

ORIGINAL ARTICLE

Asthma and Rhinitis

Novel genes and insights in complete asthma remission: A genome-wide association study on clinical and complete asthma remission

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Summary

Background: Asthma is a chronic respiratory disease without a cure, although there exists spontaneous remission. Genome-wide association (GWA) studies have pinpointed genes associated with asthma development, but did not investigate asthma remission.

Objective: We performed a GWA study to develop insights in asthma remission.

Methods: Clinical remission (ClinR) was defined by the absence of asthma treatment and wheezing in the last year and asthma attacks in the last 3 years and complete remission (ComR) similarly but additionally with normal lung function and absence of bronchial hyperresponsiveness (BHR). A GWA study on both ClinR and ComR was performed in 790 asthmatics with initial doctor diagnosis of asthma and BHR and long-term follow-up. We assessed replication of the 25 top single nucleotide polymorphisms (SNPs) in 2 independent cohorts (total n = 456), followed by

expression quantitative loci (eQTL) analyses of the 4 replicated SNPs in lung tissue and epithelium.

Results: Of the 790 asthmatics, 178 (23%) had ClinR and 55 ComR (7%) after median follow-up of 15.5 (range 3.3-47.8) years. In ClinR, 1 of the 25 SNPs, rs2740102, replicated in a meta-analysis of the replication cohorts, which was an eQTL for *POLR* in lung tissue. In ComR, 3 SNPs replicated in a meta-analysis of the replication cohorts. The top-hit, rs6581895, almost reached genome-wide significance (P -value 4.68×10^{-7}) and was an eQTL for *FRS2* and *CCT* in lung tissue. Rs1420101 was a cis-eQTL in lung tissue for *IL1RL1* and *IL18R1* and a trans-eQTL for *IL13*.

Conclusions and Clinical Relevance: By defining a strict remission phenotype, we identified 3 SNPs to be associated with complete asthma remission, where 2 SNPs have plausible biological relevance in *FRS2*, *CCT*, *IL1RL1*, *IL18R1* and *IL13*.

1 | INTRODUCTION

Asthma is a highly prevalent chronic airway disease that affects over 300 million subjects worldwide. Asthma symptoms usually start in childhood, but not all preschool wheezers will continue to have asthma symptoms in childhood. In a birth cohort of asthmatic children diagnosed before the age of 6 and followed till 12 years of age,¹ asthma remission was present in 48% of the children when defined as the absence of any asthma events (hospitalization and/or physician visit) between the ages 6 and 11 years.

In contrast, asthma remission is less common in adults. We previously defined 2 types of remission,² one being "clinical remission," where asthmatics lose their symptoms for at least 12 months without using asthma treatment. This type of remission occurred in 52% of adult asthmatics. In contrast, remission occurred only in 22% of asthmatics when defined as "complete remission." The latter type had clinical remission and additionally met the more stringent criteria of normal lung function and absence of bronchial hyperresponsiveness (BHR), a hallmark of asthma.² In an observational study in adults with and without complete asthma remission, we found that those with complete remission lacked inflammatory infiltration of the airway mucosa, suggesting the inflammatory response had faded away.³ This was supported by the observation that individuals with complete asthma remission did not develop sputum eosinophilia after inhaling adenosine monophosphate,⁴ a trigger previously shown to attract eosinophils to sputum in asthmatics, independently of the use of inhaled corticosteroids.⁵

Genome-wide association (GWA) studies have provided insights into the origins of asthma, and multiple genes have been associated with asthma development and subsequently replicated, such as the *ORMDL3* region, *IL1RL1*, *IL33* and *TSLP*.⁶ This has led to some promising novel asthma treatments like anti-TSLP.⁷ However, despite better treatments, there is still no cure for asthma. We hypothesized that genetics also plays a role in asthma remission and that the genes associated with asthma remission may differ from those

associated with the development of asthma. Therefore, we performed a GWA study on asthma remission to provide us with clues to develop a cure for asthma. We investigated a Dutch identification cohort of 790 asthmatics initially characterized by the presence of a doctor diagnosis of asthma and BHR and assessed their remission status after a median 15.5 years of follow-up. We verified our findings in 2 European cohorts using the same phenotypic criteria and finally we performed expression quantitative loci (eQTL) analyses to determine allelic effects on gene expression in lung tissue and epithelial cells.

2 | METHODS

2.1 | Study population

We included 790 subjects from a long-term follow-up cohort with a doctor diagnosis of asthma and a positive BHR test at baseline. All participants who were younger than 12 years at baseline had an additional follow-up visit during early adulthood in which the persistence of asthma was confirmed by symptoms and objective measures during a clinic visit. All subjects had at least one follow-up measurement during adulthood (>18 years) in which their asthma status was evaluated by questionnaires and in most patients additionally with spirometry and a BHR test. All subjects participated in asthma studies performed at the University Medical Center Groningen^{2,8-15} and provided written informed consent. All studies were approved by the medical ethical committee of the University Medical Center Groningen (ethics approval numbers METc 2009/007, METc 2010/378 and METc 2012/173).

2.2 | Definition of asthma remission

Clinical remission (ClinR) at follow-up visit was defined as the absence of asthma symptoms (no wheezing without having a cold in the last year and no asthma attacks in the last 3 years) and no use

of asthma medication in the last year but individuals could still have a low lung function or the presence of BHR. Complete remission (ComR) was defined as the absence of asthma symptoms and no use of asthma medication, a normal lung function (FEV_1 %predicted pre-bronchodilator $>80\%$ or FEV_1 %predicted post-bronchodilator $>90\%$) and no BHR (no 20% decrease in FEV_1 at the maximal dose of histamine, methacholine or AMP according to the protocol^{3-5,16,17}). Persistent asthma (PersA) was defined as the presence of asthma symptoms and/or the use of asthma medication. If more than one follow-up visit was available, the most recent follow-up visit with information on BHR and spirometry was used to determine asthma remission status.

2.3 | Genotyping and statistical analyses

DNA quality control is described in the Supplemental Methods. After quality control, 156 954 single nucleotide polymorphisms (SNPs) were present in all 790 subjects after combining the different genotype platforms (Illumina 317 Chip, the Illumina 370 Duo Chip, or the Omni Express Exome chip (Illumina Inc., San Diego, CA) and subsequently used in analyses. These 156 954 SNPs tagged 36%-60% of all SNPs in the genome (Table S1). All statistical analyses were performed using PLINK v1.07. Logistic regression analyses investigated the association between SNPs and asthma remission with adjustment for sex without additional covariates given the relatively low number of participants. Due to the low number of subjects with ClinR ($n = 178$) and ComR ($n = 55$), not all SNPs were tested in an additive genetic model. In the analysis on ClinR, SNPs with a minor allele frequency (MAF) below 0.25 were tested in a dominant model and SNPs with a $MAF \geq 0.25$ were tested in an additive model. For ComR, SNPs with a MAF below 0.40 were tested in a dominant model and SNPs with a $MAF \geq 0.40$ were tested in an additive model. With these cut-off values, the minimal expected number of subjects in remission having the homozygote mutant genotype in the additive models was approximately 10. Two separate analyses were performed: (1) association between SNPs and ClinR vs PersA, (2) association between SNPs and ComR vs PersA.

For each analysis, the top 25 SNPs (after excluding SNPs in LD and regardless of their P -value) were analysed in 2 replication cohorts. The results of the replication cohorts were meta-analysed using METAL.¹⁸ We assessed the direction of the effect in the identification cohort, that is a negative effect implying a lower chance of asthma remission, and a positive effect implying higher a chance of asthma remission. Significant replication was achieved if the direction of the effect in this meta-analysis was the same as in the identification cohort, and if the 1-sided P -value was $<.05$ (expected direction based on the identification analysis).

2.4 | Replication cohorts

Replication was performed in the EGEA-study¹⁹ and the SAPALDIA cohort-study.²⁰⁻²² Extensive information on these cohorts is presented in the Supporting information. All subjects with doctor-

diagnosed asthma irrespective of age of onset and a positive BHR test at the baseline visit (early 1990s) were included in the current analysis. Remission status was determined at the most recent follow-up visit according to the same definitions as used in the identification cohort.

2.5 | Gene expression analysis in lung tissue and lung epithelial cells

The 4 significantly replicated SNPs were assessed for their association with cis- and trans-acting gene expression (mRNA) in lung tissue ($n = 1087$).²³ Subsequently, significant eQTLs identified in lung tissue were analysed in an epithelial-specific eQTL database ($n = 85$) (see Supporting information).

3 | RESULTS

3.1 | Subject characteristics

Subject characteristics of the identification cohort, according to their most recent asthma status, are shown in Table 1. The median [range] follow-up was 15.5 [3.3; 47.8] years. Of the 790 included subjects, 178 (23%) had ClinR. Of these, 55 were in ComR (7% of total cohort). In 41 subjects, a BHR-test was not performed and these patients were assigned to the ClinR group. Subjects in remission were more often male, had a longer duration of follow-up, were younger at baseline and had a better lung function at baseline compared to subjects with PersA. The age of asthma onset was comparable between ClinR and PersA, while subjects in ComR had a younger age at the start of their asthma compared to PersA. Table 1 also shows the characteristics in the replication cohorts. In EGEA ($n = 351$), 23% of the subjects were in ClinR and 2% in ComR. In SAPALDIA ($n = 105$), these percentages were 51% and 13%, respectively.

3.2 | Clinical asthma remission

The results of the identification GWAS on ClinR are shown in Figure 1. There existed no inflation of data based on the lambda of 1.00 and QQ-plot (Figure S5). The results of the replication analysis (Table 2) show that 1 of the 25 SNPs tested, rs7240102, met the criteria for significant replication (see Figure S6 for regional association plot).

