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Supplemental Material

Genes Interacting with Occupational Exposures to Low Molecular Weight Agents and Irritants on Adult-Onset Asthma in Three European Studies

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Excel File Table S5. Chemical retrieved from the Comparative Toxicogenomics Database (CTD, URL: http://ctdbase.org/, Davis et al. 2014) and interacting with the five genes (PLA2G4A, PLA2R1, PRKCA, PRKD1 and RELA). In this table are indicated the LMW agents/irritants/cleaning products or disinfectants evaluated by job specific questionnaires (see Table E1) or exposures known to contain compounds with irritant properties (air pollutants and vehicle emissions).

Description of the three multicentre epidemiological European studies

The EGEA study

The EGEA study is a 12-year longitudinal survey, which combines a case-control study and a family study of asthmatic cases (https://egeanet.vjf.inserm.fr/index.php/en/). The protocol and descriptive characteristics have been described elsewhere (Kauffmann et al. 1997, 1999). Probands (388 asthmatic patients) were recruited from six chest clinics in five French cities between 1991 and 1995 and their 1,244 family members were included, either by including the proband's parents and siblings, or by including the proband's spouse and children. In addition, 415 population-based controls were recruited. The probands (asthmatics and controls) were between 7 and 70 years old at time of ascertainment. All probands and their two parents were of European ancestry and were born in France. A follow-up (EGEA2) of the initial cohort was conducted between 2003 and 2007 and included follow-up data for 1,543 participants from the initial cohort and 73 new family members. All participants responded to a questionnaire based on international standardized tools to diagnose asthma and to determine respiratory and allergic symptoms, treatments, and environmental and occupational exposures (Kauffmann et al. 1997). As a follow-up study of EGEA2, the third survey (EGEA3) was conducted in 2011 using self-completed questionnaire and 1558 questionnaires were returned (Bouzigon et al. 2015).

The present analysis uses data measured at the second stage of the study (EGEA2) in 689 adult participants with complete data with data available on genome-wide genotyping, occupational history regarding LMW agents and irritants, especially cleaning/disinfecting products, and adult-onset asthma (Figure S1). Ethical approval was obtained from the relevant institutional review board committees (Cochin Port-Royal Hospital and Necker- Enfants Malades Hospital, Paris). Written informed consent was signed by all participants.

The ECRHS study

ECRHS is a random population-based multicenter cohort study of participants aged 20–44 years at the time of recruitment (1991-93; ECRHS I). The follow-up (ECRHS II) took place approximately 9 years later (median length of follow-up, 8.9 years), during the period 2000-2002. Both surveys included an initial screening questionnaire, an extensive interviewer-led questionnaire, spirometry and a methacholine challenge test. For further details see (Burney et al. 1994; “The European Community Respiratory Health Survey II” 2002).

The SAPALDIA study

The SAPALDIA prospective population-based cohort study (Ackermann-Liebrich et al. 2005; Downs et al. 2007) recruited a random sample of inhabitants aged 18–60 years from eight areas of Switzerland representing various degrees of urbanization and air pollution exposure. Participants underwent spirometry as well as a detailed interview on respiratory health, smoking history, lifestyle factors and anthropometry at both, baseline (1991) and follow-up examination (2002). From among 6,055 SAPALDIA cohort participants participating in both examinations and agreeing to provide blood for genetic analysis, all self-reported asthma cases as well as a random sample of controls had genome wide genotyping in the framework of the European funded “GABRIEL” asthma project (Moffat et al. 2010). For the purposes of the current gene-environment interaction analysis, the case definition was restricted to cases of adult asthma (age of onset larger than 16 years) with current treatment or symptoms within the last 12 months prior to assessment. For the definition of occupational risk exposures, job history during the period between SAPALDIA-1 and SAPALDIA-2, and/or reported at the time of SAPALDIA-1 was considered. The genome wide-data set formed the basis for the candidate-pathway based genetic analysis. Overall, n=107 asthma cases and n=467 controls were included in the current pooled analysis.

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Table S1. Assessment of occupational exposure and definition of exposed and non-exposed groups

Exposed	**Exposures to asthmagenic agents (ever) evaluated by the asthma-specific JEM:** **LMW agents**: Highly reactive chemicals, cleaning/disinfecting products, metals antigenic, wood dust, isocyanates **Mixed environment with potential exposure to LMW agents**: textile production, agriculture, metal working fluids exposures, pharmaceutical drugs exposures, bioaerosol exposures **Irritants**: accidental peak exposure	**OR**	**Exposures to cleaning products and disinfectants (ever) evaluated by job specific questionnaires** (self-reported weekly exposure): **Cleaners**: bleach, ammonia, decalcifiers, solvents, formaldehyde, alcohol, quaternary ammonium compounds, and disinfection tasks **Healthcare workers**: bleach, ammonia, decalcifiers, solvents, formaldehyde, glutaraldehyde, alcohol, quaternary ammonium compounds, chloramine T, ethylene oxide, and disinfection tasks
Excluded*	**Exposures evaluated by the asthma JEM:** **HMW agents**, inducing asthma through typical allergic (IgE-mediated) response Jobs with **lower level of irritant** exposures	**OR**	**Exposures evaluated by the ALOHA JEM:** General dust, gases and fumes
Unexposed group (reference)	**Non exposed to any agent** potentially a risk for asthma or respiratory health		

JEM – Job Exposure Matrix; HMW: High Molecular Weight; LMW – Low Molecular Weight

* In order to increase specificity of both exposed and unexposed groups

Table S2. Description of SNPs interacting at the P<0.005 level with occupational exposure to LMW agents or irritants on current adult-onset asthma (463 cases/2136 controls)

