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ONLINE SUPPLEMENTAL MATERIAL

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Genes interacting with occupational exposures to low molecular weight agents and irritants on adult-onset asthma in three European studies

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- 44 **Running title**: candidate genes and occupational exposures in asthma
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47 Description of the three multicentre epidemiological European studies

48 The EGEA study

49 The EGEA study is a 12-year longitudinal survey, which combines a case-control study and a 50 family study of asthmatic cases (https://egeanet.vjf.inserm.fr/index.php/en/). The protocol and 51 descriptive characteristics have been described elsewhere (Kauffmann et al. 1997, 1999). 52 Probands (388 asthmatic patients) were recruited from six chest clinics in five French cities 53 between 1991 and 1995 and their 1,244 family members were included, either by including the 54 proband's parents and siblings, or by including the proband's spouse and children. In addition, 55 415 population-based controls were recruited. The probands (asthmatics and controls) were 56 between 7 and 70 years old at time of ascertainment. All probands and their two parents were of 57 European ancestry and were born in France. A follow-up (EGEA2) of the initial cohort was 58 conducted between 2003 and 2007 and included follow-up data for 1,543 participants from the 59 initial cohort and 73 new family members. All participants responded to a questionnaire based on 60 international standardized tools to diagnose asthma and to determine respiratory and allergic 61 symptoms, treatments, and environmental and occupational exposures (Kauffmann et al. 1997). 62 As a follow-up study of EGEA2, the third survey (EGEA3) was conducted in 2011 using self-63 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 adultonset 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.

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71 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).

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79 The SAPALDIA study

80 The SAPALDIA prospective population-based cohort study (Ackermann-Liebrich et al. 2005; 81 Downs et al. 2007) recruited a random sample of inhabitants aged 18–60 years from eight areas 82 of Switzerland representing various degrees of urbanization and air pollution exposure. 83 Participants underwent spirometry as well as a detailed interview on respiratory health, smoking 84 history, lifestyle factors and anthropometry at both, baseline (1991) and follow-up examination 85 (2002). From among 6,055 SAPALDIA cohort participants participating in both examinations 86 and agreeing to provide blood for genetic analysis, all self-reported asthma cases as well as a 87 random sample of controls had genome wide genotyping in the framework of the European 88 funded "GABRIEL" asthma project (Moffat et al. 2010). For the purposes of the current gene-89 environment interaction analysis, the case definition was restricted to cases of adult asthma (age 90 of onset larger than 16 years) with current treatment or symptoms within the last 12 months prior 91 to assessment. For the definition of occupational risk exposures, job history during the period 92 between SAPALDIA-1 and SAPALDIA-2, and/or reported at the time of SAPALDIA-1 was 93 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 pooledanalysis.
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Exposed	Exposures to asthmagenic agents (ever) evaluated	OR	Exposures to cleaning products and disinfectants (ever)
	by the asthma-specific JEM:		evaluated by job specific questionnaires (self-reported
	LMW agents: Highly reactive chemicals,		weekly exposure):
	cleaning/disinfecting products, metals antigenic, wood		Cleaners: bleach, ammonia, decalcifiers, solvents,
	dust, isocyanates		formaldehyde, alcohol, quaternary ammonium compounds,
	Mixed environment with potential exposure to		and disinfection tasks
	LMW agents: textile production, agriculture, metal		Healthcare workers: bleach, ammonia, decalcifiers,
	working fluids exposures, pharmaceutical drugs		solvents, formaldehyde, glutaraldehyde, alcohol, quaternary
	exposures, bioaerosol exposures		ammonium compounds, chloramine T, ethylene oxide, and
	Irritants: accidental peak exposure		disinfection tasks
Excluded*	Exposures evaluated by the asthma JEM:	OR	Exposures evaluated by the ALOHA JEM:
	HMW agents, inducing asthma through typical		General dust, gases and fumes
	allergic (IgE-mediated) response		
	Jobs with lower level of irritant exposures		
Unexposed group	Non exposed to any egent pet	ontially	a rick for asthma or respiratory health
(reference)	iven exposed to any agent por	cillially	a fisk for asuma of respiratory nearth
JEM – Job Exposure M	atrix; HMW: High Molecular Weight; LMW – Low Mole	ecular V	Veight
* In order to increase sp	pecificity of both exposed and unexposed groups		

Table S1. Assessment of occupational exposure and definition of exposed and non-exposed 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</th>(463 cases/2136 controls)

Chr	Gene	Region	SNP	Position ^a	Ref./Effect	EAF ^b	Marginal effect Interaction - CC		action - CC	Test for heter		ogeneity	
					allele		OR/P	-value	OR/P	-value	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

^aPosition in base pairs (bp), hg18. ^bEffect 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.

					EGEA		SAPALDIA			ECRHS			
				(12	22 cases/567 con	trols)	(107 cases/467 controls)			(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	

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

^aEffect 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) - G	LM	Results accounting for family dependency (n=2599) - GEE		Results after current smok (n=1902) - GI	excluding ers LM	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.

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

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

Chr: chromosome.

Figure S1A. Flow-chart EGEA



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 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).