Interleukin 6 polymorphisms modify the effects of smoking on the risk of adult asthma

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for important intellectual content. JJKJ had final responsibility for the decision to submit for publication. MSJ and JJKJ obtained funding and supervised the study.

Financial Disclosure: This work was supported by the Academy of Finland grants no. 138691, 266314 and 129419 the SALVE program (http://www.aka.fi/en-GB/A/), and the Ministry of Social Affairs and Health grant no. STM/1523/2012 (http://www.stm.fi/en/frontpage), and the University of Oulu Strategic Funding for CERH. The CIAs study was partly supported by the Scientific Advisory Board for Defense (MATINE) of the Ministry of Defense of Finland. The funders had no role in study design, data collection or analysis, decision to publish, or preparation of the manuscript.

Capsule summary

Our findings highlight the importance of IL-6 in the pathogenesis of asthma and imply that the GG genotype in rs1800797 and/or rs1800795 polymorphism may predispose to the adverse effects of smoking on adult asthma.

Key words: Gene-Environment Interaction, Population-Based, Case-Control Study, Odds Ratio, Meta-Analysis, Adult, Asthma

Abbreviations

FEAS, Finnish Environment and Asthma Study; CI, Confidence interval; CIAS, Cold, Infections and Asthma in Soldiers study; ERR, Excess Relative Risk; GWAS, Genome-wide association study; IL-6, Interleukin 6; LD, Linkage disequilibrium; MI, Myocardial infarction; OR, Odds ratio; RERI, Relative Excess Risk due to Interaction; SHS, Second-hand smoke exposure; SNP, Single nucleotide polymorphism

To the editor

Cytokine interleukin 6 (IL-6) may participate in the pathogenesis of asthma[1] and it is produced as a response to different stimuli, including tobacco smoke[2]. We recently reported an association between the *IL6* single nucleotide polymorphism (SNP) rs1800797 and adult-onset asthma[3]. *IL6* SNP rs1800795 and other SNPs (including rs1800797) in close linkage disequilibrium (LD) with it seem to associate with asthma especially in adults[4], but also divergent findings exist, see the Online repository (p. 1) for the details. We hypothesized that unidentified gene-environment interactions could explain some of these differences in findings. Tobacco smoking is an obvious candidate, since it is common among adults but rare among children. We have previously shown that smoking is a determinant of asthma[5] and an interaction between tobacco smoke and *IL6* SNPs on conditions other than asthma has been reported[e.g.6].

Our study population was the Finnish Environment and Asthma Study (FEAS), a population-based, incident case-control study of adult-onset asthma including 521 new cases of asthma and 932 controls. For replication, we used the Cold, Infections and Asthma in Soldiers (CIAS), a case-control study of Finnish military conscripts including 224 previously diagnosed asthma cases and 668 controls, all men. A total of 467 cases and 613 controls in FEAS, and 225 cases and 642 controls in CIAS were genotyped. We estimated the relations between rs1800797 and adult-onset asthma in FEAS, stratifying by the smoking status. In the CIAS, we analyzed association for rs1800795, which is in high LD with rs1800797. Of the tested genetic models, the recessive model was found as best fitting. We performed meta-analyses of the effect estimates from the adjusted recessive models. For cross-reference of the association results we imputed the rs1800795 in FEAS and the rs1800797 in CIAS, and analyzed their association with asthma. We tested for additive and multiplicative level

interactions between the *IL6* SNPs and smoking. See the Online Repository (p. 1-2) for the details of methods.

Characteristics of our study populations are shown in the Online repository (p. 3 and Table E1). In FEAS, rs1800797 associated with incident asthma among those with any smoking exposure (Fig 1, A and B), and a similar but weaker association was found between rs1800795 and prevalent asthma in CIAS (Fig 1, C and D). No association was seen among the never-smokers (Fig 1, A-D). In a meta-analysis the GG genotype of either polymorphism associated with an increased risk of asthma among those who reported any smoking exposure (Fig 1, E, and Online repository p. 3). More detailed categorization by smoking status revealed association among the former smokers and the current smokers and further, among the regular smokers (Fig 1 and Online repository Table E2). No gender difference in the effect of the genotypes was observed in FEAS (Data not shown). The imputed genotypes showed similar association patterns as the directly genotyped SNPs (Online repository p. 3 and Table E2).

In FEAS, an additive level interaction was seen between the rs1800797 GG genotype and any smoking (Fig 2, C), and weaker interactions were observed for former and current smoking (Fig2, F). Slightly stronger interaction of rs1800797 with≥200 cigarette-years smoked than with 1-199 cigarette-years was observed among the any smoking (Fig 2, I) and the previous smoking categories (Fig 2, L). In CIAS, the rs1800795 GG genotype showed a slightly increased, but statistically non-significant, joint effect in relation to any smoking and to current smoking (Online Repository Table E3). Whereas in FEAS, interaction was observed also at a multiplicative level (Online Repository Table E4).

We show in two independent Finnish study populations that the *IL6* SNPs rs1800797 and rs1800795 modify the effect of tobacco smoke exposure on the risk of asthma. The GG genotype of either

polymorphism was found to associate with an increased risk of asthma among those who had some tobacco smoke exposure, but not among never-smokers. The results were similar in both study populations and were strengthened in the meta-analysis of the study-specific effect estimate, as well as in the analysis of the imputed genotypes. Some differences in the results between the FEAS and the CIAS study populations were observed, please see the Online repository (p. 3-4) for the details and for validity issues (p. 3).

No previous study has reported interaction between *IL6* polymorphisms and smoking in relation to asthma. However, our results gain plausibility from previous studies on interaction between *IL6* and tobacco smoke on several other health outcomes, including e.g. coronary artery disease[6]. Likely, the same regulatory mechanisms play a role in several diseases, for example, cardiovascular diseases and allergic disorders have been linked to each other[7]. Most of the studies that have reported an interaction between smoking and rs1800795 or rs1800797 found that the C or A allele[e.g.6], carried the risk, whereas in our observation an increased risk associated with the GG genotype. However, it is plausible that a genotype may predispose to one disease and be protective for another, as the disease mechanisms are likely to be different. Also, IL-6 response to stimuli may vary by cell type and thus response in circulating levels of IL-6 may differ from that seen in locally produced IL-6. Supporting our findings, a recent meta-analysis reported a protective effect of the rs1800795 CC genotype for asthma in Caucasians and in adults[4]. See Online repository (p. 4) for further discussion.

All of the studies reporting gene-environment interaction between *IL6* and tobacco smoke have been candidate gene studies. Two genome-wide gene-smoking interaction studies have investigated asthma but they only found suggestive associations, not including *IL6*[8, 9, 9]. However, in the genome-wide studies some true positive findings may be rejected because of the rigorous significance level.

