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microRNA expression profiles and personal monitoring of exposure to particulate matter☆

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ABSTRACT

An increasing number of findings from epidemiological studies support associations between exposure to air pollution and the onset of several diseases, including pulmonary, cardiovascular and neurodegenerative diseases, and malignancies. However, intermediate, and potentially mediating, biological mechanisms associated with exposure to air pollutants are largely unknown. Previous studies on the human exposome have shown that the expression of certain circulating microRNAs (miRNAs), regulators of gene expression, are altered upon exposure to traffic-related air pollutants. In the present study, we investigated the relationship between particulate matter (PM) smaller than 2.5 μm ($PM_{2.5}$), $PM_{2.5}$ absorbance (as a proxy of black carbon and soot), and ultrafine-particles (UFP, smaller than 0.1 μm), measured in healthy volunteers by 24 h personal monitoring (PEM) sessions and global expression levels of peripheral blood miRNAs. The PEM sessions were conducted in four European countries, namely Switzerland (Basel), United Kingdom (Norwich), Italy (Turin), and The Netherlands (Utrecht). miRNAs expression levels were analysed using microarray technology on blood samples from 143 participants. Seven miRNAs, *hsa-miR-24-3p, hsa-miR-4454, hsa-miR-4763-3p, hsa-miR-425-5p, hsa-let-7d-5p, hsa-miR-502-5p,* and *hsa-miR-505-3p* were significantly (FDR corrected) expressed in association with $PM_{2.5}$ personal exposure, while no significant association was found between miRNA expression and the other pollutants. The results obtained from this investigation suggest that personal exposure to $PM_{2.5}$ is associated with miRNA expression levels, showing the potential for these circulating miRNAs as novel biomarkers for air pollution health risk assessment.

1. Introduction

Morbidity and mortality attributable to air pollution continue to be a growing problem worldwide, representing the main environmental cause of premature death (Kelly and Fussell, 2015).

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Approximately 4.2 million people died from exposure to ambient air pollution worldwide in 2016 alone (World Health Organisation (WHO), 2019). Additionally, exposure to air pollutants has been linked to several adverse health outcomes. Both short-term and long-term exposure to air pollution affect the respiratory and cardiovascular systems: inducing pulmonary inflammation, cardiovascular events, leading to increase susceptibility to respiratory infections, increase risk of cancer, and exacerbate asthma and chronic obstructive pulmonary diseases (Abelsohn and Stieb, 2011; Lai et al., 2013).

Despite the wealth of studies on air pollutant-associated health effects, the underlying molecular mechanisms by which air pollutants initiate disease remain unclear. Oxidative damage, inflammation, and endothelial dysfunction have been suggested as potential underlying mechanisms of air-pollutant associated adverse health (Demetriou et al., 2012; Münzel et al., 2018; Lodovici and Bigagli, 2011). Nonetheless, further molecular epidemiological research is warranted to completely elucidate the identity and role of involved biological pathways that underlie air pollution-associated adverse health.

There is evidence that protein-coding genes cannot account for all observed genomic effects, thus post genetic regulators such as those involved in epigenetics, potentially represent the "missing link" between environmental exposures and health-related events (Vrijens et al., 2015). As a main epigenetic regulatory mechanism, microRNAs (miRNAs) play a key role in regulating cellular processes (Marques-Rocha et al., 2015). miRNAs are endogenous, single-stranded, short non-coding RNA sequences (~22 nucleotides) that regulate gene expression at the post-transcriptional level. In most cases, miRNAs lead to the inhibition of protein synthesis either by degrading messenger-RNA transcripts or by repressing the translational process (Rider et al., 2016). These small non-coding RNAs are thus crucial for the stability and maintenance of gene expression patterns that characterize some cell types, tissues, and physiological and pathological responses (Marques-Rocha et al., 2015).

Recently, it has been highlighted that exposure to air pollutants has the potential to change the profiles of miRNAs and that these altered expression levels of miRNAs may be considered as markers of air pollution exposure or effect (Vrijens et al., 2015; Rider et al., 2016; Jardim, 2011; Krauskopf et al., 2017; Espín-Pérez et al., 2018). Investigating miRNA expression as a response to exposure to air pollution may aide in understanding the regulatory mechanisms of gene expression altered by ambient pollutants (Rider et al., 2016; Espín-Pérez et al., 2018).

