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Perturbation of metabolic pathways mediates the association of air pollutants with asthma and cardiovascular diseases

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ABSTRACT

Background: Epidemiologic evidence indicates common risk factors, including air pollution exposure, for respiratory and cardiovascular diseases, suggesting the involvement of common altered molecular pathways.
Objectives: The goal was to find intermediate metabolites or metabolic pathways that could be associated with both air pollutants and health outcomes ("meeting-in-the-middle"), thus shedding light on mechanisms and reinforcing causality.
Methods: We applied a statistical approach named 'meet-in-the-middle' to untargeted metabolomics in two independent case-control studies nested in cohorts on adult-onset asthma (AOA) and cardio-cerebrovascular diseases (CCVD). We compared the results to identify both common and disease-specific altered metabolic pathways.
Results: A novel finding was a strong association of AOA with ultrafine particles (UFP; odds ratio 1.80 [1.26, 2.55] per increase by 5000 particles/cm^3). Further, we have identified several metabolic pathways that potentially mediate the effect of air pollution on health outcomes. Among those, perturbation of Linoleate metabolism pathway was associated with air pollution exposure, AOA and CCVD.

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Conclusions: Our results suggest common pathway perturbations may occur as a consequence of chronic exposure to air pollution leading to increased risk for both AOA and CCVD.

1. Introduction

Asthmatics often suffer from comorbidities including cardiovascular diseases. Comorbidity influences the disease prognosis and control. Refractory asthma is more likely to manifest with cardiovascular comorbidity than controlled asthma (Hekking et al., 2018). Asthma and cardiovascular disease share common risk factors such as smoking, obesity, aging and air pollution exposure, consistent with common molecular pathways altered in the etiology of diseases.

Short-term effects of air pollution exposure on asthma exacerbation have long been established in adults and in children (Peel et al., 2005; Schwartz et al., 1993; Sunyer et al., 1997). The role of air pollution in asthma onset is less conclusive, particularly in adults (Anderson et al., 2013; Jacquemin et al., 2012). Only a few studies used individually assigned exposure estimates to study the effects of ambient air pollution on adult-onset asthma. The largest study sample was based on over 600,000 subjects, including 27,000 asthmatics, and demonstrated an association of PM_{10} exposure – derived from a pan-European land use regression model – with asthma prevalence (Cai et al., 2017). The 'European Study of Cohorts for Air Pollution Effects' (ESCAPE) reported a positive but not statistically significant association with asthma incidence in adults for all air pollution metrics (NO_2, NO, PM_{10}, $PM_{2.5}$, traffic load; traffic intensity) except PM_{coarse} (Jacquemin et al., 2015). In the Swiss SAPALDIA cohort, long term improvement in air pollution levels was associated with an attenuated age-related lung function decline (Downs et al., 2007), with a decreased prevalence of respiratory symptoms including wheezing and breathlessness (Schindler et al., 2009), and with a decreased onset of asthma in adults (Kunzli et al., 2009).

In addition, a growing number of epidemiological studies showed that air pollution is associated with coronary artery disease (McGuinn et al., 2016; Wolf et al., 2015), cardiovascular diseases (Brook et al., 2010; Franklin et al., 2015), and cerebrovascular diseases (Stafoggia et al., 2014) including ischemic stroke (Chung et al., 2017; Cox Jr, 2017). A recent meta-analysis within ESCAPE showed that increases in $PM_{2.5}$ and PM_{10} were associated with risks of fatal and total coronary events, respectively (Cesaroni et al., 2014), and increased risk for cerebrovascular diseases was reported for higher exposure to $PM_{2.5}$ and NO_2(Stafoggia et al., 2014).

Ultrafine particles (UFP) exposure has been less studied than exposure to larger particles, and no regulatory agencies have established guidelines for UFP so far. Compared to larger particulate matter, UFP have distinctive characteristics that may lead to higher toxicity: their extremely small size allows them to reach deeper into the tissues and evade clearance, and higher surface-to-mass ratio facilitates adhesion of larger amounts of hazardous materials. Whether this indeed translates into a higher risk of respiratory or cardiovascular diseases in humans remains to be ascertained (Herbert and Kumar, 2017).

The biological mechanisms explaining the effects of air pollution on asthma and its phenotypes and cardio- and cerebrovascular disease (CCVD) are still poorly understood. The best studied putative biological mechanism is oxidative stress caused by air pollutants, followed by pulmonary and systemic inflammation (Guarnieri and Balmes, 2014; Herbert and Kumar, 2017; Newby et al., 2015; Uzoigwe et al., 2013). Previous studies investigating the association between long-term exposure to air pollution and various inflammatory blood biomarkers reported inconsistent results, concerning specific cytokines and pro- or anti-inflammatory effects (Chuang et al., 2011; Fiorito et al., 2018; Mostafavi et al., 2015).

Large-scale profiling of small molecules in biological samples has become available recently, opening the door to the agnostic interrogation of disease processes at the molecular level in epidemiological settings. The metabolome reflects endogenous processes as well as the influences from environment and behaviors, and therefore metabolomics provides a unique opportunity to link genome, exposome, and disease. Metabolomics has been increasingly applied to investigate asthma and major adverse cardiovascular events (Kelly et al., 2017; Kordalewska and Markuszewski, 2015; Shah et al., 2012; Wurtz et al., 2015). However, few studies conducted an untargeted search for blood biomarkers of air pollution exposure (Vlaanderen et al., 2017) or asthma in adults, and none investigated the link between CCVD, asthma and air pollution.

This study was conducted in the framework of EXPOsOMICS, an EU-funded project to investigate the air- and water-borne exposome (Vineis et al., 2017). One of the research questions EXPOsOMICS addresses is the applicability of the 'meet-in-the-middle (MITM)' concept, i.e. intermediate biomarkers as evidence of causality (Vineis et al., 2013). We have applied the MITM approach within two independent case-control studies nested in cohorts: one on adult-onset asthma (AOA) within the SAPALDIA cohort, the other on CCVD within EPIC Italy cohort, and we compared the results to identify both common and disease-specific altered metabolic pathways.

2. Methods

2.1. Study population

2.1.1. Asthma in SAPALDIA

Adult-onset asthma (AOA) metabolomics was studied in a nested case-control study from the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA). A total of 9651 adults were recruited in eight cities representing different geographical and meteorological environments in Switzerland in 1991 (SAPALDIA1); 8047 and 6088 of them participated in the first follow-up in 2001–3 (SAPALDIA2) and in the second follow-up in 2010–11 (SAPALDIA3), respectively. The study protocol was described in detail previously (Ackermann-Liebrich et al., 2005; Martin et al., 1997). The present study examined blood samples from SAPALDIA3. A detailed description of the population cohort and of the study protocol was described in detail previously (Ackermann-Liebrich et al., 2005; Martin et al., 1997). Briefly, asthma cases were selected among the self-reported diagnosis of asthma occurred later than 16 years of age (n = 141) (Siroux et al., 2014) and with archived blood sample available. Controls were randomly sampled among the participants who never reported the following since SAPALDIA1: self-reported asthma; physician-diagnosed asthma; asthma attack in the last 12 months; current asthma medication; wheezing without cold in the last 12 months; three or more asthma-related symptoms in the last 12 months (symptoms considered: breathless while wheezing; woken up with a feeling of chest tightness; attack of shortness of breath after exercise; attack of shortness of breath while at rest; woken by attack of shortness of breath) (Jacquemin et al., 2015) All cases and controls had not smoked for at least 10 years before blood was drawn. Study participants were non-fasted at the time of blood collection and bench time was < 2 h for all but ten cases and five controls. Subjects' characteristics are summarized in Table 1.

2.1.2. Cardio-cerebrovascular diseases in EPIC Italy

Study participants were part of the Italian component (Turin and Varese centers) of the EPICOR study (Bendinelli et al., 2011), which is the cardiovascular section of the European Prospective Investigation

Table 1
SAPALDIA sample characteristics – adult-onset asthma.

	AOA cases	Controls	AOA cases[a]	Controls[a]
N	139	196	73	115
Age [year]	59.4 (19.4)	57.1 (15.8)	60.3 (19.1)	54.8 (15.5)
Female	87 (63%)	101 (52%)	47 (64%)	62 (54%)
BMI [kg/m^2]	25.7 (6.4)	24.4 (4.8)	27.0 (6.8)	24.7 (4.8)
Smoking[b]				
Former	54 (39%)	62 (32%)	34 (47%)	37 (32%)
Never	85 (61%)	134 (68%)	39 (53%)	78 (68%)
Education level[c]				
Low	3 (2%)	2 (1%)	1 (1%)	2 (2%)
Middle	86 (62%)	121 (62%)	46 (63%)	72 (63%)
High	50 (36%)	73 (37%)	26 (36%)	41 (36%)
Fasting time [h]	2.7 (1.2)	2.8 (1.7)	2.9 (1.8)	2.7 (1.8)
Bench time [min]	80.0 (34.5)	80.0 (28.2)	80.0 (30.0)	80.0 (28.0)
$PM_{2.5}$[d] [μg/m^3]				
t = 1	14.6 (1.9)	14.3 (1.7)	15.4 (1.5)	14.7 (2.0)
t = 2	14.7 (2.2)	14.4 (1.8)	15.7 (2.3)	14.8 (2.4)
t = 3	14.6 (2.8)	14.3 (2.2)	16.0 (2.3)	14.7 (2.4)
t = 4	16.0 (2.8)	15.6 (2.2)	16.7 (1.7)	16.2 (1.9)
t = 5	17.3 (2.3)	17.1 (2.1)	17.8 (1.8)	17.4 (1.9)
t = 6	16.5 (2.4)	16.0 (2.2)	17.2 (1.8)	16.4 (2.3)
t = 7	16.8 (3.4)	16.2 (3.0)	17.6 (3.2)	16.8 (3.2)
PNC [particles/cm^3]	–	–	13,418 (6376)	9660 (7970)
LDSA [μm^2/cm^3]	–	–	33.9 (16.1)	27.1 (16.3)
NO_2 [μg/m^3]	25.0 (14.3)	21.6 (10.9)	29.3 (11.9)	23.7 (15.0)

Data are presented as count (%) or median (interquartile range). $PM_{2.5}$: annual mean estimates derived from the PolluMap in 2010; PNC and LDSA: biennial mean estimates derived from a SAPALDIA multi-area LUR in 2011/2012; NO_2: annual mean estimates derived from a European LUR in 2010.

