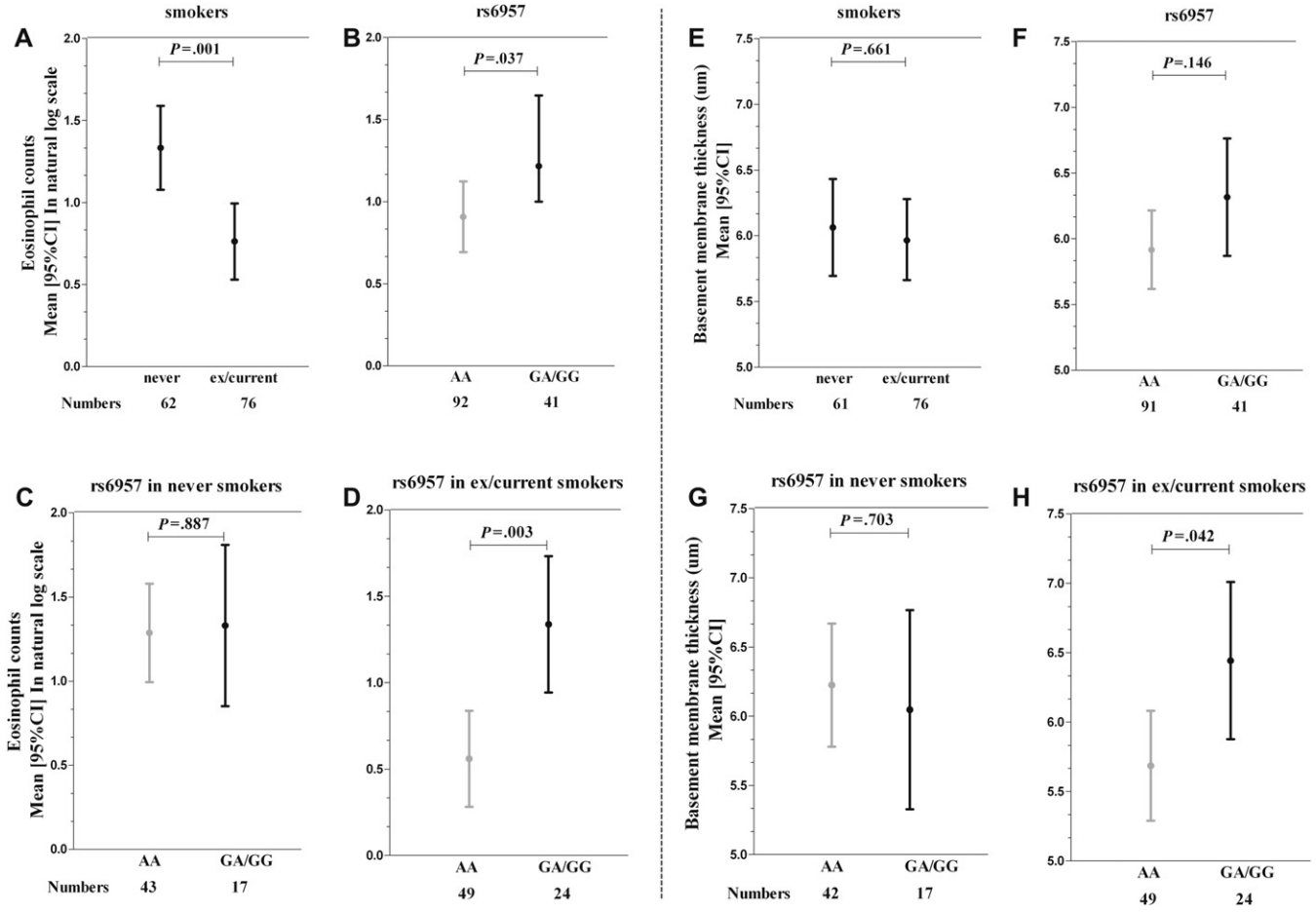

FIG 1. Associations between subepithelial eosinophil counts and smoking status **(A)**, rs6957 **(B)**, rs6957 in never-smokers **(C)** and in ever-smokers **(D)**. Associations between basement membrane thickness and smoking status **(E)**, rs6957 **(F)**, rs6957 in never-smokers **(G)** and in ever-smokers **(H)**. Mean (95% CI) eosinophil counts are given by generalized linear models adjusted for sex, age, and inhaled corticosteroid use.

in this article's Online Repository at www.jacionline.org present detailed results.

Our study shows that *TGFB1* SNPs are significantly associated with asthma severity defined by more severe AHR, airflow obstruction, and asthma remission, as well as with airway inflammation and remodeling. Of importance, there is indirect replication, because the direction of the SNP associations with asthma severity was consistent with the direction of the SNP associations with pathologic changes. We confirm results of previous studies showing that rs1800469-T is associated with airflow obstruction.[7] On the basis of its association with increased numbers of goblet cells in bronchial biopsies, we propose that mucus hypersecretion but not basement membrane thickness might be a missing link between the *TGFB1* promoter SNP and airflow obstruction in asthma.

Three (rs6957, rs1800469, and rs1800470) of the 6 SNPs in our study are known to influence TGFβ1 serum levels (see References E14-E16 in this article's Online Repository at www.jacionline.org). However, the pleiotropic nature and multiple sources of this cytokine make it difficult to determine in which compartment/cell type its expression should be measured. Because of lack of expression data, we cannot conclude whether these pathologic changes in our study are driven by high or low TGFβ1 expression and by which cellular sources. Elucidating the effects of TGFβ1 on asthma phenotypes is further complicated by the fact that its functions are controlled not only by inhaled substances and subsequently expression levels in the airways but also by the chronic response of the cells and the interaction with other growth factors and pathways in the immediate microenvironment.

We confirm that smokers have lower eosinophilic inflammation/infiltration than do nonsmokers (Fig 1).[6] However, when accounting for *TGFB1* genotypes, this effect no longer exists. Asthmatic carriers of the G-allele of rs6957 have significantly higher submucosal eosinophils (and macrophages) than the wild types, an effect that is not significant in nonsmokers. Consistent with the increase in eosinophil counts, basement membrane thickness is significantly increased in carriers of the G-allele in smokers only. Similarly, smoking is not associated with $CD8^+$ T-cells counts or AHR severity, but when accounting for the genotype, smokers carrying the A-allele of rs10417924 have significantly higher $CD8^+$ T cells and less severe AHR (Fig E2), a relationship consistent with an earlier study.[8] These data provide evidence of an interaction between *TGFB1* SNPs and smoking on asthma severity.

Our study is the first investigating the effects of *TGFB1* on FEV_1 decline in asthma (see Fig E3 in this article's Online Repository at www.jacionline.org). Rs1800469-T and rs1800470-C significantly associated with less FEV_1 decline and rs4803455-A

with accelerated FEV_1 decline. Of interest, SNPs that were associated with more airflow obstruction cross-sectionally (rs6957, rs1800469, and rs1800470) were also associated with less rapid decline in ever-smokers of our study. This is compatible with previous observations that show associations with chronic obstructive pulmonary disease protection,[9] suggesting that high TGFβ1 levels might be protective for chronic obstructive pulmonary disease, while others support that high TGFβ1 levels increase the risk for asthma.[4] These findings may infer a distinct role of *TGFB1* between severe asthma and chronic obstructive pulmonary disease.

Because we decided *a priori* to replicate and meta-analyze the results of our analysis in the 2 asthma populations, we did not adjust for multiple comparisons. Moreover, the outcomes in our analysis (clinical and pathologic phenotypes) are mutually related, suggesting that a conservative statistical approach would not do justice to their biologically linked nature.

We conclude that the *TGFB1* gene plays a role in the pathophysiology of asthma severity. We for the first time show associations of *TGFB1* polymorphisms with airway pathology and additionally link *TGFB1* with FEV_1 decline in asthma. Our data provide suggestive evidence for an interaction with smoking in asthmatic patients.

