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ABSTRACT

Background: Asthma is a heterogeneous disease in which age-of-onset plays an important role.

Objective: We sought to identify the genetic variants associated with time-to-asthma onset.

Methods: We conducted a large-scale meta-analysis of nine genome-wide association studies of time-to-asthma onset (total of 5,462 asthmatics with a broad range of age-of-asthma onset and 8,424 controls of European ancestry) performed using survival analysis techniques.

Results: We detected five regions associated with time-to-asthma onset at genome-wide significant level ($P<5x10^{-8}$). We evidenced a new locus in 16q12 region (near *cylindromatosis turban tumor syndrome* gene (*CYLD*)) and confirmed four asthma risk regions: 2q12 (*IL1RL1*), 6p21 (*HLA-DQA1*), 9p24 (*IL33*) and 17q12-q21 (*ZPBP2-GSDMA*). Conditional analyses identified two distinct signals at 9p24 (both upstream of *IL33*) and at 17q12-q21 (near *ZPBP2* and within *GSDMA*). These seven distinct loci explained together 6.0% of the variance in time-to-asthma onset. In addition, we showed that genetic variants at 9p24 and 17q12-q21 were strongly associated with an earlier onset of childhood asthma ($P\leq0.002$) whereas 16q12 SNP was associated with a later asthma onset (P=0.04). A high burden of disease risk alleles at these loci was associated with earlier age-of-asthma onset (4 years *versus* 9-12 years, $P=10^{-4}$).

Conclusion: The new susceptibility region for time-to-asthma onset at 16q12 harbors variants that correlate with the expression of *CYLD* and *NOD2* (*nucleotide-binding oligomerization domain 2*), two strong candidates for asthma. This study demonstrates that incorporating the variability of age-of-asthma onset in asthma modeling is a helpful approach in the search for disease susceptibility genes.

Key Messages:

- 16q12 genetic variants are associated with time-to-asthma onset and correlate with *CYLD* and *NOD2* expressions.
- Genetic variants at 9p24 (upstream of *IL33*) and 17q12-q21 (nearby *ZPBP2* and within *GSDMA*) are associated with an earlier asthma-onset whereas variants at 16q12 are associated with a later asthma onset.
- Taking into account the variability of age-of-asthma onset in disease modeling can increase the power of identifying new genes involved in asthma physiopathology.

Capsule summary:

This large-scale meta-analysis of nine genome-wide association studies identified 16q12 genetic variants associated with time-to-asthma onset that correlate with *CYLD* and *NOD2* expressions, two strong candidate genes implicated in inflammation.

Keywords: Asthma, age-of-onset, genetics, genome-wide association study, survival analysis, conditional analysis, *CYLD*, *NOD2*

Abbreviations:

CYLD: Cylindromatosis (turban tumor syndrome)

eQTL: Expression quantitative trait locus

GSDMA: Gasdermin A

GWAS: Genome-wide association study

HLA-DQA1: Major histocompatibility complex, class II, DQ alpha 1

HR: Hazard ratio

IL1RL1: Interleukin 1 receptor-like 1

IL33: Interleukin 33

LCL: Lymphoblastoid cell line

LD: Linkage Disequilibrium

NFkB1: Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1

NOD2: Nucleotide-binding oligomerization domain containing 2

QC: Quality control

SNP: Single nucleotide polymorphism

TAO: time-to-asthma onset

*ZPBP2***:** Zona pellucida binding protein 2

INTRODUCTION

The prevalence of asthma has dramatically increased over the past decades in high-income countries, affecting 5–16% of people worldwide.[1] It is the most common chronic disease among children and a decrease in the age-of-asthma onset has been recently documented.[2]

Asthma is a complex and heterogeneous disease with variable clinical expression over the life span.[1] It is now well recognized that asthma is not a single disease but rather a collection of different phenotypes which may represent different manifestations of a common underlying pathological process or may be separate disease entities.[3] One of the simplest characteristic that can be used to differentiate disease phenotypes is the age at onset.[4,5] Indeed, asthma displays different characteristics according to the lifetime period at which it occurs.[6] Early age of onset is more frequently associated with a family history of asthma, allergy sensitization and clinical response to triggers, whereas late-onset disease is associated with eosinophilic inflammation and obesity, is more common in women, and is generally less allergic.[3]

The risk of developing asthma has a strong genetic component, with estimated heritability ranging from 35 to 95%.[7] Genome-wide association studies (GWASs) have been successful in identifying more than 20 loci associated with asthma.[8] However, the genetic factors identified to date account only for a small part of the genetic component of the disease.[1] This hidden heritability might be linked to the phenotypic heterogeneity of asthma.[9] The vast majority of GWASs conducted until now have analyzed asthma as a binary phenotype. A few genetic studies have considered more specific definition of asthma incorporating the age of disease onset. A genome-wide linkage screen conducted for time-to-asthma onset in French families revealed two regions, 1p31 and 5q13, potentially linked to this phenotype.[10] A single GWAS has been performed on age-of-asthma onset in asthmatic children and led to the identification of two loci, not found by the previous asthma GWASs; these loci on chromosomes 3p26 and 11q24 were associated with an earlier onset of childhood asthma.[11] Moreover, the effect of

17q12-q21 genetic variants, identified by the first GWAS of asthma,[12] was found to be restricted to early-onset asthma.[13,14]

Instead of stratifying the data according to age-of-onset of disease using an arbitrary threshold, one can integrate the age-of-onset in modeling asthma risk, by using survival analysis methodology applied to both asthmatic and non-asthmatic subjects. The goal of the present study was to identify the genetic determinants underlying time-to-asthma onset in a large meta-analysis of 5,462 asthmatics and 8,424 controls from nine independent European-ancestry populations.

METHODS

Populations

We studied 13,886 individuals of European ancestry from nine independent studies (one birth cohort, five population-based and three family studies) which were part of the GABRIEL European consortium on the genetics of asthma.[14] A brief description of these studies with appropriate references is provided in the online repository and in Table E1. All of these studies had the age of asthma onset and imputed genetic data available.

For all studies, ethical approval was obtained from the appropriate institutional ethic committees and all individuals or child's legal guardians provided written informed consent.

Time-to-asthma onset definition

Definition of asthma was based on report of doctor's diagnosis and/or on standardized questionnaires (see online repository). To model time-to-asthma onset (TAO), we used age of onset or age at first wheeze for individuals who developed asthma, while in individuals who were free of disease upon examinations, we used age at last examination.

Genotyping

Genotyping, single nucleotide polymorphism (SNP) imputation process and quality control (QC) criteria (for individuals and SNPs) for each study are described in Table E1 in the online repository. All datasets were genotyped at Centre National de Génotypage (CNG, Evry, France), as part of the European GABRIEL asthma consortium.[14] Quality control and imputations were performed independently for each study. Genome-wide imputations were conducted using MACH 1.0 software,[15] with reference haplotype panels from HapMap 2. SNPs with imputation quality score (Rsq) ≥ 0.5 and minor allele frequency $\geq 1\%$ were kept for analysis. Then, to further investigate the regions associated with time-to-asthma onset at

209 the genome-wide significant level, we used imputed data from the 1000 Genomes Project and
210 applied the same SNP QC criteria.

