

ORIGINAL ARTICLE

Interleukin 6 SNP rs1800797 associates with the risk of adult-onset asthma

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Interleukin 6 (IL6) is an inflammatory cytokine that has been suggested to have an important role in the pathogenesis of asthma. *IL6* single-nucleotide polymorphisms (SNPs) have been associated with levels of IL6, and with childhood and prevalent adult asthma. A recent study also suggested that *IL6* SNPs associate especially with atopic asthma. However, association of *IL6* SNPs with adult-onset asthma has not been studied. In a population-based study of 467 incident adult-onset asthma cases and 613 disease-free controls from South Finland, we analyzed association of 6 tagging SNPs of the *IL6* locus with the risk of adult-onset asthma and with atopy. Asthma was clinically diagnosed, and atopy was defined based on Phadiatop test. *IL6* SNP rs1800797 associated with the risk of adult-onset asthma in a log additive model, with adjusted odds ratio (aOR) 1.31 (95% confidence interval 1.09–1.57), and especially with the risk of atopic adult-onset asthma when compared with non-atopic controls, aOR 1.46 (95% CI 1.12–1.90). This is the first study to show an association of *IL6* with adult-onset asthma, and especially with atopic adult-onset asthma.

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INTRODUCTION

Asthma is a disease characterized by chronic inflammation in the airways and variable expiratory airflow limitation.¹ Approximately 7.7% of the working-age population in the United States² and 7–9% of adults in Finland^{3,4} are affected by asthma. Interleukin 6 (IL6) is an inflammatory cytokine secreted by cells of innate immunity (for example, macrophages, dendritic cells, mast cells and neutrophils), endothelial cells, epithelial cells and fibroblasts, as a response to stimuli such as exposure to microbes and other cytokines.^{5,6} It activates cells either via a membrane bound IL6 receptor and gp130 signal transducer glycoprotein or via a soluble IL6 receptor and gp130.⁵ IL6 may be involved in asthma pathogenesis in many ways, such as through modulating Th1 and Th2 balance, augmenting mucus production, promoting Th17 differentiation and inhibiting regulatory T cells.^{5,6} Higher levels or increased production of IL6 have been reported among asthmatics^{7–11} and among patients with a status asthmaticus¹² when compared with controls, and increased IL6 has also been associated with exacerbation of asthma,¹¹ increased asthma severity^{10,13} and poor lung function.^{10,14–16} There is evidence on the effect of the *IL6* single-nucleotide polymorphisms (SNPs) on the circulating levels of IL6^(ref. 17) and on their associations with asthma.^{18–22} Recent studies suggested that variation in the *IL6* gene, located in 7p21, may be associated especially with atopic subtype of asthma,²³ and with asthma among adults.²⁴ However, there are no previous studies on the role of variation in *IL6* gene for the adult-onset asthma, even though one of the asthma genome-wide association studies (GWAS) did include later onset (≥ 16 years of age) asthma as an outcome.²⁵ Thus, we hypothesized that *IL6* SNPs associate with adult-onset asthma, and we studied also their role for atopy and for the development of atopic adult-onset asthma.

RESULTS

Characteristics of the study population

Characteristics of the study population according to the adult-onset asthma and atopic status are shown in Table 1. There were more women among the asthmatics than the controls, and the asthmatics were younger and less educated than the controls. Atopics were younger and had higher level of education than those without atopy, and current smoking and exposure to molds were more common among the atopics than the non-atopics.

IL6 rs1800797 associates with adult-onset asthma

Six SNPs in the *IL6* gene were genotyped and all followed the Hardy–Weinberg equilibrium with $P > 0.05$. In our data, there was considerable linkage disequilibrium (LD) between the studied SNPs as demonstrated by high D' -values (Supplementary Figure S1A); however, the low r^2 -values imply that the SNPs contain unique information (Supplementary Figure S1B). Rs1800797 associated with the adult-onset asthma under the log additive genetic model, both in an unadjusted model and in a model adjusted for age, sex, education, pets, mold exposure, smoking, recent and cumulative second-hand smoke exposures and maternal smoking during pregnancy (Table 2). This association remained statistically significant also after correction for multiple testing (Table 2). Haplotype analysis of the SNPs suggests that majority of the effect in this area is due to the rs1800797 as the haplotypes H4 (TGACCC) and H6 (TTACCC) that both include the rs1800797 A allele (underlined) had the lowest ORs and the haplotypes H1 (TGGCGC) and H2 (TGGCCC) that both include the G allele (underlined) had the highest ORs for adult-onset asthma (Supplementary Table S1). Having a T allele in rs1524107 or in rs2069861 or a C allele in rs2069824 may even out the effect of rs1800797, as the haplotypes H3, H5 and H7, respectively, did not

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Table 1. Characteristics of the study population

Characteristic Name	Category	Case-control study of adult-onset asthma		Secondary study of atopy	
		Asthmatic N (%)	Control N (%)	Atopic N (%)	Non-atopic N (%)
Total	—	465	610	352	717
Atopy ^a	—	207 (44.5)	145 (23.8)	NA	NA
Asthma	—	NA	NA	207 (58.8)	253 (35.3)
Sex	Male	155 (33.3)	271 (44.4)	146 (41.5)	276 (38.5)
	Female	310 (66.7)	339 (55.6)	206 (58.5)	441 (61.5)
Age (years)	21–29	97 (20.9)	78 (12.8)	102 (29.0)	71 (9.9)
	30–39	93 (20.0)	152 (24.9)	103 (29.3)	141 (19.7)
	40–49	115 (24.7)	176 (28.9)	76 (21.6)	215 (30.0)
	50–59	127 (27.3)	160 (26.2)	55 (15.6)	229 (31.9)
	60–64	33 (7.1)	44 (7.2)	16 (4.6)	61 (8.5)
Education ^b	No vocational schooling	96 (20.7)	96 (15.7)	52 (13.9)	141 (19.7)
	Vocational course	75 (16.1)	68 (11.2)	35 (9.3)	109 (15.2)
	Vocational institute	134 (28.8)	171 (28.0)	122 (32.5)	188 (26.2)
	College-level education	102 (21.9)	185 (30.3)	103 (27.5)	191 (26.6)
	University or corresponding	56 (12.0)	88 (14.4)	63 (16.8)	84 (11.7)
Pets at home ever	—	328 (70.5)	405 (66.7)	253 (71.9)	476 (66.4)
Visible mold or mold odor in the workplace or at home	—	110 (23.7)	142 (23.3)	96 (27.3)	155 (21.6)
Maternal smoking during pregnancy	For less than half of pregnancy	3 (0.7)	7 (1.2)	2 (3.1)	8 (1.1)
	For at least half of pregnancy	21 (4.5)	18 (3.0)	17 (4.8)	22 (3.1)
Smoking ^c	Ex-smoker	122 (26.3)	139 (22.8)	75 (21.3)	182 (25.4)
	Current smoker	124 (26.7)	154 (25.3)	108 (30.7)	169 (23.6)
SHS exposure during past 12 months	—	95 (20.4)	109 (17.9)	69 (19.6)	134 (18.5)
Lifetime cumulative SHS exposure ^d	1–99 cigarette years	89 (19.1)	153 (25.1)	74 (21.0)	167 (23.3)
	≥ 100 cigarette years	183 (39.4)	231 (37.9)	135 (38.4)	277 (38.6)

Abbreviations: NA, not applicable; SHS, second-hand smoke. ^aAtopy missing for five asthmatics and one control. ^bEducation missing for two asthmatics and two controls, and for four non-atopic individuals. ^cSmoking missing for two asthmatics and one control, and for three non-atopic individuals. ^dLifetime cumulative SHS exposure missing for 20 asthmatics and 24 controls, and for 16 atopic and 28 non-atopic individuals.

