

Original article

Polymorphisms in IL13 pathway genes in asthma and chronic obstructive pulmonary disease

Background: Asthma and chronic obstructive pulmonary disease (COPD) are chronic respiratory diseases involving an interaction between genetic and environmental factors. Interleukin-13 (IL13) has been suggested to have a role in both asthma and COPD. We investigated whether single nucleotide polymorphisms (SNPs) in the IL13 pathway may contribute to the susceptibility and severity of asthma and COPD in adults.

Methods: Twelve SNPs in IL13 pathway genes – *IL4*, *IL13*, *IL4RA*, *IL13RA1*, *IL13RA2* and *STAT6* – were genotyped in subjects with asthma ($n = 299$) and in subjects with COPD or healthy smokers ($n = 992$). Genetic association was evaluated using genotype and allele models for asthma severity, atopy phenotypes and COPD susceptibility. Linear regression was used to determine the effects of polymorphism on baseline lung function (FEV₁, FEV₁/FVC).

Results: In asthmatics, three *IL13* SNPs – rs1881457(–1512), rs1800925(–1111) and rs20541(R130Q) – were associated with atopy risk. One SNP in *IL4RA1* [rs1805010(I75V)] was associated with asthma severity, and several *IL13* SNPs showed borderline significance. *IL13* SNPs rs1881457(–1512) and rs1800925(–1111) were associated with better FEV₁ and FEV₁/FVC in asthmatics. *IL13* SNPs rs2066960(intron 1), rs20541(R130Q) and rs1295685(exon 4) were associated with COPD risk and lower baseline lung function in the recessive model. In females, but not in males, rs2250747 of the *IL13RA1* gene was associated with COPD and lower FEV₁.

Conclusion: These data suggest that *IL13* SNPs (promoter and coding region) and, to a lesser extent, *IL4RA* SNPs may contribute to atopy and asthma. We also provide tentative evidence that *IL13* SNPs in the coding region may be of significance in COPD susceptibility.

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Asthma and chronic obstructive pulmonary disease (COPD) are chronic inflammatory respiratory diseases that are most likely due to an interaction between genetic and environmental factors. Atopy is the most important risk factor of asthma (1) and tobacco smoking is the most important risk factor of COPD (2).

Interleukin-13 (IL13) is a Th2 cytokine implicated in (i) recruitment of inflammatory cells from the blood to the lung, (ii) immunoglobulin (Ig) E production by B cells, (iii) regulation of matrix metalloproteinases and (iv) induction of mucin production and secretion (3, 4). IL13 is produced by a variety of cells, including Th2 CD4⁺, Th1 CD4⁺, CD8⁺ T cells, mast cells, basophils and eosinophils. IL13 mediates its effects by interacting with a complex receptor system comprising

an IL4R α /IL13R α 1 heterodimer and the IL13R α 2 receptor (3).

The involvement of IL13 in the pathogenesis of asthma has been extensively documented (3–5). The administration of recombinant IL13 in the lungs of mice increases airway mucus secretion and causes sub-epithelial fibrosis, bronchial hyperresponsiveness, and eosinophilic airway inflammation (4). Cells expressing IL13 mRNA are increased in the bronchial tissue of atopic and nonatopic asthmatic subjects (6). Several IL13 genetic polymorphisms have been linked with susceptibility to develop atopic disease, such as bronchial asthma (7–9). Also, genetic polymorphisms in genes encoding IL4R α , IL13R α 1, or STAT6 (10–12) predispose toward development of atopy, supporting the role

of the IL13 pathway in contributing to risk of developing atopic asthma.

The involvement of IL13 and its genetic variants in COPD is less clear. In experimental studies the over-expression of IL13 in the adult murine lung caused emphysema (13), and the number of IL13+ cells was elevated in the bronchial submucosa of adult symptomatic smokers with symptoms of chronic bronchitis compared to asymptomatic smokers (14). These data are still controversial: Saha et al. (15) reported that IL13 expression was not increased in sputum and bronchial mucosa of COPD patients compared to nonsmoking controls. Finally, IL13 mRNA levels in lung homogenates from subjects with emphysema were correlated with the lung function parameter forced expiratory volume in 1 s (FEV₁) (16). Taken together, these data suggest that IL13 may be involved in the pathogenesis of asthma and COPD.

