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

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METHODS

Study populations

The present project includes nine independent studies (Table E1) among which five population-based studies: the European Community Respiratory Health Survey (ECRHS), the Swiss study on Air Pollution and Lung and Heart Disease In Adults (SAPALDIA), the Busselton Health Study (BUSSELTON), the GABRIEL Advanced Surveys (GABRIELA) and the UFA study (UFA); three familial studies: the Epidemiological study on the Genetics and Environment of Asthma (EGEA); the Saguenay-Lac-Saint-Jean Familial Collection (SLSJ) and the TOMSK study (TOMSK); and one birth cohort: the Avon Longitudinal Study of Parents and Children (ALSPAC). All of these studies were part of the GABRIEL European consortium on asthma,[1] and had the information on age of asthma onset, age at last examination and imputed genetic data available. A total of 13,886 subjects from European ancestry were included in the time-to-asthma onset GWAS meta-analysis (5,462 asthmatics and 8,424 non-asthmatics).

Study populations and Phenotype definition

ALSPAC

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based birth cohort initially comprising of 14,541 mothers and their children recruited in the former County of Avon, UK between 1991 and 1992.[2]

Asthmatics were defined by a positive response to the question: “Did you child had asthma in past 12 months?” at 81, 91, 103, 128, 157 or 166 months. Non-asthmatics were those who answered no at all surveys. In asthmatics, age of onset was defined by the first time they declared wheeze or wheezing and whistling. Wheeze was defined by a positive response to the question: “Has your child had wheezing, breathlessness or episodes of stopping breathing in past 12 months or since he was (age at last Q)?”. Wheezing and whistling were defined by a positive response to the question: “Has your child had any periods when there was wheezing with whistling on his chest when he breathed in past 12 months or since he was (age at last Q)?”. In non-asthmatics, we considered age at the last examination without any missing visit at the preceding surveys. Thus, for non-asthmatics with negative complete reports, we considered age at last examination and for non-asthmatics with negative incomplete and/or discontinued reports, we considered age until the last visit before the first missing visit. We did not include in the present analysis non-asthmatics who experienced wheeze or wheezing and whistling before 6yrs of age.

ECRHS

The ECRHS study is a European population-based study of young adults with a 8-year follow-up (ECRHS I: 1991-1993, ECRHS II: 1999-2002 and ECRHS III: 2010-ongoing).[3,4] The time-to-asthma onset GWAS is based on the two first survey data. Participants included in the meta-analysis were derived from the nested asthma case/control sample subjected to genome-wide genotyping in the context of the GABRIEL asthma GWAS.

Asthma cases were identified by participants from the random or enriched sample who said yes to the question ‘Have you ever had asthma?’ at either ECRHS I Stage 2 or at ECRHS II. Controls were a random sample (of the random sample) who answered ‘no’ to the same question in both surveys. For individuals who developed asthma, information on asthma age

at onset was obtained from age at first asthma attack at ECRHS I or II. For individuals who were free of disease upon examination, we considered age at last examination.

EGEA

Briefly, the EGEA study combines a case-control and a family-based study of asthma cases (N=2,120 subjects) with three surveys over 20 years (EGEA1: 1991-1995, EGEA2: 2003-2007 and EGEA3: 2011-2013). The whole study population included 388 asthmatic probands recruited in chest clinics and their 1,317 family members (probands' parents and/or siblings) plus 415 population-based controls.[5]

Asthma was defined in probands by a positive answer to the following four items "Have you ever had attacks of breathlessness at rest with wheezing?", "Have you ever had asthma attacks?", "Was this diagnosis confirmed by a physician?", and "Have you had an asthma attack in the last 12 months?" or on a positive self-report to two of the before mentioned items plus a medical record of asthma. Individuals were considered free of disease if they answered no at all items. Relatives of probands were defined as asthmatics if they answered positively at either survey to "Have you ever had attacks of breathlessness at rest with wheezing?" or "Have you ever had asthma attacks?" at EGEA1, EGEA2 or EGEA3.

For individuals who developed asthma, information on asthma age at onset was obtained from adult asthmatics or parents of asthmatic children who answered to the following question: "How old were you when you had your first asthma attack?" or "How old was your child when he (or she) had his (her) first asthma attack?". For individuals who were free of disease upon last examination, we considered age at last examination.

GABRIEL Advanced Surveys

GABRIELA are cross-sectional population-based surveys conducted in rural areas of Austria, Germany, and Switzerland during fall/winter 2006 and spring/summer 2007.[6]

A case was defined as a parental report of asthma diagnosed by a doctor at least once or asthmatic bronchitis diagnosed at least twice during lifetime. The reference category for asthma was no reported diagnosis of asthma ever and a diagnosis of asthmatic bronchitis no more than once. The original question on age of onset was: "How old was your child when the first symptoms of wheezing or whistling in the chest began? At the age of … years. If during the first year: At the age of … months." The variable is coded for years. Months were transferred to years. In non-asthmatics, we considered age at examination. To define atopy, we used an atopic sensitization to mite, cat, or birch upper or equal to 0.7 kU/L.

SAPALDIA

The SAPALDIA study is a cohort study with integrated biobank in the Swiss population initiated in 1991 (SAPALDIA 1: n=9,651; age 18-60 at baseline) with two follow-up assessments in 2001-2003 (SAPALDIA 2: n=8,047) and in 2010-2011 (SAPALDIA 3: n=6,200).[7,8] The time-to-asthma onset GWAS is based on the first two survey data. Participants included in the meta-analysis were derived from the nested asthma case/control sample subjected to genome-wide genotyping in the context of the GABRIEL asthma GWAS. Asthma status was defined by an affirmative answer to the question "Have you ever had asthma" at baseline and/or follow-up interview. Controls were defined by a negative answer to the same question. Age of onset was self-reported by study participants. For individuals who were free of disease upon examination, we considered age at last examination.

BUSSELTON

The Busselton Health Study is a population-based, nested, case-control panel of 1,549 individuals of European Caucasian descent from Australia[9,10] with seven cross-sectional respiratory health surveys of adults conducted between 1966 and 2005-2007 and five cross-sectional respiratory health surveys of all school children conducted between 1967 and 1983. Asthma cases were defined as those who reported doctor-diagnosed asthma at any survey that they attended from 1966 to 1994 (answer 'Yes' to 'Has your doctor ever told you that you had asthma?'). Controls are those who have consistently answered 'No' to 'Has your doctor ever told you that you had asthma?' at all previous surveys that they have attended from 1966 to 1994. Age of onset was obtained from answer to the following question "How old were you when you first developed symptoms of asthma?". For individuals who were free of disease upon examination, we considered age at last examination.

SLSJ

The Saguenay-Lac-Saint-Jean and Quebec City Familial Asthma Collection (SLSJ) consisting of a French-Canadian founder population panel of 253 multigenerational families from Saguenay-Lac-Saint-Jean region, ascertained through two asthmatic probands between 1997 and 2002.[11]

Probands were included in the study if they fulfill at least two of the following criteria: 1) a minimum of three clinic visits for acute asthma within one year; 2) two or more asthma-related hospital admissions within one year; or 3) steroid dependency, as defined by either six months of oral, or one year of inhaled corticosteroid use. Families were included in the study if at least one parent was available for phenotypic assessment, at least one parent was unaffected, and all four grandparents were of French-Canadian origin. For family members, they were considered as asthmatic: (1) if they had a reported history of asthma (validated by a

physician), or (2) if they presented asthma-related symptoms and positive PC20 (less or equal to 8mg/ml of methacholine) at recruitment. If individuals had at least one positive response on skin prick tests (wheal diameter X 3mm at 10min), they were defined as atopic. Age of onset was obtained from answers to the following questions "Have you ever had asthma attacks? How old were you when you had your first asthma attack?". When age of onset was defined below 2 years (in 41 cases), a default class of 2 years was adopted to avoid uncertainty. For non-asthmatics, we considered the age at examination.

UFA

UFA is a population-based case-control study of asthma cases and controls matched on age and sex and recruited between 1999 and the year 2007.[12] Subjects are of different ethnic origins (Russians, Tatars and Bashkirs) from Volga-Ural region of Russian Federation.

Cases are unrelated patients with physician-diagnosed asthma and controls are free of disease. Asthma patients were diagnosed by pulmonologists on the basis of clinical examination, family and medication history, objective tests of lung function. The controls were healthy subjects who met all the following criteria: (1) no symptoms or history of asthma or other pulmonary diseases; (2) no symptoms or history of atopy; and (3) absence of first-degree relatives with a history of asthma or atopy. The age of asthma onset was obtained from answer to the following question "How old were you when you had your first attack of asthma?". For non-asthmatics, we considered the age at examination.

TOMSK

TOMSK is a population-based family study conducted by the Research Institute of Medical Genetics and Siberian State Medical University (TOMSK, Russia) from 1998 onwards.[13,14]

Both nuclear families and extended pedigrees were recruited through atopic bronchial asthmatic probands. Both probands and their relatives were clinically examined to establish diagnosis of asthma and atopy using the GINA criteria (Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention. http://www.ginasthma.org). The age of onset was set as the age when asthma was first diagnosed by a doctor. For newly identified cases, it was established through their physical examination, while for other cases, it was established through the reply to a question: "What age were you when doctor first time told you that you have asthma?". For non-asthmatics, we considered the age at examination.

eQTL analysis and functional annotations

We assessed whether significant SNPs associated with time-to-asthma onset (or their proxies) were expression quantitative trait loci (eQTLs) by using publically available databases: eQTL Browser (lymphoblastoid cell lines (LCLs) from British asthma (MRCA) and eczema (MRCE) family subjects),[15] Blood eQTL Browser (non-transformed peripheral blood samples),[16] Lung eQTLs (lung tissue),[17] GTEx eQTL Browser release v4 (uploaded in July 2015 for multiple tissues including blood and lung)[18] and eQTL Chicago Browser that includes eQTL results from many sources among which Montgomery *et al*,[19] Stranger *et al*[20] and Veyrieras *et al*[21] that were performed in human LCLs.

Replication of prior asthma GWAS results

Finally, we evaluated whether previously reported susceptibility loci for asthma and asthma-related phenotypes (asthma exacerbation, asthma-plus-rhinitis comorbidity, age of asthma onset and bronchial-hyper-responsiveness) at genome-wide significance level were associated with time-to-asthma onset in our meta-analysis using data from the NHGRI Catalog of Published GWASs (last update June 2015).[22] A total of 15 GWASs mainly conducted on

European populations have reported 57 SNPs belonging to 28 independent loci (including seven loci specific to Japanese subjects or African-Americans and Latinos) associated with asthma and/or selected asthma-related phenotypes at a genome-wide significant level.

