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1 **Identification of a new locus at 16q12 associated with time-to-asthma onset**

2

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90 **ABSTRACT**

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

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

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

97 **Results:** We detected five regions associated with time-to-asthma onset at genome-wide
98 significant level ($P < 5 \times 10^{-8}$). We evidenced a new locus in 16q12 region (near *cylindromatosis*
99 *turban tumor syndrome* gene (*CYLD*)) and confirmed four asthma risk regions: 2q12
100 (*IL1RL1*), 6p21 (*HLA-DQA1*), 9p24 (*IL33*) and 17q12-q21 (*ZPBP2-GSDMA*). Conditional
101 analyses identified two distinct signals at 9p24 (both upstream of *IL33*) and at 17q12-q21
102 (near *ZPBP2* and within *GSDMA*). These seven distinct loci explained together 6.0% of the
103 variance in time-to-asthma onset. In addition, we showed that genetic variants at 9p24 and
104 17q12-q21 were strongly associated with an earlier onset of childhood asthma ($P \leq 0.002$)
105 whereas 16q12 SNP was associated with a later asthma onset ($P = 0.04$). A high burden of
106 disease risk alleles at these loci was associated with earlier age-of-asthma onset (4 years
107 versus 9-12 years, $P = 10^{-4}$).

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

113

114

115 **Key Messages:**

- 116 • 16q12 genetic variants are associated with time-to-asthma onset and correlate with
117 *CYLD* and *NOD2* expressions.
- 118 • Genetic variants at 9p24 (upstream of *IL33*) and 17q12-q21 (nearby *ZPBP2* and within
119 *GSDMA*) are associated with an earlier asthma-onset whereas variants at 16q12 are
120 associated with a later asthma onset.
- 121 • Taking into account the variability of age-of-asthma onset in disease modeling can
122 increase the power of identifying new genes involved in asthma pathophysiology.

123

124 **Capsule summary:**

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

128

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

131

132 **Abbreviations:**

133 *CYLD*: Cylindromatosis (turban tumor syndrome)

134 eQTL: Expression quantitative trait locus

135 *GSDMA*: Gasdermin A

136 GWAS: Genome-wide association study

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

138 HR: Hazard ratio

139 *IL1RL1*: Interleukin 1 receptor-like 1

- 140 *IL33*: Interleukin 33
- 141 LCL: Lymphoblastoid cell line
- 142 LD: Linkage Disequilibrium
- 143 *NFkB1*: Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
- 144 *NOD2*: Nucleotide-binding oligomerization domain containing 2
- 145 QC: Quality control
- 146 SNP: Single nucleotide polymorphism
- 147 TAO: time-to-asthma onset
- 148 *ZPBP2*: Zona pellucida binding protein 2
- 149

150 **INTRODUCTION**

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

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

163 The risk of developing asthma has a strong genetic component, with estimated heritability
164 ranging from 35 to 95%.⁷ Genome-wide association studies (GWASs) have been successful in
165 identifying more than 20 loci associated with asthma.⁸ However, the genetic factors identified
166 to date account only for a small part of the genetic component of the disease.¹ This hidden
167 heritability might be linked to the phenotypic heterogeneity of asthma.⁹ The vast majority of
168 GWASs conducted until now have analyzed asthma as a binary phenotype. A few genetic
169 studies have considered more specific definition of asthma incorporating the age of disease
170 onset. A genome-wide linkage screen conducted for time-to-asthma onset in French families
171 revealed two regions, 1p31 and 5q13, potentially linked to this phenotype.¹⁰ A single GWAS
172 has been performed on age-of-asthma onset in asthmatic children and led to the identification
173 of two loci, not found by the previous asthma GWASs; these loci on chromosomes 3p26 and
174 11q24 were associated with an earlier onset of childhood asthma.¹¹ Moreover, the effect of

175 17q12-q21 genetic variants, identified by the first GWAS of asthma,¹² was found to be
176 restricted to early-onset asthma.^{13,14}

