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Combining Genome Wide Association Study and lung eQTL analysis provides evidence for novel genes associated with asthma

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Abstract

Background—Genome wide association studies (GWAS) of asthma have identified single nucleotide polymorphisms (SNPs) that modestly increase the risk for asthma. This could be due to phenotypic heterogeneity of asthma. Bronchial hyperresponsiveness (BHR) is a phenotypic hallmark of asthma. We aim to identify susceptibility genes for asthma combined with BHR and analyse the presence of *cis*-eQTLs among replicated SNPs. Secondly, we compare the genetic association of SNPs previously associated with (doctor diagnosed) asthma to our GWAS of asthma with BHR.

Methods—A GWAS was performed in 920 asthmatics with BHR and 980 controls. Top SNPs of our GWAS were analysed in four replication cohorts and lung cis-eQTL analysis was performed on replicated SNPs. We investigated association of SNPs previously associated with asthma in our data.

Results—368 SNPs were followed up for replication. Six SNPs in genes encoding *ABI3BP, NAF1, MICA* and the 17q21 locus replicated in one or more cohorts, with one locus (17q21) achieving genome wide significance after meta-analysis. Five out of 6 replicated SNPs regulated 35 gene transcripts in whole lung. Eight of 20 asthma associated SNPs from previous GWAS were significantly associated with asthma and BHR. Three SNPs, in *IL-33* and *GSDMB,* showed larger effect sizes in our data compared to published literature.

Conclusions—Combining GWAS with subsequent lung eQTL analysis revealed disease associated SNPs regulating lung mRNA expression levels of potential new asthma genes. Adding BHR to the asthma definition does not lead to an overall larger genetic effect size than analysing (doctor's diagnosed) asthma.

Keywords

Asthma; Bronchial hyperresponsiveness; Genetics; GWAS; Gene expression

Introduction

Asthma is a chronic inflammatory disease of the airways, characterized by respiratory symptoms, reversible airflow obstruction and bronchial hyperresponsiveness (BHR). Asthma can be seen as a complex genetic disease caused by (interacting) genes and environmental factors. The heritability estimates of asthma vary between 40 and 70% (1). So far, genome wide association studies (GWAS) have robustly identified 15 loci for asthma at a genome wide significance level (2–12) (table E1).

Similarly to other complex disorders and traits, GWAS of asthma only identified risk alleles with a modest increased risk for asthma, with odds ratios (ORs) in general ranging from 1.1

to 1.4. One potential explanation for the modest contribution of genetics to asthma is its phenotypic heterogeneity. Asthma has a large variation in age of onset, severity, disease progression and presence of subphenotypes such as BHR and reversibility of airflow obstruction. Whereas most of asthma GWAS published to date rely on self-reported doctor's diagnosis of asthma (Table E1), it may be advantageous to define asthma by objective markers of the disease, such as BHR. This may result in less misclassification of asthma and less phenotypic heterogeneity, which may enhance the power to identify certain susceptibility genes (13).

It is often not clear how SNPs affect a trait under study. However, expression quantitative trait loci (eQTL) analysis has facilitated unravelling mechanisms of how disease-associated SNPs regulate gene transcription. SNPs can regulate expression of one or multiple nearby genes (*cis*-eQTL) or genes more up- or downstream (*trans*-eQTL) (14). Thus, by combining GWAS and eQTL analysis, gene transcripts can be identified that relate to the disease (15, 16). This may lead to the identification of new pathways in the pathogenesis of asthma. Here, we report the results of a GWAS of asthma with BHR in the Dutch population, and replication in four independent populations. We asses if the replicated top SNPs of our GWAS are eQTLs in lung tissue, characterize genetic pathways involved in asthma and associated top SNPs with subphenotypes. Finally, we compare our results to published GWAS of (doctor diagnosed) asthma, to investigate if differences in asthma definitions (ie. asthma with BHR vs. doctor diagnosed asthma/self-reported asthma) will lead to increased risk estimates for asthma.

