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Full length article



The relationship between residential exposure to atmospheric pollution and circulating miRNA in adults living in an urban area in northern France

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ABSTRACT

Introduction: MicroRNAs are epigenetic regulatory factors capable of silencing the expression of target genes and might mediate the effects of air pollution on health. The objective of the present population-based study was to investigate the association between microRNA expression and long-term, residential exposure to atmospheric PM₁₀ and NO₂.

Method: We included 998 non-smoking adult participants from the cross-sectional ELISABET survey (2010–2014) in the Lille urban area of France. The mean residential annual pollution levels were estimated with an atmospheric dispersion modelling system. Ten microRNAs were selected on the basis of the literature data, together with two housekeeping microRNAs (miR-93-5p and miR-191-5p) and were quantified with RT-qPCRs. Multivariate linear regression models were used to study the association between microRNAs and air pollution. The threshold for statistical significance (after correction for the FDR) was set to $p < 0.1$.

Results: The mean annual exposure between 2011 and the year of inclusion was $26.4 \pm 2.0 \mu\text{g}/\text{m}^3$ for PM₁₀ and $24.7 \pm 5.1 \mu\text{g}/\text{m}^3$ for NO₂. Each $2 \mu\text{g}/\text{m}^3$ increment in PM₁₀ exposure was associated with an 8.6% increment (95%CI [3.1; 14.3]; $p_{\text{FDR}} = 0.019$) in miR-451a expression.

A $5 \mu\text{g}/\text{m}^3$ increment in NO₂ exposure was associated with a 5.3% increment ([0.7; 10]; $p_{\text{FDR}} = 0.056$) in miR451a expression, a 3.6% decrement (95%CI [-6.1; -1.1]; $p_{\text{FDR}} = 0.052$) in miR-223-3p expression, a 3.8% decrement (95%CI [-6.8; -0.7]; $p_{\text{FDR}} = 0.079$) in miR-28-3p expression, a 4.3% decrement (95%CI [-7.7; -0.8]; $p_{\text{FDR}} = 0.055$) in miR-146a-5p expression, and a 4.0% decrement (95% CI [-7.4; -0.4]; $p_{\text{FDR}} = 0.059$) in miR-23a-5p expression. The difference between the two housekeeping microRNAs miR-93-5p and miR-191-5p was also associated with PM₁₀ and NO₂ exposure.

Conclusion: Our results suggest that circulating miRNAs are potentially valuable biomarkers of the effects of air pollution.

1. Introduction

Air pollution has a major impact on health in general and on lung, cardiovascular and metabolic diseases in particular (Brook et al., 2010;

Riant et al., 2018). The association between air pollution and health is probably mediated by a number of biological pathways, which can be investigated by monitoring biomarkers of epigenetic modification or inflammation (Darras-Hostens et al., 2022).

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Epigenetic mechanisms have attracted increasing research interest in recent years because they can explain the interactions between environmental exposures and health-related events. Epigenetics can control gene expression through various mechanisms, ranging from structural changes in chromatin to post-transcriptional regulation. MicroRNAs (miRNAs) constitute a large family of small, non-coding post-transcriptional regulators that silence the expression of target genes by pairing with the cognate mRNAs, which leads to mRNA degradation or the abrogation of translation. Given that miRNAs modulate the expression of more than 60% of the genome, they regulate a wide variety of biological processes and are believed to be involved in the pathogenesis of many diseases, including cancer (Bushati and Cohen, 2007).

The expression of miRNAs can be tissue- and organ-specific (Landgraf et al., 2007). However, highly stable protein-bound miRNAs have also been detected in the majority of biological fluids, and particularly in the bloodstream (Pozniak et al., 2022; Mitchell et al., 2008; Gilad et al., 2008; Fujimoto et al., 2019). These circulating miRNAs might be either actively secreted by cells as mediators for intercellular and interorgan communication or passively released from dead cells after apoptosis, necrosis, tumor growth or tissue damage (Pozniak et al., 2022; Hunter et al., 2008).

Several studies have reported that environmental exposure can induce alterations in the miRNA profile (Krauskopf et al., 2017; Krauskopf et al., 2018; Rodosthenous et al., 2016; Rodosthenous et al., Nov 2018; Cheng et al., 2020). Given that the level of miRNA expression varies from one tissue to another, the profile of circulating miRNAs might reflect the specific responses of tissues exposed to a harmful environment (Krauskopf et al., 2015). Circulating miRNAs (whether actively secreted or passively released) are therefore promising new non-invasive biomarkers of tissue damage (Krauskopf et al., 2015).

The association between air pollution and miRNA has been only investigated in studies of small numbers of participants. The number of adults studied ranged from 14 (Chen et al., 2022) to 153 (Fossati et al., 2014), and one study included 273 children (Li et al., 2020). Furthermore, there are few published data on the association between miRNAs and long-term exposure to air pollution. Hence, the objective of the present study was to evaluate the association between mean long-term (annual) residential exposure to PM₁₀ and NO₂ and levels of a number of miRNAs thought to be modified by air pollution and to mediate the impact of air pollution on health.

