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

Marta Rava, Ismaïl Ahmed, Manolis Kogevinas, Nicole Le Moual, Emmanuelle Bouzigon, Ivan Curjuric, Marie-Hélène Dizier, Oriane Dumas, Juan R. Gonzalez, Medea Imboden, et al.

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

3  
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164 **ABSTRACT**

165 **Background.** The biological mechanisms by which cleaning products and disinfectants - an  
166 emerging risk factor - affect respiratory health remain incompletely evaluated. Studying genes by  
167 environment interactions (GxE) may help identify new genes related to adult-onset asthma.

168 **Objectives.** To identify interactions between genetic polymorphisms of a large set of genes  
169 involved in the response to oxidative stress, and occupational exposures to low molecular weight  
170 (LMW) agents or irritants on adult-onset asthma.

171 **Methods.** Data came from three large European cohorts: EGEA, SAPALDIA, and ECRHS. A  
172 candidate pathway-based strategy identified 163 genes involved in response to oxidative stress  
173 and potentially related with exposures to LMW agents/irritants. Occupational exposures were  
174 evaluated using an asthma job-exposure matrix and job-specific questionnaires for cleaners and  
175 healthcare workers. Logistic regression models were used to detect GxE interactions, adjusted  
176 for age, sex and population ancestry in 2599 adults (Mean age: 47 years, 60% women, 36%  
177 exposed, 18% asthmatics). P-values were corrected for multiple comparisons.

178 **Results.** Ever exposure to LMW agents/irritants was associated with current adult-onset asthma  
179 (OR(95%CI)=1.28(1.04,1.58)). Eight SNP by exposure interactions at five loci were found at  
180  $p < 0.005$ : *PLA2G4A* (rs932476, chromosome 1), near *PLA2R1* (rs2667026, chromosome 2), near  
181 *RELA* (rs931127, rs7949980, chromosome 11), *PRKDI* (rs1958980, rs11847351, rs1958987,  
182 chromosome 14), and *PRKCA* (rs6504453, chromosome 17). Results were consistent across the  
183 three studies and after accounting for smoking.

184 **Conclusions.** Using a pathway-based selection process, we identified novel genes potentially  
185 involved in the adult asthma in relation with occupational exposure. These genes play a role in  
186 the NF-kB pathway involved in inflammation.

187

188 **INTRODUCTION**

189 Recent reviews regarding the role of environmental risk factors in adult-onset asthma showed  
190 that occupational exposures are important causes of asthma in adults (Le Moual et al. 2013;  
191 Beasley et al. 2015). Approximately 15% of adult asthma is likely to be attributable to  
192 occupational exposures (Toren and Blanc 2009), and occupational asthma is known to be a good  
193 model to study the pathophysiology of asthma in general (Malo et al. 2015). Exposure to  
194 cleaning agents is an emerging risk factor for adult-onset asthma. Evidence of an adverse effect  
195 of cleaning products or disinfectants in asthma mostly comes from studies on occupational risk  
196 factors (Siracusa et al. 2013), but a deleterious role of domestic cleaning exposure has also been  
197 observed (Quinn et al. 2015; Le Moual et al. 2013; Dumas et al. 2013). Some of the numerous  
198 agents contained in cleaning products and disinfectants are chemical sensitizers, but most are  
199 hypothesized to act as respiratory irritants (Siracusa et al. 2013). The biological mechanisms by  
200 which cleaning products and disinfectants affect respiratory health remain incompletely  
201 evaluated (Tarlo and Lemiere 2014; Le Moual et al. 2013; Tarlo 2014). However, inhalation of  
202 low molecular weight (LMW) agents and irritants is likely to induce the release of reactive  
203 oxygen species through the epithelium, and oxidative stress is known as one of the potential  
204 mechanisms causing epithelium injury (Mittal et al. 2014). Furthermore, there is strong evidence  
205 that an imbalance between the reducing and oxidizing systems favoring the oxidative state is  
206 present in asthma. Reactive oxygen and nitrogen species from endogenous and exogenous  
207 sources play a major role in the airway inflammation, and oxidative stress is an important  
208 pathophysiological component of asthma (Chung and Marwick 2010; Aldakheel et al. 2016).  
209 Thus, to better understand the mechanism of LMW chemical sensitizers and irritants in asthma, it

210 may be particularly relevant to focus on the oxidative pathway (Tarlo and Lemiere 2014; Tarlo,  
211 2014).

