Identification of *IL6R* and chromosome 11q13.5 as risk loci for asthma

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See **Comment** page 967

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Summary

Background We aimed to identify novel genetic variants affecting asthma risk, since these might provide novel insights into molecular mechanisms underlying the disease.

Methods We did a genome-wide association study (GWAS) in 2669 physician-diagnosed asthmatics and 4528 controls from Australia. Seven loci were prioritised for replication after combining our results with those from the GABRIEL consortium (n=26 475), and these were tested in an additional 25 358 independent samples from four in-silico cohorts. Quantitative multi-marker scores of genetic load were constructed on the basis of results from the GABRIEL study and tested for association with asthma in our Australian GWAS dataset.

Findings Two loci were confirmed to associate with asthma risk in the replication cohorts and reached genome-wide significance in the combined analysis of all available studies (n=57 800): rs4129267 (OR 1·09, combined $p=2·4\times10^{-8}$) in the interleukin-6 receptor (*IL6R*) gene and rs7130588 (OR 1·09, $p=1·8\times10^{-8}$) on chromosome 11q13.5 near the leucine-rich repeat containing 32 gene (*LRRC32*, also known as *GARP*). The 11q13.5 locus was significantly associated with atopic status among asthmatics (OR 1·33, $p=7\times10^{-4}$), suggesting that it is a risk factor for allergic but not non-allergic asthma. Multi-marker association results are consistent with a highly polygenic contribution to asthma risk, including loci with weak effects that might be shared with other immune-related diseases, such as *NDFIP1*, *HLA-B*, *LPP*, and *BACH2*.

Interpretation The *IL6R* association further supports the hypothesis that cytokine signalling dysregulation affects asthma risk, and raises the possibility that an IL6R antagonist (tocilizumab) may be effective to treat the disease, perhaps in a genotype-dependent manner. Results for the 11q13.5 locus suggest that it directly increases the risk of allergic sensitisation which, in turn, increases the risk of subsequent development of asthma. Larger or more functionally focused studies are needed to characterise the many loci with modest effects that remain to be identified for asthma.

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Introduction

Eight loci were reported to associate with asthma risk with genome-wide significance, namely the locus containing *GSDMB*, *ORMDL3*, and *GSDMA* on chromosome 17q21,[1] *PDE4D*,[2] *DENND1B*,[3] the locus containing *IL1RL1* and *IL18R1* on chromosome 2q12.1,[4] *HLA-DQ*, *IL33*, *IL2RB*, and *SMAD3*.[5] Notably, these findings point to a genetically-linked dysregulation of cytokine signalling in asthma, and provide various specific targets for the development of novel biological treatments. They also implicate previously unsuspected risk loci, such as the 17q21 region. As our understanding of the biological mechanisms underlying these associations improves, novel insights into the pathophysiology of asthma are likely to emerge.

The risk variants identified until now explain only a small fraction of the disease heritability (<1% each), indicating that many more loci remain to be identified. Because of the proven success of genome-wide association studies (GWAS) to identify common risk variants,[6] further dissection of this uncharacterised component of disease risk through well powered genetic studies represents a unique opportunity to advance our knowledge of the mechanisms that trigger asthma.

In this Article, we describe a series of genetic association analyses done to identify novel risk loci for asthma, including (1) a GWAS of physician-diagnosed asthma with data for 7197 individuals of European descent from Australia; (2) a meta-analysis of these results with findings from 26 475 individuals studied by the GABRIEL consortium;[5] and (3) testing of the most significant regions of association in a further 25 358 independent samples.

Methods

Participants

We first did a GWAS of 7197 individuals of European ancestry from Australia; throughout this paper, we refer to this analysis as the Australian GWAS. Participants were drawn from three cohorts (webappendix pp 2–6): the Australian Asthma Genetics Consortium (AAGC) cohort (n=1810), the Busselton Health Study cohort (n=1230), and the Queensland Institute of Medical Research (QIMR) GWAS cohort (n=4157). Patients were generally recruited between 1964, and 2010.

Of the 2669 asthmatic patients, 759 (28%) were diagnosed through clinical examination and 1910 (72%) reported a lifetime physician diagnosis of asthma in epidemiological questionnaires. With respect to disease onset, 1438 (54%) patients were classified as having childhood asthma (defined by an age-of-onset at or before age 16 years), 697 (26%) patients with later onset asthma (age-of-onset after the age of 16 years) and 534 (20%) with unknown age-of-onset. 1570 (59%) of asthmatics were atopic, as defined by a positive skin prick test response to at least one common allergen; 1444 (54%) had at least one first-degree relative with asthma; and 1026 (38%) reported lifetime smoking (webappendix pp 2–3).

The 4528 controls included 2701 (60%) individuals who were classified as asthma-free based on clinical examination (109 [2%]) or epidemiological questionnaires (2592 [57%]). The remaining 1827 (40%) individuals provided no information about their asthma status. As we show in the webappendix (p 7), including this group of asthma-unknown individuals in the analysis as controls improved power to detect a true genetic association. Skin prick test information and lifetime smoking status was unavailable for most controls (3903 [86%] and 3822 [84%], respectively; webappendix pp 2–3).