RESULTS

Functional annotations and effect on gene expression of main time-to-asthma onset associated SNPs

To provide some insights into the potential molecular mechanisms underlying the TAO associated variants, we queried whether the seven top SNPs (or 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 $P<5x10^{-5}$; see Table III and Table E3 for summary and complete results respectively).

The 2q12 region top SNP (rs10208293) is located within *interleukin 1 receptor-like 1* gene (*IL1RL1*) in a binding site of the nuclear factor-κB (NFkB1) and is in strong LD (D'=1 and r^2=0.57) with three *IL1RL1* missense SNPs (rs4988956, rs10192157 and rs10206753). This SNP and one of its proxy correlate with the expression of *interleukin 18 receptor 1* (*IL18R1*) and *IL18 receptor accessory protein* (*IL18RAP*) genes in blood.[16]

In 6p21 region, the strongest association was with rs9272346 located near *HLA-DQA1*. This SNP lies within an enhancer histone mark in B cells and is in strong LD (r^2>0.8) with SNPs in a predicted promoter in B cells. This SNP (or its proxies) correlates with the expression of 17 HLA class II genes in blood tissue and lymphoblastoid cell lines (LCL).[15,16,18-20] However, due to the extensive LD within HLA region, some of these associations might reflect signal inter-correlations rather than true pleiotropic effects on gene expression.

198 The two distinct SNPs (rs928413 and rs413382) associated with TAO at 9p24 are located in
199 intergenic region upstream of *IL33*. We did not find any evidence for eQTL for these SNPs or
200 their proxies.
201 The span of the SNPs associated with TAO at genome-wide significance level in 17q12-q21
202 region was approximately 389 kb. The strongest association was with a SNP (rs9901146)
203 nearby *zona pellucida binding protein 2* gene (*ZPBP2*) that is in strong LD with several SNPs
204 tagging and/or belonging to other genes in the region: *IKAROS family zinc finger 3* (*IKZF3*),
205 *ZPBP2 (including rs11557467 missense SNP)*, *gasdermin B* (*GSDMB*; among which two
206 missense SNPs rs2305480 and rs2305479) and *ORMDL sphingolipid biosynthesis regulator 3*
207 (*ORMDL3*). The rs9901146-G risk allele was positively correlated with the expression of
208 *GSDMB* and *ORMDL3*, and negatively with *IKZF3* expression in LCL and blood
209 tissue.[15,16,18,19,21] The second distinct signal at 17q12-q21 (rs3859192) is located within the
210 *gasdermin A* gene (*GSDMA*), in a GABPA (GA binding protein transcription factor, alpha
211 subunit) binding site. Moreover, this SNP is in strong LD with rs56030650 missense SNP
212 (*GSDMA*) and SNPs lying in a predicted promoter in B cells. The rs3859192-T risk allele was
213 correlated with decreased expression of *GSDMA* in the lung[17] and increased expression of
214 both *GSDMB* and *ORMDL3* in LCLs.[15]
215

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Table E1. Main characteristics of the nine studies included in the meta-analysis of time-to-asthma onset GWAS

	ALSPAC	BUSSELTON	ECRHS	EGEA	GABRIELA	SAPALDIA	SLSJ	TOMSK	UFA
Cohort information									
Country	United-Kingdom	Australia	Europe	France	Austria, Germany, Switzerland	Switzerland	Canada	Russia	Russia
Study collection type	Birth Cohort	Population-based Case/Control	Population-based case-control study	Longitudinal Case/Control and Family study	Population-based Case/Control	Population-based case-control study	Population-based family study	Population-based family study	Population-based case-control study
Sample Size									
	3,420	1,191	2,085	1,835	1,503	1,435	1,127	622	669
Main characteristics									
Sex, men (%)	1,760 (51.5)	510 (42.8)	981 (47.1)	937 (51.1)	849 (56.5)	698 (48.6)	515 (45.7)	341 (54.8)	413 (61.7)
Age in years*, mean (SD)	13.9 (0.1)	53.4 (17.3)	42.8 (7.1)	31.3 (17.0)	9.0 (1.6)	52.3 (11.2)	38.4 (21.6)	27.2 (16.7)	19.2 (13.3)
Asthma, n (%)	1,336 (39.1)	391 (32.8)	618 (29.7)	793 (43.2)	664 (44.2)	557 (38.8)	534 (47.4)	240 (38.6)	329 (49.2)
Asthma age-of-onset, median [25-75%]	1.6 [0.5-5.6]	18 [6-35]	20 [7-30]	9 [3-25]	2 [0.8-4]	21 [7-37]	9 [3-25]	5 [3-12]	5 [2-13]
Atopy**, n (%)	652 (25.1)	507 (42.6)	864 (41.5)	1,063 (57.9)	812 (54.0)	-	618 (54.8)	381 (61.3)	281 (42.0)***
IgE (UI/mL), mean (SD)	267.4 (555.6)	NA	135.9 (296.9)	282.6 (632.4)	NA	119.8 (263.8)	182.8 (202.8)	226.3 (261.4)	238.7 (373.5)
Genotyping									
Genotyping platform and SNP panel	Illumina HumanHap550Quad	Illumina 610K	Illumina 610K	Illumina 610K	Illumina 610K	Illumina 610K	Illumina 610K	Illumina 610K	Illumina 610K

	ALSPAC	BUSSELTON	ECRHS	EGEA	GABRIELA	SAPALDIA	SLSJ	TOMSK	UFA
Genotyping center	23andMe subcontracting the Wellcome Trust Sanger Institute, Cambridge, UK, and the LabCorp, Burlington, North Carolina, US	Centre National de Génotypage, Evry, France	Centre National de Génotypage, Evry, France	Centre National de Génotypage, Evry, France	Centre National de Génotypage, Evry, France	Centre National de Génotypage, Evry, France	Centre National de Génotypage, Evry, France	Centre National de Génotypage, Evry, France	Centre National de Génotypage, Evry, France
Individual QC									
Call-rate	97%	97%	97%	97%	97%	97%	97%	97%	97%
Heterozygosity	Individuals excluded if <0.320 or >0.345 for the Sanger data and <0.310 or >0.330 for the LabCorp data	Individuals excluded if <0.30 or >0.33	Individuals excluded if <0.30 or >0.33	Individuals excluded if <0.30 or >0.33	Individuals excluded if <0.30 or >0.33	Individuals excluded if <0.30 or >0.33	Individuals excluded if <0.30 or >0.33	Individuals excluded if <0.30 or >0.33	Individuals excluded if <0.30 or >0.33
Ethnic outliers	PCA based	PCA based	PCA based	PCA based	PCA based	PCA based	PCA based	PCA based	PCA based
SNP QC filters before imputation									
MAF	1%	5%	5%	5%	5%	5%	5%	5%	5%
HWE p-value	$5x10^{-7}$	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}
Call-rate	95%	97%	97%	97%	97%	97%	97%	97%	97%
Imputation - Genome									
Software	MACH 1.0	MACH 1.0	MACH 1.0	MACH 1.0	MACH 1.0	MACH 1.0	MACH 1.0	MACH 1.0	MACH 1.0
Hapmap release	Hapmap2 r22	Hapmap2 r21	Hapmap2 r21	Hapmap2 r21	Hapmap2 r21	Hapmap2 r21	Hapmap2 r21	Hapmap2 r24	Hapmap2 r21

	ALSPAC	BUSSELTON	ECRHS	EGEA	GABRIELA	SAPALDIA	SLSJ	TOMSK	UFA
SNP QC filters	Rsq ≥ 0.5 & MAF ≥ 1%	Rsq ≥ 0.5 & MAF ≥ 1%	Rsq ≥ 0.5 & MAF ≥ 1%	Rsq ≥ 0.5 & MAF ≥ 1%	Rsq ≥ 0.5 & MAF ≥ 1%	Rsq ≥ 0.5 & MAF ≥ 1%	Rsq ≥ 0.5 & MAF ≥ 1%	Rsq ≥ 0.5 & MAF ≥ 1%	Rsq ≥ 0.5 & MAF ≥ 1%
Imputation - Region									
Software	MINIMAC	IMPUTE2 v2.1.2	IMPUTE2 v2.1.2	IMPUTE2 v2.1.2	IMPUTE2 v2.1.2	IMPUTE2 v2.1.2	IMPUTE2 v2.1.2	IMPUTE2 v2.1.2	IMPUTE2 v2.1.2
1000G release	November 2010	June 2014	June 2014	June 2014	June 2014	June 2014	June 2014	June 2014	June 2014
SNP QC filters	Rsq ≥ 0.5	Info ≥ 0.5	Info ≥ 0.5	Info ≥ 0.5	Info ≥ 0.5	Info ≥ 0.5	Info ≥ 0.5	Info ≥ 0.5	Info ≥ 0.5

326 *Age at last examination

327 **Atopy defined by a positive skin prick test response to at least one aeroallergen

328 ***Available only in asthmatics

Table E2. Results of the analyses conducted in 16q12 region using 1000G CEU reference sample.

Marker	Position*	Alleles Effect/Ref†	Effect Freq	Hazard Ratio [95% CI]	P-value‡	P-Het**
rs11867101	50 847 368	T/C	0.03	1.32 [1.19-1.45]	$1.1x10^{-7}$	0.16
rs11863019	50 847 819	C/A	0.04	1.32 [1.19-1.47]	$6.3x10^{-8}$	0.12
rs4785228	50 848 914	A/G	0.03	1.32 [1.19-1.47]	$1.4x10^{-7}$	0.17
rs7199870	50 850 082	T/C	0.03	1.32 [1.19-1.47]	$9.2x10^{-8}$	0.13
rs2032688	50 850 847	T/C	0.03	1.32 [1.19-1.47]	$5.8x10^{-8}$	0.14
rs7195092	50 852 366	G/C	0.03	1.32 [1.19-1.47]	**$3.8x10^{-8}$**	0.11
rs2032687	50 852 432	T/C	0.03	1.32 [1.19-1.47]	$1.2x10^{-7}$	0.11
rs4785458	50 856 194	A/G	0.03	1.32 [1.19-1.47]	$6.2x10^{-8}$	0.10
rs1861760	50 857 693	A/C	0.04	1.28 [1.16-1.41]	$2.6x10^{-7}$	0.10

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

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

‡P-values are obtained from meta-analysis of single-SNP Cox model of time-to-asthma onset adjusted for sex and principal components.