211

212 ***Statistical analysis and strategy of analysis***

213 After the study-specific QC, a total of 13,886 individuals from the nine cohorts were included
214 in the present study. In each dataset, association between time-to-asthma onset and individual
215 SNPs was investigated under an additive genetic model using a Cox proportional-hazards
216 regression model adjusted for sex and the first four principal components to account for
217 population structure. A robust sandwich estimation of the variance[16] was used in family data
218 to take into account familial dependencies. Moreover, due to the complex sampling design of
219 the GABRIELA study, survey regression techniques were used for this study to estimate
220 robust standard errors ('svy' command in Stata). Proportional hazard assumptions for the
221 main SNP effect were tested and never rejected. Genome-wide association studies of time-to-
222 asthma onset were first conducted in each of the nine datasets separately, and then combined
223 through meta-analysis in order to increase power and to obtain more robust findings. Meta-
224 analyzed hazard-ratios (HRs) and 95% confidence intervals (CIs) were calculated using a
225 fixed-effect (inverse variance) model. To assess heterogeneity of SNP effect across studies,
226 the Cochran's Q statistic was calculated. If heterogeneity was evidenced, a random-effect
227 model was fitted. All analyses were performed using Stata® version 13.1 (STATA Corp.,
228 College Station, Texas, USA). After the meta-analysis, we only kept SNPs for which at least
229 66% of the studies (≥6 studies among the 9 studies) participated to the summary statistics in
230 the meta-analysis, to reduce the rate of false-positive findings. The meta-analysis results were
231 obtained for a total of 2,387,926 SNPs. We used the classical threshold of P-value$\leq 5 \times 10^{-8}$ to
232 declare a meta-analyzed SNP effect as genome-wide significant.

233

Conditional analysis to uncover distinct signals at TAO-associated loci

To identify distinct TAO-associated SNPs in each region harboring genome-wide significant signals, we re-analyzed separately these regions in each of the nine studies. For that purpose, we added the region top SNP into the primary Cox model as a covariate and tested the effect of each other SNP of that region. Then, the results were meta-analyzed using the same strategy as the primary GWASs. If a secondary signal was detected in a region, a second run of conditional analyses was performed to check for a third distinct signal in that region. Length of explored regions was based on regional association plots and ranged from 200 kb to 500 kb depending on recombination hotspots.

eQTL analysis and functional annotations

We queried whether significant SNPs (or their proxies) associated with time-to-asthma onset at $P \leq 5x10^{-8}$ and potentially secondary signals from conditional analysis were expression quantitative trait loci (eQTLs). We used existing eQTL databases in multiple tissues (especially blood and lung) for populations of European ancestry (see online repository).[17-23] Functional annotations of significant SNPs (or their proxies) were obtained using ENcyclopedia Of DNA Elements (ENCODE) data[24] provided by the HaploReg tool.[25]

Relationship of TAO-associated loci with age-of-asthma onset

In a first step, we investigated in asthmatics whether each of the SNPs associated with TAO were also associated with age-of-asthma onset using a non-parametric rank test followed by a non-parametric equality-of-medians test. In a second step, we assessed the cumulative effect of risk alleles of SNPs found associated with the age-of-asthma onset at step 1. For that purpose, we used either the number of risk alleles or the quintiles of a polygenic score distribution. The polygenic risk score is the weighted sum of the number of age of asthma

onset associated alleles with weight being the log of adjusted hazard ratio estimated in asthmatics only. The associations were tested in eight studies for which we had access to raw data (all datasets except ALSPAC) using a cox proportional hazard model adjusted on sex and principal components.

RESULTS

Description of populations

A total of 13,886 subjects were included in the present study (5,462 asthmatics and 8,424 non-asthmatics). Asthmatics had a mean age-of-asthma onset of 12.5 years (ranging from 0.5 to 75 years, Figure E1), a mean age of 26.8 years at examination (mean per study ranging from 9.1 to 51.3 years) and 52.6% were males. Non-asthmatics had a mean age of 32.4 years at examination (mean per study ranging from 8.9 to 55.8 years) and 49% were males (Table E1).

Genetic variants associated with time-to-asthma onset

The Manhattan and the Q-Q plots of the meta-analysis of time-to-asthma onset GWAS results are shown in Figure 1 and Figure E2 in the online repository respectively. A total of 155 SNPs were associated with time-to-asthma onset at the genome-wide significance level of $P<5x10^{-8}$. These SNPs clustered into five distinct chromosomal regions (Table I), that included a new risk locus on 16q12 (*nearby CYLD* (*cylindromatosis turban tumor syndrome*), 1 SNP) and four established risk loci for asthma: 2q12 (*IL1RL1-IL18R1*, 7 SNPs), 6p21 (*nearby HLA-DQA1*, 1 SNP), 9p24 (flanking *IL33*, 25 SNPs) and 17q12-q21 (121 SNPs spanning 389 kb, and with the main signal located near *ZPBP2*). The regional association plots for these genome-wide associated loci are shown in Figures 2 and E3, and the forest plots for the top signal in each region are shown in Figure E4. Three additional loci were associated with time-to-asthma onset at a suggestive significance threshold ($5x10^{-8}<P<10^{-6}$; Table I): *MAP4K4* (2q11-q12), *RORA* (15q22) and *IL4R* (16p12-p11).

To determine whether any of the five TAO loci harbored additional association signals, we performed conditional association analysis in each region. For this analysis, a threshold of $P\leq2.1x10^{-5}$ was used to declare significance, corresponding to a Bonferroni threshold for

2,382 independent tests. These analyses evidenced two secondary signals (Table II and Figure E5 in the online repository): 1) rs413382 in 9p24 region at 73 kb of *IL33* ($P=9.7x10^{-6}$ after conditioning on top SNP and $P=5.9x10^{-8}$ in the primary meta-analysis) and 2) rs3859192 in 17q12-q21 region within *GSDMA* ($P=4.0x10^{-6}$ after conditioning on top SNP and $P=1.5x10^{-13}$ in the primary meta-analysis). In contrast, at 2q12, 6p21 and 16q12 regions, the inclusion of the most significant time-to-asthma onset GWAS SNP as a covariate in association analysis resulted in nearly complete reduction of the association signal in these regions, suggesting that there was no evidence for a second distinct genetic factor in these regions.

To obtain a denser map of the new TAO 16q12 locus, we repeated association analyses using 1000 Genomes Project imputed SNPs. These analyses strengthened our original finding with additional signals ($3.8x10^{-8} \leq P \leq 2.6x10^{-7}$) located in an intergenic region encompassing the lead SNP rs1861760 (Table E2 and Figure E6). These SNPs were in moderate to high LD with rs1861760 ($0.71 \leq r^2 \leq 0.81$) and thus did not represent independent signals from that top hit. Similar analyses conducted in the four other TAO-associated regions also supported our original findings and did not evidence any additional independent signal in these regions.

Overall, the seven distinct SNPs (five top SNPs and two secondary SNPs) associated with TAO showed low heterogeneity between studies ($P>0.11$) and explained together 6.0% of the variance in time-to-asthma onset.

Functional annotations and effect on gene expression

To provide some insights into the potential molecular mechanisms underlying the TAO-associated variants, we queried whether the five top SNPs and two secondary signals (and their proxies) were 1) tagging potentially deleterious SNPs, 2) located in regulatory elements, and 3) reported to influence the expression of one or more of nearby genes (eQTLs at $P<5x10^{-5}$). We focused on the new TAO risk locus at 16q12 region. Functional annotations

for the remaining six loci are presented in the online repository and eQTL data are presented in Table III (main results) and in Table E3 in the online repository.