Our study investigates the relationship between 24 h personal and environmental measurements of particulate matter (PM) smaller than 2.5 μm ($PM_{2.5}$), $PM_{2.5}$ absorbance (as a proxy of black carbon and soot), and ultrafine-particulate (UFP, particulate with diameter smaller than 0.1 μm) and genome-wide expression levels in the peripheral blood miRNome.

2. Material and methods

2.1. Study population and participant data collection

This study is part of the EXPOsOMICS project, a large European exposome study (Vineis et al., 2017). One of the objectives was to characterize external exposure using high precision methods of Personal Exposure Monitoring (PEM). We estimated individual 24 h exposure to air pollutants using PEM in four European countries, namely Switzerland (Basel), United Kingdom (Norwich), Italy (Turin), and The Netherlands (Utrecht), between December 2013 and February 2015, as fully described elsewhere (Vineis et al., 2017). Briefly, non-smoking adults, with no history of pulmonary or cardiovascular disease, diabetes, or other health conditions, were recruited in the study (≥40 participants per country). Participants were invited to participate in three PEM sessions at different seasons within 12 months. Personal information, such as age, body mass index, level of education and home characteristics were collected for each participant at baseline. Questions on health, overall physical activity, smoking, dust and indoor air pollution exposure were asked at each PEM session. PEM session specific meteorological information, including average temperature and relative humidity were also collected at each session.

2.2. Exposure assessment to $PM_{2.5}$

During each PEM session, each participant was asked to carry a backpack containing air pollution sensors for 24 h, to measure individual exposure to $PM_{2.5}$. $PM_{2.5}$ was also measured outdoors at the participant's home during each PEM session. For both personal and outdoor exposure, 24 h average $PM_{2.5}$ concentration was determined by change in filter weight, corrected with average field blank concentration, following the European Study of Cohorts for Air Pollution Effects study (ESCAPE) procedures (Montagne et al., 2013; Eeftens et al., 2012). The reflectance of filters was measured using a Smoke Stain Reflectometer (Diffusion Systems Ltd, London, UK). The reflectance was transformed into $PM_{2.5}$ absorbance concentration (soot levels) absorbance according to ISO 9835, corrected with an average field blank, following ESCAPE procedures (Montagne et al., 2013; Eeftens et al., 2012). $PM_{2.5}$ measurements were not included if the elapsed time counter (ETC) showed that the pump operated for less than 66.7% of the desired 24 h and/or the end flow deviated more than 20% from the design value of 3.5 l/min (2.8−4.2 L/min).

2.3. Exposure assessment to UFP

Personal exposure to ultrafine particles (UFP) was monitored with a MiniDiSC (Testo AG, Lenzkirch, Germany). The MiniDiSC operated at a flow of 1000 ml/min measuring particles from 10 to 300 nm at 1-s intervals. We used the term UFP to refer to the median of particle number counts from the MiniDiSC over 24 h PEM session. Details on methods and quality control have been published previously (van Nunen et al., 2017).

2.4. RNA isolation and miRNA array

Each participant donated a blood sample immediately after each PEM session (within 24 ± 2 h). Blood samples were pre-analytically processed at 4 °C by adding 1600 μl of RNA later (Ambion, Life Technologies, Carlsbad, USA) to 400 μl of ETDA-buffered whole blood aliquots, and transferred to −80 °C within 2 h of venepuncture. Samples from one or two PEM sessions (depending on the blood samples availability per subject) were used for miRNAs and other omic analyses.

Total RNA was isolated using RiboPureTM-Blood (Ambion), according to the manufacturer's instructions. RNA was hybridized on Sureprint G3 Human V19 miRNA 8 × 60 K microarrays for microRNA (based on mirBase version 19). To minimize "batch effects" or systematic error introduced when samples are handled on different dates (Leek et al., 2010), the samples were randomized for isolation and hybridization. Raw data on pixel intensities were extracted using Agilent Feature Extraction Software.

Data were normalized using a quantile approach as implemented in the 'limma' R package (Ritchie et al., 2015) and transformed to log2 scale. A total of 280 miRNAs were expressed in all samples and considered for further analyses.

2.5. Statistical analysis

Since all exposure levels were not normally distributed, and values were log-transformed. Pearson's coefficient was calculated to determine correlation structures between exposures.