2. Methods

2.1. Study population

We included male and female non-smoking adults (aged from 40 to 64) having resided in the Lille urban area of northern France for the previous 5 years. These adults were participating in the population-based, cross-sectional *Enquête Littoral Souffle Air Biologie Environnement* (ELISABET) epidemiological population survey, conducted between 2010 and 2013 in the Dunkirk and Lille areas. The study's methodology has been described in detail elsewhere (Riant et al., 2018; Darras-Hostens et al., 2022; Quach et al., 2015). Briefly, the participants were selected from electoral rolls by random sampling, with stratification for sex, age and city area (Lille or Dunkirk), and were contacted in random order. Data were collected at home or (occasionally) during a consultation in a healthcare establishment. A blood sample was collected during the same visit. We excluded pregnant women, incarcerated individuals, people under legal guardianship, people unable to give their informed consent, and people lacking French social security coverage. In compliance with the French legislation on biomedical research, the study protocol was approved by the local investigational review board (CPP Nord Ouest IV, Lille, France; reference: 2010-A00065-34; [ClinicalTrials.gov](https://clinicaltrials.gov) identifier: NCT02490553). All the participants provided their written, informed consent to participation in the study.

With a view to increasing the study's statistical power for a small

number of subjects (thus limiting the cost of analysis), we included participants from the Lille urban area only because the standard deviation of the residential air PM₁₀ level was higher in Lille than in Dunkirk (1.936 µg/m³ versus 0.999 µg/m³, respectively). To limited missing data and enable subsequent genetic studies, we included participants of Caucasian origin with data on genetic variables. Lastly, we excluded individuals without acceptable spirometry data and those lacking a fasting blood sample for the biomarker assay.

2.2. Air pollution measurements

The estimation of residential air pollution exposure has been described previously (Dauchet et al. (2018); Riant et al., 2018; Havet et al., 2020). In brief, the annual mean concentrations of PM₁₀ and NO₂ over the period from 2010 to 2013 were taken from estimates produced by ATMO-Nord Pas de Calais, using an atmospheric dispersion modelling system. The latter incorporated meteorological, topographic and land-use data, pollutant emissions from natural sources and those related to human activity, and ambient air pollution data from monitoring stations. Concentration maps for PM₁₀ and NO₂ in Lille are shown on the ATMO website (Nord-Pas-de-Calais, 2015). We applied a spatial resolution of 25 × 25 m. Each participant's place of residence was located within a 25 m grid. We assessed the annual exposure levels at the place of residence as the mean value of the four closest points in the grid, weighted by the inverse square distance to each point (Riant et al., 2018; Havet et al., 2020). The residential exposure was defined as the mean annual exposure at the residential address from 2010 to the year of inclusion.

2.3. miRNAs of interest

In the present study, we assayed eight circulating miRNAs in plasma prepared from an EDTA blood sample from each study participant: miR-21-5p, miR-28-3p, miR-30b-5p, miR-126-3p, miR-132-3p, miR-146a-5p, miR-222-3p, and miR-223-3p. These miRNAs were selected on the basis of the literature data because they are reportedly deregulated after exposure to various environmental air pollutants and/or in patients with lung disease possibly related to environmental exposure. Indeed, previous studies reported changes in the expression of miR-21-5p, miR-28-3p, miR-30b-5p, miR-126-3p, miR-132-3p, miR-146a-5p, miR-222-3p, and miR-223-3p following exposure to particulate matter of various aerodynamic diameter (coarse, fine or ultrafine particles), and/or to other gaseous pollutants related to traffic or industrial/biomass combustion (Rodosthenous et al., 2016; Fossati et al., 2014; Chen et al., 2018; Krauskopf et al., 2019; Louwies et al., 2016; Quezada-Maldonado et al., Oct 2018; Ruiz-Vera et al., 2019; Fry et al., 2014; Sima et al., 2021). On the other hand, the link between the deregulation of miR-21-5p, miR-30b-5p, miR-126-3p, miR-146a-5p, miR-222-3p or miR-223-3p and the pathogenesis chronic obstructive pulmonary disease (COPD), asthma, idiopathic pulmonary fibrosis, cystic fibrosis or lung cancer has also been described (Sima et al., 2021; Ebrahimi and Sadroddiny, Oct 2015; Rupani et al., 2013; Sessa and Hata, 2013; Alipoor et al., Nov 2016; Moretti et al., 7 Nov 2017). Four additional miRNAs were assayed: miR-23a-3p and miR-451a as controls for hemolysis and blood cell contamination (Shah et al., 2016; Blondal et al., 2013), and miR-93-5p and miR-191-5p as endogenous reference genes for data normalization, as described previously (Donati et al., 5 sept 2019).

The stability of these endogenous miRNAs was confirmed using NormFinder software (Andersen et al., 1 août 2004).

2.4. miRNA assays

For miRNA extraction, 200 µL of plasma samples were incubated for 5 min at room temperature with five volumes of Qiazol Lysis Reagent (Qiagen, Courtaboeuf, France) containing 1 µg of the extraction enhancer MS2 bacteriophage RNA (Sigma-Aldrich, Saint Quentin

Fallavier, France). After mixing with 200 μL of chloroform and incubation for 2 to 3 min at room temperature, the samples were centrifuged at 12,000 g for 15 min at 4 °C. The RNAs were then extracted from the aqueous phase using the miRNeasy Serum/Plasma kit and the QIAcube instrument, according to the supplier's instructions (Qiagen). The purity and quantity of RNAs were assessed by spectrophotometric analysis (the Spark multimode microplate reader equipped with the NanoQuant Plate™; Tecan, Männedorf, Switzerland).