Chr	**Gene**	**Region**	**SNP**	**Position** [a]	**Ref./Effect allele**	**EAF** [b]	**Marginal effect OR/P-value**		**Interaction - CC OR/P-value**		**Test for heterogeneity**		
											I_2[c]	**chi-2**	**P-value**
1	*PRDX6*	/	rs10912626	171739312	A/G	0.21	1.06	0.5415	0.55	0.0014	71.5	7.67	0.02
1	*PRDX6*	/	rs6687872	171740885	A/G	0.79	0.95	0.5532	1.82	0.0014	71.5	7.66	0.02
1	*PLA2G4A*	Intronic	rs932476	185200178	A/G	0.35	1.25	**0.0036**	0.64	0.0050	**0.0**	**1.45**	**0.48**
2	*PLA2R1*	2.4 kb in 5'	rs2667026	160629801	A/G	0.83	0.89	0.2354	1.77	0.0050	**0.0**	**2.27**	**0.32**
8	*CLU*	/	rs569214	27543709	G/T	0.39	0.97	0.7048	0.58	0.0006	61.2	4.94	0.08
10	*CDK1*	3' UTR	rs10711	62224480	G/T	0.69	1.08	0.3405	1.63	0.0041	54.0	4.40	0.11
11	*RELA*	/	rs931127	65161876	A/G	0.43	1.17	**0.0350**	1.61	0.0014	**0.0**	**0.22**	**0.89**
11	*RELA*	15kb in 5'	rs7949980	65202526	C/T	0.51	1.07	0.3421	1.56	0.0030	**0.0**	**0.01**	**0.99**
14	*PRKD1*	Intronic	rs7157367	29375612	A/G	0.33	0.92	0.2926	1.59	0.0033	27.3	2.67	0.26

14	*PRKD1*	Intronic	rs1958980	29381842	A/G	0.67	1.08	0.3344	0.64	0.0042	**20.9**	**2.40**	**0.30**
14	*PRKD1*	Intronic	rs11847351	29383829	A/G	0.67	1.08	0.3429	0.64	0.0043	**21.9**	**2.43**	**0.30**
14	*PRKD1*	Intronic	rs1958987	29409562	C/T	0.68	1.07	0.3609	0.64	0.0054	**0.0**	**0.17**	**0.92**
17	*PRKCA*	Intronic	rs6504453	62166692	C/T	0.35	1.04	0.6086	0.63	0.0032	**0.0**	**1.00**	**0.61**
20	*PLCB1*	Intronic	rs227142	8367397	C/T	0.11	1.08	0.4852	1.82	0.0050	**74.7**	**8.74**	**0.01**

[a]Position in base pairs (bp), hg18. [b] Effect Allele Frequency (EAF) calculated in controls. [c] Proportion of between-study variability; **0%-24%**: little heterogeneity, 25%-49%: moderate heterogeneity, 50%-74%: large heterogeneity, and >75%: very large heterogeneity.

Chr: chromosome, CC: case-control.

Table S3: Interactions between SNPs and occupational exposure to LMW agents or irritants on current adult-onset asthma stratified by study.

				EGEA (122 cases/567 controls)			**SAPALDIA** (107 cases/467 controls)			**ECRHS** (234 cases/1102 controls)		
Chr	**Gene**	**SNP**	**Reference / Effect allele**	**EAF**[a]	**OR ± SE**	**P-value**	**EAF**[a]	**OR ± SE**	**P-value**	**EAF**[a]	**OR ± SE**	**P-value**
1	*PLA2G4A*	rs932476	A/G	0.34	0.72 ± 0.31	0.29	0.35	0.44 ± 0.37	0.02	0.34	0.71 ± 0.23	0.13
2	*PLA2R1*	rs2667026	A/G	0.82	2.76 ± 0.41	0.01	0.82	1.11 ± 0.47	0.82	0.84	1.58 ± 0.28	0.10
11	*RELA*	rs931127	A/G	0.43	1.86 ± 0.29	0.03	0.42	1.70 ± 0.33	0.11	0.44	1.57 ± 0.21	0.03
11	*RELA*	rs7949980	C/T	0.51	1.65 ± 0.28	0.08	0.50	1.63 ± 0.34	0.15	0.51	1.59 ± 0.21	0.03
14	*PRKD1*	rs1958980	A/G	0.67	0.55 ± 0.31	0.05	0.64	0.41 ± 0.36	0.01	0.68	0.77 ± 0.22	0.23
14	*PRKD1*	rs11847351	A/G	0.67	0.55 ± 0.31	0.05	0.64	0.41 ± 0.36	0.01	0.68	0.77 ± 0.22	0.24
14	*PRKD1*	rs1958987	C/T	0.67	0.66 ± 0.30	0.18	0.65	0.61 ± 0.35	0.18	0.69	0.56 ± 0.22	0.01
17	*PRKCA*	rs6504453	C/T	0.35	0.48 ± 0.31	0.02	0.36	0.67 ± 0.36	0.26	0.35	0.70 ± 0.22	0.11

[a] Effect Allele Frequency (EAF) calculated in controls

Table S4: Interactions between SNPs in *PLAG24A*, *RELA*, *PRKD1* and *PRKCA* and occupational exposure to LMW agents or irritants on current adult-onset asthma after accounting for family dependency, after excluding current smokers, and after further adjustment for smoking status (case-control approach)

Chr	**Gene**	**SNP**	**Reference /Effect Allele**	**Interaction (n=2599) - GLM**		**Results accounting for family dependency (n=2599) - GEE**		**Results after excluding current smokers (n=1902) - GLM**		**Results adjusted for smoking (n=2599) - GLM**	
				OR ± SE	P-value	**OR ± SE**	P-value	**OR ± SE**	P-value	**OR ± SE**	P-value
1	*PLA2G4A*	rs932476	A/G	0.64 ± 0.16	0.0055	0.64 ± 0.16	0.0048	0.77 ± 0.18	0.16	0.64 ± 0.16	0.0057
2	*PLA2R1*	rs2667026	A/G	1.77 ± 0.20	0.0050	1.77 ± 0.21	0.0066	1.64 ± 0.23	0.03	1.76 ± 0.20	0.0057
11	*RELA*	rs931127	A/G	1.61 ± 0.15	0.0015	1.61 ± 0.14	0.0010	1.74 ± 0.17	0.0013	1.63 ± 0.15	0.0012
11	*RELA*	rs7949980	C/T	1.56 ± 0.15	0.0030	1.56 ± 0.15	0.0029	1.64 ± 0.18	0.0045	1.56 ± 0.15	0.0032
14	*PRKD1*	rs1958980	A/G	0.64 ± 0.16	0.0042	0.63 ± 0.16	0.0038	0.68 ± 0.18	0.04	0.64 ± 0.16	0.0054
14	*PRKD1*	rs11847351	A/G	0.64 ± 0.16	0.0043	0.63 ± 0.16	0.0039	0.69 ± 0.18	0.04	0.64 ± 0.16	0.0055
14	*PRKD1*	rs1958987	C/T	0.64 ± 0.16	0.0054	0.64 ± 0.16	0.0042	0.67 ± 0.19	0.03	0.64 ± 0.16	0.0059
17	*PRKCA*	rs6504453	C/T	0.63 ± 0.16	0.0032	0.63 ± 0.16	0.0036	0.58 ± 0.19	0.0036	0.62 ± 0.16	0.0028

Chr: chromosome, CC: case-control.

