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Publication Date

2023-07-01

DOI

10.1016/j.envres.2023.115949

Peer reviewed

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Environmental Research 229 (2023) 115949

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Intermediate and long-term exposure to air pollution and temperature and the extracellular microRNA profile of participants in the normative aging study (NAS)☆

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ARTICLE INFO

Handling Editor: Jose L Domingo

Keywords:
Particulate matter
Ozone
Nitrogen dioxide
Ambient temperature
microRNA
Air pollution

ABSTRACT

Background: The molecular effects of intermediate and long-term exposure to air pollution and temperature, such as those on extracellular microRNA (ex-miRNA) are not well understood but may have clinical consequences.
Objectives: To assess the association between exposure to ambient air pollution and temperature and ex-miRNA profiles.
Methods: Our study population consisted of 734 participants in the Normative Aging Study (NAS) between 1999 and 2015. We used high-resolution models to estimate four-week, eight-week, twelve-week, six-month, and one-year moving averages of $PM_{2.5}$, O_3, NO_2, and ambient temperature based on geo-coded residential addresses. The outcome of interest was the extracellular microRNA (ex-miRNA) profile of each participant over time. We used a longitudinal quantile regression approach to estimate the association between the exposures and each ex-miRNA. Results were corrected for multiple comparisons and ex-miRNAs that were still significantly associated with the exposures were further analyzed using KEGG pathway analysis and Ingenuity Pathway Analysis.
Results: We found 151 significant associations between levels of $PM_{2.5}$, O_3, NO_2, and ambient temperature and 82 unique ex-miRNAs across multiple quantiles. Most of the significant results were associations with intermediate-term exposure to O_3, long-term exposure to $PM_{2.5}$, and both intermediate and long-term exposure to ambient temperature. The exposures were most often associated with the 75th and 90th percentile of the outcomes. Pathway analyses of significant ex-miRNAs revealed their involvement in biological pathways involving cell function and communication as well as clinical diseases such as cardiovascular disease, respiratory disease, and neurological disease.

Abbreviations: mRNA, messenger RNA; miRNA, microRNA; EV-miRNA, extracellular vesicle miRNA; ex-miRNA, extracellular miRNA.

☆ Dr. Feiby L. Nassan is a current employee and a shareholder of Biogen, Cambridge, MA. The original work on this study at Harvard T. H. Chan School of Public Health (HSPH), however, pre-dated the current employment. This manuscript does not mention any Biogen products or any of the disease states that Biogen is actively doing research in (to the coauthor's knowledge). Dr. Joel D. Schwartz has appeared as an expert witness on behalf of the US Department of Justice in cases involving violations of the Clean Air Act.

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https://doi.org/10.1016/j.envres.2023.115949
Received 1 February 2023; Received in revised form 27 March 2023; Accepted 18 April 2023
Available online 19 April 2023
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Conclusion: Our results show that intermediate and long-term exposure to all our exposures of interest were associated with changes in the ex-miRNA profile of study participants. Further studies on environmental risk factors and ex-miRNAs are warranted.

1. Introduction

The contribution of air pollution to clinical outcomes such as cardiovascular disease, respiratory disease, neurological disease, and mortality has been extensively studied (Danesh Yazdi et al., 2021a, 2021b; 2019; Di et al., 2017; Dockery et al., 1993; Klompmaker et al., 2019; Shi et al., 2020; Ward-Caviness et al., 2021; Wei et al., 2020). Recent research has shifted some of the focus on the molecular effects of deleterious environmental exposures which may eventually manifest as clinical disease. These include studies of outcomes such as DNA methylation, telomere length, and inflammatory biomarkers (Baccarelli et al., 2009; Chuang et al., 2007; Plusquin et al., 2017; Rider and Carlsten, 2019; Wang et al., 2022; Xia et al., 2015). One area of relatively newer interest has been the relationship between air pollution and microRNAs(Bollati et al., 2015; Chen et al., 2013; Danesh Yazdi et al., 2023; Fossati et al., 2014a; Mancini et al., 2020; Pergoli et al., 2017).

MicroRNAs (miRNAs) are short non-coding segments of RNA which have been identified as an important mechanism for the regulation of messenger RNA (mRNA) translation and/or stability (Bartel, 2004; Fabian et al., 2010; Roush and Slack, 2006; Saliminejad et al., 2019). MicroRNA are found both intracellularly and in extracellular spaces. Extracellular miRNAs (ex-miRNAs) can be found within extracellular vesicles (EVs) and outside of them as well. Ex-miRNAs, particularly EV-miRNAs, may also play a role in cell-to-cell communication and are of particular interest in research as they may serve as diagnostic or prognostic biomarkers (Saliminejad et al., 2019; Zhang et al., 2015).