3.3 | Complete asthma remission

The results of the identification GWAS on ComR are shown in Figure 2. The lambda of 1.01 and QQ-plot (Figure S7) gave no indication of inflation. The results of the replication analysis on ComR are presented in Table 3. Three of the 25 SNPs tested met the criteria for significant replication (see Figure S6 for regional association plots): rs6581895 (intergenic between YEATS4 and FRS2), rs12405429 (intergenic between FAM89A and TRIM67), and

TABLE 1 Characteristics of patients according to their asthma status

	Identification Cohort				EGEA				SAPALDIA			
	Persistent asthma (n = 612)	Clinical remission (n = 178)	Complete remission (n = 55)		Persistent asthma (n = 270)	Clinical remission (n = 81)	Complete remission (n = 7)		Persistent asthma (n = 51)	Clinical remission (n = 54)	Complete remission (n = 14)	
Male sex, n (%)	284 (46)	105 (59)	41 (75)		165 (61)	46 (57)	4 (57)		24 (47)	17 (32)	4 (29)	
Age at remission status, years	49.8 (41.2; 59.2)	50.3 (42.0; 59.4)	50.5 (40.9; 56.7)		37.9 (29.6; 60.5)	30.3 (24.8; 58.6)	36.1 (29.8; 55.5)		49.1 (43.1; 56.5)	53.3 (44.9; 60.0)	52.0 (44.9; 59.6)	
Duration of follow -up, years	14.4 (10.3; 39.2)	28.7 (11.7; 40.6)	32.9 (19.5; 40.4)		18.2 (14.8; 19.0)	11.0 (10.7; 12.2)	17.3 (12.1; 18.8)		10.9 (10.8; 11.0)	10.9 (10.8; 11.0)	10.9 (10.8; 11.0)	
Characteristics at first survey												
Age, years	29.7 (20.0; 38.9)	24.0 (14.0; 37.0)	23.4 (12.3; 37.8)		20.7 (11.5; 42.3)	18.8 (13.8; 46.1)	36.1 (29.8; 55.5)		38.4 (32.6; 45.6)	42.4 (33.9; 49.3)	41.2 (33.9; 48.8)	
FEV ₁ %pred pre bronchodilator	78.4 (62.6; 90.1)	83.4 (73.6; 93.1)	85.8 (75.0; 95.3)		89.1 (74.9; 100.3)	95.6 (80.1; 103.5)	90.4 (82.0; 100.7)		90.4 (82.2; 98.0)	97.7 (91.2; 106.3)	95.4 (88.6; 104.6)	
FEV ₁ %pred post bronchodilator	91.1 (79.7; 100.9)	95.5 (87.1; 103.6)	99.1 (91.7; 107.1)									
FEV ₁ %IVC pre bronchodilator	71.0 (60.0; 78.0)	75.0 (66.0; 81.3)	77.0 (66.0; 83.0)									
FEV ₁ %FVC pre bronchodilator					80.0 (69.0; 86.0)	83.0 (81.0; 91.0)	83.0 (76.0; 86.0)		73.3 (69.0; 79.1)	78.0 (75.1; 84.4)	76.2 (72.7; 82.4)	
FEV ₁ %IVC post bronchodilator	77.0 (69.0; 83.0)	80.0 (77.0; 86.0)	82.0 (78.0; 86.0)									
FEV ₁ reversibility % pred	11.8 (7.2; 19.3)	9.2 (4.5; 16.4)	11.4 (5.0; 18.2)									
Age start asthma, years	5.0 (1.0; 15.0)	4.0 (1.0; 12.3)	2.0 (0.0; 8.0)		7.0 (3.0; 19.0)	11.0 (8.0; 35.0)	7.0 (3.0; 18.0)		21 (6.0; 30.0)	5.5 (2.0; 18.0)	16.0 (5.0; 28.0)	
Start asthma before age 16, n (%)	437 (74)	130 (80)	42 (86)		195 (72)	59 (73)	5 (71)		20 (44)	25 (56)	10 (22)	

Data are presented as median (25th percentile—75th percentile) unless indicated otherwise. Bold values signify significant differences from persistent asthma with $P < 0.05$ in the identification cohort.

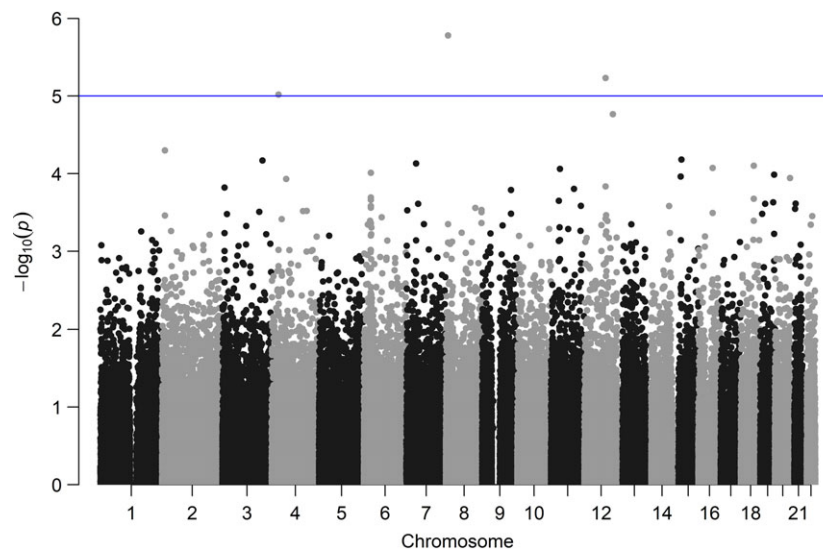


FIGURE 1 Manhattan plot of Clinical remission in the identification cohort

rs1420101 (located in *IL1RL1*). In the meta-analysis of the identification and replication cohorts, the top-hit rs6581895 had a P -value of 4.68×10^{-7} , very close to the Bonferroni-corrected genome-wide significant P -value of 3.1×10^{-7} .

3.4 | Gene expression analysis in lung tissue

To investigate whether replicated SNPs had allelic effects on gene expression in lung tissue, we assessed the association between genotype and lung tissue gene expression. For ClinR, the replicated SNP rs7240102 was associated with gene expression levels of *Polymerase (DNA) iota (POLI)*. Two of the 3 significantly replicated SNPs that were associated with ComR were identified as e-QTL measured by 9 gene expression probe sets (Table 4, Figure 3): rs6581895 was associated with expression of *Fibroblast growth factor 2 (FRS2)*, *Chaperonin containing TCP1 subunit 2 (CCT2)*, and rs1420101 located in *Interleukin 1 receptor 1 (IL1RL1)* was associated with *IL1RL1* and *Interleukin 18 Receptor 1 (IL18R1)*-expression (Table S2). In addition, rs1420101 was a trans-eQTL for *Interleukin 13 (IL13)* expression.

3.5 | Gene expression analysis in lung epithelial cells

The significant eQTLs identified in the lung tissue analysis were further explored in an epithelial specific eQTL database. None of the SNPs showed a significant association (Table S3).

4 | DISCUSSION

The goal of our study was to identify genetic markers associated with asthma remission by performing a genome-wide association study. This may provide insight into mechanisms underlying asthma remission and possibly provide us with clues to develop a cure for asthma. We applied a restrictive asthma remission phenotype and defined

complete remission by cessation of symptoms and asthma medication in addition to good lung health (normal lung function and no longer BHR present). In this way, we identified 3 consistently associated genetic loci across all 3 cohorts with in total 76 individuals in complete asthma remission. One SNP reached almost genome-wide significance in relation to complete asthma remission and also showed an association with the expression of *FRS2* and *CCT2*. The replicated SNP rs7240102 with the loose asthma remission definition, clinical remission, was associated with gene expression levels of *POLI*. Thus, our study shows that it is possible to identify gene polymorphisms that are consistently associated with a strict phenotype definition of complete asthma remission across multiple cohorts, even though none of the SNPs reached genome-wide significance in our small number of individuals. Given the functional relevance in expression (eQTL) in lung tissue, the presented data suggest that *FRS2*, *CCT2*, *IL1RL1*, *IL18R1* and *IL13* are candidate genes for further functional studies into their biological role in complete asthma remission.

This is the first genome-wide study on asthma remission, so no comparator data are available. Several of the genes identified are yet not known to be involved in asthma or inflammation. *FRS2* (*Fibroblast growth factor receptor substrate 2*) belongs to the FRS2 family of adaptor/scaffold proteins that has 2 members, FRS2alpha and FRS2-beta.²⁴ Both proteins contain N-terminal myristoylation sites for localization on the plasma membrane, a PTB domain for binding to limited species of receptor tyrosine kinases, including the FGF receptor, and a long C-terminal tail with 4 binding sites, 2 for the adaptor protein Frb2 and 2 for the tyrosine phosphatase Shp2.²⁴ Activation of these receptor tyrosine kinases allows FRS2 proteins to become phosphorylated at tyrosine residues and then bring about a cascade of events including activation of the Ras/ERK, PI3kinase and MAPK pathway.²⁵ This may among others affect neurite outgrowth.²⁶ FRS can additionally inhibit EGF signalling, resulting in inhibition of EGF-induced cell proliferation and cell transformation. An interesting finding is that FRS2 alpha is not only affecting FGF signalling pathways, but is also a critical regulator of VEGF receptor signalling which may

TABLE 2 Results of top 25 SNPs of GWAs on Clinical asthma remission. Gene annotation was performed using SCAN (www.scandb.org)