The 16q12 TAO-associated variants are located in an intergenic region delimited by two recombination hotspots on each side, near *CYLD* (22 kb downstream). The rs1861760 maps to FOXJ1 and SOX binding sites. This SNP and/or its proxies correlate with the expression of *CYLD* in both blood and human lung tissues and of *NOD2* (*nucleotide-binding oligomerization domain 2*) in blood[20] (Table III for main results and Table E3 in the online repository).[17,20]

Relationship between TAO-associated variants and age-of-asthma onset

To investigate whether TAO-associated SNPs influence the age-of-asthma onset, we compared, in asthmatics, the distribution of age-of-asthma onset according to the number of risk alleles at each of the seven main and secondary TAO-associated SNPs (Figure 3). Asthmatic subjects carrying one or two copies of risk allele at 17q12-q21 SNPs (rs9901146 and rs3859192) or at 9p24 rs928413 had a younger age-of-asthma onset than non-carriers (median 6-8 years *versus* 10 years ($P \leq 6x10^{-4}$) and median 6-8 years *versus* 9 years (P=0.002), respectively), whereas those having at least one copy of rs1861760 risk-allele at 16q12 had a later age-of-asthma onset than non-carriers (median 10 years *versus* 8 years; P=0.04). No significant difference was found for the other three SNPs. We evidenced that an increased number of risk alleles at these four SNPs was associated with a younger age-of-asthma onset (median 12 years for carrying one risk allele to 4 years for carrying 6-8 risk alleles; $P=10^{-4}$). Finally, we detected a strong association between the age-of-asthma onset and the polygenic risk score (from median 10 years in first quintile to 6 years in last quintile; $P=4x10^{-4}$).

Comparison of time-to-asthma onset GWAS results with previous asthma GWASs

To investigate the impact of taking into account the age-of-asthma onset in disease modeling through survival analysis, we explored whether the top TAO SNPs were associated with asthma modeled as a binary trait in the nine cohorts included in the present study (Table E4, see online repository). We also investigated GABRIEL top SNPs in TAO meta-analysis (Table E4).[14] We observed a strong decrease of heterogeneity of SNP effect across studies in TAO analysis ($P_{Het} \geq 0.11$) compared with asthma binary trait analyzed in the same datasets ($P_{Het} \geq 0.004$) as well as in all GABRIEL datasets ($P_{Het} \geq 0.0009$), especially in 9p24 and 17q12-q21 regions. The association signals were always more significant in TAO analysis as compared with the binary trait analysis in the same datasets. This increase of significance level was very high: 100-fold for 2q12 and 16q12, and 10^4 to 10^6-fold for 9p24 and 17q12-q21. In fact, the asthma binary trait analysis only detected two loci (*HLA* and *GSDMA*) at the genome-wide significance level out of the seven TAO-associated loci. Conversely, the present TAO analysis identified at the genome-wide significance level four of the six main published GABRIEL regions,[14] and event at higher significance for 9p24 and 17q12-q21 regions (100 to 10^4-fold) as compared to GABRIEL significance levels. The two remaining GABRIEL loci not detected by our TAO analysis were those with the weaker effects (OR=1.12 for rs744910 in 15q22 and rs2284033 in 22q13) in GABRIEL meta-analysis.[14]

Finally, we evaluated whether previously reported susceptibility loci for asthma[26] were associated with time-to-asthma onset in our meta-analysis (see online repository, Table E5). Among the 21 loci detected in European populations, 12 were replicated at 5% in our TAO meta-analysis with the same direction of effects. Among the nine non-replicated signals, three SNPs (or some proxies) were not available in our data, and the remaining six loci had been reported for specific phenotypes: asthma exacerbation, age-of-asthma onset *per se* in asthmatic children only (quantitative trait) or childhood asthma (binary trait).[11,27,28]

DISCUSSION

By taking into account the age-of-asthma onset in asthma association analysis, we identified in this large meta-analysis including both asthmatic and non-asthmatic individuals, adults and children, a new susceptibility locus at 16q12 associated with time-to-asthma onset and confirmed the involvement of six other distinct loci belonging to four regions in asthma pathogenesis (2q12, 6p21, 9p24 and 17q12-q21). Genetic variants at 9p24 and 17q12-q21 were strongly associated with an earlier onset of childhood asthma whereas the 16q12 lead SNP was associated with a risk of later onset asthma.

The most significant 16q12 genetic variant (rs1861760) is located nearby *CYLD* and *NOD2* and also maps to a binding site of FOXJ1, a transcription factor associated with allergic rhinitis.[29] Genetic variants located in a 130-kb region around rs1861760 were reported associated with immune-related diseases: inflammatory bowel diseases (Crohn's disease) and leprosy.[30-35] Interestingly, haplotype reconstruction (Figure 4) showed that the TAO rs1861760-A risk allele was always associated with SNP alleles that conferred a decreased risk of Crohn's disease (rs17221417-C, rs5743289-C and rs2076756-A located in *NOD2* and rs12324931-A located in *CYLD)*[30-32,35,36] and of leprosy (rs16948876-G located in intergenic region at 2 kb from rs1861760).[33] Indeed, GWASs revealed common genetic susceptibility loci for asthma and other immune-related disorders, suggesting shared molecular pathways involved in their etiology; however opposite alleles appear to be at risk.[37] Interestingly, an opposite effect of rs1861760-A allele is also observed at the gene expression level. Thus, TAO risk-allele at rs1861760 correlated with both the expression of *CYLD* and *NOD2* in blood but with an opposite effect.[20] However, this TAO risk allele was only associated with increased *CYLD* expression in lung tissue.[17] *CYLD* encodes a deubiquitinating enzyme that regulates diverse physiological processes including immune response and inflammation.[38] *CYLD* mainly acts as a negative regulator of nuclear factor-κB (*NFkB1*) to protect the host

from an over-reactive inflammatory response.[38] Conversely *NOD2,* which plays an important role in the innate immune response to intracellular bacterial lipopolysaccharides (LPS), activates the *NFkB1* pathway.[39] NFkB1 is a pleiotropic transcription factor that acts as a key regulator of immune and inflammatory genes, and activation of the NFkB1 pathway has been implicated in airway inflammation and asthma.[40,41] Moreover, FOXJ1 transcription factor that binds to the genomic region encompassing 16q12 TAO-associated SNP (rs1861760) was described to inhibit NFkB1 activity.[42] Recently, *CYLD* has been shown to regulate lung fibrosis in mice by inhibiting transforming growth factor-β-signaling (TGF-β) through a decrease of SMAD3 protein stability.[43] Of interest, *SMAD3* has been reported to be associated with asthma by previous GWAS.[14]

Defining the phenotype is an important consideration because phenotypic heterogeneity can reduce power of GWAS.[44] In the present analyses, we studied the variability of time-to-asthma onset in both asthmatics and non-asthmatics based on survival analysis methods. The information used for such analysis was the age-of-onset in asthmatics and the age at last examination or death in non-asthmatics. In such a model, unaffected subjects represent censored observations as they are still at risk for disease, being perhaps too young to exhibit the trait. This approach, which allowed combining the age-of-asthma onset together with the disease status (affected/unaffected) led to decrease the genetic heterogeneity across studies and increase the power to detect association signals (upon 10^6-fold increase as compared to the disease status only analysis. More specifically, increased evidence of association was observed in regions where age-of-asthma onset explained at least in part the genetic heterogeneity such as the 17q12-q21 locus for which a restricted SNP effect to a particular group of age-of-onset (early childhood onset asthma) was demonstrated.[13] Moreover, this analysis led to the identification of a new locus at 16q12 near *CYLD* and of an additional signal in the 9p24 region. These results support the hypothesis that a better consideration of