To estimate the association between each exposure ($PM_{2.5}$ and UFP) and the expression of miRNAs, we applied mixed linear models with a random intercept for each subject, to account for the correlation among repeated measurements of exposure within subjects. To account for technical factors of the miRNA laboratory assessments, a random intercept for batch effects was also included in the model. We ran three different models to estimate the sensitivity of potential confounders associated with the exposure and the outcome.

All models were adjusted for a set of baseline potential confounders, including age, sex, and body mass index. The primary model only adjusted for these baseline confounders (Model 0). We further adjusted for education level, country, season, and physical activity (Model 1). Finally, we additionally adjusted for average temperature and relative humidity (Model 2).

Multiple testing adjustments, to control for the expected proportion of false discovery rate (FDR), on the resulting p-values were performed setting alpha to a level of 0.05.

To explore the impact of the country of residence on our estimates, we ran a two sensitivity analyses. First, we fit Model 2 excluding one country at a time. Second, we fit Model 2, stratifying the population based on the country of residence.

All analyses were performed with R version 3.4.3.

2.6. miRNA target genes, gene enrichment and pathway analysis

For all relevant miRNAs associated with the exposures in our study, we searched for validated target genes using the miRWalk 3.0 database (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2). Resulting lists of target genes were tested (either for each individual miRNA or altogether) using the Enrichr software (http://amp.pharm.mssm.edu/Enrichr/), for their over-representation in biological pathways. Enrichr is an integrative web-based software application that includes gene-set libraries, available for analysis and download. Particular for the present study, we investigated: KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/) pathways. The relevance of each gene set enrichment was assessed by a *p*-value adjusted for multiple testing based on the hypergeometric distribution. Gene sets with probability <0.05 were considered as significantly overrepresented.

3. Results

The main characteristics of the study population, including information on meteorological data and air pollution exposure levels, for all individuals and by study area are summarized in Table 1. Overall, 143 subjects, of whom 48 participated in only one PEM session and 95 participated in two PEM sessions (for a total of 238 observations) donated peripheral blood samples after each PEM sessions for which they participated in. Our study population included 86 women (60%), and the median age was 61 years.

The overall median (min-max) personal $PM_{2.5}$ exposure to was 8.7 $\mu g/m^3$ (0.2–75.0), with the highest levels measured in Italy (median: 10.7 $\mu g/m^3$) and the lowest levels measured in Switzerland (median: 7.3 $\mu g/m^3$). Similarly, the median (min-max) outdoor exposure levels to $PM_{2.5}$ was 9.0 $\mu g/m^3$ (1.0–70.9) with the highest levels measured in Italy (median: 12.8 $\mu g/m^3$) and the lowest levels in Switzerland (median: 6.8 $\mu g/m^3$).

The overall median (min-max) exposure to UFP was 6318 particles/cm^3 (785–22536), with higher levels measured in Italy (median: 9149 particles/cm^3) and lower levels measured in Switzerland (median: 4937 particles/cm^3).

The correlation between personal and outdoor exposure levels for $PM_{2.5}$ was moderately high ($r = 0.6$), and was higher between personal and outdoor for $PM_{2.5}$ absorption coefficient ($r = 0.8$). The correlation between all $PM_{2.5}$ exposure levels and UFP was relatively low ($r < 0.4$).

3.1. Association between exposure to air pollution and miRNA expression levels

Three miRNAs, namely *hsa-miR-24-3p*, *hsa-miR-4454*, *hsa-miR-4763-3p*, were associated with $PM_{2.5}$ personal exposure levels, after FDR correction, consistently for each of the main analyses (Model 0, 1, 2). In particular, *hsa-miR-24-3p* was negatively associated ($\beta = -9.3$, *p*-value = 0.01), while both *hsa-miR-4454* and *hsa-miR-4763-3p* were positively associated ($\beta = 4.2$, *p*-value = 0.02; $\beta = 2.1$, *p*-value<0.01, respectively) with $PM_{2.5}$ personal exposure levels (Table 2, Fig. 1).

