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

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ABSTRACT

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

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

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

Results. Ever exposure to LMW agents/irritants was associated with current adult-onset asthma (OR(95%CI)=1.28(1.04,1.58)). Eight SNP by exposure interactions at five loci were found at $p<0.005$: *PLA2G4A* (rs932476, chromosome 1), near *PLA2R1* (rs2667026, chromosome 2), near *RELA* (rs931127, rs7949980, chromosome 11), *PRKD1* (rs1958980, rs11847351, rs1958987, chromosome 14), and *PRKCA* (rs6504453, chromosome 17). Results were consistent across the three studies and after accounting for smoking.

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

INTRODUCTION

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

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

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

interactions.

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

METHODS

Study population

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

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

Current adult-onset asthma

In all cohorts, current asthma was defined as ever diagnosis of asthma (Moffatt et al. 2010; Smit et al. 2014) and presence of respiratory symptoms (wheeze; nocturnal chest tightness; attacks of 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 ≥ 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≥97%, minor allele
301 frequency≥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≥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

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

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

Statistical analysis strategy

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

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

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

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.

397

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 $1.58x10^{-31}$ (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 ($P=0.05/3297=1.5.10^{-5}$).
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 *PRKD1* (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 *PRKD1* 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

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

Associations between SNPs and current adult-onset asthma in unexposed and exposed participants are reported in Figure 1. "Flip-Flop" interactions were observed. Near *RELA*, the risk of current adult-onset asthma was increased in G carriers of rs931127 and in T carriers of rs7949980 among exposed participants (OR=1.54, $P=2x10^{-4}$, and OR=1.40, P=0.005 respectively), whereas inverse but not significant effects were observed among unexposed participants. The risk was also increased - although not statistically significant - among exposed participants in G carriers of rs2667042 near *PLA2R1*, whereas inverse and significant effects (OR=0.74, P=0.009) were observed among unexposed participants. On the contrary, the risk of current adult-onset asthma was decreased but not significantly among exposed participants in G carriers in *PLA2G4A*, in G carriers of rs1958980 or G carriers of rs11847351 or T carriers of rs1958987 in *PRKD1*, and in T carriers of rs6504453 in *PRKCA*, and significantly in T carriers of rs6504453 in *PRKCA* (OR=0.79, P=0.05), whereas inverse and significant effects were observed among unexposed participants (OR=1.25 to 1.50, P=0.01 to $3x10^{-4}$).

PRKD1 and *PRKCA* are involved together in the "NRF2-mediated Oxidative Stress Response" pathway, in association with *RELA* in three other pathways: "Xenobiotic Metabolism Signaling", "Production of Nitric Oxide and Reactive Oxygen Species in Macrophages", and "N-formyl-methionyl-leucyl-phenylalanine (fMLP) signaling in neutrophils", or in association with *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, PRKD1* 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 *PRKD1* 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 *PRKD1* 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 *PRKD1* mapped to marks of active regulatory elements notably in
469 B cells, small airways epithelial cells, and lymphoblastoïds 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 *PRKD1* mRNA and
478 methylation of *PLA2R1* (Excel Table S5).
479

DISCUSSION

This study identified interactions between genetic variants near or within five genes, *PLA2G4A*, *PLA2R1*, *RELA*, *PRKD1* and *PRKCA,* and occupational exposures to LMW agents or irritants for current adult-onset asthma. The evidence rests on the results obtained in pooled data of three large European epidemiological studies and the consistency of results across these studies. Functional annotations of the interacting SNPs at these loci and functional supports specific for the GxE interactions detected suggest that a few of these SNPs might be involved in regulatory mechanisms.

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

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

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

To our knowledge, none of our findings have been reported previously by published GWASs of asthma (GWAS-Catalog of Published Genome-Wide Association Studies, http://genome.gov/gwastudies, Hindorff et al.), or by GEWIS studies in asthma. Differences in length of microsatellite sequences in the promoter region of *PLA2G4A* were reported between patients with severe asthma and healthy controls, with a direct impact on mRNA and protein expression, suggesting a role in asthma pathogenesis (Sokolowska et al. 2010). Scarce candidate G x occupational exposure interaction studies have been published for asthma (Kauffmann et al. 2010; Kogevinas 2014; Smit et al. 2014; Cherry et al. 2015). Focusing on occupational 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_1 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. *PRKD1* encodes a
559 serine/threonine kinase, called PKD1 that activates NF-κB 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 *PRKD1* 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 *PRKD1* 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*, *PRKD1* 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.
591

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Table 1. Characteristics of adult participants in the three studies

	All (N=2599)	ECRHS (N=1336)	SAPALDIA (N=574)	EGEA (N=689)
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 (%)[a]	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)

[a]% 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 [a]	Cases/Controls N/N	Marginal effect OR/P-value		Interaction - CC OR/P-value	
1	*PLA2G4A*	rs932476	A/G	0.35	463/2136	1.25	0.0036	0.64	0.0050
2	*PLA2R1*	rs2667026	A/G	0.83	463/2136	0.89	0.2354	1.77	0.0050
11	*RELA* [b]	rs931127	A/G	0.43	462/2135	1.17	0.0350	1.61	0.0014
11	*RELA*	rs7949980	C/T	0.51	463/2133	1.07	0.3421	1.56	0.0030
14	*PRKD1* [b]	rs1958980	A/G	0.67	463/2136	1.08	0.3344	0.64	0.0042
14	*PRKD1*	rs11847351	A/G	0.67	463/2133	1.08	0.3429	0.64	0.0043
14	*PRKD1*	rs1958987	C/T	0.68	459/2127	1.07	0.3609	0.64	0.0050
17	*PRKCA*	rs6504453	C/T	0.35	462/2134	1.04	0.6086	0.63	0.0032

Chr: chromosome, CC: case-control. [a] Effect Allele Frequency (EAF) calculated in controls.
[b]The two SNPs near *RELA* were in moderate Linkage Disequilibrium (LD) with r^2=0.65, whereas the three SNPs in *PRKD1* 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.