In conclusion, carrying the GG genotype in either rs1800797 and/or rs1800795 may increase susceptibility to the adverse effects of smoking on the risk of asthma. This could explain some of the controversies observed in previous studies regarding *IL6* polymorphisms and asthma.

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Figure legends

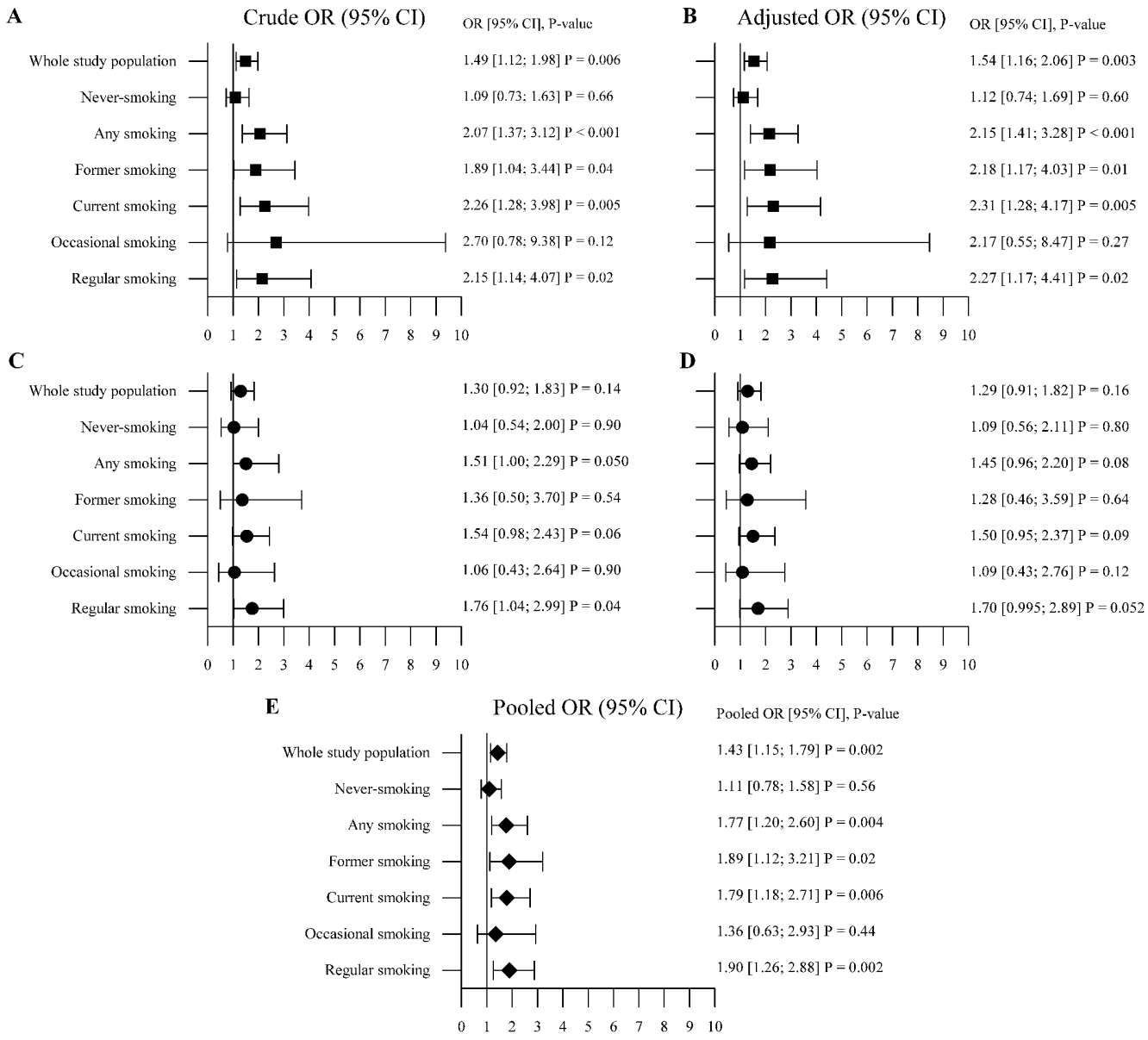

Fig 1. Association of the *IL6* polymorphisms with asthma, under the recessive model, and according to the smoking exposure. The effect of the GG genotype when compared to the effect of AA/GA (FEAS) or CC/GC (CIAS) genotypes in each exposure stratum is shown. **A,** Unadjusted and **B**, adjusted associations of rs1800797 with asthma in FEAS. **C,** Unadjusted and **D**, adjusted associations of rs1800795 with asthma in CIAS. **E**, Random effects meta-analysis of the pre-calculated, adjusted effect

estimates. **B, D and E**, Adjusted for age, gender and education in FEAS and for age and education in CIAS.

CI, Confidence interval; CIAS, Cold, Infections and Asthma in Soldiers study; FEAS, Finnish Environment and Asthma Study; OR, Odds ratio. Square, estimates for FEAS study; Circle, estimates for CIAS study; Rhomb, meta-analysis estimates.