Table S5. Gene-based interaction results obtained with VEGAS at the P-value<.05.

Chr	Gene	Number of SNPs	Start Position	Stop Position	Test	P-value
11	***RELA***	6	65178392	65186951	28.5	**0.009**
14	*FOS*	9	74815283	74818665	23.4	0.024
1	*PRDX6*	8	171713108	171724569	26.8	0.030
10	*MGMT*	71	131155455	131455358	146.7	0.030
22	*GSTT2B*	1	22629600	22633368	4.4	0.035
6	*ARG1*	5	131936057	131947161	14.8	0.036
14	***PRKD1***	50	29115437	29466650	109.9	**0.036**
22	*NDUFA6*	4	40811475	40816834	13.8	0.047

Chr: chromosome.

Figure S1A. Flow-chart EGEA

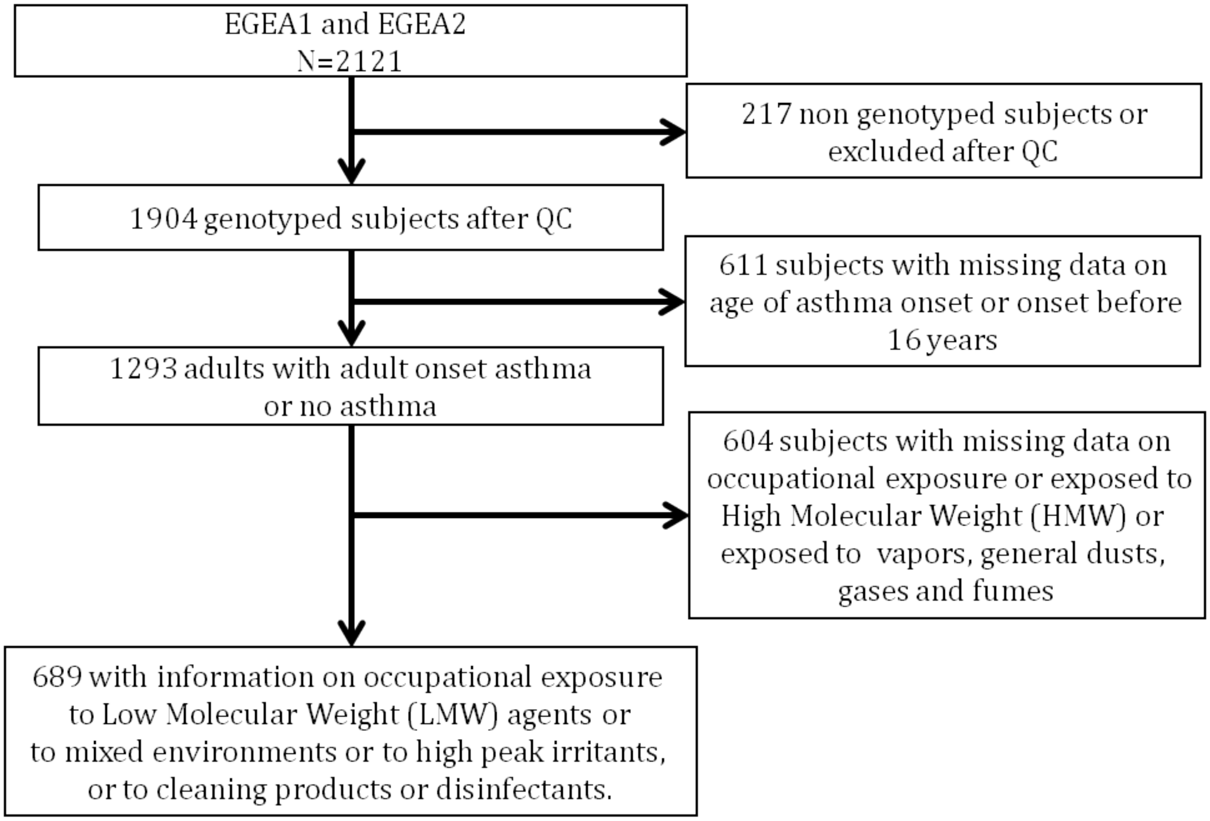


Figure S1B. Flow-chart SAPALDIA

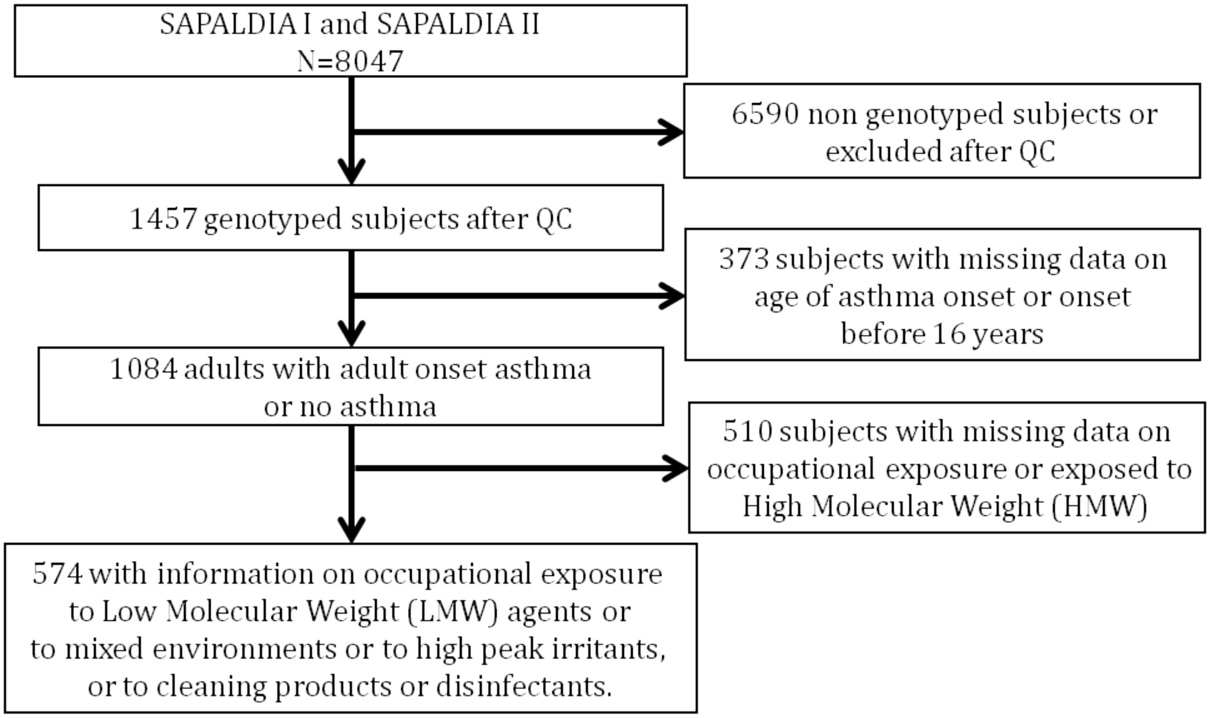


Figure S1C. Flow-chart ECRHS

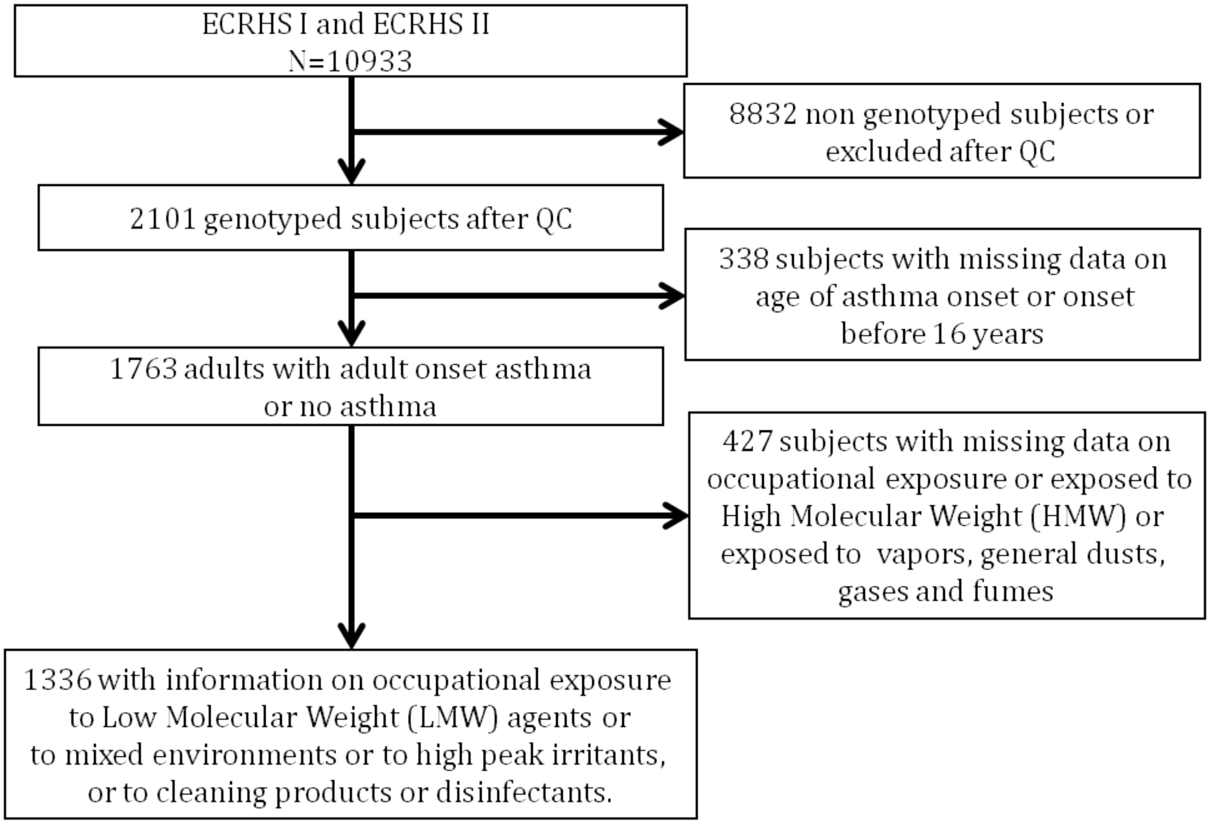
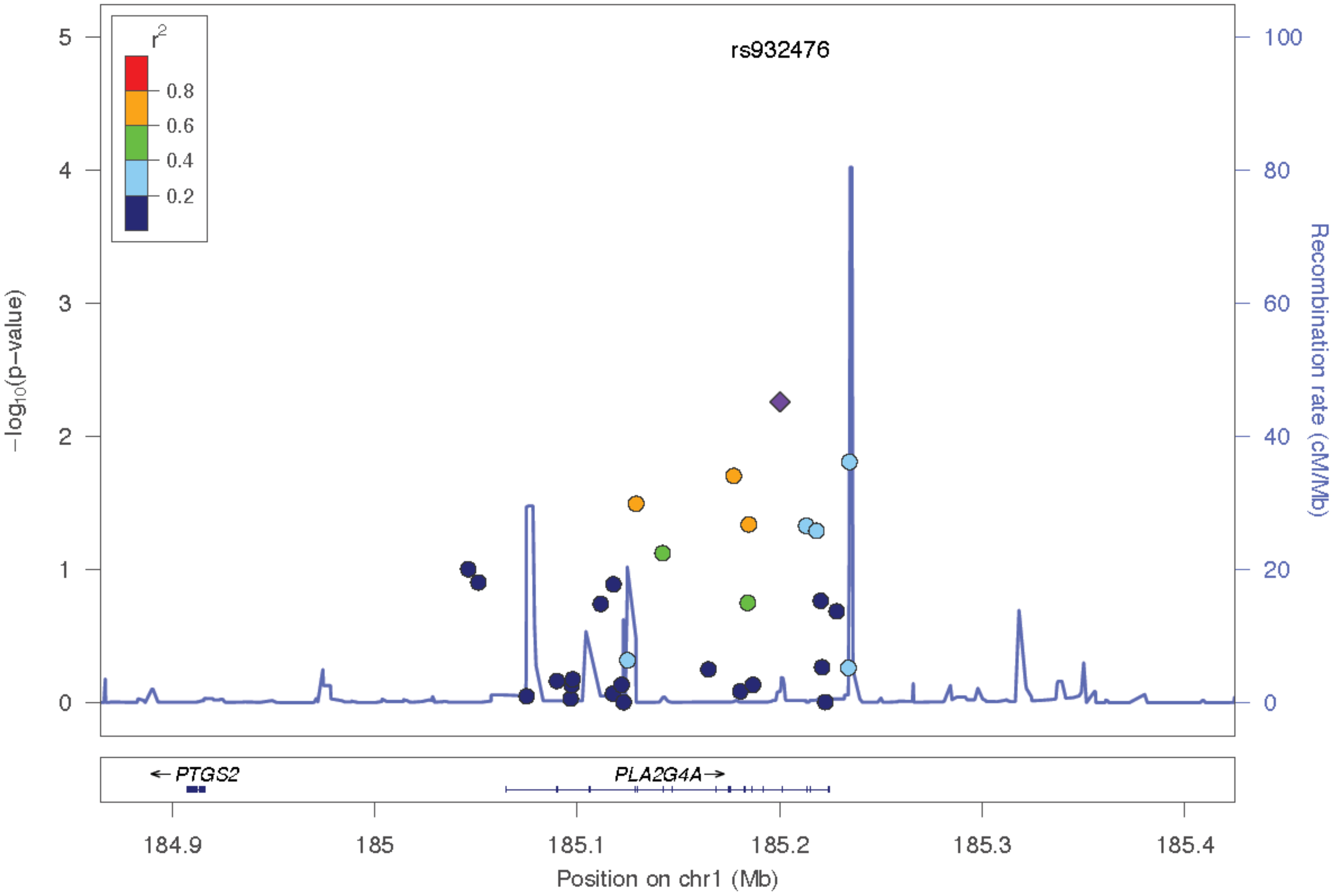