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

183

184 **METHODS**

185 *Populations*

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

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

193

194 *Time-to-asthma onset definition*

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

199

200 *Genotyping*

201 Genotyping, single nucleotide polymorphism (SNP) imputation process and quality control
202 (QC) criteria (for individuals and SNPs) for each study are described in Table E1 in the online
203 repository. All datasets were genotyped at Centre National de Génotypage (CNG, Evry,
204 France), as part of the European GABRIEL asthma consortium.¹⁴ Quality control and
205 imputations were performed independently for each study. Genome-wide imputations were
206 conducted using MACH 1.0 software,¹⁵ with reference haplotype panels from HapMap 2.
207 SNPs with imputation quality score (Rs_q) ≥ 0.5 and minor allele frequency $\geq 1\%$ were kept
208 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 variance¹⁶ 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 $P\text{-value} \leq 5 \times 10^{-8}$ to
232 declare a meta-analyzed SNP effect as genome-wide significant.

233

234 ***Conditional analysis to uncover distinct signals at TAO-associated loci***

235 To identify distinct TAO-associated SNPs in each region harboring genome-wide significant
236 signals, we re-analyzed separately these regions in each of the nine studies. For that purpose,
237 we added the region top SNP into the primary Cox model as a covariate and tested the effect
238 of each other SNP of that region. Then, the results were meta-analyzed using the same
239 strategy as the primary GWASs. If a secondary signal was detected in a region, a second run
240 of conditional analyses was performed to check for a third distinct signal in that region.
241 Length of explored regions was based on regional association plots and ranged from 200 kb to
242 500 kb depending on recombination hotspots.

243

244 ***eQTL analysis and functional annotations***

245 We queried whether significant SNPs (or their proxies) associated with time-to-asthma onset
246 at $P \leq 5 \times 10^{-8}$ and potentially secondary signals from conditional analysis were expression
247 quantitative trait loci (eQTLs). We used existing eQTL databases in multiple tissues
248 (especially blood and lung) for populations of European ancestry (see online repository).¹⁷⁻²³
249 Functional annotations of significant SNPs (or their proxies) were obtained using
250 ENcyclopedia Of DNA Elements (ENCODE) data²⁴ provided by the HaploReg tool.²⁵

251

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

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

259 onset associated alleles with weight being the log of adjusted hazard ratio estimated in
260 asthmatics only. The associations were tested in eight studies for which we had access to raw
261 data (all datasets except ALSPAC) using a cox proportional hazard model adjusted on sex and
262 principal components.
263

264 **RESULTS**

265 *Description of populations*

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

272

273 *Genetic variants associated with time-to-asthma onset*

274 The Manhattan and the Q-Q plots of the meta-analysis of time-to-asthma onset GWAS results
275 are shown in Figure 1 and Figure E2 in the online repository respectively. A total of 155
276 SNPs were associated with time-to-asthma onset at the genome-wide significance level of
277 $P < 5 \times 10^{-8}$. These SNPs clustered into five distinct chromosomal regions (Table I), that
278 included a new risk locus on 16q12 (nearby *CYLD* (cylindromatosis turban tumor syndrome),
279 1 SNP) and four established risk loci for asthma: 2q12 (*IL1RL1-IL18R1*, 7 SNPs), 6p21
280 (nearby *HLA-DQA1*, 1 SNP), 9p24 (flanking *IL33*, 25 SNPs) and 17q12-q21 (121 SNPs
281 spanning 389 kb, and with the main signal located near *ZPBP2*). The regional association
282 plots for these genome-wide associated loci are shown in Figures 2 and E3, and the forest
283 plots for the top signal in each region are shown in Figure E4. Three additional loci were
284 associated with time-to-asthma onset at a suggestive significance threshold ($5 \times 10^{-8} < P < 10^{-6}$;
285 Table I): *MAP4K4* (2q11-q12), *RORA* (15q22) and *IL4R* (16p12-p11).