Material and methods

Study subjects

The Dutch Asthma GWAS (DAG) cohort consists of in total 920 asthma cases and 980 controls, all from the northern of the Netherlands. The DAG cohort was genotyped in two phases and meta-analysed afterwards. For the first phase, 468 cases were selected from a trio and family study. The 469 controls were non-asthmatic spouses or pseudo-controls of untransmitted alleles in our trio design (GWAS I) (17–20). For the second phase (GWAS II), 452 asthmatics were selected from previous clinical and genetic studies performed by our research institute (17, 20–24). The 511 controls were selected from the COPACETIC study, a geographically matched population-based study on lung cancer screening in male smokers (25).

All asthmatics had a physician's diagnosis of asthma, asthma symptoms, and BHR to either histamine or methacholine. BHR was measured with a metacholine or histamine challenge test, and defined as PC_{20} histamine. Controls had no asthma or COPD, nor any evidence of significant airway obstruction. All studies were approved by the medical ethical committee.

Genotyping

DNA of subjects from GWAS I was genotyped on the Illumina 317 Chip (Illumina Inc, San Diego, CA). The 452 cases of GWAS II were genotyped with the Illumina 370 Duo Chip

and the 511 controls with Illumina Human Hap 600 (Illumina Inc, San Diego, CA). Details of the genotyping procedure and quality control are provided in the online supplement.

The discovery GWAS (DAG cohort) included 294,932 SNPs and were analysed using a logistic regression with an additive genetic model, and GWAS I and II were meta-analysed using Plink v1.07 (26). Potential population stratification was investigated with EIGENSTRAT (27) and OR and standard error used for meta-analysis were derived from logistic regression adjusted for 6 and 4 top eigenvectors in GWAS I and II respectively. Only overlapping SNPs between all platforms were used for the meta-analysis.

GWAS

SNPs with p-values $p<5*10^{-8}$ in our GWAS were considered genome wide significant. P-values $<1*10^{-3}$ were considered to provide suggestive evidence of association and were selected for replication in other populations. Subsequently, top results were meta-analysed together with four replication cohorts using Plink in fixed and random effect models. SNPs that replicated in one or more cohorts with a p-value of at least 10^{-5} after meta-analysis were selected for *cis*-eQTL.

Replication populations

Replication was performed in the MAGICS (n=1352), ALSPAC (n=5562), TENOR (n=2365) and SARP/CSGA/CAG (n=2377) studies (6, 28–30). All studies used a physician diagnosis of asthma (online methods supplement).

eQTL mapping in lung tissue

EQTLs were analysed per selected SNP in a large scale lung eQTL dataset (16) using linear regression in an additive genetic model using R 2.14.0, described in the online methods supplement. P-values $p<6*10^{-5}$ were considered significant, based on Bonferroni correction.

Subphenotype association

The subphenotyping was performed using the SNPs selected after replication. We selected subphenotypes which reflect the clinical heterogeneity of asthma. This resulted in 11 subphenotypes; age of onset before 4 and before 16 years, inhaled corticosteroid use, atopy, eosinophil counts, FEV_1% predicted, total IgE levels, severity of bronchial hyperresponsiveness, and neutrophils, $CD4^+$ and eosinophils in airway wall biopsies (31). The above subphenotypes were tested for association with SNPs using SPSS statistical software ver.20.0 (SPSS Inc., Chicago, IL). Detailed methods are described in the online methods supplement.

A p-value of $p<0.05$ was considered significant in the subphenotype analysis.

Asthma definition

A look-up in our data was performed on SNPs associated with asthma in previous GWA studies. If SNPs were not available, proxy SNPs with linkage disequilibrium of $r^2 \geq 0.8$ were selected. ORs were compared between our cohort and the previous studies.

Network analysis

The literature search, GWAS on asthma and BHR and subsequent eQTL analyses revealed several genes. All these genes were entered in a pathway analysis using STRING (32). Entered genes were checked for enrichment on protein-protein interaction. If enrichment is seen, FDR corrected significant Go biological processes and KEGG pathways were shown. As a sensitivity analysis, only GWAS genes nominally significant in our dataset and genome wide significant in previous studies, combined with replicated and eQTL genes were analysed.