414 the phenotypic heterogeneity of asthma may help to disentangle the genetic heterogeneity of
415 asthma.
416 Our study included both children and adult asthmatic subjects. Age-of-disease onset may be
417 subject to recall bias, especially among individuals who are the furthest from the time of the
418 first symptoms (e.g. adults who suffered of asthma in childhood) because it is often defined in
419 a retrospective manner. However, high accuracy of the self-reported year of asthma onset by
420 adult subjects has been shown by two independent studies among which the European
421 Community Respiratory Health Survey, which was part of the present study.[45,46] Erroneous
422 recall of the age-of-asthma onset is unlikely to have significantly affected the results since we
423 observed little genetic heterogeneity across studies (e.g. childhood onset asthma reported by
424 either adults or children).
425 It was suggested that some genetic variants might influence asthma in an age-specific manner.
426 Among TAO-associated SNPs, we confirmed the association of 17q12-q21 SNPs with an
427 early age-of-asthma onset[13,14] and evidenced for the first time that the top 9p24 genetic variant
428 near *IL33* was also associated with early childhood-onset asthma (median age-of-onset of 6-8
429 years in risk-allele carriers). Indeed, in GABRIEL meta-analysis, 9p24 SNPs were more
430 strongly associated with early-onset asthma (before 16 years) than late-onset asthma (after 16
431 years), but this difference was not significant.[14] Conversely, genetic variants at the new
432 susceptibility locus, 16q12, conferred a risk of later onset asthma (median age-of-onset of 10
433 years in risk-allele carriers). Moreover, we evidenced that a high burden of disease risk alleles
434 at these loci is associated with earlier age-of-asthma onset (4 years *versus* 9-12 years). This
435 difference in asthma onset may reflect difference in patterns of onset of disease.[47] Indeed, we
436 evidenced in the GABRIELA study that persistent early wheezing subjects carried more risk
437 alleles than transient early wheezing subjects, and we confirmed previous association between
438 persistent early wheezing and 9p24 and 17q12-q21 loci (data not shown). The 17q12-q21

genetic variants were reported associated with persistent childhood wheeze phenotype whereas 9p24 were mostly associated with intermediate onset wheeze but also with persistent early wheeze.[48,49] Moreover, 17q12-q21 SNPs were associated with fractional exhaled Nitric Oxide (FeNO) levels in children but not in adults, childhood severe asthma and allergic rhinitis, and 9p24 SNPs were associated with childhood severe asthma, asthma-plus-rhinitis, atopic asthma, allergy and eosinophil counts.[50-55]

In summary, we identified five regions harboring seven distinct signals that were associated with time-to-asthma onset, among which the 16q12 region that is reported for the first time. Several lines of evidence suggest that *CYLD* and *NOD2* located in that region are strong candidate genes for asthma. This study demonstrates that incorporating the variability of age-of-asthma onset in disease modeling is a useful strategy to uncover new disease genes.

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REFERENCES

1. Martinez FD, Vercelli D. Asthma. Lancet 2013; 382:1360-72.
2. Radhakrishnan DK, Dell SD, Guttmann A, Shariff SZ, Liu K, To T. Trends in the age of diagnosis of childhood asthma. J Allergy Clin Immunol 2014; 134:1057-62 e5.
3. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012; 18:716-25.
4. Siroux V, Basagana X, Boudier A, Pin I, Garcia-Aymerich J, Vesin A, et al. Identifying adult asthma phenotypes using a clustering approach. Eur Respir J 2011; 38:310-7.
5. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010; 181:315-23.
6. Szefler SJ, Chmiel JF, Fitzpatrick AM, Giacoia G, Green TP, Jackson DJ, et al. Asthma across the ages: knowledge gaps in childhood asthma. J Allergy Clin Immunol 2014; 133:3-13; quiz 4.
7. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. Immunol Rev 2011; 242:10-30.
8. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res 2014; 42:D1001-6.
9. Siroux V, Gonzalez JR, Bouzigon E, Curjuric I, Boudier A, Imboden M, et al. Genetic heterogeneity of asthma phenotypes identified by a clustering approach. Eur Respir J 2014; 43:439-52.
10. Bouzigon E, Ulgen A, Dizier MH, Siroux V, Lathrop M, Kauffmann F, et al. Evidence for a pleiotropic QTL on chromosome 5q13 influencing both time to asthma onset and asthma score in French EGEA families. Hum Genet 2007; 121:711-9.

11. Forno E, Lasky-Su J, Himes B, Howrylak J, Ramsey C, Brehm J, et al. Genome-wide association study of the age of onset of childhood asthma. J Allergy Clin Immunol 2012; 130:83-90 e4.
12. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature 2007; 448:470-3.
13. Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. N Engl J Med 2008; 359:1985-94.
14. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010; 363:1211-21.
15. Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. Annu Rev Genomics Hum Genet 2009; 10:387-406.
16. Williams RL. A note on robust variance estimation for cluster-correlated data. Biometrics 2000; 56:645-6.
17. Hao K, Bosse Y, Nickle DC, Pare PD, Postma DS, Laviolette M, et al. Lung eQTLs to help reveal the molecular underpinnings of asthma. PLoS Genet 2012; 8:e1003029.
18. Liang L, Morar N, Dixon AL, Lathrop GM, Abecasis GR, Moffatt MF, et al. A cross-platform analysis of 14,177 expression quantitative trait loci derived from lymphoblastoid cell lines. Genome Res 2013; 23:716-26.
19. Montgomery SB, Sammeth M, Gutierrez-Arcelus M, Lach RP, Ingle C, Nisbett J, et al. Transcriptome genetics using second generation sequencing in a Caucasian population. Nature 2010; 464:773-7.

20. Westra HJ, Peters MJ, Esko T, Yaghootkar H, Schurmann C, Kettunen J, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet 2013; 45:1238-43.
21. Stranger BE, Nica AC, Forrest MS, Dimas A, Bird CP, Beazley C, et al. Population genomics of human gene expression. Nat Genet 2007; 39:1217-24.
22. Veyrieras JB, Kudaravalli S, Kim SY, Dermitzakis ET, Gilad Y, Stephens M, et al. High-resolution mapping of expression-QTLs yields insight into human gene regulation. PLoS Genet 2008; 4:e1000214.
23. The Genotype-Tissue Expression (GTEx) project. Nat Genet 2013; 45:580-5.
24. Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M. An integrated encyclopedia of DNA elements in the human genome. Nature 2012; 489:57-74.
25. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res 2012; 40:D930-4.
26. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A 2009; 106:9362-7.
27. Himes BE, Hunninghake GM, Baurley JW, Rafaels NM, Sleiman P, Strachan DP, et al. Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. Am J Hum Genet 2009; 84:581-93.
28. McGeachie MJ, Wu AC, Tse SM, Clemmer GL, Sordillo J, Himes BE, et al. CTNNA3 and SEMA3D: Promising loci for asthma exacerbation identified through multiple genome-wide association studies. J Allergy Clin Immunol 2015.