212 Asthma is a heterogeneous disease, and it is now well established that it is due to a complex  
213 interplay of environmental and genetic factors (Kauffmann and Demenais 2012). There have  
214 been considerable efforts to characterize the genetic determinants of asthma (Holloway et al.  
215 2010), however, the identified genetic factors explain only a small part of the genetic component  
216 of asthma. One of the reasons is that many genetic factors are likely to be involved in the  
217 development, the activity and the severity of asthma, and that they act primarily through complex  
218 mechanisms that involve interactions with environmental factors (GxE) and with other genes  
219 (GxG), notably through pathways and networks. Furthermore, the effect of such genetic factors  
220 may be missed if genes are considered individually, regardless of the biological functions they  
221 share with other genes or the pathways they are involved in (Liu et al. 2012). Candidate GxE  
222 interaction studies conducted on genes involved in the response to oxidative/nitrosative stress  
223 and their interaction with environmental exposures in asthma focused more on children than in  
224 adults and mostly on outdoor air pollution and smoking (Romieu et al. 2010; Minelli et al. 2011).  
225 Furthermore, they have explored a limited number of genes (Kauffmann and Demenais 2012;  
226 Kogevinas 2014; Rava et al. 2015). In order to widen the number of genes to be investigated, we  
227 recently proposed a candidate pathway-based strategy to select an enriched gene-set for GxE  
228 interaction studies (Rava et al. 2013). This gene selection process integrates information on the  
229 biological processes shared by genes, the canonical pathways to which genes belong and the  
230 biological knowledge related to the environmental exposure under study. This approach  
231 represents a powerful alternative strategy between genome wide and candidate approaches to  
232 detect relevant associations of environmental exposures with biological markers as well as GxE

233 interactions.

234 In the present paper, we hypothesized that genes involved in the response to oxidative stress  
235 modify the associations of exposure to LMW agents and irritants with current asthma. We first  
236 applied the candidate pathway-based strategy to select oxidative stress related genes that may  
237 interact with occupational exposures to LMW agents and irritants in current adult-onset asthma.  
238 We then tested for interactive effect of single nucleotide polymorphisms (SNPs) of these genes  
239 and LMW agents and irritants on current adult-onset asthma in 2599 participants from the French  
240 Epidemiological family-based study of the Genetics and Environment of Asthma (EGEA), the  
241 Swiss Cohort Study on Air Pollution and Lung and Heart Disease in Adults (SAPALDIA), and  
242 the European Community Respiratory Health Survey (ECRHS).

243

244 **METHODS**

245 **Study population**

246 Data came from three multicentre epidemiological European studies: the French Epidemiological  
247 family-based study of the Genetics and Environment of Asthma ([EGEA], Kauffmann et al.  
248 1997; Kennedy et al. 2000) (see Figure S1A), and two population-based studies: the Swiss  
249 Cohort Study on Air Pollution and Lung and Heart Disease in Adults ([SAPALDIA], Downs et  
250 al. 2007; Mehta et al. 2012; Ackermann-Lieblich et al. 2005) (see Figure S1B), and the European  
251 Community Respiratory Health Survey ([ECRHS], ECRHS 2002; Kogevinas et al. 2007) (see  
252 Figure S1C). All three cohorts applied comparable study design and highly comparable  
253 questionnaires. Participants included in the analyses were derived from the entire study  
254 population for EGEA and from the nested case-control samples within ECRHS (Smit et al. 2014)  
255 and SAPALDIA cohorts (Curjuric et al. 2012). Participants had genome-wide SNP data,  
256 occupational history regarding LMW agents and irritants, especially cleaning/disinfecting  
257 products, and data on adult-onset asthma and relevant covariates such as age, sex and smoking  
258 status.

259 Ethical approval was obtained in each study from the appropriate institutional ethics committees,  
260 and written informed consent was obtained from each participant. Detailed cohort descriptions  
261 are given in the online supplemental material.

262

263 **Current adult-onset asthma**

264 In all cohorts, current asthma was defined as ever diagnosis of asthma (Moffatt et al. 2010; Smit  
265 et al. 2014) and presence of respiratory symptoms (wheeze; nocturnal chest tightness; attacks of  
266 breathlessness after activity, at rest, or at night; asthma attacks) or using asthma medications in

267 the last 12 months. Participants without asthma were those without asthma at baseline and at  
268 follow-up. Participants with ever asthma, but without symptoms or treatment in the last 12  
269 months were excluded. Since we were interested in participants who may have developed asthma  
270 due to occupational exposure, we restricted the current adult-onset asthma definition to  
271 asthmatics with an age of onset  $\geq$  age 16.

272

### 273 **Occupational exposures to LMW agents and irritants**

274 In all cohorts, occupational history was recorded by interview and job codes were linked to an  
275 asthma-specific job-exposure matrix (JEM) evaluating exposure to 22 agents, and including a  
276 local expert re-evaluation step (Kennedy et al. 2000). Healthcare workers and cleaners were  
277 further asked to answer a job-specific questionnaire regarding exposure to cleaning/disinfecting  
278 products.

