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Examination of the relationship between variation at 17q21 and childhood wheeze phenotypes

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Abstract

Background—Genome-wide association studies have identified associations of genetic variants at 17q21 near *ORMDL3* with childhood asthma.

Objectives—To find out whether associations in this region are specific to particular asthma phenotypes and specific to *ORMDL3*.

Methods—We examined associations between 244 independent single nucleotide polymorphisms (SNPs) plus 13 previously identified asthma-related SNPs in the region between 34 and 36 Mb on chromosome 17 and early wheezing phenotypes, doctor-diagnosed asthma and atopy at 7½ years, bronchial hyper-responsiveness and lung function at 8½ years in 7,045 children from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study. With this, cis expression quantitative trait loci (eQTL) signals for the same SNPs were assessed in 875 samples across genes in the same region.

Results—The strongest evidence for phenotypic association was seen for persistent wheezing (rs8076131 near *ORMDL3*, relative risk ratio (RRR) 1.60 (95% CI 1.40, 1.84), $p=1.4\times10^{-11}$, rs2305480 near *GSDML* 1.60; 1.39-1.83, $p=1.5\times10^{-11}$ and rs9303277 near *IKZF3* 1.57; 1.37-1.79, $p=4.4\times10^{-11}$). Similar, but less precisely estimated effects were seen for intermediate-onset

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ONLINE REPOSITORY
The Online Repository contains additional methods and results, 10 figures including all conditional analysis and 9 tables.

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wheeze, but there was little evidence of associations with other wheezing phenotypes. There was some evidence of associations with bronchial hyper responsiveness. SNPs across the whole region show strong evidence of association with differential levels of expression at *GSDML*, *IKZF3* and *MED24*, as well as *ORMDL3*.

Conclusions—Associations of SNPs in the 17q21 locus are specific to asthma and to specific wheezing phenotypes, and are not explained by associations with intermediate phenotypes, such as atopy or lung function.

Keywords

ALSPAC; wheezing phenotypes; chromosome 17; *ORMDL3*; gene expression

INTRODUCTION

Asthma is a complex disorder resulting from interactions of genes and environmental factors [12]. Associations of candidate genes with asthma have been reported, but few were replicated in independent studies [3]. Lack of replication may be explained in part by difficulties in defining the asthma phenotype, and by gene-environment interactions leading to variable genetic expression in differing settings [4]. Recently, a genome wide association study (GWAS) identified multiple single nucleotide polymorphisms (SNPs) on locus 17q21 that were associated with asthma in several populations of European descent [5]. The association of SNPs in this region with asthma has been replicated in similar [6-9] and ethnically diverse [1011] populations. Most [6-8], but not all [12], studies found that associations were stronger in asthma beginning in early childhood.

Heterogeneity in early childhood wheezing illness and its association with subsequent asthma [1314] led us to describe [15] and replicate [16] phenotypes of wheezing during the first seven years of childhood, based on data from population-based birth cohort studies. These phenotypes are differentially associated with physician-diagnosed asthma, atopy, lung function and bronchial responsiveness [15]. Investigation of the biological origins of well-characterized asthma-related phenotypes is crucial to the better understanding of this often ill-defined clinical entity. Evidence points to early childhood as a critical period in asthma development [17] so we aimed to assess the association of asthma-related phenotypes with a comprehensive set of common, independent variants within the previously identified asthma-associated region of chromosome 17, and to examine the association of these variants with gene expression in the same region.

SUBJECTS AND METHODS

Subjects

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a longitudinal, population-based birth cohort study that recruited pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992. The study methodology has been described in detail previously [18]. Ethical approval was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

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Asthma outcomes and wheezing phenotypes

Reports of physician-diagnosed asthma were obtained from questionnaires sent to the mother when the child was aged 7½ years. Skin prick testing (SPT) was performed in all participants at a research clinic at age 7½ years [19]. A positive response was defined as a mean weal diameter of >2 mm with no response to negative control solution, and atopy was defined as a positive response to one or more of house dust mite, cat or mixed grass. Spirometry in all participants and bronchial responsiveness to methacholine was measured in a research clinic at age 8½ years [20]. Forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and forced expiratory flow 25–75% (FEF_{25_75}) were converted to sex-, age- and height-adjusted z-scores. Bronchial hyper-responsiveness (BHR) was defined as being in the highest tertile of the dose-response slope. Responses to the question, “In the past 12 (6 for first questionnaire) months has (your child) had wheezing/wheezing with whistling on the chest?” at 6, 18, 30, 42, 54, 69 and 81 months after birth were used in a longitudinal latent class analysis to define phenotypes of childhood wheezing [15], in children with at least two questionnaire responses. The best fitting model resulted in six phenotypes: 1.”never/infrequent wheeze “ with approximately 10% prevalence of wheezing at 6 months and declining prevalence of sporadic wheeze thereafter, including children who never reported wheeze; 2.”transient early wheeze” with 50–60% prevalence of wheeze up to 18 months, declining to low prevalence from 42 months; 3.”prolonged early wheeze” with peak prevalence of wheeze around 65% at 30 months, declining to low prevalence from 69 months; 4.”intermediate onset wheeze” with low prevalence of wheeze up to 18 months, rising rapidly to high prevalence from age 42 months; 5.”late onset wheeze” with approximately 20% prevalence of wheeze up to 42 months, rising to more than 50% prevalence thereafter; and 6.”persistent wheeze” (7%) with 65% prevalence of wheeze at 6 months and approximately 90% prevalence thereafter. Trajectories of prevalence of wheeze for each phenotype are presented in Figure 1 of Henderson et al.[15] and Figure 1 of Savenjie & Granell et al. [16].

Genetic data

Details on genotyping, quality control and exclusions can be found in the online repository.

Previously reported SNPs associated with childhood asthma are located in the region containing *ORMDL3* and 14 nearby genes (Online Table A1 & Figure 1), between 35.0 and 35.5Mb on chromosome 17 [5]. To allow for associations outside this locus, SNPs within the region between 34.0 and 36.0 Mb were extracted in the form of allelic dosages. To avoid redundancy, linkage disequilibrium (LD) based data pruning was undertaken based on a pairwise tagging approach. Using analysis software Haploview v4.2 [21] and after taking best genotypes from the imputation process (best guess genotypes were only used for the variant pruning process), this removed SNPs correlated with remaining tags at $r^2 \geq 0.80$. From an initial set of 749 SNPs across the region, pruning yielded a working data set of 257 variants including 13 SNPs at the 17q21 locus (35.2 to 35.4 Mb) previously reported to be associated with asthma. As a sensitivity analysis and to further explore the patterns of association with gene expression, a further pruned SNP set was generated using a more stringent threshold $r^2 \geq 0.20$, yielding a set of 63 variants.