29. Li CS, Chae SC, Lee JH, Zhang Q, Chung HT. Identification of single nucleotide polymorphisms in FOXJ1 and their association with allergic rhinitis. J Hum Genet 2006; 51:292-7.
30. Kenny EE, Pe'er I, Karban A, Ozelius L, Mitchell AA, Ng SM, et al. A genome-wide scan of Ashkenazi Jewish Crohn's disease suggests novel susceptibility loci. PLoS Genet 2012; 8:e1002559.
31. Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447:661-78.
32. Cleynen I, Vazeille E, Artieda M, Verspaget HW, Szczypiorska M, Bringer MA, et al. Genetic and microbial factors modulating the ubiquitin proteasome system in inflammatory bowel disease. Gut 2014; 63:1265-74.
33. Zhang F, Liu H, Chen S, Low H, Sun L, Cui Y, et al. Identification of two new loci at IL23R and RAB32 that influence susceptibility to leprosy. Nat Genet 2011; 43:1247-51.
34. Liu H, Irwanto A, Fu X, Yu G, Yu Y, Sun Y, et al. Discovery of six new susceptibility loci and analysis of pleiotropic effects in leprosy. Nat Genet 2015; 47:267-71.
35. Kugathasan S, Baldassano RN, Bradfield JP, Sleiman PM, Imielinski M, Guthery SL, et al. Loci on 20q13 and 21q22 are associated with pediatric-onset inflammatory bowel disease. Nat Genet 2008; 40:1211-5.
36. Elding H, Lau W, Swallow DM, Maniatis N. Dissecting the genetics of complex inheritance: linkage disequilibrium mapping provides insight into Crohn disease. Am J Hum Genet 2011; 89:798-805.
37. Li X, Ampleford EJ, Howard TD, Moore WC, Torgerson DG, Li H, et al. Genome-wide association studies of asthma indicate opposite immunopathogenesis direction from autoimmune diseases. J Allergy Clin Immunol 2012; 130:861-8 e7.

603 38. Sun SC. CYLD: a tumor suppressor deubiquitinase regulating NF-kappaB activation and
604 diverse biological processes. Cell Death Differ 2010; 17:25-34.
605 39. Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G. Nod2, a Nod1/Apaf-1
606 family member that is restricted to monocytes and activates NF-kappaB. J Biol Chem
607 2001; 276:4812-8.
608 40. Poynter ME, Cloots R, van Woerkom T, Butnor KJ, Vacek P, Taatjes DJ, et al. NF-kappa
609 B activation in airways modulates allergic inflammation but not hyperresponsiveness. J
610 Immunol 2004; 173:7003-9.
611 41. Kurakula K, Vos M, Logiantara A, Roelofs JJ, Nieuwenhuis MA, Koppelman GH, et al.
612 Nuclear Receptor Nur77 Attenuates Airway Inflammation in Mice by Suppressing NF-
613 kappaB Activity in Lung Epithelial Cells. J Immunol 2015.
614 42. Lin L, Spoor MS, Gerth AJ, Brody SL, Peng SL. Modulation of Th1 activation and
615 inflammation by the NF-kappaB repressor Foxj1. Science 2004; 303:1017-20.
616 43. Lim JH, Jono H, Komatsu K, Woo CH, Lee J, Miyata M, et al. CYLD negatively
617 regulates transforming growth factor-beta-signalling via deubiquitinating Akt. Nat
618 Commun 2012; 3:771.
619 44. Ioannidis JP, Thomas G, Daly MJ. Validating, augmenting and refining genome-wide
620 association signals. Nat Rev Genet 2009; 10:318-29.
621 45. Pattaro C, Locatelli F, Sunyer J, de Marco R. Using the age at onset may increase the
622 reliability of longitudinal asthma assessment. J Clin Epidemiol 2007; 60:704-11.
623 46. Toren K, Palmqvist M, Lowhagen O, Balder B, Tunsater A. Self-reported asthma was
624 biased in relation to disease severity while reported year of asthma onset was accurate. J
625 Clin Epidemiol 2006; 59:90-3.
626 47. Dijk FN, de Jongste JC, Postma DS, Koppelman GH. Genetics of onset of asthma. Curr
627 Opin Allergy Clin Immunol 2013; 13:193-202.

628 48. Granell R, Henderson AJ, Timpson N, St Pourcain B, Kemp JP, Ring SM, et al.
629 Examination of the relationship between variation at 17q21 and childhood wheeze
630 phenotypes. J Allergy Clin Immunol 2013; 131:685-94.
631 49. Savenije OE, Mahachie John JM, Granell R, Kerkhof M, Dijk FN, de Jongste JC, et al.
632 Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with
633 wheezing phenotypes and asthma in childhood. J Allergy Clin Immunol 2014; 134:170-7.
634 50. van der Valk RJ, Duijts L, Timpson NJ, Salam MT, Standl M, Curtin JA, et al. Fraction
635 of exhaled nitric oxide values in childhood are associated with 17q11.2-q12 and 17q12-
636 q21 variants. J Allergy Clin Immunol 2014; 134:46-55.
637 51. Bonnelykke K, Sleiman P, Nielsen K, Kreiner-Moller E, Mercader JM, Belgrave D, et al.
638 A genome-wide association study identifies CDHR3 as a susceptibility locus for early
639 childhood asthma with severe exacerbations. Nat Genet 2014; 46:51-5.
640 52. Fuertes E, Soderhall C, Acevedo N, Becker A, Brauer M, Chan-Yeung M, et al.
641 Associations between the 17q21 region and allergic rhinitis in 5 birth cohorts. J Allergy
642 Clin Immunol 2015; 135:573-6.
643 53. Ferreira MA, Matheson MC, Tang CS, Granell R, Ang W, Hui J, et al. Genome-wide
644 association analysis identifies 11 risk variants associated with the asthma with hay fever
645 phenotype. J Allergy Clin Immunol 2014; 133:1564-71.
646 54. Hinds DA, McMahon G, Kiefer AK, Do CB, Eriksson N, Evans DM, et al. A genome-
647 wide association meta-analysis of self-reported allergy identifies shared and allergy-
648 specific susceptibility loci. Nat Genet 2013; 45:907-11.
649 55. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, et
650 al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial
651 infarction. Nat Genet 2009; 41:342-7.

652 56. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, et al. LocusZoom:
653 regional visualization of genome-wide association scan results. Bioinformatics 2010;
654 26:2336-7.
655 57. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and
656 haplotype maps. Bioinformatics 2005; 21:263-5.
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Table I. Top SNPs in main loci associated with time-to-asthma onset at genome-wide ($P \leq 5x10^{-8}$) and suggestive significance levels ($5x10^{-8} < P < 10^{-6}$)

						Time to asthma onset - N=13,886		
Chromosome	**Marker**	**Position***	**Nearest gene(s) (kb distance)**	**Effect/ Ref Alleles†**	**Effect Freq**	**Hazard Ratio [95% CI]**	**P‡**	**P-Het****
Loci with genome-wide significance - $P \leq 5x10^{-8}$								
2q12	rs10208293	102966310	*IL1RL1****	G/A	0.73	1.14 [1.08-1.19]	$3.1x10^{-8}$	0.26
6p21	rs9272346	32604372	*HLA-DQA1 (0.8)*	A/G	0.59	1.13 [1.08-1.17]	$1.6x10^{-8}$	0.12
9p24	rs928413	6213387	*IL33 (2)*	G/A	0.25	1.19 [1.13-1.25]	$6.5x10^{-16}$	0.15
16q12	rs1861760	50857693	*CYLD (22)*	A/C	0.04	1.28 [1.17-1.40]	$4.2x10^{-8}$	0.11
17q12-q21	rs9901146	38043343	*ZPBP2 (9)* *GSDMB (17)*	G/A	0.51	1.18 [1.13-1.22]	$1.9x10^{-16}$	0.17
Suggestive loci - $5x10^{-8} < P < 10^{-6}$								
2q11-q12	rs12468899	102426140	*MAP4K4****	G/A	0.69	1.12 [1.09-1.16]	$1.7x10^{-7}$	0.89
15q22	rs11071559	61069988	*RORA****	C/T	0.85	1.16 [1.10-1.24]	$8.3x10^{-7}$	0.96
16p12-p11	rs1805013	27373980	*IL4R****	T/C	0.05	1.22 [1.13-1.32]	$8.0x10^{-7}$	0.37

*Position in base pairs (bp) – build 37.3 NCBI

[†]For the calculation of the hazard ratios, effect alleles were designated as risk alleles. Effect Freq denotes frequency of the effect allele, CI confidence interval, and Ref reference allele.