Overall, the mean age of participants was 39 years (SD 18·5, range 2–92) and 3986 (55%) were women. This dataset includes 4259 samples that have not been previously included in any asthma GWAS, 1708 that were included in the study by Ferreira and colleagues,[7] and 1230 samples from the Busselton cohort included in the GABRIEL study.[5]

Next, to prioritise loci for replication, results from the Australian GWAS were combined with those published and made publicly available by the GABRIEL consortium.[5] After exclusion of overlapping samples between the two studies, the meta-analysis was based on results from 12 475 physician-diagnosed asthmatic patients and 19 967 controls.

Lastly, the most significant regions of association were tested for replication in four additional cohorts (3322 cases and 22 036 controls) that contributed in-silico results for analysis: the Western Australian Pregancy Cohort (Raine) study (654 asthmatic and 621 control patients); the QIMR follow-up cohort (602 asthmatic and 2206 control patients), consisting of individuals unrelated to those included in the QIMR GWAS cohort; the Netherlands Twin Registry (NTR) study (350 asthmatic and 2321 control patients); and the Analysis in Population-based Cohorts of Asthma Traits (APCAT) consortium (1716 asthmatic and 16 888 control patients). The APCAT consortium included six population-based cohorts from Finland and USA: the Helsinki Birth Cohort (123 asthmatic and 1533 control patients), Health 2000 (153 asthmatic and 1841 control patients), Finrisk (160 asthmatic and 1705 control patients), the Northern Finland Birth Cohort 1966 (364 asthmatic and 3502 control patients), the Young Finns Study (119 asthmatic and 1844 control patients), and the Framingham Heart Study (797 asthmatic and 6463 control patients). The webappendix (pp 8–10) contains a detailed description of every cohort. All participants gave written informed consent and the study protocols were reviewed and approved by the appropriate review committees.

Procedures

The Australian GWAS included data from 7197 individuals of whom 5523 (77%) were genotyped with Illumina 610K (Illumina, Sand Diego, CA, USA) array and 1674 (23%) with Illumina 370K array as part of four genotyping projects that are summarised in the webappendix (p 11): the AAGC (n=1810), QIMR_610K (n=2483), QIMR_370K (n=1674), and Busselton (n=1230) projects. The same single-nucleotide polymorphism (SNP) quality control filters were applied to each project individually, including the removal of SNPs with call rate lower than 95%, minor allele frequency (MAF) lower than 0·01, and Hardy-Weinberg equilibrium test p lower than 10^{-6}. Autosomal SNPs passing quality control were then used to impute 7·8 million variants available from the combined 1000 genomes (60 individuals with northern and western European ancestry from the Centre d'Etude du Polymorphisme Humain collection [CEU], March, 2010 release) and HapMap 3 (955 individuals from 11 populations, February, 2009 release) reference panels with Impute2.[8] The AAGC and QIMR_610K datasets were merged before imputation as both were genotyped with the same array, were available concurrently for analysis, and had no evidence for systematic allele frequency differences between controls (genomic inflation factor λ=1·014 for AAGC controls *vs* QIMR_610K controls) nor between asthmatic patients (λ=1·001 for AAGC cases *vs* QIMR_610K cases). We nonetheless removed a small subset of 1104 SNPs (0·2%) that had significant (p<0·001) allele frequency differences between the two datasets, as these probably indicated technical artifacts. The genomic inflation factor from a case-control association analysis in the resulting AAGC+QIMR_610K dataset (n=4293) was 1·014, further indicating that batch effects between the AAGC and QIMR_610K datasets did not have a systematic effect on the results.

Imputation and subsequent SNP quality control were done as three separate analyses, corresponding to the

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See Online for webappendix

AAGC+QIMR_610K, QIMR_370K, and Busselton datasets. The QIMR_370K and Busselton datasets were imputed separately because the QIMR_370K was genotyped with a different array and because the Busselton dataset only became available for analysis at a later stage. After imputation, we excluded SNPs with low imputation accuracy (information <0·3), MAF lower than 0·01, or Hardy-Weinberg equilibrium test p lower than 10^{-6}. To minimise the effect of potential dataset-specific technical artifacts, we also excluded from analysis SNPs with significant (p<0·001) allele frequency differences between the three imputation analysis groups (from case–case and control–control comparisons, as described above). After quality control, genotype data for 5·7 million common SNPs (webappendix p 12) were merged across the analysis datasets of AAGC+QIMR_610K, QIMR_370K, and Busselton. All people included were confirmed to be unrelated and of European ancestry (webappendix p 6) through the analysis of genome-wide allele sharing. Comparable procedures were used for the replication cohorts (webappendix pp 8–10).