Expression data

mRNA-quantified expression data were collected for 947 unrelated ALSPAC participants from lymphoblastoid cell lines (LCLs) established from blood samples taken when the children were 9 years old. Of these, 875 also had genotypic data available and 815 also had data on asthma status at 7½ years. For more technical details see the online repository.

Children with expression data were selected on the basis of availability of cell lines and completeness of phenotypic data across a wide range of outcomes, neither of which was associated with asthma outcome.

Statistical analyses

Multinomial logistic regression was used to estimate relative risk ratios (RRR), also known as “multinomial odds ratios”, with 95% confidence intervals (CI) and p-values for associations of SNP dosages with wheezing phenotypes (never/infrequent wheeze was used as the reference group). Posterior probabilities of phenotype membership for each child were used as weights in the regression models, in order to account for uncertainty in phenotype membership. Logistic, ordinal logistic and linear regression models were used to estimate associations of SNP dosages with asthma, atopy, BHR and lung function outcomes. We included terms for interaction with atopy in logistic regression models of the association of the top-ranked SNPs with asthma. The direction of association depends on the arbitrary assignment of one allele (that with greatest population frequency) as “wild-type”. Therefore, RRRs and odds ratios (ORs) less than 1 were inverted, and mean differences were coded as absolute values, so all associations are reported as positive and reported risk alleles are swapped accordingly. Conditional analyses were performed by adjusting for the dosages of 13 SNPs previously reported as asthma-related[52223] (Online Table A1). Q-Q plots were used to assess overall evidence for associations. The cumulative incidence of wheezing from 6 to 81 months was estimated using life tables in a survival-time data framework. We estimated the population-attributable risk fraction (PARF) to quantify the reduction in prevalence of a wheezing phenotype if the genotype in all subjects was changed to “no risk alleles”:

$$\mathrm{PARF}=1-\frac{\text{Average probability of wheezing phenotype in the }''\text{no risk alleles}''\text{ group}}{\text{Average probability of wheezing phenotype across all genotypes}}$$

Normalized and transformed expression levels at each of the 15 genes (*STARD3* to *MED24*) in the region 35.0 to 35.5Mb were regressed on SNP dosages in a linear regression model assuming an additive genetic model as for other analyses. The variance in expression levels explained by genotypic variation (r^2) was reported along with p-values adjusted for 3855 independent tests (945 when the reduced SNP set based on a linkage disequilibrium (LD) pruning threshold of $r^2 \geq 0.8$ was used) using Bonferroni-corrections.

All analyses were performed using Stata Version 11.0 (Stata Corp, College Station, Texas).

RESULTS

A total of 7,045 children with central-European ancestry had at least two questionnaire responses on wheezing between 6 and 81 months and data on SNP dosages. Of these children, 3595 (51.0%) were males, 1085 (19.7%) had asthma, 3140 (46.7%) had an asthmatic or allergic mother, 1516 (22.3%) were exposed to maternal smoking during pregnancy and 948 (20.1%) were atopic (Table 1). The estimated numbers (%)with each wheezing phenotype were never/infrequent (4815, 68.4%), transient early (715, 10.2%), prolonged early (526, 7.5%), intermediate-onset (162, 2.3%), late-onset (335, 4.8%) and persistent wheezing (492, 7.0%)

Associations of *ORMDL3* region SNPs with doctor-diagnosed asthma, atopy, BHR and lung function

Q-Q plots showed substantial deviation from expected values for doctor-diagnosed asthma ever at 7½ years, and small deviation for BHR but not for the other phenotypes (Online Figure A1). Consistent with previously reported GWAS, the strongest evidence of association with doctor-diagnosed asthma was for variants in the region between 35.0 and 35.5 Mb (Figure 2). Corresponding conditional p-values are plotted in the Online Figure A2 which shows that the 13 previously asthma-related SNPs explain the majority of the observed associations. The strongest evidence for association with doctor-diagnosed asthma was for the previously asthma-related SNPs: rs9303277 near *IKZF3* (OR = 1.30; 95% CI 1.19-1.43, p =4.6×10^{-8}) and rs2290400 near *GSDML* (OR = 1.30; 1.18-1.43, p=4.7×10^{-8} (Online Table A2 & A3). The strongest evidence of association with BHR was for rs1042658 near *CSF3* (1.20; 1.10-1.31, p=7.5×10^{-5}) and for the previously asthma-related SNP rs3859192 near *GSDM1* (1.19; 1.09-1.30, p=8.2×10^{-5}) (Online Table A3). Corresponding figures for crude (Online Figure A3) and conditional (Online Figure A4) ORs and mean differences for the various asthma outcomes can be found in the online repository. No evidence of effect modification by atopy was observed in the association between top 10 ranked SNPs and asthma (Online Table A4). Conditioning on each of the top SNPs did not show evidence of independent associations with doctor-diagnosed asthma ever (See Online Repository and Online Table A5).

Associations of *ORMDL3* region SNPs with wheezing phenotypes

For the intermediate-onset and persistent wheezing phenotypes, Q-Q plots showed substantial deviations from expected values (Online Figure A5). The strongest evidence of association was for SNPs in the region between 35.2 and 35.4 Mb with persistent wheezing, although there was some evidence of association for SNPs in the flanking regions between 35.0 and 35.2 and between 35.4 and 35.6 Mb (Figure 3). For the other phenotypes, there was little evidence of any association.

SNPs near the *IKZF3*, *ZPBP2*, *GSDML*, *ORMDL3* and *GSDM1* genes were associated with intermediate-onset (p-values ≤0.001) and persistent wheezing (p-values ≤1.5×10^{-9}) (Table 2). The magnitude of associations appeared similar for these two phenotypes (RRR in the range 1.46 to 1.60), and were generally larger than the corresponding odds ratios for association with doctor-diagnosed asthma (OR<1.30). The strongest evidence of association

was for persistent wheezing and rs8076131 near *ORMDL3* (RRR 1.60; 95% CI 1.40-1.84, $p=1.4\times10^{-11}$), rs2305480 near *GSDML* (1.60; 1.39-1.83, $p=1.5\times10^{-11}$) and rs9303277 near *IKZF3* (1.57; 1.37-1.79, $p=4.4\times10^{-11}$) (Table 2). Respective associations for the less frequent (162 children, prevalence 2.3%) intermediate-onset wheezing phenotype were: 1.60; 1.27-2.02, $p=7.5\times10^{-5}$, 1.58; 1.25-1.99, $p=1.1\times10^{-4}$ and 1.52; 1.21-1.91, $p=2.8\times10^{-4}$. In contrast, associations with other wheezing phenotypes were smaller (RRR < 1.20) (Table 2). Similarly, genes in the flanking regions were only associated with intermediate-onset and persistent wheezing, but the magnitude of associations was smaller. A list of the top crude associations for intermediate-onset and persistent wheezing can be found in the Online Table A3.