Table 2. Association of the *IL6* SNPs with adult-onset asthma (465 asthmatics, 610 controls)

SNP ID	Location	Minor allele/ major allele	MAF All	MAF Asthmatic	MAF Control	Crude OR (95% CI) ^a	P-value	FDR BH P-value	Adjusted OR (95% CI) ^{a,b,c}	P-value	FDR BH P-value
rs2069824 ^d	5' Near-gene	C/T	0.096	0.098	0.094	1.05 (0.78–1.41)	0.762	0.915	0.96 (0.71–1.31)	0.813	0.813
rs2069827	5' Near-gene	T/G	0.203	0.187	0.215	0.84 (0.67–1.04)	0.107	0.214	0.81 (0.65–1.02)	0.071	0.141
rs1800797	5' Near-gene	G/A	0.494	0.530	0.467	1.30 (1.09–1.55)	0.003	0.020	1.31 (1.09–1.57)	0.004	0.025
rs1524107 ^e	Intronic	T/C	0.038	0.038	0.038	1.00 (0.64–1.55)	0.988	0.988	1.06 (0.67–1.68)	0.812	0.813
rs2069840	Intronic	G/C	0.271	0.290	0.257	1.19 (0.98–1.45)	0.078	0.214	1.22 (1.00–1.49)	0.054	0.141
rs2069861	3' Near-gene	T/C	0.087	0.090	0.085	1.07 (0.79–1.44)	0.678	0.915	1.08 (0.79–1.48)	0.617	0.813

Abbreviations: CI, confidence interval; FDR BH, Benjamini and Hochberg false discovery rate correction for multiple testing; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism. Statistically significant ($P < 0.05$) P-values and estimates are bolded. ^aLog additive model. ^bAdjusted for age, sex, education, pets, visible mold or mold odor at home or at work, personal smoking, recent second-hand smoke (SHS) exposure, cumulative SHS exposure and maternal smoking during pregnancy. ^cInformation missing for two asthmatics and one control. ^dGenotype missing for one asthmatic. ^eGenotype missing for one control.

show different distributions between the asthmatics and the controls.

IL6 rs1800797 associates with atopic adult-onset asthma

We then studied whether atopy, defined as having a positive Phadiatop score in a blood test, has a role in the associations of *IL6* SNPs with adult-onset asthma, by performing a stratified association analysis and testing for heterogeneity related to atopy. No heterogeneity in the associations with adult-onset asthma was detected between the atopic and non-atopic groups (Supplementary Table S2). Rs1800797 G allele associated with adult-onset asthma also in the model controlling for atopy, but the association was weaker in this way and did not withstand multiple testing (Supplementary Table S2). When the atopic and non-atopic groups were analyzed separately, the association of this SNP with adult-onset asthma was stronger among the non-atopic group.

In addition, correction for multiple testing weakened its statistical significance (Supplementary Table S2). However, when atopic asthmatics were compared with non-atopic controls the rs1800797 G allele again showed a strong association, which remained statistically significant even after correction for multiple testing (Table 3). Closer examination of the atopic and asthmatic groups showed that the G allele frequency of rs1800797 was highest among the atopic asthmatics, next highest among the non-atopic asthmatics, then among the atopic controls and lowest among the non-atopic controls (Supplementary Table S2).

IL6 SNPs do not associate with atopy

We also analyzed whether the *IL6* SNPs associate with atopy. All of the studied SNPs followed Hardy–Weinberg equilibrium also with regard to atopy as the outcome of interest. In an analysis stratified by asthma, the rs2069824 C allele first associated with atopy

Table 3. Association of the *IL6* SNPs with the atopic adult-onset asthma (207 atopic asthmatics and 464 non-atopic controls)

SNP ID	MAF Atopic asthma	MAF Non-atopic control	Crude OR (95% CI) ^a	P-value	FDR BH P-value	Adjusted OR (95% CI) ^{a,b,c}	P-value	FDR BH P-value
rs2069824 ^d	0.112	0.085	1.35 (0.92–1.98)	0.126	0.188	1.37 (0.90–2.07)	0.146	0.292
rs2069827	0.179	0.218	0.77 (0.57–1.05)	0.097	0.188	0.74 (0.53–1.02)	0.066	0.198
rs1800797	0.541	0.457	1.43 (1.13–1.83)	0.004	0.021	1.46 (1.12–1.90)	0.005	0.030
rs1524107 ^e	0.024	0.039	0.62 (0.30–1.25)	0.178	0.213	0.85 (0.41–1.78)	0.668	0.668
rs2069840	0.297	0.251	1.27 (0.98–1.66)	0.073	0.188	1.20 (0.90–1.60)	0.214	0.321
rs2069861	0.097	0.086	1.13 (0.76–1.68)	0.540	0.540	1.25 (0.82–1.92)	0.306	0.367

Abbreviations: CI, confidence interval; FDR BH, Benjamini and Hochberg false discovery rate correction for multiple testing; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism. Statistically significant ($P < 0.05$) P -values and estimates are bolded. ^aLog additive model. ^bAdjusted for age, sex, education, pets, visible mold or mold odor at home or at work, personal smoking, recent second-hand smoke (SHS) exposure, cumulative SHS exposure and maternal smoking during pregnancy. ^cInformation missing for one non-atopic control. ^dGenotype missing for one atopic asthmatic. ^eGenotype missing for one non-atopic control.

Table 4. Association of the *IL6* SNPs with atopy (among 352 atopic and 717 non-atopic individuals)

SNP ID	MAF Atopic	MAF Non-atopic	Crude OR (95% CI) ^a	P-value	FDR BH P-value	Adjusted OR (95% CI) ^{a,b}	P-value	FDR BH P-value	Adjusted OR (95% CI) ^{a,c,d}	P-value	FDR BH P-value
rs2069824 ^e	0.117	0.086	1.42 (1.05–1.92)	0.023	0.139	1.43 (1.05–1.95)	0.025	0.148	1.50 (1.07–2.09)	0.019	0.112
rs2069827	0.190	0.210	0.88 (0.70–1.11)	0.281	0.337	0.91 (0.72–1.16)	0.454	0.545	0.92 (0.71–1.19)	0.523	0.656
rs1800797	0.524	0.479	1.21 (1.00–1.45)	0.047	0.140	1.14 (0.95–1.38)	0.167	0.334	1.12 (0.92–1.37)	0.262	0.524
rs1524107 ^f	0.028	0.043	0.67 (0.40–1.11)	0.116	0.232	0.65 (0.39–1.10)	0.107	0.320	0.72 (0.42–1.26)	0.251	0.524
rs2069840	0.287	0.262	1.14 (0.93–1.40)	0.207	0.311	1.10 (0.89–1.36)	0.363	0.544	1.01 (0.81–1.27)	0.908	0.908
rs2069861	0.091	0.086	1.06 (0.77–1.45)	0.732	0.732	1.04 (0.75–1.45)	0.809	0.809	1.11 (0.79–1.56)	0.547	0.656