[a] Data set used for UFP MWASs, number of observation smaller due to limited availability of UFP estimates.

[b] Former smokers had not smoked for at least 10 years before blood was drawn.

[c] Education level low: primary school; middle: secondary/middle school or apprenticeship; high: college or university.

[d] 365 days average t − 1 years before the examination.

into Cancer and Nutrition (EPIC) cohort (Palli et al., 2003). We designed a case-control study nested in the cohort including 386 samples (193 matched case-control pairs), using the incident density sampling method (Richardson, 2004). Criteria for cases and controls selection and matching, and outcome classification and relevant covariates acquisition were described previously (Fiorito et al., 2018) and summarized in Supplementary material. Cases and controls were never smokers or former smokers for at least one year. All subjects were fasting at the time of blood collection and bench time was always lower than 2 h for cases and controls. Table 2 summarizes the subjects' characteristics.

This study complies with the Declaration of Helsinki principles, and conforms to ethical requirements. All volunteers signed an informed consent form at enrolment. The study protocol of SAPALDIA was approved by the Swiss Academy of Medical Sciences and the regional committees for each study center and the one of EPIC by the Ethics Committees at the International Agency for Research on Cancer (Lyon, France) and at the Human Genetics Foundation (now IIGM, Turin, Italy) for EPIC.

2.2. Metabolome analyses

Serum samples were analyzed with a UHPLC-QTOF-MS system (Agilent Technologies, Palo Alto, CA, USA) in randomized order as a single batch within study. The total number of molecular features was 11,909 and 5290 for SAPALDIA and EPIC Italy respectively. A detailed description of laboratory and preprocessing procedures can be found in Supplementary material. The features with non-missing values for at least 60% of the total sample were retained. The final data set contained 7089 and 2790 features for SAPALDIA and EPIC Italy respectively (1452 were in common). In EPIC Italy, additional missing values were imputed using the procedure implemented in the R package imputeLCMD.

2.3. Air pollution exposure estimates

In SAPALDIA, annual mean exposure to $PM_{2.5}$ in 2010 (SAPALDIA3 survey) of study participants was estimated by using PolluMap, a national air pollution dispersion model for Switzerland (FOEN, 2013). Lagged estimates up to 7 years before SAPALDIA3 were obtained by interpolation from Meteotest (FOEN, 2014). Biennial mean exposure to UFP was estimated based on multi-area land use regression (LUR) models derived from SAPALDIA specific-measurement campaigns conducted in 2011/2012 and covering 4 out of 8 SAPALDIA study areas (Eeftens et al., 2016). In EPIC Italy, $PM_{2.5}$ exposure was estimated by a newly developed European LUR model derived from measurements in 2010 (de Hoogh et al., 2016). UFP exposure in Turin was estimated by a local LUR model derived from measurements in 2014/2015 (van Nunen et al., 2017). Both SAPALDIA and EPIC Italy used the NO_2 exposure estimates provided by the aforementioned European LUR model (de Hoogh et al., 2016). In addition to particle number concentration (PNC), lung deposited surface area (LDSA) was used as UFP metric in SAPALDIA. As we relied on LUR models developed to cover limited areas, UFP estimates were available for a subset of samples, 75 AOA cases and 115 controls, and 71 CCVD cases and 73 controls. Each subject was assigned air pollution exposure estimates by geocoding the residential address. In the case of SAPALDIA this was the address at the time point of the SAPALDIA 3 survey. In the case of EPIC Italy this was the address at the time of blood sample collection.

Table 2
EPIC Italy sample characteristics – cardio-cerebrovascular diseases.

	CCVD cases	Controls	CCVD cases[a]	Controls[a]
N	166	155	71	73
Center				
Turin	71 (43%)	73 (47%)	71 (100%)	73 (100%)
Varese	95 (57%)	82 (53%)	–	–
Age [years]	56.16 (9.56)	55.55 (9.44)	58.19 (8.94)	56.95 (9.90)
Female	107 (64%)	95 (61%)	12 (17%)	13 (18%)
BMI [kg/m^2]	26.34 (4.91)	26.09 (4.91)	26.34 (3.98)	26.03 (4.01)
Smoking[b]				
Former	52 (31%)	54 (35%)	38 (53%)	38 (52%)
Never	114 (69%)	101 (65%)	33 (47%)	35 (48%)
Education level[c]				
Low	103 (69%)	84 (56%)	32 (45%)	22 (30%)
Middle	48 (32%)	44 (29%)	29 (41%)	31 (43%)
High	12 (8%)	22 (15%)	10 (14%)	20 (27%)
$PM_{2.5}$ [μg/m^3]	21.27 (2.19)	21.27 (2.16)		
PNC [particles/cm^3]			14,483 (2335)	14,227 (2497)
NO_2 [μg/m^3]	50.30 (14.95)	49.62 (16.48)		

Data are presented as count (%) or median (interquartile range). $PM_{2.5}$: annual mean estimates derived a European LUR in 2010; PNC: annual mean estimates derived from a local LUR in 2014/2015; NO_2: annual mean estimates derived from a European LUR in 2010.

[a] Data set used for UFP MWASs, number of observation smaller due to limited availability of UFP estimates.

[b] Former smokers had not smoked for at least 1 year before blood was drawn.

[c] Education level: low (primary school or none), middle (vocational or another secondary school), and high (university or vocational postsecondary school).

2.4. Statistical analyses

2.4.1. Association of air pollution exposure with AOA

We assessed the effect of air pollution exposure on AOA by fitting logistic regression models. AOA was regressed, with non-asthmatics as the reference, on air pollution exposure after adjustment for age, sex, education level, body mass index (BMI), and study area as random effect. For $PM_{2.5}$, the main predictors were two polynomial lag terms defined as $u_0 = \Sigma_{t=1}{}^{7}PM_{2.5}(t)$ and $u_1 = \sum_{t=1}^{7} t \bullet PM_{2.5}(t)$, where $PM_{2.5}(t)$ is average exposure to $PM_{2.5}$ of 365 days $t-1$ years before SAPALDIA3 examination. For UFP and NO_2, the main predictors were biennial and annual mean estimates respectively. The association was also assessed in the entire SAPALDIA subjects (N = 3011; 272 AOA cases).

2.4.2. Association of air pollution exposure with CCVD

The association of exposure to air pollution with CCVD was assessed in the nested case-control study by logistic regression models adjusting for age at recruitment, center of recruitment, sex, BMI, smoking status, and education level (see Supplementary material for details). In addition, we conducted Cox proportional hazard regression to assess the association between air pollution exposure and the risk of future CCVD among all EPIC subjects (Turin and Varese centers; N = 18,982; 948

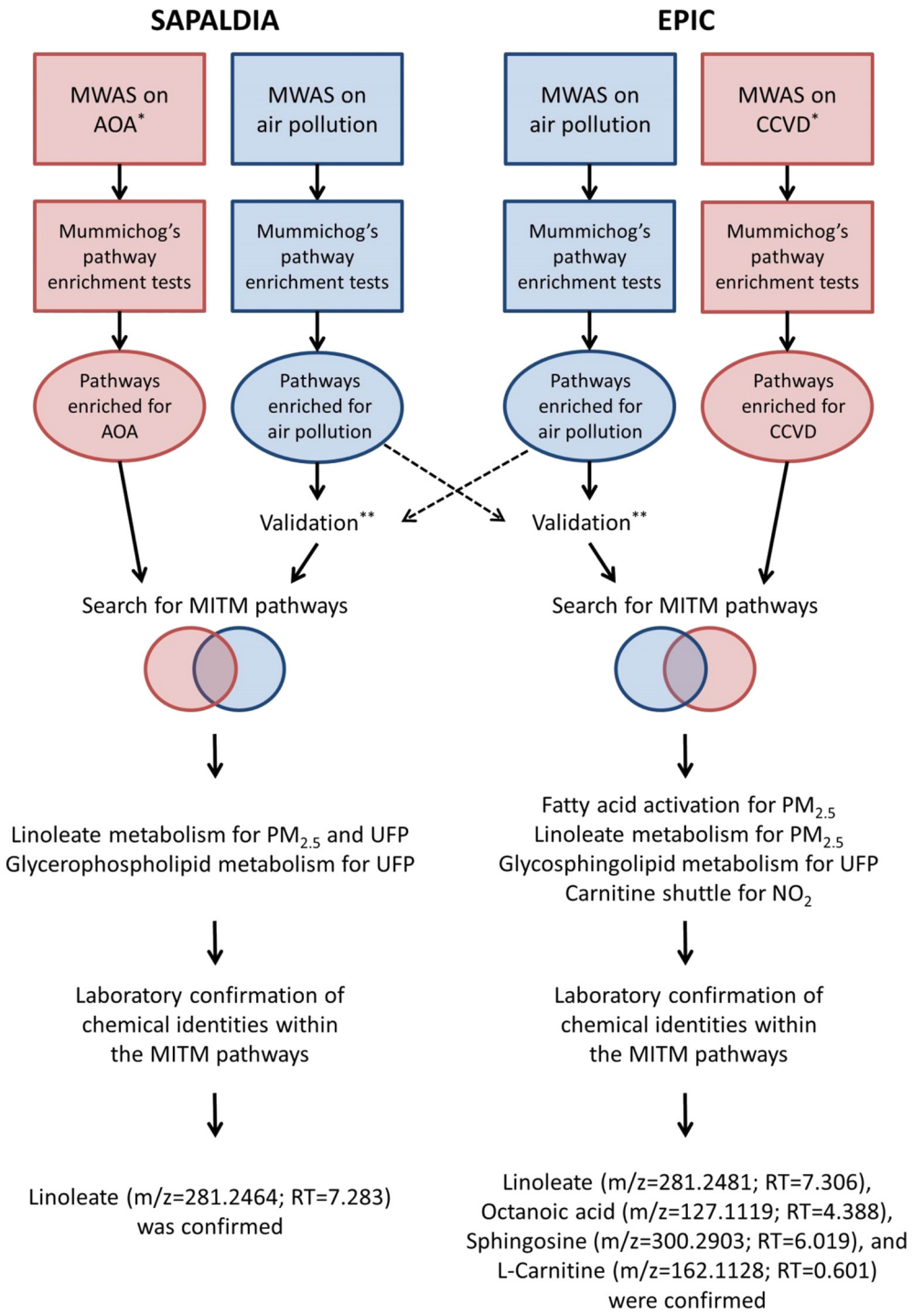

Fig. 1. Search for the MITM pathways.
*Adjusted for the corresponding air pollutant; ** by excluding the pathways not enriched in the other cohort.