Conditional analyses including the 13 previously asthma-related SNPs essentially removed any evidence for association (Online Figure A6) and demonstrated that these original signals explain the majority of observed signal across the region. Corresponding figures for crude (Online Figure A7) and conditional (Online Figure A8) RRRs for the wheezing phenotypes can be found in the online repository.

Online Figure A9 shows a low cumulative incidence of wheezing up to approximately 6 months with a large increment between 6 and 18 months. Stratification by top SNPs rs8076131 near *ORMDL3* and rs2305480 near *GSDML* shows the lack of association with wheeze at 6 months, and emergence of association from age 18 months, together with possible departures from a per-allele genetic model (particularly for rs2305480). The estimated PARF for the top SNP rs8076131 near *ORMDL3* was 35% for intermediate-onset and 40% for persistent wheezing.

Associations of *ORMDL3* region SNPs with expression

Expression of *ORMDL3*, *GSDML*, *IKZF3* and *MED24* genes showed evidence for association with genetic variation in the region between 35.0 and 35.5 Mb (Figure 4). Expression of other genes showed little evidence for association (Online Figure A10). Of those SNPs showing strongest evidence for association with the wheezing phenotypes (as reported in Table 2), greatest evidence for association was exhibited by the SNPs: rs9303277 (in *IKZF3*) for *ORMDL3*, *IKZF3* and *GSDML* expression ($r^2=0.20$, Bonferroni corrected p-value $=1.39\times10^{-44}$, $r^2=0.13$, $p=2.53\times10^{-27}$ and $r^2=0.19$, $p=6.09\times10^{-44}$ respectively), rs11557467 (in *ZPBP2)* for *ORMDL3*, *IKZF3* and *GSDML* expression ($r^2=0.20$, $p=6.75\times10^{-45}$, $r^2=0.13$, $p=1.78\times10^{-26}$ and $r^2=0.20$, $p=3.64\times10^{-45}$ respectively), rs2290400 (in *GSDML*) for *ORMDL3*, *IKZF3* and *GSDML* expression ($r^2=0.20$, $p=3.96\times10^{-45}$, $r^2=0.12$, $p=2.43\times10^{-25}$ and $r^2=0.19$, $p=4.17\times10^{-43}$ respectively) and rs4378650 (in *ORMDL3*) for *ORMDL3*, *IKZF3* and *GSDML* expression ($r^2=0.18$, $p=8.00\times10^{-41}$, $r^2= 0.12$, $p=3.39\times10^{-23}$ and $r^2=0.16$, $p=8.39\times10^{-36}$ respectively) amongst other associations (Table 3 and online Table A6). The strongest evidence for variable expression at *MED24* was seen for rs1042658 (in *CSF3*) ($r^2=0.07$, $p=2.62\times10^{-12}$) which was the SNP showing the strongest association with BHR. A list of the top 10 crude associations for expression of *ORMDL3*, *GSDML*, *IKZF3* and *MED24* genes can be found in the online Table A7. In a reduced set of 63 SNPs based on a more stringent LD pruning threshold of $r^2\geq0.2$, associations with expression were still concentrated on the loci *IKZF3*, *GSDML*,

ORMDL3, *MED24* and *ZPBP2* (Online Table A8). We found no evidence of associations between expression and wheezing phenotypes (See online repository and online Table A9).

DISCUSSION

Common SNPs in the 17q21 locus are associated with childhood asthma. Based on a birth cohort study with detailed information on phenotypes of asthma and allergic disease we found that these SNPs were strongly associated with persistent and intermediate-onset childhood wheezing phenotypes, characterized by onset before age 30 months [15]. These SNPs were also strongly associated with a diagnosis of asthma in mid-childhood as previously reported[52223], however we found that the magnitude of these associations was smaller and the p-values were weaker than for persistent wheeze. Little evidence of associations with early wheezing that resolved, or with late-onset wheezing were found. We did not find evidence that SNPs in the 17q21 locus were associated with atopy or lung function in mid-childhood, but found some evidence of associations with BHR. SNPs in the 17q21 locus were associated with expression of the *ORMDL3*, *GSDML*, *IKZF3* and *MED24* genes but no association between asthma phenotypes and expression data was found. Our analyses were well-powered to detect associations of modest strength within phenotype groups. We have previously shown that these phenotypes have face validity in their associations with other markers of asthma and its intermediate phenotypes and can be replicated in independent data [16]. The phenotypes reported here have some consonance with those described by Stein & Martinez in the Tucson study [1314]. The transient early phenotype is similar in both studies, the ALSPAC prolonged early wheezing phenotype corresponds approximately to the Tucson “non-atopic wheezers”, and our intermediate-onset, late-onset and persistent wheezing phenotypes, all of which were associated with atopy and bronchial hyper-responsiveness, correspond approximately to the Tucson “IgE-associated wheeze/asthma” phenotype, although with earlier age of onset. Our study provides further evidence for the distinct nature of these phenotypes, based on their differential associations with genetic variants in the 17q21 region.

Studies reporting associations of SNPs in the 17q21 locus with early-onset asthma have used varying definitions of this outcome. Bouzigon et al. used ordered subset regression to classify early-onset asthma (<4 years), which was strongly associated with four SNPS in the 17q21 locus [7]. Flory et al. reported that associations did not differ according to age of onset [12], while Bisgaard et al. reported evidence of a stronger association of SNPs in the 17q12-21 region with asthma that began below the age of 3 years [6] and, consistent with our results, a lack of association with allergic sensitization. They suggested that these associations reflected increased susceptibility to non-atopic asthma. However, the situation may be more complicated than a simple dichotomy between atopic and non-atopic disease. Galanter et al. reported stronger associations between *ORMDL3* SNPs and asthma when the latter was associated with raised serum IgE concentrations [10].