Figure S1. Flow-charts of the selected participants in each European study (**A**: EGEA, **B**: SAPALDIA and **C**: ECRHS).

Figure S2A



rs932476
r2
0.8
0.6
0.4
0.2
5
4
3
2
1
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
← PTGS2
PLA2G4A →
184.9
185
185.1
185.2
185.3
185.4
Position on chr1 (Mb)

Figure S2B

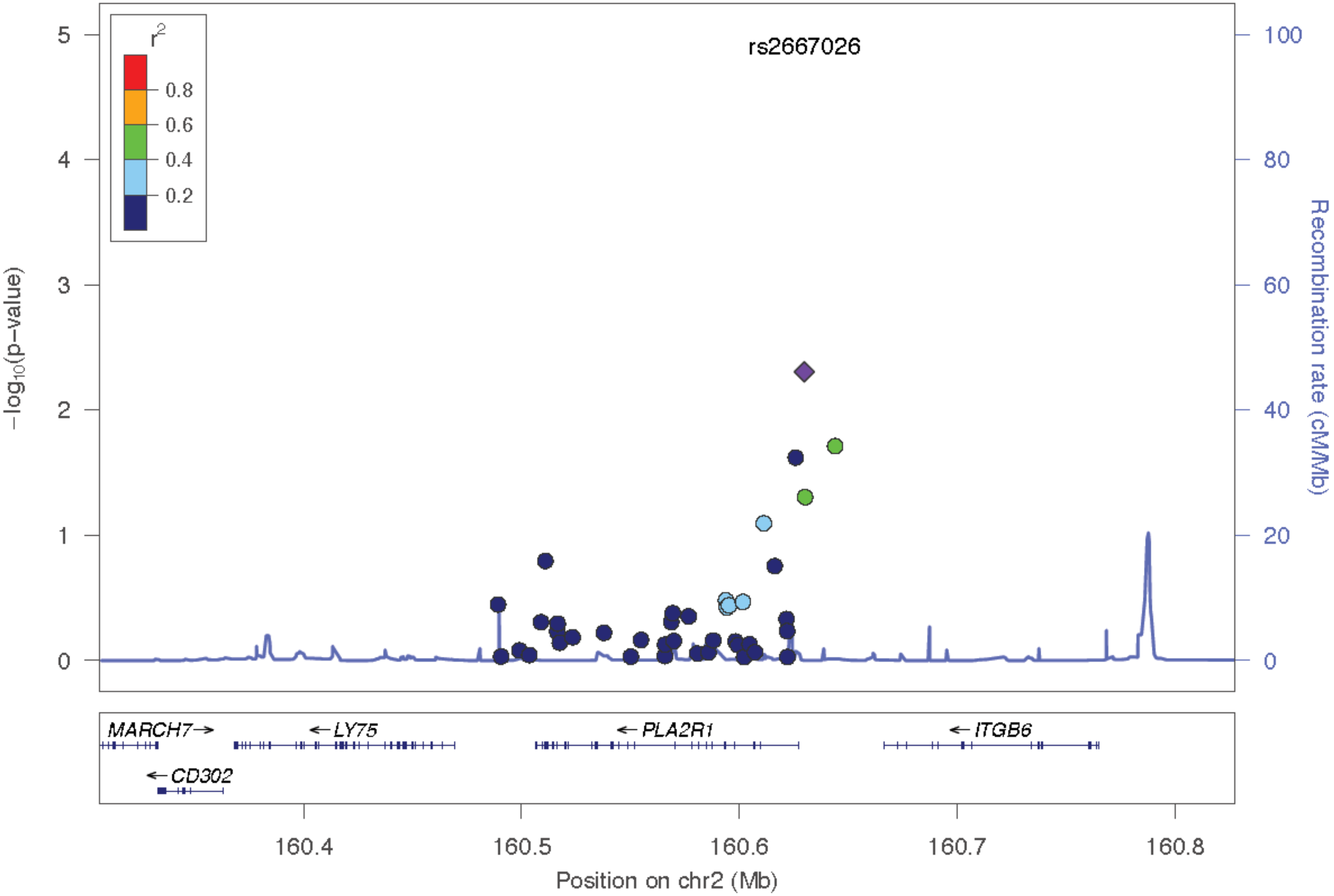
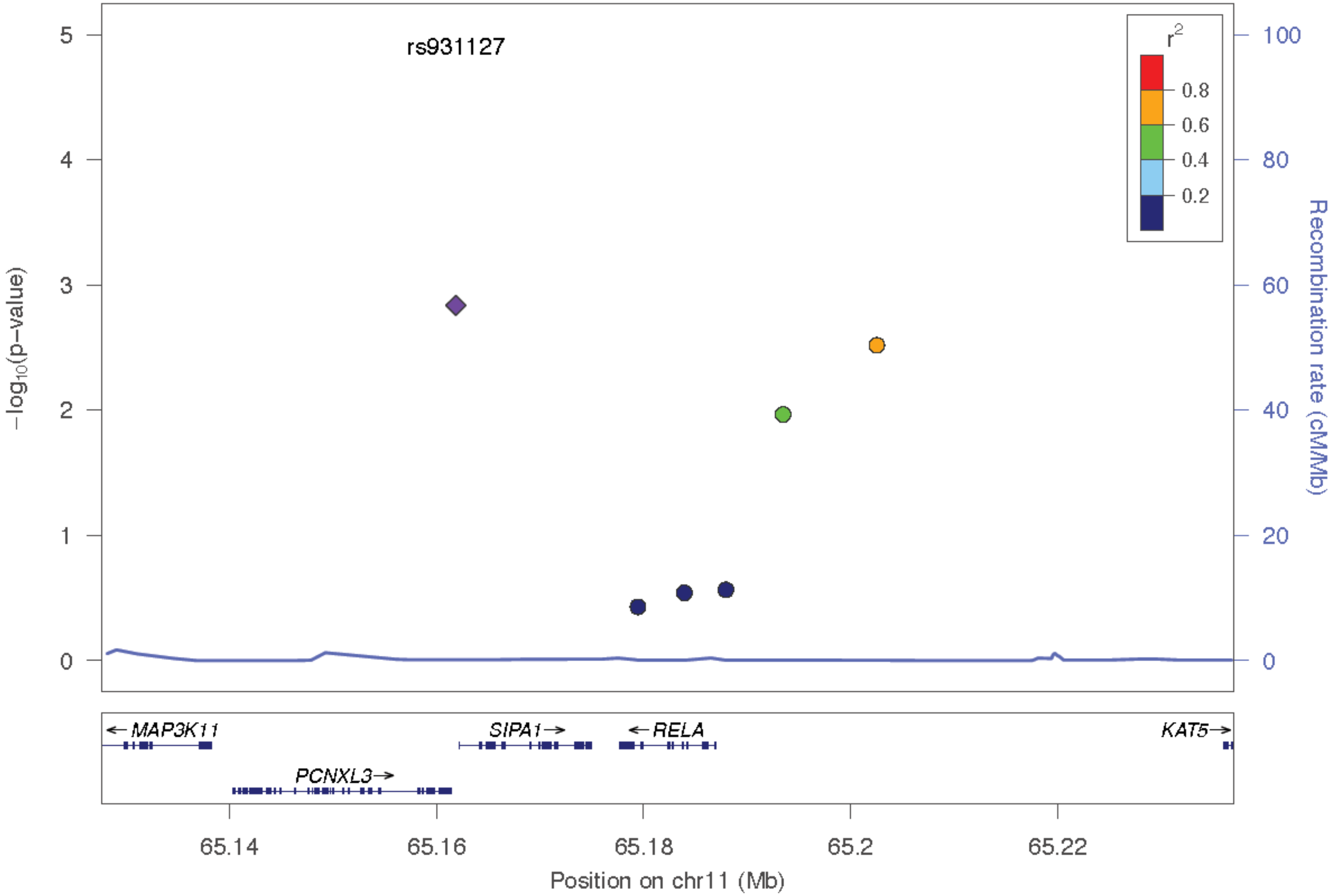

r2
0.8
0.6
0.4
0.2
rs2667026
5
4
3
2
1
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
MARCH7→
← LY75
← PLA2R1
← ITGB6
← CD302
160.4
160.5
160.6
160.7
160.8
Position on chr2 (Mb)