CCVD events).

In both studies, odds ratios (OR), hazard ratios (HR), and 95% confidence intervals (CI) refer to an increase of 5 μg/m^3 $PM_{2.5}$, 5000 particles/cm^3 PNC, 10 μm^2/cm^3 LDSA, and 10 μg/m^3 NO_2.

2.4.3. Metabolome-wide association study (MWAS) on AOA

We conducted logistic regression analyses of AOA on each of the 7089 features after adjustment for age, sex, study area, bench time, fasting time, sine and cosine functions of venipuncture time with periods of 24 and 12 h, and their multiplicative interaction terms with fasting time. We did not adjust for smoking because all subjects were non-smokers since 10 years. Feature intensity, age, bench time, and fasting time were scaled to have mean equal 0 and standard deviation equal 1. We applied the Firth's bias-reduction method (Firth, 1993; Perry, 2017) to obtain less biased estimates and the Benjamini-Hochberg method to correct for multiple testing (Benjamini and Hochberg, 1995).

2.4.4. MWAS on CCVD

For each of the 2790 features, we tested for their association with incident CCVD by logistic regression models adjusting for age at recruitment, center of recruitment, sex, BMI, smoking status, and education level.

2.4.5. MWAS on air pollution

In SAPALDIA and EPIC Italy separately, each feature was regressed on $PM_{2.5}$, UFP, or NO_2 after adjustment for the same covariates as in AOA MWAS and in CCVD MWAS, respectively. In SAPALDIA, a binary indicator for perfect geocoding quality was additionally included as a potential modifier of the effect of air pollution exposure on the metabolite level. Geocoding was declared perfect if the matching was possible at the level of residential address. As in the association of air pollution with AOA, first and second order polynomial lag terms were used for $PM_{2.5}$ while biennial and annual mean exposures were used for UFP and NO_2, respectively. In EPIC Italy, annual average exposure was used as the proxy for long-term exposure for each pollutant.

2.4.6. Link and variance functions

In EPIC Italy, feature intensities were Box-Cox transformed before regression (Han and Kronmal, 2004). In SAPALDIA, the best link and variance were sought for each feature and semi-partial pseudo-R^2 was computed as a measure of effect size (see Supplementary material for details).

2.5. Meet-in-the-middle (MITM) approach

2.5.1. Search for MITM features

We examined if any of the features associated with air pollution overlapped with the features associated with AOA or CCVD as an attempt to search for MITM features. As no single feature showed metabolome-wide significant association with AOA or CCVD, we found no single MITM features. Instead, we searched for MITM pathways as described below. The history of our analyses in this study is summarized as flowcharts in Supplementary materials (Fig. S1: MITM features; Fig. 1: MITM pathways).

2.5.2. Functional annotation and pathway enrichment tests using Mummichog

Mummichog is an algorithm developed to predict functional activities of metabolites (Li et al., 2013). Taking untargeted MWAS results as input, Mummichog searches for chemical identities by matching the measured mass (m/z) of the features to a reference metabolic model, integrated from KEGG (Kanehisa et al., 2006), UCSD BiGG (Duarte et al., 2007), and Edinburgh human metabolic network (Ma et al., 2007). Based on this putative annotation, it conducts pathway enrichment tests using Fisher's exact test. The statistical significance of pathway enrichment is estimated by permutation, where the features are randomly selected and mapped to each of the possible annotations to produce null distribution. We customized the types of ions that Mummichog searches for chemical identities, to match with the UHPLC-QTOF-MS method used. Cut-off p-value was chosen to have a reasonable number of significant features to ensure for the algorithm to conduct pathway enrichment analysis. We first used the 10th percentile of the p-values from each MWAS result as the cut-off and then the 5th percentile as a sensitivity analysis (Table S1).

2.5.3. Search for MITM pathways

Pathways found enriched (empirical p-value < 0.05) from Mummichog were listed. The pathways with overlap size – the number of features that contributed to the enrichment – smaller than 4 were ignored. This is an attempt to reduce the false positive findings as Mummichog annotates features only by matching m/z and hence matches are subject to error. The pathways that were not enriched for the same air pollution metric in both SAPALDIA and EPIC Italy were excluded. If the pathway enriched for air pollution metric was also enriched for AOA or CCVD after adjustment for the same metric, they were declared as “MITM” pathways (Figs. S3–S5). The MITM pathways were evaluated by confirmation of the putative annotation which Mummichog used to compute pathway enrichment (see Supplementary material for details).

3. Results

3.1. Exposure to UFP is associated with AOA

From logistic regression of AOA (n = 73) with non-asthmatics as the reference group (n = 115), we found a strong association of UFP exposure with AOA (Table 3). The odds ratios were 1.80 [95% CI 1.26, 2.55] for an increase in particle number concentration (PNC) by 5000 particles/cm^3, and 1.73 [95% CI 1.27, 2.36] for an increase in lung deposited surface area (LDSA) by 10 μm^2/cm^3. On the contrary, $PM_{2.5}$ and NO_2 did not show a significant association with AOA. The estimated risk for AOA due to UFP exposure is still significant after the inclusion of the other pollutants in the regression model, supporting the independence of the effect, although ORs were lower when estimated in the whole cohort (Table S2).

3.2. Weak but consistent association of air pollution with CCVD

We have observed a positive association of exposure to $PM_{2.5}$, PNC, and NO_2 with the risk of CCVD (OR = 1.34 [95% CI 0.72, 2.52] for 10 μg/m^3 increase in $PM_{2.5}$; OR = 1.09 [95% CI 0.60, 2.00] for 5000 particles/cm^3 increase in PNC; OR = 1.03 [95% CI 0.89, 1.18] for μg/cm^3 increase in NO_2), though the associations did not reach statistical significance (Table 3). However, when we expanded the analyses to the whole EPIC Turin-Varese subjects (N = 18,982; 948 CCVD events), the associations became stronger and significant (HR = 1.29 [95% CI 1.08, 1.55] for 10 μg/m^3 increase in $PM_{2.5}$; HR = 1.16 [95% CI 0.97, 1.39] for 5000 particles/cm^3 increase in PNC (Turin subjects; N = 8753); HR = 1.12 [95% CI 0.99, 1.27] for μg/cm^3 increase in NO_2).

3.3. MWAS: no single metabolites are associated with both air pollution and AOA or CCVD

None of the 7089 features in SAPALDIA or 2790 features in EPIC Italy showed a significant association with AOA or CCVD after multiple testing corrections, respectively (Fig. S1). The air pollution MWAS in SAPALDIA showed 237, three, six and one features significantly associated with $PM_{2.5}$, PNC, LDSA, and NO_2, respectively (Fig. 2). One of the three PNC associated features coincided with the LDSA associated features. Five out of the eight UFP associated features were not

Table 3
Association of air pollution with AOA and CCVD.

Air pollution metric	AOA		CCVD	
	OR[a] [95% CI]	OR[b] [95% CI]	OR[c] [95% CI]	HR[d] [95% CI]
$PM_{2.5}$[e]	1.05 [0.57, 1.95]	1.00 [0.65, 1.56]	1.34 [0.72, 2.52]	1.29 [1.08, 1.55]
PNC[f]	1.80 [1.26, 2.55]	1.39 [1.03, 1.87]	1.09 [0.60, 2.00]	1.16 [0.97, 1.39]
LDSA[g]	1.73 [1.27, 2.36]	1.36 [1.04, 1.79]	–	–
NO_2[h]	1.12 [0.81, 1.55]	1.16 [0.94, 1.43]	1.03 [0.89, 1.18]	1.12 [0.99, 1.27]

Note: Odds ratios are reported for all cross-sectional analyses (meet-in-the-middle/metabolome subsample) while hazard ratio is reported for the longitudinal analysis on larger CCVD samples; sample size is smaller for UFP than other pollutants because the LUR models were derived only for 4 out of 8 study areas in SAPALDIA and for Turin but not for Varese in EPIC Italy.

[a] Odds ratio adjusted for age, sex, education level, BMI, and study area as random effect (N = 335 for $PM_{2.5}$ and NO_2; N = 188 for UFP).

[b] Odds ratio adjusted for age, sex, education level, BMI, and study area as random effect (N = 3011 for $PM_{2.5}$ and NO_2; N = 1555 for UFP).

[c] Odds ratio adjusted for age, center of recruitment, sex, BMI, smoking status, and educational level (N = 321 for $PM_{2.5}$ and NO_2; N = 144 for UFP).

[d] Hazard ratio adjusted for age, center of recruitment, sex, BMI, smoking status, and educational level (N = 18,982 for $PM_{2.5}$ and NO_2; N = 8753 for UFP).