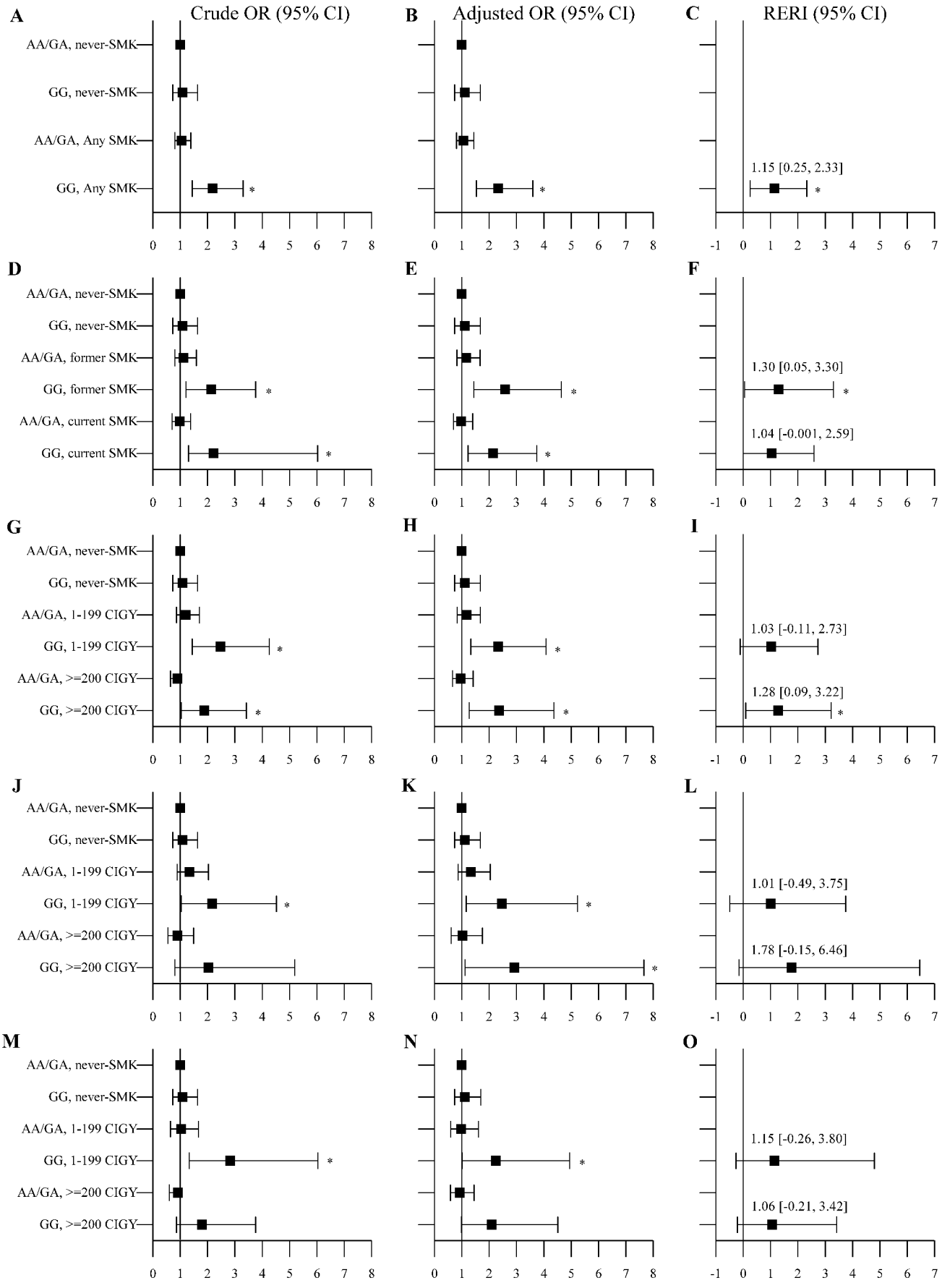
A
Crude OR (95% CI)
AA/GA, never-SMK
GG, never-SMK
AA/GA, Any SMK
GG, Any SMK
B
Adjusted OR (95% CI)
C
RERI (95% CI)
1.15 [0.25, 2.33]
D
AA/GA, never-SMK
GG, never-SMK
AA/GA, former SMK
GG, former SMK
AA/GA, current SMK
GG, current SMK
E
F
1.30 [0.05, 3.30]
1.04 [-0.001, 2.59]
G
AA/GA, never-SMK
GG, never-SMK
AA/GA, 1-199 CIGY
GG, 1-199 CIGY
AA/GA, >=200 CIGY
GG, >=200 CIGY
H
I
1.03 [-0.11, 2.73]
1.28 [0.09, 3.22]
J
AA/GA, never-SMK
GG, never-SMK
AA/GA, 1-199 CIGY
GG, 1-199 CIGY
AA/GA, >=200 CIGY
GG, >=200 CIGY
K
L
1.01 [-0.49, 3.75]
1.78 [-0.15, 6.46]
M
AA/GA, never-SMK
GG, never-SMK
AA/GA, 1-199 CIGY
GG, 1-199 CIGY
AA/GA, >=200 CIGY
GG, >=200 CIGY
N
O
1.15 [-0.26, 3.80]
1.06 [-0.21, 3.42]

Fig 2. Additive level interaction between *Interleukin 6* polymorphism rs1800797 and smoking status or cigarette-years in FEAS, under the recessive model. **A-C**, rs1800797 genotype and smoking categorized as never-smokers and any smoking exposure. **D-F,** rs1800797 genotype and smoking categorized as never-smokers, former smokers and current smokers. **G-I,** rs1800797 genotype and cigarette years in never-smokers and in those with any smoking exposure. **J-L,** rs1800797 genotype and cigarette years in never-smokers and in former smokers. **M-O,** rs1800797 genotype and cigarette years in never-smokers and in current smokers. **B, C**, **E, F, H, I, K, L, N, O,** analysis adjusted for age, gender and education. **A-O**, the category AA/GA and never-smoker is the reference category.

CI; Confidence interval; CIGY, Cigarette-years; OR, Odds ratio; RERI, Relative excess risk due to interaction; SMK, smoker. **P*<0.05.

Online repository Tables

Table E1. Characteristics of the study populations

Characteristic Name	Category	FEAS New Asthma N (%)	FEAS Controls N (%)	CIAS Prevalent Asthma N (%)	CIAS Controls N (%)
Total	-	465	610	224	640
Gender	Male	155 (33.3)	271 (44.4)	224 (100.0)	640 (100.0)
	Female	310 (66.7)	339 (55.6)	NA	NA
Age (years)	21-29	97 (20.9)	78 (12.8)	NA	NA
In FEAS	30-39	93 (20.0)	152 (24.9)	NA	NA
	40-49	115 (24.7)	176 (28.9)	NA	NA
	50-59	127 (27.3)	160 (26.2)	NA	NA
	60-64	33 (7.1)	44 (7.2)	NA	NA
In CIAS	17-19	NA	NA	177 (79.0)	519 (81.1)
	20-30	NA	NA	47 (21.0)	121 (18.9)
Education [1]	No vocational schooling	98 (21.1)	98 (16.0)	NA	NA
In FEAS	Vocational course	75 (16.1)	68 (11.2)	NA	NA
	Vocational institute	134 (28.8)	171 (28.0)	NA	NA
	College-level education	102 (21.9)	185 (30.3)	NA	NA
	University or corresponding	56 (12.0)	88 (14.4)	NA	NA
In CIAS	Comprehensive schooling	NA	NA	20 (8.9)	51 (8.0)
	Vocational course/institute	NA	NA	110 (49.1)	312 (48.8)
	High school	NA	NA	77 (34.4)	236 (36.9)
Pets at home ever	Yes	328 (70.5)	405 (66.7)	NA	NA
Visible mold or mold odor in the workplace or at home	Yes	110 (23.7)	142 (23.3)	NA	NA
Smoking [2]	Never smoker	217 (46.9)	315 (51.7)	74 (33.0)	171 (26.7)
	Former smoker	122 (26.2)	140 (23.0)	27 (12.1)	93 (14.5)
	Former smoker, quit over one year ago	95 (20.5)	130 (21.4)	NA	NA
	Former smoker, quit less than one year ago	27 (5.8)	9 (1.5)	NA	NA
	Current smoker	124 (26.7)	154 (25.3)	118 (52.7)	365 (57.0)
	Occasional smoker	27 (5.8)	32 (5.3)	34 (15.2)	83 (13.0)
	Regular smoker	97 (21.0)	122 (20.0)	84 (37.5)	282 (44.1)
Smoking, cig. years [3]	0.1 - 49	59 (13.1)	60 (10.1)	61 (27.2)	199 (31.1)
	50 - 99	30 (6.7)	27 (4.6)	33 (14.7)	96 (15.0)
	100 -199	41 (9.1)	48 (8.1)	13 (5.8)	50 (7.8)
	≥ 200	103 (22.9)	144 (24.2)	4 (1.8)	3 (0.5)
Maternal smoking during pregnancy	For less than half of pregnancy	3 (0.7)	7 (1.2)	NA	NA
	For at least half of pregnancy	21 (4.5)	18 (3.0)	NA	NA
SHS exposure during the past 12 months	Yes	95 (20.4)	109 (17.9)	NA	NA
Lifetime cumulative SHS exposure (in cig. years) [4]	1 - 99	89 (19.1)	153 (25.1)	NA	NA
	≥ 100	183 (39.4)	231 (37.9)	NA	NA