Figure S2C


rs931127
r²
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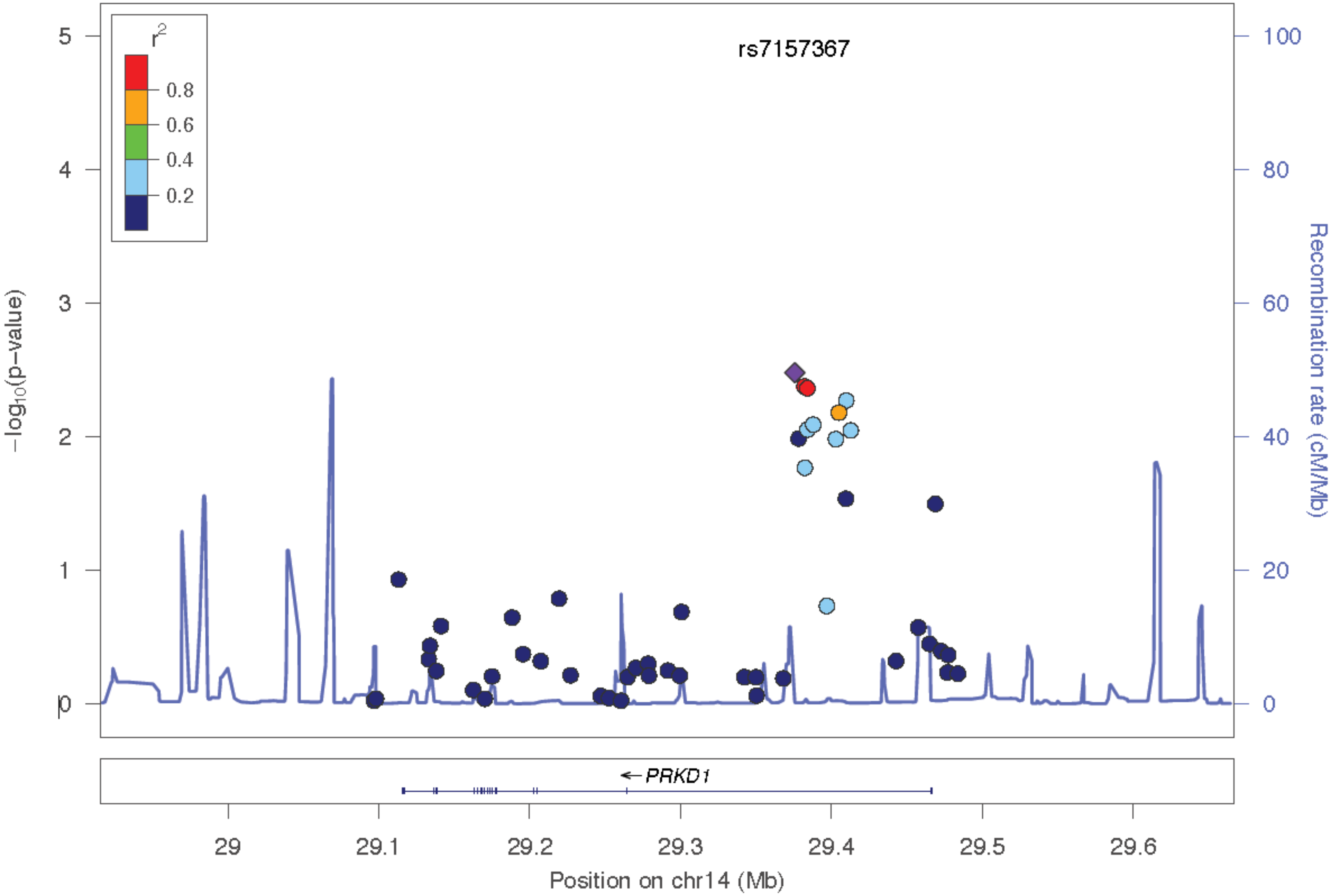
0.6
0.4
0.2
5
4
3
2
1
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
MAP3K11
PCNXL3
SIPA1
RELA
KAT5
65.14
65.16
65.18
65.2
65.22
Position on chr11 (Mb)

Figure S2D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0.2
5
4
3
2
1