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Online Repository

Transforming Growth Factor beta-1 polymorphisms and asthma severity, airway inflammation and remodelling

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Definitions for terms used in tables of the online repository

Strata: never and ever(ex/current)-smokers

Interaction: genotype*ever-smokers

Airway hyperresponsiveness: PC_{20} histamine < 16 mg/ml (population 1) and PC_{20} AMP < 320 mg/ml (population 2)

Complete asthma remission: former diagnosis of asthma, PC_{20} AMP > 320 mg/ml and/or PC_{20} histamine > 16 mg/ml, post-bronchodilation (BD) FEV_1%predicted > 90%, no asthma symptoms, no asthma medication

Severe asthma: pre-BD FEV_1%predicted < 80% and use of inhaled corticosteroids

The number of positively stained inflammatory cells was counted in a total area of 0.1 mm^2 in the submucosa, 100μm under the basement membrane

Goblet cell numbers were counted on PAS-stained biopsy sections and expressed per 1000 μm of basement membrane

Abbreviations used in tables of the online repository

AA1: mast cell tryptase

B: regression coefficient

CI: confidence interval

EPX: eosinophilic peroxidase

FEV_1: forced expiratory volume in 1 second

ln natural logarithm

OR: odds ratio

NA: not applicable

48 NT: not tested interaction (when strata effect difference <40%)

49 VC: vital capacity

50

Table E1. Characteristics of populations 1 and 2

	Population 1 N = 243	**Population 2 N =137**
Males	146 (60.1)	70 (51.1)
Age, years	51 (8.8)	47 (12.6)
Never/ex/current smokers	100/74/69(41/31/28)	61/47/29 (44/34/21)
Inhaled corticosteroid use	125 (51.4)	57 (41.6)
Airway hyperresponsiveness	163 (76.9)	72 (52.6)
Complete asthma remission	30 (12.3)	20 (15)
Severe asthma	100 (41.3)	17 (12.5)
FEV_1% predicted	70.6 (23.8)	91.6 (18.0)
FEV_1/ VC%	60.4 (14.3)	70.6 (11.5)

Data are expressed as numbers (%) or mean (SD)

Population 1: longitudinal clinical follow up. Population 2: cross-sectional clinical evaluation and airway wall biopsy collection.

Table E2a: Minor allele frequency

TGFB1 **tag SNPs**	**Chr 19 position**	**MAF (%) population 1**	**MAF (%) population 2**	**Major : Minor**
rs6957	3'UTR	0.14	0.16	A:G
rs10417924	3'UTR	0.15	0.17	C:T
rs7254679	3'UTR	0.13	0.13	A:G
rs4803455	Intron 2	0.50	0.46	C:A
rs1800470[#]	Exon 1	0.38	0.36	T:C
rs1800469*	Promoter	0.30	0.30	C:T

[#]formerly known as rs1982073

* tags rs2241715, rs2317130, rs1982072 (*TGFB1* genomic region +/- 5kb; MAF <0.1; HapMap.org)

Table E2b: Genotype distribution in populations 1 and 2

		Population 1 (n=243)			Population 2 (n=137)		
***TGFB1* SNPs**	**Genotype**	**Total**	**Never smokers**	**Ever smokers**	**Total**	**Never smokers**	**Ever smokers**
rs6957	AA	178	78	100	92	43	49
	GA	60	21	39	40	17	23
	GG	3	1	2	1	0	1
rs10417924	CC	170	70	100	92	45	47
	TC	56	21	35	37	14	23
	TT	6	1	5	4	1	3
rs7254679	AA	179	77	102	99	46	53
	GA	61	20	41	36	15	21
	GG	1	1	0	0	0	0
rs4803455	CC	58	20	38	37	16	46
	CA	125	58	67	75	35	15
	AA	58	21	37	25	10	0
rs1800470	TT	93	38	55	59	30	29
	TC	110	50	60	57	25	32
	CC	37	9	28	20	6	14
rs1800469	CC	121	53	68	68	31	37
	CT	97	42	55	51	25	26
	TT	25	5	20	15	4	11

Table E3a-E3b: Associations with FEV_1 decline in population 1

E3a		**FEV_1 Decline (ml/year)**			
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**
rs6957	AA	reference			
	GA/GG	-0.16	-8.24	7.93	0.97
rs10417924	CC	reference			
	TC/TT	-0.29	-8.32	7.73	0.94
rs1800469	CC	reference			
	CT/TT	6.29	-0.36	12.95	**0.06**
rs1800470	TT	reference			
	TC/CC	10.73	3.81	17.65	**0.002**
rs4803455	CC	reference			
	CA/AA	-11.37	-18.74	-4.01	**0.003**

E3b		**FEV_1 Decline (ml/year)**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**P-value**
rs6957	AA	reference				reference				reference	
	GA/GG	-14.95	-29.21	-0.69	**0.04**	7.52	-2.97	18.00	0.16	19.13	**0.02**
rs10417924	CC	reference				reference				reference	
	TC/TT	-3.74	-17.60	10.11	0.60	2.33	-7.42	12.08	0.64	5.88	0.47
rs1800469	CC	reference				reference				reference	
	CT/TT	4.89	-7.05	16.83	0.42	8.85	0.78	16.91	**0.03**	3.97	0.56
rs1800470	TT	reference				reference				reference	
	TC/CC	10.27	-1.88	22.42	0.10	10.99	2.41	19.58	**0.01**	NT	NT
rs4803455	CC	reference				reference				reference	
	CA/AA	-4.68	-18.74	9.38	0.51	-15.69	-24.09	-7.29	**<0.001**	-9.68	0.22

Table E4a-E4d: Associations with FEV_1 % predicted in populations 1 and 2

E4a		**FEV_1% predicted pre bronchodilation**							
		Population 1				**Population 2**			
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
rs6957	AA	reference				reference			
	GA/GG	-6.60	-12.26	-0.93	**0.02**	-8.11	-14.66	-1.56	**0.02**
rs10417924	CC	reference				reference			
	TC/TT	-0.60	-6.53	5.34	0.84	8.29	1.66	14.92	**0.02**
rs1800469	CC	reference				reference			
	CT/TT	-1.60	-6.64	3.45	0.53	-4.45	-10.62	1.72	0.16
rs1800470	TT	reference				reference			
	TC/CC	-3.26	-8.45	1.92	0.22	-5.20	-11.20	0.80	**0.09**
rs4803455	CC	reference				reference			
	CA/AA	2.24	-3.66	8.14	0.46	4.42	-2.38	11.22	0.20

E4b		**FEV_1% predicted pre bronchodilation**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
		B	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**P-value**
***TGFB1* SNPs**	**Genotype**	**Population 1**									
rs6957	AA	reference				reference				reference	
	GA/GG	-3.73	-12.61	5.15	0.41	-8.16	-15.46	-0.86	**0.03**	-3.97	0.50
rs10417924	CC	reference				reference				reference	
	TC/TT	-4.25	-13.44	4.95	0.36	1.58	-6.12	9.27	0.69	-4.08	0.41
rs1800469	CC	reference				reference				reference	
	CT/TT	1.82	-5.58	9.21	0.63	-4.29	-11.18	2.59	0.22	-6.20	0.23
rs1800470	TT	reference				reference				reference	
	TC/CC	-1.09	-8.85	6.68	0.78	-4.98	-11.98	2.02	0.16	-3.70	0.49
rs4803455	CC	reference				reference				reference	
	CA/AA	0.83	-8.42	10.07	0.86	3.21	-4.49	10.91	0.41	2.59	0.68
		Population 2									
rs6957	AA	reference				reference				reference	
	GA/GG	-3.86	-12.65	4.93	0.38	-12.07	-21.63	-2.50	**0.01**	-8.31	0.21
rs10417924	CC	reference				reference				reference	
	TC/TT	4.80	-4.19	13.78	0.29	11.09	1.61	20.58	**0.02**	5.85	0.39
rs1800469	CC	reference				reference				reference	
	CT/TT	1.75	-6.23	9.74	0.62	-9.90	-18.92	-0.88	**0.03**	-11.99	**0.052**
rs1800470	TT	reference				reference				reference	
	TC/CC	0.01	-7.82	7.85	1.00	-9.85	-18.71	-0.99	**0.03**	-10.20	**0.09**
rs4803455	CC	reference				reference				reference	
	CA/AA	3.16	-5.68	12.00	0.48	4.83	-5.19	14.84	0.34	NT	NT