Identification cohort										EGEA				SAPALDIA				META-analysis				META-analysis				Gene—annotation	
SNP	CHR	TA	TEST	MAF	Cases: 178		Controls: 612		Cases: 81		Controls: 270		Cases: 54		Controls: 51		EGEA+SAPALDIA		EGEA+SAPALDIA		P		P		Gene	Left gene/Right gene	
					OR	2-sided P	OR	2-sided P	OR	1-sided P	OR	1-sided P	OR	1-sided P	OR	1-sided P	OR	1-sided P	OR	2-sided P	Dir	OR	2-sided P	Dir			
rs565169	8	C	DOM	0.15	2.37	1.7 × 10 ⁻⁶	1.06	4.2 × 10 ⁻¹	1.06	4.2 × 10 ⁻¹	1.06	4.2 × 10 ⁻¹	1.50	2.1 × 10 ⁻¹	1.50	2.1 × 10 ⁻¹	1.15	2.9 × 10 ⁻¹	1.15	2.9 × 10 ⁻¹	++	1.84	2.7 × 10 ⁻⁵	+++	NA	CLDN23/MFHAS1	
rs1471239	12	G	ADD	0.37	1.78	5.9 × 10 ⁻⁶	0.98	5.5 × 10 ⁻¹	0.98	5.5 × 10 ⁻¹	0.98	5.5 × 10 ⁻¹	0.96	5.6 × 10 ⁻¹	0.96	5.6 × 10 ⁻¹	0.97	5.7 × 10 ⁻¹	0.97	5.7 × 10 ⁻¹	--	1.40	6.5 × 10 ⁻⁴	+--	NA	LOC100128335/SLC6A15	
rs6850690	4	G	ADD	0.27	1.79	9.6 × 10 ⁻⁶	1.31	8.0 × 10 ⁻²	1.31	8.0 × 10 ⁻²	1.31	8.0 × 10 ⁻²	0.81	7.5 × 10 ⁻¹	0.81	7.5 × 10 ⁻¹	1.15	2.0 × 10 ⁻¹	1.15	2.0 × 10 ⁻¹	+-	1.51	7.0 × 10 ⁻⁵	++-	NA	LOC391642/LOC645641	
rs7137598	12	G	DOM	0.13	2.23	1.7 × 10 ⁻⁵	0.81	7.6 × 10 ⁻¹	0.81	7.6 × 10 ⁻¹	0.81	7.6 × 10 ⁻¹	0.84	6.5 × 10 ⁻¹	0.84	6.5 × 10 ⁻¹	0.82	7.9 × 10 ⁻¹	0.82	7.9 × 10 ⁻¹	--	1.56	3.0 × 10 ⁻³	+--	NA	RBM19/LOC255480	
rs4669688	2	A	ADD	0.38	0.58	5.0 × 10 ⁻⁵	0.87	2.2 × 10 ⁻¹	0.87	2.2 × 10 ⁻¹	0.87	2.2 × 10 ⁻¹	1.13	6.6 × 10 ⁻¹	1.13	6.6 × 10 ⁻¹	0.94	3.4 × 10 ⁻¹	0.94	3.4 × 10 ⁻¹	+-	0.71	7.9 × 10 ⁻⁴	--+	NA	KCNF1/LOC645054	
rs8041207	15	A	ADD	0.43	1.66	6.6 × 10 ⁻⁵	1.02	4.6 × 10 ⁻¹	1.02	4.6 × 10 ⁻¹	1.02	4.6 × 10 ⁻¹	1.01	4.9 × 10 ⁻¹	1.01	4.9 × 10 ⁻¹	1.02	4.6 × 10 ⁻¹	1.02	4.6 × 10 ⁻¹	++	1.36	1.6 × 10 ⁻³	+++	NA	GOLGA8B/GJD2	
rs1515735	3	A	DOM	0.15	2.06	6.8 × 10 ⁻⁵	0.73	8.6 × 10 ⁻¹	0.73	8.6 × 10 ⁻¹	0.73	8.6 × 10 ⁻¹	0.56	8.8 × 10 ⁻¹	0.56	8.8 × 10 ⁻¹	0.68	9.4 × 10 ⁻¹	0.68	9.4 × 10 ⁻¹	--	1.42	1.7 × 10 ⁻²	+--	NA	OTOL1/LOC100132484	
rs12701705	7	G	ADD	0.46	1.66	7.4 × 10 ⁻⁵	1.23	1.2 × 10 ⁻¹	1.23	1.2 × 10 ⁻¹	1.23	1.2 × 10 ⁻¹	0.97	5.4 × 10 ⁻¹	0.97	5.4 × 10 ⁻¹	1.15	1.8 × 10 ⁻¹	1.15	1.8 × 10 ⁻¹	+-	1.42	3.1 × 10 ⁻⁴	++-	POU6F2	VPS41/C7orf36	
rs7240102	18	G	DOM	0.24	1.99	7.9 × 10 ⁻⁵	1.54	4.6 × 10 ⁻²	1.54	4.6 × 10 ⁻²	1.54	4.6 × 10 ⁻²	1.19	3.3 × 10 ⁻¹	1.19	3.3 × 10 ⁻¹	1.43	4.9 × 10 ⁻²	1.43	4.9 × 10 ⁻²	++	1.75	3.9 × 10 ⁻⁵	+++	NA	LOC100130003/C18orf26	
rs2967137	16	G	ADD	0.41	0.61	8.4 × 10 ⁻⁵	1.02	5.4 × 10 ⁻¹	1.02	5.4 × 10 ⁻¹	1.02	5.4 × 10 ⁻¹	1.86	9.8 × 10 ⁻¹	1.86	9.8 × 10 ⁻¹	1.19	8.7 × 10 ⁻¹	1.19	8.7 × 10 ⁻¹	++	0.80	2.1 × 10 ⁻²	--+	NA	LOC388282/CNGB1	
rs1518746	11	A	ADD	0.43	0.61	8.7 × 10 ⁻⁵	1.34	9.5 × 10 ⁻¹	1.34	9.5 × 10 ⁻¹	1.34	9.5 × 10 ⁻¹	0.85	2.9 × 10 ⁻¹	0.85	2.9 × 10 ⁻¹	1.18	8.7 × 10 ⁻¹	1.18	8.7 × 10 ⁻¹	+-	0.80	2.2 × 10 ⁻²	--	NA	C11orf74/LOC100129825	
rs203888	6	A	ADD	0.27	1.68	9.7 × 10 ⁻⁵	0.92	6.4 × 10 ⁻¹	0.92	6.4 × 10 ⁻¹	0.92	6.4 × 10 ⁻¹	0.97	5.3 × 10 ⁻¹	0.97	5.3 × 10 ⁻¹	0.94	6.4 × 10 ⁻¹	0.94	6.4 × 10 ⁻¹	--	1.37	3.2 × 10 ⁻³	+--	NA	OR2B7P/OR1F12	
rs10403164	19	A	ADD	0.49	0.61	1.0 × 10 ⁻⁴	0.99	4.7 × 10 ⁻¹	0.99	4.7 × 10 ⁻¹	0.99	4.7 × 10 ⁻¹	1.10	6.3 × 10 ⁻¹	1.10	6.3 × 10 ⁻¹	1.02	5.4 × 10 ⁻¹	1.02	5.4 × 10 ⁻¹	+-	0.75	3.1 × 10 ⁻³	--+	HSPBP1	SAPS1/HSPBP1	
rs2219507	15	A	DOM	0.22	1.95	1.1 × 10 ⁻⁴	0.67	9.4 × 10 ⁻¹	0.67	9.4 × 10 ⁻¹	0.67	9.4 × 10 ⁻¹	0.54	9.3 × 10 ⁻¹	0.54	9.3 × 10 ⁻¹	0.63	9.8 × 10 ⁻¹	0.63	9.8 × 10 ⁻¹	--	1.27	7.8 × 10 ⁻²	+--	OTUD7A	LOC100128855/DEPDC1P1	
rs4925184	20	G	DOM	0.05	2.60	1.1 × 10 ⁻⁴	1.12	3.8 × 10 ⁻¹	1.12	3.8 × 10 ⁻¹	1.12	3.8 × 10 ⁻¹	3.02	6.2 × 10 ⁻¹	3.02	6.2 × 10 ⁻¹	1.37	1.7 × 10 ⁻¹	1.37	1.7 × 10 ⁻¹	++	2.05	2.6 × 10 ⁻⁴	+++	GATA5	C20orf151/GATA5	
rs7679186	4	C	ADD	0.30	0.58	1.2 × 10 ⁻⁴	0.91	3.2 × 10 ⁻¹	0.91	3.2 × 10 ⁻¹	0.91	3.2 × 10 ⁻¹	0.81	2.4 × 10 ⁻¹	0.81	2.4 × 10 ⁻¹	0.87	2.1 × 10 ⁻¹	0.87	2.1 × 10 ⁻¹	--	0.68	5.0 × 10 ⁻⁴	---	NA	LOC255130/SRIL	
rs12828146	12	G	DOM	0.07	2.33	1.5 × 10 ⁻⁴	0.60	8.5 × 10 ⁻¹	0.60	8.5 × 10 ⁻¹	0.60	8.5 × 10 ⁻¹	0.63	7.6 × 10 ⁻¹	0.63	7.6 × 10 ⁻¹	0.61	8.9 × 10 ⁻¹	0.61	8.9 × 10 ⁻¹	--	1.70	6.5 × 10 ⁻³	+--	NA	LOC100128335/SLC6A15	
rs1488793	3	A	ADD	0.33	1.61	1.5 × 10 ⁻⁴	0.86	7.9 × 10 ⁻¹	0.86	7.9 × 10 ⁻¹	0.86	7.9 × 10 ⁻¹	0.91	6.2 × 10 ⁻¹	0.91	6.2 × 10 ⁻¹	0.87	8.0 × 10 ⁻¹	0.87	8.0 × 10 ⁻¹	--	1.28	1.2 × 10 ⁻²	+--	NA	GRM7/LMCD1	
rs1579874	11	A	DOM	0.06	2.58	1.6 × 10 ⁻⁴	0.97	5.3 × 10 ⁻¹	0.97	5.3 × 10 ⁻¹	0.97	5.3 × 10 ⁻¹	0.51	8.0 × 10 ⁻¹	0.51	8.0 × 10 ⁻¹	0.84	6.8 × 10 ⁻¹	0.84	6.8 × 10 ⁻¹	--	1.83	3.8 × 10 ⁻³	+--	NA	LOC100128230/CCDC67	
rs1411605	9	A	DOM	0.16	1.97	1.6 × 10 ⁻⁴	0.93	6.0 × 10 ⁻¹	0.93	6.0 × 10 ⁻¹	0.93	6.0 × 10 ⁻¹	1.36	2.6 × 10 ⁻¹	1.36	2.6 × 10 ⁻¹	1.03	4.6 × 10 ⁻¹	1.03	4.6 × 10 ⁻¹	+-	1.55	2.1 × 10 ⁻³	+--	ZFP37	LOC169834/LOC100128385	
rs10835893	11	A	ADD	0.28	0.58	2.2 × 10 ⁻⁴	0.79	1.3 × 10 ⁻¹	0.79	1.3 × 10 ⁻¹	0.79	1.3 × 10 ⁻¹	1.60	9.4 × 10 ⁻¹	1.60	9.4 × 10 ⁻¹	0.98	4.6 × 10 ⁻¹	0.98	4.6 × 10 ⁻¹	+-	0.73	4.2 × 10 ⁻³	--+	NA	RCN1/WT1	
rs3810114	19	A	DOM	0.17	1.92	2.3 × 10 ⁻⁴	1.34	1.4 × 10 ⁻¹	1.34	1.4 × 10 ⁻¹	1.34	1.4 × 10 ⁻¹	0.67	8.2 × 10 ⁻¹	0.67	8.2 × 10 ⁻¹	1.10	3.4 × 10 ⁻¹	1.10	3.4 × 10 ⁻¹	+-	1.57	1.5 × 10 ⁻³	++-	NA	SIGLEC12/SIGLECP11	
rs2825209	21	A	DOM	0.18	1.91	2.4 × 10 ⁻⁴	0.93	6.2 × 10 ⁻¹	0.93	6.2 × 10 ⁻¹	0.93	6.2 × 10 ⁻¹	3.28	7.2 × 10 ⁻¹	3.28	7.2 × 10 ⁻¹	1.16	2.3 × 10 ⁻¹	1.16	2.3 × 10 ⁻¹	+-	1.53	1.2 × 10 ⁻³	+--	NA	PRSS7/PP1AL3	
rs2043293	19	C	DOM	0.19	1.90	2.4 × 10 ⁻⁴	1.06	4.1 × 10 ⁻¹	1.06	4.1 × 10 ⁻¹	1.06	4.1 × 10 ⁻¹	1.07	4.4 × 10 ⁻¹	1.07	4.4 × 10 ⁻¹	1.06	3.9 × 10 ⁻¹	1.06	3.9 × 10 ⁻¹	++	1.52	2.4 × 10 ⁻³	+++	GMIP	LPAR2/ATP13A1	
rs1554495	7	A	ADD	0.46	1.58	2.5 × 10 ⁻⁴	0.97	5.6 × 10 ⁻¹	0.97	5.6 × 10 ⁻¹	0.97	5.6 × 10 ⁻¹	1.01	4.9 × 10 ⁻¹	1.01	4.9 × 10 ⁻¹	0.98	5.5 × 10 ⁻¹	0.98	5.5 × 10 ⁻¹	+-	1.31	5.6 × 10 ⁻³	+--	PKD11	FLJ21075/HUS1	