In the first report of the association of the 17q21 locus with asthma, Moffatt et al. suggested *ORMDL3* as a promising candidate on the basis of gene expression studies in EBV-transformed lymphoblastoid cell lines [5]. However, the function of *ORMDL3* remains to be fully elucidated and it is possible that other genes in this region, or more distant genes,

contain the true causal variants [24]. We confirmed that SNPs at the 17q21 locus were associated with expression of *ORMDL3* but, additionally, identified associations with *GSDML*, *IKZF3* and *MED24*. *ORMDL3* encodes a member of a family of transmembrane proteins that are anchored in the endoplasmic reticulum, ubiquitously expressed in adult and foetal tissues and show evidence of functional conservation between species [25]. It has recently been reported to bind and inhibit sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) activity, which may contribute to airway remodelling in asthma [26] and provides a plausible mechanisms whereby *ORMDL3* may contribute to non-allergic mechanisms of airway inflammatory responses [27].

The loci, other than *ORMDL3*, implicated in this study have a number of possible connections to the persistent wheeze phenotype and to measureable changes in gene expression. Firstly, *GSDML*, *IKZF3* and *ZPBP2* (and *MED24/THRAP4* by way of regional association) have all been directly highlighted in GWAS for conditions that have an aetiological contribution from autoimmunity, including ulcerative colitis [28], rheumatoid arthritis [29], biliary cirrhosis [30] and type 1 diabetes [31]. Whilst not providing direct evidence that observed associations are underpinned by autoimmunity, this does confirm the likely importance of immunological processes in the development of wheezing phenotypes. Secondly, these loci have been shown to play roles in the action and control of transcription and gene expression. For example, *IKZF3* encodes a member of the IKAROS family of zinc finger proteins which are haemopoietic-specific transcription factors involved in the regulation of lymphocyte development [32]. Lastly, observed associations between genetic variation here and expression patterns may be the result of more global patterns of transcriptional activity. Variation at *MED24/THRAP4* encodes a component of the mediator complex which is thought to be required for the expression of almost all genes [3334], which may be related to non-specific differential expression at multiple loci but which has downstream effects that contribute to the overall risk of persistent wheeze. Taken together, these aspects make it entirely plausible that variation at multiple loci contribute to observed phenotypes but considerable effort will be required to elucidate precise mechanisms. Although associations of wheezing phenotype related SNPs with expression data were found in this study, the absence of a direct association of expression profiles with asthma phenotypes is likely to be due to measurement error, confounding and sample size limitation.

The results of this study suggest that associations of SNPs in the 17q21 locus with susceptibility to asthma in early and mid childhood are specific to asthma and to specific wheezing phenotypes, and are not explained by associations with intermediate phenotypes, such as atopy or lung function. Elucidation of causal mechanisms has the potential to facilitate disease prediction in children with wheezing in preschool years, contribute to separation of discrete phenotypes of childhood asthma, and hence identify risk factors that may be targets for primary or secondary disease prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ALSPAC	Avon longitudinal study of parents and children
BHR	Bronchial hyper-responsiveness
GWAS	genomewide association study
FEV_1	force expiratory volume per second
FEF_{25-75}	forced expiratory flow 25–75%
FVC	forced vital capacity
LSDRS	least square dose response slope
Mb	Megabase
OR	odd ratio
RRR	relative risk ratio
SNP	single nucleotide polymorphism

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KEY MESSAGES

- Asthma-related genetic variants are associated with intermediate-onset and persistent wheezing but not with early-onset wheezing
- These associations are not explained by associations with intermediate phenotypes, such as atopy or lung function
- Elucidation of causal mechanisms has the potential to identify risk factors that may be targets for primary or secondary disease prevention

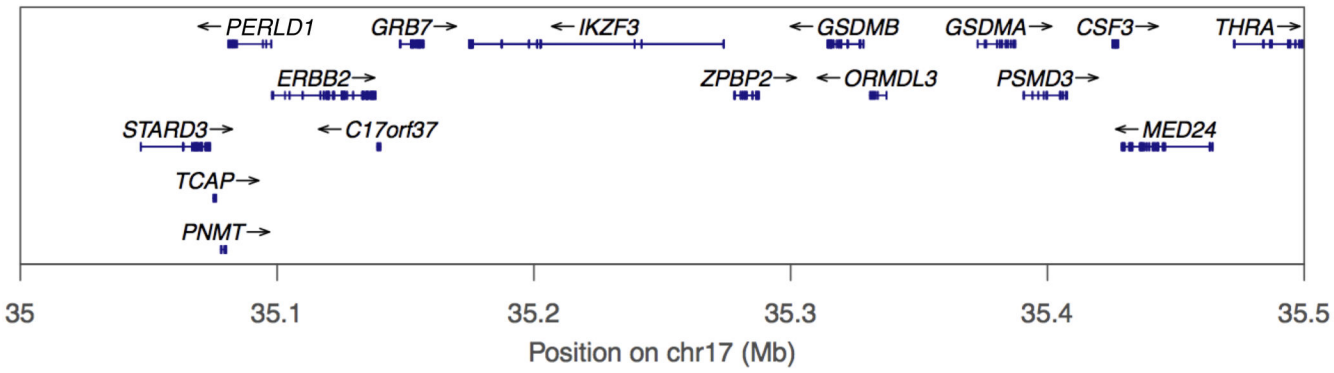

Figure 1.

Schematic of chromosome 17 between 35.0 and 35.5 Mb. Vertical red lines delineate region between 35.2 and 35.4 Mb around *ORMDL3* (marked in green) containing 13 asthma-related SNPs [5, 22, 23].