Abbreviations: CI, confidence interval; FDR BH, Benjamini and Hochberg false discovery rate correction for multiple testing; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism. Statistically significant ($P < 0.05$) P -values and estimates are bolded. ^aLog additive model. ^bAdjusted for personal asthma. ^cAdjusted for personal asthma, age, sex, education, pets, visible mold or mold odor at home or at work, personal smoking, recent second-hand smoke (SHS) exposure, cumulative SHS exposure and maternal smoking during pregnancy. ^dInformation missing for two atopic and one non-atopic individuals. ^eGenotype missing for one atopic individual. ^fGenotype missing for one non-atopic individual.

among the controls, as well as in the analysis controlling for asthma, and the rs1524107 T associated with atopy among the asthmatics in the adjusted model, but for both associations the statistical significance disappeared when correction was made for multiple testing (Supplementary Table S3). No heterogeneity regarding atopy as an outcome was observed between the asthmatics and the controls (Supplementary Table S3); and thus, we conducted an analysis of the whole study population that was adjusted for asthmatic status. In the unadjusted analysis, the *IL6* SNP rs1800797 showed borderline significant association with atopy, but this association was not statistically significant when adjusting for asthma (Table 4). Similarly to the stratified analysis, rs2069824 C allele first showed an association with atopy, even after adjusting for asthma, but this became non-significant when correcting for multiple testing (Table 4).

DISCUSSION

We show in a large population-based study of incident asthma cases and randomly selected controls that *IL6* SNP rs1800797 G allele is associated with adult-onset asthma, and especially with atopic adult-onset asthma. The associating allele is common in our Finnish study population, with an estimated minor allele frequency (MAF) of 0.494 for the source population that produced the asthma cases. The association in our study was consistent after adjusting for confounding, and statistically significant even after correction for multiple testing. It is also supported by the haplotype analysis of the studied SNPs.

Validity issues

The strengths of this study include recruitment of well-defined (Supplementary Table S5) new cases of adult-onset asthma and

controls that were randomly selected from the same, uniform, source population, as well as careful exposure assessment. The response rates in our study were high, 86% for the new cases of adult-onset asthma and 80% for the control subjects who had been randomly selected from the population that produced the cases, that is, the working-age population (21–63 years of age) living in the Pirkanmaa Hospital District in South Finland. This incident case–control study of asthma enables us to estimate the incidence rate ratios (rather than risk ratios) of adult-onset asthma by ORs; and thus, the rare disease assumption is not needed. Currently, there are no many data sets available for adult-onset asthma and our study is among the largest. The present study with the incident case–control design corresponds to a follow-up of approximately 100 000 adults for 5 years if we assume a realistic asthma incidence of one case per 1000 person-years. The good response rates among both cases and controls minimize the likelihood of selection bias; and since the study subjects originate from the same geographic area of Finland,²⁶ it reduces the likelihood of the effect of population stratification on our results. In addition, environmental factors are likely to be more homogenous in such a population. The estimation of the genetic determinants of atopy used the data from the case–control study, but applied an approach using case–control data for an additional outcome.²⁷ We first focused separately on adults with and without incident asthma and then combined the cases and controls adjusting the effect estimates for 'caseness', that is, asthma status. Restricting the study to adult-onset asthma and further considering the role of atopy reduces heterogeneity of the phenotype studied.

The sample size was based on the primary objectives of the case–control study,³ which provided a relatively good power

for detecting effects of common polymorphisms as shown by the 95% CIs in Table 2.

Synthesis with previous knowledge

Our finding of the association of *IL6* polymorphism rs1800797 with the adult-onset asthma is novel, as we were not able to find in our systematic literature search any previous study concerning *IL6* SNPs and adult-onset asthma. However, there are such studies focusing on children,^{18,28} prevalent adult asthma^{20–22,29} or asthma in populations including both children and adults.^{19,23,30} In addition, one GWAS included later onset (≥ 16 years of age) asthma as an outcome.²⁵

In summary, of the SNPs genotyped in our study, rs1800797 had been genotyped in four of the previous studies,^{19,21,22,30} rs2069827 in one²³ and rs2069840 in two^{23,28} (Supplementary Table S4). In one of the studies regarding rs1800797 this SNP was monomorphic²² and in two studies no association with asthma was found.^{19,30} Supportive to our results, one study reported a borderline statistically significant protective effect of the A allele on asthma.²¹ Most of the studies had genotyped rs1800795,^{18–22,29,30} which in our study should be tagged by rs1800797 in such a way that the G alleles of these SNPs usually occur together ($r^2 = 0.966$, Supplementary Figure S1C). These studies were recently meta-analyzed and reviewed,²⁴ except for the study by Padrón-Morales *et al.*,³⁰ with the conclusion that the rs1800795 CC genotype when compared with the GG may be a protective factor for asthma in Caucasian populations and in adults (Supplementary Table S4). Thus, the results of this meta-analysis²⁴ support our results. Padrón-Morales *et al.*³⁰ found no association with asthma as such but reported gene–gene interactions between *IL6* and other cytokine genes (Supplementary Table S4). One recent study had specifically looked at atopic asthma as an outcome in an Indian population, in a case–control study as well as in a family study.²³ They reported a protective effect of *IL6* rs2069832 A allele on atopic asthma in both study populations when compared with non-asthmatics that were free of atopy.²³ These results are highly consistent with our finding of atopic asthma, and since rs1800797 in our study should tag for rs2069832 with the G alleles occurring together ($r^2 = 0.933$, Supplementary Figure S1C), the study of Kumar *et al.*²³ strongly supports our findings. Furthermore, similarly to our study, they found the rs2069827 A allele to show a tendency for a protective effect on atopic asthma, while no association was found with rs2069840. One study that had genotyped three *IL6* SNPs, namely rs13447445, rs2069840 and rs2069845, found no association with asthma.²⁸ With regard to rs2069840, their result is consistent with our result, while the results of rs2069845 are contrasting as this SNP should be tagged by rs1800797 in our study ($r^2 = 0.884$, Supplementary Figure S1D), though with a weaker LD than rs1800795 and rs2069832.

All of the studies reporting findings regarding to *IL6* are candidate gene studies, none of the GWAS of asthma has reported findings in the *IL6* locus. Searching the GRASP v2.0.0.0 database³¹ with *IL6*, asthma and the lowest *P*-value threshold results in two SNPs, rs17147267 ($P = 0.021$) and rs6949149 ($P = 0.046$). The latter SNP is in LD with rs1524107 ($r^2 = 0.82$) that shows no association in our study. In the GWAS Central (www.gwascentral.org)³² a search of *IL6* with the lowest *P*-value threshold returns five SNPs, rs2069837 ($P = 0.50$), rs2066922 ($P = 0.25$), rs1554606 ($P = 0.26$) that are in LD with rs1800797 ($r^2 = 0.89$), rs10242595 ($P = 0.50$) and rs11766273 ($P = 0.63$). All these are results of the whole study population from the study by Moffat *et al.*²⁵ This is a large GWAS asthma study that also analyzed associations with several subtypes of asthma, including later onset asthma (a disease that developed at 16 years of age or older, including 1947 cases and 3669 controls). No GWAS significant association between the *IL6* SNPs and later onset asthma was reported in that study, and to

our knowledge, the statistically not GWAS significant results of this subtype analysis from Moffat *et al.* are not publicly available. The differences in findings between the study of Moffat *et al.*²⁵ and our study may partially be explained by the different cutoff ages for later onset asthma (≥ 16 years of age) and adult-onset asthma (≥ 21 years of age).