Circulating miRNAs were reverse-transcribed using 2 μL of extracted RNAs and the TaqMan® Advanced miRNA cDNA Synthesis Kit, according to manufacturer's instructions (ThermoFisher Scientific, Courtaboeuf, France). Briefly, the synthesis of cDNAs started by 3' poly(A) tailing and 5' ligation of an adaptor sequence to extend the miRNAs at the 5' and 3' ends. The miRNAs were then reverse-transcribed and pre-amplified (using universal primers) to uniformly increase the amount of cDNA for each target while maintaining the relative differential levels. The universal primers recognize the universal sequences added to every miRNA and help to ensure the absence of amplification bias. All the target miRNAs were quantified using the TaqMan™ Fast Advanced Master Mix and the following TaqMan Advanced miRNA Assays: hsa-miR-21-5p_477975; hsa-miR-23a-3p_478532; hsa-miR-28-3p_477999; hsa-miR-30b-5p_478007; hsa-miR-93-5p_478210; hsa-miR-126-3p_477887; hsa-miR-132-3p_477900; hsa-miR-146a-5p_478399; hsa-miR-191-5p_477952; hsa-miR-222-3p_477982; hsa-miR-223-3p_477983; hsa-miR-451a_478107 (ThermoFisher Scientific). The samples were amplified using the QuantStudio™ 12 K Flex Real-Time PCR System (Applied Biosystems, ThermoFisher Scientific), and the results were calculated using the comparative cycle threshold (Ct) method and normalized against the signals detected for miR-93-5p and miR-191-5p.

2.5. miRNA analysis

Using the raw extraction values and the quartiles of the distribution of each miRNA, we determined an interval [min; max] outside of which values were considered to be outliers: min = quartile 1 - (1.5 * [interquartile range (IQR)]) and max = quartile 3 + (1.5 * [IQR]).

The lowest raw miRNA extraction values (expressed as $-\Delta\text{Ct}$) corresponded to a very high number of RT-PCR cycles and thus an almost undetectable amount of miRNA. We replaced the low outlier values by reassigning them with the corresponding min value.

The presence of hemolysis or red blood cell contamination in the sample was evaluated in two ways. Firstly, four medical biologists independently determined the presence or absence of hemolysis by eye. Secondly, we calculated the difference between the levels of the two hemolysis control miRNAs (miR-23a-3p and miR-45a) (Blondal et al., 2013). If the difference was greater than five, the sample was potentially contaminated by red blood cells. If the difference was greater than seven, the samples were considered to be hemolyzed.

We calculated the extraction values (normalized using the ΔCt method) by subtracting the mean value of the two housekeeping miRNAs [miRNA - mean (miR-93-5p and miR-191-5p)] from the value for each miRNA. The relative expression of the miRNAs (i.e. the variation between the different samples) was expressed as $2^{-\Delta\text{Ct}}$.

2.6. Inflammatory biomarker assays

The inflammatory biomarker assays have been described previously (Darras-Hostens et al., 2022). Briefly, the serum concentration of high-sensitivity C-reactive protein (hs-CRP) was measured in a nephelometric assay (BN ProSpec System, Siemens) with a detection range of 0.17 to 10 mg/L. Values below the limit of detection (LOD) were computed as $0.12 \text{ (LOD}/\sqrt{2}) \text{ mg/L}$. For cytokine measurements, we used MesoScale Discovery® electrochemiluminescent immunoassays (Meso Scale Diagnostics LLC, Rockville, MA, USA): multiplex assays for interleukin (IL)-1 β , IL-6, IL-8, IL-10, IL-17A, and tumor necrosis factor alpha (TNF α), and an individual assay for IL-22. Values below the lower

limit of quantification (the lowest concentration on the standard curve which gave a percentage coefficient of variation of <20%) were counted as real concentrations.

2.7. Statistical analysis

We used multivariate linear regression models to estimate the effect of PM₁₀ and NO₂ exposure on the expression levels of selected miRNAs (ΔCt). We adjusted our models for age, sex, body mass index, and year of inclusion. We adjusted for BMI because obesity is strongly related to many metabolism pathways including inflammation (de Heredia et al., 2012). We have adjusted for the year of inclusion because the construction of the exposure variable was strongly dependent of the inclusion year which may be associated to temporal confusion bias.

Effect estimates and their 95% confidence intervals were expressed as a percentage of the change in miRNA expression level for an increment in a defined unit of pollutant or biomarker, using the equation $(2 \text{ (estimate coefficient} \times \text{range of increment)} - 1) \times 100$. The increments of 2 $\mu\text{g}/\text{m}^3$ and 5 $\mu\text{g}/\text{m}^3$ were used because they were close to the IQR of exposure (Darras-Hostens et al., 2022). We studied the associations between miRNAs (ΔCt) and biomarkers of inflammation: cytokines (IL-1 β , IL-6, IL-8, IL-10, IL-17A, IL-22, TNF α , and hs-CRP).

Due to the large number of comparisons, we applied Benjamini-Hochberg false discovery rate (FDR) method to correct p-values for multiple comparisons. The threshold for statistical significance (after correction for the FDR) was set to $p < 0.1$.

We performed a stratified analysis on gender and test interaction for air pollution/gender interaction.

For the sensitivity analyses, we carried out the same analyses but excluded the hemolyzed samples determined by eye or by calculation. Lastly, we conducted sensitivity analyses using other miRNA normalization techniques: either by subtracting the mean of the three most stable miRNAs (miR-93-5p and miR-191-5p and miR-23a-3p) or the mean of all the other miRNAs from the level of each individual miRNA. The normalization method based on the mean of all the other miRNAs usually requires the measurement of dozens of miRNA and so could not be applied to our main analyses; however, we felt that its use could be justified in the sensitivity analysis (with 12 miRNAs measured (Khan et al., 2017)).

All statistical analyses were performed using R software (version 4.2.0).