[e] Per 5 μg/m^3 increase in biennial (SAPALDIA) or annual (EPIC Italy) mean $PM_{2.5}$.

[f] Per increase by 5000 particles/cm^3 in biennial (SAPALDIA) or annual (EPIC Italy) mean PNC.

[g] Per increase by 10 μm^2/cm^3 in biennial mean LDSA.

[h] Per increase by 10 μg/m^3 in annual mean NO_2.

associated with any other air pollutant. The only NO_2 associated feature was also associated with $PM_{2.5}$ (Fig. S2). In EPIC Italy, no single feature showed a significant association with air pollution exposure, nor with CCVD after multiple testing corrections (Fig. 3). The top 100 signals from each of the air pollution MWASs in both cohorts are summarized in Supplementary material with putative annotation.

3.4. Several metabolic pathways are commonly associated with air pollution in both cohorts

Various pathways were associated with air pollution varying with the air pollutant and the cohort examined (Fig. 1, Tables S3–S9). The pathways that were enriched for the same air pollutant in both cohorts are summarized in Table 4 and Figs. S3–S5: Linoleate metabolism and Fatty acid activation were enriched for $PM_{2.5}$; Linoleate metabolism, glycerophospholipid metabolism, and glycosphingolipid metabolism for UFP; carnitine shuttle and pyrimidine metabolism for NO_2. No overlap was found looking at the list of features that contributed to the enrichment in the two studies (Table S10). We then repeated the same enrichment analysis using the 5th percentile p-value as the cut-off, as a sensitivity analysis. Linoleate metabolism and glycerophospholipid metabolism, associated to UFP, were confirmed in both cohorts. All the pathways associated to NO_2, carnitine shuttle and pyrimidine metabolism, were also confirmed.

3.5. Pathways enrichment and MITM analysis for AOA and CCVD

We found various altered metabolic pathways associated with AOA and CCVD (Fig. 1, Tables 5 and 6). The majority of the enriched pathways did not overlap between AOA and CCVD. Pathways associated with AOA and CCVD, respectively, after adjustment for single air pollution metrics to identify MITM pathways are presented in Tables S11–17.

3.6. Linoleate metabolism is a common MITM pathway linking air pollution to AOA and CCVD

Linoleate metabolism was enriched for $PM_{2.5}$ and UFP in both cohorts and for AOA after adjustment for $PM_{2.5}$ or UFP (Tables S11–S13) as well as for CCVD after adjustment for $PM_{2.5}$ (Table S15). Therefore, we considered Linoleate metabolism as MITM linking $PM_{2.5}$ and UFP to AOA and $PM_{2.5}$ to CCVD. Similarly, we considered glycerophospholipid metabolism as MITM linking UFP to AOA (Table S13); fatty acid activation, glycosphingolipid metabolism, and carnitine shuttle as MITM linking $PM_{2.5}$, UFP, or NO_2 to CCVD, respectively (Tables S15–S17).

Linoleate metabolism and glycerophospholipid metabolism were confirmed as MITM pathways linking UFP to AOA after the sensitivity analysis (5th percentile of p-values as the cut-off), as well as glycosphingolipid metabolism linking UFP to CCVD, and carnitine shuttle linking NO_2 to CCVD.

3.7. Confirmed annotation of metabolites in MITM pathways

A total of 108 features mapping to the aforementioned MITM pathways were selected for confirmation of the putative annotation. Table 7 summarizes all the features whose annotation was confirmed using chemical standards and fragmentation spectra. Linoleate was confirmed in both cohorts with confidence level 1 according to the classification of the Chemical Analysis Working Group (CAWG) (Sumner et al., 2007). In SAPALDIA, linoleate was considered as a signal for the AOA MWAS further adjusted for UFP and contributed to the enrichment of linoleate metabolism and glycerophospholipid metabolism. In EPIC Italy, linoleate was considered as a signal for the $PM_{2.5}$ MWAS and contributed to the enrichment of linoleate metabolism. Also confirmed were octanoic acid, sphingosine, and L-carnitine, contributing in EPIC Italy to the enrichment of fatty acid activation for $PM_{2.5}$, glycosphingolipid metabolism for UFP, and carnitine shuttle for CCVD adjusted for NO_2, respectively. Five additional features were confirmed for their chemical classes with confidence level 3 for the CAWG (Sumner et al., 2007).

3.8. Additional sensitivity analyses

For consistency between the two studies, we performed further sensitivity analyses on AOA. Additional adjustment for education level resulted in a non-relevant change of the results, while adjustment for BMI slightly changed the results (Table S18). In the pathway enrichment analyses, glycerophospholipid metabolism remained as MITM linking UFP to AOA after adjustment for BMI or for education level. Linoleate metabolism remained as MITM linking UFP to AOA after adjustment for education level but not after adjustment for BMI.

4. Discussion

In short-term studies, UFP exposure has been reported to have cardio-respiratory effects that were stronger than for larger particles. Peters et al. reported that UFP exposure had a stronger effect on peak expiratory flow than larger particles (Peters et al., 1997). Exposure to UFP but not to larger particles was associated with asthma exacerbations in children (Evans et al., 2014). However, a recent in vitro study showed that coarse particles might have stronger effects on airway epithelium, possibly due to the higher iron content in coarse particles (Kumar et al., 2015). Studies investigating the long-term cardio-respiratory effects of UFP exposure remain very limited. In the California Teachers Study cohort, UFP exposure derived from a chemical transport model was associated with all-cause and ischemic heart disease mortality (Ostro et al., 2015). In the SAPALDIA cohort, UFP exposure was associated with carotid-intima media thickness, a marker of subclinical

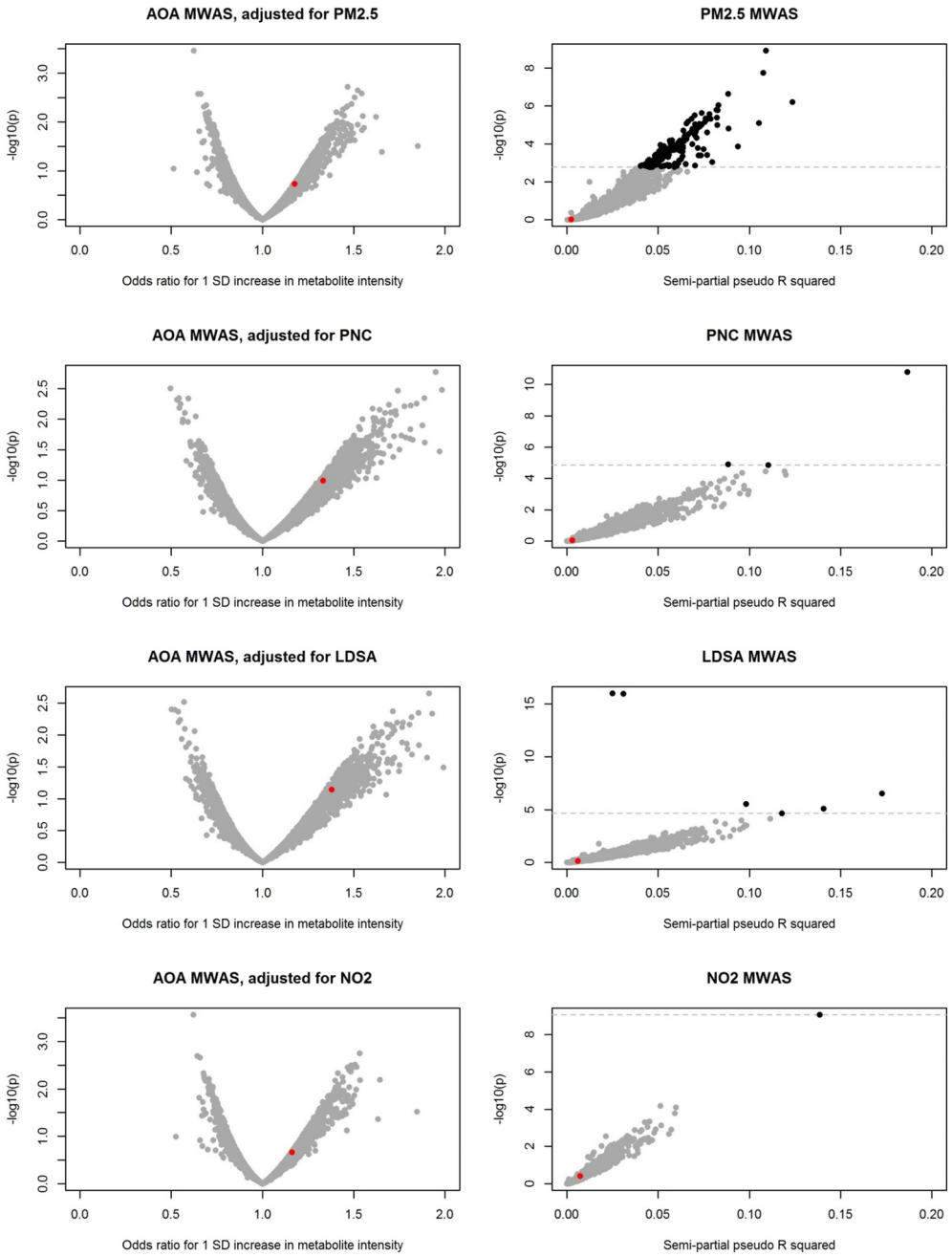

Fig. 2. Volcano plots of MWAS results in SAPALDIA.
Note the asymmetric distribution of points in air pollution MWASs due to the positive nature of semi-partial pseudo-R^2 used as a measure of effect size. Linoleate (*m/z* = 281.2464; RT = 7.283) whose annotation was confirmed with confidence level 1 is highlighted in red; Metabolome-wide signals after Benjamini-Hochberg correction in black. Dotted line depicts Benjamini-Hochberg adjusted p = 0.05.

atherosclerosis (Aguilera et al., 2016). UFP exposure derived from a city-specific LUR model in Toronto linked to health registry data of 1.1 million adult city residents found no positive association of UFP exposure with respiratory disease incidence including AOA (Weichenthal et al., 2017). This is in contrast to our findings, which are based on individual reports of asthma and which provide evidence of UFP effects being stronger than, and independent of, those of larger particles. Traffic-related pollutants contribute mainly to the fine or ultrafine particles, while specks of dust of geological origin including metals link to the coarse particles (Kelly and Fussell, 2012; Yamada et al., 2005). Particulates of various sizes may have different toxicity dependent on their composition (Kumar et al., 2015; Schwarze et al., 2007).