**P-Het value reflect test of heterogeneity across studies using Cochran's Q test.

Table E3. Cis-eQTLs results for the top SNPs (and their proxies) in genome-wide associated regions from the meta-analysis of time-to-asthma onset. We focused our search on eQTLs measured in blood, LCLs and lung tissue.

Chr	SNP	Position (build 37.3)	LD (D'/r^2) with main SNP	Alleles† (Ref/Effect)	Z score / LOD	P-value	Gene	FDR	Source	Tissue	Reference
2	**rs10208293***	102 966 310	-	G/A	34.06	$9.8x10^{-198}$	*IL18RAP*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
				G/A	11.65	$2.5x10^{-13}$	*IL18R1*	NA	eQTL Browser	LCLs (eczema)	Liang *et al*, 2013
	rs3771167	102 986 188	rs10208293 (D'=1, r^2=0.56)	G/A	7.34	$2.1x10^{-13}$	*IL18R1*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
6	**rs9272346***	32 604 372	-	NA	NA	$2.1x10^{-21}$	*HLA-DQA1*	NA	eQTL_Chicago	LCLs	Stranger et al, 2007
				NA	NA	$4.6x10^{-21}$	*HLA-DQA1*	NA	eQTL_Chicago	LCLs	Montgomery *et al*, 2010
				NA	NA	$8.3x10^{-18}$	*HLA-DQB1*	NA	eQTL_Chicago	LCLs	Montgomery *et al*, 2010
				NA	NA	$1.1x10^{-7}$	*HLA-DRB1*	NA	eQTL_Chicago	LCLs	Montgomery *et al*, 2010
				G/A	-10.61	$1.4x10^{-26}$	*HLA-DQA1*	NA	GTEx	Lung	GTEx consortium, 2013
				G/A	-12.57	$1.6x10^{-36}$	*HLA-DQA1*	NA	GTEx	Whole_Blood	GTEx consortium, 2013
				G/A	8.98	$1.4x10^{-19}$	*HLA-DQA2*	NA	GTEx	Whole_Blood	GTEx consortium, 2013
				G/A	6.63	$1.7x10^{-11}$	*HLA-DQA2*	NA	GTEx	Lung	GTEx consortium, 2013
				G/A	-8.84	$4.6x10^{-19}$	*HLA-DQB1*	NA	GTEx	Lung	GTEx consortium, 2013
				G/A	-12.16	$2.4x10^{-34}$	*HLA-DQB1*	NA	GTEx	Whole_Blood	GTEx consortium, 2013

Chr	SNP	Position (build 37.3)	LD (D'/r²) with main SNP	Alleles† (Ref/Effect)	Z score / LOD	P-value	Gene	FDR	Source	Tissue	Reference
				G/A	-8.72	$1.4x10^{-18}$	*HLA-DQB1-AS1*	NA	GTEx	Lung	GTEx consortium, 2013
				G/A	-11.34	$4x10^{-30}$	*HLA-DQB1-AS1*	NA	GTEx	Whole_Blood	GTEx consortium, 2013
				G/A	10.06	$4x10^{-24}$	*HLA-DQB2*	NA	GTEx	Whole_Blood	GTEx consortium, 2013
				G/A	-6.85	$7.5x10^{-12}$	*HLA-DRA*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
				G/A	-5.85	$2.5x10^{-9}$	*HLA-DRB1*	NA	GTEx	Lung	GTEx consortium, 2013
				G/A	-7.43	$5.3x10^{-14}$	*HLA-DRB1*	NA	GTEx	Whole_Blood	GTEx consortium, 2013
				G/A	-23.43	$2.1x10^{-121}$	*HLA-DRB5*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
				G/A	-5.15	$1.3x10^{-7}$	*HLA-DRB5*	NA	GTEx	Whole_Blood	GTEx consortium, 2013
				G/A	4.70	$1.3x10^{-6}$	*HLA-DRB6*	NA	GTEx	Whole_Blood	GTEx consortium, 2013
				G/A	-6.60	$4.1x10^{-11}$	*TAP2*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
	rs3129889	32 413 545	rs9272346 (D'=1, r^2=0.41)	G/A	-6.68	$2.9x10^{-8}$	*HLA-DRB1*	NA	eQTL Browser	LCLs (asthma)	Liang *et al*, 2013
	rs9272723	32 609 427	rs9272346 (D'=1, r^2=0.97)	T/C	-10.10	$5.7x10^{-24}$	*TAP2*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
					7.23	$4.8x10^{-13}$	*HLA-DOB*	$<10^{-5}$			
					-25.04	$2.3x10^{-138}$	*HLA-DRB5*	$<10^{-5}$			

Chr	SNP	Position (build 37.3)	LD (D'/r²) with main SNP	Alleles† (Ref/Effect)	Z score / LOD	P-value	Gene	FDR	Source	Tissue	Reference
	rs9273325	32 623 193	rs9272346 (D'=1, r²=0.02)	A/G	-4.50	$6.9x10^{-6}$	*TAP1*	NA	eQTLs_Lung	Lung	Hao *et al*, 2012
	rs2859579	32 784 073	rs9272346 (D'=1, r²=0.007)	T/G	19.10	$6.8x10^{-21}$	*TAP2*	NA	eQTL Browser	LCLs (asthma)	Liang *et al*, 2013
	rs9277725	33 091 543	rs9272346 (D'=1, r²=0.05)	T/A	15.60	$2.3x10^{-17}$	*HLA-DPB2*	NA	eQTL Browser	LCLs (asthma)	Liang *et al*, 2013
	rs2395357	33 101 006	rs9272346 (D'=1, r²=0.05)	A/G	7.80	$6.2x10^{-15}$	*HSD17B8*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
					-6.66	$2.8x10^{-11}$	*HLA-DPB1*	$<10^{-5}$			
					-5.12	$3.1x10^{-7}$	*HLA-DMA*	10^{-4}			
16	**rs1861760***	50 857 693	-	C/A	6.62	$3.6x10^{-11}$	*NOD2*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
	rs5743266 (now rs2076752)	50 731 096	rs1861760 (D'=1, r²=0.02)	A/G	-5.85	$5.0x10^{-9}$	*CYLD*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
					23.31	$3.2x10^{-120}$	*NOD2*	$<10^{-5}$			
	rs7205760	50 844 773	rs1861760 (D'=1, r²=0.005)	C/G	4.69	$2.8x10^{-6}$	*CYLD*	NA	eQTLs_Lung	Lung	Hao *et al*, 2012
					7.85	$4.0x10^{-15}$	*NOD2*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
17	**rs9901146***	38 043 343	-	A/G	36.54	$9.8x10^{-198}$	*GSDMB*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013

Chr	SNP	Position (build 37.3)	LD (D'/r²) with main SNP	Alleles† (Ref/Effect)	Z score / LOD	P-value	Gene	FDR	Source	Tissue	Reference
				A/G	6.00	$9.9x10^{-10}$	*GSDMB*	NA	GTEx	Whole_Blood	GTEx consortium, 2013
				A/G	8.55	$1.3x10^{-17}$	*GSDMB*	NA	eQTL Browser	LCLs (asthma)	Liang *et al*, 2013
				A/G	33.50	$2.2x10^{-35}$	*GSDMB*	NA	eQTL Browser	LCLs (eczema)	Liang *et al*, 2013
				A/G	36.41	$9.8x10^{-198}$	*ORMDL3*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
				A/G	11.16	$6.2x10^{-29}$	*ORMDL3*	NA	eQTL Browser	LCLs (asthma)	Liang *et al*, 2013
				A/G	42.30	$3.3x10^{-44}$	*ORMDL3*	NA	eQTL Browser	LCLs (eczema)	Liang *et al*, 2013
				NA	NA	$1.3x10^{-10}$	*ORMDL3*	NA	eQTL_Chicago	LCLs	Veyrieras *et al*, 2008
				NA	NA	$3.8x10^{-6}$	*ORMDL3*	NA	eQTL_Chicago	LCLs	Montgomery *et al*, 2010
				A/G	4.57	$2.4x10^{-6}$	*ORMDL3*	NA	GTEx	Whole_Blood	GTEx consortium, 2013
	rs9896940	37 895 975	rs9901146 (D'=1, r²=0.07)	G/A	-15.81	$2.6x10^{-56}$	*IKZF3*	$<10^{-5}$	Blood eQTLs	Blood	Westra *et al*, 2013
17	**rs3859192***	38 128 648	-	C/T	-6.91	$2.5x10^{-12}$	*GSDMA*	NA	GTEx	Lung	GTEx consortium, 2013
				C/T	6.10	$1.1x10^{-7}$	*GSDMB*	NA	eQTL Browser	LCLs (eczema)	Liang *et al*, 2013
				C/T	8.10	$1.1x10^{-9}$	*ORMDL3*	NA	eQTL Browser	LCLs (eczema)	Liang *et al*, 2013

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

343 †Haplotype reconstruction was done using Haploview.[23] The effect allele of the top SNP is always transmitted with the indicated effect allele of its proxy

Table E4. Comparison of the main results of time-to-asthma onset (TAO, in bold) GWAS meta-analysis ($P \leq 5x10^{-8}$) with asthma (binary trait) GWAS meta-analysis results obtained in the same nine studies and in the whole GABRIEL dataset (25 studies, N=26,475)[1]