No one obvious reason can be identified for the differences between the results of the *IL6* studies. The effect of *IL6* may be more pronounced in asthma that begins in adulthood, as our study and most of the studies supporting our result included adults. Childhood-onset asthma and adult-onset asthma seem to be different phenotypes.³³ For example, early-onset (before the age of 12 years) and late-onset (after the age of 12) severe asthma differ with regard to the presence of allergies, eosinophilia and other inflammatory processes.³⁴ In addition, *IL6* is a multi-functional cytokine and it is produced as a response to several different stimuli.³⁵ Thus, it may interact with many environmental exposures that are difficult to control adequately in the studies, which may explain why asthma GWAS with large combined populations have found no associations for *IL6*. Our study design was population based and our study population originated from a geographically-defined area in South Finland. Thus, our study population was likely to be relatively homogenous with regard to many environmental factors. In addition, our results suggest that atopy may modify the association between *IL6* SNPs and asthma, which is plausible as *IL6* is involved in inflammatory pathways that are important for the development of both atopy and asthma.⁵ Rs1800797 was found to associate with the adult-onset asthma in the whole study population, as well as with atopic adult-onset asthma when compared with controls without atopy, that is, in a comparison over the two phenotypes asthma and atopy. No statistically significant heterogeneity was observed for the association with asthma between the atopic and non-atopic groups, but the association was clearly stronger among the non-atopic group. Furthermore, rs1800797 showed no association with atopy, but the G allele frequency increased steadily across the groups from non-atopic controls to atopic asthmatics. Together, these results suggest that the rs1800797 G allele is associated with adult-onset asthma, but for some reason the G allele is also enriched among adults with atopy. Either the rs1800797 G allele associates also with atopy but our study has no power to detect it, or it may be in LD with a variant that associates with atopy. One study that applied Phadiatop test as a marker of allergen sensitization found an inverse association between *IL6* rs1800795 GC genotype and atopy when compared with the reference genotype GG, thus supporting our findings.³⁶ However, they could not replicate this finding in a larger study population.³⁷

Possible mechanisms

The SNP rs1800797 that showed an association with (atopic) adult-onset asthma is located in the 5' near-gene area of the *IL6* gene. Thus, it may predispose to development asthma and atopy via regulation of *IL6* gene expression as a response to different stimuli directly, or it may be in LD with regulatory polymorphism(s). Indeed, rs1800797 is located in a region that, according to VaDE v1.6,³⁸ has suggested enhancer and promoter functions in 36 and 51 cell types, respectively, has DNase hypersensitivity in 59 experimental settings and is a transcription factor binding motif recognized by CAC-binding protein. In addition, rs1800797 is a proxy ($r^2 \geq 0.8$) for several SNPs with reported functional properties in VaDE v1.6^(ref. 38) and in HaploReg v4.1.³⁹ These SNPs include, for example, rs1800795 (*IL6* -174 G/C) that has been reported functional also in the literature.^{17,40–43}

The observed associations may also be mediated by epigenetic mechanisms such as DNA methylation. For example, rs1800795 may cause a change in a methylation site (CpG) and it is located in the 5' near-gene area. DNA methylation in the *IL6* promoter has

been shown to regulate its activity,³⁹ and has been associated with rheumatoid arthritis³⁹ as well as with increased fractional exhaled nitric oxide in children with asthma.⁴⁴ Additionally, the observed associations may be due to regulation of other genes by the *IL6* polymorphisms. According to the GTEx Portal,⁴⁵ rs1800797 is an expression quantitative trait loci in several tissue types, including lung tissue, for AC073072.5 ($P=2.0e-13$, effect size -0.70) a suggested RNA gene, and for STEAP family member 1B (STEAP1B, $P=2.4e-7$, effect size -0.56), a gene with two putative protein isoforms.⁴⁶ Functions of these two genes are currently unknown. In addition to rs1800797, there are 17 SNPs in the *IL6* locus that are suggested expression quantitative trait loci, mainly for AC073072.5 and STEAP1B (Supplementary Table S6). All these but three are either directly genotyped in our study or are in LD ($r^2 \geq 0.80$) with a genotyped SNP. One of the SNPs (rs1554606) in LD with rs1800797 has also been reported as an expression quantitative trait loci for *IL6* in osteoblasts treated with dexamethasone.

In conclusion, we report a novel finding of a significant association of the *IL6* SNP rs1800797 with the risk of adult-onset asthma, and especially with the atopic adult-onset asthma, in a population originating from South Finland. These findings suggest that *IL6* gene and its polymorphisms have a role in the pathogenesis of both adult-onset asthma and atopy.

MATERIALS AND METHODS

Recruitment of cases and controls

This study was a single population-based incident case-control study of asthma.³ We expanded the analyses to study atopy as an additional outcome.²⁷ The source population for this study consisted of adults 21–63 years of age living in a geographically-defined area of Pirkanmaa in South Finland. The population of the area was 447 051 in 2000. We systematically recruited all new cases of asthma in 1997–2000 at all health-care facilities diagnosing asthma in the Pirkanmaa Hospital District.^{3,47,48} In addition, the National Social Insurance Institution of Finland invited all patients whose reimbursement rights for asthma medication began during the study period, but who had not yet participated. To ensure that all the cases had asthma diagnosed for the first time at recruitment, the medical records of all cases were checked and we selected as cases only those with no previously diagnosed asthma or long-term use of any asthma medication. A total of 521 cases participated (response rate 86%).

The controls were randomly drawn from the source population, using the Population Register Centre. The general eligibility criteria (living in the Pirkanmaa area, age 21–63 years) were applied. Recruitment of controls took place at regular intervals during the study period. A total of 1016 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.

The study was approved by the ethics committees of the Finnish Institute of Occupational Health, the Tampere University Hospital and the Oulu University Hospital District. Informed consent was obtained from all participants.

Diagnosis of asthma, lung function measurements and definition of atopy

The diagnostic criteria for asthma, including lung function measurements, followed the Finnish National Asthma Guidelines.⁴⁹ The detailed description of diagnosis of asthma and lung function measurements is available in Supplementary Table S5. Atopy was defined as a positive (score ≥ 1) Phadiatop test result (Supplementary information).

Selection of SNPs, laboratory methods and statistical analysis

To yield full genetic information of the gene locus, six tagging SNPs (tagSNP) (Table 2, Supplementary Figure S1A and B) in the *IL6* gene were picked using HapMap Tagger⁵⁰ and based on HapMap CEU (Utah residents with ancestry from northern and western Europe) population (Data Rel 27, Phase II+III, Feb09) (<http://hapmap.ncbi.nlm.nih.gov/>). Pairwise tagging method with limits of $r^2 \geq 0.8$ and MAF ≥ 0.030 was used. The selected

tagSNPs for *IL6* gene were rs2069824, rs2069827, rs1800797 also known as -597A/G, rs1524107, rs2069840 and rs2069861. Rs2069837 was also picked as a tagSNP, but it was not possible to include this SNP in the genotyping array due to technical reasons.