Results

Patient characteristics of DAG cohort

The mean age of the included asthmatics was 34 years, 47% being males (table E2). Asthmatics in GWAS I and II had comparable characteristics. Most participants (77%) had asthma onset in childhood (median age of onset being 6 years), 63% showed reversibility of airflow obstruction (>9% of predicted) and 74% were atopic as evidenced by one or more positive skin prick tests.

GWAS results DAG cohort

A flow chart of all analyses is shown in figure 1. Figure E3 shows the Manhattan plot of the discovery GWAS. There was no evidence for population stratification after meta-analysis of the two discovery populations ($\lambda = 1.01$, QQ plot figure E4). No SNP met the genome wide significant threshold, yet 368 SNPs had suggestive results ($p<10^{-4}$) (table E12) and were selected for follow-up in 4 replication populations.

Replication of DAG cohort results

After replication and meta-analysis of the 368 suggestive SNPs, 7 SNPs at the 17q12 locus met genome wide significance (p-values between $1.43*10^{-14}$ and $2.55*10^{-20}$) (table 1). Two of these SNPs (rs11557467, rs2305480) were nonsynonymous. The 7 genome wide significant SNPs that passed Bonferroni correction significance threshold, can be tagged with 2 SNPs: rs2305480 and rs2290400 (figure E5).

Four other SNPs, not at the 17q12 locus, replicated at nominal p-value in one or more of the replication populations with a p-value of at least 10^{-5} after meta-analysis, yet they did not reach the Bonferroni significance threshold for the meta-analysis of the replication cohorts (chromosome 3; rs13091963 and rs1449302 near *ABI family, member 3 (NESH) binding protein* (*ABI3BP)*, chromosome 4; rs4132177 near *Nuclear Assembly Factor 1 ribonucleoprotein* (*NAF1)* and chromosome 6; rs2596560 near *MHC class I polypeptide-related sequence A (MICA))*. Table E7 provides information on function of the genes.

Lung eQTL analysis

The 2 tagging SNPs at the 17q12 locus and the 4 SNPs most strongly associated in the replication studies were selected for eQTL analysis. Five of the 6 SNPs showed strong *cis*-eQTL effects, including genes not previously implicated with asthma; among others *ABI3BP, SFTA2, RNF5, TUBB and ZFP57* (figure 2 and tables E8 and E9).

The two tagging SNPs of the 17q12 locus (rs2290400 and rs2305480) showed an overlap in eQTLs likely due to significant LD between SNPs (r^2=0.8). Out of the 7 significant eQTLs for rs2305480 and 9 significant eQTLs for rs2290400, 7 transcripts, covering 5 genes, showed an effect in the same direction (Figure 2).

Both SNPs on chromosome 3 (LD between SNPs r^2=0.49) regulate *ABI3BP* gene transcript, the presence of a minor allele being associated with down regulation of this transcript. Rs2596560 on chromosome 6, is an eQTL for 16 gene transcripts, covering 13 different genes. The top result is located in the *ZFP57* gene transcript (p-value: $2.66*10^{-19}$).

Association with selected subphenotypes within asthma patients

Our subphenotype analysis within the group of asthmatics revealed several associations in all selected SNPs associated with asthma, yet these p-values did not pass Bonferroni corrected significance threshold (table E10).

The risk alleles of the two SNPs on chromosome 17q12 were associated with a higher level of $CD4^+$ T-cells in asthma airway wall biopsies of asthmatics (rs2290400 T allele: Beta=0.34, p=0.04 and rs2305480 G allele: Beta=0.40 p=0.02). Rs2305480 was associated with a higher number eosinophils in airway wall biopsies of asthmatics (G allele: Beta=0.31 p=0.04). The risk alleles of both SNPs on chromosome 3 near *ABI3BP* were associated with lower levels of total IgE measured in blood within asthmatics (rs1449302 T allele: Beta= −0.19 p=0.02 and rs13091963 G allele: Beta=−0.22 p=<0.0095). The minor allele of rs4132177 on chromosome 4 near *NAF1*, associated with a lower risk of atopy within asthmatics (A allele: odds ratio=0.58, p=0.03). The risk allele of rs2596560 on chromosome 6 near *MICA,* associated with a higher risk of atopy within asthmatics (C allele: odds ratio=1.50, p=0.03).