In the sensitivity analysis, after excluding either Italy or United Kingdom, these *hsa-miR-24-3p, hsa-miR-4454* and *hsa-miR-4763-3p* were still associated with $PM_{2.5}$ personal exposure levels. However, when excluding The Netherlands from the analysis only *hsa-miR-24-3p* and *hsa-miR-4763-3p* were significantly associated with the exposure. Finally, when excluding Switzerland from the analysis only *hsa-miR-4763-3p* was still associated to $PM_{2.5}$ levels (Table 2).

After stratifying by country, there was no statistically significant association found when assessing only Italy, or The Netherlands, or United Kingdom, while five miRNAs were associated to $PM_{2.5}$ personal exposure levels when assessing only Switzerland: *hsa-let-7d-5p, hsa-miR-24-3p, hsa-miR-425-5p, hsa-miR-502-5p, hsa-miR-505-3p* (Table 2).

The correlations between *hsa-let-7d-5p, hsa-miR-24-3p, hsa-miR-425-5p, hsa-miR-502-5p, hsa-miR-505-3p, hsa-miR-4454* and *hsa-miR-4763-3p* were overall weak and are summarized in Fig. 2.

No miRNA was associated with $PM_{2.5}$ outdoor exposure levels after FDR correction.

Twelve miRNAs were associated with $PM_{2.5}$ personal exposure absorption coefficient in Model 0, but these associations were not statistically significant after adjusting for education level, country, season, and physical activity (Model 1) and for average temperature and relative humidity (Model 2) (data not shown). Similar results were observed when investigating the association with $PM_{2.5}$ outdoor exposure absorption coefficient: a statistically significant association was highlighted with five miRNAs in Model 0, but no significancy remained once adjusted for other potential confounding factors with Models 1 and 2 (data not shown).

No statistically significant association was found between miRNA expression and $PM_{2.5}$ outdoor exposure levels, $PM_{2.5}$ personal exposure absorption coefficient, and $PM_{2.5}$ outdoor exposure absorption coefficient when excluding one country at a time (data not shown). No association was found when performing stratified analysis, with the exception of Switzerland, for which one miRNA was associated with $PM_{2.5}$ personal exposure absorption coefficient, and United Kingdom, for which one miRNA was associated with $PM_{2.5}$ outdoor exposure levels, seven miRNA with $PM_{2.5}$ personal exposure absorption coefficient, and twenty-three with $PM_{2.5}$ outdoor exposure absorption coefficient (Supplementary Table S1).

No miRNA was associated with UFP personal exposure levels after FDR correction. When including only United Kingdom in the model, one miRNA, namely *hsa-miR-451a* was inversely associated to UFP exposure (Supplementary Table S1).

Table 1
Characteristics of the study population (n = 143) according to the city of residence. For each continuous variable the median (standard deviation) value is reported.

	All participants (n = 143)	Switzerland (n = 43)	United Kingdom (n = 20)	Italy (n = 43)	The Netherlands (n = 37)	P value[a]
Age (years)	61.0 (10.0)	62.8 (14.9)	63.1 (5.9)	59.9 (7.1)	61.0 (12.4)	0.21
BMI (kg/m^2)	24.8 (5.1)	23.9 (5.2)	27.5 (4.4)	24.8 (4.0)	24.4 (5.1)	0.01
Physical activity (MET-hours/day)	1.6 (0.3)	1.5 (0.2)	1.6 (0.3)	1.6 (0.2)	1.7 (0.3)	<0.001
Women (n)	86	23	12	21	30	
Season (n)						
Autumn	34	4	4	0	26	
Spring	86	27	14	32	13	
Summer	64	13	13	14	24	
Winter	54	23	0	31	0	
Temperature (°C)	+14.1 (8.9)	+10.4 (11.2)	+13.6 7.7)	+14.7 (11.1)	+14.0 (5.0)	0.05
Relative Humidity (%)	75.9 (17.7)	69.9 (16.2)	73.2 (13.8)	65.3 (18.0)	82.0 (13.8)	<0.001
PM 2.5 personal (μg/m^3)	8.7 (6.4)	7.3 (5.6)	8.3 (6.1)	10.7 (9.4)	9.0 (5.4)	0.002
PM 2.5 personal Absorbance (μg/m^3)	0.9 (1.0)	0.7 (0.8)	0.5 (0.3)	1.7 (1.2)	0.7 (1.0)	<0.001
PM 2.5 outdoor (μg/m^3)	9.0 (9.7)	6.8 (7.2)	7.1 (5.3)	12.8 (13.5)	9.5 (8.4)	<0.001
PM 2.5 outdoor Absorbance (μg/m^3)	1.1 (1.4)	0.8 (0.9)	0.7 (0.5)	2.3 (1.8)	1.0 (1.0)	<0.001
UFP median (particles/cm^3)	6318 (3842)	4937 (3107)	5378 (2556)	9149 (4006)	5900 (3519)	<0.001

[a] Kruskal-Wallis test.