Several past studies have found associations between exposure to air pollution and other environmental risk factors and alterations in the miRNA profile of individuals (Bollati et al., 2015; Cecconi et al., 2022; Chen et al., 2022, 2020; Cong et al., 2022; Danesh Yazdi et al., 2023; Espín-Pérez et al., 2018; Fossati et al., 2014b; Krauskopf et al., 2019, 2018; Li et al., 2020; Liu et al., 2019; Mancini et al., 2020; Motta et al., 2016; Rodosthenous et al., 2018, 2016). However, very few studies have focused on ex-miRNAs or EV-miRNAs and most of those have looked only at short-term exposure to these pollutants (Chen et al., 2022, 2020; Danesh Yazdi et al., 2023; Krauskopf et al., 2019, 2018; Pergoli et al., 2017). Very few have utilized multi-pollutant models to account for confounding by other exposures (Chen et al., 2020; Danesh Yazdi et al., 2023; Rodosthenous et al., 2018). Moreover, while some have included ambient temperature as a covariate, most have not looked at this variable as an exposure of interest (Chen et al., 2020, 2022; Cong et al., 2022; Mancini et al., 2020; Motta et al., 2016; Pergoli et al., 2017). Furthermore, most of these studies were conducted in very small groups of individuals (Bollati et al., 2015; Cecconi et al., 2022; Chen et al., 2022, 2020; Espín-Pérez et al., 2018; Fossati et al., 2014b; Krauskopf et al., 2018, 2019).

To address these gaps, we conducted a study assessing the intermediate- and long-term effects of exposure to air pollutants - specifically $PM_{2.5}$, nitrogen dioxide (NO_2), and ozone (O_3) - as well as ambient temperature on the ex-miRNA profiles of subjects in the Normative Aging Study (NAS) from 1999 to 2015. We hypothesized that these exposures would be significantly associated with alterations in levels of ex-miRNA. We used a longitudinal quantile regression approach to examine this association. We then conducted pathway analyses to assess the potential biological consequences of changes in the counts of ex-miRNA.

2. Materials and methods

2.1. Study population

NAS is a cohort established in 1963 which recruited male veterans living in the Greater Boston Area who had no chronic health conditions and utilized the services of the US Department of Veteran's Affairs (VA). These individuals have been followed for the past six decades, with follow-up visits occurring every three to five years. The follow-up visits included physical exams, sample collection, and behavioral and health questionnaires. Greater detail about the NAS can be found elsewhere (Bell et al., 1966). Our study population included individuals who provided samples for analysis between 1999 and 2015 and lived in the contiguous United States during the study follow-up.

The VA Boston Health Care System and Harvard TH Chan School of Public Health Institutional Review Boards approved this study. All participants provided written consent for inclusion in the cohort.

2.2. Exposure assessment

Our exposures of interest included intermediate to long-term exposure to average ambient temperature and air pollutants, namely: $PM_{2.5}$, NO_2, and O_3. Intermediate-term exposure was defined as moving averages of four weeks, eight weeks, and twelve weeks of exposure and long-term exposure was defined as moving averages of six months and one year of exposure. Levels of these exposures were assigned based on the participants' geo-coded residential addresses from fine-scale high-resolution spatiotemporal models.

For air pollutants, the exposure models used predictors such as meteorological variables, chemical transport models, land use variables, and remote sensing data as input for three machine learners: a random forest, a gradient boosting machine, and a neural network. The predictions from the machine learning models were then incorporated into a geographically weighted generalized additive ensemble model which in turn generated the final predictions. Pollution levels were estimated on a daily temporal scale and a 1 km by 1 km spatial scale. These predictions were validated against monitored levels using ten-fold cross-validation. The cross-validated R^2 for daily levels was 0.86 for $PM_{2.5}$, 0.79 for NO_2, and 0.90 for O_3. The cross-validated R^2 for annual levels was 0.89 for $PM_{2.5}$, 0.84 for NO_2, and 0.86 for O_3 (Di et al., 2019a, 2019b; Requia et al., 2020). This air pollution data was modeled for the entire contiguous United States from 2000 to 2016.