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
← PRKD1
29
29.1
29.2
29.3
29.4
29.5
29.6
Position on chr14 (Mb)

Figure S2E

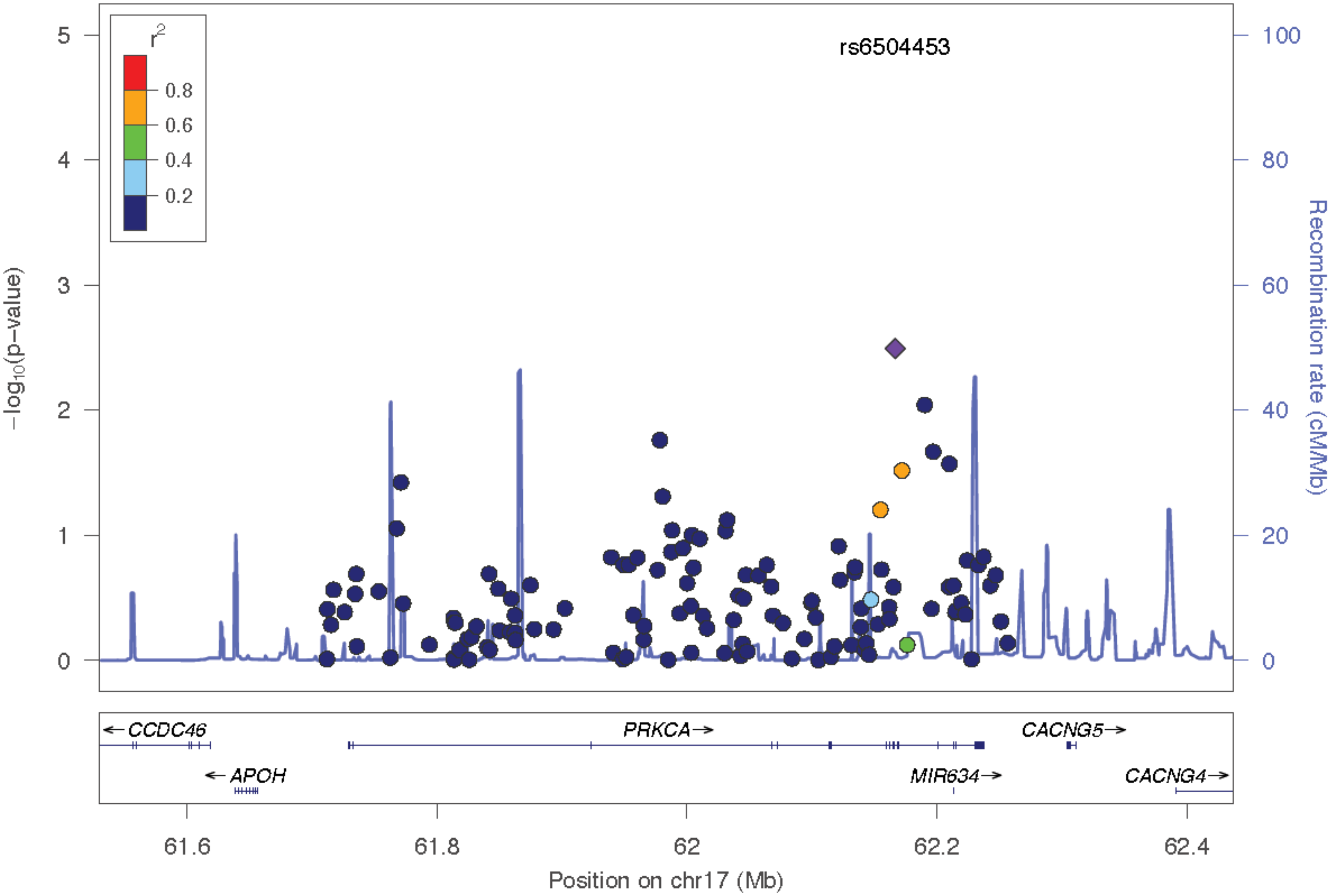
r^2
0.8
0.6
0.4
0.2
rs6504453
5
4
3
2
1
0
$-\log_{10}$(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
← CCDC46
← APOH
PRKCA →
MIR634 →
CACNG5 →
CACNG4 →
61.6
61.8
62
62.2
62.4
Position on chr17 (Mb)