E4c		**FEV_1% predicted post bronchodilation**							
		Population 1				**Population 2**			
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
rs6957	AA	reference				reference			
	GA/GG	-5.58	-11.15	-0.02	**0.049**	-8.44	-14.60	-2.27	**0.01**
rs10417924	CC	reference				reference			
	TC/TT	-0.45	-6.28	5.39	0.88	7.75	1.40	14.09	**0.02**
rs1800469	CC	reference				reference			
	CT/TT	-2.56	-7.50	2.38	0.31	-2.47	-8.36	3.41	0.41
rs1800470	TT	reference				reference			
	TC/CC	-4.87	-9.93	0.19	**0.059**	-4.50	-10.23	1.23	0.12
rs4803455	CC	reference				reference			
	CA/AA	2.51	-3.29	8.30	0.40	1.63	-4.85	8.11	0.62

E4d		**FEV_1% predicted post bronchodilation**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
		B	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**P-value**
***TGFB1* SNPs**	**Genotype**	**Population 1**									
rs6957	AA	reference				reference				reference	
	GA/GG	-2.80	-11.35	5.75	0.52	-6.81	-14.08	0.47	**0.07**	-3.45	0.55
rs10417924	CC	reference				reference				reference	
	TC/TT	-3.08	-11.99	5.83	0.49	0.79	-6.88	8.42	0.84	3.60	0.55
rs1800469	CC	reference				reference				reference	
	CT/TT	0.10	-7.01	7.20	0.98	-4.66	-11.48	2.15	0.18	-4.90	0.34
rs1800470	TT	reference				reference				reference	
	TC/CC	-4.10	-11.47	3.26	0.27	-5.82	-12.77	1.13	0.10	NT	NT
rs4803455	CC	reference				reference				reference	
	CA/AA	0.34	-8.57	9.25	0.94	3.72	-3.93	11.36	0.34	3.65	0.55
		Population 2									
rs6957	AA	reference				reference				reference	
	GA/GG	-7.23	-14.84	0.37	**0.06**	-9.98	-19.35	-0.61	**0.04**	NT	NT
rs10417924	CC	reference				reference				reference	
	TC/TT	4.86	-2.97	12.69	0.22	9.55	0.17	18.93	**0.046**	3.75	0.56
rs1800469	CC	reference				reference				reference	
	CT/TT	2.40	-4.66	9.46	0.50	-6.64	-15.55	2.26	0.14	-9.71	0.10
rs1800470	TT	reference				reference				reference	
	TC/CC	0.30	-6.64	7.24	0.93	-8.84	-17.57	-0.10	**0.047**	-9.76	**0.09**
rs4803455	CC	reference				reference				reference	
	CA/AA	2.08	-5.76	9.92	0.60	1.91	-7.86	11.68	0.70	NT	NT

Table E5a-E5d: Associations with FEV_1/VC in populations 1 and 2

E5a		**FEV_1/VC pre bronchodilation**							
		Population 1				**Population 2**			
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
rs6957	AA	reference				reference			
	GA/GG	0.20	-3.24	3.63	0.91	-1.20	-4.61	2.21	0.49
rs10417924	CC	reference				reference			
	TC/TT	-0.32	-3.85	3.22	0.86	2.00	-1.40	5.41	0.25
rs1800469	CC	reference				reference			
	CT/TT	-0.72	-3.72	2.28	0.64	-1.59	-4.73	1.55	0.32
rs1800470	TT	reference				reference			
	TC/CC	-0.95	-4.07	2.17	0.55	-1.95	-5.03	1.13	0.21
rs4803455	CC	reference				reference			
	CA/AA	0.71	-2.77	4.20	0.69	3.32	-0.08	6.72	**0.056**

E5b		**FEV_1/VC pre bronchodilation**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
		B	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**P-value**
***TGFB1* SNPs**	**Genotype**	**Population 1**									
rs6957	AA	reference				reference				reference	
	GA/GG	0.36	-5.00	5.72	0.89	-0.14	-4.63	4.35	0.95	-0.64	0.86
rs10417924	CC	reference				reference				reference	
	TC/TT	-1.06	-6.62	4.50	0.71	0.22	-4.36	4.80	0.92	0.88	0.82
rs1800469	CC	reference				reference				reference	
	CT/TT	-0.33	-4.79	4.13	0.88	-1.41	-5.51	2.70	0.50	-1.37	0.66
rs1800470	TT	reference				reference				reference	
	TC/CC	-0.81	-5.61	4.00	0.74	-1.30	-5.48	2.89	0.54	NT	NT
rs4803455	CC	reference				reference				reference	
	CA/AA	0.20	-5.28	5.68	0.94	1.04	-3.52	5.61	0.65	0.96	0.79
		Population 2									
rs6957	AA	reference				reference				reference	
	GA/GG	3.67	-1.76	9.09	0.18	-4.94	-9.70	-0.19	**0.04**	-8.25	**0.02**
rs10417924	CC	reference				reference				reference	
	TC/TT	1.30	-4.03	6.63	0.63	3.05	-1.56	7.66	0.19	1.53	0.66
rs1800469	CC	reference				reference				reference	
	CT/TT	0.14	-4.56	4.84	0.95	-3.68	-8.12	0.77	0.10	-4.15	0.19
rs1800470	TT	reference				reference				reference	
	TC/CC	-1.19	-5.78	3.39	0.60	-2.74	-7.15	1.68	0.22	-1.81	0.56
rs4803455	CC	reference				reference				reference	
	CA/AA	4.08	-1.05	9.21	0.12	1.92	-2.85	6.68	0.43	-2.34	0.50

E5c		**FEV_1/VC post bronchodilation**							
		Population 1				**Population 2**			
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
rs6957	AA	reference				reference			
	GA/GG	-0.45	-3.58	2.69	0.78	-1.54	-4.81	1.72	0.35
rs10417924	CC	reference				reference			
	TC/TT	-0.24	-3.47	3.00	0.89	3.09	-0.18	6.36	**0.06**
rs1800469	CC	reference				reference			
	CT/TT	-1.86	-4.58	0.87	0.18	-0.85	-3.87	2.17	0.58
rs1800470	TT	reference				reference			
	TC/CC	-1.90	-4.74	0.94	0.19	-2.13	-5.11	0.86	0.16
rs4803455	CC	reference				reference			
	CA/AA	0.82	-2.38	4.02	0.61	2.37	-0.93	5.67	0.16

E5d		**FEV_1/VC post bronchodilation**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
		B	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**P-value**
***TGFB1* SNPs**	**Genotype**	**Population 1**									
rs6957	AA	reference				reference				reference	
	GA/GG	-0.27	-5.33	4.78	0.91	-0.63	-4.61	3.35	0.75	-0.26	0.94
rs10417924	CC	reference				reference				reference	
	TC/TT	-0.61	-5.91	4.70	0.82	-0.22	-4.29	3.86	0.92	-0.10	0.98
rs1800469	CC	reference				reference				reference	
	CT/TT	-2.41	-6.60	1.77	0.26	-1.89	-5.52	1.74	0.31	0.19	0.95
rs1800470	TT	reference				reference				reference	
	TC/CC	-2.74	-7.22	1.74	0.23	-1.79	-5.52	1.94	0.34	NT	NT
rs4803455	CC	reference				reference				reference	
	CA/AA	-0.02	-5.24	5.19	0.99	1.20	-2.86	5.26	0.56	1.44	0.67
		Population 2									
rs6957	AA	reference				reference				reference	
	GA/GG	1.15	-3.90	6.19	0.65	-3.98	-8.51	0.54	**0.08**	-4.67	0.17
rs10417924	CC	reference				reference				reference	
	TC/TT	3.52	-1.23	8.27	0.14	3.30	-1.11	7.72	0.14	NT	NT
rs1800469	CC	reference				reference				reference	
	CT/TT	0.82	-3.46	5.09	0.70	-2.11	-6.35	2.13	0.32	-3.84	0.20
rs1800470	TT	reference				reference				reference	
	TC/CC	-1.36	-5.53	2.80	0.52	-2.71	-7.00	1.58	0.21	-1.60	0.59
rs4803455	CC	reference				reference				reference	
	CA/AA	4.08	-1.05	9.21	0.12	1.92	-2.85	6.68	0.43	-2.23	0.50