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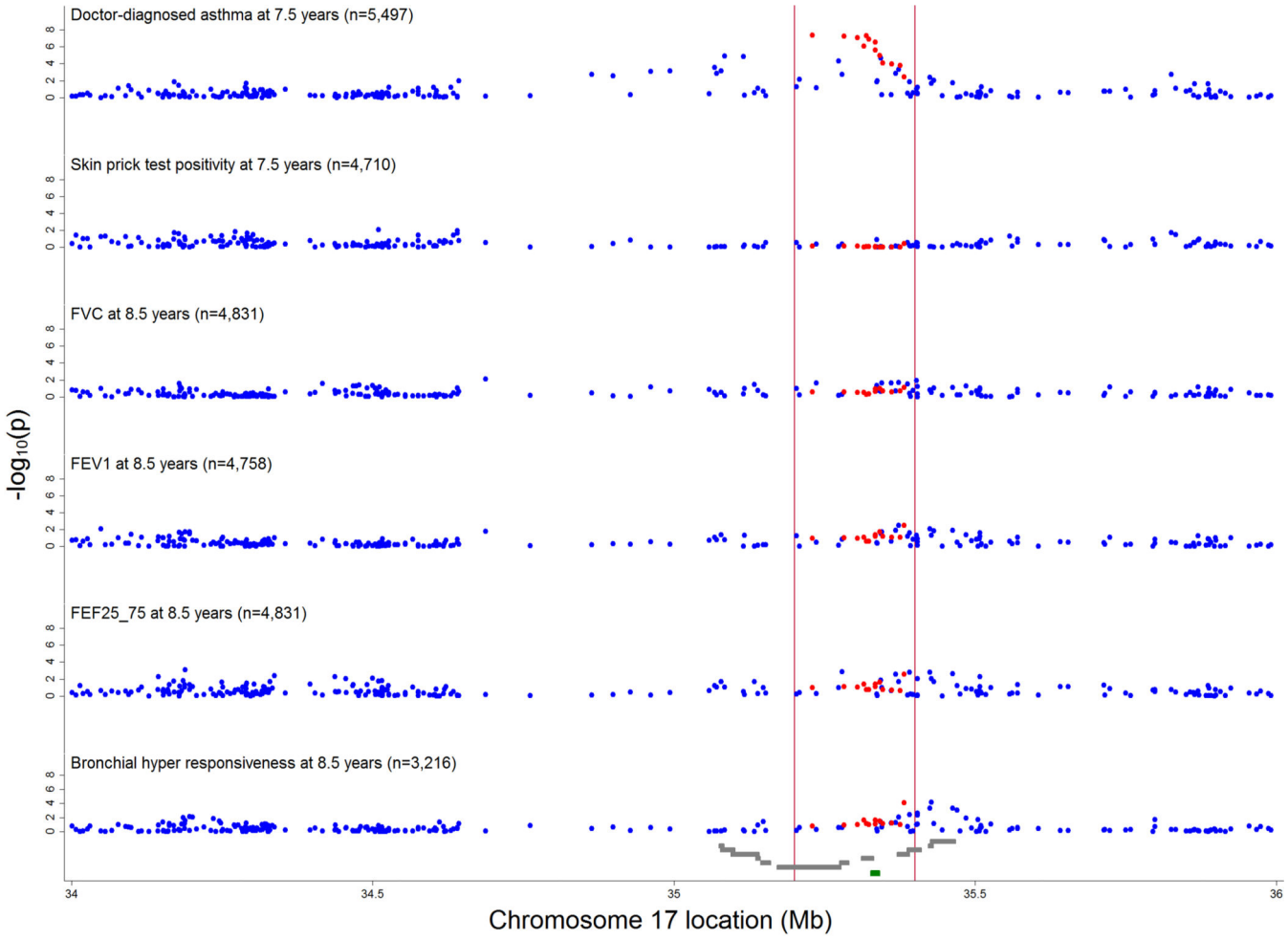

Figure 2.

Inverse log10 transformed P-values for crude associations of 257 independent SNPs between 34 and 36 Mb on chromosome 17 with doctor-diagnosed asthma ever, skin prick test positivity, bronchial hyper- responsiveness, FVC, FEV_1 and FEF_{25_75}. Vertical red lines delineate region between 35.2 and 35.4 Mb around *ORMDL3* (marked in green) containing 13 asthma-related SNPs [5, 22, 23] (highlighted in red). Grey gene tracks show loci in the order *PNMT, PERLD1, ERBB2, C17orf37, GRB7, IKZF3, ZPBP2, GSDML, GSDM1, PSMD3, CSF3, MED24.*

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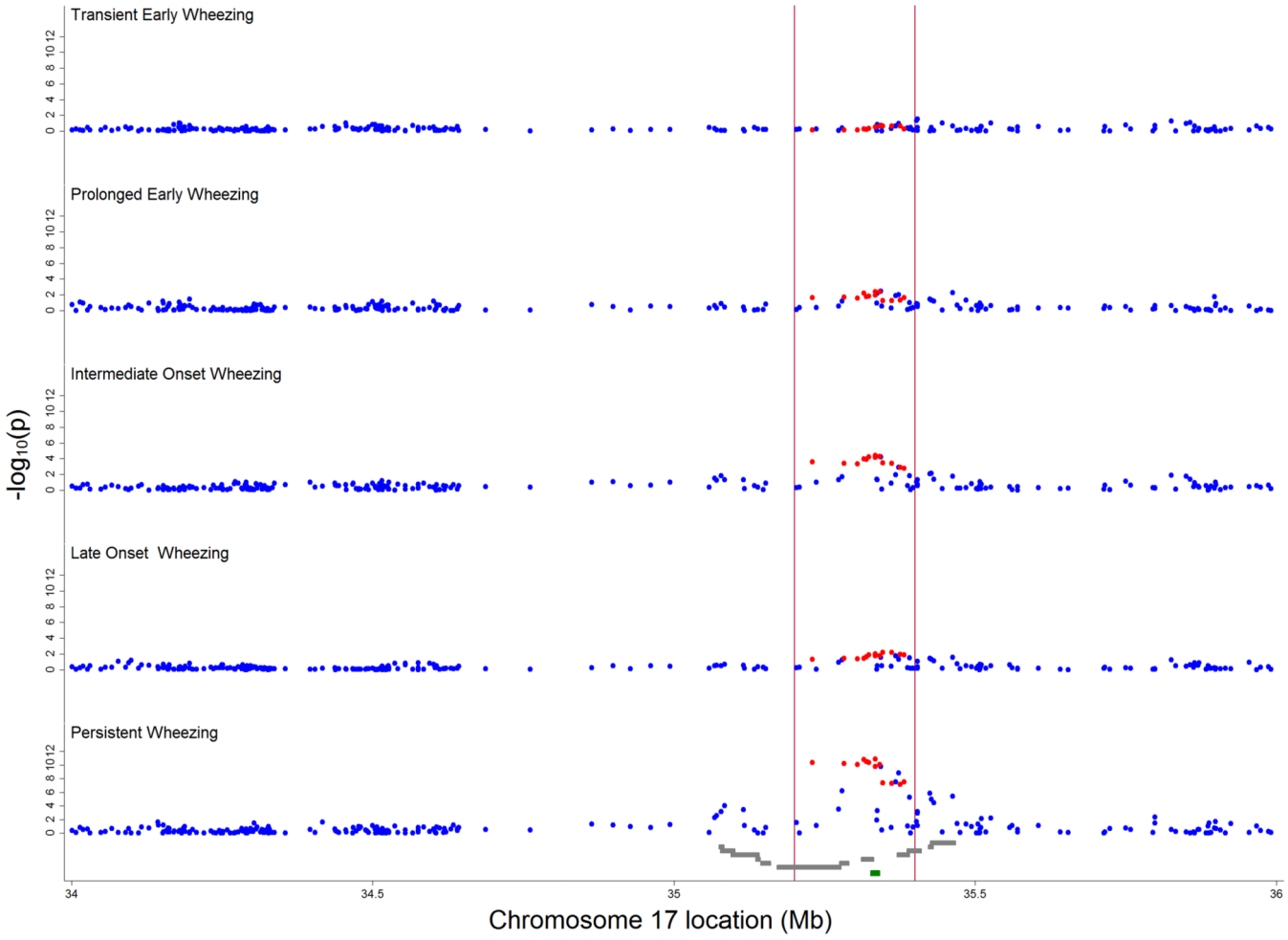