3. Results

3.1. Characteristics of the study population

The ELISABET study included 1668 participants in the Lille urban area, of whom 1335 were nonsmokers (either never-smokers or people having stopped smoking at least 12 months previously). We selected the 1125 participants estimated to be Caucasian on the basis of the available genetic data. We then excluded 72 participants without acceptable spirometry data and 26 participants who had not fasted before the blood sample collection. Ultimately, biomarkers could be assayed for 1004 of the remaining participants. Measurements of the normalization housekeeping miRNAs (miR-93-5p or miR-191-5p) were missing for 6 participants, leaving 998 in the main analysis (454 (45.5%) men; median age: 54) (Table 1). In this sample, four values were missing for miR-28-3p, 6 for miR-132-3p and 5 miR-30b-5p. There were no missing data for the other miRNAs.

Between 2011 and the year of inclusion, the mean PM₁₀ exposure was $26.4 \pm 2.0 \mu\text{g}/\text{m}^3$ and the mean NO₂ exposure was $24.7 \pm 5.1 \mu\text{g}/\text{m}^3$. The correlation between PM₁₀ and NO₂ exposure levels was strong ($r_{\text{pearson}} = 0.79$; 95%CI [0.77; 0.81]; $p = < 2.2\text{e-}16$). The mean and Sd of fold log₂ fold change of the levels of miRNA are reported in supplemental table 1.

Main analysis: the association between air pollution exposure and

Table 1
Characteristics of the study participants (n = 998).

Variable	Value
Age (year)	53.8 (7.2)
Male sex, n (%)	454 (45.5)
Height (cm)	169.6 (8.9)
BMI (kg/m ²)	26.8 (5.0)
Mean annual levels of PM ₁₀ pollution between 2011 and the inclusion year (µg/m ³)	26.4 (2.0)
Mean annual levels of NO ₂ pollution between 2011 and the inclusion year (µg/m ³)	24.7 (5.1)
hs-CRP (mg/L)	0.9 (0.5; 1.9)
IL1b (pg/mL)	0.0 (0.0; 0.1)
IL6 (pg/mL)	0.4 (0.3; 0.7)
IL8 (pg/mL)	2.7 (2.1; 3.8)
IL10 (pg/mL)	0.2 (0.1; 0.3)
IL17a (pg/mL)	0.8 (0.5; 1.3)
IL22 (pg/mL)	0.2 (0.1; 0.4)
TNFα (pg/mL)	2.4 (1.9; 2.9)
DBP (mmHG)	126 (115; 139.4)
HbA1c (%)	5.5 (5.2; 5.9)
Glycemia (g/L)	0.9 (0.9; 1)
Total cholesterol (g/L)	2.2 (1.9; 2.5)
Triglycerides (g/L)	0.9 (0.7; 1.3)
HDL (g/L)	0.6 (0.5; 0.7)
LDL (g/L)	1.4 (1.2; 1.6)

Data are expressed as the mean (SD), the median [IQR] or n (%)
 BMI: body mass index, PM10: particulate matter < 10 µm, NO2: nitrogen dioxide, HbA1c: glycosylated hemoglobin, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

miRNA expression.

After correction for the FDR ($p_{FDR} < 0.1$), we found that one miRNA (miR-451a) was positively associated with both PM₁₀ and NO₂ levels and that four miRNAs levels (miR-223-3p, miR-28-3p, miR-146a-5p, and miR-23a-5p) were negatively associated with the NO₂ level (Table 2). A 2 µg/m³ increment in PM₁₀ exposure was associated with an 8.6% increment (95% confidence interval [3.1; 14.3]; $p_{FDR} = 0.019$) in miR-451a expression (Table 2). A 5 µg/m³ increment in NO₂ exposure was associated with a 5.3% increment ([0.7; 10]; $p_{FDR} = 0.056$) in miR-451a and a 3.6% decrement ([-6.1; -1.1]; $p_{FDR} = 0.052$) in miR-223-3p, a 3.8% decrement ([-6.8; -0.7]; $p_{FDR} = 0.079$) in miR-28-3p, a 4.3% decrement ([-7.7; -0.8]; $p_{FDR} = 0.055$) in miR-146a-5p, and a 4.0% decrement ([-7.4; -0.4]; $p_{FDR} = 0.059$) in miR-23a-5p (Table 2). We also observed that the difference between the two housekeeping miRNAs (miR-93-5p normalized against miR-191-5p or vice versa) were significantly associated with PM₁₀ and NO₂.

Table 2
Association between residential PM10 and NO2 and miRNA, using the level of the miRNA minus the average of the two housekeeping miRNAs.

micro-RNA	PM ₁₀ (per 2 µg/m3 increment)			micro-RNA	% difference [95CI%]	p FDR*	
	% difference [95CI%]	p	p FDR				
miR-451a	8.6 [3.1; 14.3]	0.002	0.019	miR-223-3p	-3.6 [-6.1; -1.1]	0.005	0.052
miR-28-3p	-4.3 [-7.8; -0.6]	0.022	0.112	miR-28-3p	-3.8 [-6.8; -0.7]	0.016	0.079
miR-223-3p	-3.1 [-6.0; -0.1]	0.042	0.140	miR-146a-5p	-4.3 [-7.7; -0.8]	0.016	0.055
miR-132-3p	3.1 [-0.8; 7.2]	0.117	0.292	miR-451a	5.3 [0.7; 10.0]	0.022	0.056
miR-146a-5p	-3.0 [-7.0; 1.2]	0.164	0.328	miR-23a-5p	-4.0 [-7.4; -0.4]	0.029	0.059
miR-23a-5p	-3.0 [-7.1; 1.3]	0.166	0.277	miR-126-3p	-2.2 [-4.5; 0.2]	0.073	0.122
miR-126-3p	-2.0 [-4.7; 0.9]	0.174	0.248	miR-30b-5p	-2.3 [-5.6; 1.1]	0.175	0.249
miR-222-3p	-1.6 [-4.9; 1.9]	0.362	0.452	miR-222-3p	-1.9 [-4.7; 1.0]	0.205	0.256
miR-30b-5p	-1.7 [-5.6; 2.3]	0.395	0.438	miR-132-3p	1.5 [-1.7; 4.9]	0.359	0.399
miR-21-5p	0.5 [-2.7; 3.7]	0.781	0.781	miR-21-5p	-0.8 [-3.4; 2.0]	0.577	0.577
miR-93-5p	3.8 [1.2; 6.4]	0.004		miR-93-5p	3.5 [1.3; 5.7]	0.002	
miR-191-5p	-3.6 [-6.0; -1.2]	0.004		miR-191-5p	-3.4 [-5.4; -1.3]	0.002	