Statistical analyses

For the Australian GWAS, individual SNPs were tested for association with lifetime physician-diagnosed asthma with a Cochran-Mantel-Haenszel test as implemented in PLINK,[9] with three strata corresponding to the three imputation analysis groups described in the previous section. This analysis had adequate power (80% for α=5×10^{-8}) to detect loci with a genotype relative risk ranging from 1·23 (MAF=0·50) to 2·23 (MAF=0·01; webappendix p 7). For imputed SNPs, we analysed best-guess (ie, most likely) genotypes. We applied the Breslow-Day test of homogeneity to test for differences in SNP effects between the three groups.

To prioritise SNPs for follow-up in lower-ranked loci from the Australian GWAS, we did a fixed-effects meta-analysis of our results (after excluding the 1230 overlapping samples from Busselton) with those published and made available by the GABRIEL study,[5] which were based on 10 365 asthmatic patients and 16 110 controls genotyped with the Illumina 610K array. We restricted our analysis to 421 334 SNPs that were available in all 36 GABRIEL cohorts, had no strong evidence for significant heterogeneous effects between the 36 cohorts (p>0·01), and which were tested in our study. We used the Cochran's *Q* to identify SNPs with significant heterogeneous effects between our study and the GABRIEL study.[5] No correction for genomic inflation of test statistics was applied to either set of results before the meta-analysis.

In the replication study, SNPs were tested in every cohort separately (logistic regression with cohort-specific adjustments) and then combined by doing a fixed-effects meta-analysis with METAL.[10]

Lastly, we used a recently described[11] approach for phenotype prediction from genome-wide SNP data to address the hypothesis that hundreds or thousands of loci with individual weak effects contribute to asthma risk. Briefly, we selected groups of SNPs based on their level of association with asthma in the GABRIEL[5] analysis (for example, SNPs with a p≤5×10^{-7}) and created a quantitative score of genetic load based on these SNPs for every individual included in the Australian GWAS, after restricting the sample to 2082 patients with asthma and 2211 controls genotyped with the 610K array and who did not overlap with those included in the GABRIEL. This genetic score was calculated as the weighted sum of the number of reference alleles for each genotyped SNP, with the weight corresponding to the effect size (log of the odds ratio [OR]) estimated for that marker in the GABRIEL study.[5] We then used logistic regression to test the association between a specific genetic score and the case-control asthma status in our study. The log of the OR was selected as the weight (instead of, for example, simply counting the number of risk alleles) because it considers both the expected magnitude and direction of effect of an individual SNP. We used the OR estimated in the GABRIEL study because that study represents the largest asthma GWAS done so far, hence providing the most accurate estimates of SNP effects available for asthma. We considered seven groups of SNPs based on arbitrary thresholds of significance in the GABRIEL study, from very strict to very liberal: p≤5×10^{-7}, 5×10^{-7}<p≤10^{-4}, 10^{-4}<p≤0·01, 0·01<p≤0·1, 0·1<p≤0·2, 0·2<p≤0·5 and 0·5<p≤1. To help interpretation, we restricted our analysis to directly genotyped SNPs that were in low linkage disequilibrium (r^2<0·1) with each other, using the clump routine implemented in PLINK.[9] Results were unchanged by inclusion as covariates the first four components obtained from the multidimensional scaling analysis of identity-by-state allele sharing.

Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

To identify novel SNPs associated with asthma risk, we first did a GWAS in 2669 physician-diagnosed asthmatic patients and 4528 controls from Australia. We recognise that with this sample size, power was only adequate (≥80%) at the genome-wide level (α=5×10^{-8}) to detect loci with strong effects (eg, an OR≥1·25 for an allele frequency of 0·40). The genomic inflation factor confirmed that population substructure or other potential technical artifacts did not have a systematic impact on the results (λ=1·014; webappendix p 13).

In the Australian GWAS, five independent SNPs (linkage disequilibrium r^2 <0·1) were associated with asthma risk at a predefined cutoff of p≤5×10^{-6}