[‡]P-value obtained from single-SNP Cox model for time-to-asthma onset adjusted for sex and principal components (fixed-effect model when there was no significant evidence of heterogeneity or random-effect model otherwise)

[**]P-Het reflects P-value of Cochran's Q statistic across studies

[***]SNP is located within reported gene

Table II. Secondary signals associated with time-to-asthma onset after stepwise conditional analysis in 9p24 and 17q12-q21 regions. This table contains, for these two regions, the top time-to-asthma onset SNP in bold (rs928413 and rs9901146 respectively) and the most significant SNP in the conditional analysis, after fitting the lead SNP in the region.

						Single SNP analysis			Fitted SNP(s)		
Chr	**Marker**	**Nearest gene (kb distance)**	**Position***	**Effect/Ref Alleles†**	**Effect Freq**	**Hazard Ratio [95% CI]**	**P‡**	**P-Het****	**Hazard Ratio [95% CI]**	**P‡**	**P-Het****
9p24 region									**rs928413**		
9	rs413382	*IL33 (73)*	6142948	A/C	0.80	1.15 [1.08-1.22]	$5.9x10^{-8}$	0.84	1.13 [1.06-1.20]	$9.7x10^{-6}$	0.80
9	**rs928413**	*IL33 (2)*	6213387	G/A	0.25	1.19 [1.13-1.25]	$6.5x10^{-16}$	0.15	-	-	-
17q12-q21 region									**rs9901146**		
17	**rs9901146**	*ZPBP2 (9)*	38043343	G/A	0.51	1.18 [1.13-1.22]	$1.9x10^{-16}$	0.17	-	-	-
17	rs3859192	*GSDMA****	38128648	T/C	0.48	1.16 [1.12-1.21]	$1.5x10^{-13}$	0.90	1.11 [1.06-1.15]	$4.0x10^{-6}$	0.74

*Position: Position in base pairs (bp) - build 37.3 NCBI

673 [†]For the calculation of the hazard ratio, effect alleles were designated as risk alleles. Effect Freq denotes frequency of the effect allele, CI

674 confidence interval, and Ref reference allele

675 [‡]P-values are obtained from Cox model of time-to-asthma onset adjusted for sex and principal components

676 [**]P-Het reflects P-value of Cochran's Q statistic across studies

677 [***]SNP is located within reported gene

678

Table III. Main cis-eQTLs results for the top SNPs in genome-wide associated regions from the meta-analysis of time-to-asthma onset. We focused on eQTLs measured in blood, lymphoblastoïd cell lines (LCLs) and lung tissue.

Locus	SNP* (LD with top SNP)	Alleles (Ref/Effect)	Gene(s)	Range of P-values	Tissue	Source††
2q12	**rs10208293**	G/A	*IL18RAP, IL18R1*	$2.5x10^{-13}$-$9.8x10^{-198}$	Blood, LCLs	Blood eQTLs, eQTL Browser
6p21	**rs9272346**	G/A	*HLA-DQA1/DQA2/DQAS1/ DQB1/DQB2,HLA-DRA/DRB1/ DRB5/DRB6, TAP2*	$1.3x10^{-6}$-$2.1x10^{-121}$	LCLs, Lung, Blood	eQTL_Chicago,GTEx, Blood eQTLs
16q12	**rs1861760**	C/A	*NOD2*	$3.6x10^{-11}$	Blood	Blood eQTLs
	rs5743266† (D'=1, r^2=0.02)		*CYLD, NOD2*	$5.0x10^{-9}$-$3.2x10^{-120}$	Blood	Blood eQTLs
	rs7205760† (D'=1, r^2=0.005)		*CYLD, NOD2*	$2.8x10^{-6}$-$4.0x10^{-15}$	Lung, Blood	Lung eQTLs, Blood eQTLs
17q12-q21	**rs9901146**	A/G	*GSDMB, ORMDL3*	$3.8x10^{-6}$-$9.8x10^{-198}$	Blood, LCLs	Blood eQTLs, GTEx, eQTL Browser, eQTL_Chicago
	rs3859192	C/T	*GSDMA, GSDMB, ORMDL3*	$1.1x10^{-7}$-$2.5x10^{-12}$	Lung, LCLs	GTEx, eQTL Browser

* Top genome-wide significant SNPs in time-to-asthma onset meta-analysis and secondary associations identified by conditional analyses are indicated in bold.

683 [†]Haplotype reconstruction was done using Haploview; the effect allele of the top SNP (A-rs1861760) is always transmitted with the effect allele
684 of its proxy (G-rs5743266 and G-rs7205760).
685 [††]Interrogated databases: *eQTL Browser* (LCLs of British subjects with asthma or eczema),[18] *Blood eQTL Browser* (non-transformed peripheral
686 blood sample),[20] *Lung eQTLs* (lung),[17]*GTEx eQTL Browser v4* (several tissues among which blood and lung),[23] and *eQTL Chicago Browser*
687 (LCLs).[19,21,22]