Table 2
miRNAs significantly associated to $PM_{2.5}$ personal exposure levels after FDR correction.

PM2.5 personal exposure levels		
miRNA	Adjusted *p*-value[a]	Beta-Coefficient
	Model 2	
hsa-miR-24-3p	0.011	−9.26
hsa-miR-4454	0.017	4.16
hsa-miR-4763-3p	<0.001	2.05
	Model 2 without Italy	
hsa-miR-24-3p	<0.001	−1.16
hsa-miR-4454	0.016	4.39
hsa-miR-4763-3p	0.001	2.25
hsa-miR-502-5p	0.016	1.56
	Model 2 without The Netherlands	
hsa-miR-24-3p	<0.001	−1.46
hsa-miR-4763-3p	0.018	2.01
	Model 2 without Switzerland	
hsa-miR-4763-3p	0.005	2.17
	Model 2 without United Kingdom	
hsa-miR-24-3p	0.02	−1.46
hsa-miR-4454	0.048	3.83
hsa-miR-4763-3p	0.003	2.01
hsa-miR-502-5p	0.027	4.24
	Model 2 only Switzerland	
hasa-let-7d-5p	0.04	4.98
hsa-miR-24-3p	<0.001	−2.80
hsa-miR-425-5p	0.029	6.55
hsa-miR-502-5p	0.0001	2.17
hsa-miR-505-3p	0.029	1.57
	Model 2 only Italy	
–	–	–
	Model 2 only United Kingdom	
–	–	–
	Model 2 only The Netherlands PM	
–	–	–

Model 2: adjusted for age, sex, and body mass index, education level, city, season, and physical activity, average temperature and relative humidity.
[a] After FDR correction.

3.2. miRNA target genes, gene enrichment and pathway analysis

The list of the experimentally validated target genes for the seven miRNAs associated with $PM_{2.5}$ personal exposure levels, namely, *hsa-miR-24-3p, hsa-miR-4454, hsa-miR-4763-3p, hsa-miR-425-5p, hsa-let-7d-5p, hsa-miR-502-5p,* and *hsa-miR-505-3p*, retrieved in the miRWalk 3.0 database, is reported in Supplementary Table S2. Three-hundred sixty-eight target genes were identified for the above miRNAs. Gene enrichment analysis provided statistically significant over-representation and KEGG terms identified several significant pathways, among which p53 signaling pathways (hsa04115), pathways in cancer (hsa05200) and JAK-STAT signaling pathway (hsa04630) (Table 3).

4. Discussion

This study suggests that personal exposure to $PM_{2.5}$ is associated with miRNA expression. More specifically *hsa-miR-24-3p* was down-regulated in association with $PM_{2.5}$ personal exposure, while *hsa-miR-4454, hsa-miR-4763-3p, hsa-miR-425-5p, hsa-let-7d-5p, hsa-miR-502-5p,* and *hsa-miR-505-3p* were all up-regulated in association with $PM_{2.5}$ personal exposure. The associations found