	Cases (n)	Controls (n)	IL6R (rs4129267,T)				11q13.5 (rs7130588,G)			
			MAF	OR (95% CI)	Association p value	Heterogeneity test p value* (*k*, I^2, 95% CI)	MAF	OR (95% CI)	Association p value	Heterogeneity test p value* (*k*, I^2, 95% CI)
Discovery panels										
Australian GWAS†	2110	3857	0·41	1·09 (1·01–1·18)	0·0372	0·0417 (2; 76, 0–100)	0·36	1·12 (1·03–1·21)	0·0111	0·3523 (2; 0, 0–100)
GABRIEL‡	10 365	16 110	0·37	1·09 (1·05–1·13)	$1·9×10^{-5}$	0·1734 (36; 18, 0–46)	0·34	1·10 (1·06–1·14)	$3·2×10^{-6}$	0·1602 (36; 19, 0–47)
Combined§	12 475	19 967	..	1·09 (1·06–1·13)	$2·0×10^{-6}$	0·1839 (38; 17, 0–44)	..	1·10 (1·07–1·14)	$1·2×10^{-7}$	0·1894 (38; 17, 0–44)
Follow-up panels										
APCAT	1716	16 888	0·35	1·08 (1·00–1·15)	0·0671	0·6792 (3; 0, 0–90)	0·31	1·08 (1·00–1·16)	0·0749	0·3611 (3; 2, 0–90)
Raine	654	621	0·39	1·07 (0·92–1·25)	0·4005	..	0·36	0·96 (0·82–1·12)	0·6013	..
QIMR	602	2206	0·40	1·10 (0·97–1·23)	0·1458	..	0·35	1·15 (1·02–1·28)	0·0343	..
NTR	350	2321	0·39	1·16 (1·00–1·32)	0·0723	0·2040 (2; 38, 0–100)	0·37	1·01 (0·85–1·18)	0·8685	0·7099 (2; 0, 0–100)
Combined§	3322	22 036	..	1·09 (1·03–1·15)	0·0033	0·7911 (7; 0, 0–71)	..	1·07 (1·01–1·13)	0·0328	0·4630 (7; 0, 0–71)
Overall										
All samples§	15 797	42 003	..	1·09 (1·06–1·12)	$2·3×10^{-8}$	0·3251 (45; 8, 0–36)	..	1·09 (1·06–1·13)	$1·8×10^{-8}$	0·2184 (45; 14, 0–41)

MAF is reported for non-asthmatic individuals. Both SNPs were directly genotyped in the discovery panels, APCAT (except FHS cohort, imputed with $r^2 > 0·90$), Raine and QIMR studies; both were imputed with high confidence (information > 0·95) in NTR. MAF=minor allele frequency. OR=odds ratio. *k*=number of studies. *Results for a test of heterogeneity (Breslow-Day test for the Australian GWAS and NTR analyses; Cochran's Q test for all other analyses) across studies are provided for panels that incorporated multiple cohorts. †Samples that overlapped with the GABRIEL (n=1230) were removed in this analysis. ‡Results from the GABRIEL consortium[5] were obtained from the website of the Centre National de Génotypage. The MAF of each SNP corresponds to the mean MAF across all 36 individual studies. §For the combined analysis, the association p value corresponds to a fixed-effects meta-analysis of the results from the individual panels. Results remained unchanged under a random-effects model.

Table: **Association results for two variants in *IL6R* and chromosome 11q13.5 in the discovery, follow-up, and overall analyses**

(webappendix p 14). Among these regions were two loci previously reported[1,4] to associate with asthma—chromosome 17q21 near *GSDMA* and 5q22.1 near *WDR36*—and three novel loci: chromosomes 12q24.31 near *CABP1*, 16q24.1 in *KCNG4* and 8q22.1 in *CDH17*.

The 12q24.31 locus corresponds to an uncommon variant (chr12:119562269; MAF=0·02) from the 1000 Genomes Project imputed in our data with modest confidence (imputation information of 0·86 for the 610K array and 0·52 for the 370K array). However, no supporting evidence existed for association from neighbouring markers (webappendix p 15) nor from haplotype analyses done after exclusion of the chr12:119562269 variant (data not shown), suggesting that this association probably represents a technical artefact. The 16q24.1 and 8q22.1 associations were supported by multiple markers (webappendix p 15) and so were selected for follow-up in an independent replication panel, as described later in this Article.

Next, to prioritise additional loci for follow-up, we did a fixed-effects meta-analysis of our results with those published and made publicly available by the GABRIEL consortium.[5] After exclusion of overlapping samples, the combined analysis was based on results from 12 475 asthmatic patients and 19 967 controls genotyped at 421 334 autosomal SNPs. The genomic inflation factor for this analysis was 1·068 (webappendix p 16).

19 independent (r^2<0·1) SNPs had a meta-analysis association p≤$5×10^{-6}$ (listed in the webappendix, p 17). Of these, 14 were not considered for further analysis because they were not associated with asthma in the Australian GWAS (n=2) or because they were located in regions previously reported to associate with asthma risk (n=12). The remaining five SNPs were nominally significant (p≤0·05) in our dataset and were located in novel regions for asthma; as such, they were also selected for follow-up (webappendix p 17).

Thus, in total, we identified seven putative novel asthma risk loci (p≤$5×10^{-6}$) that we sought to follow up in an independent panel of 3322 asthmatic patients and 22 036 controls previously genotyped as part of four studies (APCAT, Raine, QIMR, and NTR). Power to replicate each individual SNP association at a Bonferroni-corrected threshold (α≤0·05/7=0·007) ranged between 64% and 100%.