4.1. Meet-in-the-middle (MITM) approach

We applied the 'meet-in-the-middle (MITM)' approach, which helps in developing a causal hypothesis and improve biological understanding for air pollution-cardio-respiratory health associations, making use of high-resolution metabolomic data. In the MITM approach, one searches for intermediate biomarkers that are associated with both the exposure and the outcome (Vineis et al., 2013). Ideally, this applies to longitudinal studies where the exposure precedes the biomarker measurement, and the biomarker measurement precedes the outcome, e.g. incidence of cardiovascular events, as we did for CCVD in EPIC Italy. It is much less straightforward to define incident cases for asthma than for CCVD. Asthma is a complex chronic disease phenotype

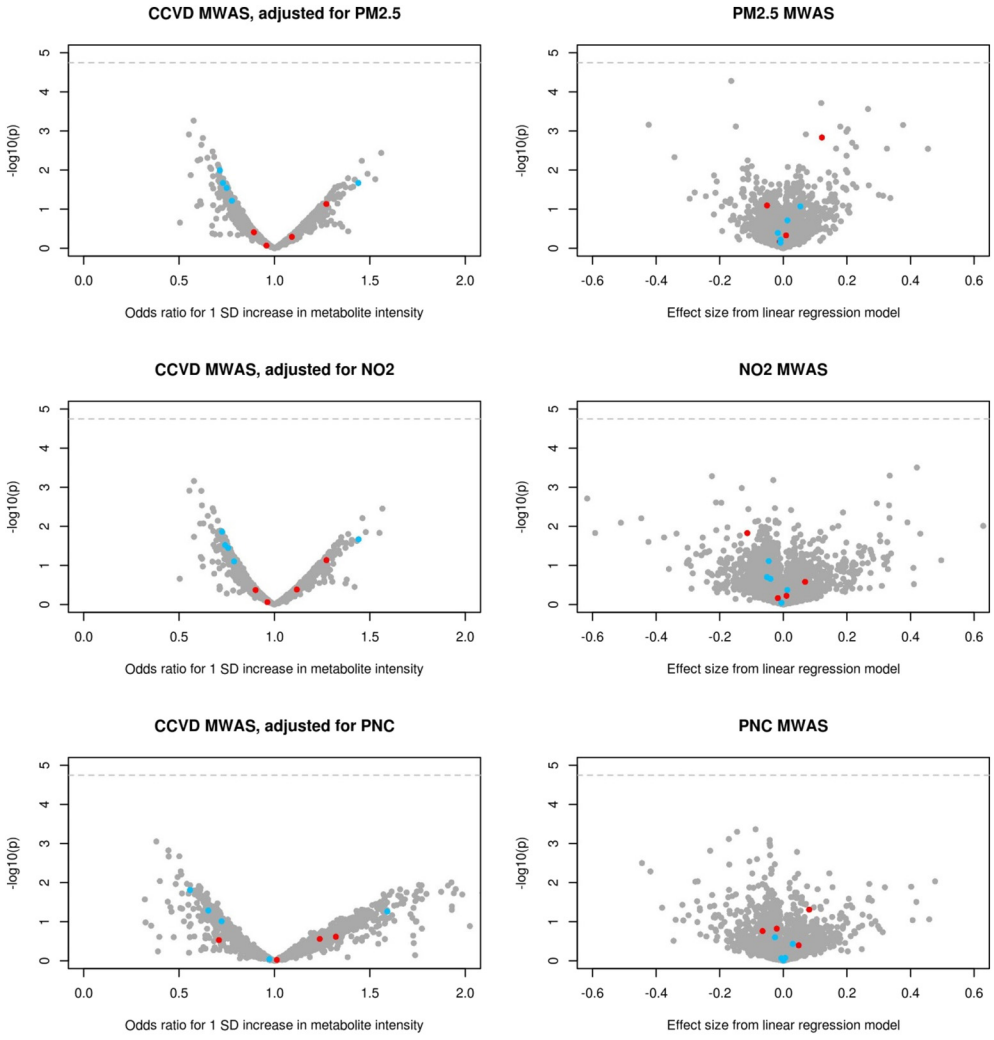

Fig. 3. Volcano plots of MWAS results in EPIC Italy.
Metabolites whose annotation was confirmed with confidence level 1 are highlighted in red: Linoleate (m/z = 281.2481; RT = 7.306), octanoic acid (m/z = 127.1119; RT = 4.388), sphingosine (m/z = 300.2903; RT = 6.019), and L-carnitine (m/z = 162.1128; RT = 0.601); metabolites whose annotation was confirmed with confidence level 3 are in blue: α-Linolenic acid (m/z = 279.2321; RT = 7.166), D-glucose (m/z = 145.0495; RT = 0.646), linoelaidyl carnitine (m/z = 424.3428; RT = 6.199), octadecenoyl carnitine (m/z = 426.3590; RT = 6.337), and stearoylcarnitine (m/z = 428.373; RT = 6.479). No metabolome-wide signals after Benjamini-Hochberg correction.

that develops over a long period of time, can go unnoticed for years if not for decades, and can also disappear as well as resurface. This difficulty inherent to asthma research is complicating the assessment of causality to identified risks such as air pollution. Realizing this difficulty, we pursued the MITM approach for asthma even though our study is by design cross-sectional. For all these reasons, we restricted the outcome to adult (after the 16 years of age) onset of asthma which is less susceptible to reverse causation bias and exposure misclassification.

4.2. MWAS analyses

At the level of single metabolites, we found no intermediate biomarkers among the 7089 and 2790 features investigated in SAPALDIA and EPIC Italy respectively, due to lacking metabolome-wide significant associations. Multiple testing corrections can be too stringent, given the highly inter-correlated nature of the metabolome. The effective number of tests (ENT) computed for the SAPALDIA metabolome was 2728, indicating a high degree of dependency in the data. Given this highly correlated, high dimensional data structure, our study likely suffers from low power to detect subtle differences related to chronic diseases,

Table 4
Pathways associated to air pollution in both SAPALDIA and EPIC Italy.

Air pollutant	Pathway	SAPALDIA			EPIC Italy		
		Overlap size	Pathway size	p-Value	Overlap size	Pathway size	p-Value
$PM_{2.5}$	Linoleate metabolism[b,c]	17	21	0.0007	6	20	0.0249
	Fatty acid activation[c]	10	21	0.0054	5	15	0.0180
UFP[a]	Linoleate metabolism[b]	12	21	0.0007	7	20	0.0084
	Glycerophospholipid metabolism[b]	12	36	0.0023	13	35	0.0022
	Glycosphingolipid metabolism[c]	8	26	0.0079	6	21	0.0367
NO_2	Carnitine shuttle[c]	10	26	0.0063	6	19	0.0040
	Pyrimidine metabolism	12	33	0.0074	8	28	0.0035

[a] Either PNC or LDSA in SAPALDIA and PNC in EPIC Italy.
[b] Also enriched for AOA after further adjustment for the corresponding air pollutant.
[c] Also enriched for CCVD after further adjustment for the corresponding air pollutant.

Table 5
Pathways associated to AOA unadjusted for air pollution exposure.

Pathway	Overlap size	Pathway size	p-Value
Tryptophan metabolism	20	54	0.0009
Vitamin B6 (pyridoxine) metabolism	4	6	0.0017
Biopterin metabolism	6	13	0.0021
TCA cycle	4	8	0.0041
Hexose phosphorylation	5	12	0.0048
Fatty acid metabolism	5	14	0.0101
De novo fatty acid biosynthesis	7	22	0.0102
Drug metabolism - cytochrome P450	12	42	0.0102
Valine, leucine and isoleucine degradation	7	23	0.0137
Urea cycle/amino group metabolism	9	32	0.0169
Fatty acid activation	6	21	0.0258
Leukotriene metabolism	13	51	0.0278
Butanoate metabolism	5	17	0.0284
Glycosphingolipid metabolism	7	26	0.0312
Lysine metabolism	6	22	0.0342
Drug metabolism - other enzymes	5	18	0.0387
Arginine and proline metabolism	6	23	0.0447
Starch and sucrose metabolism	4	14	0.0458
Pentose and glucuronate interconversions	4	14	0.0458
Vitamin E metabolism	8	32	0.0461

Mummichog pathway enrichment test on the results from AOA MWAS adjusted for age, sex, study area, bench time, fasting time, sine and cosine functions of venipuncture time with periods of 24 and 12 h, and their multiplicative interaction terms with fasting time.

Table 6
Pathways associated to CCVD unadjusted for air pollution exposure.

Pathway	Overlap size	Pathway size	p-Value
De novo fatty acid biosynthesis	9	14	0.0011
Hexose phosphorylation	8	12	0.0012
Phosphatidylinositol phosphate metabolism	6	10	0.0031
Carnitine shuttle	9	19	0.0047
Starch and sucrose metabolism	6	11	0.0051
Linoleate metabolism	9	20	0.0070
Glycosphingolipid metabolism	9	21	0.0105
Glutamate metabolism	5	10	0.0139
Caffeine metabolism	5	11	0.0249
Fatty acid activation	6	15	0.0398
Glycolysis and gluconeogenesis	4	9	0.0479
Fructose and mannose metabolism	4	9	0.0479

Mummichog pathway enrichment test on the results from CCVD MWAS adjusted for age at recruitment, center of recruitment, sex, BMI, smoking status, and education level.

and in particular to asthma, where distinguishing sub-phenotypes may be essential for understanding risk and etiology of the disease (Jeong et al., 2017; Siroux et al., 2014; Wenzel, 2012). Therefore, heterogeneity and misclassification might have attenuated the associations with biomarkers. Distinguishing further sub-phenotypes requires larger data in future metabolome studies. Given the above, we focused on pathway enrichment analyses.