						TAO meta-analysis 9 studies			AST (binary) meta-analysis 9 studies														AST (binary) meta-analysis All GABRIEL			
									ALL					Childhood-onset					Adult-onset					P Het				
Chr	**Marker**	**Position***	**Closest Gene (kb distance)**	**Effect allele Freq**	**Effect/ Ref Alleles†**	**HR**	**P‡**	**Phet****	**OR fix**	**P fix**	**OR ran**	**P ran**	**Phet**	**OR fix**	**P fix**	**OR ran**	**P ran**	**Phet**	**OR fix**	**P fix**	**OR ran**	**P ran**	**P het**		**OR**	**P ran**	**P fix**	**P het**
2	**rs10208293**	102.97	*IL1RL1*	0.27	A/G	0.88	$3.1x10^{-8}$	0.26	0.88	$4.0x10^{-5}$	0.88	$4.7x10^{-3}$	0.03	0.84	$1.4x10^{-5}$	0.84	$1.4x10^{-5}$	0.84	0.94	$2.7x10^{-1}$	0.97	$7.8x10^{-1}$	0.004	0.07				
2	rs3771166	102.99	*IL18R1*	0.38	A/G	0.89	$5.0x10^{-8}$	0.57	0.88	$5.5x10^{-6}$	0.88	$4.2x10^{-4}$	0.10	0.84	$6.0x10^{-7}$	0.84	$6.0x10^{-7}$	0.53	0.96	$3.4x10^{-1}$	0.97	$6.1x10^{-1}$	0.13	0.02	1.15	$3.4x10^{-9}$	$3.5x10^{-12}$	0.18
6	**rs9272346††**	32.60	*HLA-DQA1 (0.8)*	0.58	A/G	1.13	$1.6x10^{-8}$	0.12	1.17	$4.9x10^{-8}$	1.17	$1.2x10^{-7}$	0.38	1.13	$6.1x10^{-4}$	1.14	$2.5x10^{-3}$	0.21	1.25	$6.3x10^{-6}$	1.25	$6.3x10^{-6}$	0.83	0.12	1.18	$7.0x10^{-14}$	$7.0x10^{-14}$	0.50
9	**rs413382**	6.14	*IL33 (73)*	0.80	A/C	1.16	$5.9x10^{-8}$	0.84	1.20	$3.3x10^{-7}$	1.22	$1.9x10^{-4}$	0.01	1.19	$2.2x10^{-4}$	1.19	$5.8x10^{-3}$	0.06	1.23	$3.7x10^{-4}$	1.26	$1.8x10^{-2}$	0.02	0.63				
9	rs1342326	6.19	*IL33 (26)*	0.84	A/C	0.84	$1.6x10^{-12}$	0.43	0.80	$2.1x10^{-9}$	0.80	$6.9x10^{-6}$	0.05	0.74	$4.8x10^{-11}$	0.73	$7.1x10^{-9}$	0.26	0.93	$1.9x10^{-1}$	0.92	$1.9x10^{-1}$	0.41	0.003	1.20	$9.2x10^{-10}$	$8.7x10^{-12}$	0.22
9	**rs928413**	6.21	*IL33 (2)*	0.76	A/G	0.84	$6.5x10^{-16}$	0.15	0.80	$2.2x10^{-12}$	0.80	$2.5x10^{-7}$	0.04	0.75	$5.4x10^{-13}$	0.75	$4.7x10^{-9}$	0.16	0.90	$3.0x10^{-2}$	0.90	$4.7x10^{-2}$	0.33	0.006				
15	rs744910	6.74	*SMAD3*	0.51	A/G	0.93	$3.2x10^{-4}$	0.60	0.92	$1.9x10^{-3}$	0.92	$1.9x10^{-3}$	0.87	0.90	$2.0x10^{-3}$	0.90	$2.0x10^{-3}$	0.81	0.95	$2.9x10^{-1}$	0.95	$2.9x10^{-1}$	0.74	0.31	1.12	$3.9x10^{-9}$	$3.9x10^{-9}$	0.85
16	**rs1861760**	50.86	*CYLD (22)*	0.04	A/C	1.28	$4.2x10^{-8}$	0.11	1.36	$3.8x10^{-6}$	1.37	$3.3x10^{-5}$	0.22	1.35	$3.8x10^{-4}$	1.36	$1.1x10^{-2}$	0.05	1.37	$3.1x10^{-3}$	1.37	$3.1x10^{-3}$	0.79	0.93				
17	**rs9901146**	38.04	*ZPBP2 (9)*	0.48	A/G	0.85	$1.9x10^{-16}$	0.17	0.85	$4.0x10^{-9}$	0.85	$7.3x10^{-5}$	0.01	0.78	$3.0x10^{-12}$	0.78	$3.0x10^{-12}$	0.61	0.98	$5.9x10^{-1}$	0.96	$5.4x10^{-1}$	0.15	0.0002				
17	rs2305480	38.06	*GSDMB*	0.42	A/G	0.85	$8.1x10^{-16}$	0.14	0.85	$1.7x10^{-8}$	0.86	$3.7x10^{-4}$	0.004	0.77	$4.1x10^{-13}$	0.77	$4.1x10^{-13}$	0.83	1.01	$8.3x10^{-1}$	1.00	$9.7x10^{-1}$	0.20	$4.9x10^{-6}$	1.18	$9.6x10^{-8}$	-	0.0009
17	rs3894194	38.12	*GSDMA*	0.47	A/G	1.16	$1.4x10^{-13}$	0.89	1.17	$1.7x10^{-8}$	1.17	$1.4x10^{-6}$	0.19	1.25	$8.6x10^{-11}$	1.25	$8.6x10^{-11}$	0.88	1.04	$4.3x10^{-1}$	1.04	$4.3x10^{-1}$	0.55	$9.3x10^{-4}$	1.17	$4.6x10^{-9}$	-	0.02
17	**rs3859192**	38.13	*GSDMA*	0.54	C/T	0.86	$1.5x10^{-13}$	0.90	0.86	$2.7x10^{-8}$	0.86	$2.7x10^{-8}$	0.64	0.81	$1.7x10^{-9}$	0.81	$1.7x10^{-9}$	0.95	0.95	$2.2x10^{-1}$	0.95	$2.2x10^{-1}$	0.82	0.009				
22	rs2284033	37.53	*IL2RB*	0.47	A/G	0.94	$2.1x10^{-3}$	0.36	0.91	$1.1x10^{-3}$	0.91	$1.1x10^{-3}$	0.66	0.94	$7.9x10^{-2}$	0.94	$7.9x10^{-2}$	0.46	0.87	$2.0x10^{-3}$	0.87	$2.0x10^{-3}$	0.86	0.16	1.12	$1.2x10^{-8}$	$1.2x10^{-8}$	0.92

LD between TAO main SNPs and GABRIEL main SNPs in genome-wide associated regions:

2q12: rs10208293 & rs3771166 (r^2=0.53, D'=0.95); **6p21**: rs9272346 & rs9273349 (r^2=D'=1.0); **9p24**: rs928413 & rs1342326 (r^2=0.51, D'=1.0), rs413382 - rs1342326 (r^2=0.0, D'=0.23); **17q12-21**: rs9901146 & rs2305480 (r^2=0.82, D'=1.0), rs3859192 & rs3894194 (r^2=0.43, D'=0.71)

*Position in megabases (Mb) – 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

†† main GABRIEL SNP in 6p21 (rs9273349) was not imputable

‡‡ Additional distinct SNPs detected with conditional analyses

357 Table E5. Published genome-wide associations with asthma compared with time-to-asthma onset GWAS meta-analysis results

				Meta-analysis of time-to-asthma onset GWASs				GW results reported in GWAS for asthma and asthma-related traits NCBI GWAS Catalog, June 2015							
Chr	**SNP or proxy***	**Pos (Mb)**	**Gene Symbol**	**Effect/Ref Alleles†**	**Effect Freq**	**HR [95% CI]**	**p‡**	**Mapped Genes**	**Effect Allele**	**Effect Freq in ctrls**	**P**	**OR [95% CI]**	**Trait**	**References**	**Pop**
1	rs4129267	154.43	*IL6R*	C/T	0.57	0.97 [0.94-1.01]	0.19	*IL6R*	T	0.37	$2.0x10^{-8}$	1.09 [1.06-1.12]	Asthma	Ferreira, Lancet, 2011[24]	European
1	rs1101999	158.93	*PYHIN1*	NA	NA	NA	NA	*PYHIN1*	NA	NA	$4.0x10^{-9}$	NA	Asthma	Torgerson, Nat Genet, 2011[25]	African American & Latinos
1	rs2786098	197.33	*CRB1*	G/T	0.78	1.03 [0.98-1.08]	0.25	*CRB1-DENND1B*	NA	0.85	$2.0x10^{-13}$	1.43 [NA]	Asthma	Sleiman, N Engl J Med, 2010[26]	European
2	rs3771180	102.95	*IL1RL1*	G/T	0.86	1.16 [1.1-1.23]	**$5.9x10^{-7}$**	*IL1RL1*	NA	NA	$2.0x10^{-15}$	NA	Asthma	Torgerson, Nat Genet, 2011[25]	Multi-ethnic
2	rs13408661	102.96	*IL1RL1*	A/G	0.14	0.86 [0.81-0.91]	**$5.9x10^{-7}$**	*IL1RL1*	G	0.84	$1.0x10^{-9}$	1.23 [1.15-1.31]	Asthma	Ramasamy, PLoS One, 2012[27]	European
2	rs10197862	102.97	*IL1RL1*	A/G	0.86	1.16 [1.09-1.23]	**$9.8x10^{-7}$**	*IL1RL1*	A	0.85	$4.0x10^{-11}$	1.24 [1.16-1.32]	Asthma & hay fever	Ferreira, J Allergy Clin Immunol, 2014[28]	European
2	rs3771166	102.99	*IL18R1*	A/G	0.35	0.89 [0.86-0.93]	**$5.0x10^{-8}$**	*IL18R1*	G	0.62	$3.0x10^{-9}$	1.15 [1.10-1.20]	Asthma	Moffatt, N Engl J Med, 2010[1]	European
3	rs9815663	3.61	.	C/T	0.82	1 [0.95-1.05]	0.96	*CRBN - LRRN1*	T	0.182	$2.0x10^{-8}$	0.84 [NA]	Childhood Asthma	**Forno, J Allergy Clin Immunol, 2012[29]**	European