Considering the biological interaction between IL13 and the IL4 receptor, we sought to examine the major component of the IL13 pathway by genotyping 12 known single nucleotide polymorphisms (SNPs) in *IL13*, *IL4*, *IL13RA1*, *IL13RA2*, *IL4RA1* and *STAT6* in asthmatics, subjects with COPD and healthy smokers. Thus, the aim of our study was to investigate whether SNPs in the IL13 pathway may contribute to the susceptibility and severity of asthma and COPD in adults.

Subjects and methods

Subjects

Three cohorts were included in this study. Adult asthmatics were recruited from Nottingham, UK ($n = 176$) and Padova, Italy ($n = 123$). The following inclusion criteria were used: physician-diagnosed asthma, Caucasian, age 16–60 years, <10 pack-year cigarette smoking history, documented asthma for >1 year, and no other respiratory diseases. Comprehensive phenotype data were collected, including details of current medication, co-existing atopic disorders such as hay fever and/or eczema, and family history of asthma and atopy. Each subject underwent a lung function test. Skin prick tests for common inhaled allergens (*Dermatophagoides*, grass, tree, cat, dog) were determined in the Italian population. Asthma severity was evaluated according to British Thoracic Society guidelines (1). Blood samples (10 ml) were obtained from all subjects, and genomic DNA was isolated using the Puregene DNA extraction kit (Gentra, QIAGEN, West Sussex, UK). Approval was obtained from the Nottingham and Padova local research ethics committees.

Subjects with COPD and healthy smokers (controls) were recruited from five UK centres. Smokers ($n = 537$) were recruited in Nottingham; criteria were Caucasian, age >40 years, and >10 pack-year smoking history. Criteria for subjects with spirometry-defined COPD ($n = 455$) were Caucasian, age >45 years, and >10 pack-year smoking history. The combined population ($n = 992$) recruited for smoking history or COPD diagnosis was stratified into (i) healthy smokers ($n = 176$) – postbronchodilator (postBD, salbutamol) FEV₁ >80% and postBD FEV₁/FVC >0.7 – and (ii) COPD subjects ($n = 599$) – postBD FEV₁ <80% and postBD FEV₁/FVC <0.7. Subjects missing data or those not meeting these criteria were excluded from the case-control analyses.

SNP selection, genotyping and haplotypes

SNPs were chosen for putative function, previous association with asthma phenotypes or ability to tag the haplotypes spanning the regions. SNPs were genotyped using a KASPar reagent kit (KBiosciences, Hertfordshire, UK). Hardy–Weinberg equilibrium was assessed and *IL13* and *IL4RA* haplotypes were identified using HAPLOVIEW software (17).

Statistical analyses

For the asthma cohort contingency table, analyses were completed with genotype or allele counts in the different groups using GRAPHPAD Prism 5.0. For genotype analyses, 2×3 tables were generated and differences in groups were determined by chi-square test. For allele analyses, 2×2 tables were generated and differences were evaluated by Fisher exact test and odds ratio. In the asthma cohort, dichotomous traits tested were atopy, determined by positive skin test; rhinitis questionnaire; and asthma severity, evaluated according to British Thoracic Society guidelines (1) [stage 1 (mild asthma) vs stage >3 (severe asthma)]. Using SPSS v15 (SPSS Inc., Chicago, IL, USA), logistic regression analyses were completed for COPD susceptibility using additive (e.g. AA vs AC vs CC), recessive (AA/AC vs CC) or dominant (AA vs AC/CC) models, including age, sex and smoking pack-years as covariates. By linear regression we determined the contribution of each SNP to baseline FEV₁ (liters) or FEV₁/FVC using additive, recessive or dominant models, including age, sex, height and smoking pack-years as covariates. We did not correct for multiple testing, as linkage disequilibrium (LD) suggested these were not independent tests. A P -value of <0.05 was considered significant for all analyses.