Table E6: Meta-analysis of associations with lung function in populations 1 and 2

E6 Meta-analysis	*TGFB1 SNP*	Total B [95%CI]; p-value	Never-Smokers B [95%CI]; p-value	Ever-Smokers B [95%CI]; p-value	Interaction B [95%CI]; p-value
FEV_1% predicted pre BD	rs6957-T	**-7.2446 [-11.5311; -2.9581] 0.0009**	-3.7962 [-10.0436; 2.4512] 0.2337	**-9.5953 [-15.399; -3.7916] 0.0012**	-5.8673 [-14.4843; 2.7497] 0.182
	rs10417924-G	3.7194 [-4.9841; 12.4229] 0.4023	0.3783 [-6.0475; 6.8042] 0.9081	**5.3534 [-0.6235; 11.3303] 0.0792**	5.7535 [-3.1578; 14.6649] 0.2057
	rs1800469-T	-2.7395 [-6.6455; 1.1665] 0.1692	1.7885 [-3.6371; 7.2141] 0.5182	**-6.358 [-11.83; -0.886] 0.0228**	**-8.6245 [-16.3901; -0.8589] 0.0295**
	rs1800470-C	**-4.0919 [-8.0139; -0.1699] 0.0409**	-0.5421 [-6.0571; 4.9729] 0.8472	**-6.8487 [-12.3411; -1.3564] 0.0145**	**-6.5832 [-14.4199; 1.2534] 0.090**
	rs4803455-A	3.1798 [-1.2756; 7.6352] 0.1619	2.0459 [-4.3418; 8.4335] 0.5302	3.8111 [-2.2951; 9.9173] 0.2212	2.1746 [-6.8552; 11.2045] 0.6369
FEV_1% predicted post BD	rs6957-T	**-6.864 [-10.9958; -2.7323] 0.0011**	-5.2746 [-10.9561; 0.4069] 0.0688	**-7.9979 [-13.7436; -2.2523] 0.0064**	-3.2249 [-11.5682; 5.1185] 0.4487
	rs10417924-G	3.5499 [-4.4768; 11.5766] 0.386	1.4009 [-4.4791; 7.2809] 0.6405	4.2791 [-1.6406; 10.1987] 0.1565	3.6703 [-4.9689; 12.3095] 0.405
	rs1800469-T	-2.5244 [-6.3074; 1.2585] 0.1909	1.2558 [-3.7514; 6.263] 0.623	**-5.3928 [-10.8045; 0.0189] 0.0508**	**-6.9699 [-14.5066; 0.5668] 0.0699**
	rs1800470-C	**-4.7081 [-8.5001; -0.9162] 0.015**	-1.7707 [-6.8211; 3.2797] 0.492	**-6.9913 [-12.4291; -1.5535] 0.0117**	-5.2764 [-12.8497; 2.2968] 0.1721
	rs4803455-A	2.1173 [-2.2027; 6.4373]	1.3198 [-4.5649; 7.2044]	3.0311 [-2.9906; 9.0529]	1.8245 [-6.9163; 10.5653]

		0.3368	0.6602	0.3239	0.6825
FEV_1/VC% predicted pre BD	rs6957-T	-0.5075 [-2.9279; 1.9129] 0.6811	1.995 [-1.8175; 5.8074] 0.3051	-2.4011 [-5.6656; 0.8635] 0.1494	**-4.5144 [-9.4247; 0.3960] 0.0716**
	rs10417924-G	0.8864 [-1.5654; 3.3383] 0.4786	0.1722 [-3.6753; 4.0196] 0.9301	1.6283 [-1.6215; 4.8782] 0.3261	1.2059 [-3.7477; 6.1594] 0.6333
	rs1800469-T	-1.1365 [-3.3051; 1.0321] 0.3044	-0.1074 [-3.3416; 3.1267] 0.9481	-2.4495 [-5.4622; 0.5632] 0.111	-2.7423 [-7.0703; 1.5857] 0.2143
	rs1800470-C	-1.4575 [-3.6492; 0.7342] 0.1924	-1.0092 [-4.3249; 2.3066] 0.5508	-1.9768 [-5.0156; 1.062] 0.2023	-1.3906 [-5.7747; 2.9936] 0.5342
	rs4803455-A	2.0462 [-0.3884; 4.4807] 0.0995	2.2702 [-1.4732; 6.0136] 0.2346	1.4593 [-1.8369; 4.7555] 0.3856	-0.7863 [-5.7239; 4.1513] 0.7549
FEV_1/VC% predicted post BD	rs6957-T	-0.9719 [-3.2302; 1.2864] 0.3989	0.4384 [-3.1324; 4.0092] 0.8098	-2.0899 [-5.0769; 0.8971] 0.1703	-2.3682 [-6.9653; 2.2289] 0.3126
	rs10417924-G	1.4075 [-0.8917; 3.7066] 0.2302	1.6847 [-1.8541; 5.2234] 0.3508	1.4011 [-1.5929; 4.3951] 0.359	-0.6124 [-5.229; 4.0041] 0.7949
	rs1800469-T	-1.4044 [-3.4274; 0.6185] 0.1736	-0.8322 [-3.8189; 2.1546] 0.585	-1.9823 [-4.7398; 0.7751] 0.1588	-1.6922 [-5.718; 2.3336] 0.41
	rs1800470-C	**-2.008 [-4.0656; 0.0496] 0.0558**	-2.0037 [-5.0551; 1.0477] 0.1981	-2.1879 [-5.0027; 0.627] 0.1277	-0.4977 [-4.5964; 3.601] 0.8119
	rs4803455-A	1.5721 [-0.7222; 3.8664] 0.1793	2.0653 [-1.5917; 5.7223] 0.2683	1.4995 [-1.5903; 4.5894] 0.3415	-0.4209 [-5.0432; 4.2014] 0.8584

Table E7a-b: Associations with severity of airway hyperresponsiveness in populations 1 and 2

E7a		**Airway hyperresponsiveness**							
		Population 1				**Population 2**			
***TGFB1* SNPs**	**Genotype**	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**
rs6957	AA	reference				reference			
	GA/GG	3.31	1.34	8.18	**0.009**	1.18	0.53	2.61	0.68
rs10417924	CC	reference				reference			
	TC/TT	0.69	0.32	1.47	0.33	0.63	0.28	1.40	0.26
rs1800469	CC	reference				reference			
	CT/TT	0.81	0.41	1.58	0.53	1.23	0.60	2.55	0.57
rs1800470	TT	reference				reference			
	TC/CC	0.66	0.32	1.35	0.25	1.08	0.53	2.22	0.84
rs4803455	CC	reference				reference			
	CA/AA	1.01	0.46	2.20	0.99	0.71	0.31	1.59	0.40