				Meta-analysis of time-to-asthma onset GWASs				GW results reported in GWAS for asthma and asthma-related traits NCBI GWAS Catalog, June 2015							
Chr	**SNP or proxy***	**Pos (Mb)**	**Gene Symbol**	**Effect/Ref Alleles†**	**Effect Freq**	**HR [95% CI]**	**P‡**	**Mapped Genes**	**Effect Allele**	**Effect Freq in ctrls**	**P**	**OR [95% CI]**	**Trait**	**References**	**Pop**
4	rs4833095	38.80	*TLR1*	C/T	0.27	0.95 [0.9-0.99]	0.02	*TLR1*	T	0.74	$5.0x10^{-12}$	1.2 [1.14-1.26]	Asthma & hay fever	Ferreira, J Allergy Clin Immunol, 2014[28]	European
4	rs17218161	59.21	.	NA	NA	NA	NA	*SRIP1 - MIR548AG1*	NA	NA	$2.0x10^{-8}$	NA	Childhood Asthma	Ding, Hum Genomics, 2013[30]	European
4	rs7686660	144.00	.	G/T	0.24	0.99 [0.94-1.03]	0.50	*FLJ44477 - USP38*	T	0.27	$2.0x10^{-12}$	1.16 [1.11-1.21]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
4	rs3805236	144.36	*GAB1*	A/G	0.30	0.99 [0.95-1.04]	0.80	*GAB1*	G	0.25	$7.0x10^{-8}$	1.20 [1.14-1.26]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
5	rs1588265	59.37	.	A/G	0.70	0.99 [0.94-1.03]	0.50	*PDE4D*	C	0.29	$3.0x10^{-8}$	1.18 [1.08-1.30]	Asthma	Himes, Am J Hum Genet, 2009[32]	European
5	rs1837253	110.40	.	C/T	0.77	1.13 [1.05-1.2]	**$5.6x10^{-4}$**	*SLC25A46 - TSLP*	C	0.35	$1.0x10^{-16}$	1.17 [1.13-1.22]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
			.	C/T	0.77	1.13 [1.05-1.2]	**$5.6x10^{-4}$**	*SLC25A46 - TSLP*	NA	NA	$1.0x10^{-14}$	NA	Asthma	Torgerson, Nat Genet, 2011[25]	Multi-ethnic
			.	C/T	0.77	1.13 [1.05-1.2]	**$5.6x10^{-4}$**	*SLC25A46 - TSLP*	C	0.71	$1.0x10^{-9}$	1.17 [1.12-1.24]	Asthma & hay fever	Ferreira, J Allergy Clin Immunol, 2014[28]	European
5	rs1438673	110.47	.	C/T	0.54	1.08	**$4.5x10^{-5}$**	*WDR36 - RPS3AP21*	C	0.49	$3.0x10^{-11}$	1.16	Asthma & hay fever	Ferreira, J Allergy Clin Immunol, 2014[28]	European

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Chr	**SNP or proxy***	**Pos (Mb)**	**Gene Symbol**	**Effect/Ref Alleles†**	**Effect Freq**	**HR [95% CI]**	**P‡**	**Mapped Genes**	**Effect Allele**	**Effect Freq in ctrls**	**P**	**OR [95% CI]**	**Trait**	**References**	**Pop**
						[1.04-1.13]						[1.11-1.21]			
6	rs204993	32.16	*PBX2*	A/G	0.76	0.96 [0.92-1.01]	0.08	*PBX2*	A	0.58	$2.0x10^{-15}$	1.17 [1.12-1.21]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
6	rs404860	32.18	*NOTCH4*	C/T	0.18	1 [0.91-1.11]	0.95	*NOTCH4*	A	0.5	$4.0x10^{-23}$	1.21 [1.16-1.25]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
6	rs3129943	32.34	*C6orf10*	A/G	0.75	1.01 [0.97-1.06]	0.62	*C6orf10*	T	0.62	$3.0x10^{-15}$	1.17 [1.12-1.21]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
6	rs3117098	32.36	.	A/G	0.66	1.02 [0.98-1.07]	0.29	*HNRNPA1P2 - BTNL2*	G	0.25	$5.0x10^{-12}$	1.16 [1.11-1.21]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
6	rs9268516	32.38	.	C/T	0.68	0.93 [0.87-0.99]	0.02	*BTNL2-HLA-DRA*	T	0.24	$1.0x10^{-8}$	1.15 [1.10-1.21]	Asthma	Ramasamy, PLoS One, 2012[27]	European
6	rs3129890 rs9268856*	32.43	.	A/C	0.22	0.97 [0.92-1.01]	0.15	*HLA-DRA - HLA-DRB9*	T	0.61	$5.0x10^{-13}$	1.15 [1.11-1.20]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
6	rs9272346	32.60	.	A/G	0.61	1.13 [1.08-1.17]	**$1.6x10^{-8}$**	*HLA-DQA1*	NA	NA	$2.0x10^{-8}$	NA	Asthma	Lasky-Su, Clin Exp Allergy, 2012[33]	European
6	rs9273349 rs9272346*	32.60	.	A/G	0.61	1.13 [1.08-1.17]	**$1.6x10^{-8}$**	*HLA-DQA1 - HLA-DQB1*	C	0.58	$7.0x10^{-14}$	1.18 [1.13-1.24]	Asthma	Moffatt, N Engl J Med, 2010[1]	European

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Chr	**SNP or proxy***	**Pos (Mb)**	**Gene Symbol**	**Effect/Ref Alleles†**	**Effect Freq**	**HR [95% CI]**	**p‡**	**Mapped Genes**	**Effect Allele**	**Effect Freq in ctrls**	**P**	**OR [95% CI]**	**Trait**	**References**	**Pop**
6	rs9273373	32.63	.	NA	NA	NA	NA	*HLA-DQA1 - HLA-DQB1*	G	0.54	$4.0x10^{-14}$	1.24 [1.17-1.30]	Asthma & hay fever	Ferreira, J Allergy Clin Immunol, 2014[28]	European
6	rs7775228	32.66	.	C/T	0.16	1.05 [0.98-1.13]	0.18	*HLA-DQB1 - HLA-DQA2*	A	0.63	$5.0x10^{-15}$	1.17 [1.12-1.21]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
6	rs9275698	32.69	.	A/G	0.60	1.02 [0.98-1.07]	0.29	*HLA-DQB1 - HLA-DQA2*	T	0.79	$5.0x10^{-12}$	1.18 [1.12-1.24]	Asthma	Hirota, Nat Genet, 201 [31]	Japanese
6	rs9500927	32.96	.	A/G	0.16	1 [0.95-1.05]	0.98	*BRD2 - HLA-DOA*	T	0.26	$4.0x10^{-9}$	1.13 [1.09-1.18]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
6	rs987870	33.04	.	A/G	0.82	0.97 [0.92-1.02]	0.28	*HLA-DPA1; HLA-DPB1*	C	0.14	$2.0x10^{-10}$	1.4 [1.26-1.55]	Asthma	Noguchi, PLoS Genet, 2011[34]	Japanese
7	rs6967330	105.66	*FLJ23834*	A/G	0.18	1.13 [1.07-1.19]	**$1.4x10^{-5}$**	*CDHR3*	A	0.19	$3.0x10^{-14}$	1.26 [1.18-1.33]	Childhood Asthma	Bonnelykke, Nat Genet, 2014[35]	European
8	rs7009110	81.29	.	C/T	0.59	0.93 [0.9-0.97]	**$7.6x10^{-4}$**	*RPS5P5 - ZBTB10*	T	0.36	$4.0x10^{-9}$	1.14 [1.09-1.19]	Asthma & hay fever	Ferreira, J Allergy Clin Immunol, 2014[28]	European
8	rs3019885	118.03	.	G/T	0.48	1.02 [0.98-1.06]	0.40	*SLC30A8*	G	0.31	$5.0x10^{-13}$	1.34 [1.24-1.45]	Asthma	Noguchi, PLoS Genet, 2011[34]	Japanese
9	rs72699186	6.18	.	NA	NA	NA	NA	*RANBP6 - IL33*	T	0.15	$2.0x10^{-9}$	1.26	Asthma & hay fever	Ferreira, J Allergy	European

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												[1.16-1.35]		Clin Immunol, 2014[28]	
9	rs1342326	6.19	.	A/C	0.79	0.84 [0.8-0.88]	**$1.6x10^{-12}$**	*RANBP6 - IL33*	C	0.16	$9.0x10^{-10}$	1.2 [1.13-1.28]	Asthma	Moffatt, N Engl J Med, 2010[1]	European
9	rs2381416	6.19	.	A/C	0.68	0.85 [0.81-0.89]	**$3.6x10^{-14}$**	*RANBP6 - IL33*	NA	NA	$2.0x10^{-12}$	NA	Asthma	Torgerson, Nat Genet, 2011[25]	Multi-ethnic
9	rs928413	6.21	.	A/G	0.70	0.84 [0.8-0.88]	**$6.5x10^{-16}$**	*IL33*	G	0.28	$9.0x10^{-13}$	1.24 [1.17-1.32]	Childhood severe Asthma	Bonnelykke, Nat Genet, 2014[35]	European
9	rs16929097	12.52	.	A/G	0.04	1.04 [0.91-1.19]	0.55	*PTPRD - TYRP1*	NA	NA	$8.0x10^{-9}$	NA	Childhood Asthma	Ding, Hum Genomics, 2013[30]	European
10	rs7915695	68.44	*CTNNA3*	NA	NA	NA	NA	*CTNNA3*	C	0.09	$2.2x10^{-8}$	NA	Asthma exacerbations	McGeachie, J Allergy Clin Immunol,2015[36]	European
10	rs12570188	100.86	*HPSE2*	NA	NA	NA	NA	*HPSE2*	NA	NA	$5.0x10^{-8}$	NA	Childhood Asthma	Ding, Hum Genomics, 2013[30]	European
10	rs10508372	8.97	.	A/G	0.08	0.95 [0.88-1.02]	0.16	*KRT8P16 - TCEB1P3*	C	0.433	$2.0x10^{-15}$	1.16 [1.12-1.21]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
11	rs7130588	76.27	.	A/G	0.63	0.95 [0.91-0.99]	$9.2x10^{-3}$	*C11orf30 - LRRC32*	G	0.34	$2.0x10^{-8}$	1.09 [1.06-1.13]	Asthma	Ferreira, Lancet, 2011[24]	European
11	rs215521	76.59		G/T	0.51	0.93	$7.6x10^{-4}$	*C11orf30 - LRRC32*	T	0.48	$5.0x10^{-11}$	1.16	Asthma & hay fever	Ferreira, J Allergy	European