* p value corrected for the false discovery rate adjusted for age, sex, body mass index, and year of inclusion
 PM₁₀: particulate matter < 10 µm, NO₂: nitrogen dioxide

3.2. Sensitivity analyses

Stratified analysis on gender are presented in supplemental table 2 and 3. none of the interaction test between air pollution and gender were Significant after FDR correction. miR-23a-5p, miR-223-3p and miR-451a were significantly associated with NO₂ and PM10 were significantly associated with miR-451a and miR-132-3p after FDR correction in women. no association with NO₂ or PM10 were significant in men.

A total of 130 (12.0%) samples with hemolysis were excluded: 26 by eye and 104 using the calculation method (Supplemental Table 4). Exclusion of the samples with hemolysis did not greatly modify the results, except that the association between PM₁₀ and miR-28-3p became statistically significant. We also observed fewer significant associations with NO₂ exposure after correction for the FDR.

When subtracting the mean level of the three most stable miRNAs (miR-93-5p, miR-191-5p and miR-23a-3p) from each miRNA, we found that two of the other miRNAs (miR-451a and miR-132-3p) were positively associated with PM₁₀ and one (miR-28-3p) was negatively associated with PM₁₀ (Supplemental table 5) and NO₂. However, only the association with miR-451a remained significant after correction for the FDR (Supplemental Table 6).

When subtracting the mean of all the other miRNAs from each miRNA, we found that three miRNAs were positively associated with PM₁₀ (miR-451a, miR-93-5p and miR-132-3p), three (miR-191-5p, miR-28-3p,miR-223-3p) were negatively associated with PM₁₀ (Supplemental Table 3), three (miR-451a, miR-93-5p and miR-132-3p) were positively associated with NO₂, and five (miR-146a-5p, miR-23a-5p, miR-223-3p, miR-28-3p and miR-191-5p) were negatively associated with NO₂.

3.3. Additional analyses

Association between inflammatory biomarkers and miRNA.

By considering the difference with the mean of the two-housekeeping miRNA, we found positive associations between inflammatory biomarkers and miRNAs. MiR-223-3p and miR-21-5p were associated with hs-CRP, IL-6 and IL-10; miR-30b-5p was associated with hs-CRP; miR-126-3p was associated with IL-6 and IL-10; and miR-23a-5p and miR-132-3p were associated with IL-10. No significant associations were observed for IL-1β, IL-8, IL-17a, TNFα, and IL-22.

4. Discussion

The results of our population-based study showed that long-term exposure to residential air pollution was associated with plasma miRNA levels and thus suggested that air pollution impacts gene

Table 3

Association between inflammatory biomarkers and miRNA, using the miRNA level minus the average of the two housekeeping miRNAs.

	microRNA	% difference	[95%CI]	Pr(> t)	p FDR*
hs-CRP	miR-223-3p	9.3	[3.1; 15.9]	0.003	0.028
	miR-21-5p	9.3	[2.8; 16.2]	0.004	0.022
	miR-30b-5p	10.5	[2.3; 19.5]	0.012	0.040
	miR-146a-5p	8.3	[-0.3; 17.7]	0.058	0.145
	miR-126-3p	5.3	[-0.3; 11.3]	0.063	0.126
	miR-28-3p	6.9	[-0.5; 14.9]	0.070	0.117
	miR-23a-5p	7.1	[-1.4; 16.3]	0.106	0.151
	miR-222-3p	4.3	[-2.4; 11.6]	0.213	0.267
	miR-451a	-5.8	[-14.8; 4.2]	0.250	0.277
	miR-132-3p	3.9	[-3.7; 12.0]	0.324	0.324
	miR-93-5p	-6.4	[-10.8; -1.7]	0.008	
	miR-191-5p	6.8	[1.8; 12.1]	0.008	
	IL6	miR-223-3p	7.1	[2.1; 12.3]	0.005
miR-21-5p		6.6	[1.4; 12.0]	0.013	0.064
miR-126-3p		5.7	[1.1; 10.5]	0.015	0.049
miR-30b-5p		5.0	[-1.5; 11.8]	0.133	0.332
miR-146a-5p		4.7	[-2.1; 11.9]	0.181	0.361
miR-23a-5p		3.9	[-2.8; 11.1]	0.263	0.438
miR-222-3p		1.6	[-3.8; 7.3]	0.566	0.809
miR-132-3p		0.3	[-5.5; 6.6]	0.915	1.144
miR-28-3p		0.2	[-5.5; 6.2]	0.942	1.047
miR-451a		0.1	[-7.8; 8.6]	0.983	0.983
miR-93-5p		-1.9	[-5.7; 2.0]	0.331	
miR-191-5p		2.0	[-1.9; 6.0]	0.331	
IL10		miR-23a-5p	26.5	[10.5; 44.8]	0.001
	miR-21-5p	14.6	[3.6; 26.7]	0.008	0.042
	miR-223-3p	13.1	[2.7; 24.6]	0.012	0.041
	miR-126-3p	10.4	[0.9; 20.8]	0.031	0.077
	miR-132-3p	13.0	[0.1; 27.7]	0.049	0.098
	miR-146a-5p	13.8	[-0.5; 30.3]	0.060	0.100
	miR-30b-5p	11.8	[-1.6; 26.9]	0.087	0.125
	miR-28-3p	10.4	[-1.9; 24.1]	0.101	0.126
	miR-451a	-8.6	[-22.6; 7.8]	0.284	0.316
	miR-222-3p	5.3	[-5.6; 17.6]	0.355	0.355
	miR-93-5p	-7.5	[-14.5; 0.1]	0.053	
	miR-191-5p	8.1	[-0.1; 17.0]	0.053	