279 In the present study, we considered only exposures to substances hypothesized to cause irritant-  
280 asthma, or to cause asthma through mechanisms induced by LMW agents. Exposure to LMW  
281 agents was evaluated by the JEM, and included products typically classified as LMW agents  
282 (e.g., highly reactive chemicals, metals), but also mixed environments with potential exposure to  
283 high molecular weight (HMW) and LMW agents (e.g., agriculture, textile). Exposure to irritants  
284 was evaluated 1) by the JEM, for high peaks irritant exposure, and 2) using self-reported  
285 exposure to cleaning/disinfecting products, with a focus on those that are more likely to be  
286 respiratory irritants (see Supplemental Material Table S1 for more details). Participants who had  
287 ever been exposed to any of the LMW agents, mixed environments, irritants or any specific  
288 cleaning/disinfecting products were classified as “exposed”. Unexposed participants were those  
289 who were never exposed to any of the 22 agents of the asthma JEM (including HMW agents) or

290 to other agents potentially at risk for respiratory health (vapors, general dusts, gases and fumes)  
291 evaluated by another JEM (ALOHA JEM, Matheson et al. 2005; de Jong et al. 2015). The three  
292 cohorts used same definitions.

293

## 294 **Genotyping**

295 The three cohorts (EGEA, SAPALDIA and ECRHS) were part of the European Gabriel  
296 consortium (<http://www.gabriel-fp6.org/>) for asthma genetics (Moffatt et al. 2010), and  
297 constitutes the ESE consortium. Participants were genotyped using Illumina 610 Quad array  
298 (Illumina, San Diego, CA) at the Centre National de Génotypage (CNG, Evry, France). Stringent  
299 quality criteria, as detailed by Imboden et al. (2012), were used to select both individuals and  
300 SNPs for analysis. The quality control (QC) criteria were call rate $\geq$ 97%, minor allele  
301 frequency $\geq$ 5%, and Hardy-Weinberg (HW) P-value $>10^{-4}$ .

302 Gene coverage, which indicates the fraction of common HapMap markers successfully tagged by  
303 the set of selected SNPs, was obtained with Haploview 4.2 (Barrett et al. 2005). We specified  
304 that all HapMap markers being captured by the set of tags should be correlated at  $r^2 \geq 0.8$  with at  
305 least one marker in the set.

306

## 307 **Gene selection through a candidate pathway-based strategy**

308 For this study, a large set of genes was selected according to the candidate pathway-based  
309 strategy previously published (Rava et al. 2013). Briefly, the selection process followed three  
310 steps: **Step1- Gene selection:** we used the Gene Ontology (GO) database (Gene Ontology  
311 Consortium (Ashburner et al. 2000, <http://amigo2.berkeleybop.org/amigo>, version 1.8) to select  
312 genes involved in the "response to oxidative stress" (GO:0006979). This list was further enlarged

313 by literature reviews of asthma related genome-wide association studies, and biological studies  
314 on response to oxidative stress related to environmental exposures of interest; **Step 2 - Pathway**  
315 **enrichment:** using Ingenuity Pathway Analysis (IPA, <http://www.ingenuity.com/>) we identified  
316 the canonical pathways that contained at least 5 genes out of the set of the genes selected in step  
317 1 and which were significantly enriched in these genes ( $p < 0.05$ ); **Step 3 - Environment**  
318 **integration:** we selected the subset of pathways identified at step 2 that contained genes selected  
319 at step 1 expected to be involved in the response to oxidative stress potentially caused by  
320 occupational exposure to LMW agents or and irritants. This strategy is fully detailed in Rava et  
321 al. (2013).

322 For each of the genes belonging to the selected pathways, we examined all SNPs passing the  
323 quality control QC process and lying from 20 kb upstream to 20 kb downstream of the gene  
324 (UCSC genome browser hg18 assembly; Build 37.1).

325

### 326 **Statistical analysis strategy**

327 The three ESE cohorts were pooled to increase statistical power as done before (Smit et al. 2012,  
328 2014); this also allowed assessing consistency of results across cohorts. SNP-occupational  
329 exposure interactions were investigated using a logistic regression model that included the SNP  
330 effect assumed to be additive, a binary exposure (E) variable (1=exposed, 0=unexposed) and a  
331 multiplicative term for SNPxE interaction. All models were adjusted for age, sex and the four  
332 first principal components (PCs) to account for population stratification as previously done (Smit  
333 et al. 2014). No additional adjustment for study was done since PCs are capturing any possible  
334 variability caused by geographical location. Smoking status was further included as a potential  
335 confounder.



336 Test of SNPxE interaction was based on a Wald test. To account for multiple testing, the  
337 Benjamini and Hochberg procedure (1995) was implemented. For interactions belonging to the  
338 top 1% of P-values distribution, consistency of interaction effect estimates across studies was  
339 assessed by use of the Cochran Q test statistic and the extent of heterogeneity was measured by  
340  $I^2$ , which ranges from 0% to 100%. The  $I^2$  statistic describes the percentage of variation across  
341 studies that is due to heterogeneity rather than chance (Higgins and Thompson 2002; Higgins et  
342 al. 2003) and  $I^2 = 100\% \times (Q - \text{degree of freedom}) / Q$ .  $I^2$  values of 0%-24% suggest little  
343 heterogeneity, of 25%-49% reflect moderate heterogeneity, of 50%-74% reflect large  
344 heterogeneity, and of >75% reflect very large heterogeneity (Viechtbauer and cheung 2010). As  
345 smoking may also induce oxidative stress, a sensitivity analysis excluding current smokers was  
346 performed. The robustness of the results to the family dependency existing in the EGEA study  
347 was investigated by using generalized estimating equations (GEE) with an exchangeable  
348 working correlation matrix to take into account potential clustering within families.