4.3. Pathway enrichment analyses

Metabolomics, given the high dimensionality and high dependency, benefits much from multivariate systems approaches like pathway enrichment tests. Yet, the challenge unique to metabolomics in this context is annotation. Unlike other omics, annotation of the features obtained from untargeted metabolomics requires laborious manual work. The Mummichog software offers an opportunity to bypass this step and to conduct pathway enrichment tests directly from untargeted MWAS results. Using Mummichog, we found various pathways enriched for AOA, CCVD, and air pollution exposures. Air pollution MWASs and pathway enrichment tests conducted in two cohorts served as each other's validation. Although we found no single overlapping features between the two cohorts when comparing validated pathways, lack of such overlap does not exclude the possibility that the pathways truly reflect air pollution-induced metabolic changes, involving different molecules. The specific molecules affected in a pathway may, for example, depend on the particle composition which can vary across different areas (Kelly and Fussell, 2012).

4.4. Linoleate metabolism is a common MITM pathway for AOA and CCVD

AOA and CCVD were mostly associated with different sets of pathways and hence MITM pathways linking air pollution exposure to both chronic diseases differed. The two chronic diseases may involve different biological mechanisms and the same environmental insults may act through different pathways. One exception was linoleate metabolism pathway, which was found not only as MITM pathway linking $PM_{2.5}$ and UFP to AOA but also linking $PM_{2.5}$ to CCVD. Laboratory analysis confirmed the annotation of linoleate in both cohorts. The feature confirmed as linoleate showed a positive association with AOA, while it did not show statistically significant association with UFPs exposure and did not contribute to the pathway enrichment for UFPs. Still, the lineolate MITM-pathway finding seems biologically interesting. Linoleate was reported in an in vitro experiment to regulate the pro-inflammatory cytokine IL8 (Maruyama et al., 2014) and induce smooth muscle contraction via the free fatty acid receptor 1 (FFAR1) (Mizuta et al., 2015). Another in vitro study demonstrated that α1-antitrypsin bound to linoleate reduced the expression and secretion of IL1β in LPS-stimulated neutrophils, while free α1-antitrypin did not (Aggarwal et al., 2016). In observational studies in children, eczema was positively associated with linoleate intake (Miyake et al., 2011) and atopy with circulating linoleate (Yen et al., 2008). A recent targeted metabolomic study investigated 64 lipid metabolites and reported Linoleate metabolism and Arachidonic acid metabolism as the top pathways albeit not statistically significantly associated with asthma control (McGeachie et al., 2015). Few studies associated linoleate with CCVD, although in general ω−6 fatty acids have long been believed to have pro-inflammatory effects in the cardiovascular system. An early in vitro study suggested that linoleate may lead to atherogenesis by NFκB signaling mediated vascular adhesion molecule-1 (VCAM-1) expression (Dichtl et al., 2002).

4.5. CCVD specific MITM pathways

Glycosphingolipid metabolism was found as MITM pathway linking exposure to UFP and CCVD and annotation of sphingosine was confirmed as one of the modulated metabolites in this pathway. Sphingolipids are structural components of cell membrane but known to play a crucial role in apoptosis, cell growth, senescence, and cell cycle control (Yang et al., 2004). Sphingolipids in blood have been associated with cardiovascular diseases including acute coronary syndrome (Pan et al., 2014) and myocardial infarction (Park et al., 2015). A recent clinical trial reported a strong association between blood sphingolipids and incident cardiovascular diseases (Wang et al., 2017). Sphingolipids have also been associated with asthma (Petrache and Berdyshev, 2016) in contrast to our findings. Perturbation of sphingolipid metabolism may be more relevant for allergic or child-onset asthma (Ono et al., 2015).

Carnitine shuttle pathway was identified as a MITM pathway linking exposure to NO_2 and CCVD. Carnitines facilitate the transport of long-chain fatty acids from the cytosol into the mitochondria and play an important role in fatty acid metabolism and carbohydrate utilization. The role of L-carnitine in CCVD has been extensively described, reporting protective effects of L-carnitine administration for various cardiovascular diseases including coronary artery disease, congestive heart failure, and hypertension (Ferrari et al., 2004). A recent meta-

Table 7
MWAS results for features with confirmed annotation.

Metabolite	Putative annotation from Mummichog	Level of confidence (Sumner et al., 2007)	Pathway	MWAS	Regression model	Coefficient	p-Value	Pseudo-R^{2a}
m/z = 281.2464 RT = 7.283	Linoleate	Level 1	Linoleate metabolism; glycerophospholipid metabolism	AOA, PNC adjusted	Logistic	0.29	0.10	–
				AOA, LDSA adjusted	Logistic	0.32	0.071	–
				PNC in SAPALDIA	Gamma with log link	7.9e−7	0.86	0.0030
				LDSA in SAPALDIA	Gamma with log link	−0.00026	0.69	0.0059
m/z = 281.2481 RT = 7.306	Linoleate	Level 1	Fatty acid activation; linoleate metabolism	CCVD, $PM_{2.5}$ adjusted	Logistic	1.05	0.40	–
				$PM_{2.5}$ in EPIC Italy	Linear	0.06	0.001	–
m/z = 127.1119 RT = 4.388	Octanoic acid	Level 1	Fatty acid activation	CCVD, $PM_{2.5}$ adjusted	Logistic	0.93	0.17	–
				$PM_{2.5}$ in EPIC Italy	Linear	−0.03	0.08	–
m/z = 300.2903 RT = 6.019	Sphingosine	Level 1	Glycosphingolipid metabolism	CCVD, PNC adjusted	Logistic	0.65	0.30	–
				PNC in EPIC Italy	Linear	0.0003	0.08	–
m/z = 162.1128 RT = 0.601	L-Carnitine	Level 1	Carnitine shuttle	CCVD, NO_2 adjusted	Logistic	3.24	0.07	–
				NO_2 in EPIC Italy	Linear	0.001	0.59	–
m/z = 279.2321 RT = 7.166	α-Linolenic acid; γ-linolenic acid	Level 3	Linoleate metabolism; fatty acid activation	CCVD, $PM_{2.5}$ adjusted	Logistic	0.51	0.01	–
				$PM_{2.5}$ in EPIC Italy	Linear	0.03	0.08	–
m/z = 145.0495 RT = 0.646	D-Glucose; galactose	Level 3	Glycosphingolipid metabolism	CCVD, PNC adjusted	Logistic	1.59	0.05	–
				PNC in EPIC Italy	Linear	0.0001	0.97	–
m/z = 424.3428 RT = 6.199	Linoelaidyl carnitine; linoleyl carnitine	Level 3	Carnitine shuttle	CCVD, NO_2 adjusted	Logistic	0.79	0.08	–
				NO_2 in EPIC Italy	Linear	−0.007	0.20	–
m/z = 426.3590 RT = 6.337	Octadecenoyl carnitine; vaccenyl carnitine; elaidic carnitine	Level 3	Carnitine shuttle	CCVD, NO_2 adjusted	Logistic	0.75	0.03	–
				NO_2 in EPIC Italy	Linear	−0.005	0.22	–
m/z = 428.373 RT = 6.479	Stearoylcarnitine	Level 3	Carnitine shuttle	CCVD, NO_2 adjusted	Logistic	0.34	0.03	–
				NO_2 in EPIC Italy	Linear	−0.006	0.08	–

Level 1: identified compounds; Level 3: putatively characterized compound class.
[a] Semi-partial pseudo R^2 as a measure of effect size for air pollution MWASs in SAPALDIA: for details see Supplementary information.

analysis of randomized controlled trials demonstrated the efficacy of L-carnitine against chronic heart failure (Song et al., 2017). In an experimental study in rats, inflammation accompanied with hypertension was attenuated by L-carnitine administration (Miguel-Carrasco et al., 2008). In this study, however, L-carnitine was associated with increased risk of CCVD.

4.6. Strengths and limitations

Strengths of our study include its prospective nature (nested in longitudinal cohorts), the individual assessment of exposure to air pollution, the accurate diagnoses for the outcomes, the agnostic nature of our metabolome-wide measurements, and the application of 'meet-in-the-middle' as a novel approach helping in the causal interpretation of the results. We focused on biological pathways that were associated with air pollution (mostly UFP) in both studies, supporting the robustness and replicability of our findings. Limitations include the small sample size for metabolome-wide analyses; we focused on pathways enrichment but we were not able to identify single features associated with both air pollution and at least one disease due to the lack of statistical power. Also, we used slightly different statistical methods (including the set of confounders) in the two studies, mainly due to the nature of the outcomes and the quite different estimation of exposure in the two studies. For example, unlike in EPIC Italy, we did not adjust for BMI in SAPALDIA. Air pollution exposure can increase the risk of obesity (Eze et al., 2015; Wei et al., 2016) and obesity may have a causal effect on asthma (Wenzel, 2012), therefore adjustment for BMI can lead to missing some signals. Given the smaller sample size and expected subtle effects, parsimony was more strongly sought in AOA MWAS. And a previous study observed less strong association between socioeconomic status and air pollution exposure in Switzerland than in Italy (Temam et al., 2017). Sensitivity analysis showed that the additional adjustment did not change the results.

5. Conclusions

In summary, we successfully applied a MITM approach in untargeted metabolomics to produce evidence of common and disease-specific pathway perturbations in the etiological relationship between air pollution exposure, AOA, and CCVD. Our findings need to be confirmed in future targeted and untargeted studies.

Data availability

Raw metabolomic data that support the findings of this study are available from EXPOsOMICS but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of EXPOsOMICS.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.06.025.