Figure S2. Summary of the SNP-occupational exposure interaction results around rs932476 in *PLA2G4A* (**A**), rs2667026 in *PLA2R1* (**B**), rs931127 in *RELA*(**C**) , rs1958980 in *PRKD1* (**D**) and rs6504453 in *PRKCA* (**E**) using the LocusZoom software (Pruim et al. 2010).

The left Y-axis shows the -log10 P-values. The position of the SNPs in megabases (build 36.3) is shown on the X-axis. rs932476, rs2667026, rs931127, rs1958980 and rs6504453 are colored in purple. The degree of LD (from the hg/1000 Genomes reference panel) between these SNPs and the SNPs we selected in the same region is reflected by the color of the dots (red being the highest degree of LD). The blue line represents the recombination rate (right Y-axis).

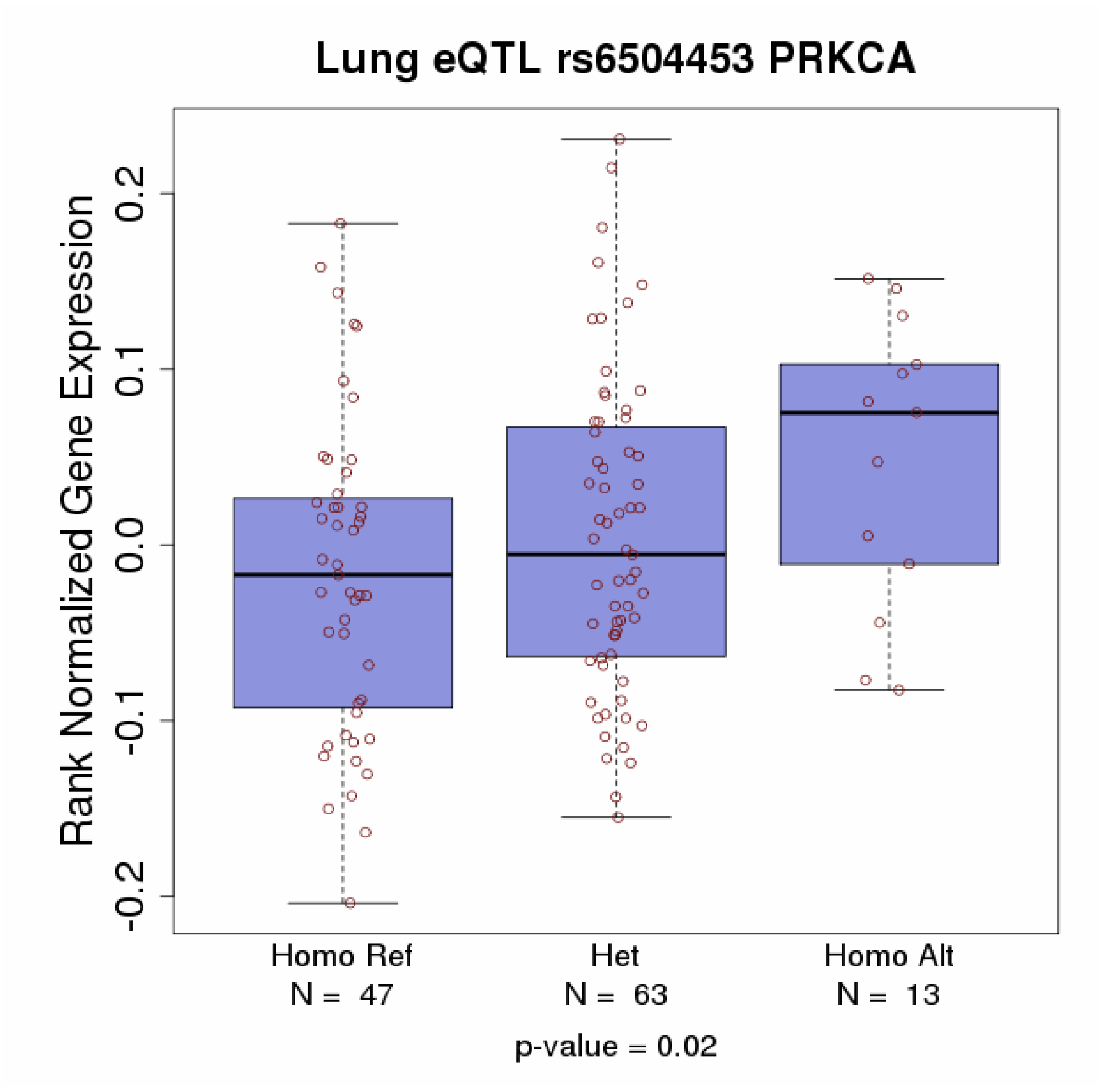

Figure S3. Association between T allele at rs6504453 in *PRKCA* and gene expression in lung tissue (eQTL browser GTEx, Gibson et al. 2015).