A total of 467 cases and 613 controls gave a blood sample for DNA analyses. The detailed description of DNA extraction and genotyping are available in S2. Association testing and haplotype analysis of the *IL6* SNPs on adult-onset asthma was performed using PLINK v1.07 (S2).⁵¹

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on Genes and Immunity website (<http://www.nature.com/gene>)

1 **S1**

2 **Additional Methods**

3 **Diagnosis of asthma and Lung Function Measurements**

4 The diagnostic criteria for asthma were: 1. occurrence of at least one asthma-like symptom, and 2.
5 demonstration of significant reversibility in airways obstruction in lung function investigations (S2
6 Table S5). The same diagnostic protocol was applied for all patients with suspicion of asthma. The
7 details of lung function measurements have been described elsewhere (1,2). Shortly, spirometry
8 and bronchodilatation test were recorded with a pneumotachograph spirometer using disposable
9 flow transducer (Medikro 905, Medikro, Kuopio, Finland) according to the standards of the
10 American Thoracic Society (3). Presence of obstruction was defined based the reference values
11 derived from the Finnish population (4).

12 **Assessment of atopy**

13 Blood samples were collected at the Tampere University Hospital Laboratory Center. Serum was
14 separated by centrifugation and stored at -20°C before shipping to Finnish Institute of
15 Occupational Health for analysis. Specific IgE antibodies to common aeroallergens, including birch,
16 timothy, grass, mugwort, cat, dog, horse, *Dermatophagoides pteronyssinus*, and *Aspergillus*
17 *fumigatus* were measured using immunoassay system Pharmacia UniCAP (Pharmacia Diagnostics
18 Ab, Uppsala, Sweden; www.unicapinvitrosight.com) for determining the Phadiatop score (5).
19 Atopy was defined as a positive score (≥ 1) in the Phadiatop test which corresponds to the limit of
20 ≥ 0.35 kU/L. Those having a score 0 (0–0.34 kU/ L) were defined as non-atopic.

1 **DNA extraction**

2 DNA was extracted from EDTA blood samples stored at -70°C, by using Omega Biotek E.Z.N.A.®
3 Blood DNA Midi Kit according to the manufacturer's instructions. The amount of starting material
4 was 10 ml. DNA extraction was performed at BioSer Oulu, Department of Biology, University of
5 Oulu, Finland.

6 **Genotyping**

7 Genotyping was performed at FIMM Technology Centre, University of Helsinki, using Illumina's
8 GoldenGate Genotyping Assay, iScan System, and with standard reagents and protocols provided
9 by Illumina Inc., San Diego. The genotypes were read and quality checked with Illumina's
10 GenomeStudio v. 2011.1 software, in-house developed database tools, and PLINK v1.07 toolset
11 (<http://pngu.mgh.harvard.edu/purcell/plink/>) (6).

12 In the whole dataset there were altogether 1080 individuals with blood sample available for DNA
13 extraction, two samples were discarded due to too low DNA concentration, thus leaving a total of
14 1078 DNA samples, 36 of which were analyzed as duplicates for quality control. Altogether 384
15 SNPs were genotyped and 30 (7.8%) of these were excluded due to clustering morphology or low
16 success rate. After these exclusions only three samples had low success rate (<95%) and were
17 discarded.

18 Six SNPs in the *IL6* gene, located in 7p21, were genotyped. Closer examination of genotyping
19 results of the *IL6* SNPs revealed that clustering morphology for rs1524107 and rs2069861 was
20 somewhat unclear (two genotype clusters located relatively close to each other). In order to
21 validate the genotyping results, sequencing of the area where the polymorphisms are located was
22 performed for 34 of the DNA samples. Sequencing was performed at the FIMM, using ABI3730xl
23 and applying Sequencher 4.8 for analyzing the results. All the samples gave identical results with

1 respect to the genotyping, and thus the SNPs were included in the study. All the studied SNPs
2 followed Hardy-Weinberg equilibrium with $p > 0.05$. In our data, there was considerable linkage
3 disequilibrium between the SNPs as demonstrated by high D' -values, however the relatively low
4 r^2 -values imply that the SNPs are not proxies for each other (S3 Figs. A and B).

5 **Statistical methods**

6 Association testing and haplotype analysis of the six SNPs on adult-onset asthma was done by
7 using PLINK v1.07 (6). Genetic associations were tested under log additive model, using
8 unadjusted and adjusted logistic regression. The results were corrected for multiple testing by
9 using Benjamini & Hochberg step-up false discovery rate (FDR) control. For the haplotype analysis,
10 we used the likelihood ratio-based test which compares the alternate model with the null model (-
11 -chap command). In the first phase, the haplotype TGACCC (H4) was found to have the lowest OR
12 for adult-onset asthma and it was thus set as the reference haplotype. This model for each
13 haplotype having a unique effect was then compared against the null model (no haplotypes having
14 any different effect). In the second phase, haplotype TTACCC (H6) that had an OR close to 1.0 was
15 also included to the reference category and haplotypes TGGCCC (H2) and TGGCGC (H1) that
16 showed the highest ORs for adult-onset asthma were combined to form one category, whereas
17 the other haplotypes were assumed to have a unique effect. Again, this model was compared to
18 the null model (no haplotypes having any different effect). Linkage disequilibrium (LD) and
19 haplotype blocks of the investigated SNPs and HapMap data were identified and visualized using
20 Haploview v4.2 (7).

21 We adjusted our analyses for environmental and life-style factors including both recent (past 12
22 months prior the entry to the study) and life-time cumulative second-hand smoke exposure,
23 personal smoking, the presence of pets in the home and mold problems (visible mold or mold

1 odor) at home or at work, that we have previously reported to be determinants of adult-onset
2 asthma in our study population (1,8). In addition, the analyses were adjusted for potential
3 confounders including age, sex, education as an indicator for socioeconomic status, and maternal
4 smoking during pregnancy.

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5

Table S1. Haplotype analysis of *IL6* SNPs on risk of adult-onset asthma. Statistically significant ($p < 0.05$) p-values and estimates are bolded.

Haplotype number ^a	Haplotype rs2069824 rs2069827 rs1800797 rs1524107 rs2069840 rs2069861	F (cases)	F (controls)	OR (95% CI)	Adjusted OR (95% CI) ^b	OR (95% CI)	Adjusted OR (95% CI) ^b
H4	TGACCC	0.195	0.235	reference	reference	reference	reference
H6	TTACCC	0.187	0.214	1.04 (0.79-1.36)	1.00 (0.76-1.33)		
H1	TGGCGC	0.290	0.257	1.36 (1.06-1.75)	1.37 (1.06-1.78)	1.39 (1.14-1.69)	1.42 (1.16-1.75)
H2	TGGCCC	0.104	0.078	1.58 (1.13-2.20)	1.57 (1.12-2.21)		
H3	TGGTCC	0.038	0.038	1.20 (0.75-1.92)	1.25 (0.77-2.05)	1.18 (0.75-1.86)	1.25 (0.78-2.01)
H7	CGGCCC	0.097	0.094	1.23 (0.88-1.74)	1.13 (0.79-1.62)	1.21 (0.89-1.66)	1.13 (0.81-1.57)
H5	TGACCT	0.089	0.084	1.25 (0.88-1.76)	1.24 (0.87-1.78)	1.23 (0.89-1.69)	1.24 (0.89-1.73)
Likelihood ratio test ^c		-	-	p=0.06	p=0.05	p=0.03	p=0.02

Abbreviations: F, Frequency; OR, Odds ratio; CI, Confidence interval

^a7 common haplotypes (frequency ≥ 0.01) from the 64 possible

^bAdjusted for age, sex, education, pets, visible mold or mold odor at home or at work, smoking, recent SHS exposure, cumulative SHS exposure, and maternal smoking during pregnancy

^cComparison of the alternate model with the null model (no haplotypes having any specific effect).