For observations from 1999, for which modeled levels were not available, we filled in missing values using data from monitors located at Countway Library at Harvard University in Boston, Massachusetts. First, we regressed modeled values of pollution levels at each address against monitored values and daily maximum temperature and daily minimum temperature for the year 2000 and extracted the regression coefficients. We then used Countway monitored values and temperature to predict address-specific levels for 1999. Predicted daily values were averaged to obtain levels for intermediate- and long-term moving averages. If Countway data was not measured on a particular date, it was considered to be missing. Moving averages with any missing days over that length of time were considered missing. The number of missing observations for each exposure time window is available in Table S1.

Ambient temperature was derived from the gridMET model. This model estimated daily levels of maximum and minimum ambient temperature across the United States from 1998 to 2015 on an approximately 4 km by 4 km scale (Abatzoglou, 2013). We averaged the maximum and minimum values to obtain average ambient temperature

levels for each day.

2.3. Outcome measurement

Our outcome in this study was the ex-miRNA profile of participants in NAS. The process for extracting and sequencing the RNA data has been more extensively described elsewhere (Eckhardt et al., 2022). Participants provided fasting blood samples during their follow-up visits which were centrifuged to separate the plasma layer and subsequently stored at −80 °C. Samples were thawed, centrifuged, and filtered prior to processing. Plasma/Serum Circulating and Exosomal RNA Purification Kits, Slurry Format (Norgen Biotek Corp, Canada) were used to isolate RNAs from the samples (Gandhi et al., 2017; Laurent, 2015). Sequencing was done in accordance with procedures described previously on a HiSeq2500 system. (Srinivasan et al., 2019). The small RNAseq libraries were made using the NEBNext® Small RNA Library Prep Set for Illumina® (Multiplex Compatible). Data were mapped using the exceRpt Small RNA-seq Pipeline for exRNA Profiling on the Genboree Workbench (http://genboree.org/site/exrna_toolset/). The parameters used for the mapping included a minimum read length of 15 with no mismatches allowed and default settings for all other parameters. Samples were further removed if they failed library preparation (<10,000 total input reads) or had low miRNA reads (<100,000 total miRNA reads). Similar sequences that did not identify a unique miRNA were collapsed into a single category. If multiple samples were collected from the same individuals on the same visit day and both samples were processed, ex-miRNA counts were averaged to obtain final values. Ex-miRNA values were reported as counts per million. We only included ex-miRNAs in the analyses that were detectable in at least 40% of our total samples. This left us with 567 ex-miRNAs for analysis.

2.4. Covariate data

Covariate data were obtained by self-report and questionnaires collected during the follow-up visits. We included as covariates participant characteristics such as: age, body mass index (BMI), physician-diagnosed diabetes, maximum number of years of education, cigarette smoking status (ever smoked: yes/no), cumulative pack-years of smoking, and drinking behavior (at least two drinks a day: yes/no). We further adjusted for sequencing batch pool to capture batch effects. For the model using a one-year moving average as exposure, we adjusted for secular long-term trends by including a linear year term. For all other models, we adjusted for secular long-term trends by including the number of days since January 1st, 1995. This date was chosen arbitrarily to precede the beginning of the follow-up. Moreover, we adjusted for seasonality by including the sine and cosine of the day of the year in the model.

2.5. Statistical analysis

We used a longitudinal quantile regression approach to examine the exposure-outcome relationship. A quantile regression is advantageous in this case as it is not subject to the distributional assumptions of other models which may not be met by miRNA distributions and is robust to the presence of outliers. Since we had repeated measurements from the individuals in our study, we employed a longitudinal approach that approximates a random intercept for each person. The equation for our model was as follows:

$$E(Q_{ij}) = \beta_0 + \beta_1 X_{ij} + \beta_2 C_{ij} + \delta_i + \varepsilon_{ij}$$

Where Qij is the quantile of interest for the ex-miRNA of interest in individual i in measure made during visit j, Xij is a vector of exposures, Cij is a vector of covariates, δ_i is a random intercept approximation by individual, and ε_{ij} is the residual. We ran a separate model for each moving average which included all exposures in the same model to adjust for potential confounding by co-pollutant. We looked at the 10th, 25th, 50th, 75th, and 90th quantiles of each ex-miRNA. Standard errors were calculated using a bootstrap approach with 1000 iterations. To account for multiple comparisons, we adjusted the p-values obtained for each regression using a false discovery rate (FDR) approach and the number of ex-miRNAs studied.