Definition of abbreviations: CIAS = Cold, Infections and Asthma in Soldiers study; FEAS = Finnish Environment and Asthma Study; NA = Not applicable; SHS = second-hand smoke exposure

[1] Education missing for 17 asthmatic subjects and 41 controls in CIAS.

[2] Smoking missing for 2 asthmatic subjects and 1 control in FEAS, and for 5 asthmatic subjects and 11 controls in CIAS.

[3] Cigarette-years missing for 13 cases and 15 controls in the FEAS, and for 29 cases and 106 controls in CIAS.

[4] Lifetime cumulative SHS exposure missing for 20 asthmatic subjects and 24 controls in FEAS.

Table E2. Details of the smoking stratified association analyses for the directly genotyped SNPs and P-values for the analyses of the imputed SNPs.

	Analysis of directly genotyped SNPs									Analysis of imputed SNPs			
		FEAS rs1800797			CIAS rs1800795			Random effects meta-analysis		FEAS, rs1800795 imputed		CIAS, rs1800797 imputed	
Stratum	**Genotype**	**N cases**	**N controls**	**% weight in meta-analysis**	**N cases**	**N controls**	**% weight in meta-analysis**	**I^2**	**P-value [1]**	**P-value Unadjusted**	**P-value Adjusted [2]**	**P-value Unadjusted**	**P-value Adjusted [2, 3]**
Whole study population [4]	Total	465	610	58.9	224	640	41.1			**0.007**	**0.004**	0.15	0.16
	A_1A_1/GA_1	336	485		162	494		0.0%	0.43				
	GG	129	125		62	146							
Never-smoking	Total	217	315	72.0	74	171	28.0			0.72	0.67	0.94	0.85
	A_1A_1/GA_1	161	239		57	133		0.0%	0.95				
	GG	56	76		17	38							
Any smoking [5]	Total	246	294	49.8	145	458	50.2			**<.001**	**<.001**	**0.050**	0.08
	A_1A_1/GA_1	174	245		100	353		41.1%	0.19				
	GG	72	49		45	105							
Former smoking	Total	122	140	73.6	27	93	26.4			**0.04**	**0.01**	0.56	0.65 [6]
	A_1A_1/GA_1	89	117		20	74		0.0%	0.39				
	GG	33	23		7	19							
Current smoking	Total	124	154	40.6	118	365	59.4			**0.003**	**0.004**	0.06	0.08
	A_1A_1/GA_1	85	128		80	279		22.6%	0.26				
	GG	39	26		38	86							
Occasional smoking	Total	27	32	31.8	34	83	68.2			0.11	0.26	0.89	0.84
	A_1A_1/GA_1	18	27		25	62		0.0%	0.41				
	GG	9	5		9	21							
Regular smoking	Total	97	122	39.3	84	282	60.7			**0.01**	**0.01**	**0.04**	0.051
	A_1A_1/GA_1	67	101		55	217		0.0%	0.50				
	GG	30	21		29	65							

Definition of abbreviations: A_1 = major allele (A allele for rs1800797 in FEAS and C allele for rs1800795 in CIAS); CIAS = Cold, Infections and Asthma in Soldiers study; FEAS = Finnish Environment and Asthma Study; I^2 = variation in estimate attributable due to heterogeneity

[1] P-value for heterogeneity in the meta-analyses.

[2] Adjusted for age, gender and education in FEAS, and for age and education in CIAS.

[3] Education missing for 17 asthmatics and 41 controls in CIAS. In CIAS a separate category for missing education was included in the analyses.

[4] Genotyping of rs1800797 in FEAS is missing for 2 cases and 3 controls and genotyping of rs1800795 in CIAS is missing for 1 case and 2 controls.

[5] Smoking missing for 2 asthmatics and 1 control in FEAS, and for 5 asthmatics and 11 controls in CIAS.

[6] In CIAS adjustment could not be carried out for the three category education and thus, a two category education (comprehensive schooling or vocational course/institution vs. high school) was applied instead.

Table E3. Additive level interaction between *Interleukin 6* (*IL6*) polymorphism rs1800795 and smoking status in CIAS, under the recessive model.

Determinant	N (%) Cases	N (%) Controls	OR (95% CI)	Adjusted OR (95% CI) [1,2]	ERR (95% CI) [1,2]	RERI (95% CI) [1,2]
Total [3]	219	629	NA	NA	NA	NA
rs1800795 genotype and smoking status categorized as never-smokers and any smoking exposure [3]						
CC /GC only (never-smoker)	57 (26.0)	133 (21.1)	ref	ref	ref	NA
GG only (never-smoker)	17 (7.8)	38 (6.0)	1.04 (0.55-2.00)	1.06 (0.55-2.03)	0.06 (-0.45 to 1.03)	NA
(CC /GC and) any smoking	100 (45.7)	356 (56.1)	**0.66 (0.45-0.97)**	**0.65 (0.44-0.95)**	**-0.35 (-0.56 to -0.05)**	NA
GG and any smoking	45 (20.6)	105 (16.7)	1.00 (0.63-1.60)	0.98 (0.61-1.56)	-0.02 (-0.39 to 0.56)	0.27 (-0.76 to 0.91)
rs1800795 genotype and smoking status categorized as never-smokers, ex-smokers, and current smokers [3]						
CC/GC only (never-smoker)	57 (26.0)	133 (21.1)	ref	ref	ref	NA
GG only (never-smoker)	17 (7.8)	38 (6.0)	1.04 (0.55-2.00)	1.06 (0.55-2.03)	0.06 (-0.45 to 1.03)	NA
(CC/GC and) ex-smoker	20 (9.1)	74 (11.8)	0.63 (0.35-1.13)	0.62 (0.35-1.12)	-0.38 (-0.65 to 0.12)	NA
GG and ex-smoker	7 (3.2)	19 (3.0)	0.86 (0.34-2.16)	0.85 (0.34-2.14)	-0.15 (-0.66 to 1.14)	0.17 (-1.03 to 1.48)
(CC/GC and) current smoker	80 (36.5)	279 (44.4)	**0.67 (0.45-0.996)**	**0.66 (0.44-0.98)**	**-0.34 (-0.56 to -0.02)**	NA
GG and current smoker	38 (17.4)	86 (13.7)	1.03 (0.63-1.69)	1.00 (0.61-1.65)	0.00 (-0.38 to 0.65)	0.28 (-0.75 to 0.98)