SNP, single nucleotide polymorphism; CHR, chromosome; TA, tested allele; TEST: DOM, dominant model; ADD, additive model; MAF, minor allele frequency; OR, Odds Ratio; Dir, direction of the effect; “+/-” indicates positive/negative association between the tested allele and remission. In bold; significantly replicated SNPs.

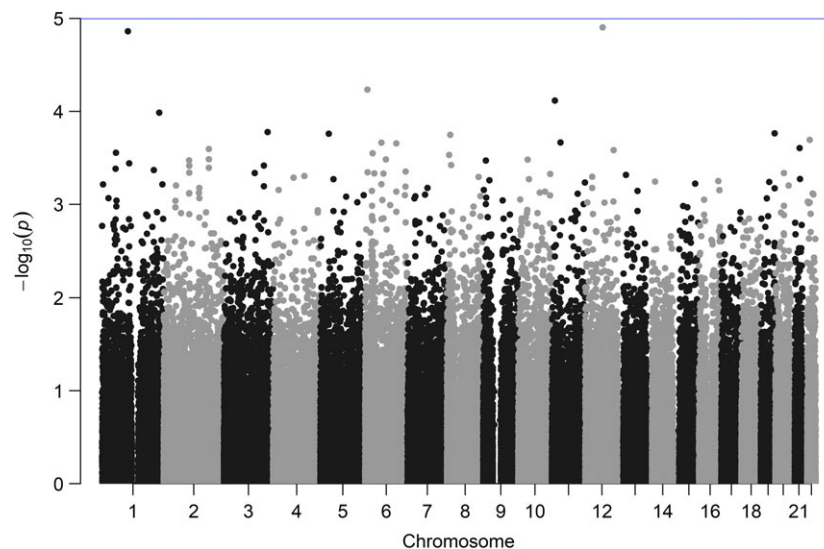


FIGURE 2 Manhattan plot of Complete remission in the identification cohort

affect angiogenesis as well as actin reorganization.²⁵ If the latter is impaired, this might contribute to a reduced load and resolution of inflammation.²⁵ Finally, changes in this receptor may affect the function of multiple FGFs, some previously associated with lung development or disease, including FGF10²⁷ and FGF7.²⁸

The protein encoded by *CCT2* (*Chaperonin Containing TCP1, Subunit 2*) is a molecular chaperone that is a member of the chaperonin containing TCP1 complex (CCT), also known as the TCP1 ring complex (TRiC). This complex consists of 2 identical stacked rings, each containing 8 different proteins. CCT2 assists the folding of polypeptides that enter the central cavity of the complex in an ATP-dependent manner, including actin and tubulin. This is of importance as tissue responses to injury fundamentally involve reorganization of the actin cytoskeleton of participating cells, including epithelial cells, fibroblasts, endothelial cells and macrophages. Mutation analysis in plants has shown that this may affect cell growth.²⁹ CCT2 has additionally been associated with primary cilia, non-motile microtubule-based organelles that function as central antennae that sense a wide variety of signals during development and tissue homeostasis, including involvement in the maintenance of cell proliferation.³⁰

We found that the A-allele of rs1420101, a SNP located in *IL1RL1*, is associated with a lower chance for complete asthma remission in our adult asthma patients. This seems remarkable, as this rs1420101 A-allele has been identified previously as a risk for asthma in GWAS.³¹ The A-allele of this *IL1RL1* variant was associated with lower *IL1RL1* and *IL18R1* lung tissue expression³² and higher *IL13* lung tissue expression in our study. Moreover, it was also associated with higher numbers of eosinophils in childhood asthma and with a higher risk for asthma in a previous study.³³ This suggests that higher *IL1RL1* expression might eventually, and perhaps in association with other factors, contribute to remission of asthma by negatively regulating Type 2 inflammation as reflected by lower lung *IL13* mRNA levels. *IL1RL1* encodes 3 splice variants, and the synonymous asthma SNP rs1420101 is located in exon 5E,

which is only retained in the transcript encoding the truncated *IL1RL1*-c, also called ST2V.³⁴ This might indicate an effect of rs1420101 on this specific transcript. However, this variant is not supported by refseq annotation and the function of this transcript is yet unknown.

We found 1 SNP to be associated with the more loose definition of asthma remission, clinical remission. This SNP associated with *POLI* (*Polymerase iota*) expression in lung tissue. Human DNA polymerase iota (Pol*i*) belongs to the Y family of specialized DNA polymerases and is one of the most error-prone enzymes involved in DNA synthesis.³⁵ The functions of Pol*i* in human cells remain largely unknown, and it is not clear which might be the role of this gene in clinical asthma remission.

There are strengths and weaknesses to the current study. A strength of our approach is that both active asthma and asthma remission was defined using objective characteristics of the disease, that is bronchial hyperresponsiveness, airway obstruction, in combination with the presence or absence of patient reported symptoms and medication use. Due to the use of different genotyping arrays, we investigated “only” 156,954 SNPs that tagged 36%–60% of all SNPs in the genome. The term “genome-wide” is thus not really applicable to our study. Our study has a rich characterization of the asthmatics collected over a follow-up period of 3–47 years. Because of the detailed characterization, which included among others hyper-responsiveness both at baseline and follow-up visits, a strict asthma remission definition could be used. This reduced the number of asthmatics with complete remission to 55 in the identification cohort and 7 and 14 in the replication cohorts, EGEA and SALPALLIA, respectively. This might theoretically lead to false-negative or spurious findings, but the fact that all 3 cohorts had the same direction and magnitude of effect of the identified SNPs in relation to complete asthma remission and that these genes had biologically plausible eQTLs may suggest that the study has identified promising new leads for further research on the mechanisms of asthma remission.

TABLE 3 Results of top 25 SNPs of GWAs on Complete asthma remission. Gene annotation was performed using SCAN (www.scandb.org)