E7b		**Airway hyperresponsiveness**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
		OR	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**	**OR**	**P-value**
***TGFB1* SNPs**	**Genotype**	**Population 1**									
rs6957	AA	reference				reference				reference	
	GA/GG	5.84	0.67	51.23	0.11	3.26	1.11	9.53	**0.03**	NT	NT
rs10417924	CC	reference				reference				reference	
	TC/TT	0.47	0.12	1.86	0.28	0.76	0.29	1.96	0.57	1.57	0.59
rs1800469	CC	reference				reference				reference	
	CT/TT	1.07	0.34	3.41	0.91	0.79	0.33	1.89	0.59	0.96	0.96
rs1800470	TT	reference				reference				reference	
	TC/CC	0.38	0.09	1.52	0.17	0.96	0.39	2.39	0.94	3.51	0.13
rs4803455	CC	reference				reference				reference	
	CA/AA	1.88	0.49	7.19	0.36	0.84	0.31	2.27	0.73	0.45	0.33
		Population 2									
rs6957	AA	reference				reference				reference	
	GA/GG	0.20	0.05	0.80	**0.02**	4.74	1.45	15.49	**0.01**	21.44	**0.001**
rs10417924	CC	reference				reference				reference	
	TC/TT	1.58	0.48	5.22	0.45	0.24	0.08	0.72	**0.01**	0.16	**0.03**
rs1800469	CC	reference				reference				reference	
	CT/TT	1.19	0.42	3.36	0.74	1.52	0.57	4.00	0.40	1.34	0.68
rs1800470	TT	reference				reference				reference	
	TC/CC	0.91	0.33	2.53	0.86	1.24	0.46	3.31	0.67	1.42	0.62
rs4803455	CC	reference				reference				reference	
	CA/AA	0.74	0.23	2.41	0.62	1.00	0.35	2.87	0.99	1.43	0.66

Table E8: Meta-analysis of associations with severity of airway hyperresponsiveness in populations 1 and 2

E8 Meta-analysis	*TGFB1 SNP*	Total B [95%CI]; p-value	Never-Smokers B [95%CI]; p-value	Ever-Smokers B [95%CI]; p-value	Interaction B [95%CI]; p-value
AHR	rs6957-T	**1.8512 [1.0197; 3.3607] 0.043**	0.9674 [0.0353; 26.5039] 0.9843	**3.8577 [1.7413; 8.5466] 0.0009**	3.5884 [0.0928; 138.7111] 0.4932
	rs10417924-G	0.6575 [0.3782; 1.1431] 0.1373	0.9373 [0.3812; 2.3046] 0.8878	**0.4631 [0.2256; 0.9506] 0.0359**	0.4977 [0.0533; 4.6485] 0.5405
	rs1800469-T	0.9815 [0.5991; 1.6079] 0.9408	1.1363 [0.5246; 2.4608] 0.746	1.0573 [0.5514; 2.0273] 0.8669	1.1372 [0.4181; 3.0931] 0.8012
	rs1800470-C	0.8417 [0.5072; 1.3968] 0.5049	0.6694 [0.294; 1.5241] 0.339	1.0811 [0.5552; 2.1053] 0.8185	2.0904 [0.7176; 6.09] 0.1765
	rs4803455-A	0.8497 [0.4842; 1.491] 0.5702	1.1134 [0.4599; 2.6951] 0.8118	0.9108 [0.4423; 1.8754] 0.7998	0.8170 [0.2628; 2.5400] 0.7269

Table E9a-b: Associations with complete asthma remission in populations 1 and 2

E9a		**Complete asthma remission**							
		Population 1				**Population 2**			
***TGFB1* SNPs**	**Genotype**	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**
rs6957	AA	reference				reference			
	GA/GG	0.23	0.06	0.82	**0.02**	0.86	0.28	2.71	0.80
rs10417924	CC	reference				reference			
	TC/TT	2.30	0.96	5.49	**0.06**	2.17	0.78	6.07	0.14
rs1800469	CC	reference				reference			
	CT/TT	1.29	0.57	2.97	0.54	1.38	0.51	3.77	0.53
rs1800470	TT	reference				reference			
	TC/CC	1.46	0.61	3.47	0.39	1.36	0.50	3.69	0.54
rs4803455	CC	reference				reference			
	CA/AA	0.79	0.31	2.01	0.62	0.88	0.30	2.57	0.81

E9b		**Complete asthma remission**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
		OR	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**	**OR**	**P-value**
***TGFB1* SNPs**	**Genotype**	**Population 1**									
rs6957	AA	reference				reference				reference	
	GA/GG	NA	NA	NA	NA	0.29	0.08	1.06	**0.06**	NA	NA
rs10417924	CC	reference				reference				reference	
	TC/TT	1.39	0.20	9.78	0.74	2.55	0.96	6.79	**0.06**	1.62	0.65
rs1800469	CC	reference				reference				reference	
	CT/TT	1.02	0.18	5.98	0.98	1.38	0.54	3.53	0.50	1.25	0.82
rs1800470	TT	reference				reference				reference	
	TC/CC	0.92	0.14	6.09	0.93	1.50	0.57	3.97	0.41	1.35	0.77
rs4803455	CC	reference				reference				reference	
	CA/AA	0.54	0.08	3.64	0.52	0.93	0.32	2.70	0.90	1.74	0.61
		Population 2									
rs6957	AA	reference				reference				reference	
	GA/GG	0.59	0.10	3.36	0.55	1.24	0.27	5.79	0.79	2.24	0.49
rs10417924	CC	reference				reference				reference	
	TC/TT	1.08	0.23	4.96	0.92	4.86	1.03	23.01	**0.046**	4.19	0.19
rs1800469	CC	reference				reference				reference	
	CT/TT	1.56	0.41	6.01	0.52	1.13	0.25	5.12	0.87	0.74	0.77
rs1800470	TT	reference				reference				reference	
	TC/CC	2.00	0.50	7.96	0.32	0.87	0.21	3.69	0.85	0.43	0.40
rs4803455	CC	reference				reference				reference	
	CA/AA	1.98	0.35	11.11	0.44	0.42	0.10	1.82	0.25	0.22	0.18

Table E10: Meta-analysis of associations with complete asthma remission in populations 1 and 2

E10 Meta-analysis	***TGFB1 SNP***	**Total B [95%CI]; p-value**	**Never-Smokers B [95%CI]; p-value**	**Ever-Smokers B [95%CI]; p-value**	**Interaction B [95%CI]; p-value**
Asthma remission	rs6957-T	**0.4795 [0.2038; 1.1285] 0.0923**	Not applicable	0.5281 [0.1953; 1.4280] 0.2084	Not applicable
	rs10417924-G	**2.2431 [1.1538; 4.3608] 0.0172**	1.1862 [0.3563; 3.9487] 0.7808	**3.0632 [1.3355; 7.026] 0.0082**	2.5732 [0.5715; 11.5868] 0.2183
	rs1800469-T	1.3278 [0.7004; 2.5172] 0.385	1.3356 [0.4575; 3.899] 0.5965	1.3081 [0.5901; 2.8994] 0.5084	1.2231 [0.7937; 1.8849] 0.3613
	rs1800470-C	1.4164 [0.7368; 2.7228] 0.2965	1.5276 [0.5013; 4.6553] 0.4561	1.2679 [0.566; 2.8403] 0.564	1.2306 [0.6979; 2.1700] 0.4734
	rs4803455-A	0.8274 [0.4087; 1.6748] 0.5985	1.1032 [0.3061; 3.9762] 0.8807	0.7086 [0.2998; 1.6746] 0.4324	0.6476 [0.1373; 3.0549] 0.5831

Table E11a-b: Associations with severe asthma in populations 1 and 2

E11a		**Severe asthma**							
		Population 1				**Population 2**			
***TGFB1* SNPs**	**Genotype**	**OR**	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**
rs6957	AA	reference				reference			
	GA/GG	1.03	0.55	1.92	0.93	1.79	0.56	5.72	0.33
rs10417924	CC	reference				reference			
	TC/TT	1.23	0.66	2.32	0.52	1.18	0.36	3.84	0.79
rs1800469	CC	reference				reference			
	CT/TT	1.01	0.59	1.74	0.97	3.85	1.09	13.61	**0.04**
rs1800470	TT	reference				reference			
	TC/CC	0.87	0.50	1.54	0.64	3.48	0.90	13.43	**0.07**
rs4803455	CC	reference				reference			
	CA/AA	0.66	0.35	1.25	0.20	0.48	0.15	1.54	0.22