Asthma definition

We compared the effect sizes of our results with the published GWAS results (table 2). Of the 24 SNPs previously reported, 20 SNPs (83%) were present in our genotyped dataset with either the same SNP or a proxy SNP (r^2>0.93). Of these 20 SNPs, 8 (40%) had the same direction of effect with p-values p≤0.05. The other 12 SNPs were not significantly associated, yet odds ratios were in the same directions as described in the literature. The SNPs in the genes *GSDMB* and *IL-33* had a larger effect size in the DAG cohort than reported in the literature. Other SNPs showed similar or smaller effect sizes.

Network analysis

After the GWAS analysis (table 1), the literature search (table 2) and eQTL analyses (table E9), 37 different genes were included in STRING. The proteins encoded by these genes showed significant protein-protein interaction enrichment (figure E6). In total 43 interactions were observed, where 5.4 interactions were expected ($p<1.0*10^{-12}$). 101 Significant GO biological processes and 24 significant KEGG pathways were found (table E110). Of our novel replicated genes found in eQTL analysis, *ABI3BP* was involved in the (positive) regulation of cell adhesion ($1.23*10^{-3}$). The sensitivity analysis excluding GWAS genes not significant in our dataset (28 genes) showed similar enrichment for protein-protein

interaction (number of protein-protein interactions observed: 20; expected 2.34, enrichment p value: $1.16*10^{-12}$).

Discussion

We associate asthma with presence of BHR to four regions of the genome after replication in four independent cohorts: the 17q12 locus (being genome wide significant), and provide evidence for association with three other loci: *ABI3BP, NAF1,*and *MICA*. We confirm previous observations, that the 17q12 locus is associated with a doctor's diagnosis of asthma and extend these observations by showing that it is also associated with asthma combined with BHR. Analyses of the top associated SNPs revealed that 5 out of 6 SNPs were strong eQTLs, which led to the identification of new gene transcripts of interest for asthma. The combination of the suggestive findings from our GWAS, the significant nominal replication in one or more replication cohorts as well as the functional effects on gene transcription in the lung provides suggestive evidence for the role of these genes in asthma. Furthermore, adding BHR as an objective marker of asthma does not result in larger genetic effect sizes when compared to published studies that use a doctor's diagnosis of asthma.

Seven SNPs on chromosome 17q provided genome wide significant associations with asthma in our analysis. The 17q12 locus is replicated in many studies and is one of the strongest loci for asthma (3, 5, 6, 8, 33, 34). Previous reports indicated that the 17q12 SNPs regulate gene expression of 6 genes: *ORMDL3, GSDMA, IKZF3, CRKRS, GSDMB* and *ZPBP2* in lung tissue (16) and lymphoblastoid cells (35, 36). We found *cis*-eQTLs in lung tissue for two additional genes; *PNMT* and *PERLD1.*

The function of the 17q12 locus is still not known. To get more inside in the possible function of the locus, we performed additional association analyses of our top SNPs with phenotypes related to asthma, allergy and airway inflammation that are available in our study (table E10). In these analyses we found that risk alleles of our tagging SNPs at the 17q12 locus were associated with an increased number of $CD4^+$ cells in the airway wall biopsies of asthmatics, suggesting involvement of these genes in the Th2 pathway in asthma. Moreover, a role of the 17q21 genes in the relation of eosinophils with asthma was suggested by the association of rs2305480 with the number of eosinophils in airway wall biopsies in asthmatics. Recently, Ormdl3 was shown to promote eosinophil trafficking to the sites of inflammation in a mouse model, and our data in asthma patients are consistent with this observation (37).

One SNP on chromosome 6 near *MICA* associated with atopy within asthmatics. This last finding is of interest, since *MICA* has been associated previously with allergic diseases in a GWAS study (38). In this previous study, having the risk allele of the SNP rs9266772 in MICA was associated with a higher risk of atopy (LD between rs9266772 and rs2596560 (our SNP) $r^2= 0.1$). In our study, we found that having the risk allele associates with a higher chance of being atopic within asthma. Furthermore, another study showed that levels of soluble MICA in blood is five times higher in paediatric children with a history of respiratory allergic symptoms after house dust mite exposure, compared to healthy children of the same age (39).