Results

Clinical characteristics and allele frequencies

Characteristics of the study subjects are reported in Table 1. As anticipated, FEV₁ and FEV₁/FVC of the smoking control subjects ($n = 176$) and COPD patients ($n = 599$) were significantly different ($P < 0.0001$). Comparison of other baseline features identified significant differences in age, sex and smoking pack-years; therefore, in subsequent COPD susceptibility analyses we adjusted for these variables. Allele frequencies were comparable across the cohorts and showed good concordance with data in the SNP database (dbSNP) (Table 2). All SNPs were tested for deviation from the Hardy–Weinberg equilibrium in asthma ($n = 299$) and in the smoking/COPD cohort ($n = 992$), and there was no deviation ($P > 0.05$, data not shown).

Haplotype structure

Haplotype analyses of SNPs genotyped in the 5q locus (*IL4/IL13*) and the 16p locus (*IL4RA*) are shown for the different populations in Fig. 1. There is some redundancy in the IL4/IL13 genotyping in asthmatics (Fig. 1A, B); i.e., rs1881457-rs1800925 are in high LD (0.97), as are rs20541-rs1295685. In the *IL4RA1* locus, rs1805015 and rs1801275 are in high LD. Similar findings were observed in the smoking cohort (Fig. 1C, D).

Table 1. Characteristics of the study subjects

	Asthmatics (<i>n</i> = 299)	All smokers (<i>n</i> = 992)	Healthy smokers (controls) (<i>n</i> = 176)	Smokers with COPD (<i>n</i> = 599)
Age (years)	39.24 ± 12.31	63.33 ± 10.28 (992)	54.38 ± 9.52	65.96 ± 9.01
Female (%)	65.6	43.8 (984)	56.3	40.4
FEV ₁ (% predicted)	92.37 ± 20.57	56.01 ± 28.17 (975)	96.03 ± 12.15	40.31 ± 15.63
FEV ₁ /FVC	75.94 ± 11.58	55.3 ± 17.4 (971)	77.3 ± 5.9	46.3 ± 12.6
PostBD FEV ₁ (% predicted)	ND	59.08 ± 27.14 (885)	99.48 ± 11.72	44.65 ± 15.52
PostBD FEV ₁ /FVC	ND	55.6 ± 17.7 (873)	79.1 ± 5.5	46.2 ± 12.0
Smoking pack-years	2.98 ± 6.32	43.52 ± 26.05 (969)	32.74 ± 20.04	47.61 ± 27.01

Values are mean ± SD. FEV₁, forced expiratory volume in 1 s

FVC, forced vital capacity; BD, bronchodilator; ND, not determined.

Values in parentheses are the number of individuals with data available. Control subjects were defined as having postBD (salbutamol) FEV₁ >80% and postBD FEV₁/FVC > 0.7. Subjects with COPD were defined as having postBD FEV₁ <80% and postBD FEV₁/FVC <0.7. Individuals not meeting these criteria were excluded from the case-control analyses. Variables between control and COPD groups were compared by independent *t*-test ([continuous] variables or Pearson chi-square test [sex]). *P* < 0.0001 for all variables.

IL13 pathway SNPs and atopy/asthma associations

In asthmatics, *IL13* SNPs rs1881457(–1512) (odds ratio [OR] 2.1, confidence interval [CI] 1.09–4.4, *P* = 0.017), rs1800925(–1111) (OR 2.2, CI 1.13–4.40, *P* = 0.013) and rs20541(R130Q) (OR 2.3, CI 1.02–5.0, *P* = 0.029) were significantly associated with atopy (defined as a positive skin test), while rs2250747 (*IL13RA1*, tagging SNP) had a borderline association in females, but not in males (Table 3). While no single *IL13* pathway SNP showed a significant association with hay fever, all the borderline associations were within the *IL13* gene and included risk alleles rs1881457C(–1512), rs1800925T(–1111) and rs20541T(R130Q), with a strong concordance with the skin prick test analyses. The *IL4RA* SNP rs1805010(I75V) was associated with more severe asthma (OR 1.8, CI 1.1–3.3, *P* = 0.032). Multiple borderline associations (*P* ~ 0.06) were found between *IL13* SNPs and asthma severity, including risk alleles rs1881457C

(–1512), rs1800925T(–1111), rs20541T(R130Q) and rs1295685T(exon 4) (data not shown).