Table S2. Association of the *IL6* SNPs with the adult-onset asthma, in an analysis stratified by atopy. Statistically significant (p<0.05) p-values and estimates are bolded.

SNP ID	Model	Association among atopics (207 atopic asthmatics and 145 atopic controls)					Association among non-atopics (253 non-atopic asthmatics and 464 non-atopic controls) ¹					Cochran-Mantel-Haenszel test, controlling for atopy { disease x SNP cluster}			Breslow-Day test for heterogeneity
		MAF Atopic asthma	MAF Atopic control	OR (95% CI)	p-value	FDR BH p-value	MAF Non-atopic asthma	MAF Non-atopic control	OR (95% CI)	p-value	FDR BH p-value	OR (95% CI)	p-value	FDR BH p-value	p-value
rs2069824 ²	U	0.112	0.124	0.87 (0.53-1.43)	0.59	0.59	0.089	0.085	1.05 (0.71-1.54)	0.81	0.96	0.98 (0.73-1.32)	0.89	0.89	0.58
	A ³			0.81 (0.48-1.38)	0.44	0.53			0.92 (0.61-1.39)	0.68	0.82	0.95 (0.71-1.29)	0.76	0.76	0.69
rs2069827	U	0.179	0.207	0.83 (0.57-1.22)	0.35	0.59	0.196	0.218	0.87 (0.66-1.14)	0.31	0.54	0.86 (0.69-1.07)	0.18	0.36	0.84
	A ³			0.82 (0.54-1.23)	0.33	0.53			0.85 (0.64-1.14)	0.29	0.54	0.87 (0.70-1.09)	0.22	0.45	0.78
rs1800797	U	0.541	0.500	1.18 (0.87-1.60)	0.28	0.59	0.520	0.457	1.30 (1.04-1.63)	0.02	0.12	1.25 (1.05-1.49)	0.01	0.08	0.64
	A ³			1.15 (0.83-1.59)	0.41	0.53			1.31 (1.03-1.65)	0.03	0.16	1.24 (1.04-1.48)	0.02	0.10	0.69
rs1524107 ⁴	U	0.024	0.034	0.69 (0.28-1.69)	0.41	0.59	0.049	0.039	1.27 (0.76-2.11)	0.36	0.54	1.09 (0.70-1.72)	0.69	0.83	0.24
	A ³			0.68 (0.25-1.80)	0.43	0.53			1.29 (0.75-2.21)	0.36	0.54	1.10 (0.70-1.73)	0.68	0.76	0.23
rs2069840	U	0.297	0.272	1.14 (0.81-1.60)	0.46	0.59	0.281	0.251	1.17 (0.91-1.49)	0.22	0.54	1.15 (0.95-1.40)	0.16	0.36	0.89
	A ³			1.13 (0.78-1.63)	0.53	0.53			1.24 (0.96-1.61)	0.10	0.31	1.15 (0.94-1.40)	0.17	0.45	0.89
rs2069861	U	0.097	0.083	1.19 (0.70-2.04)	0.52	0.59	0.087	0.086	1.01 (0.69-1.49)	0.96	0.96	1.07 (0.78-1.46)	0.68	0.83	0.63
	A ³			1.21 (0.68-2.15)	0.51	0.53			0.97 (0.64-1.46)	0.87	0.87	1.06 (0.77-1.44)	0.74	0.76	0.59

Abbreviations: MAF, Minor allele frequency; OR, Odds ratio; CI, Confidence interval; FDR BH, Benjamini and Hochberg false discovery rate correction for multiple

testing; U, unadjusted log additive model; A, adjusted log additive model.

¹In adjusted analyses information missing for two non-atopic asthmatics and one non-atopic control.

²Genotype missing for 1 atopic asthmatic.

³Adjusted for age, sex, education, pets, visible mold or mold odor at home or at work, personal smoking, recent secondhand smoke (SHS) exposure, cumulative SHS exposure, and maternal smoking during pregnancy.

⁴Genotype missing for 1 non-atopic control.

Table S3. Association of the *IL6* SNPs with atopy, in an analysis stratified by adult-onset asthma. Statistically significant (p<0.05) p-values and estimates are bolded.

SNP ID	Model	Association among asthmatics (207 atopic asthmatics and 253 nonatopic asthmatics) ^a					Association among controls (145 atopic controls and 464 nonatopic controls) ^b					Cochran-Mantel-Haenszel test, controlling for asthma { disease x SNP cluster}			Breslow- Day test for heteroge neity p-value
		MAF Atopic asthma	MAF Non- atopic asthma	OR (95% CI)	p- value	FDR BH p- value	MAF Atopic control	MAF Non- atopic control	OR (95% CI)	p- value	FDR BH p- value	OR (95% CI)	p- value	FDR BH p- value	
rs2069824	U	0.112	0.089	1.32 (0.84-2.08)	0.23	0.60	0.124	0.085	1.53 (1.00-2.34)	0.05	0.29	1.40 (1.04-1.89)	0.03	0.17	0.58
	A ^c			1.41 (0.84-2.38)	0.19	0.39			1.62 (1.03-2.54)	0.04	0.21	1.43 (1.06-1.94)	0.02	0.12	0.69
rs2069827	U	0.179	0.196	0.89 (0.64-1.25)	0.51	0.60	0.207	0.218	0.93 (0.67-1.31)	0.69	0.86	0.92 (0.73-1.16)	0.46	0.56	0.84
	A ^c			0.94 (0.64-1.38)	0.74	0.81			0.94 (0.66-1.34)	0.73	0.73	0.92 (0.73-1.16)	0.46	0.56	0.78
rs1800797	U	0.541	0.520	1.09 (0.84-1.42)	0.52	0.60	0.500	0.457	1.20 (0.91-1.58)	0.19	0.56	1.14 (0.94-1.37)	0.17	0.35	0.64
	A ^c			0.97 (0.72-1.30)	0.81	0.81			1.24 (0.93-1.65)	0.14	0.43	1.14 (0.95-1.37)	0.17	0.34	0.69
rs1524107	U	0.024	0.049	0.50 (0.24-1.03)	0.06	0.37	0.034	0.039	0.88 (0.43-1.80)	0.73	0.86	0.65 (0.39-1.09)	0.10	0.29	0.24
	A ^c			0.41 (0.18-0.94)	0.04	0.22			1.17 (0.55-2.51)	0.69	0.73	0.65 (0.39-1.09)	0.09	0.28	0.23
rs2069840	U	0.297	0.281	1.09 (0.81-1.45)	0.58	0.60	0.272	0.251	1.12 (0.83-1.52)	0.46	0.86	1.10 (0.89-1.35)	0.37	0.55	0.89
	A ^c			0.92 (0.66-1.27)	0.60	0.81			1.08 (0.78-1.49)	0.65	0.73	1.10 (0.89-1.35)	0.38	0.56	0.89
rs2069861	U	0.097	0.087	1.13 (0.71-1.81)	0.60	0.60	0.083	0.086	0.96 (0.60-1.53)	0.86	0.86	1.04 (0.75-1.44)	0.81	0.81	0.63

A ^c	1.49	0.14	0.39	0.91	0.70	0.73	1.05	0.78	0.78	0.59
	(0.88-2.54)			(0.56-1.48)			(0.76-1.45)			

MAF, Minor allele frequency; OR, Odds ratio; CI, Confidence interval; FDR BH, Benjamini and Hochberg false discovery rate correction for multiple testing; U unadjusted log additive model; A adjusted log additive model.