FIGURE LEGENDS

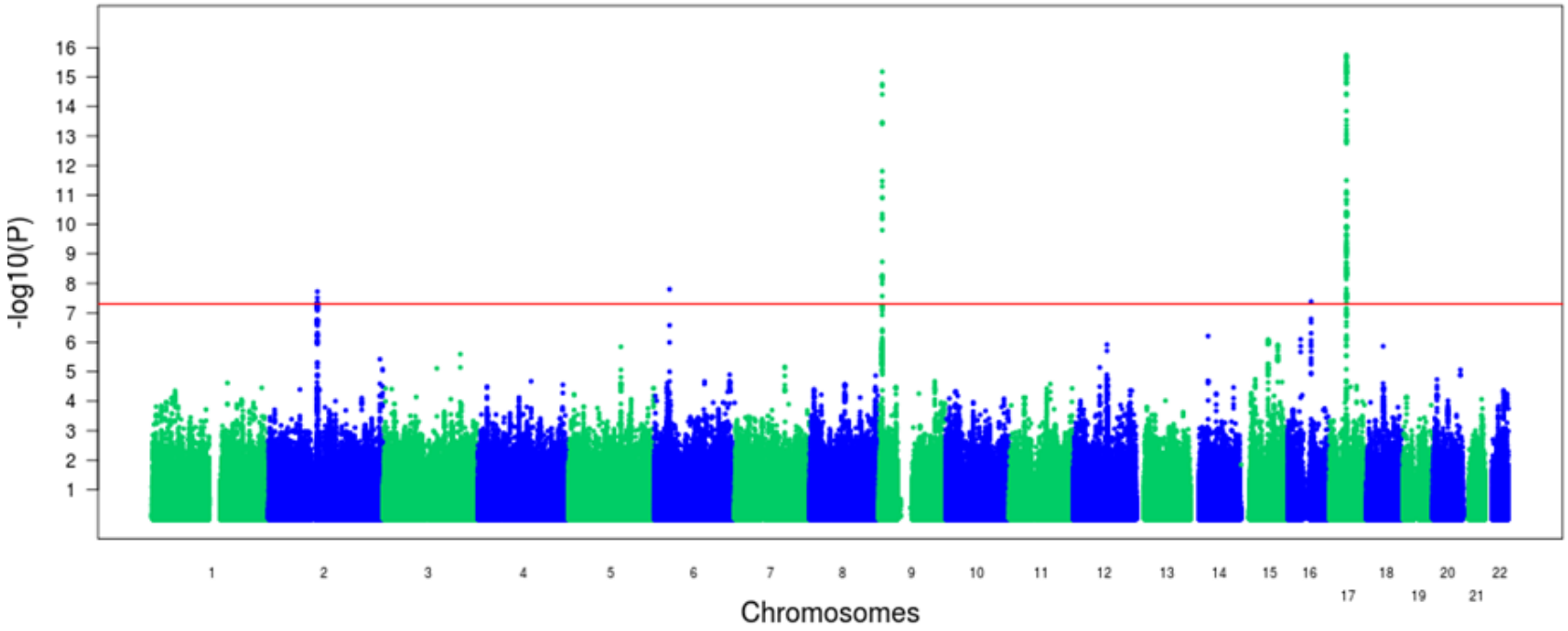
Figure 1. Manhattan plot showing the association P-values of the genome-wide association results for time-to-asthma onset from the meta-analysis. The $-\log_{10}$ of the P-value for each of 2,400,368 SNPs (y-axis) is plotted against the genomic position (x-axis). The solid red line indicates the genome-wide significance threshold of $P=5x10^{-8}$.

Figure 2. Regional association plot of the 16q12 region using Locuzoom software.[56] SNPs are plotted with their P-values ($-\log_{10}$ values, left y-axis) as a function of genomic position (x-axis). Estimated recombination rates (right y-axis) taken from 1000G (EUR) are plotted to reflect the local LD structure around the top associated SNP (purple circle) and correlated proxies (according to a blue to red scale from $r^2=0$ to 1).

Figure 3. Relationship between time-to-asthma onset associated SNPs and age-of-asthma onset. Part A) Association between age-of-asthma onset and genotypes at individual locus; Part B) Median of age-of-asthma onset as a function of the number of individual's risk allele burden; Part C) Median of age-of-asthma onset by quintile of genetic risk score.

Figure 4. Map of 16q12 region (build 37.3 position: 50,723,355-50,860,722) and haplotype reconstruction for SNPs found associated with inflammatory bowel disease (among which Crohn's disease, blue), leprosy (green) or asthma (red) or with the expression of *CYLD* or *NOD2* (black). Linkage disequilibrium plot was obtained using Hapmap2 CEU reference sample from Haploview[57] (values and colors reflect r^2 and D' respectively). The 16q12 top SNP (rs1861760) associated with time-to-asthma onset is indicated in bold.

Figure 1.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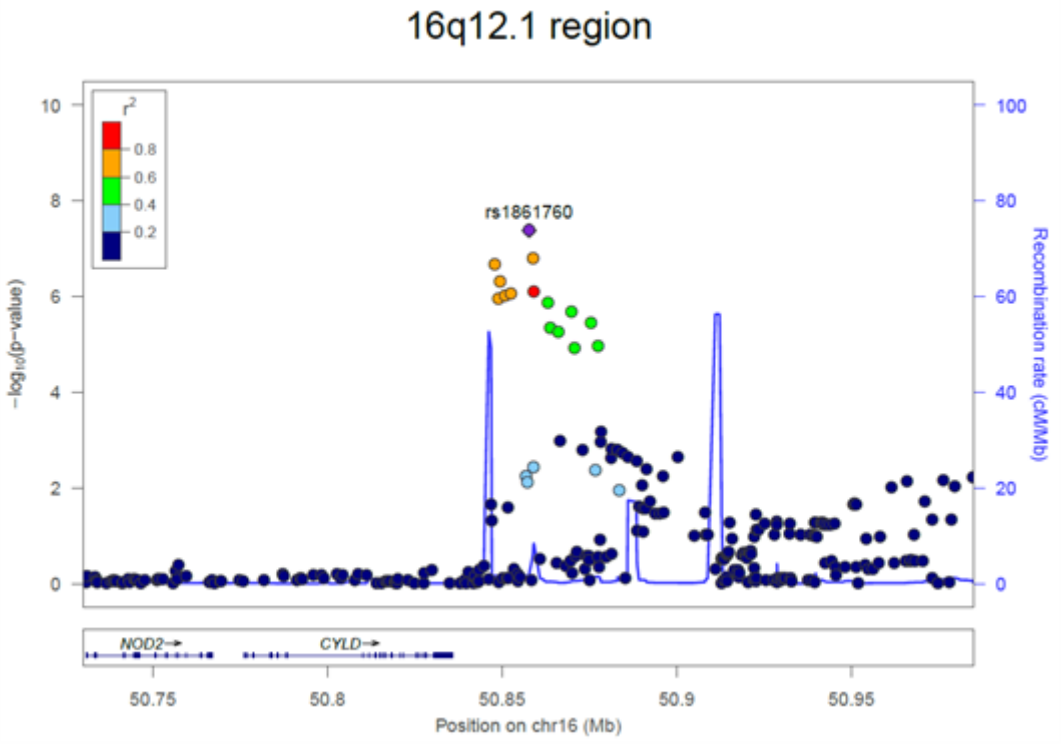
Chromosomes

712 **Figure 2.**



713

714

Figure 3.