Follow-up analyses replicated at p≤0·007 the association with one SNP located in the interleukin-6 receptor (*IL6R*) gene on chromosome 1q21.3 (rs4129267, uncorrected p=0·0033; table). In the overall analysis of the discovery and follow-up panels, this variant was highly associated with asthma risk (OR=1·09, p=$2·3×10^{-8}$; table and figure 1A). A second SNP, rs7130588 on chromosome 11q13.5, replicated less strongly in the follow-up panels (combined p=0·0328) but reached genome-wide significance in the overall analysis of all samples (OR=1·09, p=$1·8×10^{-8}$; table and figure 1B). The remaining five regions of association (*PCDH20*, *PRKG1*, *KCNG4*, *IGHMBP2*, *CDH17*) were not significantly associated with asthma in the follow-up study (p>0·05; webappendix pp 19–20). Thus, genome-wide association analyses followed by replication identified variants in *IL6R* and on chromosome 11q13.5 as novel risk loci for asthma.

To further characterise the association between the *IL6R* and 11q13.5 variants and asthma risk, we tested both loci for association with nine asthma subphenotypes measured in up to 2669 asthmatic individuals

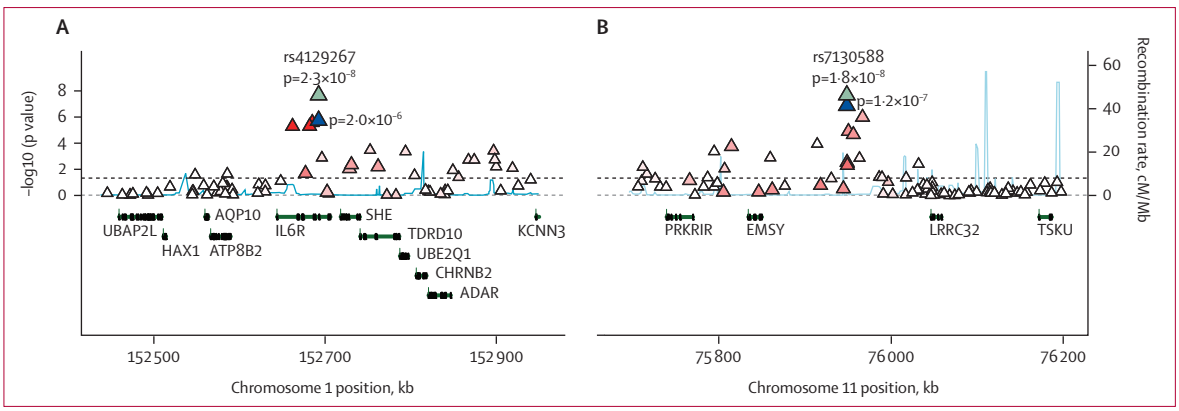

Figure 1: **Regional association plots for chromosomes 1q21.3 and 11q13.5**
Association results (–$\log_{10}$ p-value, *y*-axis) from a meta-analysis of the Australian GWAS and the GABRIEL[5] studies for the 1q21.3 (A) and 11q13.5 (B) loci, focusing on a subset of SNPs genotyped in both studies. Results for rs4129267 ($p=2{\cdot}0\times10^{-6}$) and rs7130588 ($p=1{\cdot}2\times10^{-7}$) in this analysis are represented by the blue triangle in each plot. The green triangle corresponds to results for rs4129267 ($p=2{\cdot}3\times10^{-8}$) and rs7130588 ($p=1{\cdot}8\times10^{-8}$) in the meta-analysis of our study, the GABRIEL study,[5] and the follow-up panels (cf table), representing 15 797 asthmatic patients and 42 003 controls. The recombination rate (second *y*-axis) is plotted in light blue and is based on the CEU HapMap population. Exons for every gene are represented by vertical bars. Regional association plots for both loci in the Australian GWAS are presented in the webappendix (p 18). GWAS=genome-wide association studies. SNP=single-nucleotide polymorphism. CEU=individuals from northern and western European ancestry from the Centre d'Etude du Polymorphisme Humain.