IL13 pathway SNPs and COPD

Three SNPs in *IL13* – rs2066960A(intron 1), rs20541T(R130Q), and rs1295685T(exon 4) – were associated with COPD risk in smokers (OR ~4.49–9.21), but only in the recessive model (Table 4). rs2250747G(intron 1) in *IL13RA1* was associated with COPD risk in females, but not in males (OR 10.5, CI 1.21–91.4, *P* = 0.033) (Table 4).

IL13 pathway SNPs and lung function

In the asthmatic cohort, *IL13* SNPs rs1881457(–1512) and rs1800925(–1111) were associated with higher FEV₁ and FEV₁/FVC using regression analyses (Fig. 2A–D). In the smoking cohort, in the recessive model, rs2066960

Table 2. Gene location and minor allele frequencies of genotyped IL13 pathway SNPs

SNP	Gene	Location	Location	Alleles	Selected for:	Asthmatics (<i>n</i> = 299)	All smokers (<i>n</i> = 992)	Smoking controls (<i>n</i> = 176)	COPD patients (<i>n</i> = 599)
rs2243250	<i>IL4</i>	5:132037053	IL4-589	C/T	Prom.	0.12	0.13	0.12	0.13
rs1881457	<i>IL13</i>	5:132020308	IL13-1512	A/C	Prom.	0.21	0.19	0.17	0.19
rs1800925	<i>IL13</i>	5:132020708	IL13-1111	C/T	Prom.	0.22	0.19	0.17	0.18
rs2066960	<i>IL13</i>	5:132022334	IL13 Intron 1	C/A	htSNP	0.10	0.10	0.12	0.09
rs20541	<i>IL13</i>	5:132023863	IL13 Exon 4	C/T	R/Q 130	0.20	0.18	0.16	0.18
rs1295685	<i>IL13</i>	5:132024344	IL13 Exon 4	C/T	htSNP	0.20	0.18	0.16	0.17
rs1805010	<i>IL4RA</i>	16:27263704	IL4RA Exon 5	A/G	I/V 75	0.47	0.45	0.46	0.46
rs1805015	<i>IL4RA</i>	16:27281681	IL4RA Exon 11	T/C	P/S 503	0.16	0.16	0.16	0.16
rs1801275	<i>IL4RA</i>	16:27281901	IL4RA Exon 11	A/G	R/Q 576	0.22	0.21	0.21	0.22
rs2250747 (males)	<i>IL13RA1</i>	X:117747776	IL13RA1 Intron 1	A/G	Tagging SNP	0.08	0.08	0.08	0.09
rs2250747 (females)	<i>IL13RA1</i>	X:117747776	IL13RA1 Intron 1	A/G	Tagging SNP	0.13	0.17	0.17	0.17
rs5946040 (males)	<i>IL13RA2</i>	X:114154022	IL13RA2 Intron 5	G/T	Tagging SNP	0.14	0.09	0.09	0.08
rs5946040 (females)	<i>IL13RA2</i>	X:114154022	IL13RA2 Intron 5	G/T	Tagging SNP	0.21	0.16	0.16	0.16
rs324011	<i>STAT6</i>	12: 55788449	STAT6	C/T	Intron	0.40	0.42	0.42	0.43