349 For each of the genes belonging to the selected pathways, interactions with occupational  
350 exposure for current adult-onset asthma were also investigated at the gene level by using the  
351 versatile gene-based test (VEGAS, Liu et al. 2010). This gene-based statistic sums up the chi-  
352 square test statistics of SNPxE interaction (square of the Wald test statistics) for all SNPs of a  
353 gene. The correlation ( $r^2$ ) between these statistics is taken into account by computing an  
354 empirical P-value through Monte-Carlo simulations using the linkage-disequilibrium pattern of  
355 HapMap Utah residents with ancestry from northern and western Europe (CEU) reference  
356 sample; this empirical P-value is estimated by the proportion of simulated test statistics that  
357 exceeds the observed gene-based test statistic. The empirical P-values were then adjusted  
358 for multiple testing using the method of Benjamini and Hochberg.

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**eQTL analysis, functional annotation and chemical–gene/protein interactions**

We investigated whether the SNPs (or their proxies,  $r^2 \geq 0.8$ ) found to interact with occupational exposures to LMW agents or irritants were cis-expression quantitative trait loci (cis-eQTLs). We used the eQTL browser (<http://www.gtexportal.org/home/>) that includes e-QTL data from many tissues; the Genotype-Tissue Expression project (GTEx, Gibson 2015). Furthermore, functional annotations of these SNPs (or proxies) were done using the HaploReg tool (<http://www.broadinstitute.org/mammals/haploreg/haploreg.php>). HaploReg annotates SNPs in terms of predicted ROADMAP and ENcyclopedia Of DNA Elements (ENCODE), chromatine states (promoter and enhancer histone modification signals), DNase I hypersensitivity sites, and transcription factor (TF) and protein binding sites.

Furthermore, curated [chemical–gene interactions|chemical–disease|gene–disease] data were retrieved from the Comparative Toxicogenomics Database (CTD, Davis et al. 2014, MDI Biological Laboratory, Salisbury Cove, Maine, and NC State University, Raleigh, North Carolina. World Wide Web, URL: <http://ctdbase.org/>). [April, 2016]. CTD is a robust, publicly available database that aims to advance understanding about how environmental exposures affect human health. It provides manually curated information about chemical–gene/protein interactions, chemical–disease and gene–disease relationships.

## 381 **RESULTS**

### 382 **Data description**

383 The study population included 2599 participants with a mean age of 46.7 years and 60% of  
384 women (Table 1). ECRHS participants were younger, and the proportion of women was lower in  
385 SAPALDIA. Almost half of the participants were never smokers. The proportion of current  
386 smokers varied from 18.6% (EGEA) to 31.4% (ECRHS), and 463 had current adult-onset  
387 asthma. Among the 927 exposed participants, 25.4% were exposed to LMW agents only, 4.4%  
388 were exposed to irritants only, 23.7% were health care workers or cleaners (exposure to cleaning  
389 products), 12.6% were exposed to mixed environment only, and 33.9% had combined exposures  
390 (i.e. two or more of the aforementioned exposures).

391 A positive and significant association was found between lifetime occupational exposure to  
392 LMW agents or irritants and current adult-onset asthma: age and sex adjusted pooled Odds-Ratio  
393 (ORa)=1.28; 95% Confidence Interval (95%CI) 1.04-1.58). Across the three cohorts, the  
394 associations between exposure and asthma were: age and sex adjusted ORa=1.09 (95%CI: 0.72-  
395 1.65; n=122/689, cases/all) in EGEA, 0.89 (95%CI: 0.56-1.42; n=107/574) in SAPALDIA, and  
396 1.55 (95%CI: 1.15-2.08; n=234/1336) in ECRHS.

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### 398 **Genes selected with the candidate pathway-based strategy**

399 **Step1- Gene selection:** 387 genes were selected through GO and further enriched by literature  
400 reviews and biological studies to get a list of 411 genes; **Step 2 - Pathway enrichment:** we  
401 identified 277 pathways that contained at least 5 genes out of the 411 genes selected at step 1 and  
402 which were enriched in these genes ( $p < 0.05$ ); **Step 3 - Environment integration:** 17 of the 277  
403 pathways were further selected because they included genes involved in response to oxidative

404 stress and potentially related with exposures to LMW agents or irritants. These pathways had  
405 pathway enrichment P-values ranging from 0.03 to  $1.58 \times 10^{-31}$  (Excel Table S1) and included  
406 from 5 up to 47 genes (15-20 genes on average); more than 50% of the genes were involved in  
407 more than one pathway. The final analyzed set included a total of 163 unique genes (Excel  
408 Tables S2) and 3297 SNPs.