* p value corrected for the false discovery rate.

hs-CRP: high-sensitivity C-reactive protein.

regulation processes. Plasma levels of miR-223-3p, miR-28-3p, miR-146a-5p and miR-23a-5p were negatively associated with NO₂ exposure, and whereas the level of miR-451a was positively associated with NO₂ exposure. No significant interaction between gender and air pollution were observed. We didn't highlight any difference or air pollution effect between gender. When studying PM₁₀, only one association (with miR-451a) achieved statistical significance after correction for the FDR. Nevertheless, PM₁₀ and NO₂ were strongly correlated, and so it is hard to identify specific effects of one or the other. The standard deviations of NO₂ were higher than those of PM₁₀, which might have resulted in greater statistical power in the analysis of the association with NO₂.

Previous studies of the association between air pollution and these miRNAs had smaller samples and gave disparate results. These studies are described in supplemental table 7 Most of previous studies focus on short term exposure: five studies had cross sectional design (Krauskopf et al., 2018; Chen et al., 2018; Krauskopf et al., 2019; Khan et al., 2017; Broz and Dixit, 2016) including one with experimental exposure to diesel exhaust (Khan et al., 2017), six were small cohort with repeated measurement of exposure and miRNA (Rodosthenous et al., 2016; Louwies et al., 2016; Bauernfeind et al., 15 juill 2009; Olivieri et al., 2021; Bauernfeind et al., 15 Oct 2012; Haneklaus et al., 15 Oct 2012) two were cross-sectional studies (Li et al., 2020; Herberth et al., 2014). Long term exposure was less studied, Only Rodosthenous et al (Rodosthenous et al., 2016) studied short- and long-term exposure (6month-and1year) with repeated measurement. The association with benzo[a]

pyrene-r-7,t-8,t-9,c-10-tetrahydrotetrol and serum albumin(BPDE-Alb), a biomarker of PM exposure was studied in children (Li et al., 2020) and a cohort of pregnant women studied exposure in pregnancy associated to miRNA in placenta at birth.

The negative association we observed between NO₂ and miR-23a-5p was consistent with negative association observed at short term with traffic air pollution (TRAP) in chinees students (Broz and Dixit, 2016) (supplemental table 8). But conversely to our find a positive association for long term exposure were observed in the study of Rodosthenous et al (Rodosthenous et al., 2016). The negative association we observed between NO₂ and miR-28-3p has not been found in previous study. One positive association were observed at short term in a cross-sectional study (Krauskopf et al., 2019). The negative association between NO₂ and miR-146a-5p were consistent with previous association observed with short term exposure (Fossati et al., 2014; Chen et al., 2018; Haneklaus et al., 15 Oct 2012) but conversely positive association at short term (Rodosthenous et al., 2016; Krauskopf et al., 2019) and positive association with BPDE-Alb (Li et al., 2020) were also observed. The negative association with miR-223-3p were inconsistent with positive association at short (Rodosthenous et al., 2016; Khan et al., 2017) and long term (Rodosthenous et al., 2016) observed previously. The negative association at long term we observed with miR-451a were inconstant with the positive short-term association observed by Rodosthenous et al (Rodosthenous et al., 2016). Although miR-191-5p and mir-93-5p were used as housekeeping miRNAs the results suggest that at least on of the may be associated to air pollution, indeed Rodousthenous et al have previous found significant association between air pollution these miRNAs. Finally, Other association have been observed for the miRNAs not associated to air pollution in our study and are listed in supplemental table 8 with biological effect or evaluated miRNAs. The inconsistency of result may be explained by difference population in population, exposure and methodology. More population base study with large sample are needed to confirm these results.

4.1. Physiological pathways

Inflammasomes are cytosolic protein complexes responsible for the activation of inflammatory pathways in response to a wide range of pathogens and cell damage signals (Broz and Dixit, 2016). Transcription of the most-studied inflammasome (NLR family pyrin domain containing 3 (NLRP3)) can be induced by Toll-like receptor (TLR) agonists and some inflammatory cytokines in an NF- κ B-dependent manner (Bauernfeind et al., 15 juill 2009). Through activation of caspase 1, activation of the NLRP3 inflammasome allows the conversion of the proinflammatory cytokines IL-1 β and IL-18 into their mature forms, together with the induction of gasdermin-D-mediated pyroptotic cell death.

In order to avoid inappropriate activation, basal levels of inflammasome components are rather low in most cells. It is now well established that inflammasome functionalization can be modulated by a subset of miRNAs called inflammamiRs (Olivieri et al., 2021). Three of the miRNAs negatively correlated with pollution exposure in the present study are classified as inflammamiRs; miR-223-3p, miR-146a-5p, miR-23a-3p.