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						[0.90-0.97]						[1.11-1.21]		Clin Immunol, 2014[28]	
11	rs7927044	127.76	.	A/G	0.01	1.09 [0.89-1.33]	0.43	*NCRNA00288 - ETS1*	A	0.0134	$7.0x10^{-9}$	0.85 [NA]	Childhood Asthma	Forno, J Allergy Clin Immunol, 2012[29]	European
12	rs2069408	56.36	*CDK2*	A/G	0.66	0.98 [0.94-1.02]	0.31	*CDK2*	C	0.23	$1.0x10^{-10}$	1.15 [1.10-1.20]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
12	rs1701704	56.41	.	G/T	0.35	1.03 [0.99-1.08]	0.10	*SUOx - IKZF4*	G	0.18	$2.0x10^{-13}$	1.19 [1.14-1.25]	Asthma	Hirota, Nat Genet, 2011[31]	Japanese
15	rs744910	67.45	*SMAD3*	A/G	0.49	0.93 [0.9-0.97]	**$3.2x10^{-4}$**	*SMAD3*	G	0.49	$4.0x10^{-9}$	1.12 [1.09-1.16]	Asthma	Moffatt, N Engl J Med, 2010[1]	European
15	rs17294280	67.47	*SMAD3*	A/G	0.71	0.93 [0.88-0.98]	$4.9x10^{-3}$	*SMAD3*	G	0.23	$4.0x10^{-9}$	1.18 [1.11-1.25]	Asthma & hay fever	Ferreira, J Allergy Clin Immunol, 2014[28]	European
16	rs62026376	11.23	*CLEC16A*	NA	NA	NA	NA	*CLEC16A*	C	0.72	$1.0x10^{-8}$	1.17 [1.11-1.24]	Asthma & hay fever	Ferreira, J Allergy Clin Immunol, 2014[28]	European
17	rs2305480	38.06	*GSDMB*	A/G	0.41	0.85 [0.82-0.88]	**$8.1x10^{-16}$**	*GSDMB*	G	0.60	$6.0x10^{-23}$	1.32 [1.23-1.39]	Childhood severe Asthma	Bonnelykke, Nat Genet, 2014[35]	European
				A/G	0.41	0.85 [0.82-0.88]	**$8.1x10^{-16}$**	*GSDMB*	A	0.45		0.85 [0.81-0.90]	Asthma	Moffatt, N Engl J Med, 2010[1]	European

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Chr	**SNP or proxy***	**Pos (Mb)**	**Gene Symbol**	**Effect/Ref Alleles†**	**Effect Freq**	**HR [95% CI]**	**p‡**	**Mapped Genes**	**Effect Allele**	**Effect Freq in ctrls**	**P**	**OR [95% CI]**	**Trait**	**References**	**Pop**
17	rs11078927	38.06	*GSDMB*	C/T	0.59	1.18 [1.13-1.22]	**$6.8x10^{-16}$**	*GSDMB*	NA	NA	$2.0x10^{-16}$	NA	Asthma	Torgerson, Nat Genet, 2011[25]	Multi-ethnic
17	rs7216389	38.07	*GSDMB*	C/T	0.46	0.86 [0.81-0.91]	**$3.1x10^{-8}$**	*GSDMB*	T	0.52	$9.0x10^{-11}$	1.45 [1.17-1.81]	Asthma	Moffatt, Nature, 2007[37]	European
17	rs4794820	38.09	.	A/G	0.40	0.86 [0.82-0.89]	**$1.0x10^{-13}$**	*ORMDL3 - GSDMA*	NA	NA	$1.0x10^{-8}$	1.33 [1.20-1.45]	Asthma	Wan, Thorax, 2012[38]	European
17	rs3894194	38.12	*GSDMA*	A/G	0.49	1.16 [1.11-1.2]	**$1.4x10^{-13}$**	*GSDMA*	A	0.45	$5.0x10^{-9}$	1.17 [1.11-1.23]	Asthma	Moffatt, N Engl J Med, 2010[1]	European
				A/G	0.49	1.16 [1.11-1.2]	**$1.4x10^{-13}$**	*GSDMA*	A	NA	$3.0x10^{-21}$	1.59 [1.44-1.76]	Childhood severe Asthma	Bonnelykke, Nat Genet, 2014[35]	European
17	rs7212938	38.12	*GSDMA*	G/T	0.50	1.18 [1.13-1.23]	**$1.1x10^{-15}$**	*GSDMA*	G	0.46	$4.0x10^{-10}$	1.16 [1.11-1.20]	Asthma & hay fever	Ferreira, J Allergy Clin Immunol, 2014[28]	European
22	rs2284033	37.53	*IL2RB*	A/G	0.41	0.94 [0.9-0.98]	$2.1x10^{-3}$	*IL2RB*	G	0.56	$1.0x10^{-8}$	1.12 [1.08-1.16]	Asthma	Moffatt, N Engl J Med, 2010[1]	European

* The SNP with the strongest LD with the reported SNP in the literature was used if the SNP reported in the literature was not available in the imputed data

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

361 ‡P-values are shown for tests of association under a fixed-effect model when there was no significant evidence of heterogeneity or under a random-effect model otherwise.

362 Associations with P-values ≤ 0.001 in the time-to-asthma onset meta-analysis are indicated in bold

363 NA: Not available

FIGURE LEGENDS

Figure E1. Distribution of age-of-asthma onset

Figure E2. Quantile-quantile (QQ) plots of 2,387,926 SNPs of nine GWAS (N = 13,886) after quality control (Rsq≥0.50, MAF≥0.01, ≥6 contributing studies) under a fixed-effect model (inflation factor, λ_{GC}=1.04). The dots represent the distribution of observed Chi-Square values against the theoretical model distribution of expected Chi-Square values. The red line represents the theoretical model distribution of expected Chi-Square values under the null distribution.

Figure E3. Regional association plots of the genome-wide associated regions using Locuszoom[39] software: 2q12, 6p21, 9p24, 17q12-q21. 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 1000 Genomes (March 2012 EUR) are plotted in cyan to reflect the local LD structure. SNPs surrounding the most significant SNP (purple circle) are color-coded according to LD with lead SNP (pairwise r2, according to a blue to red scale from r^2=0 to 1). In 9p24 and 17q12-q21 regions, additional SNP detected by conditional analyses is indicated by an arrow (Part A). Two additional plots show SNPs color-coded according to LD with additional SNP detected in conditional analysis (Part B).

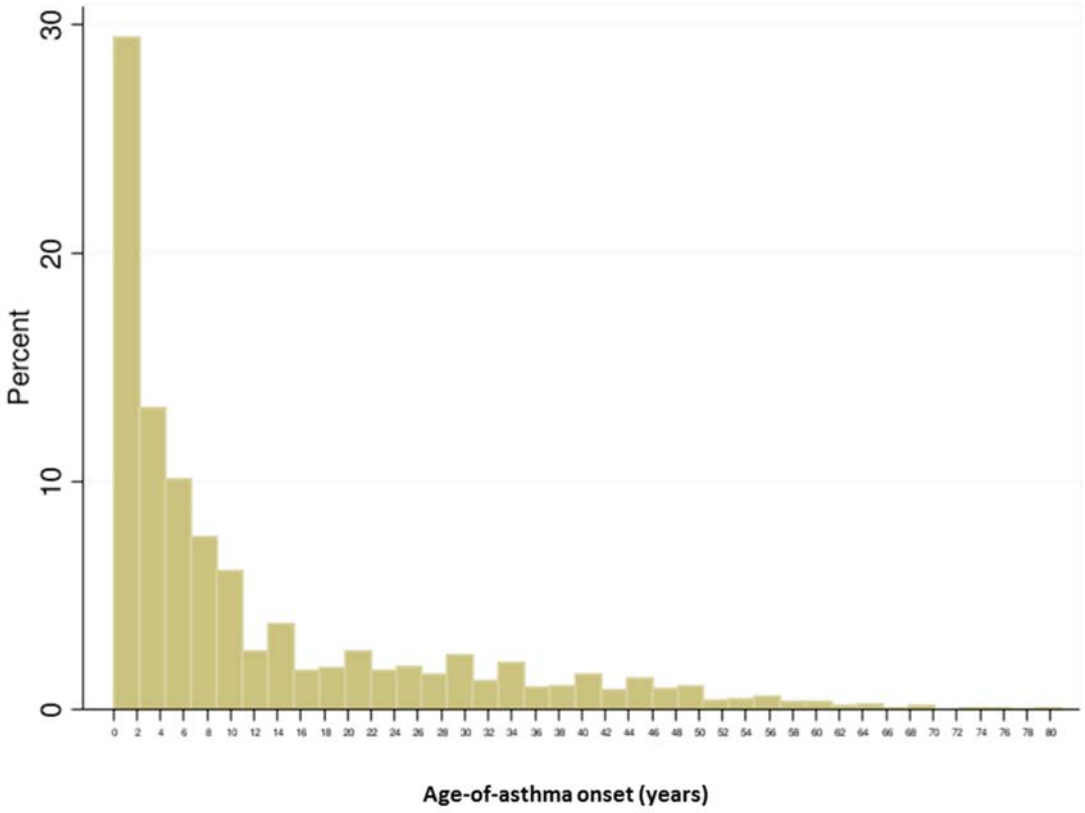
Figure E4. Forest plots of hazard ratios for SNPs associated with time-to-asthma onset at genome-wide significant level ($P \leq 5x10^{-8}$) and two additional SNPs detected by conditional analyses in 9p24 and 17q12-q21 regions. The hazard ratios and 95% confidence intervals for seven loci show distinct effect on time-to-asthma onset. In each plot, the diamond indicates the effect size and the 95% CI derived from the meta-analysis of nine studies.