409

### 410 **Analysis of SNPs x occupational exposure interactions**

411 At the SNP level, none of the interactions with LMW/irritants on current adult-onset asthma  
412 reached the significance level after correction for multiple testing ( $P=0.05/3297=1.5 \cdot 10^{-5}$ ).  
413 However, we selected 14 interactions belonging to the top 1% of P-values distribution ranked  
414 from lowest (top) to highest (bottom) (Supplemental Material Table S2). Among these 14  
415 interactions, 8 interactions at five loci showed little heterogeneity ( $I^2 < 24\%$ ) between the three  
416 studies (Table 2 and Supplemental Table S3): rs932476 in *PLA2G4A* (phospholipase A2, group  
417 IVA (cytosolic, calcium-dependent) gene, chromosome 11,  $P=0.005$ ), rs2667026 near *PLA2R1*  
418 (phospholipase A2 Receptor 1, chromosome 2,  $P=0.005$ ), rs931127 and rs7949980 near *RELA*  
419 (V-Rel Avian Reticuloendotheliosis Viral Oncogene Homolog A gene, chromosome 11,  $P=0.001$   
420 and  $P=0.003$  respectively), rs1958980, rs11847351, and rs1958987 in *PRKDI* (protein kinase  
421 D1, chromosome 14, P-values ranging from 0.004 to 0.005), and rs6504453 in *PRKCA* (protein  
422 kinase C, alpha, chromosome 17,  $P=0.003$ ). The two SNPs near *RELA* were in moderate linkage  
423 disequilibrium (LD,  $r^2=0.65$ ), whereas the three SNPs in *PRKDI* were in strong LD ( $r^2 > 0.8$ , see  
424 Figures S2A to S2E). Further, rs932476 in *PLA2G4A* and rs931127 in *RELA* were also  
425 marginally associated with asthma ( $P=0.0036$  and  $P=0.035$  respectively, Table 2). Similar  
426 interactive and marginal estimates were obtained by taking into account family dependency

427 (Supplemental Material Table S4) or by adjusting for study/centre (data not shown). Excluding  
428 current smokers from the analysis showed consistent results except for the *PLA2G4A* gene  
429 (Supplemental Material Table S4). Finally, adjusting for smoking gave similar estimates  
430 (Supplemental Material Table S4).

431 Associations between SNPs and current adult-onset asthma in unexposed and exposed  
432 participants are reported in Figure 1. "Flip-Flop" interactions were observed. Near *RELA*, the risk  
433 of current adult-onset asthma was increased in G carriers of rs931127 and in T carriers of  
434 rs7949980 among exposed participants (OR=1.54,  $P=2 \times 10^{-4}$ , and OR=1.40,  $P=0.005$   
435 respectively), whereas inverse but not significant effects were observed among unexposed  
436 participants. The risk was also increased - although not statistically significant - among exposed  
437 participants in G carriers of rs2667042 near *PLA2R1*, whereas inverse and significant effects  
438 (OR=0.74,  $P=0.009$ ) were observed among unexposed participants. On the contrary, the risk of  
439 current adult-onset asthma was decreased but not significantly among exposed participants in G  
440 carriers in *PLA2G4A*, in G carriers of rs1958980 or G carriers of rs11847351 or T carriers of  
441 rs1958987 in *PRKDI*, and in T carriers of rs6504453 in *PRKCA*, and significantly in T carriers  
442 of rs6504453 in *PRKCA* (OR=0.79,  $P=0.05$ ), whereas inverse and significant effects were  
443 observed among unexposed participants (OR=1.25 to 1.50,  $P=0.01$  to  $3 \times 10^{-4}$ ).

444 *PRKDI* and *PRKCA* are involved together in the "NRF2-mediated Oxidative Stress Response"  
445 pathway, in association with *RELA* in three other pathways: "Xenobiotic Metabolism Signaling",  
446 "Production of Nitric Oxide and Reactive Oxygen Species in Macrophages", and "N-formyl-  
447 methionyl-leucyl-phenylalanine (fMLP) signaling in neutrophils", or in association with  
448 *PLA2G4A* in the "CCR3 signaling in eosinophils" pathway (Excel Table S3). Furthermore, *RELA*

449 and *PRKCA* are involved together in the "Apoptosis signaling" pathway, and *RELA*, *PLA2G4A*  
450 and *PLA2R1* are involved together in the "Antioxidant Action of Vitamin C" pathway.  
451 Gene coverage for the SNPs in *PLA2G4A*, *PLA2R1*, *PRKDI* and *PRKCA* were quite high: 55%  
452 ( $r^2=0.98$ ), 76% ( $r^2=0.97$ ), 74% ( $r^2=0.96$ ) and 68% ( $r^2=0.96$ ) respectively. A low coverage was  
453 observed for *RELA* (<10%).