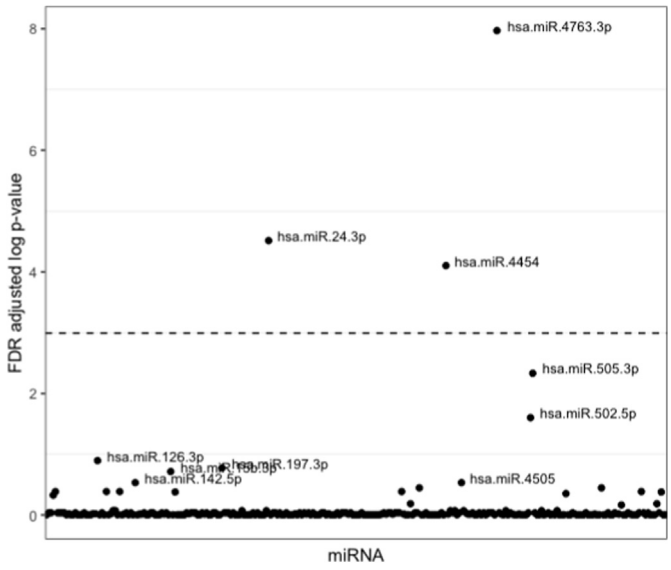

Fig. 1. FDR adjusted log p-values of the association between PM2.5 personal exposure levels and miRNAs expression after adjusting for potential confounders (age, sex, and body mass index, education level, city, season, and physical activity, average temperature and relative humidity).

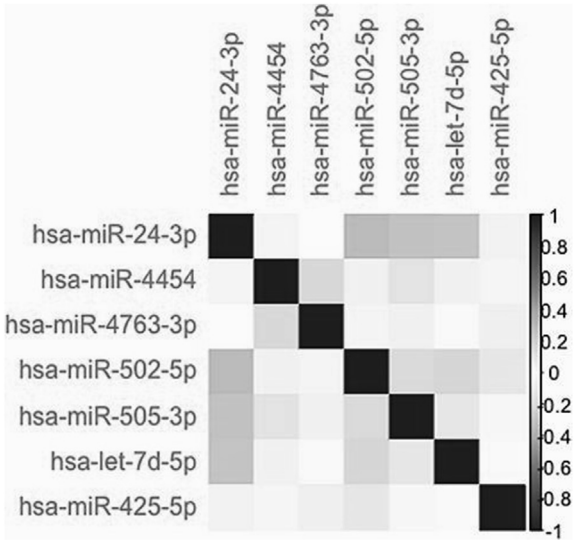

Fig. 2. Pearson's correlation coefficient between expression levels of miRNA significantly associated with air pollution: *hsa-miR-24-3p, hsa-miR-4454, hsa-miR-4763-3p, hsa-miR-425-5p, hsa-let-7d-5p, hsa-miR-502-5p,* and *hsa-miR-505-3p*.

between personal exposure to $PM_{2.5}$ and miRNA expression levels show fair consistency throughout the different study populations living in the four European countries included in the study. Likely, these estimates are not influenced by the country specific levels of air pollution, while there were differences in the levels of exposure found across the four countries, the overall variability was not very large, with lowest median exposure levels being in Switzerland (Basel: 7.3 $\mu g/m^3$), and the highest in Italy (Turin: 10.7 $\mu g/m^3$).

In contrast, we found no association between $PM_{2.5}$ outdoor exposure levels and miRNA expression levels. The lack of association between $PM_{2.5}$ outdoor exposure levels and miRNA expression levels, although could be due to the limited statistical power of the study, could also support the idea that, in comparison to exposure measurements carried out with personal devices, environmental measurements do not accurately reflect the individual's real exposure.

Notably, in our study UFP personal exposure did not result associated to miRNA expression levels.

Several studies have previously investigated the association between miRNA expression and the exposure to air pollution, leading to different results (Vrijens et al., 2015). This inconsistency of the scientific findings may be due to several reasons such as the use of different methods to estimate the exposure (e.g. personal *vs* environmental), or from measuring miRNA in different tissues (e.g. serum *vs.* blood). Additionally, the field of research on miRNAs has evolved where the number of miRNAs analysed among studies has changed, along with changes in nomenclature and methodological approaches.

Similar to our study, Espín-Pérez et al. (2018) investigated blood microRNA expression levels in relation to personal exposure to air pollution, although their study considered short-term exposure (2 h) from two studies (Oxford Street and TAPAS). The authors found nine miRNAs associated to $PM_{2.5}$ exposure in the Oxford Street Study (59 volunteers), while no miRNA was associated with exposure to UFP after FDR correction. In the TAPAS study (30 volunteers), the authors found ten miRNAs associated to $PM_{2.5}$ and one miRNA to UFP exposure after FDR correction, although no overlap was present with the results of Oxford Street Study. There is no overlap between miRNAs associated to $PM_{2.5}$ personal exposure in our study and those reported by Espín-Pérez and colleagues.