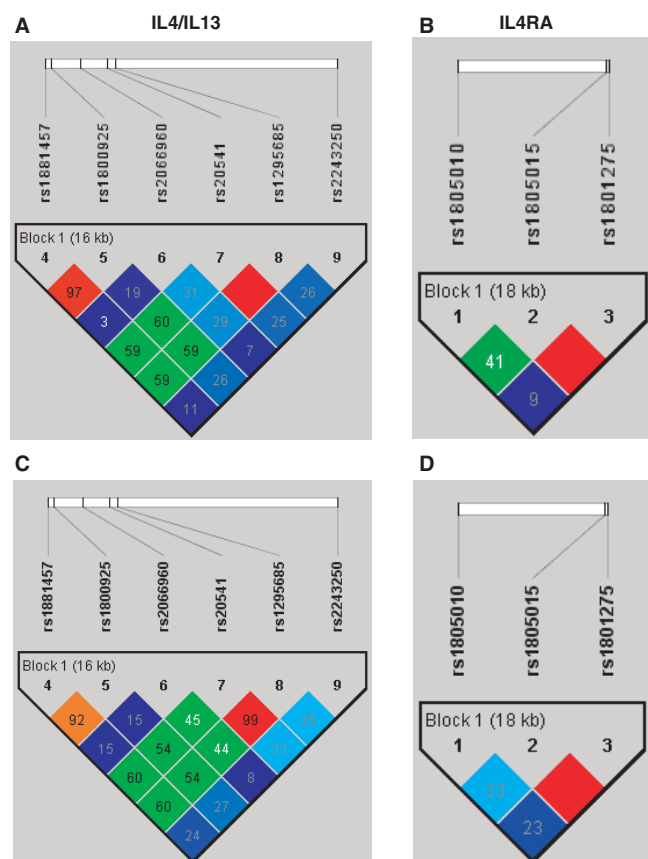


Figure 1. Haplotype block structure of *IL13* and *IL4RA* SNPs in asthmatics (Panels A and B, $n = 299$) and smokers (Panels C and D, $n = 992$). The location of each tested SNP is indicated. The intensity of shading represents D' (a measure of LD), and numerical values were generated using HAPLOVIEW software (17).

(intron 1), rs20541(R130Q) and rs1295685(exon 4) were associated with lower FEV₁, while rs2250747 was associated with lower FEV₁ only in females (Fig. 2E–I).

Discussion

This study confirms that SNPs in the *IL13* pathway genes, particularly those spanning the *IL13* promoter/coding region, may influence atopy development and, to a lesser extent, asthma severity. Our data also suggest a possible role for *IL13* intron/exon polymorphisms in COPD development in smokers. Finally, our results show that genetic variants in the *IL13* pathway may interact to affect baseline lung function in both asthmatics and smokers.

To further validate our findings, we completed data mining of the general 1958 birth cohort online data set (available at <http://www.b58cgene.sgul.ac.uk/>). Analyses identified an association between *IL13* SNPs rs1881457 (–1512), rs1800925(–1111) and rs20541(R130Q) and increased total IgE at age 44–45. These data show excellent concordance with identification of these SNPs as atopy risk alleles in the current study. Similarly, rs1800925(–1111) and rs2066960(intron 1) showed an association with increased FEV₁ (at age 44–45) in this general population, confirming the importance of these SNPs in determining lung function as observed in our analyses in smokers. It is interesting to note that, in the general vs smoking populations, the direction of effect was reversed.

Overall our results are consistent with those from previous studies reporting the association of *IL13* SNPs and atopy phenotypes. We replicated the association of

Table 3. Skin prick test and *IL13* pathway SNPs in asthmatics

SNP	Negative ($n = 39$)				Positive ($n = 70$)				Genotype	Allele	Allele	95% CI
	0	1	2	MAF	0	1	2	MAF				
rs2243250	31	8	0	0.10	54	15	1	0.12	0.7465	0.4283	1.209	0.4965–2.946
rs1881457	29	9	2	0.16	36	29	7	0.30	0.0688	0.0166	2.194	1.097–4.388
rs1800925	28	10	2	0.18	32	31	7	0.32	0.0481	0.0127	2.233	1.134–4.396
rs2066960	34	6	0	0.08	57	15	0	0.10	0.3113	0.322	1.434	0.5333–3.856
rs20541	31	5	2	0.12	41	27	3	0.23	0.0247	0.0294	2.254	1.015–5.004
rs1295685	30	8	2	0.15	41	26	4	0.24	0.1723	0.0782	1.784	0.8641–3.683
rs1805010	13	17	10	0.46	18	38	16	0.49	0.5596	0.4212	1.099	0.6357–1.901
rs1805015	27	13	0	0.16	47	23	1	0.18	0.752	0.476	1.101	0.5283–2.296
rs1801275	26	14	0	0.18	42	28	2	0.22	0.4958	0.2547	1.347	0.6702–2.707
rs2250747 (males)	10	0	0	0.00	30	3	0	0.05	0.4421	0.4471	2.26	0.1119–45.64
rs2250747 (females)	27	3	0	0.05	28	11	0	0.14	0.0567	0.0678	3.119	0.8292–11.73
rs5946040 (males)	8	2	0	0.10	21	12	0	0.18	0.2873	0.3133	2.00	0.4080–9.803
rs5946040 (females)	14	12	4	0.33	16	16	7	0.38	0.8373	0.3297	1.25	0.6179–2.529
rs324011	16	19	5	0.36	29	37	5	0.33	0.6193	0.3701	0.8701	0.4898–1.546