454

#### 455 **Analysis of Gene x occupational exposure interactions**

456 At the gene level, *RELA* and *PRKDI* were among the top gene interaction with occupational  
457 exposures to LMW/irritants that were detected by the gene-based test among all 163 studied  
458 genes (P-value=0.009 and P=0.04 respectively, Supplemental Material Table S5), but none of  
459 them reached the significance level after correction for multiple testing.

460

#### 461 **eQTL, functional annotations and chemical–gene/protein interactions**

462 Using the eQTL browser GTEx, we found that the T allele at rs6504453 in *PRKCA* was  
463 associated with increased gene expression in lung tissue (see Figure S3, P=0.017). No eQTL was  
464 found among the SNPs (or proxies) interacting with exposures at *PLA2G4A*, *PLA2R1*, *RELA* and  
465 *PRKDI* loci.

466 Using the functional annotation HaploReg tool v3, we found that the SNPs rs932476 in  
467 *PLA2G4A*, rs2667026 near *PLA2R1*, rs931127 and rs7949980 near *RELA*, and rs1958980,  
468 rs11847351 and rs1958987 in *PRKDI* mapped to marks of active regulatory elements notably in  
469 B cells, small airways epithelial cells, and lymphoblastoids cell lines. These marks included  
470 enhancer histone marks, DNase hypersensitivity sites, and binding protein sites for NFkB,

471 Histone Deacetylase 2 (HDAC2), and Nuclear factor erythroid 2-related factor 2 (Nrf2) (Excel  
472 Table S4).

473 Further, from the Comparative Toxicogenomics Database, we found that chlorine, formaldehyde  
474 and hydrogen peroxide have been reported to modify the localization of *PRKCA* protein, the  
475 expression of *PLA2R1* or *PLA2G4A* mRNA, and the expression and the activity of *RELA* protein  
476 (Excel Table S5). We also found that exposures known to contain compounds with irritant  
477 properties (air pollutants and vehicle emissions) modified the expression of *PRKDI* mRNA and  
478 methylation of *PLA2R1* (Excel Table S5).

479

480 **DISCUSSION**

481 This study identified interactions between genetic variants near or within five genes, *PLA2G4A*,  
482 *PLA2R1*, *RELA*, *PRKDI* and *PRKCA*, and occupational exposures to LMW agents or irritants for  
483 current adult-onset asthma. The evidence rests on the results obtained in pooled data of three  
484 large European epidemiological studies and the consistency of results across these studies.  
485 Functional annotations of the interacting SNPs at these loci and functional supports specific for  
486 the GxE interactions detected suggest that a few of these SNPs might be involved in regulatory  
487 mechanisms.

488 Up to now, a limited number of genes were explored in GxE interaction studies conducted with  
489 candidate gene approaches. The most commonly studied genes were those coding for the  
490 enzymes NAD(P)H dehydrogenase [quinine] 1 (*NQO1*), the glutathione S-transferases (*GSTs*),  
491 the heme oxygenase 1 (*HMOX-1*), the catalase (*CAT*) and the superoxide dismutase (*SOD*)  
492 (Minelli et al. 2011). Our study relies on an original strategy to select and enlarge the list of  
493 candidate genes. Supported by biological knowledge, we think this approach allows a good  
494 tradeoff between GEWIS and candidate gene approaches. It is interesting to note that our set of  
495 163 genes included the few genes mentioned previously and studied in interaction with other  
496 exposures related to oxidative stress (smoking, outdoor air pollution) in asthma following a  
497 candidate gene approach. We cannot exclude that our selection may have overrepresented the  
498 anti-oxidative defense, and may lose a number of relevant genes that are not targeted by our  
499 analysis. However, we were able to highlight that genes modulating exposure to LMW agents  
500 and irritants have all a prominent role in the NF-kappa-B pathway and our strategy had also the  
501 capacity to generate new hypotheses. One of the difficulties in GxE studies is the need of large  
502 studies or consortia to detect significant interaction, which in turn might be affected by



503 heterogeneity in both outcome and exposure definition of the participating studies. To overcome  
504 these limitations, definition of adult-onset asthma as well as those of occupational exposures to  
505 LMW agents or irritants were harmonized across the three epidemiological studies, and  
506 genotyping was performed identically in the three studies in the framework of the European  
507 Gabriel consortium asthma GWAS (Moffatt et al. 2010). Despite the fact that the three studies  
508 were pooled, we obtained 463 exposed participants with adult onset asthma to detect GxE  
509 interactions. This small number of exposed cases may have hampered our findings, and we  
510 acknowledge that the lack of replication is a limitation. However, replication is very difficult  
511 because EGEA, SAPALDIA and ECRHS are, to the best of our knowledge, the only three  
512 cohorts having such specific information on occupational exposures (the asthma-specific JEM  
513 with the expertise step that increases the precision of exposure assessment, and the specific  
514 questionnaires in cleaners and health care workers). By adding other studies using only the  
515 asthma-specific JEM, we would lose part of the specificity of our analysis. None of our GxE  
516 interaction tests reached the significance level after correction for multiple testing, so we focused  
517 on SNPs with P-values for SNPsxE in top 1% of the distribution, and reduced false positives by  
518 only selecting consistent interactions across the three studies. As regards the method used,  
519 various study designs and statistical methods have been proposed to investigate GxE interactions  
520 (Liu et al. 2012). We used the classical GxE interaction test based on a case-control design,  
521 which may not be the most powerful approach. Indeed, when one can assume independence  
522 between exposure and SNPs, it has been shown that case-only based approaches (Mukherjee et  
523 al. 2008) are better alternatives. However, these approaches could not be applied to our study  
524 because our gene-selection process aimed at selecting genes potentially associated with the  
525 environmental exposure due to their biological function. We further repeated the analyses using a