References

Ackermann-Liebrich, U., Kuna-Dibbert, B., Probst-Hensch, N.M., Schindler, C., Dietrich, D.F., Stutz, E.Z., et al., 2005. Follow-up of the Swiss Cohort Study on air pollution and lung diseases in adults (SAPALDIA 2) 1991–2003: methods and characterization of participants. Soz. Praventivmed. 50, 245–263.
Aggarwal, N., Korenbaum, E., Mahadeva, R., Immenschuh, S., Grau, V., Dinarello, C.A., et al., 2016. A-linoleic acid enhances the capacity of α1-antitrypsin to inhibit lipopolysaccharide-induced IL-1β in human blood neutrophils. Mol. Med. 22, 680–693.
Aguilera, I., Dratva, J., Caviezel, S., Burdet, L., de Groot, E., Ducret-Stich, R.E., et al., 2016. Particulate matter and subclinical atherosclerosis: associations between different particle sizes and sources with carotid intima-media thickness in the SAPALDIA study. Environ. Health Perspect. 124, 1700–1706.
Anderson, H.R., Favarato, G., Atkinson, R.W., 2013. Long-term exposure to air pollution and the incidence of asthma: meta-analysis of cohort studies. Air Qual. Atmos. Health 6, 47–56.
Bendinelli, B., Masala, G., Saieva, C., Salvini, S., Calonico, C., Sacerdote, C., et al., 2011. Fruit, vegetables, and olive oil and risk of coronary heart disease in Italian women: the EPICOR Study. Am. J. Clin. Nutr. 93, 275–283.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 289–300.
Brook, R.D., Rajagopalan, S., Pope 3rd, C.A., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., et al., 2010. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121, 2331–2378.
Cai, Y., Zijlema, W.L., Doiron, D., Blangiardo, M., Burton, P.R., Fortier, I., et al., 2017. Ambient air pollution, traffic noise and adult asthma prevalence: a BioSHaRE approach. Eur. Respir. J. 49.
Cesaroni, G., Forastiere, F., Stafoggia, M., Andersen, Z.J., Badaloni, C., Beelen, R., et al., 2014. Long term exposure to ambient air pollution and incidence of acute coronary events: prospective cohort study and meta-analysis in 11 European cohorts from the ESCAPE Project. BMJ (Clin. Res. Ed.) f7412, 348.
Chuang, K.J., Yan, Y.H., Chiu, S.Y., Cheng, T.J., 2011. Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. Occup. Environ. Med. 68, 64–68.
Chung, J.W., Bang, O.Y., Ahn, K., Park, S.S., Park, T.H., Kim, J.G., et al., 2017. Air pollution is associated with ischemic stroke via cardiogenic embolism. Stroke 48, 17–23.
Cox Jr., L.A., 2017. Socioeconomic and air pollution correlates of adult asthma, heart attack, and stroke risks in the United States, 2010–2013. Environ. Res. 155, 92–107.
de Hoogh, K., Gulliver, J., Donkelaar, A.V., Martin, R.V., Marshall, J.D., Bechle, M.J., et al., 2016. Development of West-European PM2.5 and NO2 land use regression models incorporating satellite-derived and chemical transport modelling data. Environ. Res. 151, 1–10.
Dichtl, W., Ares, M.P., Jonson, A.N., Jovinge, S., Pachinger, O., Giachelli, C.M., et al., 2002. Linoleic acid-stimulated vascular adhesion molecule-1 expression in endothelial cells depends on nuclear factor-kappab activation. Metab. Clin. Exp. 51, 327–333.
Downs, S.H., Schindler, C., Liu, L.J., Keidel, D., Bayer-Oglesby, L., Brutsche, M.H., et al., 2007. Reduced exposure to PM10 and attenuated age-related decline in lung function. N. Engl. J. Med. 357, 2338–2347.
Duarte, N.C., Becker, S.A., Jamshidi, N., Thiele, I., Mo, M.L., Vo, T.D., et al., 2007. Global reconstruction of the human metabolic network based on genomic and bibliomic data. Proc. Natl. Acad. Sci. U. S. A. 104, 1777–1782.
Eeftens, M., Meier, R., Schindler, C., Aguilera, I., Phuleria, H., Ineichen, A., et al., 2016. Development of land use regression models for nitrogen dioxide, ultrafine particles, lung deposited surface area, and four other markers of particulate matter pollution in the Swiss SAPALDIA regions. Environ. Health 15, 1–14.
Evans, K.A., Halterman, J.S., Hopke, P.K., Fagnano, M., Rich, D.Q., 2014. Increased ultrafine particles and carbon monoxide concentrations are associated with asthma exacerbation among urban children. Environ. Res. 129, 11–19.
Eze, I.C., Schaffner, E., Foraster, M., Imboden, M., von Eckardstein, A., Gerbase, M.W., et al., 2015. Long-term exposure to ambient air pollution and metabolic syndrome in adults. PLoS One 10, e0130337.
Ferrari, R., Merli, E., Cicchitelli, G., Mele, D., Fucili, A., Ceconi, C., 2004. Therapeutic effects of L-carnitine and propionyl-L-carnitine on cardiovascular diseases: a review. Ann. N. Y. Acad. Sci. 1033, 79–91.
Fiorito, G., Vlaanderen, J., Polidoro, S., Gulliver, J., Galassi, C., Ranzi, A., et al., 2018. Oxidative stress and inflammation mediate the effect of air pollution on cardio- and cerebrovascular disease: a prospective study in nonsmokers. Environ. Mol. Mutagen. 59 (3), 234–246.
Firth, D., 1993. Bias reduction of maximum likelihood estimates. Biometrika 80, 27–38.
FOEN, 2013. PM10 and PM2.5 ambient concentrations in Switzerland. Modelling results for 2005, 2010 and 2020. In: Environmental Studies No. 1304. Federal Office for the Environment, Bern (83 pp.).
FOEN, 2014. Karten der luftbelastung schweiz. Dokumentation zu datengrundlagen, berechnungsverfahren und resultaten. Aktualisierung 2013/2014.
Franklin, B.A., Brook, R., Arden Pope 3rd, C., 2015. Air pollution and cardiovascular disease. Curr. Probl. Cardiol. 40, 207–238.
Guarnieri, M., Balmes, J.R., 2014. Outdoor air pollution and asthma. Lancet 383, 1581–1592.
Han, C., Kronmal, R., 2004. Box-Cox transformation of left-censored data with application to the analysis of coronary artery calcification and pharmacokinetic data. Stat. Med. 23, 3671–3679.
Hekking, P.W., Amelink, M., Wener, R.R., Bouvy, M.L., Bel, E.H., 2018. Comorbidities in difficult-to-control asthma. J Allergy Clin Immunol Pract 6 (1), 108–113.