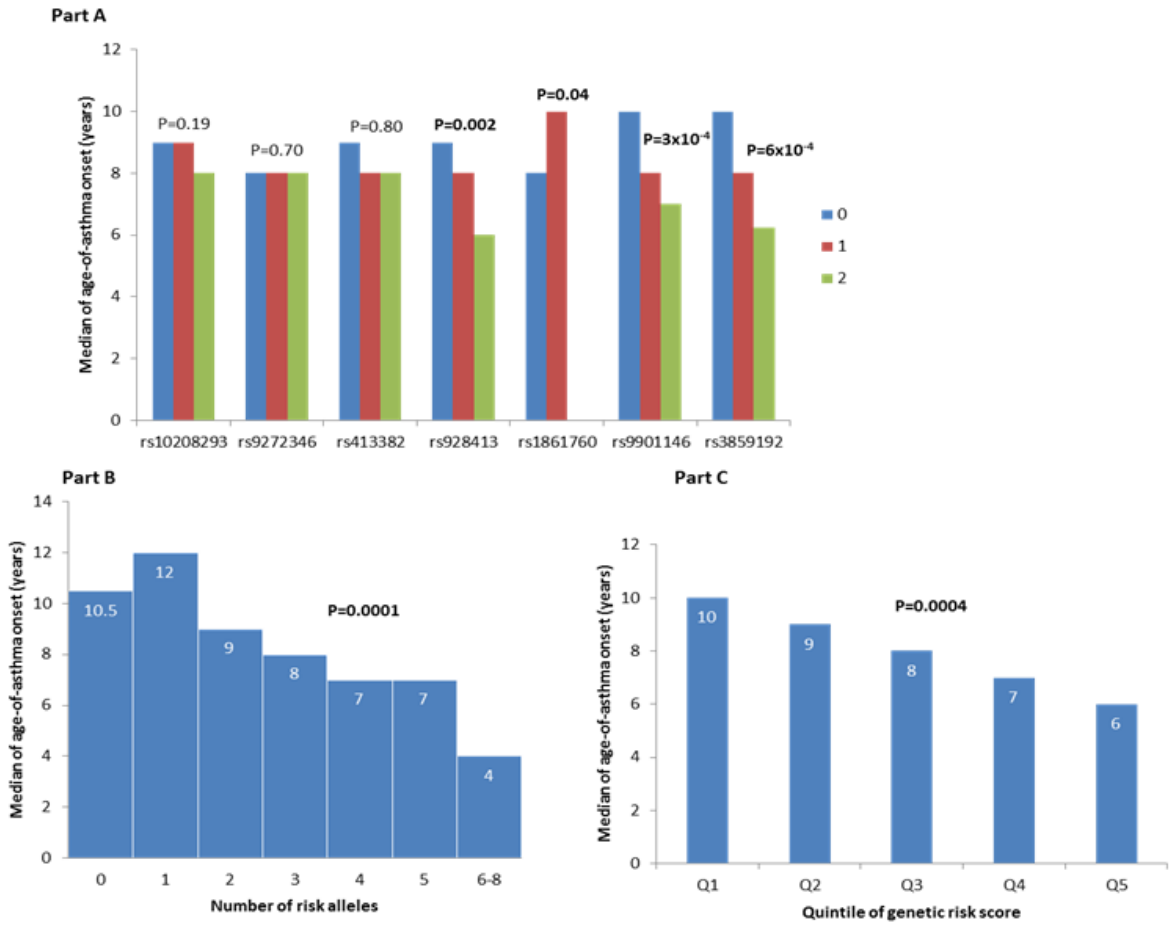
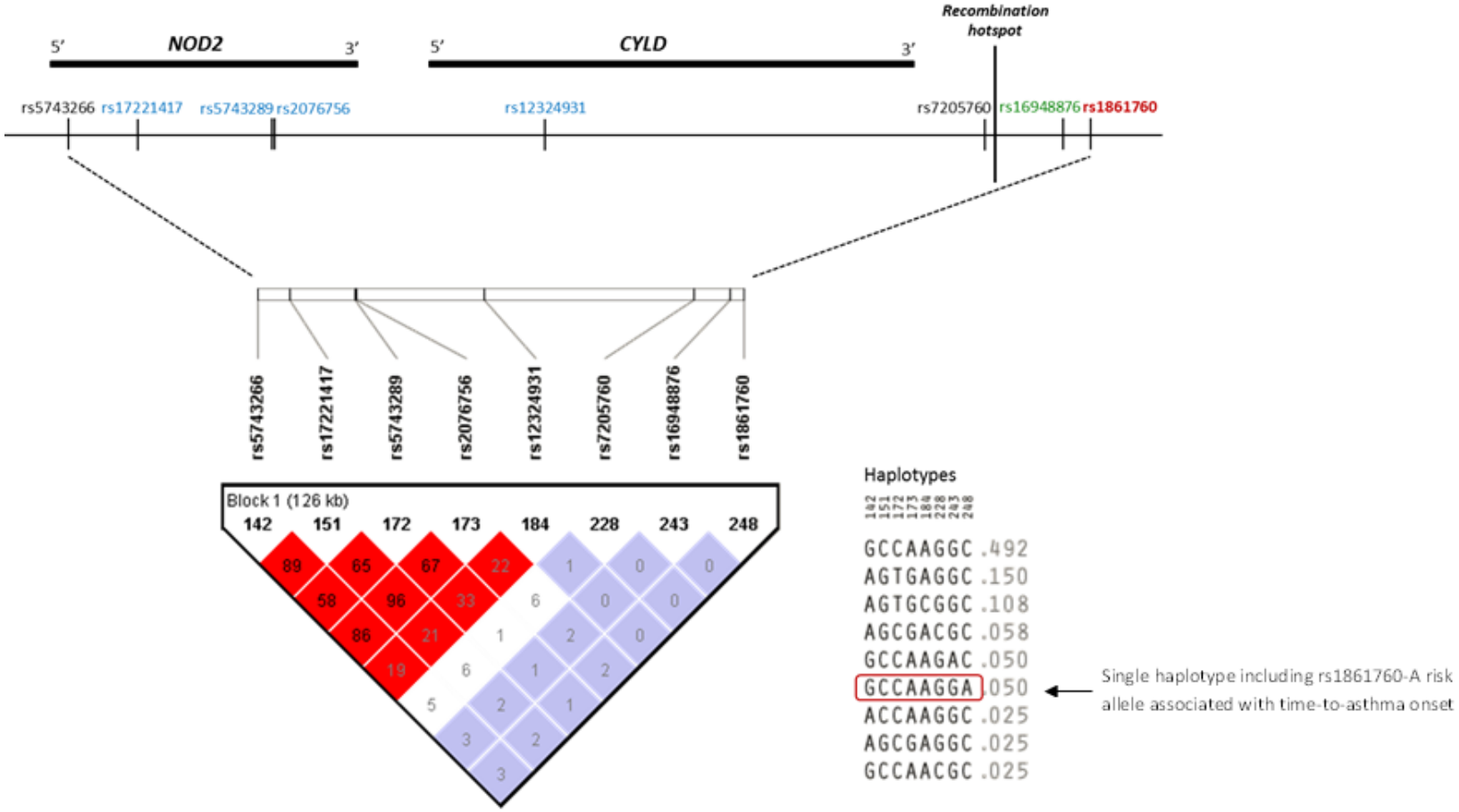

Part A
Median of age-of-asthma onset (years)
P=0.19
P=0.70
P=0.80
P=0.002
P=0.04
P=3x10-4
P=6x10-4
0
1
2
rs10208293
rs9272346
rs413382
rs928413
rs1861760
rs9901146
rs3859192
Part B
Median of age-of-asthma onset (years)
10.5
12
9
8
7
7
4
P=0.0001
Number of risk alleles
Part C
Median of age-of-asthma onset (years)
10
9
8
7
6
P=0.0004
Q1
Q2
Q3
Q4
Q5
Quintile of genetic risk score

Figure 4.


Recombination hotspot
5′ NOD2 3′
5′ CYLD 3′
rs5743266 rs17221417 rs5743289 rs2076756 rs12324931 rs7205760 rs16948876 rs1861760
rs5743266
rs17221417
rs5743289
rs2076756
rs12324931
rs7205760
rs16948876
rs1861760
Block 1 (126 kb)
142 151 172 173 184 228 243 248
89 65 67 22 1 0 0
58 96 33 6 0 0
86 21 1 2 0
19 6 1 2
5 2 1
3 2
3
Haplotypes
142 151 172 173 184 228 243 248
GCCAAGGC .492
AGTGAGGC .150
AGTGCGGC .108
AGCGACGC .058
GCCAAGAC .050
GCCAAGGA .050
ACCAAGGC .025
AGCGAGGC .025
GCCAACGC .025
Single haplotype including rs1861760-A risk allele associated with time-to-asthma onset