Our GWAS revealed *ABI3BP* as a new candidate gene involved in asthma. In the GO biological processes analysis *ABI3BP* was involved in (positive) regulation of cell adhesion. A study using *Abi3b* knock-out mice showed that *Abi3bp* controls mesenchymal stem cell proliferation and differentiation in bone marrow, lung and liver, interacting with Integrin-beta-1 (40). Thus, we speculate that *ABI3BP* may be involved in matrix remodelling in asthma, which could be subject of further study.

Five out of 6 top SNPs acted as eQTLs in lung tissue for one or several gene transcripts. In addition to the eQTL effects of the chromosome 17q12 SNPs, we also identified one SNP in in *MICA* to be associated with among others four new gene transcripts on chromosome 6q21 (*SFTA2, RNF5, TUBB* and *ZFP57)*. In the GO biological processes *RNF5* was involved in responses to bacteria, external stimulus, biotic organism, organic substance and chemical stimulus. The KEGG pathway analysis showed that *TUBB* was involved in the endoplasmic reticulum mediated phagocytosis. So far, no link with asthma is known.

Furthermore, rs2596560 on chromosome 6 regulates *HLA-DQB1*, which is involved in antigen presentation and has been associated with asthma in multiple studies (9, 29). Moreover, the SNP regulates *NOTCH4*, a potent regulator of *SMAD3*, another asthma gene found by GWAS (41). *NOTCH4* has already been associated with lung function in asthma (42, 43).

Combining GWAS and eQTL analysis revealed new candidate genes in our study. We identified a strong enrichment of *cis*-eQTLs among our top SNPs, which is consistent with the literature (14). We confirm that eQTL analysis can be used as a tool to associate different gene transcripts to a disease SNP, thereby providing insight into new candidate genes and possibly functional mechanisms in asthma (16).

We hypothesized that an asthma definition combined with the presence of BHR could lead to stronger effects of the SNPs on the disease risk compared to a doctor's diagnosis of asthma. However, our data did not support this general notion, as we only found three SNPs with an increased risk estimate compared to the published literature. This may indicate that BHR itself is a complex trait, regulated by multiple genetic and environmental factors, such as smoking and corticosteroid use. Another explanation could be that not only the effect of a single SNP is involved in asthma, but epistasis of different SNPs associate with asthma. If this is the case, SNPs by themselves could only have a small influence on the risk estimate, as seen in our study.

There are several strengths and limitations to be considered when interpreting our work. We analysed asthmatics with rich phenotypic measurements, including airway wall biopsies in 98 asthmatics. Although most asthmatics came from the same geographical region, ascertainment schemes were different (families, clinical cases) and patients of varying ages and asthma severity were combined in our analysis. Second, although we gathered almost 1,000 well characterized asthmatics, by current standards our sample size is limited.

Although we identified several associations of our top SNPs to different asthma subphenotypes, these associations would not survive a strict correction for multiple testing. To increase the sample size of the control population, we selected geographically matched

controls that had normal lung function and no respiratory symptoms, although they had significant smoking history. Nevertheless, we found several hits that were replicated in several populations.

Our replication cohorts did not include BHR in the diagnosis of asthma. Therefore it could be that our finding (asthma diagnosis with BHR) is diluted in the replication phase. GWA studies tend to overestimate the ORs, this phenomenon is called the ‘winners curse’ and has been described in literature (44). Since we compared the GWAS results from the literature to our results, it could be that the ORs of SNPs from literature have an upwards bias. It could therefore be that the differences we find in our paper are smaller due to the ‘winners curse’. Nowadays, GWAS’s are often performed on imputed data to expand the coverage of the genome. Although we only present a GWAS with genotype data, we did perform a GWAS on the hapmap 2 version 21 imputed data, but did not find any different region compared to the genotyped analysis.

EQTLs are known to be tissue specific. A drawback of our approach may be that whole lung tissue was used, which consists of different cellular subpopulations. Moreover, strong LD between SNPs (for example on chromosome 17) may further complicate the interpretation of our results, since a causal SNP is hard to determine in a region of strong LD. Nevertheless, our positive associations of SNPs with gene expression in lung tissue, relevant to asthma as a disease, provides further guidance for future studies of these transcripts in asthma.