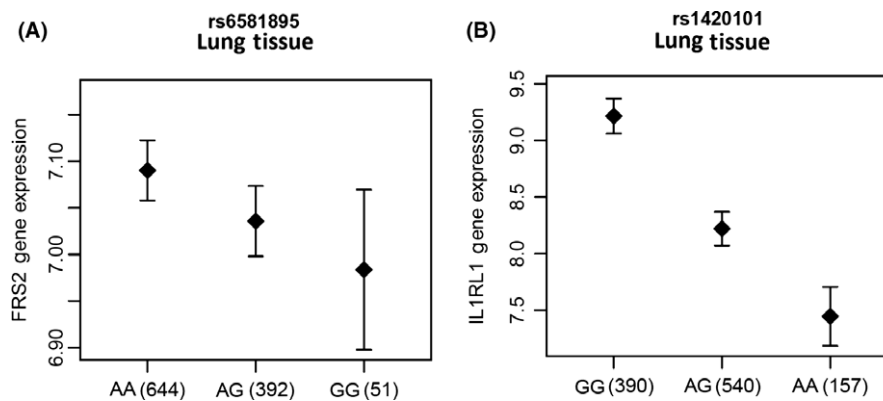
Identification cohort					EGEA		SAPALDIA		META-analysis		META-analysis		GENE-ANNOTATION		
					Cases: 7		Cases: 14		EGEA+SAPALDIA		PALDIA		SA-		
					Controls: 612	Controls: 270	Controls: 51	OR	1-sided P	OR	1-sided P	OR	2-sided P	Dir	Gene
SNP	CHR	TA	TEST	MAF	OR	2-sided P	OR	1-sided P	OR	1-sided P	OR	2-sided P	Dir	Gene	Left gene/Right gene
rs6581895	12	G	DOM	0.24	3.83	1.3×10^{-5}	3.74	6.0×10^{-2}	3.75	2.5×10^{-2}	3.75	6.0×10^{-3}	++	NA	YEATS4/FRS2
rs7552867	1	G	DOM	0.18	3.57	1.4×10^{-5}	1.05	4.8×10^{-1}	0.89	5.7×10^{-1}	0.94	5.5×10^{-1}	+-	NA	LOC642337/LOC100130867
rs9379986	6	C	DOM	0.38	0.31	5.9×10^{-5}	2.10	8.1×10^{-1}	0.82	3.8×10^{-1}	1.15	6.1×10^{-1}	+-	NA	LOC389365/OFCC1
rs1866714	11	G	DOM	0.33	4.74	7.7×10^{-5}	3.38	1.3×10^{-1}	1.85	2.0×10^{-1}	2.23	9.5×10^{-2}	++	TEAD1	PARVA/RASSF10
rs12405429	1	G	DOM	0.15	3.10	1.0×10^{-4}	2.51	1.2×10^{-1}	2.65	9.2×10^{-2}	2.58	3.8×10^{-2}	++	NA	FAM89A/TRIM67
rs7628387	3	C	DOM	0.14	2.99	1.7×10^{-4}	1.45	3.3×10^{-1}	NA	NA	1.45	3.3×10^{-1}	??	NA	LOC730168/TBL1XR1
rs10403164	19	A	ADD	0.49	0.44	1.7×10^{-4}	0.38	7.2×10^{-2}	0.85	3.7×10^{-1}	0.63	1.3×10^{-1}	--	HSPBP1	SAPS1/HSPBP1
rs299596	5	A	DOM	0.33	0.31	1.8×10^{-4}	1.64	7.2×10^{-1}	1.58	7.6×10^{-1}	1.60	8.2×10^{-1}	++	C1QTNF3	AMACR/LOC643373
rs919590	8	A	ADD	0.41	0.42	1.8×10^{-4}	0.85	3.9×10^{-1}	1.07	5.6×10^{-1}	0.97	4.7×10^{-1}	-+	DLC1	C8orf79/C8orf48
rs713702	22	G	DOM	0.26	3.12	2.0×10^{-4}	0.45	8.3×10^{-1}	1.23	3.7×10^{-1}	0.87	6.1×10^{-1}	-+	NA	LOC100129936/LOC100130624
rs3751031	11	G	DOM	0.07	3.33	2.2×10^{-4}	1.93	2.2×10^{-1}	1.34	3.7×10^{-1}	1.62	2.2×10^{-1}	++	CD44	PDHX/SLC1A2
rs6915644	6	A	ADD	0.44	2.16	2.2×10^{-4}	0.35	9.5×10^{-1}	0.49	9.3×10^{-1}	0.43	9.8×10^{-1}	--	NA	LOC100130393/LOC727977
rs1353203	6	A	DOM	0.37	4.29	2.2×10^{-4}	0.94	5.3×10^{-1}	2.32	1.2×10^{-1}	1.52	2.1×10^{-1}	-+	NA	HDDC2/LOC643623
rs1041868	21	A	DOM	0.16	2.88	2.5×10^{-4}	NA	NA	1.22	3.9×10^{-1}	1.22	3.9×10^{-1}	??	IFNAR1	IL10RB/IFNGR2
rs10497579	2	A	ADD	0.44	2.18	2.5×10^{-4}	0.57	8.2×10^{-1}	0.61	8.4×10^{-1}	0.60	9.1×10^{-1}	--	NA	LOC729026/LOC100127923
rs7137598	12	G	DOM	0.13	2.96	2.6×10^{-4}	1.06	4.8×10^{-1}	0.50	8.0×10^{-1}	0.72	7.1×10^{-1}	+-	TBX5	RBM19/LOC255480
rs12137498	1	A	DOM	0.11	2.94	2.8×10^{-4}	1.57	3.0×10^{-1}	0.75	6.5×10^{-1}	1.03	4.8×10^{-1}	+-	NA	LOC127406/PPAP2B
rs3094694	6	G	DOM	0.22	2.89	2.8×10^{-4}	1.07	4.7×10^{-1}	0.84	6.0×10^{-1}	0.92	5.6×10^{-1}	+-	NA	LOC646520/RANP1
rs565169	8	C	DOM	0.15	2.87	3.0×10^{-4}	0.42	7.9×10^{-1}	2.17	1.4×10^{-1}	1.32	3.2×10^{-1}	-+	NA	CLDN23/MFHAS1
rs6909889	6	A	DOM	0.19	2.85	3.3×10^{-4}	0.91	5.5×10^{-1}	2.11	1.4×10^{-1}	1.51	2.2×10^{-1}	-+	NA	TPBG/UBE2CBP
rs6243359	10	A	DOM	0.25	2.96	3.3×10^{-4}	0.94	5.3×10^{-1}	1.11	4.4×10^{-1}	1.04	4.7×10^{-1}	-+	ZNF33A	ZNF25/ZNF33A
rs1420101	2	A	ADD	0.41	0.44	3.4×10^{-4}	0.39	7.1×10^{-2}	0.60	1.3×10^{-1}	0.52	3.9×10^{-2}	--	IL1RL1	LOC100129822/IL18R1
rs2784609	9	C	DOM	0.11	2.91	3.4×10^{-4}	0.72	6.2×10^{-1}	1.96	1.7×10^{-1}	1.45	2.7×10^{-1}	-+	PTPRD	RN7SLP2/LOC646087
rs650985	1	G	DOM	0.07	3.25	3.6×10^{-4}	2.84	1.1×10^{-1}	1.05	4.9×10^{-1}	2.01	1.6×10^{-1}	++	GSTM4	LOC441896/GSTM2
rs11203826	8	A	DOM	0.21	2.82	3.8×10^{-4}	0.92	5.4×10^{-1}	1.34	3.4×10^{-1}	1.15	4.0×10^{-1}	-+	EFHA2	FGF20/LOC100130392

SNP, single nucleotide polymorphism; CHR, chromosome; TA, tested allele; TEST: DOM, dominant model; ADD, additive model; MAF, minor allele frequency; OR, Odds Ratio; Dir, direction of the effect; “+/-” indicates positive/negative association between the tested allele and remission. In bold; significantly replicated SNPs.

TABLE 4 Significant associations between SNPs and gene expression in lung tissue

SNP	CHR	TA	Cis/Trans	Number of tested probes	Probe	Gene	B	SE	P
SNP associated with ClinR									
rs7240102	18	G	Cis	27	100130406_TGI_at	POLI	0.041	.010	5.86×10^{-5}
					100313174_TGI_at	POLI	0.051	.012	2.23×10^{-5}
					100157415_TGI_at	POLI	0.055	.012	2.11×10^{-6}
SNP associated with ComR									
rs6581895	12	G	Cis	53	100133437_TGI_at	FRS2	−0.043	.013	8.88×10^{-4}
					100306086_TGI_at	FRS2	0.029	.009	8.77×10^{-4}
					100156179_TGI_at	CCT2	−0.029	.006	4.65×10^{-6}
rs1420101	2	A	Cis	42	100302151_TGI_at	IL1RL1	−0.061	.011	1.10×10^{-7}
					100302783_TGI_at	IL1RL1	−0.860	.049	5.10×10^{-69}
					100148210_TGI_at	IL1RL1	−0.741	.043	2.82×10^{-65}
					100148162_TGI_at	IL1RL1	−0.578	.035	9.61×10^{-63}
					100312840_TGI_at	IL1RL1	−0.548	.033	1.07×10^{-63}
					100136977_TGI_at	IL18R1	−0.221	.022	9.88×10^{-23}
					100125222_TGI_at	IL13	0.100	.020	3.69×10^{-7}
				Trans	49 236				

SNP, single nucleotide polymorphism; CHR, chromosome; TA, tested allele; Cis: association with gene-expression of a gene within a 1 Mb distance of the SNP; Trans: association with gene-expression of a gene anywhere in the genome; B, Beta; SE, standard error; P, P-value.

**FIGURE 3** Lung eQTL associated with complete asthma remission. A, Association between SNP rs6581895 and gene expression of *FRS2* (probe set: 100133437_TGI_at), (B) association between SNP rs1420101 and gene expression of *IL1RL1* (probe set: 100302783_TGI_at)³²

Of interest, none of the replicated SNPs was an eQTL in epithelial cells, while these cells are thought to be fundamental to asthma development.³⁶ This suggests that either larger groups of patients should be studied to ensure that epithelial cells do not contribute to asthma remission, or that different inflammatory or resident cells in the lung may be important for asthma remission compared to those for asthma inception. The prevalence of asthma remission in the SAPALDIA study is higher than in the other 2 cohorts. This may be the result of the fact that the SAPALDIA study is a general population-based cohort while the other 2 cohorts are hospital-based studies that generally attract patients with more severe asthma.

In conclusion, we found suggestive evidence that a set of genes may contribute to asthma remission in a subset of asthmatics, using a strict definition of complete asthma remission. The study points to the value of deep phenotyping for the identification of novel drug targets through genetic epidemiology complemented with functional

characterization of associated SNPs. We invite the scientific community to contribute to further characterization of asthmatics in a similar way to increase the numbers of asthmatics with complete remission to be studied with a genome-wide screen, thus allowing gene-gene interaction and epigenetic assessments as well, or even better to do so with whole genome sequencing as this may additionally elucidate rare variants and pinpoint genes that associate with such a rare, but extremely important phenotype of asthma: full remission!