Definition of abbreviations: CI = Confidence interval; OR = Odds ratio: SHS = Second-hand smoke exposure.

[1] Adjusted for age and education.

[2] Education missing for 17 asthmatics and 41 controls in CIAS, a separate category for missing education was included in the analyses.

[3] Smoking missing for 5 asthmatics and 11 controls in CIAS.

Statistically significant estimates are bolded.

Table E4. Multiplicative level interaction between *Interleukin 6* (*IL6*) polymorphism rs1800797 and smoking status, under the recessive model and organized according to the different types of smoking exposures in FEAS

Determinant	**Main effects model OR (95% CI) [1]**	**P-value [1]**	**Model with interaction parameters OR (95% CI) [1]**	**P-value**	**Main effects model OR (95% CI) [2]**	**p-value**	**Model with interaction parameters OR (95% CI) [2]**	**P-value [2]**
rs1800797 genotype and smoking status categorized as non-smokers and any smoking exposure [3]								
AA/GA genotype	ref	NA	ref	NA	ref	NA	ref	NA
GG genotype	**1.55 (1.16-2.07)**	**0.003**	1.12 (0.74-1.68)	0.59	**1.55 (1.15-2.08)**	**0.004**	1.10 (0.73-1.66)	0.65
non-smoker	ref	NA	ref	NA	ref	NA	ref	NA
any smoking	1.26 (0.97-1.63)	0.08	1.07 (0.80-1.44)	0.64	1.21 (0.93-1.58)	0.15	1.02 (0.76-1.38)	0.89
Genotype x any smoking=0	NA	NA	ref	NA	NA	NA	ref	NA
Genotype x any smoking=1	NA	NA	**1.96 (1.09-3.52)**	**0.03**	NA	NA	**2.02 (1.12-3.65)**	**0.02**
rs1800797 genotype and smoking status categorized as non-smokers, ex-smokers, and current smokers [3]								
AA/GA genotype	ref	NA	ref	NA	ref	NA	ref	NA
GG genotype	**1.55 (1.16-2.08)**	**0.003**	1.12 (0.74-1.68)	0.59	**1.55 (1.16-2.08)**	**0.003**	1.10 (0.73-1.66)	0.66
non-smoker	ref	NA	ref	NA	ref	NA	ref	NA
ex-smoker	**1.37 (1.00-1.88)**	**0.047**	1.17 (0.82-1.67)	0.39	1.35 (0.98-1.86)	0.07	1.14 (0.80-1.66)	0.48
current smoker	1.17 (0.85-1.58)	0.36	0.98 (0.69-1.40)	0.92	1.09 (0.79-1.50)	0.60	0.91 (0.63-1.32)	0.62
Genotype x smoking=0	NA	NA	ref	NA	NA	NA	ref	NA
Genotype x smoking=1 (GG x ex-smoker)	NA	NA	1.98 (0.95-4.13)	0.07	NA	NA	2.04 (0.97-4.29)	0.06
Genotype x smoking=2 (GG x current smoker)	NA	NA	1.95 (0.96-3.99)	0.07	NA	NA	2.03 (0.99-4.16)	0.054

Definition of abbreviations: CI = Confidence interval; OR = Odds ratio: SHS = Second-hand smoke exposure

[1] Adjusted for age, gender, and education.

[2] Adjusted for age, gender, education, pets, molds, recent SHS, cumulative SHS, and maternal smoking during pregnancy.

[3] Smoking missing for 2 asthmatics and 1 control in FEAS

Statistically significant estimates and p-values are bolded.

Table E5. Association of the *IL6* polymorphism rs1800795 (CIAS) with highly sensitive C-reactive protein (hsCRP) levels ≥ 3rd quartile at the beginning of the service (≥3.211 mg/l), and at the end of the service (≥1.412 mg/l) according to the smoking exposure. The results are shown for the participants without asthma.

Genotype	N hsCRP < 3rd quartile	N hsCRP ≥ 3rd quartile	Unadjusted OR (95% CI)	p-value	adjusted OR (95% CI)	p-value
Beginning of the service						
All	474	166				
CC/GC	356	138	ref		ref	
GG	118	28	**0.61 (0.39-0.97)**	**0.035**	**0.58 (0.37-0.93)** [1]	**0.02**
Never-smoking [2]	127	44				
CC/GC	98	35	ref		ref	
GG	29	9	0.87 (0.37-2.02)	0.744	0.91 (0.39-2.14) [1]	0.83
Any smoking [2]	337	121				
CC/GC	250	103	ref		ref	
GG	87	18	**0.50 (0.29-0.88)**	**0.015**	**0.45 (0.26-0.80)** [1]	**0.006**
Former smoking	72	21				
CC/GC	54	20	ref		ref	
GG	18	1	0.15 (0.02-1.20)	0.074	0.12 (0.02-1.03) [1]	0.054
Current smoking	265	100				
CC/C	196	83	ref		ref	
GG	69	17	0.58 (0.32-1.05)	0.072	**0.52 (0.29-0.95)** [1]	**0.03**
End of the service						
All [3]	428	153				
CC/GC	324	129	ref		ref	
GG	104	24	**0.58 (0.36-0.94)**	**0.029**	**0.55 (0.34-0.91)** [4]	**0.02**
Never-smoking [5]	108	46				
CC/GC	82	37	ref		ref	
GG	26	9	0.77 (0.33-1.80)	0.542	0.68 (0.28-1.65) [4]	0.40
Any smoking [5]	310	107				
CC/GC	234	92	**ref**		**ref**	
GG	76	15	**0.50 (0.27-0.92)**	**0.025**	**0.49 (0.27-0.91)** [4]	**0.02**
Former smoking	68	18				
CC/GC	53	16	ref		ref	
GG	15	2	0.44 (0.09-2.14)	0.310	0.72 (0.13-3.83) [4]	0.70
Current smoking	242	89				
CC/C	181	76	**ref**		**ref**	
GG	61	13	**0.51 (0.26-0.98)**	**0.043**	**0.47 (0.24-0.92)** [4]	**0.03**

Definition of abbreviations: CI, Confidence interval; hsCRP, highly sensitive C-reactive protein; OR, Odds ratio

[1]Adjusted for age, education with missing as one category, and for the intake group (July 2004 or January 2005).