Figure E5. Regional plots of 9p24 (Part-a) and 17q12-q21 (Part-b) regions for distinct association signals using sequential conditional analysis and time-to-asthma onset as an outcome variable: original meta-analysis (A), adjusted for lead SNP (B) and additionally

389 adjusted for the secondary signal (C). Signals above the red line ($P<10^{-5}$) were considered to
390 exhibit evidence of association in the regions. SNPs are colored according to their pairwise
391 LD r^2 with the lead SNP. r^2 was estimated from 1000 Genomes (March 2012 EUR).
392 **Figure E6.** Association plots of the fine-mapping conducted in 16q12 region using 1000G
393 CEU reference sample. SNPs are plotted with their P-values ($-\log_{10}$ values, left y-axis) as a
394 function of genomic position (x-axis). Estimated recombination rates (right y-axis) taken from
395 1000G are plotted to reflect the local LD structure around the top associated SNP (purple
396 circle) and correlated proxies (according to a blue to red scale from $r^2 = 0$ to 1).
397

398 Figure E1.

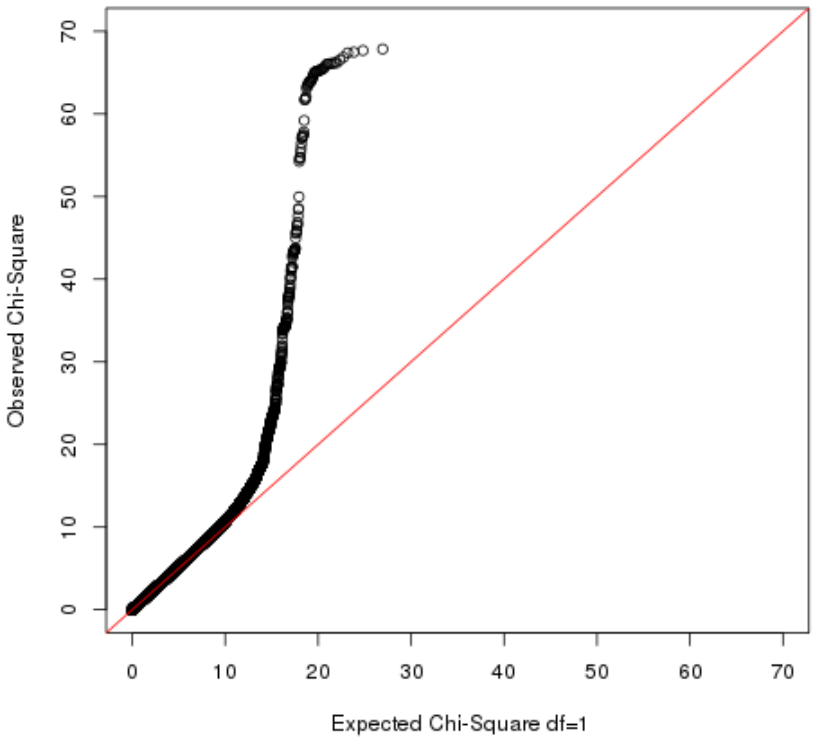
399



400

401 Figure E2.

402



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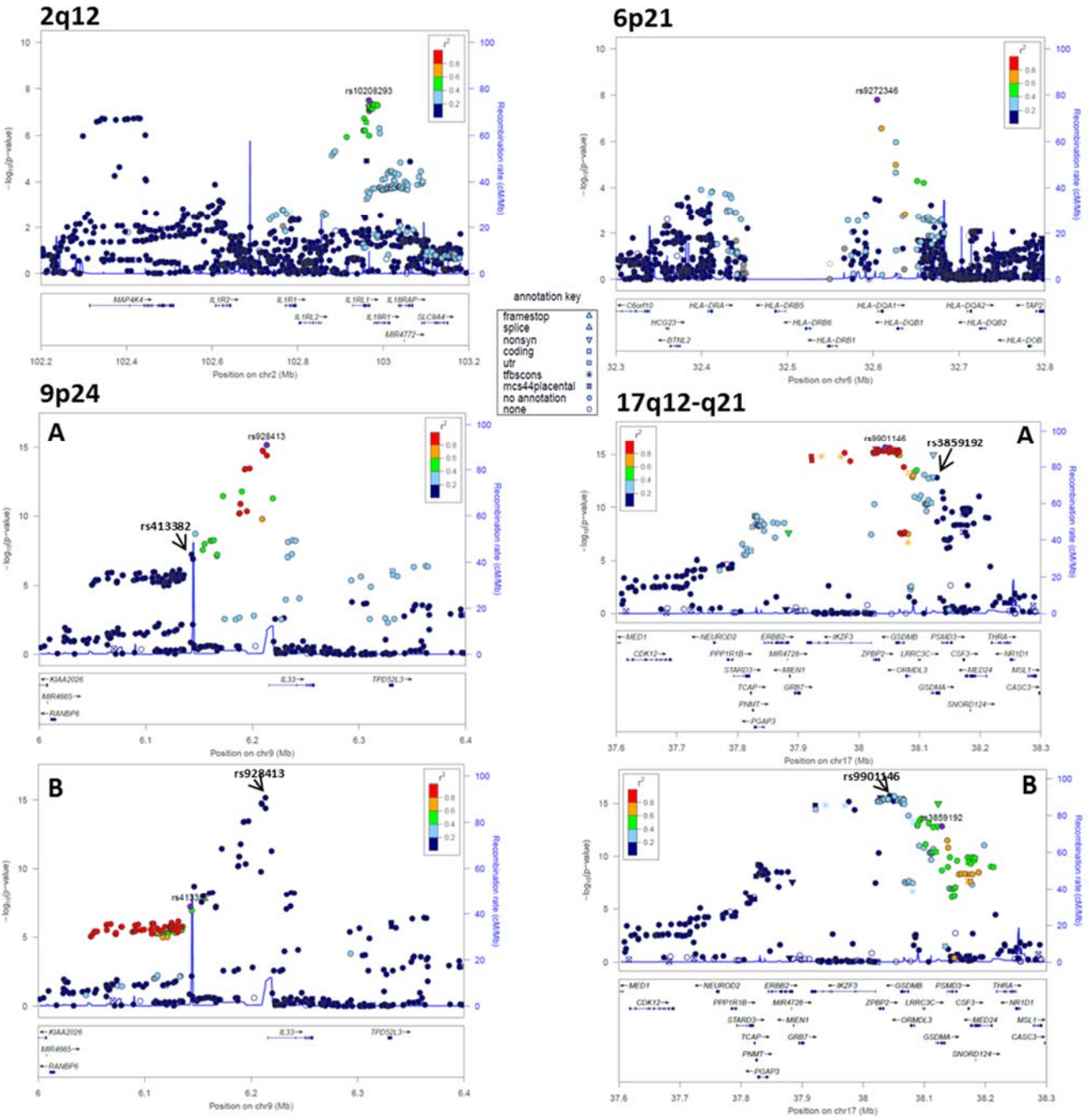
404 Figure E3.



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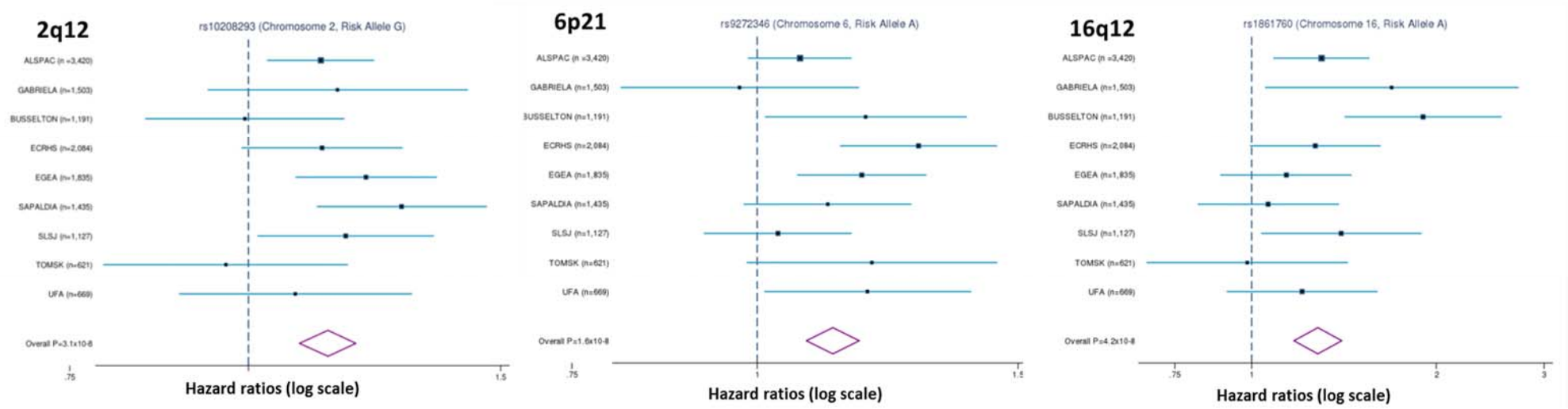
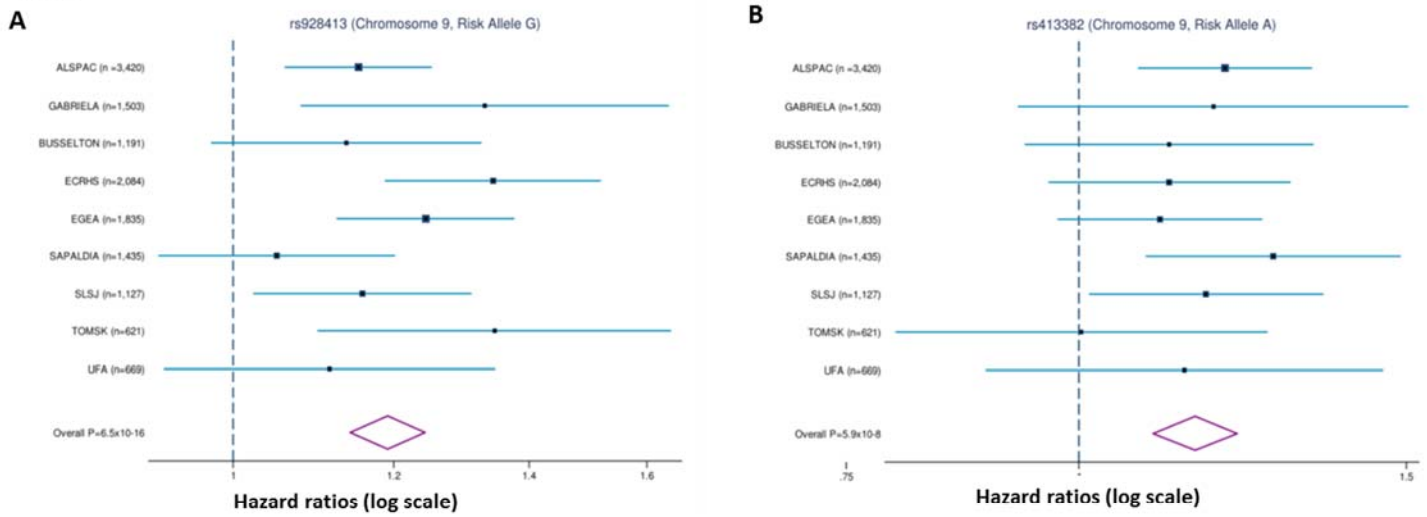
406

Figure E4.