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CONFLICT OF INTEREST

Dr. Siroux reports personal fees from TEVA, AstraZeneca, and Novartis, outside the submitted work. Dr. Sin reports grants and personal fees from AstraZeneca, Sanofi-Aventis, Regeneron, Merck, Boehringer Ingelheim, and Novartis, outside the submitted work. Dr. van den Berge reports research grants paid to University from GlaxoSmithKline, TEVA, Chiesi, and AstraZeneca, outside the submitted work. Dr. Koppelman reports research grants paid to the University from TEVA and the TETRI foundation, outside the submitted work. Dr. Postma reports grants and fees for consultancies paid to the University from AstraZeneca, Chiesi, Genentec, Boehringer Ingelheim, Chiesi, GSK, Takeda, TEVA and Roche, outside the submitted work. Dr. Vonk, Dr. Nieuwenhuis, Dr. Dijk, Dr. Boudier, Dr. Bouzigon, Dr. Probst-Hensch, Dr. Imboden, Dr. Keidel, Dr. Bossé, Dr. Hao and Dr. Faiz report no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Online Supplementary Material

Novel genes and insights in complete asthma remission

A Genome Wide Association Study on Clinical and Complete Asthma Remission

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Supplemental Methods

Identification and replication cohorts

	Identification cohort	EGEA	SAPALDIA
Number of participants	790	351	105
Study design cohort	Case-control	Longitudinal case-control and family study	Population-based cohort study, nested Case-control
Ethnicity	Caucasian	Caucasian	Caucasian
Population included in analysis	Asthmatics + BHR	Asthmatics + BHR	Asthmatics + BHR
Definition of current asthma	Asthma doctor diagnosis + BHR	Asthma doctor diagnosis + BHR	Asthma doctor diagnosis + BHR
Covariates used	Gender	Gender	Gender
CHIP	Illumina 370K DUO Chip; Illumina 317K Chip; Omni Express Exome	Illumina Human 610quad BeadChip	Illumina Human 610quad BeadChip

BHR: Bronchial Hyperresponsiveness

Genotyping

DNA was genotyped with the Illumina 317 Chip (n=363), the Illumina 370 Duo Chip (n= 332), or the Omni Express Exome chip (Illumina Inc, San Diego, CA) (n= 95). Quality control was applied to each platform separately; subjects were removed from the analysis if they were not of Caucasian descent, had a low genotyping call rate (<95%) or had discrepant or ambiguous genetic sex data. Single Nucleotide Polymorphisms (SNPs) were removed if the call rates were low (<95%), not in Hardy-Weinberg Equilibrium ($p < 1 \times 10^{-4}$), or if the minor allele frequency was <0.05. After this platform-specific quality control overlapping SNPs were merged. A second quality control was applied to this merged dataset: SNPs not in Hardy Weinberg Equilibrium ($p < 1 \times 10^{-4}$) and SNPs with a low genotyping call rate were (<95%) were removed. Finally, 156,954 SNPs remained for analysis. A principal component analysis on this final dataset did not show any remaining clustering (figure E4). With the help of Dr. V. Guryev (European Institute of the Biology of Aging, University Medical Center Groningen) we calculated the percentage of tagged SNPs in the GoNL database (www.nlgenome.nl). Of the 156,954 SNPs we analyzed, 721 were not present in GoNL. Depending on the R^2 used, the remaining 156,233 SNPs tagged between 36% and 60% of the 5,551,734 SNPs in Go NL with a MAF > 5% (see table E1).

Table E1. Percentage of tagged GoNL SNPs with the 156,233 SNPs used in our analysis

R ²	# tagged SNPs	% tagged SNPs
0.5	3,348,389	60.31
0.6	2,897,685	52.19
0.7	2,046,001	43.82
0.8	2,006,022	36.13

Statistical analysis in the identification cohort

~~Given the relatively small number of subjects with ComR (n=55) and ClinR (n=178) not all SNPs were tested in an additive genetic model. For ComR, SNPs with a MAF below 0.40 were tested in a dominant model and SNPs with a MAF ≥ 0.40 were tested in an additive model. For ClinR, SNPs with a MAF below 0.25 were tested in a dominant model and SNPs with a MAF ≥ 0.25 were tested in an additive model. With these cut-off values the minimal expected number of subjects in remission having the homozygote mutant genotype in the additive models was approximately 10.~~

Gene expression analysis in lung tissue

Lung tissue was obtained from patients that underwent lung resection surgery at three participating sites; University of Groningen (GRN), Laval University (Laval) and University of British Columbia (UBC)¹. The final dataset included 1,087 subjects. A cis-eQTL was defined as a SNP that was significantly associated with expression levels of a probe set (gene) within 1 Mb distance of that SNP. Significant p-values were based on a Bonferroni-corrected threshold ($p = 0.05/\text{number of probe sets within the defined window}$). Trans-eQTLs were SNPs associated with expression levels of a probe set anywhere in the entire genome and the Bonferroni-corrected p-value was $0.05/49236 = 1 \times 10^{-6}$. In each cohort separately, linear regression analysis (using software package R) was performed to test for association between the SNPs and 2-log transformed gene expression levels using an additive genetic model with adjustment for disease status (alpha-1 antitrypsin deficiency, idiopathic pulmonary fibrosis, pulmonary hypertension, cystic fibrosis, COPD, other disease), age, gender, smoking status and the cohort specific number of principal components. SNP effect estimates of the three cohorts were meta-analysed using fixed effects models with effect estimates weighted by the reciprocal of the estimated variance.

1 *Gene expression analysis in lung epithelial cells*

2 DNA samples were genotyped on the Illumina OmniExpress Exome genotyping arrays.
3 Gene expression profiles were obtained from bronchial brushing of controls (n=85) without a
4 lung disease, FEV₁%predicted >90% and FEV₁/FVC >70% and absence of BHR, using a
5 Human genome ST v1.0 array. Linear regression analysis was used to test for association
6 between the SNPs and 2-log transformed gene expression levels. SNPs were tested in a
7 dominant genetic model adjusted for age, sex, and smoking status

1 Table E2. Association between SNPs and gene expression in lung tissue.

SNPs associated with ComR						
SNP	Tested Allele	Gene name	Probe	eqtl meta estimate (B)	eqtl meta standard error (SE)	eqtl meta p-value
rs6581895	G		100127694_TGI_at	0.01	0.01	0.39
		<i>MDM2</i>	100135208_TGI_at	0.01	0.02	0.45
		<i>RAB3IP</i>	100309946_TGI_at	0.01	0.01	0.52
			100123262_TGI_at	0.01	0.03	0.85
		<i>RAB3IP</i>	100160044_TGI_at	0.05	0.01	1.00E-03
		<i>CPM</i>	100132227_TGI_at	0.00	0.01	0.85
		<i>MDM2</i>	100302926_TGI_at	0.01	0.02	0.65
		<i>NUP107</i>	100301885_TGI_at	0.00	0.01	0.61
		<i>FRS2</i>	100123359_TGI_at	-0.03	0.01	0.05
		<i>CNOT2</i>	100140473_TGI_at	0.00	0.00	0.95
		<i>CPSF6</i>	100135750_TGI_at	-0.02	0.01	0.09
		<i>CNOT2</i>	100306942_TGI_at	0.00	0.01	0.50
			100129073_TGI_at	-0.01	0.00	0.11
		<i>SLC35E3</i>	100126417_TGI_at	0.00	0.02	1.00
		<i>CNOT2</i>	100311239_TGI_at	0.01	0.02	0.71
		<i>LRRC10</i>	100132996_TGI_at	0.01	0.01	0.55
		<i>LYZ</i>	100303765_TGI_at	0.06	0.05	0.23
		<i>NUP107</i>	100148420_TGI_at	-0.01	0.01	0.36
		<i>CPSF6</i>	100307209_TGI_at	0.00	0.01	0.86
		<i>CNOT2</i>	100313600_TGI_at	0.00	0.01	0.73
		<i>CPSF6</i>	100314036_TGI_at	0.00	0.01	0.69
		<i>FRS2</i>	100306086_TGI_at	0.03	0.01	8.77E-04
			100312478_TGI_at	0.01	0.01	0.28
			100312009_TGI_at	0.00	0.02	0.98
		<i>BEST3</i>	100141637_TGI_at	-0.01	0.02	0.72
		<i>CCT2</i>	100148427_TGI_at	-0.02	0.01	0.14
		<i>RAB3IP</i>	100304839_TGI_at	0.01	0.01	0.61
		<i>CPM</i>	100307202_TGI_at	0.01	0.01	0.63
		<i>KCNMB4</i>	100302305_TGI_at	0.01	0.02	0.78
		<i>RAB3IP</i>	100125891_TGI_at	-0.02	0.02	0.30
		<i>C12orf28</i>	100122524_TGI_at	-0.08	0.03	0.01
		<i>CPSF6</i>	100129713_TGI_at	0.00	0.01	0.84
			100157144_TGI_at	-0.01	0.01	0.60
			100310737_TGI_at	0.02	0.01	0.20
			100149036_TGI_at	0.00	0.02	0.93
			100154265_TGI_at	0.01	0.01	0.24
		<i>SLC35E3</i>	100301951_TGI_at	-0.01	0.01	0.38
			100308513_TGI_at	0.01	0.01	0.34
		<i>hCG_1757335</i>	100307048_TGI_at	0.00	0.01	0.67
		<i>CPSF6</i>	100303866_TGI_at	0.06	0.02	3.13E-03