In conclusion, the combination of GWAS and lung eQTL analysis led to the identification of potential new genes for asthma; *ABI3BP, NAF1* and *MICA*. Future studies are necessary to investigate the mechanisms of the new genes found that may potentially contribute to asthma development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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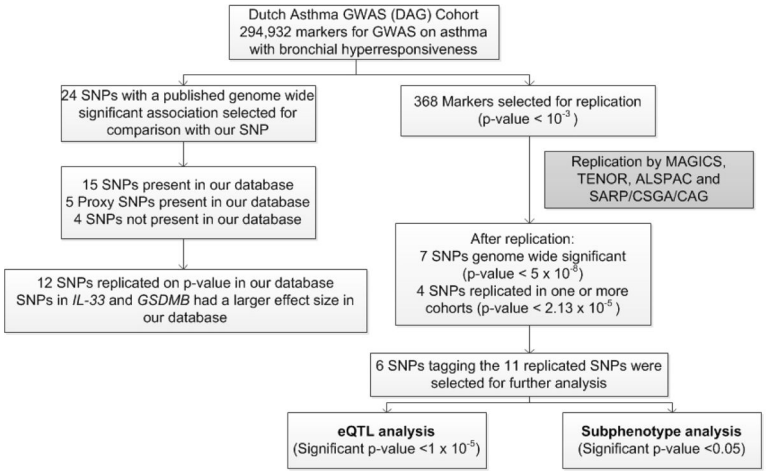

Figure 1.
Flow chart of all analysis in the Dutch Asthma GWAS.