526 joint test of gene and gene-environment interaction (Dai et al. 2012) but similar results were  
527 obtained (data not shown).

528 Irritant-induced asthma is usually described as a separate, “nonsensitizing”, type of occupational  
529 asthma (Maestrelli et al. 2009; Tarlo and Lemiere 2014). On the other side, low molecular  
530 weight agents are generally classified as sensitizers, although most of them are not associated  
531 with the production of specific IgE (Tarlo and Lemiere 2014). The precise mechanisms linking  
532 irritants and LMW chemicals to asthma are poorly known, and it is therefore challenging to  
533 classify most asthmogenic chemicals (*e.g.*, cleaning products) into definite categories. However,  
534 both occupational exposures to LMW chemicals and irritants may result in oxidative stress  
535 (Dumas et al. 2015). We could thus investigate a relatively broad spectrum of exposures by  
536 carefully selecting genes through our pathway-based strategy integrating hypotheses about the  
537 environment. Smoking is also known to be related to oxidative stress. Our results remained  
538 almost consistent after running analysis without current smokers or after accounting for smoking,  
539 suggesting that the detected interactions were not due to the effect of smoking.

540 To our knowledge, none of our findings have been reported previously by published GWASs of  
541 asthma (GWAS-Catalog of Published Genome-Wide Association Studies,  
542 <http://genome.gov/gwastudies>, Hindorff et al. ), or by GEWIS studies in asthma. Differences in  
543 length of microsatellite sequences in the promoter region of *PLA2G4A* were reported between  
544 patients with severe asthma and healthy controls, with a direct impact on mRNA and protein  
545 expression, suggesting a role in asthma pathogenesis (Sokolowska et al. 2010). Scarce candidate  
546 G x occupational exposure interaction studies have been published for asthma (Kauffmann et al.  
547 2010; Kogevinas 2014; Smit et al. 2014; Cherry et al. 2015). Focusing on occupational  
548 exposures, *CTNNA3* (catenin alpha 3, alpha-T catenin) was reported by GWAS as the strongest

549 candidate gene for toluene diisocyanate (TDI)-induced asthma in Korean patients (Kim et al.  
550 2009), and only one genome-wide study of interaction (GEWIS) was published that identified  
551 novel susceptibility loci for occupational exposure to biological dust, mineral dust, and gases and  
552 fumes in relation to FEV<sub>1</sub> levels (de Jong et al. 2015).

553 Interestingly, all the genes we detected play a role in the NF-kappa-B pathway. NF-kappa-B is  
554 an ubiquitous transcription factor involved into the mechanism whereby oxidants affect the  
555 pathophysiology of asthma (Schuliga 2015). The genetic variants interacting with exposure do  
556 not belong to protein-coding regions, but are more likely to have a regulatory function, as  
557 indicated by the functional annotations of a few of these SNPs. *RELA* encodes the RelA protein  
558 that is complexed with NFKB1, the most abundant form of NF-kB. *PRKDI* encodes a  
559 serine/threonine kinase, called PKD1 that activates NF-kB in response to oxidative stress  
560 conditions (Sundram et al. 2011; Storz 2007). Exposure to photochemically altered air pollutant  
561 mixture, was associated with a decrease in expression of *PRKDI* mRNA in human lung  
562 epithelial cells (Rager et al. 2011). On the contrary, exposure to Zinc Oxide nanoparticles, that is  
563 associated with acute pulmonary oxidative stress and inflammation (Vandebriel and de Jong  
564 2012), was reported to activate NF-kB in human bronchial epithelial cells through a mechanism  
565 that involves RelA-NF-kB phosphorylation (Wu et al. 2010). Interestingly, in a similar opposite  
566 manner, we found negative associations between genetic variants in *PRKDI* and adult-onset  
567 asthma (decreased risk), and positive associations between genetic variants near *RELA* and adult-  
568 onset asthma (increased risk) in participants exposed to LMW or irritant agents. All these effects  
569 are "Flip-flop effects", and we can only speculate on the mechanism behind an opposite effect  
570 among the exposed and unexposed subjects. Finally, the protein encoded by *PRKCA* was  
571 suggested as a regulator of NF-kB-induced expression of genes involved in inflammatory