Herbert, C., Kumar, R.K., 2017. Ambient air pollution and asthma. Eur. Respir. J. 49.
Jacquemin, B., Schikowski, T., Carsin, A.E., Hansell, A., Kramer, U., Sunyer, J., et al., 2012. The role of air pollution in adult-onset asthma: a review of the current evidence. Semin. Respir. Crit. Care Med. 33, 606–619.
Jacquemin, B., Siroux, V., Sanchez, M., Carsin, A.E., Schikowski, T., Adam, M., et al., 2015. Ambient air pollution and adult asthma incidence in six European cohorts (ESCAPE). Environ. Health Perspect. 123, 613–621.
Jeong, A., Imboden, M., Hansen, S., Zemp, E., Bridevaux, P.O., Lovison, G., et al., 2017. Heterogeneity of obesity-asthma association disentangled by latent class analysis, the SAPALDIA cohort. Respir. Med. 125, 25–32.
Kanehisa, M., Goto, S., Hattori, M., Aoki-Kinoshita, K.F., Itoh, M., Kawashima, S., et al., 2006. From genomics to chemical genomics: new developments in KEGG. Nucleic Acids Res. 34, D354–D357.
Kelly, F.J., Fussell, J.C., 2012. Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter. Atmos. Environ. 60, 504–526.
Kelly, R.S., Dahlin, A., McGeachie, M.J., Qiu, W., Sordillo, J., Wan, E.S., et al., 2017. Asthma metabolomics and the potential for integrative omics in research and the clinic. Chest 151, 262–277.
Kordalewska, M., Markuszewski, M.J., 2015. Metabolomics in cardiovascular diseases. J. Pharm. Biomed. Anal. 113, 121–136.
Kumar, R.K., Shadie, A.M., Bucknall, M.P., Rutlidge, H., Garthwaite, L., Herbert, C., et al., 2015. Differential injurious effects of ambient and traffic-derived particulate matter on airway epithelial cells. Respirology 20, 73–79.
Kunzli, N., Bridevaux, P.O., Liu, L.J., Garcia-Esteban, R., Schindler, C., Gerbase, M.W., et al., 2009. Traffic-related air pollution correlates with adult-onset asthma among never-smokers. Thorax 64, 664–670.
Li, S., Park, Y., Duraisingham, S., Strobel, F.H., Khan, N., Soltow, Q.A., et al., 2013. Predicting network activity from high throughput metabolomics. PLoS Comput. Biol. 9, e1003123.
Ma, H., Sorokin, A., Mazein, A., Selkov, A., Selkov, E., Demin, O., et al., 2007. The Edinburgh human metabolic network reconstruction and its functional analysis. Mol. Syst. Biol. 3, 135.
Martin, B.W., Ackermann-Liebrich, U., Leuenberger, P., Kunzli, N., Stutz, E.Z., Keller, R., et al., 1997. SAPALDIA: methods and participation in the cross-sectional part of the Swiss study on air pollution and lung diseases in adults. Soz. Praventivmed. 42, 67–84.
Maruyama, H., Takahashi, M., Sekimoto, T., Shimada, T., Yokosuka, O., 2014. Linoleate appears to protect against palmitate-induced inflammation in Huh7 cells. Lipids Health Dis. 13, 78.
McGeachie, M.J., Dahlin, A., Qiu, W., Croteau-Chonka, D.C., Savage, J., Wu, A.C., et al., 2015. The metabolomics of asthma control: a promising link between genetics and disease. Immun. Inflammation Dis. 3, 224–238.
McGuinn, L.A., Ward-Caviness, C.K., Neas, L.M., Schneider, A., Diaz-Sanchez, D., Cascio, W.E., et al., 2016. Association between satellite-based estimates of long-term PM2.5 exposure and coronary artery disease. Environ. Res. 145, 9–17.
Miguel-Carrasco, J.L., Mate, A., Monserrat, M.T., Arias, J.L., Aramburu, O., Vazquez, C.M., 2008. The role of inflammatory markers in the cardioprotective effect of L-carnitine in L-NAME-induced hypertension. Am. J. Hypertens. 21, 1231–1237.
Miyake, Y., Tanaka, K., Sasaki, S., Arakawa, M., 2011. Polyunsaturated fatty acid intake and prevalence of eczema and rhinoconjunctivitis in Japanese children: the Ryukyus Child Health study. BMC Public Health 11, 358.
Mizuta, K., Zhang, Y., Mizuta, F., Hoshijima, H., Shiga, T., Masaki, E., et al., 2015. Novel identification of the free fatty acid receptor FFAR1 that promotes contraction in airway smooth muscle. Am. J. Phys. Lung Cell. Mol. Phys. 309, L970–L982.
Mostafavi, N., Vlaanderen, J., Chadeau-Hyam, M., Beelen, R., Modig, L., Palli, D., et al., 2015. Inflammatory markers in relation to long-term air pollution. Environ. Int. 81, 1–7.
Newby, D.E., Mannucci, P.M., Tell, G.S., Baccarelli, A.A., Brook, R.D., Donaldson, K., et al., 2015. Expert position paper on air pollution and cardiovascular disease. Eur. Heart J. 36, 83–93b.
Ono, J.G., Worgall, T.S., Worgall, S., 2015. Airway reactivity and sphingolipids-implications for childhood asthma. Mol. Cell. Pediatr. 2, 13.
Ostro, B., Hu, J., Goldberg, D., Reynolds, P., Hertz, A., Bernstein, L., et al., 2015. Associations of mortality with long-term exposures to fine and ultrafine particles, species and sources: results from the California Teachers Study cohort. Environ. Health Perspect. 123, 549–556.
Palli, D., Berrino, F., Vineis, P., Tumino, R., Panico, S., Masala, G., et al., 2003. A molecular epidemiology project on diet and cancer: the EPIC-Italy Prospective Study. Design and baseline characteristics of participants. Tumori 89, 586–593.
Pan, W., Yu, J., Shi, R., Yan, L., Yang, T., Li, Y., et al., 2014. Elevation of ceramide and activation of secretory acid sphingomyelinase in patients with acute coronary syndromes. Coron. Artery Dis. 25, 230–235.
Park, J.Y., Lee, S.H., Shin, M.J., Hwang, G.S., 2015. Alteration in metabolic signature and lipid metabolism in patients with angina pectoris and myocardial infarction. PLoS One 10, e0135228.
Peel, J.L., Tolbert, P.E., Klein, M., Metzger, K.B., Flanders, W.D., Todd, K., et al., 2005. Ambient air pollution and respiratory emergency department visits. Epidemiology 16, 164–174.
Perry, P.O., 2017. Fast moment-based estimation for hierarchical models. J. R. Stat. Soc. Ser. B Stat Methodol. 79 (1), 267–291.
Peters, A., Wichmann, H.E., Tuch, T., Heinrich, J., Heyder, J., 1997. Respiratory effects are associated with the number of ultrafine particles. Am. J. Respir. Crit. Care Med. 155, 1376–1383.
Petrache, I., Berdyshev, E.V., 2016. Ceramide signaling and metabolism in pathophysiological states of the lung. Annu. Rev. Physiol. 78, 463–480.
Richardson, D.B., 2004. An incidence density sampling program for nested case-control analyses. Occup. Environ. Med. 61, e59.
Schindler, C., Keidel, D., Gerbase, M.W., Zemp, E., Bettschart, R., Brandli, O., et al., 2009. Improvements in PM10 exposure and reduced rates of respiratory symptoms in a cohort of Swiss adults (SAPALDIA). Am. J. Respir. Crit. Care Med. 179, 579–587.
Schwartz, J., Slater, D., Larson, T.V., Pierson, W.E., Koenig, J.Q., 1993. Particulate air pollution and hospital emergency room visits for asthma in Seattle. Am. Rev. Respir. Dis. 147, 826–831.
Schwarze, P.E., Ovrevik, J., Hetland, R.B., Becher, R., Cassee, F.R., Lag, M., et al., 2007. Importance of size and composition of particles for effects on cells in vitro. Inhal. Toxicol. 19 (Suppl 1), 17–22.
Shah, S.H., Kraus, W.E., Newgard, C.B., 2012. Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function. Circulation 126, 1110–1120.
Siroux, V., Gonzalez, J.R., Bouzigon, E., Curjuric, I., Boudier, A., Imboden, M., et al., 2014. Genetic heterogeneity of asthma phenotypes identified by a clustering approach. Eur. Respir. J. 43, 439–452.
Song, X., Qu, H., Yang, Z., Rong, J., Cai, W., Zhou, H., 2017. Efficacy and safety of L-carnitine treatment for chronic heart failure: a meta-analysis of randomized controlled trials. Biomed. Res. Int. 2017, 6274854.
Stafoggia, M., Cesaroni, G., Peters, A., Andersen, Z.J., Badaloni, C., Beelen, R., et al., 2014. Long-term exposure to ambient air pollution and incidence of cerebrovascular events: results from 11 European cohorts within the ESCAPE project. Environ. Health Perspect. 122, 919–925.
Sumner, L.W., Amberg, A., Barrett, D., Beale, M.H., Beger, R., Daykin, C.A., et al., 2007. Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). Metabolomics 3, 211–221.
Sunyer, J., Spix, C., Quenel, P., Ponce-de-Leon, A., Ponka, A., Barumandzadeh, T., et al., 1997. Urban air pollution and emergency admissions for asthma in four European cities: the APHEA project. Thorax 52, 760–765.
Temam, S., Burte, E., Adam, M., Anto, J.M., Basagana, X., Bousquet, J., et al., 2017. Socioeconomic position and outdoor nitrogen dioxide (NO2) exposure in western Europe: a multi-city analysis. Environ. Int. 101, 117–124.
Uzoigwe, J.C., Prum, T., Bresnahan, E., Garelnabi, M., 2013. The emerging role of outdoor and indoor air pollution in cardiovascular disease. N. Am. J. Med. Sci. 5, 445–453.
van Nunen, E., Vermeulen, R., Tsai, M.Y., Probst-Hensch, N., Ineichen, A., Davey, M., et al., 2017. Land use regression models for ultrafine particles in six European areas. Environ. Sci. Technol. 51, 3336–3345.
Vineis, P., van Veldhoven, K., Chadeau-Hyam, M., Athersuch, T.J., 2013. Advancing the application of omics-based biomarkers in environmental epidemiology. Environ. Mol. Mutagen. 54, 461–467.
Vineis, P., Chadeau-Hyam, M., Gmuender, H., Gulliver, J., Herceg, Z., Kleinjans, J., et al., 2017. The exposome in practice: design of the EXPOsOMICS project. Int. J. Hyg. Environ. Health 220 (2), 142–151.
Vlaanderen, J.J., Janssen, N.A., Hoek, G., Keski-Rahkonen, P., Barupal, D.K., Cassee, F.R., et al., 2017. The impact of ambient air pollution on the human blood metabolome. Environ. Res. 156, 341–348.
Wang, D.D., Toledo, E., Hruby, A., Rosner, B.A., Willett, W.C., Sun, Q., et al., 2017. Plasma ceramides, Mediterranean diet, and incident cardiovascular disease in the PREDIMED trial (Prevencion con Dieta Mediterranea). Circulation 135, 2028–2040.
Wei, Y., Zhang, J.J., Li, Z., Gow, A., Chung, K.F., Hu, M., et al., 2016. Chronic exposure to air pollution particles increases the risk of obesity and metabolic syndrome: findings from a natural experiment in Beijing. FASEB J. 30, 2115–2122.
Weichenthal, S., Bai, L., Hatzopoulou, M., Van Ryswyk, K., Kwong, J.C., Jerrett, M., et al., 2017. Long-term exposure to ambient ultrafine particles and respiratory disease incidence in Toronto, Canada: a cohort study. Environ. Health 16, 64.
Wenzel, S.E., 2012. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat. Med. 18, 716–725.
Wolf, K., Stafoggia, M., Cesaroni, G., Andersen, Z.J., Beelen, R., Galassi, C., et al., 2015. Long-term exposure to particulate matter constituents and the incidence of coronary events in 11 European cohorts. Epidemiology 26, 565–574.
Wurtz, P., Havulinna, A.S., Soininen, P., Tynkkynen, T., Prieto-Merino, D., Tillin, T., et al., 2015. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. Circulation 131, 774–785.
Yamada, E., Funoki, S., Abe, Y., Umemura, S., Yamaguchi, D., Fuse, Y., 2005. Size distribution and characteristics of chemical components in ambient particulate matter. Anal. Sci. 21, 89–94.
Yang, J., Yu, Y., Sun, S., Duerksen-Hughes, P.J., 2004. Ceramide and other sphingolipids in cellular responses. Cell Biochem. Biophys. 40, 323–350.
Yen, C.H., Dai, Y.S., Yang, Y.H., Wang, L.C., Lee, J.H., Chiang, B.L., 2008. Linoleic acid metabolite levels and transepidermal water loss in children with atopic dermatitis. Ann Allergy Asthma Immunol 100, 66–73.