0, 1, and 2 represent the number of major, heterozygote and minor genotypes, respectively. For minor allele frequencies in controls vs COPD patients, see Table 2. MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

*We did not correct for multiple testing as tested genes that have been previously reported to be associated with respiratory disease/phenotypes.

Table 4. Risk of COPD in smokers and IL13 pathway SNPs

SNP	Smoking controls (n = 176)			COPD patients (n = 599)			Additive			Recessive			Dominant		
	0	1	2	0	1	2	P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI
rs2243250	133	41	1	445	132	11	0.906	0.98	0.64–1.49	0.472	2.23	0.25–19.9	0.744	0.93	0.58–1.47
rs1881457	121	47	6	397	171	24	0.280	1.22	0.85–1.74	0.281	1.75	0.63–4.85	0.407	1.20	0.79–1.84
rs1800925	121	46	6	395	174	22	0.178	1.29	0.89–1.85	0.579	1.34	0.47–3.81	0.169	1.35	0.88–2.08
rs2066960	134	38	1	494	94	8	0.927	0.98	0.63–1.53	0.046	9.21	1.04–81.2	0.411	0.81	0.50–1.33
rs20541	118	49	3	400	149	26	0.128	1.33	0.92–1.92	0.023	4.49	1.23–16.3	0.442	1.19	0.77–1.84
rs1295685	123	49	3	412	154	26	0.104	1.36	0.94–1.96	0.021	4.55	1.25–16.52	0.373	1.22	0.79–1.89
rs1805010	50	87	37	166	299	117	0.920	0.99	0.74–1.31	0.999	1.00	0.61–1.63	0.877	0.97	0.62–1.51
rs1805015	121	49	4	419	161	14	0.894	0.97	0.66–1.44	0.682	1.37	0.30–6.19	0.787	0.94	0.61–1.46
rs1801275	111	58	7	362	207	24	0.728	1.06	0.75–1.51	0.557	1.38	0.47–4.06	0.863	1.04	0.69–1.56
rs2250747 (males)	65	12	0	285	62	0	0.837	1.08	0.51–2.33	NA	NA	NA	0.837	1.08	0.51–2.33
rs2250747 (females)	65	32	1	168	58	11	0.637	1.13	0.68–1.88	0.033	10.5	1.21–91.4	0.636	0.87	0.48–1.57
rs5946040 (males)	63	14	0	282	54	0	0.819	0.91	0.43–1.97	NA	NA	NA	0.819	0.91	0.43–1.97
rs5946040 (females)	67	28	1	161	64	4	0.762	0.92	0.52–1.62	0.631	1.93	0.13–28.1	0.656	0.87	0.47–1.60
rs324011	58	87	31	187	302	105	0.474	0.90	0.68–1.20	0.523	0.85	0.50–1.42	0.590	0.89	0.58–1.36

OR, odds ratio; CI, confidence interval; ND, not determined (minor allele frequency too low); NA, not applicable (i.e., recessive model in males for X-linked gene).

0, 1, and 2 represent the number of major, heterozygote and minor genotypes, respectively. For minor allele frequencies in controls vs COPD patients, see Table 2. Logistic regression was used to compare genotype frequencies using the additive, recessive, and dominant models, with the covariates age, sex and pack-years included in the model.