E11b		**Severe asthma**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
		OR	**95%CI**		**P-value**	**OR**	**95%CI**		**P-value**	**OR**	**P-value**
***TGFB1* SNPs**	**Genotype**	**Population 1**									
rs10417924	CC	reference				reference				reference	
	TC/TT	1.53	0.53	4.38	0.43	1.05	0.48	2.31	0.91	1.55	0.50
rs6957	AA	reference				reference				reference	
	GA/GG	0.79	0.29	2.16	0.64	1.22	0.56	2.67	0.62	0.69	0.58
rs1800469	CC	reference				reference				reference	
	CT/TT	1.15	0.50	2.64	0.74	0.96	0.47	1.95	0.90	0.80	0.69
rs1800470	TT	reference				reference				reference	
	TC/CC	1.33	0.55	3.20	0.53	0.61	0.29	1.28	0.19	0.44	0.15
rs4803455	CC	reference				reference				reference	
	CA/AA	0.35	0.12	1.04	**0.058**	0.90	0.41	1.99	0.80	2.48	0.18
		Population 2									
rs6957	AA	reference				reference				reference	
	GA/GG	1.05	0.08	13.92	0.97	1.92	0.53	7.02	0.32	1.56	0.76
rs10417924	CC	reference				reference				reference	
	TC/TT	1.79	0.14	22.33	0.65	1.14	0.30	4.26	0.85	0.71	0.81
rs1800469	CC	reference				reference				reference	
	CT/TT	2.14	0.18	25.69	0.55	3.66	0.89	15.16	**0.08**	1.88	0.66
rs1800470	TT	reference				reference				reference	
	TC/CC	2.07	0.17	24.62	0.57	4.02	0.80	20.26	**0.09**	2.01	0.64
rs4803455	CC	reference				reference					
	CA/AA	0.53	0.04	7.12	0.63	0.40	0.11	1.43	0.16	NT	NT

Table E12: Meta-analysis of associations with severe asthma in populations 1 and 2

E12 Meta- Analysis	***TGFB1 SNP***	**Total B [95%CI]; p-value**	**Never-Smokers B [95%CI]; p-value**	**Ever-Smokers B [95%CI]; p-value**	**Interaction B [95%CI]; p-value**
Severe Asthma	rs6957-T	1.165 [0.6737; 2.0146] 0.5848	0.8171 [0.3196; 2.0893] 0.6733	1.3773 [0.7051; 2.6902] 0.3487	1.5487 [0.4849; 4.9465] 0.4604
	rs10417924-G	1.2203 [0.6984; 2.1322] 0.4844	1.5638 [0.592; 4.1307] 0.367	1.0705 [0.5435; 2.1086] 0.8439	0.6966 [0.2133; 2.2755] 0.5494
	rs1800469-T	1.7374 [0.4809; 6.2769] 0.3993	1.2226 [0.5568; 2.6844] 0.6166	1.2514 [0.6624; 2.3642] 0.4896	0.8958 [0.3248; 2.4707] 0.8316
	rs1800470-C	1.5143 [0.4020; 5.7034] 0.5397	1.3947 [0.6086; 3.1961] 0.4317	1.3585 [0.2200; 8.3867] 0.7415	0.5332 [0.1854; 1.5333] 0.2433
	rs4803455-A	**0.6149 [0.3522; 1.0735] 0.0872**	**0.3742 [0.1383; 1.0125] 0.0529**	0.7184 [0.3658; 1.4108] 0.3368	2.0748 [0.6217; 6.9242] 0.2352

Table E13a-b: Associations with airway wall inflammation in population 2

E13a		**Number of EPX+ eosinophils/0.1mm^2 (ln)**				**Number of CD68+ Macropahes/0.1mm^2 (ln)**				**Number of AA1+ Mast cells/0.1mm^2 (ln)**			
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
rs6957	AA	reference				reference				reference			
	GA/GG	0.45	0.07	0.84	**0.02**	0.26	0.01	0.51	**0.045**	-0.05	-0.34	0.23	0.71
rs10417924	CC	reference				reference				reference			
	TC/TT	0.26	-0.14	0.66	0.20	0.14	-0.12	0.40	0.30	0.06	-0.23	0.35	0.68
rs1800469	CC	reference				reference				reference			
	CT/TT	0.19	-0.17	0.55	0.30	0.09	-0.15	0.33	0.46	-0.10	-0.36	0.16	0.46
rs1800470	TT	reference				reference				reference			
	TC/CC	0.21	-0.15	0.57	0.24	0.13	-0.11	0.36	0.28	0.01	-0.25	0.27	0.94
rs4803455	CC	reference				reference				reference			
	CA/AA	-0.15	-0.55	0.25	0.46	-0.07	-0.33	0.19	0.58	0.16	-0.13	0.45	0.27

		Number of CD4+ T-cells /0.1mm^2 (ln)				**Number of CD8+ T-cells/0.1mm^2 (ln)**			
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
rs6957	AA	reference				reference			
	GA/GG	0.17	-0.19	0.52	0.36	0.15	-0.17	0.48	0.36
rs10417924	CC	reference				reference			
	TC/TT	-0.06	-0.42	0.30	0.74	0.37	0.06	0.69	**0.02**
rs1800469	CC	reference				reference			
	CT/TT	0.02	-0.31	0.35	0.90	-0.01	-0.32	0.29	0.93
rs1800470	TT	reference				reference			
	TC/CC	0.06	-0.26	0.39	0.70	0.04	-0.26	0.34	0.78
rs4803455	CC	reference				reference			

	CA/AA	0.15	-0.21	0.51	0.41	0.07	-0.26	0.41	0.67

E13b		**Number of EPX+eosinophils/0.1mm^2 (ln)**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**P-value**
rs6957	AA	reference				reference				reference	
	GA/GG	0.04	-0.57	0.66	0.89	0.78	0.27	1.29	**0.003**	0.72	**0.07**
rs10417924	CC	reference				reference				reference	
	TC/TT	0.34	-0.27	0.95	0.27	0.25	-0.29	0.79	0.36	NT	NT
rs1800469	CC	reference				reference				reference	
	CT/TT	0.31	-0.21	0.83	0.23	0.10	-0.41	0.61	0.70	-0.25	0.49
rs1800470	TT	reference				reference				reference	
	TC/CC	0.24	-0.28	0.76	0.35	0.25	-0.27	0.77	0.34	NT	NT
rs4803455	CC	reference				reference				reference	
	CA/AA	-0.03	-0.63	0.57	0.92	-0.35	-0.89	0.20	0.21	-0.37	0.37
		Number of CD68+Macropahes/0.1mm^2 (ln)									
rs6957	AA	reference				reference				reference	
	GA/GG	0.06	-0.25	0.37	0.71	0.48	0.11	0.84	**0.01**	0.47	**0.07**
rs10417924	CC	reference				reference				reference	
	TC/TT	-0.05	-0.36	0.26	0.74	0.32	-0.06	0.70	0.09	0.36	0.17
rs1800469	CC	reference				reference				reference	
	CT/TT	0.10	-0.17	0.36	0.46	0.11	-0.26	0.47	0.56	NT	NT
rs1800470	TT	reference				reference				reference	
	TC/CC	0.05	-0.21	0.31	0.68	0.25	-0.12	0.61	0.18	0.14	0.55
rs4803455	CC	reference				reference				reference	