2.6. Pathway analysis

We further examined significant relationships, defined as adjusted p-values <0.05 using pathway analysis. We linked significant ex-miRNA in each exposure time window to relevant genes and biological pathways using the Kyoto Encyclopedia for Genes and Genomes (KEGG) Pathway Analysis run through the DIANA-miRPath version 3 web application (Vlachos et al., 2015). We then looked at relevant mRNA targets, pathways, and clinical diseases using the MicroRNA Target Filter tool in QIAGEN's Ingenuity Pathway Analysis Software (QIAGEN Inc., https://digitalinsights.qiagen.com/IPA) (Krämer et al., 2014).

All data cleaning and statistical analysis were done in R Statistical Software (Version 3.6.3). The "rqpd" package was used to run the statistical models (Koenker and Bache, 2014).

3. Results

3.1. Study population characteristics

The characteristics of the study population have been previously reported and are shown in Table 1(Danesh Yazdi et al., 2023). After sequencing, we had 1508 samples from 734 individuals in the study. The cohort is fairly homogeneous; most are white and elderly. Across visits, about fifteen percent of participants had a physician diagnosis of diabetes and approximately nineteen percent reported drinking at least two drinks per day. Over two-thirds were former or current smokers with an average of fifteen years of education and a BMI of 28.0 kg/m^2. We had at least two samples from two-thirds of the subjects. Sixteen observations had missing covariate data. Any observations with missing exposure or covariate data were dropped from the final analyses.

3.2. Exposure distribution and characteristics

Table 2 shows the exposure distribution of the twelve-week moving average. The exposures appear to be approximately normally distributed. Mean values for the twelve-week moving average of $PM_{2.5}$, NO_2, O_3, and temperature are 9.63 $\mu g/m^3$, 23.50 ppb, 35.23 ppb, and 285.34 K (12.19 °C), respectively. These represent fairly low values of these pollutants. The correlation between the twelve-week moving averages of the exposures is shown in Fig. 1. The correlations ranged from weak (−0.04) to medium (0.57).

3.3. Regression results

We found a total of 151 significant results between our exposures of interest and our outcomes. Of these, there were134 significant relationships between our exposures of interest and 82 unique ex-miRNAs, with some showing significant results in multiple quantiles. The distribution of significant associations of the exposure time windows and quantiles is shown in Fig. 2a and b. Most of the significant results we found were for O_3, $PM_{2.5}$ and temperature. For $PM_{2.5}$ and temperature, these were largely concentrated between the 6-month and 1-year time windows. For O_3, most of the significant effects were between 8 and 12 weeks. In terms of quantiles of the outcome most of the associations to be significant were between O_3, $PM_{2.5}$ and temperature and the 75th and 90th percentiles. NO_2 represented a much smaller portion of the significant results. NO_2 was associated with significant effects during the 1-year exposure time window and the 90th percentile of the ex-miRNA outcomes. Of all the significant relationships, 78 ex-miRNA levels

Table 1
Study population characteristics.

Variable		All Visits (N=1508)	Visit 1 (N=734)	Visit 2 (N=460)	Visit 3 (N=254)	Visit 4 (N=58)	Visit 5 (N=2)
Age (years), mean ± SD		74.7 ± 6.9	72.7 ± 6.9	75.6 ± 6.3	77.9 ± 6.2	78.7 ± 6.0	81 ± 5.7
Body Mass Index (kg/m^2), mean ± SD		28.0 ± 4.1	28.2 ± 4.1	27.8 ± 4.2	27.6 ± 4.0	27.5 ± 4.4	26.0 ± 0.08
Education (years), mean ± SD		15.0 ± 2.9	15.0 ± 2.9	15.2 ± 2.9	15.1 ± 2.9	15.2 ± 3.0	14.0 ± 2.8
Diabetes, n (%)	Yes	227 (15.1%)	102 (13.9%)	71 (15.4%)	47 (18.5%)	7 (12.1%)	0 (0%)
	No	1281 (84.9%)	632 (86.1%)	389 (84.6%)	207 (81.5%)	51 (87.9%)	2 (100%)
Alcohol consumption (≥2/Day), n (%)	Yes	286 (19.0%)	136 (18.5%)	91 (19.8%)	43 (16.9%)	15 (25.9%)	1 (50%)
	No	1222 (81.0%)	598 (81.5%)	369 (80.2%)	211 (83.1%)	43 (74.1%)	1 (50%)
Smoking, n (%)	Never	482 (32.0%)	224 (30.5%)	153 (33.3%)	81 (31.9%)	23 (39.7%)	1 (50%)
	Current or Former	1019 (67.6%)	504 (68.7%)	306 (66.5%)	173 (68.1%)	35 (60.3%)	1 (50%)
Smoking pack-years, mean ± SD		20.0 ± 24.6	21.3 ± 26.6	19.2 ± 23.5	18.6 ± 21.5	16.6 ± 20.4	10.0 ± 14.1
Race, n (%)	White	1468 (97.3%)	713 (97.1%)	448 (97.4%)	247 (97.2%)	58 (100%)	2 (100%)
	Black	24 (1.6%)	14 (1.9%)	7 (1.5%)	3 (1.2%)	0 (0%)	0 (0%)
	Hispanic White	12 (0.8%)	5 (0.7%)	4 (0.9%)	3 (1.2%)	0 (0%)	0 (0%)
	Hispanic Black	3 (0.2%)	1 (0.1%)	1 (0.2%)	1 (0.4%)	0 (0%)	0 (0%)