MiR-223-3p was the first human miRNA found to directly target NLRP3 (Bauernfeind et al., 15 Oct 2012; Haneklaus et al., 15 Oct 2012) and thus exhibits significant anti-inflammatory and antipyroptotic effects. In a number of studies, miR-223-3p expression is associated with exposure to multiple airborne contaminants. Nevertheless, the results of these studies are not always consistent and appear to vary as a function of the nature of the pollutant or the biological matrix analyzed. In a pilot study with 22 participants, Rodosthenous et al. (Herberth et al., 2014) found a significant association between long-term ambient PM_{2.5} exposure and circulating levels of extracellular vesicle-encapsulated miR-223-3p. In accordance with these results, several studies have described increases in expression levels of this miRNA in response to other environmental challenges (Fry et al., 2014; Schembri et al., 17 févr

2009). In contrast, a significant downregulation of miR-223 was observed in human and rat lungs after tobacco smoke exposure (Izzotti et al., 2009; Carberry et al., 2022). Lastly, in line with our present results, the plasma miR-223-3p level was low in female CD-1 mice exposed to smoke condensate samples collected from different biomass burn scenarios (Hou et al., 2021).

As with miR-223, miR-146a has an anti-inflammatory role because it can downregulate the NF- κ B-mediated priming of the NLRP3 inflammasome. Indeed, miR-146a targets TLR4, myeloid differentiation primary response gene 88 (MyD88), interleukin-1 receptor-associated kinase 1 (IRAK1), and TNF-receptor-associated factor (TRAF6) – all key proteins in the canonical NF- κ B pathway (Olivieri et al., 2021; Etzrodt et al., 19 avr 2012). Moreover, miR-146a downregulates RelB, a pivotal factor in the non-canonical NF- κ B pathway (McMillan et al., 2013; Boldin et al., 6 juin 2011). The anti-inflammatory role of miR-146a was also evidenced by the fact that miR-146a knock-out mice develop NF- κ B-driven low-grade inflammation (Zhao et al., 31 mai 2011.; Bollati et al., 2010). In agreement with our findings, several researchers have observed a negative correlation between PM exposure and miR-146a-5p levels (Fossati et al., 2014; Chen et al., 2018; Tsamou et al., 2018; Chang et al., 2021).

In contrast, a 24-participant cross-over study of personal air pollution exposure assessment gave discordant results: the circulating level of miR-146a-5p was positively correlated with exposure to airborne ultrafine particles (Krauskopf et al., 2019).

Lastly, miR-23a-3p can also help to downregulate the inflammasome by targeting the NLRP3-activating factors NIMA-related kinase 7 (NEK7) (Pan et al., 31 janv 2018) or the CXCR4 chemokine receptor involved in stabilization of thioredoxin interacting protein (TXNIP) (Yang et al., Oct 2011). Initially, miR-23a-3p was selected (along with miR-451a) to evaluate the level of hemolysis in biological samples. Very few studies have linked the expression of this miRNA with exposure to air pollution. In their pilot study of 22 participants, Rodosthenous et al. (Herberth et al., 2014) observed a significant association between long-term ambient PM_{2.5} exposures and circulating miR-23a-3p levels. However, as with the two previously mentioned anti-inflammatory miRNAs (i.e. miR-223-3p and miR-146a-5p), we observed a negative correlation between the plasma miR-23a-3p level and pollution exposure. Taken as a whole, these data suggest that organisms adapt to and control the inflammatory response induced by exposure to air pollutants. The association between short-term exposure to atmospheric air pollution and low-grade systemic inflammation previously observed in our study population might reflect this phenomenon (Darras-Hostens et al., 2022).

Although not described as inflammasomes, the two other miRNAs correlated with pollution exposure in our study (i.e. miR-28-3p and miR-451a) regulate inflammatory signaling pathways. Yang et al. showed that miR-28 directly targets NRF2 (Jain et al., 2010), a major regulator of antioxidant and anti-inflammatory responses (Ichimura et al., 2013; Hur et al., 2015). Hence, miR-28-3p could be considered as a pro-inflammatory miRNA. In the present study, we observed a negative correlation between miR-28-3p levels and exposure to PM₁₀ and NO₂. Krauskopf et al.'s recent preliminary study of early biomarkers in only 30 participants showed that circulating miR-28-3p was positively correlated with a mixture of compounds found in traffic-related air pollution, including black carbon, CO₂, nitrogen oxides, and ultrafine particles (Krauskopf et al., 2019). However, Krauskopf et al. did not find an association with PM_{2.5}.

Based on the literature data, miR-451a is a potential anti-inflammatory miRNA. Indeed, several studies have described how miR-451a inhibits the NF- κ B pathway by reducing the expression of CAB39 (Li et al., Nov 2013), IKKB (Xie et al., 2016) and HMGB1 (Barney-Verdier et al., 2009; Motta et al., 2016). Our present study evidenced a positive correlation between the plasma level of miR-451a and long-term pollution exposure. Motta et al. (Schwartz et al., 15 déc 2005) did not find a significant association with PM₁₀ levels after 24 or 48 h of exposure. However, Motta et al.'s study included 90 overweight or obese

adults, and obesity has already been shown to induce variability in the effects of PM (Dubowsky et al., 2006; Deng et al., 2019). Similarly, Rodosthenous et al. found no association between short- and long-term PM_{2.5} exposure and the miR-451a level. (Herberth et al., 2014). In contrast, Deng et al. observed low miR-451a plasma levels in coke oven workers exposed to both metals and polycyclic aromatic hydrocarbons (Boxberger et al., 15 avr 2019).