2q12
rs10208293 (Chromosome 2, Risk Allele G)
ALSPAC (n =3,420)
GABRIELA (n=1,503)
BUSSELTON (n=1,191)
ECRHS (n=2,084)
EGEA (n=1,835)
SAPALDIA (n=1,435)
SLSJ (n=1,127)
TOMSK (n=621)
UFA (n=669)
Overall P=3.1x10-8
.75
1.5
Hazard ratios (log scale)
6p21
rs9272346 (Chromosome 6, Risk Allele A)
ALSPAC (n =3,420)
GABRIELA (n=1,503)
BUSSELTON (n=1,191)
ECRHS (n=2,084)
EGEA (n=1,835)
SAPALDIA (n=1,435)
SLSJ (n=1,127)
TOMSK (n=621)
UFA (n=669)
Overall P=1.6x10-8
.75
1
Hazard ratios (log scale)
16q12
rs1861760 (Chromosome 16, Risk Allele A)
ALSPAC (n =3,420)
GABRIELA (n=1,503)
BUSSELTON (n=1,191)
ECRHS (n=2,084)
EGEA (n=1,835)
SAPALDIA (n=1,435)
SLSJ (n=1,127)
TOMSK (n=621)
UFA (n=669)
Overall P=4.2x10-8
.75
1
2
3
Hazard ratios (log scale)

9p24



409

17q12-q21

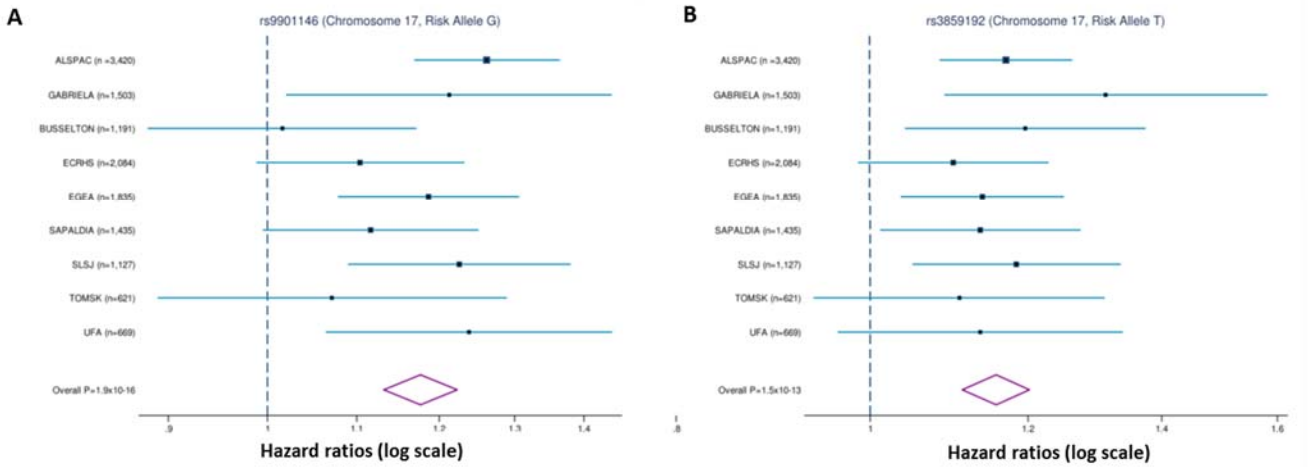


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Figure E5.

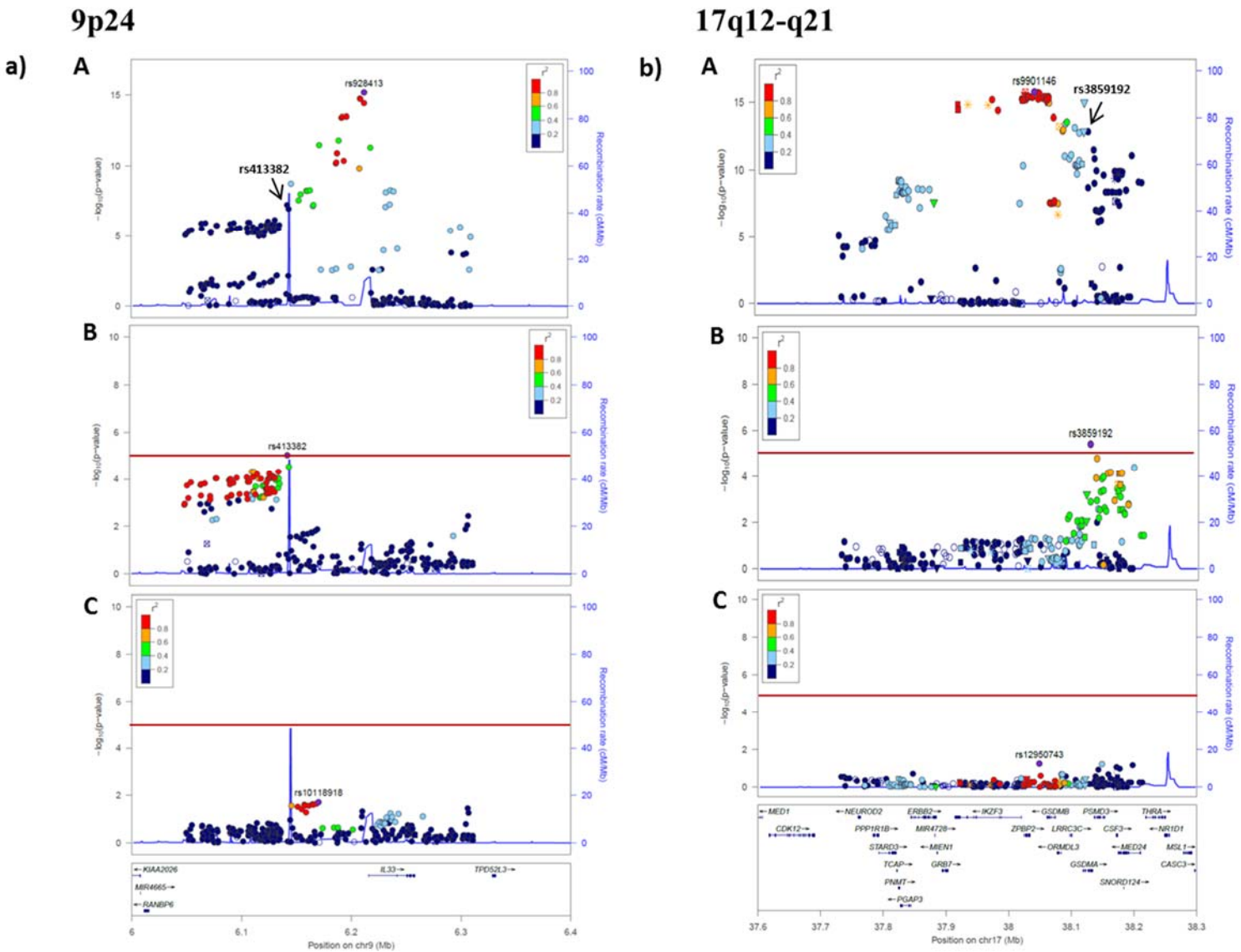
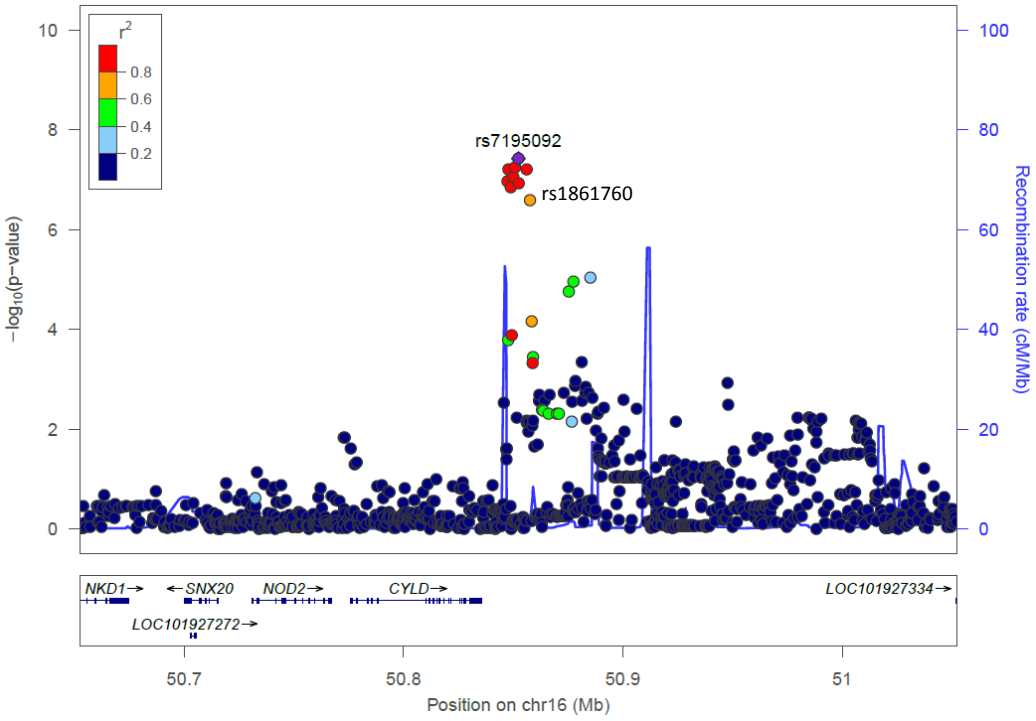

9p24
a)
A
rs928413
rs413382
B
rs413382
C
rs10118918
KIAA2026
MIR4665
RANBP6
IL33
TPD52L3
Position on chr9 (Mb)
17q12-q21
b)
A
rs9901146
rs3859192
B
rs3859192
C
rs12950743
MED1
CDK12
NEUROD2
PPP1R1B
ERBB2
MIR4728
IKZF3
STARD3
MIEN1
TCAP
GRB7
PNMT
PGAP3
GSDMB
PSMD3
THRA
ZPBP2
LRRC3C
CSF3
NR1D1
ORMDL3
MED24
MSL1
GSDMA
CASC3
SNORD124
Position on chr17 (Mb)
Recombination rate (cM/Mb)

Figure E6.


r²
0.8
0.6
0.4
0.2
rs7195092
rs1861760
−log10(p−value)
Recombination rate (cM/Mb)
NKD1
SNX20
NOD2
CYLD
LOC101927334
LOC101927272
Position on chr16 (Mb)