Figure 3.

Inverse log10 transformed P-values for crude associations of 257 independent SNPs between 34 and 36 Mb on chromosome 17 with wheezing phenotypes (compared with never/infrequent wheeze). Vertical red lines delineate region between 35.2 and 35.4 Mb around *ORMDL3* (marked in green) containing 13 asthma-related SNPs [5, 22, 23] (highlighted in red). Grey gene tracks show loci in the order *PNMT*, *PERLD1*, *ERBB2*, *C17orf37*, *GRB7*, *IKZF3*, *ZPBP2*, *GSDML*, *GSDM1*, *PSMD3*, *CSF3*, *MED24*.

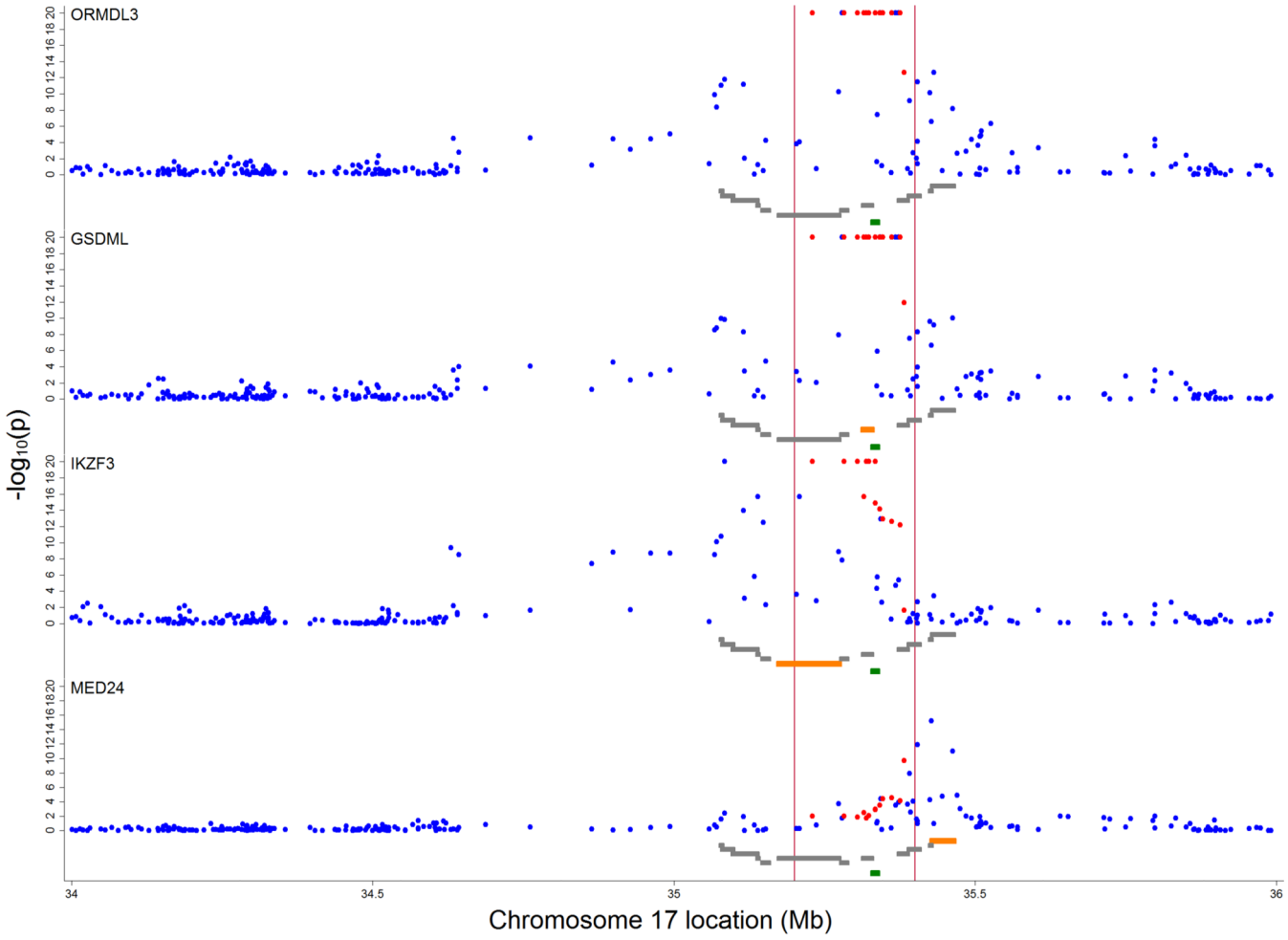

Figure 4.

Inverse log10 transformed P-values for crude associations of 257 independent SNPs between 34 and 36 Mb on chromosome 17 with gene specific expression levels. Vertical red lines delineate region between 35.2 and 35.4 Mb around *ORMDL3* (marked in green) containing 13 asthma-related SNPs [5, 22, 23] (highlighted in red). Grey gene tracks show loci in the order *PNMT*, *PERLD1*, *ERBB2*, *C17orf37*, *GRB7*, *IKZF3*, *ZPBP2*, *GSDML*, *GSDM1*, *PSMD3*, *CSF3*, *MED24* with the reported marker in orange. Figure shows results for *ORMDL3*, *GSDML*, *IKZF3* and *MED24*.