[2] For the 640 participants without asthma and with genotyping available, smoking is missing for 10 participants with hsCRP <3rd quartile and for 1 participant with hsCRP≥3rd quartile.

[3] For the 640 participants without asthma and with genotyping available, hsCRP measurement at the end of the service is missing for 59 participants.

[4]Adjusted for age, education with missing as one category and for the intake group (July 2004 or January 2005) and the duration of service.

[5] For the 581 participants without asthma and with genotyping and hsCRP measurement at the end of the service available, smoking is missing for 10 participants with hsCRP <3rd quartile.

Statistically significant estimates and p-values are bolded.

Online Repository: *Interleukin 6* polymorphisms modify the effects of smoking on the risk of adult asthma

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Review of the previous studies assessing *IL6* polymorphisms and asthma

We recently reported an association between the *IL6* SNP rs1800797 and adult-onset asthma [E1]. Based on previous studies, rs1800795 and other *IL6* SNPs (including rs1800797) in close linkage disequilibrium (LD) with it seem to associate with asthma in adults [E2-5] but not in children [E6]. However, one study found no association in adults [E7] while two reported association in children [E8, 9]. Furthermore, among the studies using populations comprised of children and adults, two found an association [E10, 11] while one did not [E12]. Regarding the effect of tobacco smoke, of the previous studies that included adults, most did not take smoking into account [E2-5, 11, 12], while one excluded subjects with a history of smoking [E10] and one excluded those with a history of ≥10 pack years [E7]. There has also been discrepancy about which alleles are the risk associated ones but this may be explained by different LD patterns in populations of different ethnicity, as demonstrated e.g. by Ivanova and others [E13].

Supplementary methods

Study populations
FEAS is a population-based, incident case-control study of adult-onset asthma, described in detail elsewhere [E14]. Shortly, we systematically recruited all new adult (21 to 63 years of age) cases of asthma at 1997-2000 in the Pirkanmaa Hospital District, South Finland. The response rate among cases was 86%, and a total of 521 cases participated. The controls were randomly drawn from the source population, the general eligibility criteria (living in the Pirkanmaa area, age 21-63 years) were applied. A total of 1,016 controls participated (response rate 80%). After excluding those with previous or current asthma (n=76), those older than 63 years (n=6), and those with incomplete questionnaires (n=2), our study population included 932 controls. CIAS is a case-control study conducted among Finnish military conscripts aged from 17.4 to 29.6 years, in the Kainuu Brigade, Kajaani, Northern Finland at 2004-2006, also described in detail elsewhere [E15]. The participants belonged to two intake groups: one entered service in July 2004 and the other in January 2005. The total number of recruits in the intake groups was 1,836 and 1,861, with response rates of 82% and 75%, respectively. All men with previous diagnosis of asthma as well as randomly selected controls without asthma were recruited. 45 men were discharged from the service and the study population due to medical problems other than asthma. Altogether 224 men with asthma and 668 controls participated. The FEAS study protocol was approved by the ethics committees of the Finnish Institute of Occupational Health, the Tampere University Hospital and the Oulu University Hospital District, and the CIAS study protocol by the ethics committees of the Kainuu Central Hospital and the Oulu University Hospital District. Informed consent was obtained from all participants of both studies.

Diagnosis of asthma and lung function measurements
In FEAS, the diagnostic criteria for asthma followed the Finnish National Asthma Guidelines [E16]. The criteria were: 1. occurrence of at least one asthma-like symptom, and 2. demonstration of significant reversibility in airways obstruction in lung function investigations [E14]. Spirometry and bronchodilatation test were recorded with a pneumotachograph spirometer using a disposable flow transducer (Medikro 905, Medikro, Kuopio, Finland) according to the standards of the American Thoracic Society [E17]. In CIAS, the information on asthma was obtained with a self-administered questionnaire. Asthma was defined based on answering "yes" to the questions "Have you ever had asthma?" and "Has your asthma been diagnosed by a physician?" Data on military service-related health and call-up examinations was used to confirm the participant's own opinion. In Finland, the military recruits are first examined by a local practitioner, and then, a re-examination is performed at call-up by an army physician.

Exposure assessment
In both studies, smoking was assessed based on a self-administered questionnaire that the participants filled in at the beginning of the study [E15, 18]. Smoking was defined as follows: regular smoker = at least one cigarette or cigar a day or 25 g of pipe tobacco a month (FEAS) or smoking daily (CIAS); occasional smoker = less than one cigarette a day or 25 g of pipe tobacco a month (FEAS) or smoking less frequently than daily (CIAS); current smoker = regular and occasional smoker; former smoker = had quit smoking; any smoking = current smokers and former smokers. In FEAS, the cumulative life-time cigarette-years were calculated as the average smoking rate (cigarettes/cigars/pipefuls per day) x duration of smoking.

DNA extraction, SNP selection and genotyping

In FEAS, a DNA sample was available for 467 cases and 613 controls. DNA extraction, SNP selection and genotyping have been previously described [E1]. Shortly, DNA was extracted from EDTA blood samples stored at -70°C at BioSer Oulu, Department of Biology, University of Oulu, Finland. Six tagging SNPs, namely rs2069824, rs2069827, rs1800797 (-597 A/G), rs1524107, rs2069840, and rs2069861, in the *IL6* gene were genotyped at FIMM Technology Centre, University of Helsinki, using Illumina's GoldenGate Genotyping Assay, iScan System, and with standard reagents and protocols provided by Illumina Inc., San Diego. The detailed association analysis of these SNPs with adult-onset asthma is described in our previous study, where rs1800797 was found to associate with adult-onset asthma [E1]. In CIAS, a DNA sample was available for 225 cases and 642 controls. DNA was extracted from isolated and homogenized leukocytes [E19]. Tagging SNPs or potentially functional SNPs in genes important for immune system and infections were genotyped at the Mutation Analysis Facility (MAF), Karolinska Institute, Stockholm, Sweden. Genotyping was performed by MALDI-TOF mass spectrometry (matrix-assisted laser desorption/ ionization-time of flight: Sequenom GmbH, San Diego, CA, USA).