572 responses (Nakashima 2002), and was associated with generation of reactive oxygen species  
573 through a biological interaction with other genes including member of the mammalian PLA2  
574 family (Chi et al. 2014). A role of the secretory phospholipase A2 receptor in the development of  
575 asthma was recently reported in animal models of asthma and in human lung cells (Murakami et  
576 al. 2014; Leslie 2015). It is noteworthy that the SNPs interacting with exposure identified by this  
577 study mapped to protein binding sites that included NFkB, Histone Deacetylase 2 (HDAC2)  
578 whose activity is regulated by oxidative stress and Nuclear factor erythroid 2-related factor 2  
579 (Nrf2) which plays a crucial role in the cellular defense against oxidative stress. Lastly, chlorine,  
580 formaldehyde and hydrogen peroxide were reported to affect the localization of the *PRKCA*  
581 protein or to modify the expression of *PLA2G4A* and *PLA2R1* mRNA, or the activity or  
582 expression of *RELA* protein (CTD, <http://ctdbase.org/>, Davis et al. 2014). Overall, all these data  
583 suggest that *PLA2G4A*, *PLA2R1*, *RELA*, *PRKDI* or *PRKCA* may play a role in risk of asthma in  
584 adults in relationship with exposure to LMW agents or irritants.

585

## 586 **CONCLUSIONS**

587 In conclusion, the present study identified new promising candidate genes interacting with  
588 occupational exposures to LMW agents or irritants in current adult-onset asthma. More  
589 generally, this study highlights the interest to perform GxE interaction analysis to identify new  
590 genes and mechanisms of asthma occurrence related to specific environmental exposures.

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**Table 1.** Characteristics of adult participants in the three studies

	<b>All (N=2599)</b>	<b>ECRHS (N=1336)</b>	<b>SAPALDIA (N=574)</b>	<b>EGEA (N=689)</b>
Age, year, mean (SD)	46.7 (11.3)	43.1 (7.1)	53.4 (10.9)	48.0 (14.9)
Sex, women, n (%)	1563 (60.1)	822 (61.5)	311 (54.2)	430 (62.4)
Smoking habits, n (%)				
Never smokers	1167 (44.9)	569 (42.6)	248 (43.2)	350 (50.8)
Ex-smokers	735 (28.3)	337 (25.2)	191 (33.3)	207 (30.0)
Current smokers	682 (26.2)	419 (31.4)	135 (23.5)	128 (18.6)
Missing	15 (0.6)	11 (0.8)	0 (0.0)	4 (0.6)
Occupational exposure, n (%) <sup>a</sup>	927 (35.7)	440 (32.9)	175 (30.5)	312 (45.3)
Current adult onset asthma, n (%)	463 (17.8)	234 (17.5)	107 (18.6)	122 (17.7)

<sup>a</sup>% ever exposed to Low Molecular Weight (LMW) agents or to mixed environments or to high pick irritants, or to specific cleaning products or disinfectants in the population selected for the analyses, *i.e.*; after exclusion of adults with occupational exposures to other potentially asthmagenic agents (High Molecular Weight (HMW) agents).

**Table 2.** Interactive effects of SNPs by occupational exposure to LMW agents or irritants on current adult-onset asthma

Chr	Gene	SNP	Reference /Effect Allele	EAF <sup>a</sup>	Cases/Controls N/N	Marginal effect OR/P-value	Interaction - CC OR/P-value
1	<i>PLA2G4A</i>	rs932476	A/G	0.35	463/2136	1.25 0.0036	0.64 0.0050
2	<i>PLA2R1</i>	rs2667026	A/G	0.83	463/2136	0.89 0.2354	1.77 0.0050
11	<i>RELA</i> <sup>b</sup>	rs931127	A/G	0.43	462/2135	1.17 0.0350	1.61 0.0014
11	<i>RELA</i>	rs7949980	C/T	0.51	463/2133	1.07 0.3421	1.56 0.0030
14	<i>PRKDI</i> <sup>b</sup>	rs1958980	A/G	0.67	463/2136	1.08 0.3344	0.64 0.0042
14	<i>PRKDI</i>	rs11847351	A/G	0.67	463/2133	1.08 0.3429	0.64 0.0043
14	<i>PRKDI</i>	rs1958987	C/T	0.68	459/2127	1.07 0.3609	0.64 0.0050
17	<i>PRKCA</i>	rs6504453	C/T	0.35	462/2134	1.04 0.6086	0.63 0.0032

Chr: chromosome, CC: case-control. <sup>a</sup> Effect Allele Frequency (EAF) calculated in controls.

<sup>b</sup>The two SNPs near *RELA* were in moderate Linkage Disequilibrium (LD) with  $r^2=0.65$ , whereas the three SNPs in *PRKDI* are in strong LD ( $r^2>0.8$ ).

## Figure legends

**Figure 1.** Associations between SNPs that showed an interactive effect with occupational exposure to LMW agents or irritants on current adult-onset asthma in unexposed (grey) and exposed (black) participants.