	CA/AA	-0.01	-0.31	0.29	0.95	-0.18	-0.57	0.21	0.35	-0.24	0.37
		Number of AA1+Mast cells/0.1mm^2 (ln)									
rs6957	AA	reference				reference				reference	
	GA/GG	-0.01	-0.42	0.41	0.98	-0.09	-0.49	0.32	0.66	-0.07	0.82
rs10417924	CC	reference				reference				reference	
	TC/TT	0.00	-0.43	0.43	1.00	0.06	-0.34	0.46	0.77	NT	NT
rs1800469	CC	reference				reference				reference	
	CT/TT	0.09	-0.28	0.46	0.62	-0.19	-0.57	0.19	0.31	-0.34	0.20
rs1800470	TT	reference				reference				reference	
	TC/CC	0.05	-0.32	0.41	0.80	-0.02	-0.41	0.37	0.91	-0.12	0.66
rs4803455	CC	reference				reference				reference	
	CA/AA	0.20	-0.21	0.61	0.34	0.26	-0.15	0.67	0.20	NT	NT
		Number of CD4+T-cells /0.1mm^2 (ln)									
rs6957	AA	reference				reference				reference	
	GA/GG	-0.06	-0.59	0.47	0.83	0.31	-0.19	0.80	0.22	0.27	0.46
rs10417924	CC	reference				reference				reference	
	TC/TT	-0.05	-0.57	0.47	0.86	-0.03	-0.53	0.46	0.90	NT	NT
rs1800469	CC	reference				reference				reference	
	CT/TT	-0.07	-0.52	0.39	0.77	0.10	-0.38	0.58	0.68	0.12	0.72
rs1800470	TT	reference				reference				reference	
	TC/CC	-0.12	-0.56	0.32	0.59	0.28	-0.19	0.76	0.24	0.38	0.26
rs4803455	CC	reference				reference				reference	
	CA/AA	0.61	0.13	1.10	**0.01**	-0.32	-0.83	0.18	0.20	-0.97	**0.01**
		Number of CD8+T-cells/0.1mm^2 (ln)									
rs6957	AA	reference				reference				reference	
	GA/GG	0.07	-0.44	0.58	0.79	0.23	-0.21	0.67	0.31	0.15	0.66
rs10417924	CC	reference				reference				reference	

	TC/TT	0.27	-0.21	0.76	0.27	0.46	0.04	0.88	**0.03**	NT	NT
rs1800469	CC	reference				reference				reference	
	CT/TT	0.20	-0.23	0.64	0.35	-0.17	-0.59	0.26	0.44	-0.44	0.15
rs1800470	TT	reference				reference				reference	
	TC/CC	0.18	-0.25	0.60	0.41	-0.03	-0.46	0.40	0.88	-0.26	0.39
rs4803455	CC	reference				reference				reference	
	CA/AA	0.06	-0.44	0.55	0.83	0.05	-0.41	0.51	0.83	NT	NT

Table E14a-b: Associations with airway wall remodeling in population 2

E14a		**Basement membrane thickness (um)**				**Number of goblet cells per 1000um BM (ln)**			
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**
rs6957	AA	reference				reference			
	GA/GG	0.39	-0.17	0.95	0.17	-0.08	-0.32	0.16	0.52
rs10417924	CC	reference				reference			
	TC/TT	0.00	-0.57	0.57	1.00	0.06	-0.17	0.30	0.58
rs1800469	CC	reference				reference			
	CT/TT	0.22	-0.28	0.71	0.39	0.23	0.03	0.43	**0.03**
rs1800470	TT	reference				reference			
	TC/CC	0.01	-0.50	0.53	0.96	0.07	-0.14	0.28	0.50
rs4803455	CC	reference				reference			
	CA/AA	0.06	-0.52	0.63	0.85	-0.16	-0.39	0.07	0.18

E14b		**Basement membrane thickness (um)**									
		NEVER SMOKERS				**EVER SMOKERS**				**Interaction**	
***TGFB1* SNPs**	**Genotype**	**B**	**95%CI**		**P-value**	**B**	**95%CI**		**P-value**	**B**	**P-value**
rs6957	AA	reference				reference				reference	
	GA/GG	-0.18	-1.11	0.75	0.70	0.76	0.03	1.49	**0.04**	0.86	0.13
rs10417924	CC	reference				reference				reference	
	TC/TT	0.40	-0.50	1.31	0.37	-0.32	-1.06	0.43	0.40	-0.72	0.22
rs1800469	CC	reference				reference				reference	
	CT/TT	0.67	-0.03	1.36	**0.06**	-0.10	-0.81	0.60	0.77	-0.82	0.10
rs1800470	TT	reference				reference				reference	
	TC/CC	0.47	-0.30	1.24	0.22	-0.38	-1.09	0.34	0.30	-0.90	0.10
rs4803455	CC	reference				reference				reference	
	CA/AA	0.18	-0.71	1.07	0.69	0.04	-0.72	0.81	0.91	-0.15	0.80
		Number of goblet cells per 1000um BM (ln)									
rs6957	AA	reference				reference				reference	
	GA/GG	-0.21	-0.64	0.22	0.33	0.07	-0.31	0.44	0.72	0.27	0.33
rs10417924	CC	reference				reference				reference	
	TC/TT	0.26	-0.12	0.63	0.17	-0.14	-0.48	0.20	0.42	-0.38	0.13
rs1800469	CC	reference				reference				reference	
	CT/TT	0.29	-0.04	0.62	**0.08**	0.27	-0.04	0.57	**0.08**	NT	NT
rs1800470	TT	reference				reference				reference	
	TC/CC	0.12	-0.21	0.45	0.47	0.08	0.16	-0.25	0.40	NT	NT
rs4803455	CC	reference				reference				reference	
	CA/AA	-0.16	-0.53	0.21	0.39	-0.04	-0.39	0.31	0.81	0.08	0.74

Online Repository

Transforming Growth Factor beta-1 polymorphisms and asthma severity, airway inflammation and remodelling

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Abbreviations used

AA1: mast cell tryptase

AMP: adenosine 5'-monophosphate

BD: bronchodilation

BM: basement membrane

EPX: anti-eosinophilic peroxidase

FEV_1: forced respiratory volume in 1 second

ICS: inhaled corticosteroids

LD: linkage disequilibrium

LME: Linear Mixed Effect models

MAF: minor allele frequency

SNP(s): single nucleotide polymorphism(s)

TGFβ1: Transforming Growth Factor beta-1

TGFB1: Transforming Growth Factor beta-1 gene

VC: vital capacity

METHODS

Subjects

All participants had doctor's diagnosed asthma, were atopic and hyperresponsive at first clinical assessment, and originated from the northern region of The Netherlands.

Population 1: 281 asthma patients who had entered a cohort study between 1966 and 1975 were re-examined during the period 1991–1999.[E1] Before testing, participants had to be in stable condition without any exacerbation in the last 6 weeks and subjects were allowed to use maintenance medication before testing. Patients filled in a questionnaire on respiratory symptoms and underwent lung function testing, histamine challenge (30 seconds tidal breathing method) and skin prick tests. The clinical assessment was performed as previously published.[E2] The subjects' medical records provided information on lung function and corticosteroid use during their annual routine check-ups. DNA samples have been collected from 243 subjects.

Population 2: 138 asthma patients, previously investigated by our research group[E3-5] were re-examined during the period 2002-2006. Main inclusion criteria were: FEV_1 ≥1.2 L, absence of bronchiectasis, upper respiratory tract infection (e.g. colds) and/or use of antibiotics or oral corticosteroids within the last 2 months before inclusion. Only 36.8% (21/57) of subjects on ICS treatment had stopped ICS medication for a minimum of four weeks before entering the study. Lung function testing and adenosine 5'-monophosphate (AMP) provocation test were performed followed 1-2 weeks later by a bronchoscopy. Subjects with a negative AMP provocation test (PC_{20} AMP > 320 mg/ml) were subjected to a histamine provocation test >3

weeks later.[E6-8] DNA samples and bronchial biopsies were available in 137 subjects. Thirty two subjects of population 2 were derived from population 1.

The study was approved by the medical ethics committee of the University Medical Center Groningen, and all participants signed an informed consent.