286 To determine whether any of the five TAO loci harbored additional association signals, we
287 performed conditional association analysis in each region. For this analysis, a threshold of
288 $P \leq 2.1 \times 10^{-5}$ was used to declare significance, corresponding to a Bonferroni threshold for

289 2,382 independent tests. These analyses evidenced two secondary signals (Table II and Figure
290 E5 in the online repository): 1) rs413382 in 9p24 region at 73 kb of *IL33* ($P=9.7\times 10^{-6}$ after
291 conditioning on top SNP and $P=5.9\times 10^{-8}$ in the primary meta-analysis) and 2) rs3859192 in
292 17q12-q21 region within *GSDMA* ($P=4.0\times 10^{-6}$ after conditioning on top SNP and $P=1.5\times 10^{-13}$
293 in the primary meta-analysis). In contrast, at 2q12, 6p21 and 16q12 regions, the inclusion of
294 the most significant time-to-asthma onset GWAS SNP as a covariate in association analysis
295 resulted in nearly complete reduction of the association signal in these regions, suggesting
296 that there was no evidence for a second distinct genetic factor in these regions.

297 To obtain a denser map of the new TAO 16q12 locus, we repeated association analyses using
298 1000 Genomes Project imputed SNPs. These analyses strengthened our original finding with
299 additional signals ($3.8\times 10^{-8}\leq P\leq 2.6\times 10^{-7}$) located in an intergenic region encompassing the
300 lead SNP rs1861760 (Table E2 and Figure E6). These SNPs were in moderate to high LD
301 with rs1861760 ($0.71\leq r^2\leq 0.81$) and thus did not represent independent signals from that top
302 hit. Similar analyses conducted in the four other TAO-associated regions also supported our
303 original findings and did not evidence any additional independent signal in these regions.

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

307

308 ***Functional annotations and effect on gene expression***

309 To provide some insights into the potential molecular mechanisms underlying the TAO-
310 associated variants, we queried whether the five top SNPs and two secondary signals (and
311 their proxies) were 1) tagging potentially deleterious SNPs, 2) located in regulatory elements,
312 and 3) reported to influence the expression of one or more of nearby genes (eQTLs at
313 $P<5\times 10^{-5}$). We focused on the new TAO risk locus at 16q12 region. Functional annotations

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

316 The 16q12 TAO-associated variants are located in an intergenic region delimited by two
317 recombination hotspots on each side, near *CYLD* (22 kb downstream). The rs1861760 maps to
318 FOXJ1 and SOX binding sites. This SNP and/or its proxies correlate with the expression of
319 *CYLD* in both blood and human lung tissues and of *NOD2* (*nucleotide-binding*
320 *oligomerization domain 2*) in blood²⁰ (Table III for main results and Table E3 in the online
321 repository).^{17,20}

322

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

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

337

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

339 To investigate the impact of taking into account the age-of-asthma onset in disease modeling
340 through survival analysis, we explored whether the top TAO SNPs were associated with
341 asthma modeled as a binary trait in the nine cohorts included in the present study (Table E4,
342 see online repository). We also investigated GABRIEL top SNPs in TAO meta-analysis
343 (Table E4).¹⁴ We observed a strong decrease of heterogeneity of SNP effect across studies in
344 TAO analysis ($P_{\text{Het}} \geq 0.11$) compared with asthma binary trait analyzed in the same datasets
345 ($P_{\text{Het}} \geq 0.004$) as well as in all GABRIEL datasets ($P_{\text{Het}} \geq 0.0009$), especially in 9p24 and 17q12-
346 q21 regions. The association signals were always more significant in TAO analysis as
347 compared with the binary trait analysis in the same datasets. This increase of significance
348 level was very high: 100-fold for 2q12 and 16q12, and 10^4 to 10^6 -fold for 9p24 and 17q12-
349 q21. In fact, the asthma binary trait analysis only detected two loci (*HLA* and *GSDMA*) at the
350 genome-wide significance level out of the seven TAO-associated loci. Conversely, the present
351 TAO analysis identified at the genome-wide significance level four of the six main published
352 GABRIEL regions,¹⁴ and even at higher significance for 9p24 and 17q12-q21 regions (100 to
353 10^4 -fold) as compared to GABRIEL significance levels. The two remaining GABRIEL loci
354 not detected by our TAO analysis were those with the weaker effects (OR=1.12 for rs744910
355 in 15q22 and rs2284033 in 22q13) in GABRIEL meta-analysis.¹⁴
356 Finally, we evaluated whether previously reported susceptibility loci for asthma²⁶ were
357 associated with time-to-asthma onset in our meta-analysis (see online repository, Table E5).
358 Among the 21 loci detected in European populations, 12 were replicated at 5% in our TAO
359 meta-analysis with the same direction of effects. Among the nine non-replicated signals, three
360 SNPs (or some proxies) were not available in our data, and the remaining six loci had been
361 reported for specific phenotypes: asthma exacerbation, age-of-asthma onset *per se* in
362 asthmatic children only (quantitative trait) or childhood asthma (binary trait).^{11,27,28}