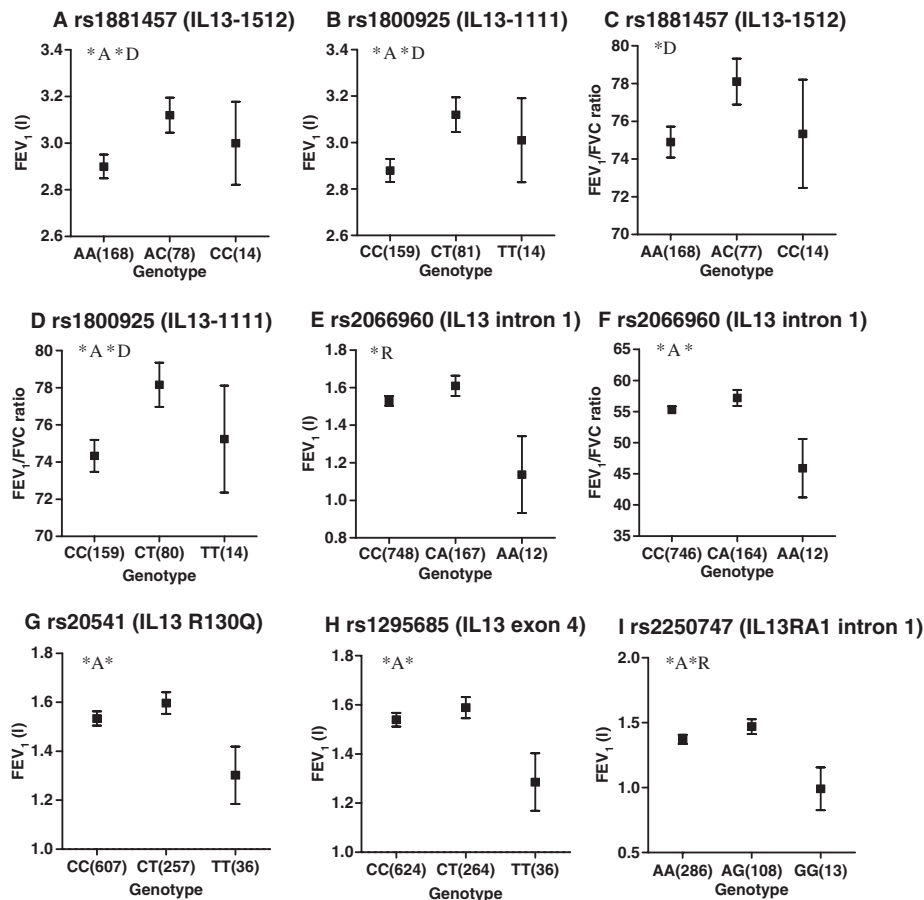


Figure 2. IL13 SNPs are associated with baseline FEV₁ and FEV₁/FVC in asthmatics (Panels A–D) and smokers (Panels E–I). Regression analysis was used to investigate the association between SNPs and baseline FEV₁ and FEV₁/FVC using the additive (A), recessive (R) and dominant (D) models. Data represent mean ± SE. Covariates included in the model were age, sex, height, and pack-years. **P* ≤ 0.05.

the IL13-1111T variant reported by Howard et al. (8) with a positive skin test and the association of IL13-1512C and IL13-1111T variants with atopy, defined as increased total serum IgE levels reported in three different allergic cohorts of more than 3000 children (18).

Howard et al. (8) found that the IL13-1111T variant was associated with asthma *per se*; we did not formally test this, but we observed a borderline association between four *IL13* SNPs, including the IL13-1111CT variant, and asthma severity. In addition, it would be difficult to identify the IL13-1111CT variant as a true risk polymorphism because of the strong LD observed between the four SNPs (-1512, -1111, R130Q and exon 4). In asthmatics, two of the SNPs associated with asthma severity were also associated with better lung function (higher FEV₁ and FEV₁/FVC). However, severity was established according to the pattern of medication required to keep asthma under control (1), and thus lung function in treated patients may not reflect severity.

DeMeo et al. (19), who analyzed 685 asthmatic children and their parents enrolled in the Childhood Asthma Management Program, reported a positive association between the *IL13* R130Q polymorphism and various atopic phenotypes, including eosinophils, serum IgE and positive skin test, but not airway responsiveness, asthma diagnosis or asthma severity. This is not completely unexpected, as *IL13* genetic variants have been associated with airway responsiveness but not with asthma in Chinese children (12). Furthermore, in a Costa Rican study, *IL13* Arg130Gln was associated with asthma exacerbations only in children receiving treatment with inhaled steroids (20).