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Table 1

Characteristics of children included in analyses

	Number of children with data available	Number (%) of children with characteristic[a]	Sub-sample with expression data (n=947)	Number (%) with characteristic in subsample[a]
Male sex	7045	3595 (51.0)	875	413 (47.2)
Maternal history of asthma or allergy	6723	3140 (46.7)	855	423 (49.5)
Paternal history of asthma or allergy	4963	1994 (40.2)	671	267 (39.8)
Prenatal maternal smoking	6786	1516 (22.3)	857	136 (15.9)
Maternal lower education level[b]	6853	4012 (58.5)	864	445 (51.5)
Wheezing phenotype[c]	7045		947	
Never/Infrequent		4815 (68.4)		682 (72.0)
Transient Early		715 (10.2)		81 (8.6)
Prolonged Early		526 (7.5)		58 (6.1)
Intermediate Onset		162 (2.3)		20 (2.1)
Late Onset		335 (4.8)		48 (5.1)
Persistent		492 (7.0)		58 (6.1)
Doctor-diagnosed asthma ever by 7½ years	5497	1085 (19.7)	815	149 (18.3)
Skin prick test sensitivity at 7.5 years	4710	948 (20.1)	728	123 (16.9)
Mean [IQR] FVC[d] at 8.5 years (l)	4831	0.02 [1.26]	833	0.03 [1.23]
Mean [IQR] FEV_1[d] at 8.5 years (l)	4758	0.02 [1.29]	821	0.04 [1.20]
Mean [IQR] FEF_{25_75}[d] at 8.5 years (l/s)	4831	0.02 [1.33]	833	0.02 [1.21]
BHR[d] at 8.5 years	3216		875	
No BHR (LSDRS<0)		616 (19.2)		93 (15.5)
Positive BHR (1st tertile)		869 (27.0)		152 (25.3)
Positive BHR (2nd tertile)		867 (27.0)		184 (30.7)
Positive BHR (3rd tertile)		864 (27.0)		171 (28.5)

[a] Unless labeled as Mean [IQR]

[b] Educated to GCE level (school leaving certificate) or lower

[c] Estimated using posterior probabilities

[d] FVC= forced vital capacity (adjusted z-scores), FEV_1= forced expiratory volume in 1 s (adjusted z-scores), FEF_{25_75}= forced 6 expiratory flow 25–75% (adjusted z-scores), BHR=bronchial hyper-responsiveness, LSDRS=least square dose response slope

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Table 2

Association of variants in the region of chromosome 17 between 35.0 and 35.5 Mb (around 17q21) with wheezing phenotypes in 7,045 children

Nearby gene [a]	SNP [b]	Position (Mb)	MAF [c]	Overall P-value	Ph [d]	Risk allele±	RRR (95%CI) [e]	P-value for Ph	Other SNPs (overall p-value) [f]
Region between 35.0 and 35.2 Mb [g]									
PNMT	rs876493	35.078071	0.41	0.002	TE	A (common)	1.02 (0.91, 1.14)	0.79	NA
					PE	G	1.10 (0.97, 1.25)	0.13	
					IO	G	1.35 (1.06, 1.73)	0.02	
					LO	G	1.08 (0.93, 1.26)	0.33	
					P	G	1.29 (1.11, 1.48)	6.52×10^{-4}	
PERLD1	rs2941504	35.084426	0.31	0.001	TE	G (common)	1.01 (0.91, 1.13)	0.81	NA
					PE	A	1.06 (0.94, 1.20)	0.36	
					IO	A	1.27 (1.00, 1.60)	0.05	
					LO	A	1.10 (0.95, 1.28)	0.21	
					P	A	1.31 (1.14, 1.51)	9.98×10^{-5}	
ERBB2	rs2952155	35.229995	0.24	0.004	TE	C (common)	1.03 (0.91, 1.16)	0.64	*No signals*
					PE	T	1.06 (0.93, 1.22)	0.39	
					IO	T	1.29 (1.00, 1.67)	0.05	
					LO	T	1.10 (0.93, 1.29)	0.27	
					P	T	1.31 (1.13, 1.53)	3.68×10^{-4}	
Region between 35.2 and 35.4 Mb									
IKZF3	**rs9303277**	35.229995	0.48	2.24×10^{-11}	TE	T (common)	1.02 (0.92, 1.12)	0.77	rs9635726 (0.004)
					PE	C	1.14 (1.02, 1.28)	0.024	
					IO	C	1.52 (1.21, 1.91)	2.81×10^{-4}	
					LO	C	1.15 (1.00, 1.32)	0.05	
					P	C	1.57 (1.37, 1.79)	4.43×10^{-11}	
ZPBP2	**rs11557467**	35.282160	0.48	3.52×10^{-11}	TE	T (common)	1.02 (0.92, 1.13)	0.73	**rs8067378** (6.37×10^{-11})
					PE	G	1.14 (1.02, 1.28)	0.02	rs12150079 (3.57×10^{-6})
					IO	G	1.50 (1.20, 1.89)	4.11×10^{-4}	
					LO	G	1.16 (1.01, 1.33)	0.04	