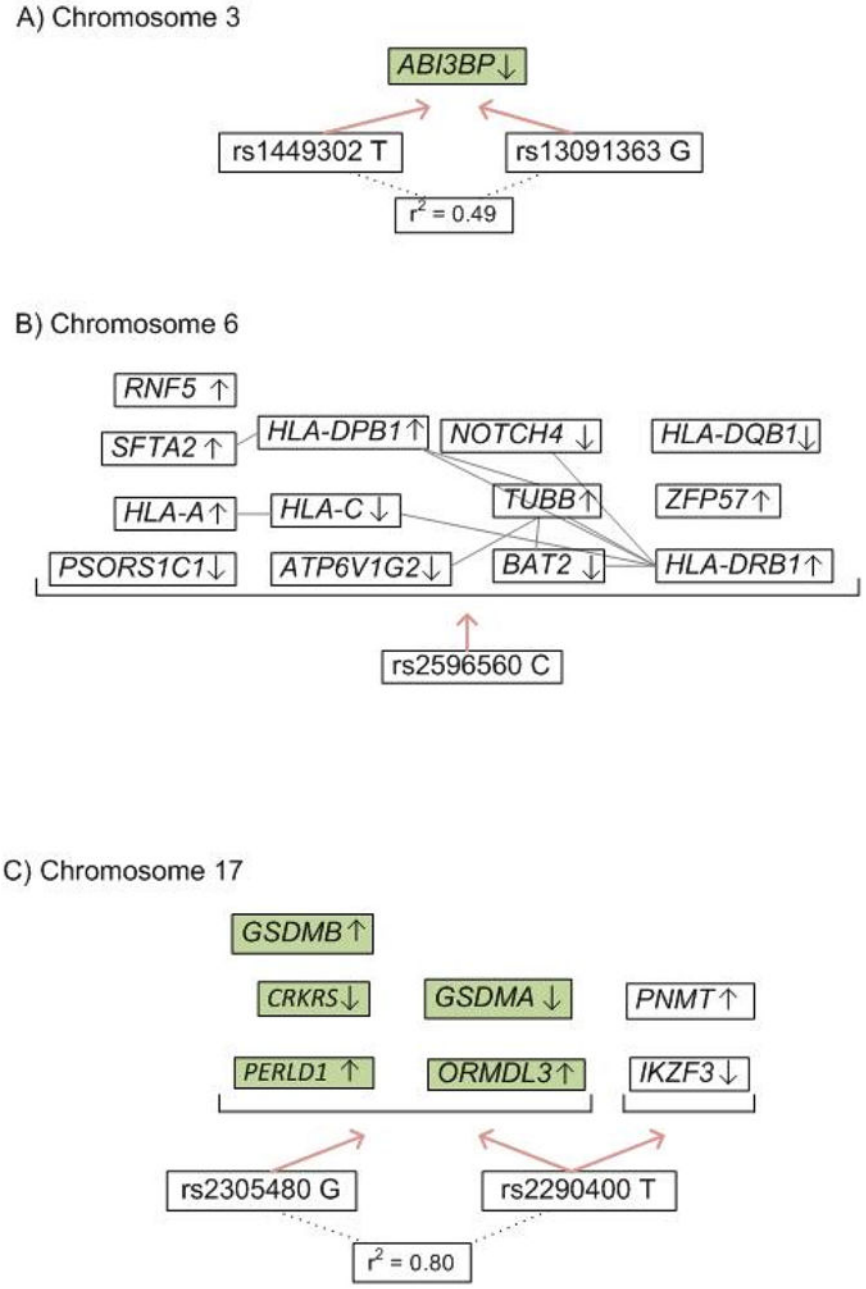

Figure 2.
Significant results of *cis*-eQTL analysis with top SNPs

Table 1

Top hits of Dutch Asthma GWAS, replication results in 4 replication populations and meta-analysis.

			Meta-analysis							Discovery		Replication cohorts								
			DAG + Replication cohorts				Replication cohorts			DAG Cohort n ca= 920 n co=980		Magics n ca= 680 n co= 672		Alspac n ca= n co=		Tenor n ca= 473 n co= 1892		SARP/ CSGA/CAG n ca= 813 n co= 1564		Annotation
CHR	BP	SNP	A1	OR	P	I	OR	P	I	OR	P	OR	P	OR	P	OR	P	OR	P	Closest gene
3	102212341	rs1449302	C	1.13	1.56E-05	28	1.10	2.52E-03	0	1.27	2.91E-04	1.03	6.96E-01	1.06	2.02E-01	1.20	2.26E-02	1.16	2.37E-02	*ABI3BP*
3	102220701	rs13091963	A	1.15	1.77E-05	58	1.11	3.24E-03	0	1.32	1.95E-04	1.09	3.09E-01	1.07	1.47E-01	1.11	2.57E-01	1.19	2.11E-02	*ABI3BP*
4	164177106	rs4132177	A	1.45	1.37E-05	42	1.31	7.93E-03	0	1.79	1.27E-04	1.10	6.79E-01	1.39	5.28E-03					*NAF1*
6	31463297	rs2596560	C	1.20	2.13E-05	0	1.16	5.25E-03	25	1.27	7.41E-04	1.34	4.98E-03			1.07	4.95E-01	1.12	1.46E-01	*MICA*
17	35229995	rs9303277	T	1.31	1.43E-14	57	1.34	2.70E-12	83	1.25	8.15E-04	1.61	3.38E-08	1.27	8.51E-07					*IKZF3*
17	35282160	rs11557467	T	1.32	3.29E-15	60	1.35	9.71E-13	84	1.25	5.22E-04	1.64	1.41E-08	1.27	5.78E-07					*ZPBP2*
17	35304874	rs8067378	G	1.32	3.27E-15	64	1.35	8.31E-13	86	1.25	5.88E-04	1.64	6.35E-09	1.27	7.32E-07					*GSDMB*
17	35315722	rs2305480	A	1.28	3.63E-17	60	1.28	5.92E-14	76	1.28	1.35E-04	1.69	1.82E-09	1.25	4.24E-06	1.15	9.13E-02	1.22	3.19E-03	*GSDMB*
17	35319766	rs2290400	C	1.31	2.55E-20	56	1.31	6.78E-17	73	1.30	7.79E-05	1.72	6.68E-10	1.28	3.80E-07	1.20	2.51E-02	1.26	5.56E-04	*GSDMB*
17	35323475	rs7216389	C	1.34	2.86E-16	69	1.36	5.29E-13	89	1.30	9.71E-05	1.69	1.15E-09	1.27	1.06E-06					*GSDMB*
17	35341943	rs4795405	T	1.26	1.90E-15	64	1.26	3.32E-12	77	1.30	1.19E-04	1.69	2.21E-09	1.20	7.75E-05	1.18	5.53E-02	1.19	1.02E-02	*ORMDL3*

RA: Risk allele in GWAS, P: P-value fixed effect, OR: Odds ratio fixed effect, DAG cohort: Dutch Asthma GWAS cohort, meta-analysis: meta-analysis of the replication cohorts I: measurement of heterogeneity between cohorts. Random effects of meta-analysis are shown in Table E12.