363

364 **DISCUSSION**

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

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

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

399 Defining the phenotype is an important consideration because phenotypic heterogeneity can
400 reduce power of GWAS.⁴⁴ In the present analyses, we studied the variability of time-to-
401 asthma onset in both asthmatics and non-asthmatics based on survival analysis methods. The
402 information used for such analysis was the age-of-onset in asthmatics and the age at last
403 examination or death in non-asthmatics. In such a model, unaffected subjects represent
404 censored observations as they are still at risk for disease, being perhaps too young to exhibit
405 the trait. This approach, which allowed combining the age-of-asthma onset together with the
406 disease status (affected/unaffected) led to decrease the genetic heterogeneity across studies
407 and increase the power to detect association signals (upon 10^6 -fold increase as compared to
408 the disease status only analysis. More specifically, increased evidence of association was
409 observed in regions where age-of-asthma onset explained at least in part the genetic
410 heterogeneity such as the 17q12-q21 locus for which a restricted SNP effect to a particular
411 group of age-of-onset (early childhood onset asthma) was demonstrated.¹³ Moreover, this
412 analysis led to the identification of a new locus at 16q12 near *CYLD* and of an additional
413 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

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

445 In summary, we identified five regions harboring seven distinct signals that were associated
446 with time-to-asthma onset, among which the 16q12 region that is reported for the first time.
447 Several lines of evidence suggest that *CYLD* and *NOD2* located in that region are strong
448 candidate genes for asthma. This study demonstrates that incorporating the variability of age-
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- 658

659 Table I. Top SNPs in main loci associated with time-to-asthma onset at genome-wide ($P \leq 5 \times 10^{-8}$) and suggestive significance levels ($5 \times 10^{-8} < P < 10^{-6}$)
660 $^{\circ} < P < 10^{-6}$)

Chromosome	Marker	Position *	Nearest gene(s) (kb distance)	Effect/ Ref Alleles †	Effect Freq	Time to asthma onset - N=13,886		
						Hazard Ratio [95% CI]	P ‡	P-Het **
Loci with genome-wide significance - $P \leq 5 \times 10^{-8}$								
2q12	rs10208293	102966310	<i>IL1RL1</i> ***	G/A	0.73	1.14 [1.08-1.19]	3.1×10^{-8}	0.26
6p21	rs9272346	32604372	<i>HLA-DQA1</i> (0.8)	A/G	0.59	1.13 [1.08-1.17]	1.6×10^{-8}	0.12
9p24	rs928413	6213387	<i>IL33</i> (2)	G/A	0.25	1.19 [1.13-1.25]	6.5×10^{-16}	0.15
16q12	rs1861760	50857693	<i>CYLD</i> (22)	A/C	0.04	1.28 [1.17-1.40]	4.2×10^{-8}	0.11
17q12-q21	rs9901146	38043343	<i>ZBP2</i> (9) <i>GSDMB</i> (17)	G/A	0.51	1.18 [1.13-1.22]	1.9×10^{-16}	0.17
Suggestive loci - $5 \times 10^{-8} < P < 10^{-6}$								
2q11-q12	rs12468899	102426140	<i>MAP4K4</i> ***	G/A	0.69	1.12 [1.09-1.16]	1.7×10^{-7}	0.89
15q22	rs11071559	61069988	<i>RORA</i> ***	C/T	0.85	1.16 [1.10-1.24]	8.3×10^{-7}	0.96
16p12-p11	rs1805013	27373980	<i>ILAR</i> ***	T/C	0.05	1.22 [1.13-1.32]	8.0×10^{-7}	0.37