		<i>KCNMB4</i>	100139316_TGI_at	0.02	0.02	0.38
			100149542_TGI_at	0.02	0.02	0.45
			100124872_TGI_at	-0.01	0.02	0.67
			100124952_TGI_at	0.00	0.01	0.79
		<i>FRS2</i>	100133437_TGI_at	-0.04	0.01	8.88E-04
		<i>YEATS4</i>	100125980_TGI_at	-0.01	0.01	0.50
		<i>CCT2</i>	100156179_TGI_at	-0.03	0.01	4.65E-06
		<i>BEST3</i>	100304246_TGI_at	0.01	0.01	0.34
		<i>CPSF6</i>	100127368_TGI_at	0.05	0.02	2.78E-03
		<i>CNOT2</i>	100161969_TGI_at	-0.01	0.01	0.17
		<i>CPM</i>	100160696_TGI_at	0.00	0.02	0.96
			100156613_TGI_at	-0.01	0.02	0.74
			100134709_TGI_at	-0.01	0.01	0.47
rs12405429	G	<i>GNPAT</i>	100142725_TGI_at	0.00	0.01	0.64
		<i>AGT</i>	100143203_TGI_at	0.07	0.04	0.10
		<i>C1orf131</i>	100307787_TGI_at	0.00	0.01	0.85
		<i>TTC13</i>	100151838_TGI_at	0.02	0.01	0.04
		<i>COG2</i>	100311024_TGI_at	-0.01	0.02	0.42
		<i>ARV1</i>	100151323_TGI_at	0.01	0.01	0.45
		<i>GALNT2</i>	100157180_TGI_at	0.05	0.02	2.84E-03
			100136530_TGI_at	-0.01	0.02	0.66
			100303421_TGI_at	0.00	0.01	0.83
		<i>FAM89A</i>	100129264_TGI_at	0.01	0.02	0.73
		<i>CAPN9</i>	100161212_TGI_at	0.01	0.02	0.70
			100125499_TGI_at	0.14	0.05	3.39E-03
			100304106_TGI_at	0.01	0.02	0.48
		<i>EGLN1</i>	100313058_TGI_at	-0.03	0.01	0.02
		<i>TSNAX</i>	100141032_TGI_at	-0.02	0.02	0.24
		<i>COG2</i>	100300634_TGI_at	-0.02	0.01	0.04
		<i>AGT</i>	100155968_TGI_at	0.00	0.01	0.96
			100306993_TGI_at	0.05	0.04	0.16
			100146945_TGI_at	0.01	0.03	0.73
		<i>C1orf198</i>	100158854_TGI_at	0.00	0.02	0.78
			100156131_TGI_at	0.01	0.01	0.48
			100158580_TGI_at	0.00	0.01	0.51
		<i>AGT</i>	100145225_TGI_at	-0.02	0.01	0.19
			100311475_TGI_at	0.07	0.04	0.10
			100162356_TGI_at	0.02	0.01	0.27
		<i>TRIM67</i>	100131467_TGI_at	0.00	0.01	0.95
		<i>TSNAX</i>	100142722_TGI_at	0.00	0.01	0.67
		<i>DISC1</i>	100155369_TGI_at	-0.01	0.02	0.57
		<i>C1orf124</i>	100155018_TGI_at	-0.02	0.01	0.19
		<i>GALNT2</i>	100148868_TGI_at	0.00	0.01	0.87
		<i>EXOC8</i>	100134565_TGI_at	0.00	0.01	0.92
			100129621_TGI_at	0.01	0.02	0.63

		<i>TRIM67</i>	100156177_TGI_at	0.03	0.01	0.07
		<i>CAPN9</i>	100302502_TGI_at	0.13	0.04	1.73E-03
		<i>PGBD5</i>	100161563_TGI_at	0.00	0.03	0.98
		<i>GALNT2</i>	100155852_TGI_at	-0.01	0.01	0.41
		<i>C1orf131</i>	100146570_TGI_at	0.00	0.01	0.74
		<i>C1orf124</i>	100304229_TGI_at	0.00	0.01	0.76
			100150162_TGI_at	0.01	0.01	0.68
		<i>DISC1</i>	100313095_TGI_at	0.03	0.02	0.07
		<i>EGLN1</i>	100313721_TGI_at	0.02	0.01	0.08
		<i>ARV1</i>	100313022_TGI_at	0.02	0.01	0.01
			100121634_TGI_at	0.00	0.02	0.81
		<i>FAM89A</i>	100137456_TGI_at	0.02	0.02	0.34
			100137441_TGI_at	0.00	0.02	0.75
			100305210_TGI_at	0.04	0.02	0.16
			100155235_TGI_at	0.02	0.02	0.41
		<i>EGLN1</i>	100121808_TGI_at	-0.01	0.01	0.37
rs1420101	T		100126851_TGI_at	0.00	0.01	0.64
			100300514_TGI_at	-0.01	0.01	0.12
		<i>SLC9A2</i>	100129736_TGI_at	0.07	0.02	4.17E-03
		<i>SLC9A2</i>	100302857_TGI_at	-0.01	0.01	0.39
			100151303_TGI_at	0.00	0.01	0.71
			100135008_TGI_at	-0.01	0.02	0.67
			100139696_TGI_at	-0.01	0.01	0.13
		<i>MAP4K4</i>	100122422_TGI_at	-0.01	0.01	0.15
		<i>IL1R2</i>	100156614_TGI_at	0.06	0.03	0.04
		<i>MAP4K4</i>	100140079_TGI_at	0.01	0.01	0.48
		<i>IL1RL1</i>	100148210_TGI_at	-0.74	0.04	2.82E-65
		<i>IL18R1</i>	100136977_TGI_at	-0.22	0.02	9.88E-23
			100131830_TGI_at	0.00	0.01	0.68
		<i>IL1RL1</i>	100148162_TGI_at	-0.58	0.03	9.61E-63
			100123814_TGI_at	0.02	0.01	0.13
			100131639_TGI_at	-0.03	0.02	0.16
		<i>IL1R1</i>	100126810_TGI_at	-0.01	0.01	0.18
		<i>TMEM182</i>	100301278_TGI_at	0.00	0.01	0.97
		<i>MFSD9</i>	100149741_TGI_at	0.00	0.01	0.99
			100129894_TGI_at	0.01	0.02	0.63
		<i>TMEM182</i>	100142780_TGI_at	-0.01	0.02	0.33
		<i>CREG2</i>	100135213_TGI_at	0.00	0.02	0.98
		<i>TMEM182</i>	100310871_TGI_at	0.00	0.01	0.90
			100149533_TGI_at	0.01	0.01	0.26
			100154541_TGI_at	0.02	0.01	0.11
		<i>IL1RL1</i>	100312840_TGI_at	-0.55	0.03	1.07E-63
			100133110_TGI_at	-0.02	0.02	0.28
		<i>MFSD9</i>	100151768_TGI_at	-0.01	0.01	0.36
		<i>IL1RL1</i>	100302783_TGI_at	-0.86	0.05	5.10E-69

		<i>SLC9A4</i>	100303432_TGI_at	0.00	0.01	0.70
		<i>MAP4K4</i>	100124060_TGI_at	-0.01	0.01	0.47
		<i>IL1RL2</i>	100157422_TGI_at	-0.01	0.01	0.31
		<i>CREG2</i>	100148191_TGI_at	-0.02	0.01	4.84E-03
		<i>MAP4K4</i>	100146402_TGI_at	0.00	0.01	0.88
		<i>IL1RL1</i>	100302151_TGI_at	-0.06	0.01	1.10E-07
		<i>MAP4K4</i>	100304599_TGI_at	0.00	0.01	0.77
		<i>MAP4K4</i>	100125831_TGI_at	-0.01	0.01	0.11
		<i>UNQ9419</i>	100136468_TGI_at	0.00	0.02	0.83
		<i>MAP4K4</i>	100307128_TGI_at	0.00	0.01	0.66
		<i>IL1R1</i>	100313713_TGI_at	-0.01	0.01	0.29
		<i>IL18RAP</i>	100141328_TGI_at	0.02	0.02	0.26
		<i>MFSD9</i>	100307278_TGI_at	-0.01	0.01	0.24

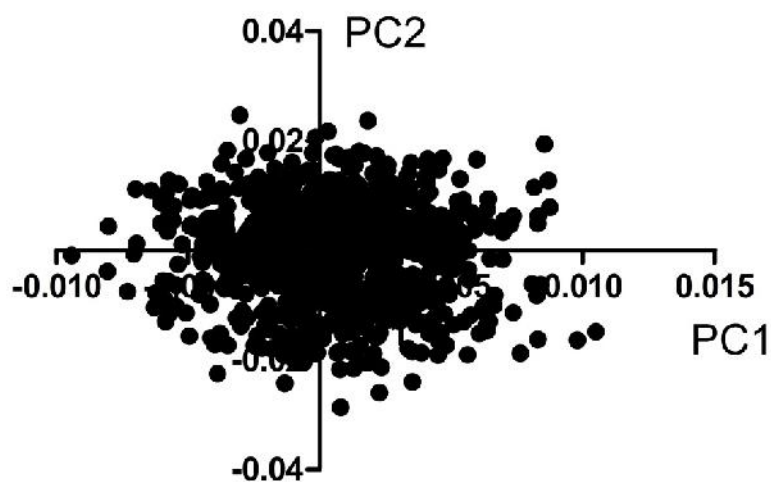
SNP associated with ClinR

SNP	Tested Allele	Gene name	Probe set	eqtl meta estimate (B)	eqtl meta standard error (SE)	eqtl meta p-value
rs7240102	G	<i>TCF4</i>	100125935_TGI_at	0.03	0.01	0.02
			100144255_TGI_at	-0.04	0.02	0.04
		<i>C18orf26</i>	100132124_TGI_at	0.01	0.01	0.32
		<i>TCF4</i>	100307627_TGI_at	-0.01	0.01	0.45
		<i>CCDC68</i>	100301640_TGI_at	0.03	0.01	0.06
			100132867_TGI_at	-0.02	0.02	0.22
			100127435_TGI_at	-0.02	0.02	0.44
		<i>TCF4</i>	100149310_TGI_at	-0.01	0.01	0.05
			100161539_TGI_at	-0.01	0.01	0.27
		<i>RAB27B</i>	100146805_TGI_at	-0.01	0.02	0.65
		<i>TCF4</i>	100161929_TGI_at	0.00	0.01	0.51
		<i>C18orf54</i>	100301048_TGI_at	0.03	0.02	0.11
		<i>TCF4</i>	100151954_TGI_at	-0.01	0.01	0.42
		<i>MBD2</i>	100302777_TGI_at	0.01	0.01	0.40
		<i>MBD2</i>	100132706_TGI_at	0.01	0.01	0.44
		<i>STARD6</i>	100147408_TGI_at	-0.01	0.01	0.21
		<i>TCF4</i>	100140856_TGI_at	-0.01	0.02	0.34
		<i>POLI</i>	100130406_TGI_at	0.04	0.01	5.86E-05
			100138730_TGI_at	0.00	0.01	0.70
		<i>C18orf54</i>	100151943_TGI_at	0.03	0.02	0.26
		<i>MBD2</i>	100160551_TGI_at	0.00	0.01	0.75
			100306550_TGI_at	-0.01	0.01	0.39
		<i>TCF4</i>	100310719_TGI_at	0.00	0.01	0.86
		<i>C18orf26</i>	100301041_TGI_at	0.02	0.01	0.20
		<i>CCDC68</i>	100139699_TGI_at	0.04	0.01	4.11E-03
		<i>POLI</i>	100313174_TGI_at	0.05	0.01	2.23E-05
		<i>POLI</i>	100157415_TGI_at	0.06	0.01	2.11E-06