Finally, we found a borderline association between *IL13* rs1800925 and the risk to develop allergic rhinitis, confirming the data reported by Huebner et al. (21) in a cohort of 923 children and by Black et al. (22) in the longitudinal 1946 birth cohort study of the UK Medical Research Council's National Survey of Health and Development.

Taken together, our findings and data from the literature (19, 23) suggest a significant role for *IL13* polymorphism in atopy in asthmatic subjects. However, we cannot exclude the possibility that the weak association of *IL13* SNPs with asthma severity in our population may be due to the inability to exclude differential LD with unevaluated variants in *IL13* or nearby genes, or the small number of subjects in this study.

While our findings are globally consistent with those of previous association studies of single SNPs in the *IL13* gene itself with atopy and, to a lesser extent, asthma, they are not consistent with the results of studies supporting the role of individual *IL4/IL4RA1* SNPs in susceptibility to both atopy and asthma. Indeed, while IL4-589CT has been widely reported to be a risk factor for both atopy and asthma phenotypes in different populations (24–26), we did not find any association between this common

SNP and any of the phenotypes of atopy (positive skin test, serum IgE), hay fever, or asthma. Similarly, no associations were found between single SNPs in the gene encoding *IL4RA1*, such as the common Gln551Arg and Ser478Pro polymorphisms, and any atopy and asthma phenotypes frequently reported to be associated with asthma and allergic disorders (27–30); only a weak association between the *IL4RA1*50V polymorphism and asthma severity was found in our asthmatic population. The lack of replication could be due to the different SNPs tested, to different ethnicity-specific effects of particular SNPs, or to the relatively small number of subjects analyzed in this and previous studies.

In the present study, we also demonstrated that three *IL13* polymorphisms in smokers – rs2066960(intron 1), rs20541(R130Q) and rs1295685(exon 4) – are associated with COPD susceptibility. Furthermore, in females, but not in males, the rs2250747 SNP in *IL13Rα1* is associated with COPD. These findings are novel and highlight a potential role for the *IL13* intron/coding region SNPs in COPD susceptibility. Importantly, these same SNPs were determinants of baseline lung function in smokers.

We did not replicate the association between the IL13-1111CT polymorphism and COPD that was previously reported in the Dutch population (31). However, in that study the positive association could be due to the relatively small sample size (151 COPD patients and 78 healthy control smokers). On the other hand, in a larger population of 1073 smokers and former smokers, IL13-1111CT was not associated with lung function measures, and there was a significant combined effect of the promoter SNP IL13-1111CT on percent predicted FEV₁, suggesting that the genetic variant may modulate the adverse effects of cigarette smoking on pulmonary function (32). In fact, IL13-1112CT has been reported to enhance *IL13* activity *in vitro* (33).

The lack of replication of this association in our study may be due to a bias in the selection of healthy smokers. Indeed, the mean age of COPD patients was higher than that of healthy smokers, although we corrected for this in the regression model. It can be anticipated that some of the healthy smokers may develop COPD later in life. Other limitations of our study are that we did not have independent replication of the genetic effects observed, and several of the analyses are based on relatively small numbers.

Interestingly, we found a positive association between *IL13* R130Q and COPD and impaired lung function that was not found in the Dutch study (31). Vladich et al. (34) reported that an R → Q replacement may amplify *IL13* activity (e.g., STAT6 phosphorylation and CD23 expression in monocytes), particularly when associated with other SNPs in the *IL13* pathway, such as -1111CT (often found in LD) or V50R551 of *IL4Rα* (35). It is important to note that, while both the asthma/atopy and COPD analyses highlighted a role for *IL13* SNPs in disease mechanisms, the asthma/atopy associations involved

SNPs in the promoter, intron and exon regions, whereas the COPD signal was localized to intronic and coding region SNPs. These findings may in part explain the lack of correlation between COPD and IL13 expression (15) clearly observed in asthma and atopy (3).

In conclusion, the data reported here confirm that genetic variants in the IL13 pathway may be involved in the development of atopy and, to a lesser extent, severe asthma. Also, the novel positive associations found with COPD suggest a role for IL13 in COPD development in smokers as well.

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