Genotyping of Single Nucleotide Polymorphisms (SNPs)

We genotyped 5 haplotype tagging SNPs (rs10417924, rs7254679, rs4803455, rs1800470, rs1800469) in the *TGFB1* gene (+/- 5kb) according to HapMap CEU genotype data (release 24/phase II Nov'08) ($r^2 < 0.8$). The cutoff value used for minor allele frequency (MAF) was 10% but we forced included (MAF<10% according to HapMap) rs1800470, a functional variant on exon-1.[E9] Rs6957 found in previous studies to be associated with decreased lung function in children with asthma[E10] and with decreased TGFβ1 serum levels[E11] was not found in HapMap thus we included it based on its dbSNP (National Center for Biotechnology Information) location. SNPs were genotyped by K-Bioscience Ltd (Herts, Hoddesdon, UK) using their competitive allele specific PCR system (KASPar) (see Figure 1 for the genotyped SNPs).

Bronchial biopsies

After local anesthesia, at least 5 bronchial biopsies were obtained from the subsegmental carinas from the left or right lower lobe using a flexible bronchoscope (Olympus BF P20 or BF XT20). Biopsies were fixed in 4% formalin, processed and embedded in paraffin and cut in 3μm thick sections. Macroscopically the best biopsy was selected for processing. Quantification was performed on the largest of three biopsy sections. Morphological features

were determined on sections stained with hematoxylin and eosin (HE) and with periodic acid Schiff. Immunohistochemical stainings were performed using the DAKO autostainer (DAKO, Glostrup, Denmark). The slides were included in a random fashion in each run to avoid group-wise staining. Immunohistochemistry was performed using antibodies: anti-CD8 and anti-CD-4 (DAKO) for T-lymphocytes, anti-CD68 (DAKO) for macrophages, anti-mast cell tryptase (AA1, DAKO) for mast cells and anti-eosinophilic peroxidase (EPX) antibody for eosinophils. In short, sections were deparaffinized and after antigen retrieval, incubated with the primary antibodies. These antibodies were detected with Envision™ Detection Kit (DAKO) and the chromogen NovaRED (Vector Labs, Burlingame, USA). EPX was detected via biotinylated anti-mouse IgG1 (Southern Biotech), alkaline phosphatase-labeled conjugate (DAKO) and permanent Red (DAKO). All stainings were quantified by a blinded observer using computer-assisted image analysis at a magnification of 200x (Qwin, Leica Microsystems Imaging Solutions, Cambridge, UK). Inflammatory cell numbers were quantified by counting the number of positively stained cells in the submucosal area, 100μm under the basement membrane (BM), in a total area of $0.1mm^2$ per biopsy sample. Goblet cell numbers were counted on PAS-stained biopsy sections and expressed per 1000 μm of BM. The length of basement membrane was variable among patients with a minimum of 973μm. The (median [min-max]) length of basement membrane covered with intact epithelium analyzed in each biopsy was 605 [0-3147] μm. BM thickness was calculated based on computer-assisted measurements of BM surface area and BM length. Reproducibility of the measurements was confirmed by a single observer performing repeated measurements in 10% of all cases.

Statistical Analysis

Normalization of the distribution of the variables was performed by natural logarithm transformation if necessary. Multiple linear regression models were used to test for associations with pre-/post-BD FEV_1%predicted (corrected for smoking status and ICS use) FEV_1/VC (corrected for age, gender, height, smoking status and ICS use), BM thickness and number of goblet and inflammatory cells (corrected for age, gender, smoking status and ICS use). Logistic regression models were used to assess the associations of SNPs with binary clinical outcomes:

1. Airway hyperresponsiveness: PC_{20}_histamine < 16mg/ml (population 1) and PC_{20}_AMP < 320mg/ml (population 2) (corrected for age, gender, smoking status and ICS use)
2. Complete asthma remission: former diagnosis of asthma, PC_{20}_AMP > 320mg/ml and PC_{20}_histamine > 16mg/ml, post-BD FEV_1%predicted > 90%, no asthma symptoms, no asthma medication (corrected for age, gender and smoking status)
3. Severe asthma: pre-BD FEV_1%predicted < 80% and ICS use (corrected for age, gender, and smoking status)

To assess whether there is an interaction of SNPs with smoking we stratified our analysis in 2 groups: never vs ever (ex/current) smokers. A common limitation for detecting gene by environment interactions is study power, therefore we decided *a priori* that we would only test for an interaction (genotype*ever-smokers) if the estimates of the SNPs showed a larger than 40% difference between the strata. We used the estimates (beta-coefficient) and standard errors of the SNPs and their interactions with smoking to meta-analyze associations with asthma clinical outcomes and lung function in the two populations. Fixed effects were used. When index of heterogeneity between studies (I^2) was high (>70) and/or Cochran's Q-test of heterogeneity was nearly significant ($p<0.07$) then random effects were used. All regression

modeling was conducted with SPSS (version 18; SPSS Inc., Chicago, Illinois, USA). Because the number of subjects with the homozygote mutant genotype was relatively low in the 2 subgroups (n≈<10) (see table E1 online repository), only dominant (heterozygotes and homozygotes mutant pooled vs wild types) models were used. Meta-analysis was done with R-programming language using the meta-package (R package 2.8.1. http://cran.r-project.org/bin/windows/base/old/2.8.1/). P-values<0.05 were considered statistically significant and 0.05≤ p-values ≤0.09 of borderline significance (tested 2-sided).

Lung function decline analysis

FEV_1 decline was analyzed with piecewise Linear Mixed Effect models (LME) as described before. [E2] In summary, following the methodology described by Naumova et al,[E12] time was defined as the time relative to the start of inhaled corticosteroid (ICS) treatment in years. Individual variations in the pre and post ICS-period declines and intercepts were accounted for by estimating the random effects for these variables. Sex was incorporated in the model by adding the interaction between time, ICS use, and sex. Other explanatory variables in the model were height (centered at 1.75 m), first available FEV_1 after age 30 (FEV_1 centered at 2.8 L, called "FEV_1 at age 30"), and their interaction with time, pack years of smoking, and oral corticosteroid use. The latter two variables were time varying. Only FEV_1 measurements after the age of 30 years were included since this is the age where the lung function is in a plateau phase or decline begins in normal subjects. [E13]

To assess whether the effect of the SNPs on the course of asthma is modified by cigarette smoking we initially stratified our analysis in 2 groups (ever vs never smokers). For the majority of the SNPs the frequency of the homozygotes mutants per strata was low (<10) thus

dominant (heterozygotes and homozygotes mutant pooled vs wild types) genetic models were used. A common limitation for detecting gene by environment interactions is study power therefore we decided a priori that we will only test for an interaction (genotype*ever-smoker*time) if the estimates of the SNPs show a more than 40% difference between the strata. LME models were conducted in S-plus 7.0 (Insightful Corp., Seattle, Washington, USA). P-values<0.05 were considered statistically significant and 0.05≤ p-values ≤0.09 of borderline significance (tested 2-sided).

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Figure E1: Analysis of the linkage disequilibrium patterns and correlation coefficients (r^2) of 6 genotyped *TGFB1* gene single nucleotide polymorphisms in the two populations using Haploview software. The location of the SNPs is given by HapMap or dbSNP

Figure E2 Associations between subepithelial CD8+T-cell counts and (a) smoking status, (b) rs10417924, rs10417924 in never (c) and in ever (d) smokers of population 2.

Associations between airway hyperresponsiveness and (e) smoking status, (f) rs10417924, rs10417924 in never (g) and in ever (h) smokers

Odds ratios (95%CI) are given by multiple logistic regression models adjusted for gender, age and inhaled corticosteroids use

Figure E3 Mean (95%CI) FEV_1 decline (ml/year) per genotype of *TGFB1* SNPs in never and in ever smokers of population 1.

Piecewise linear mixed effect models were used to test the association between *TGFB1* SNPs and FEV_1 decline. Regression Coefficient (beta) and p-value are indicated. *significance level at 0.05; ** significant smoking by SNP interaction