Rodosthenous et al. (2016) randomly selected 22 participants with available serum samples between the years 2000 and 2008. A validated spatio-temporal land-use regression model was used to estimate the ambient moving-average PM2.5 levels at each participant's home address for 6 time windows (1 day, 1 week, 1 month, 3 months, 6 months, and 1 year) preceding each blood withdrawal. Of the 31 tested extracellular vesicles miRNAs, the authors found that 16 were statistically significantly associated with either the 6-month or 1-year PM2.5 moving averages. No clear overlap was observed with the results obtained in our study.

Potential differences between study populations, including sample size, exposure levels, and confounding factors, among others, could explain the lack of overlap between air pollutant-associated miRNA among the different studies, whereby future studies will need to consider factors such. Nevertheless, this is still early research and the reasons for inconsistency need to be further explored.

miRNAs are considered important regulators of gene expression, and more generally, for cellular processes. In the present study, we have identified 368 predicted genes targets that are potentially regulated by the seven miRNAs associated with $PM_{2.5}$ personal exposure that are involved in several important pathways, which share several miRNA targets, such as p53 signaling pathway, JAK-STAT signaling pathway, TGF-beta signaling pathway, Hippo signaling pathway and a more general Pathways in cancer and others.

The tumor suppressor *p53* is a key mediator of cell proliferation, apoptosis, senescence, and transformation in response to cellular damage; indeed, *p53* is the most frequently altered gene in human cancers (Rivlin et al., 2011). Previous studies have highlighted that several miRNAs are involved in the post-transcriptional regulation of *p53* (Le et al., 2009; Bommer et al., 2007; He et al., 2007). Our results suggest that personal exposure to $PM_{2.5}$ deregulates miRNA expression, potentially leading to an inhibition of genes that regulate *p53* expression. This mechanism could contribute to explaining the carcinogenic properties attributed to $PM_{2.5}$ exposure (Loomis et al., 2013), though will need to be further validated. Because PM2.5 can translocate from the lungs to extrapulmonary

Table 3
Functional enrichment analysis for the validated target genes of deregulated miRNAs in association with PM2.5 personal exposure levels. The significant enriched KEGG pathways, the adjusted p-value, and the genes present in our dataset and involved in the listed pathways are provided. In bold the shared target genes between pathways.

Term	Overlap	P-value	Adjusted P-value	Genes
p53 signaling pathway	10/72	7.66E-07	2.36E-04	*STEAP3;COP1;**CCND2**;RRM2;**CHEK1**;**MDM2**;MDM4;**THBS1**;* ***BBC3**;**BCL2L1***
Pathways in cancer	26/530	6.28E-06	9.66E-04	***CXCL8**;**TGFA**;XIAP;**BBC3**;**IGF1R**;RASGRP3;**RPS6KA5**;**CCND2**;* ***BCL2L1**1;**MYC**;**DVL3**;VHL;**IL6R**;**WNT4**;NCOA1;**PDGFRB**;**SMAD2**;* ***STAT2**;ZBTB16;**IL4**;RAD51;**SP1**;**MDM2**;**CALM3**;**CALM1**;**BCL2L1***
Signaling pathways regulating pluripotency of stem cells	11/139	5.39E-05	0.006	***SMAD2**;RIF1;**MYC**;PCGF3;SMARCAD1;**DVL3**;JARID2;**ACVR1B**;* ***MAPK14**;**IGF1R**;WNT4*
Cellular senescence	11/160	1.90E-04	0.015	***PPP1CB**;**SMAD2**;**CCND2**;**CXCL8**;RBBP4;**MYC**;**CHEK1**;**MDM2**;* ***CALM3**;**CALM1**;**MAPK14***
Hippo signaling pathway	11/160	1.90E-04	0.012	***PPP1CB**;**SMAD2**;**PPP2CB**;**CCND2**;**MYC**;**DVL3**;**PPP2R2A**;LLGL1;* *NKD1;**WNT4**;**BBC3***
JAK-STAT signaling pathway	10/162	8.58E-04	0.044	***PDGFRB**;**IL4**;**CCND2**;SOCS1;**MYC**;**STAT2**;IFNLR1;**IL6R**;SOCS7;* ***BCL2L1***
Bladder cancer	5/41	8.92E-04	0.039	***CXCL8**;**RPS6KA5**;**MYC**;**MDM2**;**THBS1***
TGF-beta signaling pathway	7/90	0.001	0.052	***SMAD2**;**PPP2CB**;ZFYVE9;**SP1**;**MYC**;**ACVR1B**;**THBS1***
Glioma	6/75	0.002	0.079	***PDGFRB**;**MDM2**;**TGFA**;**CALM3**;**CALM1**;**IGF1R***
PI3K-Akt signaling pathway	15/354	0.002	0.085	***PDGFRB**;**TGFA**;**PPP2R2A**;**THBS1**;EREG;**IGF1R**;**IL4**;**PPP2CB;*** *NR4A1;**CCND2**;**BCL2L11**;**MYC**;**MDM2**;**IL6R**;**BCL2L1***