The tight control over inflammasome assembly and signaling is crucial for enabling the immune system to mount sufficiently strong antimicrobial and inflammatory responses to environmental challenges while avoiding the excessive tissue damage that could result from an uncontrolled reaction. Thus, deregulated inflammasome activation has been linked to the development of various diseases, from inflammatory diseases to cancers. In this context, any variation in the expression of inflammation-related miRNAs might reflect or cause pathological processes. It is therefore not surprising that the deregulation of the five miRNAs correlated with pollution exposure in our study has been linked to the pathogenesis of many diseases. In particular, the inflammasome-modulating miRNAs miR-23a-3p, miR-146a-5p and miR-223-3p are reportedly dysregulated in a wide range of immune inflammatory diseases (such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, type 1 diabetes, and asthma (Mortazavi-Jahromi et al., Nov 2020; Roffel et al., 2020), various non-immune inflammatory diseases (such as COPD and atopic, metabolic, neuroinflammatory and cardiovascular diseases (Roffel et al., 2020; Specjalski and Jassem, 2019; Aslani et al., Oct 2021; Vickers et al., 2014; Zhang et al., 2020; Grieco et al., 2022; Kaur et al., 2022; Iacona and Lutz, 2019), and several types of cancer (Favero et al., Nov 2021; Wang et al., 20 déc 2018;; Silva et al., 24 mars 2021). The expression of the remaining miRNAs (miR-28a-3p and miR-451a) is reportedly altered in tumoral diseases; these miRNAs have been suggested as promising cancer biomarkers with therapeutic potential (Huang et al., 2017; Khordadmehr et al., 2019; Ghafouri-Fard et al., Nov 2021). Lastly, abnormal levels of miR-451a have been observed in patients with cardiac fibrosis (Ghafouri-Fard et al., 2021), cardiovascular diseases (Wang et al., 17 déc 2021;; Churov et al., Nov 2015) and rheumatoid arthritis (Kyyaly et al., 2021), and serum concentrations of miR-28a-3p are potential biomarkers in severe asthma (Akbas et al., 2012) and COPD (Du et al., 2022).

4.2. Strengths and weaknesses

The present study had several strengths. Firstly, to the best of our knowledge, this is the first population-based present study to have measured the association between air pollution and miRNAs. Secondly, our study included a large sample (n = 998) of non-smokers, which is more than in previous studies. Our study has greater sample size than previous ones it had enough power to detect association with similar effect size than those previously observed. For example, Fossati et al. (Brook et al., 2010) observed a -19.4% decrease of miR-126 for 3.02 $\mu\text{g}/\text{m}^3$ increase of 28 days exposure to PM_{2.5}. In our study the standard deviation of PM₁₀ exposure were 2 $\mu\text{g}/\text{m}^3$. to take in account that standard deviation(SD) of PM₁₀ which include PM_{2.5} are by definition higher than SD of PM_{2.5} we multiply the SD of PM₁₀ by 0.7(Riant et al., 2018) and considering a SD of exposure at 1.4 $\mu\text{g}/\text{m}^3$ for PM exposure. The SD of difference of Ct between miRNA and average of the two housekeeping miRNAs were 0.604. according to these data with an alpha risk at 0.005 (to take in account FDR correction). the power our study was estimate at 98%. Thirdly, most previous studies looked at short-term exposure; we provided new data on long-term exposure with a fine-scale model (25x25m).

Our study also had some limitations. Firstly, we chose housekeeping miRNAs because of their known stability, which was confirmed here. Nevertheless, the observed association between housekeeping miRNAs and exposure might have influence the interpretation of our data. The association between housekeeping miRNAs and pollution can be explore

indirectly only if several housekeeping miRNAs have been studied. This validity check is not generally described in the literature on housekeeping miRNA (Donati et al., 5 sept 2019;; Chen et al., 2 mai 2020;; Bohatá et al., 2021). Here, we identified a significant association between air pollution and the difference between the two main housekeeping miRNAs; this prevented us from drawing any definite conclusions about the effect size of the observed associations. Nevertheless, our results are consistent because the associations observed in the main analysis were also seen in the sensitivity analyses.

5. Conclusion

We observed associations between residential long-term air pollution exposure and a number of miRNAs in large population-based sample of urban inhabitants. The miRNAs significantly associated with air pollution are mostly involved in inflammation - suggesting that inflammation is a major pathway. Our results suggest that circulating miRNAs are potentially valuable biomarkers of the environmental risk.

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The data are not publicly available due to restrictions their contained information that could compromise the privacy of research participants. Demand of data transfer agreement can be done writing to the corresponding author LD, it needs agreement of the investigators and the sponsor and in the respect of the French law.

CRedit authorship contribution statement

AudreyHubert: Formal analysis, Writing - original draft, **Djamal Achour:** Resources, Writing - review & editing. **Céline Grare:** Resources, Writing - review & editing. **Gianni Zarcone:** Resources, Writing - review & editing. **Manon Muntaner:** Data curation, Formal analysis, Writing - review & editing. **Aghiles Hamroun:** Statistical analysis, Writing - review & editing. **Victoria Gauthier:** Formal analysis, Writing - review & editing. **Philippe Amouyel:** Funding acquisition, Conceptualization, Writing - review & editing. **Régis Matran:** Funding acquisition, Conceptualization, Writing - review & editing. **Farid Zerimech:** Resources, Writing - review & editing. **Jean-Marc Lo Guidice:** Resources, Conceptualization, Writing - review & editing. **Luc Dauchet:** Funding acquisition, Conceptualization, Writing - review & editing, Supervision.

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.

Data availability

The authors do not have permission to share data.

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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.2023.107913>.

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