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Nearby gene [a]	SNP [b]	Position (Mb)	MAF [c]	Overall P-value	Ph [d]	Risk allele±	RRR (95%CI) [e]	P-value for Ph	Other SNPs (overall p-value) [f]
					P	G	1.56 (1.37, 1.78)	5.99×10^{-11}	
GSDML	**rs2305480**	35.323475	0.48	1.69×10^{-12}	TE	T (common)	1.03 (0.93, 1.15)	0.51	**rs7216389** (4.82×10-12)
					PE	T (common)	1.17 (1.05, 1.31)	0.006	**rs2290400** (6.66×10-12)
					IO	T (common)	1.58 (1.25, 1.99)	1.11×10^{-4}	
					LO	T (common)	1.16 (1.01, 1.34)	0.03	
					P	T (common)	1.60 (1.39, 1.83)	1.5×10^{-11}	
Region between 35.2 and 35.4 Mb									
ORMDL	**rs8076131**	35.334438	0.48	8.55×10^{-13}	TE	A (common)	1.05 (0.95, 1.17)	0.31	**rs4795405** (3.08×10^{-12})
					PE	A (common)	1.18 (1.06, 1.33)	0.004	**rs4378650** (6.76×10^{-12})
					IO	A (common)	1.60 (1.27, 2.02)	7.52×10^{-5}	rs4794820 (7.04×10^{-12})
					LO	A (common)	1.19 (1.03, 1.37)	0.02	**rs8079416** (1.06×10^{-8})
					P	A (common)	1.60 (1.40, 1.84)	1.41×10^{-11}	rs3744246 (5.57×10^{-4})
GSDM1	rs3902025	35.372780	0.44	1.20×10^{-9}	TE	T (common)	1.09 (0.98, 1.20)	0.12	**rs4795408** (1.38×10^{-8})
					PE	T (common)	1.17 (1.04, 1.31)	0.009	**rs3859192** (2.23×10^{-8})
					IO	T (common)	1.46 (1.16, 1.85)	0.001	rs7219080 (5.34×10^{-8})
					LO	T (common)	1.15 (1.00, 1.33)	0.05	**rs3894194** (6.36×10^{-8})
					P	T (common)	1.53 (1.33, 1.75)	1.46×10^{-9}	
Region between 35.4 and 35.5 Mb									
PSMD3	rs2227321	35.424820	0.37	1.60×10^{-6}	TE	G (common)	1.00 (0.90, 1.11)	0.95	rs8075668 (6.80×10^{-6})
					PE	G (common)	1.14 (1.01, 1.28)	0.04	rs2241245 (0.003)
					IO	G (common)	1.38 (1.09, 1.76)	0.009	
					LO	G (common)	1.17 (1.01, 1.35)	0.04	
					P	G (common)	1.42 (1.23, 1.64)	1.36×10^{-6}	
CSF3	rs1042658	35.427428	0.38	3.45×10^{-5}	TE	T	1.05 (0.95, 1.16)	0.36	NA
					PE	T	1.12 (1.00, 1.26)	0.05	
					IO	T	1.36 (1.08, 1.70)	0.008	
					LO	T	1.15 (1.00, 1.32)	0.05	

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Nearby gene [a]	SNP [b]	Position (Mb)	MAF [c]	Overall P-value	Ph [d]	Risk allele±	RRR (95%CI) [e]	P-value for Ph	Other SNPs (overall p-value) [f]
					P	T	1.34 (1.18, 1.53)	1.13×10^{-5}	
MED24	rs8065443	35.462466	0.41	5.84×10^{-6}	TE	A	1.07 (0.96, 1.18)	0.22	rs2302776 (1.77×10^{-4})
					PE	A	1.18 (1.05, 1.32)	0.006	
					IO	A	1.31 (1.05, 1.65)	0.02	
					LO	A	1.17 (1.02, 1.35)	0.03	
					P	A	1.36 (1.2, 1.56)	4.15×10^{-6}	

[a] From online database SNP Function Prediction (FuncPred): http://manticore.niehs.nih.gov/snpfunc.htm

[b] SNP with the smallest overall p-value, only for genes for which there is evidence of association for at least one variant (p<0.001). A complete list of results for all SNPs is available from the authors on request. SNPs highlighted in bold are the 13 previously reported asthma-related SNPs[5 22 23].

[c] MAF=Minor allele frequency, RRR=Relative risk ratio

[d] Ph= Phenotype: TE=Transient early, PE=Prolonged early, IO=Intermediate onset, LO=Late onset, P=Persistent

[e] Risk allele is rare allele unless indicated otherwise

[f] Overall p-values for other SNPs within the same gene which are also associated with intermediate onset and persistent wheezing only.

Table 3

Association of variants in the region of chromosome 17 between 35.0 and 35.5 Mb (around 17q21) with expression quantitative traits for local genes in 875 children

Nearby gene	SNP [a]	*ORMDL3 expression* (r^2) [b]	p[c]	Other eQTL[d]	expression (r^2) [b]	p[c]
Region between 35.0 and 35.2 Mb						
PNMT	rs876493	0.05	3.38×10^{-8}	*IKZF3*	0.05	6.28×10^{-8}
				GSDML	0.05	4.50×10^{-7}
PERLD1	rs2941504	0.05	6.10×10^{-9}	*IKZF3*	0.08	1.47×10^{-14}
				GSDML	0.05	5.37×10^{-7}
ERBB2	rs2952155	0.05	2.48×10^{-8}	*IKZF3*	0.43	4.08×10^{-11}
				GSDML	−0.33	1.93×10^{-5}
Region between 35.2 and 35.4 Mb						
IKZF3	**rs9303277**	0.20	1.39×10^{-44}	*IKZF3*	0.13	2.53×10^{-27}
				ZPBP2	0.02	0.04
				GSDML	0.19	6.09×10^{-44}
ZPBP2	**rs11557467**	0.20	6.75×10^{-45}	*IKZF3*	0.13	1.78×10^{-26}
				ZPBP2	0.02	0.09
				GSDML	0.20	3.64×10^{-45}
GSDML	**rs2305480**	0.16	1.78×10^{-35}	*IKZF3*	0.07	1.15×10^{-12}
				GSDML	0.17	6.33×10^{-36}
ORMDL3	**rs8076131**	0.16	4.05×10^{-34}	*IKZF3*	0.07	4.86×10^{-12}
				GSDML	0.16	4.03×10^{-33}
GSDM1	rs3902025	0.10	1.40×10^{-19}	*IKZF3*	0.02	0.02
				GSDML	0.10	1.83×10^{-20}
				MED24	0.02	0.46
Region between 35.4 and 35.5 Mb						
PSMD3	rs2227321	0.05	2.79×10^{-7}	*GSDML*	0.04	0.001
				MED24	0.02	0.20
CSF3	rs1042658	0.03	0.001	*GSDML*	0.03	0.001
				MED24	0.07	2.62×10^{-12}
MED24	rs8065443	0.04	2.59×10^{-5}	*GSDML*	0.05	3.77×10^{-7}
				MED24	0.05	3.84×10^{-8}

[a] SNP near each gene with the smallest overall p-value from primary analyses with wheeze phenotypes (Table 2) and their relationships with expression quantitative traits for genes in the *ORMDL3* region of chromosome 17. Supplemental Table A6 shows the complete list of expression quantitative traits for the SNPs reported in this table. A complete list of SNPs and their relationship with expression quantitative traits for all loci in this region is available from the authors.

[b] r^2 refers to variance in expression explained in expression levels by SNP. For all results, 875 samples were available with both genotype and expression data.

[c] Bonferroni corrected p values (based on 3855 tests)

[d] Other eQLT, results are shown where evidence for association exceeds a threshold when taking a Bonferroni corrected p value for 3855 tests (257 independent loci*15 expression quantitative traits). All raw p values are <0.0002.

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