Missing SNPs were either not available in the database (ALSPAC) or were not analysed due to the restricted number of 18 SNPs which could be asked for replication (TENOR and SARP/SGA/CAG)

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Table 2

Results of GWAS loci significantly associated with asthma in previously published studies, compared to the results of the Dutch Asthma GWAS

First author (ref)	Number of cases included	Chr	Gene	Rs number	Risk allele	OR, 95% CI	OR Dutch Asthma GWAS	P value
Torgerson (3)	5,416	1	*PYHIN1*	rs1102000	T	1.32 (1.17–1.46)	NA	NA
		2	*IL1RL1*	rs10173081*	C	1.20 (1.11–1.29)	1.14	0.24
		5	*TSLP*	rs1837235*	C	1.19 (1.12–1.27)	1.11	0.13
		9	*IL33*	rs2381416*	C	1.18 (1.08–1.28)	1.25	$2.81*10^{-3}$
		17	*GSDMB*	rs11078927*	C	1.27 (1.20–1.34)	1.29	$1.35*10^{-4}$
Fereira(4)	2,669	1	*IL6R*	rs4129267	T	1.09 (1.06–1.12)	1.09	0.21
		11	11q13.5	rs7130588	G	1.09 (1.06–1.13)	1.11	0.13
Himes(2)	345	5	*PDE4D*	rs1588265	A	G: 0.85 (0.77–0.93)	0.85	0.02
Moffatt(6)	10,365	17	*ORMDL3*	rs7216398	T	NA	NA	NA
		17	*GSDMA*	rs3894194	A	1.17 (1.11–1.23)	1.14	0.04
		17	*GSDMB*	rs2305480	G	1.18 (1.11–1.23)	1.29	$1.35*10^{-4}$
		6	*HLA-DQ*	rs9273349	C	1.18 (1.13–1.24)	NA	NA
		22	*IL2RB*	rs2284033	G	1.12 (1.08–1.16)	1.14	0.05
		2	*IL18R1/IL1RL1*	rs3771166*	G	1.15 (1.10–1.20)	1.11	0.15
		9	*IL33*	rs1342326	C	1.20 (1.13–1.28)	1.36	$6.88*10^{-4}$
		15	*SMAD3*	rs744910	G	1.12 (1.09–1.16)	1.06	0.35
Sleiman(5)	793	1	*DENND1B*	rs2786098	C	1.42 (1.28–1.59)	NA	NA
Noguchi(7)	938	6	*HLA-DP*	rs987870	G	1.40 (1.26–1.55)	1.20	0.05
Hirota(8)	7,171	4	No gene annotated	rs7686660	T	1.16 (1.11–1.21)	1.03	0.68
		6	*HLA-DQ*	rs404860	A	1.21 (1.16–1.25)	1.03	0.77
		10	Gene desert, *GATA3*	rs10508372	C	1.16 (1.12–1.21)	1.08	0.63
		12	a.o. *IKZF4*	rs1701704	G	1.19 (1.14–1.25)	1.13	0.08
Anantharaman(10)	490	17	*PERLD1*	rs2941504	G	0.78 (0.65–0.94)	0.90	0.14
Bonnelykke (12)	1,173	7	*CDHR3*	rs6967330	A	1.26 (1.18–1.33)	1.01	0.91

*Results of proxy a SNP: rs10173081 proxy rs13431828 (r^2=1); rs1837235 proxy rs1461241 (r^2=1); rs2381416 proxy rs992969 (r^2=0.93); rs11078927 proxy rs2305480 (r^2=1); rs3771166 proxy rs10206753 (r^2=0.97)