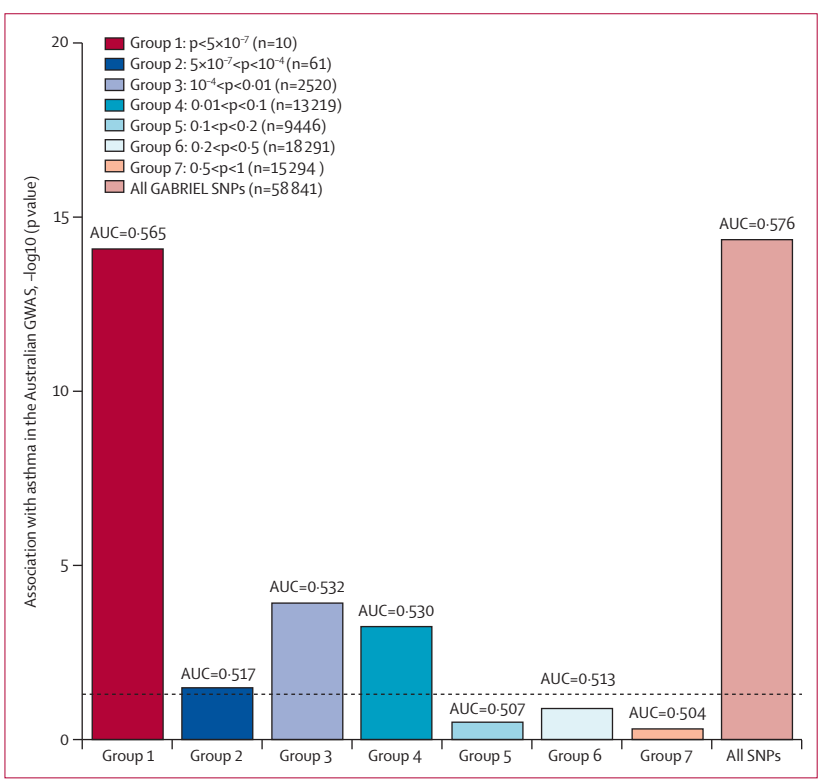

Figure 2: **Multi-SNP prediction of asthma case-control status**
Quantitative scores of genetic load were computed with information for seven non-overlapping groups of independent (r^2<0·1, except rs2305480 and rs3894194 in group 1, r^2 0·5) SNPs identified in the GABRIEL analysis[5] and tested for association with asthma case-control status in our study with logistic regression. The rightmost vertical bar represents the results obtained when including all seven quantitative scores as predictors in the logistic regression model. The horizontal dashed black line represents a p=0·05. The AUC, estimated from the logistic regression analysis, is shown above every bar. AUC=area under the receiver operator curve. GWAS=genome-wide association study.

(webappendix p 21). The rs7130588:G predisposing variant on 11q13.5 was more common in atopic asthmatic patients (defined by a positive skin prick test to at least one common allergen) than non-atopic asthmatic patients (G allele frequency 38% *vs* 32%, p=0·0007; webappendix p 21–22). Consistent with this result, there was no evidence for an increased risk of asthma associated with this allele when considering only non-atopic individuals (webappendix p 22); however, because of the modest sample size for this secondary analysis, these findings require confirmation by independent studies.

We also sought to validate the genome-wide significant associations reported in the GABRIEL study,[5] Sleiman and colleagues,[3] and Himes and colleagues.[2] We replicated (same SNP, p≤0·05, and same direction of effect) the association with *GSDMB* (and *GSDMA*), *IL18R1*, *IL33*, and *IL2RB* (webappendix p 23). A consistent but non-significant effect was recorded for *HLA-DQ* and *SMAD3*. There was no support for an association with *PDE4D* or *DENND1B* (webappendix pp 23–24).

Next, we investigated the hypothesis that hundreds or potentially thousands of common variants with weak effects influence asthma risk. A multi-SNP score computed based on data for the ten most associated loci reported in the GABRIEL study[5] was significantly associated ($p=8{\cdot}2\times10^{-15}$) with asthma status in our study (figure 2). Multi-SNP scores based on loci with less remarkable levels of association with asthma in the GABRIEL study[5] were also associated with asthma status in our study. For example, a score computed based on 2520 largely independent SNPs (r^2 <0·1) that had individual p values between 10^{-4} and 0·01 in the GABRIEL study[5] significantly associated with asthma case-control status ($p=1{\cdot}2\times10^{-4}$), indicating that many of these SNPs probably represent genuine asthma risk loci with modest effects. We stress, however, that these results have little clinical relevance, since the multi-SNP scores tested had very poor discriminative ability, with values for the area

under the receiver operator characteristic curve (AUC) not exceeding 0·576 (figure 2).

Three confirmed asthma loci are shared with Crohn's disease, namely *ORMDL3*, *IL1RL1,* and now chromosome 11q13.5. We postulated that loci with modest effects on asthma risk might also be shared with other inflammatory or immune diseases. To explore this possibility, we identified 356 SNPs listed in the catalog of published GWAS[12] that were previously reported to associate at $p \leq 5 \times 10^{-8}$ with 54 such traits or diseases excluding asthma (webappendix p 25). Importantly, this list did not include any SNP located near (<500 kb) the ten GABRIEL loci,[5] *PDE4D*,[2] *DENND1B*,[3] *IL6R*, or 11q13.5. After exclusion of redundant markers ($r^2 \geq 0{\cdot}1$), results for 207 largely independent SNPs from the original list were available in the meta-analysis of the Australian GWAS and the GABRIEL,[5] either directly or through a proxy SNP ($r^2 \geq 0{\cdot}8$). Of these, 16 (8%) were associated with asthma risk with a $p \leq 0{\cdot}01$ (webappendix p 26), when only about two were expected at this threshold under the null hypothesis of no association between the 207 SNPs and asthma (Fisher's exact test p=0·001). Four SNPs survived a Bonferroni correction for multiple testing ($p \leq 0{\cdot}00024$): rs11167764 near *NDFIP1* (OR=1·11, $p = 4{\cdot}6 \times 10^{-6}$) and rs1847472 in *BACH2* (OR=1·07, p=0·00023), two variants associated with Crohn's disease;[13] rs2596560 near *HLA-B* (OR=0·92, $p = 6{\cdot}5 \times 10^{-5}$), a proxy SNP ($r^2$=1·00) for a variant (rs3134792) reported to associate with psoriasis;[14] and rs13076312 in *LPP* (OR=0·93, p=0·00016), a proxy SNP (r^2=1·00) for a variant (rs1464510) reported to associate with coeliac disease.[15] These variants represent putative novel associations for asthma that require further confirmation and draw attention to molecular pathways that are probably shared between asthma and other inflammatory or immune diseases.