^aIn adjusted analyses information missing for two nonatopic asthmatics.

^bIn adjusted analyses information missing for one nonatopic control.

^cAdjusted for age, sex, education, pets, personal smoking, recent secondhand smoke (SHS) exposure, and cumulative SHS exposure.

Table S4. Previous studies regarding *IL6* polymorphisms and asthma.

Findings of the study related to our findings	Study population	Asthma definition	N Asthmatics/ N Controls	<i>IL6</i> SNP studied, minor allele and its frequency	Associations	Reference
Consistent with our finding of a significant association between <i>IL6</i> rs1800797 G allele and (atopic) adult-onset asthma.	Egyptian children, aged 2-13 years.	Patients treated and followed up for bronchial asthma.	69/98	-174 (rs1800795), G allele f=0.495 ^a	1. GG genotype more common among asthmatics OR=3.2 (95 % CI 1.09-10.0) and CG genotype among controls OR=0.037 (95 % CI 0.05-0.2). 2. GG genotype suggested to associate with uncontrolled OR=4.6, (95 % CI 1.2-17.0) but not with controlled asthma OR=2.5 (95 % CI 0.6-9.0).	Settin et al. 2008 (1)
	Macedonian adult (aged 37-59 years) patients with bronchial asthma, and controls, all of Macedonian origin.	Patients with bronchial asthma fulfilling the criteria of Guidelines for the diagnosis and management of asthma (National Institutes of Health, 1997).	74/301	-174 (rs1800795), C allele f=0.302 ^a ; nt565 (rs1800797), A allele f=0.287 ^a	1. -174 C allele was found as a protective factor for asthma OR=0.611 (95 % CI 0.397-0.942) and GG genotype of the same SNP as a risk factor OR=1.791 (95 % CI 1.063-3.017). 2. nt565 allele A was also a borderline statistically significant protective factor OR=0.657 (95 % CI 0.426-1.014). 3. Haplotype analysis of these two SNPs found GG combination OR=1.636 (95 % CI 1.061-2.520) as well as diplotype GG:GG as significant risk factors for asthma OR=1.791 (95 % CI 1.063-3.017).	Trajkov et al. 2008 (2)
	Adult patients (aged 18 years or older) with asthma only, patients with asthma and nasal polyposis, patients with nasal polyposis	Clinical and spirometric criteria.	45 (asthma only) 45 (asthma and nasal polyposis) 63 (nasal polyposis only)/ 81 controls	-174 (rs1800795), C allele f=0.333 ^c	1. GG genotype more common in all disease groups than in controls (nasal polyposis 61.9%, asthma 82.22%, both 71.11%, controls 38.27 %, Fisher exact test p-value for asthma vs. controls <0.001). 2. GG genotype associated with increased risk of asthma when compared to C allele carriers OR=7.46 (95% CI 3.08-18.09).	Kosugi et al. 2013 (3)

	only, and controls.				
	Case-control study: Atopic asthmatics (38.5 ± 15 years) and non-atopic controls (37.0 ± 13.5 years), Family study: atopic asthmatic probands (15.6 ± 11.2 years).	Clinically diagnosed.	Case-control study: 147/149 Family study: 1022 individuals, 247 probands with asthma	rs2069827 A allele f=0.057 ^a , f=0.039 ^d ; rs2069832 A allele f=0.217 ^a , f=0.208 ^d ; rs2069840 C allele f=0.191 ^a , f=0.162 ^d ;	1. rs2069832 A allele was found to show a protective effect for atopic asthma, both in the case-control study OR=0.54 (95 % CI 0.35-0.82) and in the family-based analysis OR=0.62 (95 % CI 0.48-0.79). Kumar et al. 2015 (4)
	Meta-analysis of the case-control association studies regarding <i>IL6</i> rs1800795 (-174G/C) polymorphism and bronchial asthma, includes the references (1-3,5-7).				1. The <i>IL6</i> rs1800795 CC genotype is protective of asthma when compared to the GG genotype OR=0.51 (95% CI 0.27-0.96, p = 0.038). 2. The risk of asthma is reduced in Caucasian, CC vs. GG OR=0.51 (95% CI 0.27-0.96, p = 0.038). 3. The risk of asthma is reduced in adults, CC vs. GG OR=0.47 (95% CI 0.23-0.97, p = 0.042). Li et al. 2015 (8)
Somewhat consistent with our findings. No association between <i>IL6</i> SNPs and asthma, but observed an interaction	Dutch children	At least 1 episode of wheeze or dyspnea in the last year and/or the use of inhaled steroids.	2927 children from three birth cohorts PIAMA, PREVASC and KOALA (934 + 229 children with doctor's diagnosis of asthma)	rs13447445, C allele f=0 ^b ; rs2069840, allele not stated f=0.38 ^b ; rs2069845, G allele f=0.42 ^b ;	1. No association with asthma at age 6-8 y, with allergic asthma or doctor's diagnosis of asthma. 2. rs2069845 AG/GG and specific serum IgE to egg at age of 1 to2 years OR=1.7 (95% CI 1.0-2.8). 3. <i>IL6</i> rs2069845 interacted with <i>TGFB2</i> rs3773663, so that among carriers of <i>TGFB2</i> A allele the carriers of <i>IL6</i> G allele had higher levels of serum IgE to egg at age of 1 to2 years, whereas among the <i>TGFB2</i> GG genotype Bottema et al. 2010 (9)

between <i>IL6</i> and other genes, on either asthma or allergic sensitization.	Asthmatics (mean age $\pm$ SD = 31 $\pm$ 26 years) and controls.	Physician-diagnosed asthma and at least 2 symptoms consistent with asthma, a positive methacholine provocation test or broncho dilatator test result, absence of other pulmonary disorders.	219/157	rs1800795, minor allele not provided; rs1800797, minor allele not provided	carriers those with <i>IL6</i> genotype AA had higher levels. Note: Associations did not withstand correction for multiple testing. 1. <i>IL6</i> SNPs alone did not associate with asthma. 2. Multifactor dimensionality reduction (MDR) analysis suggested that rs1800797 shows gene-gene interaction with <i>IL12B</i> rs3212227, <i>TGFB</i> rs1982073, and <i>IL10</i> rs1800871 on atopic asthma and with <i>TGFB</i> rs1982073 on non-atopic asthma.	Padrón-Morales et al. 2013 (10)
Somewhat inconsistent with reported association for alleles that are different compared with our findings.	Han Chinese adult (aged 18-78 years) patients with bronchial asthma and controls	Diagnosis based on the guidelines for prevention and treatment of bronchial asthma (the Respiratory Society of the Chinese Medical Association, 2003).	108/88	-174 (rs1800795), C allele f=0.0985 ^a ; nt565 (rs1800797) A allele f=0 ^a i.e. this SNP was monomorphic	1. -174 genotype CG was more common among asthmatics than among controls (cases 33.0%, controls 19.7 %, p-value 0.0339, there were no individuals with CC genotype). 2. This SNP also correlated with plasma levels of IL-6, IL-10 and IgE. 3. IL-6 levels did not associate with asthma, but IL-10 levels were lower and IgE levels higher in asthmatics than in controls.	Xiaomin et al. 2008 (7)
	Iranian asthmatics aged 1.5 to 33 years and healthy blood donors, all had Farsi ethnic origin	Diagnosis based on the National Asthma Education and Prevention Program guidelines.	60/140	-174 (rs1800795), C allele f=0.363 ^a ; nt565 (rs1800797), A allele f=0.180 ^a	1. -174 GG genotype protective of asthma, OR for being a control =4.50 (95% CI 1.58-13.83) and CG genotype a risk of asthma, OR for being a control =0.24 (95% CI 0.08-0.68). 2. No association between nt565 and asthma.	Mahdavian et al. 2009 (6)