Assessment of serum highly sensitive C-reactive protein (hsCRP)

In CIAS, the serum hsCRP level measurements were available from the beginning of the military service and from the end of the military service. HsCRP levels were measured using an Immunoenzymometric Assay (IEMA) test (Medix Biochemica, Kaunianen, Finland) from the blood samples according to the manufacturer's instructions. The range of the assay was 0.3 to 30 mgl/l, and the sensitivity was 0.08 mg/l.

Statistical analyses

Our goal was to further elaborate our previous finding of a significant association between *IL6* SNP rs1800797 and adult-onset asthma in FEAS [E1]. We first analyzed the association of rs1800797 with asthma in FEAS, stratified by smoking status. In order to reproduce our findings, we analyzed association of *IL6* gene with asthma in the CIAS study. In CIAS, the previously genotyped SNPs did not include rs1800797, and because of this the rs1800795 was selected instead for the replication analysis. This SNP is in high LD with rs1800797, for example in the Finnish 1000 Genomes sample the r^2= 0.921 [E20]. Both SNPs are located in the 5' (promoter) area of the *IL6* gene, and both have been reported as functional in literature [E21, 22] and in databases [E23, 24]. Thus, the SNPs are proxies for each other, and both may also contribute to causal mechanisms leading to asthma. Log-additive, genotypic, dominant and recessive genetic models were tested, applying logistic regression analysis. No correction for multiple testing was done since the aim was to found the best fitting model for the association of one SNP in each data and under one interaction hypothesis. Goodness of fit of the different unadjusted models was tested using Akaike's information criterion. Since the CIAS study population was all males, we also studied the potential effect of gender on our results by analyzing the associations in FEAS for men and women separately. Associations were analyzed applying PLINK v1.07 [E25] and goodness of fit was tested using the SAS statistical package (SAS Institute Inc., Cary, NC, USA) version 9.3. To combine the information from FEAS and CIAS, we performed a meta-analysis of the pre-calculated effect estimates from the adjusted recessive models using STATA 13.0 (StataCorp LP, College Station, Texas, USA), applying the "Metan" command and a random effects model.

Imputation of genotypes on FEAS and CIAS data was performed using Impute v2.3.2 software (Genetics Software Suite, The University of Oxford). All of the successfully genotyped SNPs in the *IL6* gene area were used as input information for the imputation, in FEAS this included the SNPs rs2069824, rs2069827, rs1800797, rs1524107, rs2069840, and rs2069861 and in CIAS this included the SNPs rs1800795, rs1474347, rs1554606, and rs2069845. We used the 1000 Genomes Phase 3 data available at the IMPUTE2 website (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html# reference) as a reference panel. Imputation was performed on the *IL6* gene and the nearby area, i.e. Chromosome 7, between base pair locations 22,763,000 and 22,790,000 (NCBI build 37), with default setting of the effective population size (-Ne) of 20000. We used SNPTEST v2.5.2 software (Genetics Software Suite, The University of Oxford) for association analysis of imputed data, applying the frequentist test with multiple Newton-Raphson iterations (method –ml). Association tests were performed for the SNPs rs1800797 and rs1800795 under a recessive genetic model.

Gene-environment interaction on additive level was studied by estimating the independent and joint effects of the SNPs (recessive model) and smoking on the risk of asthma, and by comparing the risk of asthma in the different determinant categories to the reference category constituting of "reference genotype and never-smoker". Estimates of the independent and joint effects were derived from the same logistic regression model. The models were first adjusted for age, gender, and education as a proxy for socioeconomic status (in FEAS), or for age and education (in CIAS). Age, gender and socioeconomic status are known to associate with the risk of asthma and with smoking habits. In addition, especially age and gender may affect manifestation of the genetic factors. In FEAS, we also performed additional adjustment for having indoor pets, presence of dampness and/or molds at home or at work, recent (past 12 months) and lifetime cumulative SHS exposure, and maternal smoking during pregnancy as potential confounders. We present the results as the Excess Relative Risks (ERR) for the independent and joint effects for the categories of genotype (A) and smoking (B). The departure from additivity of relative risks is quantified by the Relative Excess Risk due to Interaction (RERI) = ERR(AB) – ERR(A) – ERR(B). We estimated the 95% confidence intervals (CI) for RERI applying the method of variance estimates recovery [E26]. For RERI, the null value corresponds to statistical significance level of p=0.05. In FEAS, we also tested for interaction on multiplicative level. Interaction variables were calculated based on

the original variables as Interaction = Genotype x Smoking status. Logistic regression analyses with and without the interaction variable were performed. For interaction analyses we used SAS statistical package (SAS Institute Inc., Cary, NC, USA) version 9.3.

In CIAS, we have previously demonstrated an association between rs1800795 C allele and CC genotype and increased levels of serum hsCRP [E27]. Since IL-6 induces CRP production, the *IL6* SNPs affecting IL-6 levels may also affect CRP levels. Of the CIAS participants with a genotyping result, the hsCRP measurement was available for 864 participants from beginning of the service –time point and for 773 participants from end of the service –time point. Here we reanalyzed the association of rs1800795 with serum hsCRP levels in CIAS under a recessive model and stratified by smoking exposure for the cases and controls separately. Due to several samples having a measurement close to the lower detection limit of the assay, we categorized hsCRP levels to normal and elevated levels, according to the 3rd quartile of the measurements in each time point. All of the adjusted analyses took into account age, education, and the intake group (July 2004 or January 2005). In addition, we adjusted the analyses of the end of the service –time point hsCRP for the duration of service (6 months, 9 months or 12 months). The intake group and duration of the service were taken into account because of the possible seasonal variation in hsCRP levels. Association analyses were performed applying PLINK v1.07 [E25].

Supplementary results.

Characteristics of our study populations are shown in the Table E1. In FEAS, there were more women among the asthmatics than the controls, and the asthmatics were younger and less educated. Both of the SNPs, rs1800797 in FEAS and rs1800795 in CIAS, followed Hardy-Weinberg equilibrium with $p > 0.05$. The allele frequencies of the SNPs were similar, among the controls, the frequency of the rs1800797 G allele was 0.467 in FEAS, and the frequency of the rs1800795 G allele was 0.472 in CIAS (two-sided Pearson X^2-test for the allele frequency distributions $p = 0.82$).

Of the tested genetic models, the recessive model was found as best fitting in most of the analyses. In FEAS, an association was detectable under all but the dominant model, whereas in CIAS, an association was detected only under the recessive model.