organs via the systemic circulation, endothelial cells are unavoidable directly exposed to the airborne fine particles. Exposure to PM2.5 has been strongly linked to the disturbance of endothelial function either in human beings or in animal models. However, the underlying mechanism of PM2.5 on vascular endothelial cells is poorly understood. Hu and colleagues (Hu et al., 2016) studied for the first time the toxicity mechanism of PM2.5 on endothelial activation in vitro in human umbilical vein endothelial cells. They reported that PM2.5 triggers endothelial activation via upregulation of the IL-6 dependent JAK1/STAT3 signaling pathway. Interestingly, this pathway is also significantly over represented in the set of validated target genes whose expression is regulated by the seven miRNAs associated to PM2.5 exposure in the present study. Future studies integrating miRNAs as mediating mechanisms are warranted.

4.1. Strengths and limitations

There are several strengths of our study. First, advanced technological devices were used to measure personal exposure to air pollution, minimizing measurement error and misclassification of the exposure. Moreover, by repeatedly assessing participants for exposure measurements within one year, covering different seasons, and information concerning climate characteristics and other potential confounders, we were able to minimize these confounding factors, increasing the internal validity of our findings. Within the EXPOsOMICS consortium, a standardized protocol and operating procedure was applied across the four European areas, facilitating comparable results between each country. Nevertheless we cannot definitely exclude the possibility that differences in the sampling procedures within each study area could still be present and have an impact on the final results. Finally, the miRNA analyses were performed by the same laboratory for the samples collected in the different centres, thus minimizing the batch effects.

There are some limitations that may influence the interpretation of the results. A relatively limited number of individuals were included in the study, reducing statistical power, especially for the sensitivity analysis. This limitation is mainly due to the fact that both high-quality exposure assessment and molecular assessments are resource intensive, and consequently are feasibly carried out for a small number of subjects at present. Additionally, in this analysis we did not assess the relationship between miRNA expression and gene expression, which will need to be considered for future studies. However, we were able to predict genes likely regulated by miRNAs and potential pathway level effects from dysregulated miRNAs.

5. Conclusions

In conclusion, our results suggest an association between $PM_{2.5}$ personal exposure and the expression of seven miRNAs. We identified the p53 signaling pathways, pathways in cancer, and JAK-STAT signaling pathway as significantly enriched, a finding plausible with the carcinogenic properties attributed to $PM_{2.5}$ exposure. Further studies are needed to replicate these results and to investigate the molecular mechanisms by which exposure to air pollution leads to disease as well as to explore observed inconsistencies with other studies.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Francesca Romana Mancini: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Jessica E. Laine:** Conceptualization, Data curation, Writing - original draft, Writing - review & editing. **Sonia Tarallo:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **Jelle Vlaanderen:** Data curation. **Roel Vermeulen:** Conceptualization. **Erik van Nunen:** Methodology, Investigation, Data curation. **Gerard Hoek:** Conceptualization. **Nicole Probst-Hensch:** Conceptualization, Data curation. **Medea Imboden:** Data curation. **Ayoung Jeong:** Data curation. **John Gulliver:** Conceptualization, Data curation. **Marc Chadeau-Hyam:** Conceptualization,

Data curation. **Mark Nieuwenhuijsen:** Conceptualization, Data curation. **Theo M. de Kok:** Conceptualization. **Jolanda Piepers:** Methodology, Investigation, Data curation. **Julian Krauskopf:** Methodology, Investigation, Data curation, Writing - review & editing. **Jos C.S. Kleinjans:** Conceptualization, Data curation. **Paolo Vineis:** Conceptualization, Writing - original draft, Writing - review & editing, Supervision. **Alessio Naccarati:** Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114392.

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