In contrast, no association between <i>IL6</i> SNPs and asthma.	Adult asthmatics (mean age $\pm$ SD = 43.03 $\pm$ 12.76 years) and controls (age and sex matched).	Diagnosis based on the criteria of the Global Initiative for Asthma (GINA).	81/124	-174 (rs1800795), C allele f=0.202 ^a	1. No association with asthma OR=0.792 (95% CI 0.472-1.328). 2. C allele moderately associated with reduced forced expiratory flow (FEF) 25-75% values in asthmatics (C allele 49.51 $\pm$ 20.4 vs. G allele 65.86 $\pm$ 36.42, one way analysis of variance p=0.045).	Daneshmandi et al. 2012 (5)
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Abbreviations: CI, Confidence interval

^aReported frequency in the control group .

^bReported minor allele frequency.

^cFrequency in the control group calculated based on reported numbers of individuals.

^dFrequency in the family study.

Table S5. Diagnostic criteria applied for asthma.

1. Occurrence of at least one asthma-like symptom: prolonged cough, wheezing, attacks of or exercise-induced dyspnea, or nocturnal cough or wheezing

AND

2. Demonstration of significant reversibility in airways obstruction in lung function investigations:

Significant improvement in response to short-acting bronchodilating medication in a bronchodilator test after baseline spirometry or at the end of methacholine challenge. The criteria for significant changes were(11): $FEV_1 \geq 15\%$, $FVC \geq 15\%$, $PEF \geq 23\%$

AND/OR

$\geq 20\%$ daily variation^a and/or $\geq 15\%$ improvement^a in response to short-acting bronchodilating medication during at least two days in a 2-week diurnal PEF monitoring

AND/OR

Significant improvement in spirometric lung function (for % criteria see above) and/or

$\geq 20\%$ improvement in the average PEF level in response to a 2-week oral steroid treatment

FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; PEF, peak expiratory flow.

^aCalculated according to the standard practice of the Tampere University Hospital: maximum daily variation = (highest PEF value during the day - lowest PEF value during the day)/highest PEF value during the day; bronchodilator response = (highest PEF value after bronchodilating medication - highest PEF value before medication)/highest PEF value before medication.

Table S6. Expression quantitative trait loci (eQTL) hits^a in the *IL6* locus

SNP	eQTL tissue or trait	Correlated genes	P-value	In our study	N of SNPs in LD (r ² ≥0.8)	N of SNPs with an eQTL hit in LD (r ² ≥0.8)
rs2069824	Adipose_Subcutaneous	AC073072.5	1.24e-07	genotyped	1	-
rs2069827	Adipose_Subcutaneous	AC073072.5	3.10e-06	genotyped	1	1
	Adipose_Subcutaneous	STEAP1B	2.37e-09			
	Artery_Tibial	AC005682.6	8.14e-07			
	Peripheral blood monocytes	ADH6	7.80E-06			
rs1800797	22 tissues	AC073072.5	NA ^b	genotyped	26	26
		STEAP1B	NA ^b			
	Dendritic cells treated with Mycobacterium tuberculosis	TOMM7	0.0069			
rs1800796	Adipose_Visceral_Omentum	AC073072.5	5.61e-09	tagged by rs1524107	2	2
rs1800795	22 tissues	AC073072.5	NA ^b	tagged by rs1800797	27	27
		STEAP1B	NA ^b			
	Peripheral blood monocytes	TEDDM1	3.33e-06			
rs2069832	21 tissues	AC073072.5	NA ^b	tagged by rs1800797	29	29
		STEAP1B	NA ^b			
	Lung	LOC401312	7.77e-15			
rs2069833	21 tissues	AC073072.5	NA ^b	tagged by rs1800797	29	29
		STEAP1B	NA ^b			
rs1474348	21 tissues	AC073072.5	NA ^b	tagged by rs1800797	29	29
		STEAP1B	NA ^b			
rs1474347	21 tissues	AC073072.5	NA ^b	tagged by rs1800797	29	29
		STEAP1B	NA ^b			
rs1524107	Adipose_Visceral_Omentum	AC073072.5	3.94e-09	genotyped	2	2
rs2066992	Adipose_Visceral_Omentum	AC073072.5	3.99e-09	tagged by rs1524107	2	2
rs2069840	9 tissues	AC073072.5	NA ^b	genotyped	-	-

rs1554606	20 tissues	AC073072.5	NA ^b	tagged by	30	30
	Osteoblasts treated with dexamethasone	STEAP1B	NA ^b	rs1800797		
	Dendritic cells	IL6	3.74e-05			
		TOMM7	0.0096			
rs1548216	Peripheral blood monocytes	CAV1	8.80E-06	not genotyped	24	-
		DLAT	9.92E-06	not tagged		
rs2069845	20 tissues	AC073072.5	NA ^b	tagged by	30	30
		STEAP1B	NA ^b	rs1800797		
rs2069861	Adipose_Subcutaneous	AC073072.5	9.47e-09	genotyped	-	-
	Artery_Tibial	AC073072.5	6.55e-06			
	Lung	AC073072.5	1.34e-06			
	Nerve_Tibial	AC073072.5	1.23e-06			
rs60056354	Adipose_Subcutaneous	AC073072.5	1.61e-06	not genotyped	-	-
	Artery_Tibial	AC073072.5	1.87e-06	not tagged		
rs2069852	Adipose_Visceral_Omentum	AC073072.5	1.84e-06	not genotyped	-	-
				not tagged		

Abbreviations: LD, Linkage disequilibrium; NA, Not applicable

^aHaploReg v4.1 (12)

^bToo many p-values to list feasibly

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S3 Supplementary figure

Fig.S1. Pairwise linkage disequilibrium (LD) between the six examined *IL6* SNPs. In A and B, LD in our study population, as D' (A) and as r^2 (B). In C and D, LD between the *IL6* SNPs genotyped in the HapMap population of Utah residents with ancestry from northern and western Europe (CEU) in the sample of 117 singletons (version 3, release R2) (C) and 27 trios and 20 families (Data Rel 27, Phasell+III, Feb09) (D), as r^2 . In C and D the 6 tag SNPs of this study are indicated by an asterisk (note that rs2069861 is not present in the HapMap singleton data). In A values in the boxes are D' measures, in the boxes without number the $D'=1$, red color indicates strong LD. In B, C and D the values in the boxes are r^2 measures, white color indicates $r^2=0$, and the different shades of gray indicate $0 < r^2 < 1$. In C also locations of the SNPs in the *IL6* gene are shown. The haplotype blocks were generated by Haploview (1) using the default algorithm.

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