Imputation certainty of the SNP rs1800795 in FEAS and that of the SNP rs1800797 in CIAS was good, with info metrics of 0.973 and 0.938, respectively. Info typically has values between 0 and 1, with values near 1 indicating high imputation certainty. Also the concordance and correlation between the input genotypes and imputed genotypes for the SNP rs1800797 in FEAS and rs1800795 in CIAS was good, with concordance = 0.998 and $r^2 = 0.978$ for rs1800797 and concordance = 0.993 and $r^2 = 0.991$ for rs1800795.The associations observed for the imputed SNPs (Table E2) were highly similar to the findings based on analyzing two separate but in high pairwise LD SNPs (Fig 1 and Table E2).

In CIAS, among the controls, the rs1800795 genotype GG associated with a decreased risk of having hsCRP $\geq$ 3rd quartile, in the whole group, and in the any smoking and current smoking categories, both at the beginning and at the end of service (Table E5). No association was seen among the cases, data not shown.

Supplementary discussion

Validity issues

The participants of CIAS represent ordinary healthy young Finnish men. The conscripts of the Kainuu brigade mainly come from the central area of Finland. In Finland, the military service is mandatory, and all men aged 18-19 years are called up. Chronic illnesses are generally a reason for exemption, but young men with asthma in good control can be enrolled. Definition of physician-diagnosed asthma was based on the responses to the questionnaire, but the diagnosis was confirmed with data from previous medical appointments and from the first examination at service. No pulmonary function testing, except for PEF measurements, was done since all the asthmatic participants had controlled asthma. The CIAS study did not include men with severe or uncontrolled asthma, as they were exempted during the clinical examinations or were discharged during the first two weeks of service. Limitations of the CIAS study population regarding a gene-smoking interaction study include the prevalent setting of cases, lack of more severe asthma cases, as well as the young age, and thus likely a relatively short duration of the smoking exposure, of the participants.

Exposure assessment was based on questionnaires. In FEAS, the study was introduced to the participants as an investigation of environmental factors in general, and in CIAS, the primary study focused on experiences of cold and infections during the service, with no special focus on smoking mentioned in either study. These approaches reduced information bias in participants' reports. Any random misclassification of exposure would lead to underestimation of the effects. Lack of a true replication (i.e. direct genotyping) of the two SNPs in the two study populations is a limitation in our study and could be reflected in the slightly different association and interaction patterns observed in the studies.

The response rates in both of our studies were high, which reduces the likelihood of selection bias. The sample sizes were based on the primary objectives of the studies, which provided a relatively good power for detecting effects of common polymorphisms and gene-environment interactions between common variants and exposures, as is shown by the relatively narrow 95% confidence intervals of e.g. any smoking category (Fig 1 and 2). However some of the smoking sub-categories were small and in those our study lacks power.

Differences in associations and gene-environment interactions between FEAS and CIAS

The consistently stronger associations observed in FEAS than in CIAS could be due to a gender effect since the CIAS study included only men. However, analysis of the genders separately in FEAS did not support this theory. There may also be a true difference between the SNPs rs1800797 and rs1800795, since the LD between these two may not be complete. For example, in the 1000 Genomes project's populations the pairwise r^2 of these SNPs among the Finns was 0.921 [E20]. In addition, both SNPs have been reported as functional in the literature [E21, 22, 28, 29] and in databases [E23, 24] and could thus have their own effect on IL-6 production. Yet, the results from association analyses of the imputed genotypes do not support this option as they were highly similar to the associations observed for the directly genotyped SNPs. The difference between the two studies may also be due to the different type of cases; in FEAS the cases are incident, included only adult-onset asthma, and represent a population-based sample of a certain geographic area. Whereas in CIAS the cases are prevalent, and the age of asthma onset is not known. If the association is stronger in adulthood, as suggested by the recent meta-analysis [E4], FEAS would be expected to detect it better than CIAS. Also the gene-environment interaction between *IL6* SNPs and smoking would be expected to be better captured by FEAS, where the exposure is known to precede the outcome. In CIAS some of the participants are likely to have developed asthma in childhood or in teenage and then started smoking at a later age. On the other hand, people who have asthmatic symptoms or asthma in childhood would be expected to avoid smoking later in life. Such selection is also the most plausible explanation for the protective main effect of smoking on the risk of asthma that was observed in CIAS , i.e. only those subjects who have not developed symptoms (as the first sign of asthma) as a consequence of smoking, have continued to smoke. This phenomenon of selection away from smoking is to some degree seen also in FEAS, where the gene-environment interaction was stronger with former smoking than with current smoking. We have previously reported a similar phenomenon from FEAS concerning the independent effects of smoking on the risk of developing asthma: the effect estimate was somewhat higher for former smokers (OR 1.49) than for current smokers (1.33) [E18]. Those individuals, who are the most susceptible and thus develop asthma symptoms related to a smaller exposure, are more likely to quit smoking, reduce smoking, or to smoke less. The latter two behavioral reactions probably explain the observation that, in FEAS, among the current smokers, the interaction was slightly stronger with the lower exposure level of cigarette-years smoked. Finally, the study population in CIAS was on average much younger than that in FEAS, thus suggesting a clearly shorter average duration of smoking exposure, and as a consequence a smaller effect related to it in CIAS compared to FEAS. This could be reflected especially in the differing findings regarding the effect of former smoking in the two studies, where the effect observed was clearly stronger in FEAS.

Association of rs1800797 with serum hsCRP levels

Our finding that the rs1800795 genotype GG associates with a decreased risk of elevated hsCRP levels in CIAS control subjects, among any smokers and current smokers, provides evidence of a functional effect the polymorphism.

Previous studies assessing interaction between IL6 polymorphisms and tobacco-smoke

Supporting our findings of interaction between *IL6* rs1800797 and rs1800795 and tobacco smoking on asthma, are several previous studies that reported interaction on health conditions other than asthma. Interaction between *IL6* -174 G/C (i.e. rs1800795) SNP and smoking has been reported to increase the risk of for example myocardial infarction (MI) [E30], coronary artery disease [E31, 32], and bladder cancer [E33]. In addition, interaction between rs1800795 and smoking on the levels of inflammatory markers [E34] has been reported. On the other hand, other studies have not been able to show interactions between rs1800795 and smoking in relation to coronary heart disease [E35], MI [E36], or inflammatory markers [E37]. In relation to the rs1800797, an increased risk of lung cancer among the never smoking A allele carriers was recently reported [E28]. *IL6* polymorphisms have also been associated with lung function decline and COPD. One study found an association between *IL6* -572G/C (rs1800796) polymorphism and COPD[E38]. In the same study, the -174 (rs1800795) and -597 (rs1800797) SNPs were not associated with COPD but the haplotype containing a G allele of both of these SNPs was found as protective of it [E38]. Another study reported association of rs1800795 and rs1800797 C and A allele, respectively, with a rapid decline of FEV1 and an increased risk of COPD [E39]. Whereas a recent meta-analysis found that rs1800796 associates with COPD but rs1800797 does not [E40].

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