Discussion

We identified two new loci with genome-wide significant association with asthma risk: rs4129267 in *IL6R* and rs7130588 on chromosome 11q13.5 (panel). Multiple lines of evidence suggest that *IL6R* is indeed the causal gene underlying our observed association with rs4129267. First, rs4129267 is strongly associated with variation in serum concentration of the soluble form of the IL-6 receptor (sIL-6R).[18] Each copy of the rs4129267:T allele increases sIL-6R protein concentration by about 1·4-fold,[18] while it increases the risk of asthma by 1·09-fold based on our analyses. Second, the concentration of sIL-6R is increased in both the serum[19] and airways[20] of patients with asthma, and it correlates with Th2 cytokine production in the lung.[20] Lastly, selective blockade of sIL-6R in mice suppressed IL-4, IL-5, and IL-13 production and decreased eosinophil numbers in the lung; on the other hand, blockade of sIL6-R plus the membrane-bound form of the receptor (mIL-6R) caused not only the suppression of Th2 cytokine production but also the expansion of CD4+CD25+ Tregs in the lung with increased IL-10 production and suppressive capacity.[20] Thus, together with our results, these data suggest that rs4129267, or a causal variant in linkage disequilibrium with it, increases the risk of development of asthma by upregulating protein concentrations of sIL-6R or mIL-6R, or both, which in turn contributes to the development and maintenance of a Th2 immune response in the lung. An IL-6R antagonist (tocilizumab) has been approved as an effective biological drug to treat rheumatoid arthritis;[21] further studies are warranted to test the hypothesis that tocilizumab might also be effective to treat asthma, particularly for patients with the rs4129267:T risk variant.

The second locus that our analyses implicate in the causation of asthma is represented by rs7130588, which is located on chromosome 11q13.5 near a SNP recently reported to associate with two immune-related diseases, Crohn's disease[22] and atopic dermatitis.[23] The variant reported to associate with Crohn's disease and atopic dermatitis (rs7927894) is in complete linkage disequilibrium (r^2=1) with rs7130588, and the two predisposing alleles (rs7927894:T and rs7130588:G) occur on the same haplotype. This finding indicates that the same underlying causal variant is likely to explain the association between this locus and the three diseases. Each copy of the rs7130588:G allele increases the risk of atopic dermatitis by 1·22-fold,[23] Crohn's disease by 1·16-fold,[22] and asthma in our study by 1·09-fold. The association with atopic dermatitis and our finding that rs7130588:G does not seem to increase asthma risk in non-atopic individuals collectively suggests that this allele directly increases the risk of allergic sensitisation and, if this develops, it increases the risk of subsequently developing asthma. This mechanism is consistent with the epidemiological findings that sensitisation, eczema, and allergic rhinitis often precede the development of asthma symptoms.[24–27] While this work was under review, Marenholz and colleagues[28] also reported an association between 11q13.5 and asthma, although not at the genome-wide significance level. Results from that study are also consistent with an effect for the 11q13.5 locus that is specific to allergic asthma. The leucine-rich repeat containing 32 gene (*LRRC32*) is a plausible causal candidate in this region, as it is expressed in activated Treg cells,[29] of which numbers and immune suppressive function seem to be impaired in asthma.[30]

Our analyses also provided independent evidence for association with four of the eight loci previously reported to associate with asthma risk at the genome-wide significance level, specifically with the *ORMDL3* locus, *IL18R1, IL33*, and *IL2RB*.[5] The reported SNPs for *HLA-DQ,* and *SMAD3* had consistent but non-significant evidence for association in our study; these results suggest that they might represent true risk factors for asthma in the population we studied, but have an associated risk that is lower than originally reported.[5] On

Panel: **Research in context**

Systematic review

Hundreds of studies have attempted to map genes that contribute to the risk of development of asthma. Most linkage and candidate-gene association studies were underpowered to detect genetic loci with effect sizes now considered realistic for common diseases[16] and so often the identified regions or genes failed to replicate in independent studies. As a result, before 2007, more than 100 loci had been implicated in asthma causation,[17] but which, if any, represented genuine risk factors was largely unclear. Since 2007, genome-wide association studies (GWAS) have contributed significantly to change this landscape. In the largest GWAS of asthma published so far, the GABRIEL consortium[5] identified six loci associated with asthma risk at the genome-wide significance level: *IL18R1*, *HLA-DQ*, *IL33*, *SMAD3*, the *ORMDL3* locus, and *IL2RB*. Given the size of that study, these represent the most convincing genetic risk factors for asthma reported until now. However, each explains only a small fraction of the disease heritability, indicating that many more risk loci remain to be identified. This can be achieved by expanding and combining existing asthma GWAS, as we have done in this study.