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

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

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

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

667 ***SNP is located within reported gene

668

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

Chr	Marker	Nearest gene (kb distance)	Position *	Effect/Ref Alleles [†]	Effect Freq	Single SNP analysis			Fitted SNP(s)		
						Hazard Ratio [95% CI]	P [‡]	P-Het ^{**}	Hazard Ratio [95% CI]	P [‡]	P-Het ^{**}
9p24 region						rs928413					
9	rs413382	<i>IL33</i> (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	<i>IL33</i> (2)	6213387	G/A	0.25	1.19 [1.13-1.25]	6.5x10 ⁻¹⁶	0.15	-	-	-
17q12-q21 region						rs9901146					
17	rs9901146	<i>ZPBP2</i> (9)	38043343	G/A	0.51	1.18 [1.13-1.22]	1.9x10 ⁻¹⁶	0.17	-	-	-
17	rs3859192	<i>GSDMA</i> ***	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

672 *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

679 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
 680 focused on eQTLs measured in blood, lymphoblastoid cell lines (LCLs) and lung tissue.

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

681 * Top genome-wide significant SNPs in time-to-asthma onset meta-analysis and secondary associations identified by conditional analyses are
 682 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}

688 **FIGURE LEGENDS**

689 **Figure 1.** Manhattan plot showing the association P-values of the genome-wide association
690 results for time-to-asthma onset from the meta-analysis. The $-\log_{10}$ of the P-value for each of
691 2,400,368 SNPs (y-axis) is plotted against the genomic position (x-axis). The solid red line
692 indicates the genome-wide significance threshold of $P=5 \times 10^{-8}$.

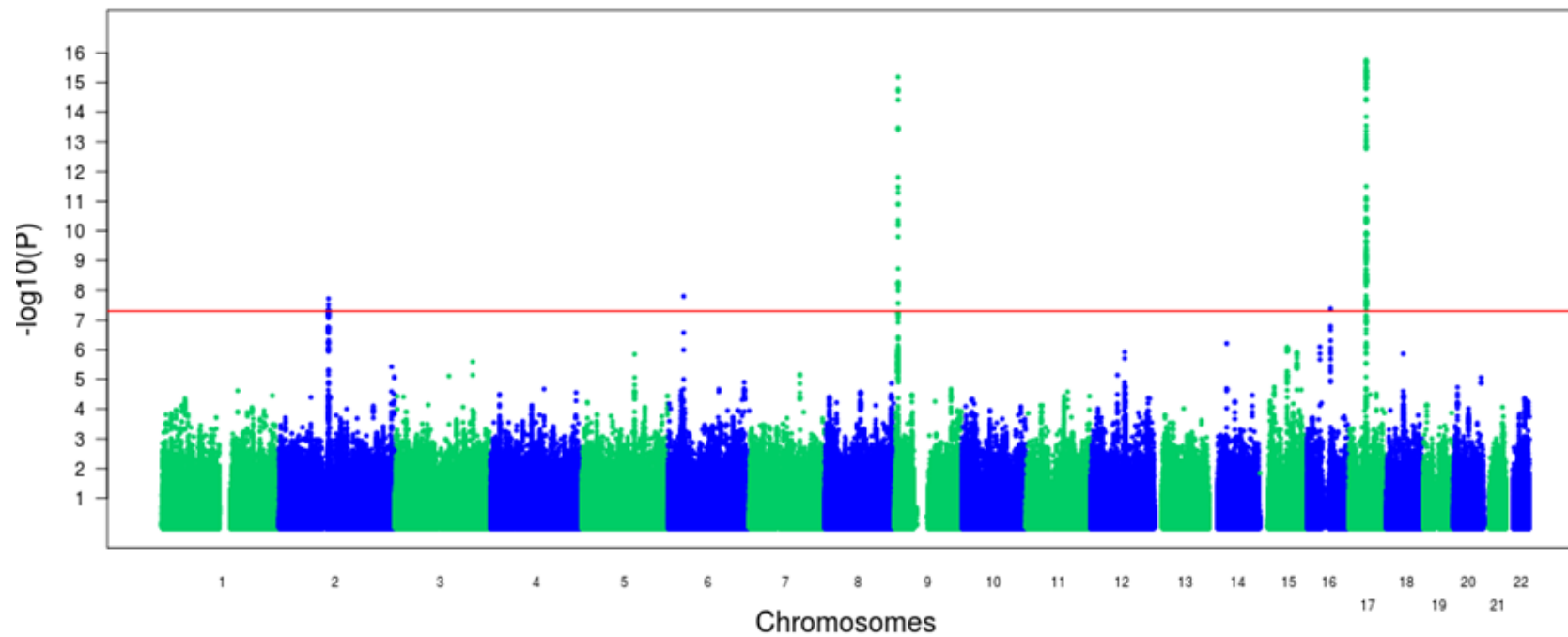
693 **Figure 2.** Regional association plot of the 16q12 region using Locuzoom software.⁵⁶ SNPs are
694 plotted with their P-values ($-\log_{10}$ values, left y-axis) as a function of genomic position (x-
695 axis). Estimated recombination rates (right y-axis) taken from 1000G (EUR) are plotted to
696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