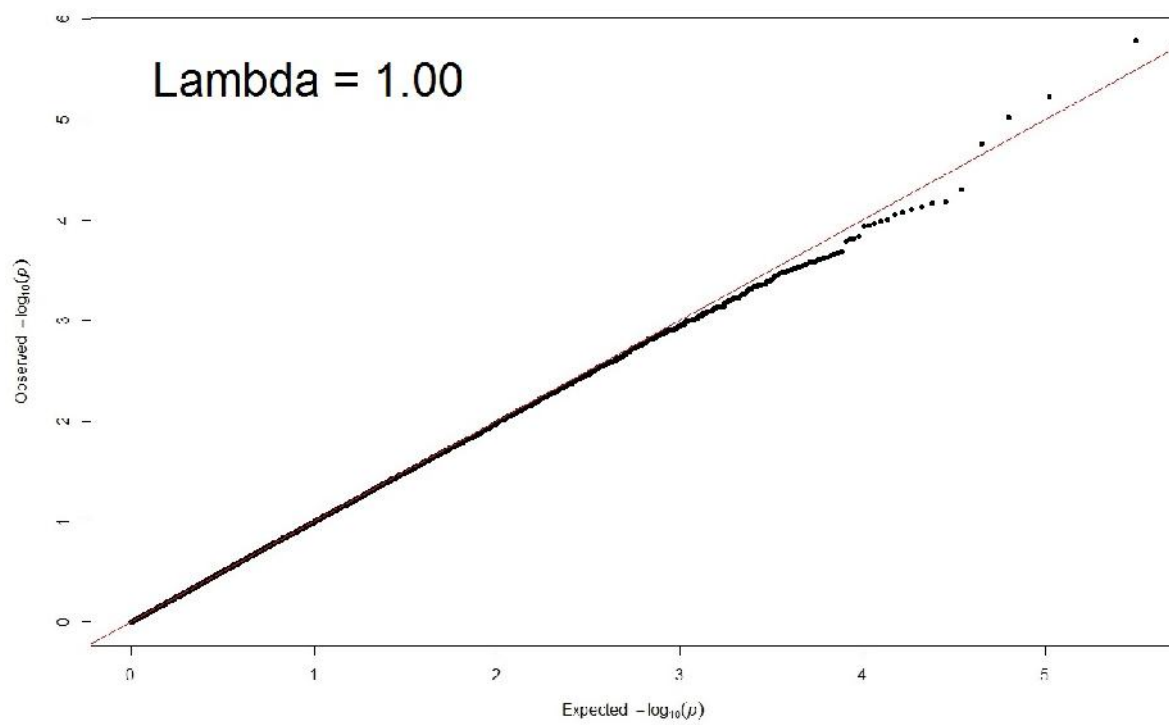
Table E3. Association between SNPs and gene expression in lung epithelial cells.

SNP	gene	Tested allele	B	SE	P
rs7240102	<i>POLI</i>	G	0.06	0.09	0.52
rs6581895	<i>FRS2</i>	G	0.06	0.08	0.46
	<i>CCT2</i>	G	0.01	0.07	0.83
rs1420101	<i>IL1RL1</i>	A	0.07	0.08	0.41
	<i>IL18R1</i>	A	-0.03	0.06	0.69
	<i>IL13</i>	A	0.10	0.07	0.17

Figure E4: Principal component analysis of the final genotype dataset



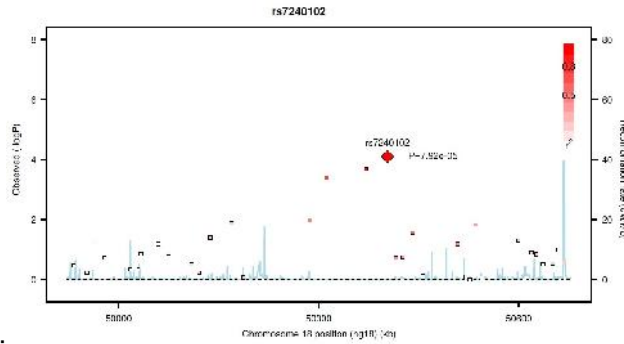
1 Figure E5: Q-Q plot of the clinical remission analysis in the identification cohort



2

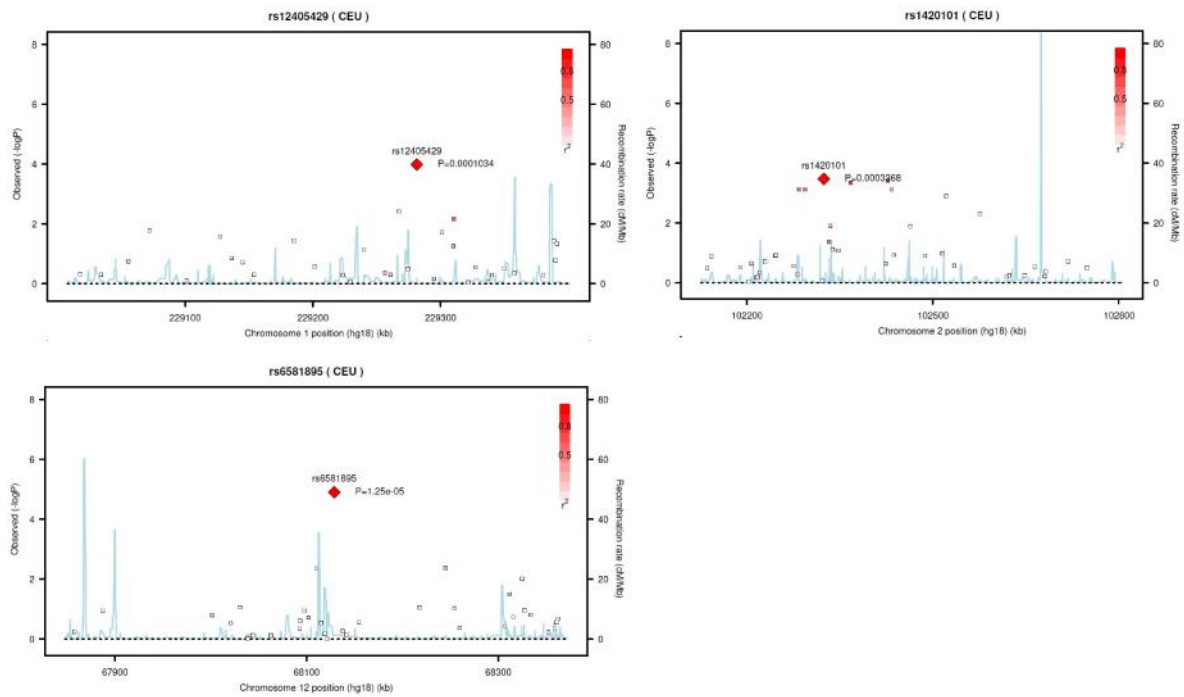
3

- 1 Figure E6: Regional association plots of replicated regions in A: Clinical asthma remission
- 2 and B. Complete asthma remission. Horizontal axis; genomic basepair position, Left vertical
- 3 axis; p-value, Right vertical axis recombination rate. Red color is strong linkage
- 4 disequilibrium between replicated SNP and regional SNP



5 A:

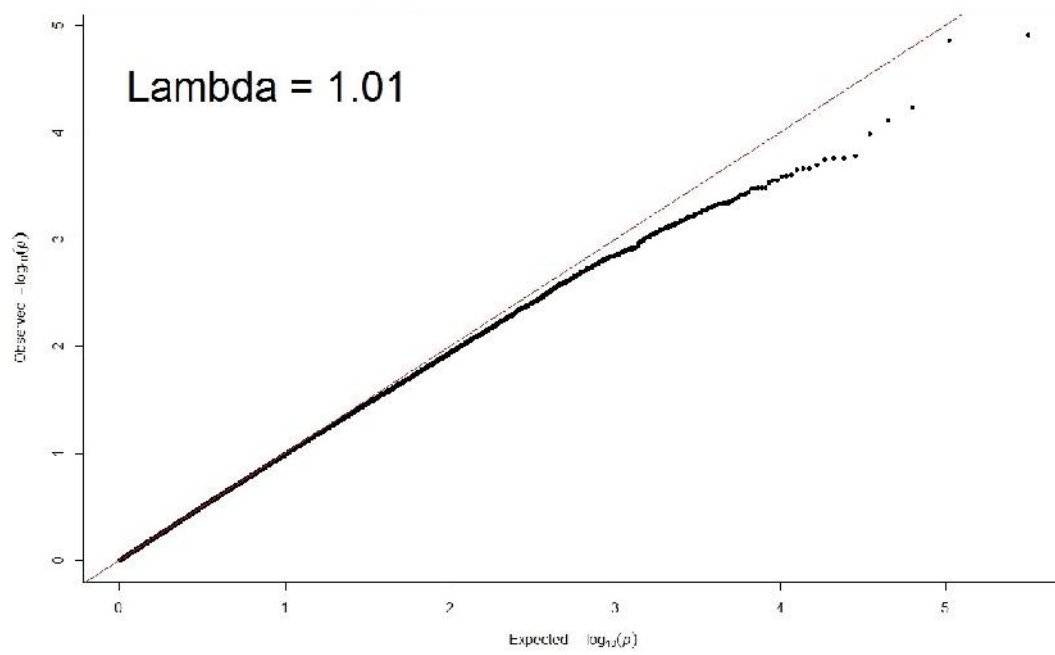
6 B:



7

1 Figure E7: Q-Q plot of the complete remission analysis in the identification cohort

2



3

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