Interpretation

Our study identifies two additional loci with genome-wide significant association with asthma risk: *IL6R* and chromosome 11q13.5. The *IL6R* finding raises the possibility that an approved IL6R antagonist could be effective to treat asthma. We also provide independent support for four of the six GABRIEL loci: *IL18R1*, *IL33*, *ORMDL3*, and *IL2RB*. Taken together, these results support the hypothesis that a genetic dysregulation of cytokine signalling increases disease risk.

the other hand, we found no supporting evidence for an association with *PDE4D*[2] or *DENND1B*[3] variants, despite appropriate power. Larger meta-analyses of available GWAS are in progress and will be able to study these loci in greater detail.

We also investigated the hypothesis that the genetic component of asthma risk includes a highly polygenic contribution, with hundreds or potentially thousands of variants that individually explain only a very small fraction of the disease heritability, as suggested for other traits such as schizophrenia[31] and height.[32] Consistent with this hypothesis, we observed that quantitative genetic scores that represented the combined effect of thousands of common SNPs—each individually influencing asthma risk only weakly (eg, median OR=1·05 for group 3 in figure 2) in the GABRIEL study[5]—were significantly associated with asthma case-control status in our study. These results thus suggest that many of these SNPs are either in high linkage disequilibrium with true causal variants that are common in the population but influence disease risk weakly, or they are in low linkage disequilibrium with causal variants that are rarer but increase disease risk more strongly. The presence of both types of variants is not mutually exclusive; very large sample sizes will be needed to identify these through GWAS with genome-wide significance. The multi-SNP scores tested here, despite being significantly associated with asthma risk, provided low discrimination in disease status (ie, low sensitivity and specificity) and so currently have little or no diagnostic utility per se, which is consistent with recent findings.[31,33,34] However, as larger asthma GWAS are done, SNP effects will be estimated more precisely, and this will improve the discrimination accuracy of genome-wide SNP scores.

Lastly, our analysis of SNPs previously reported to associate with immune or inflammatory diseases identified several loci that could represent genuine asthma risk factors with modest effects. These include variants near plausible functional candidates, such as *NDFIP1*, which causes severe inflammation of the skin and lung when knocked-out in mice[35] and *BACH2*, a B-cell-specific transcription repressor that is a key regulator of antibody response.[36] Further studies are needed to confirm these as genuine asthma risk loci.

When interpreting results from this study, it is important to recognise that we used a broad definition of asthma status, which grouped together not only clinically diagnosed and self-reported physician-diagnosed cases, but also groups of cases that were probably exposed to different environmental risk factors. Similarly, asthma controls included both asthma-free and asthma-unknown individuals, with limited information on atopic status. As a result, our primary association analysis provided improved power to detect risk loci with homogeneous effects across different asthma subtypes but was likely underpowered to detect loci with subtype-specific effects. Furthermore, a large fraction of SNPs discovered by the 1000 Genomes Project were imputed with modest confidence. For these SNPs, power to detect an association with asthma will also have been reduced.

In conclusion, we identified novel variants in *IL6R* and chromosome 11q13.5 with genome-wide significant association with asthma. The *IL6R* findings further support the hypothesis that a genetic dysregulation of cytokine signalling increases disease risk and raise the possibility that tocilizumab may be effective to treat asthma, perhaps in a genotype-dependent manner; studies that address this possibility are warranted. At this stage, it is unclear which gene underlies the association with 11q13.5. Given that no specific gene in this region has been directly implicated in allergic disease previously, further characterisation of this region of association is likely to discover novel molecular mechanisms involved in the causality of eczema, atopy, and asthma. Lastly, our results are consistent with the contribution of hundreds or potentially thousands of

variants with weak effects on asthma risk, which can be identified through larger GWAS as already shown for other diseases.[13]

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Conflicts of interest
MAF owns stock in Illumina, Roche, and Complete Genomics. PST's institution has received consulting fees from GlaxoSmithKline as a result of activities outside this work. PST has received lecture fees from GlaxoSmithKline and Novartis. GTO has received consulting fees from Novartis and Sunovion Inc, as a result of work unrelated to this study. GBM was a board member of Novartis; GBM's institution received fees for lectures and preparation of educational presentations from Boehringer Ingelheim and AstraZeneca. CFR received consulting fees from GlaxoSmithKline and Merck as a result of activities outside this work. ASK's institution received speaker's fees from Nutricia. All other authors declare that they have no conflicts of interest.

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