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

702 **Figure 4.** Map of 16q12 region (build 37.3 position: 50,723,355-50,860,722) and haplotype
703 reconstruction for SNPs found associated with inflammatory bowel disease (among which
704 Crohn's disease, blue), leprosy (green) or asthma (red) or with the expression of *CYLD* or
705 *NOD2* (black). Linkage disequilibrium plot was obtained using Hapmap2 CEU reference
706 sample from Haploview⁵⁷ (values and colors reflect r^2 and D' respectively). The 16q12 top
707 SNP (rs1861760) associated with time-to-asthma onset is indicated in bold.

708

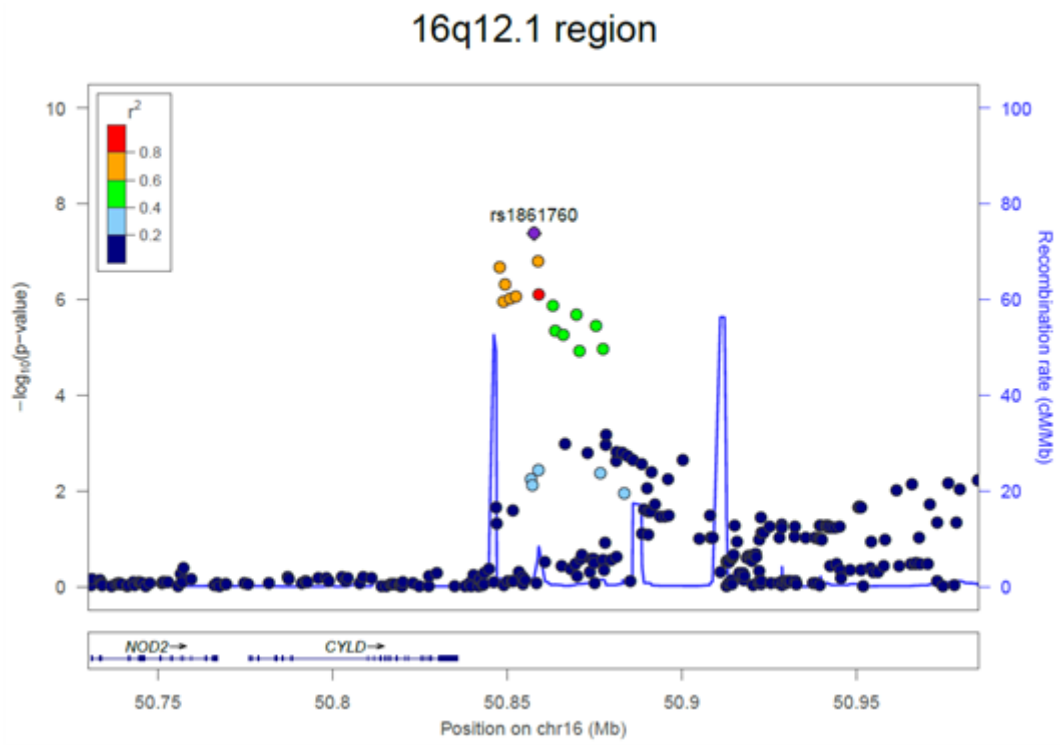
709 **Figure 1.**



710

711

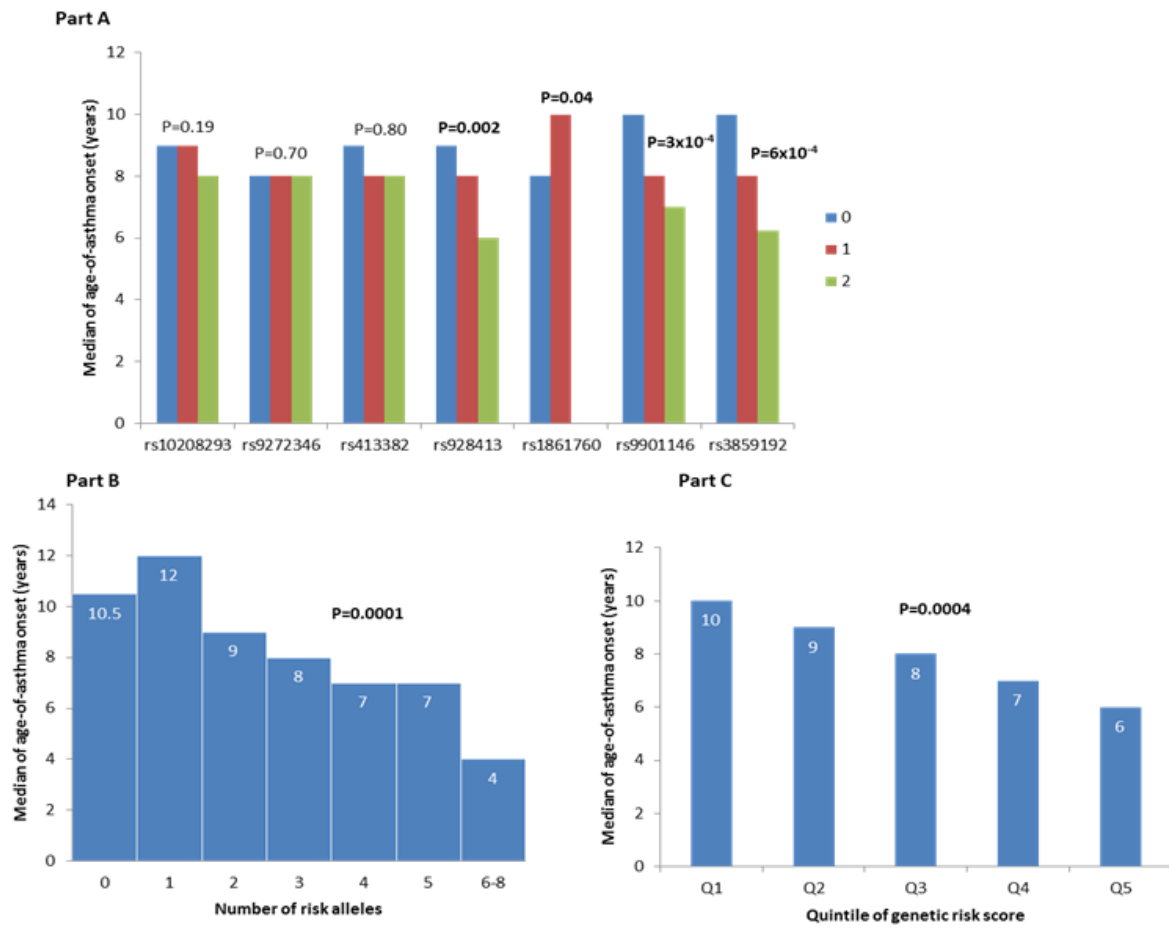
712 **Figure 2.**



713

714

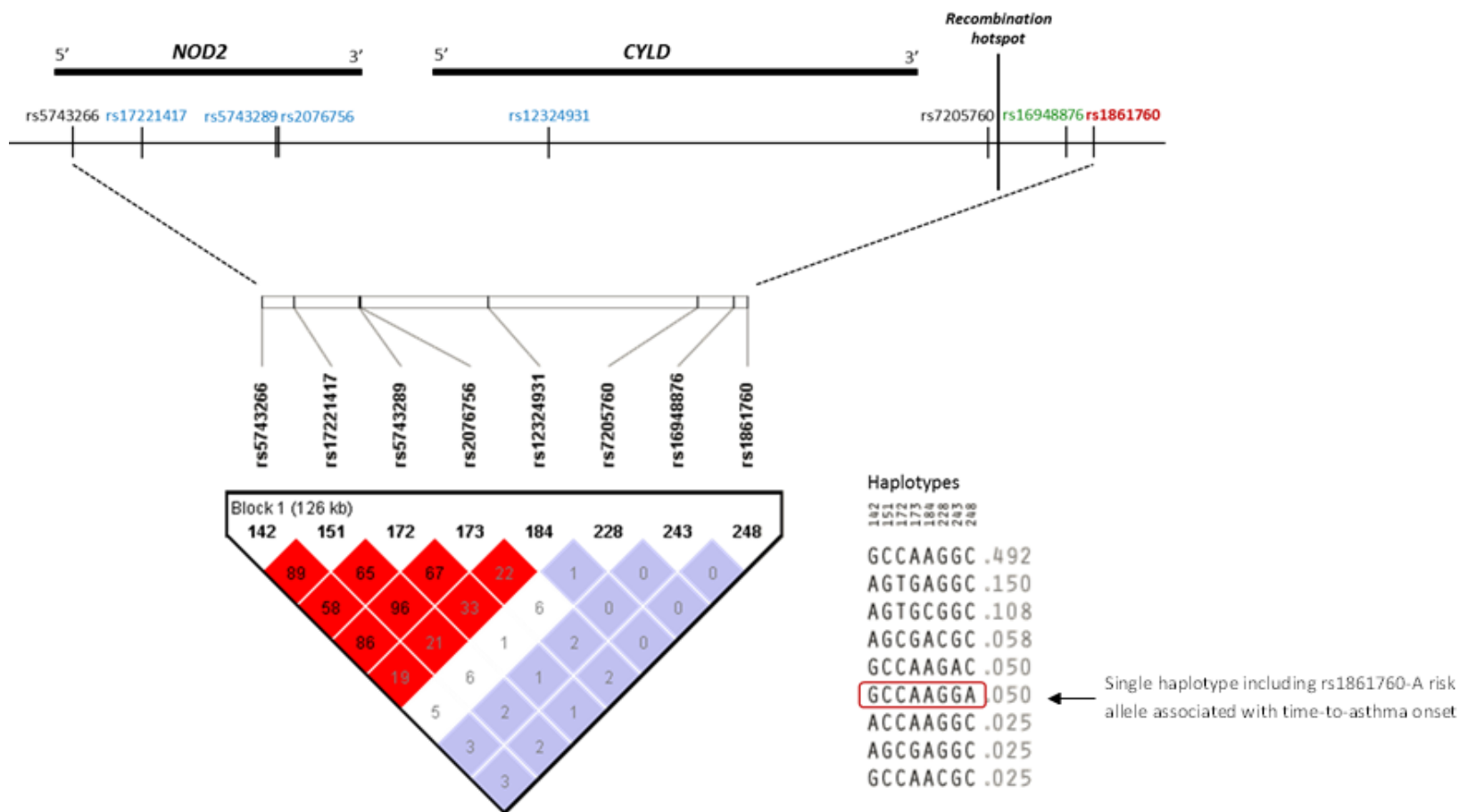
715 **Figure 3.**



716

717

718 **Figure 4.**



719