Table 2
Twelve-week moving average exposure distribution.

Variable	Minimum	10th Percentile	25th Percentile	Mean	Median	75th Percentile	90th Percentile	Maximum
PM_{25} (μg/m^3)	3.24	6.79	8.05	9.63	9.55	11.08	12.68	22.19
NO_2 (ppb)	2.16	10.40	15.92	23.50	23.51	31.21	36.34	48.53
O_3 (ppb)	12.93	21.98	28.56	35.23	36.58	42.43	45.59	64.14
Temperature (K)	268.28	273.33	278.08	285.34	286.27	292.56	294.74	302.63

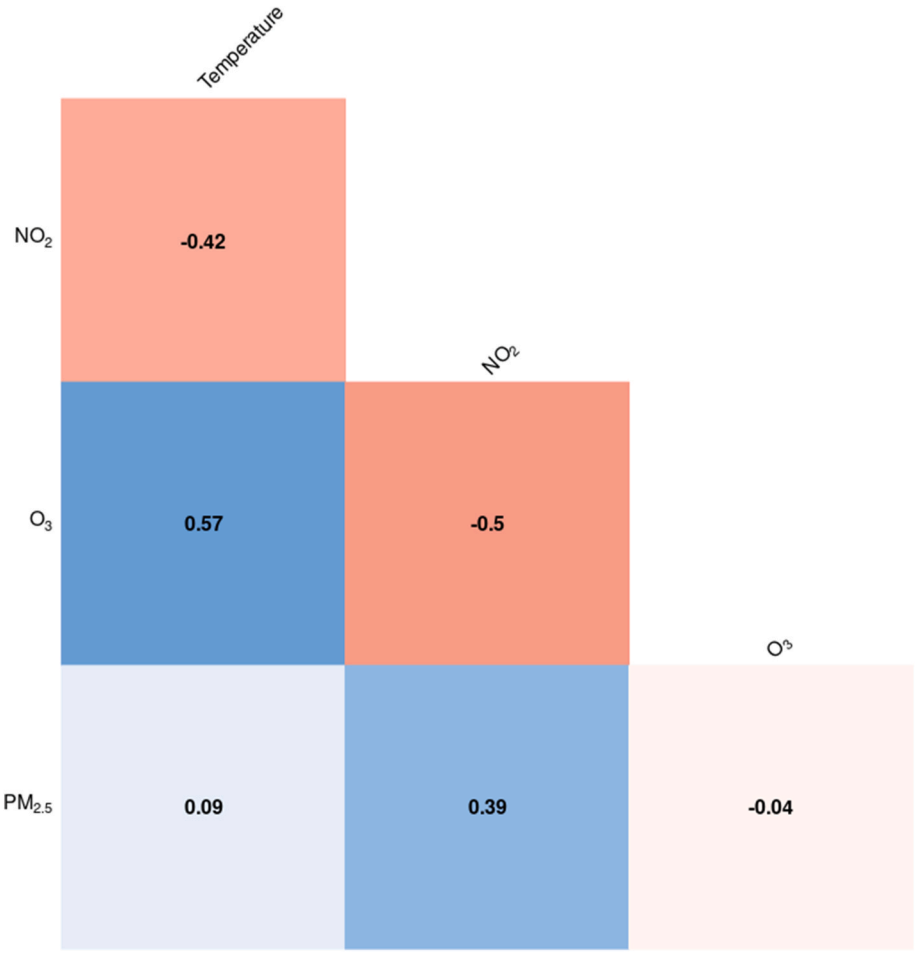

Fig. 1. Correlations between 12-week moving averages of exposures of interest.

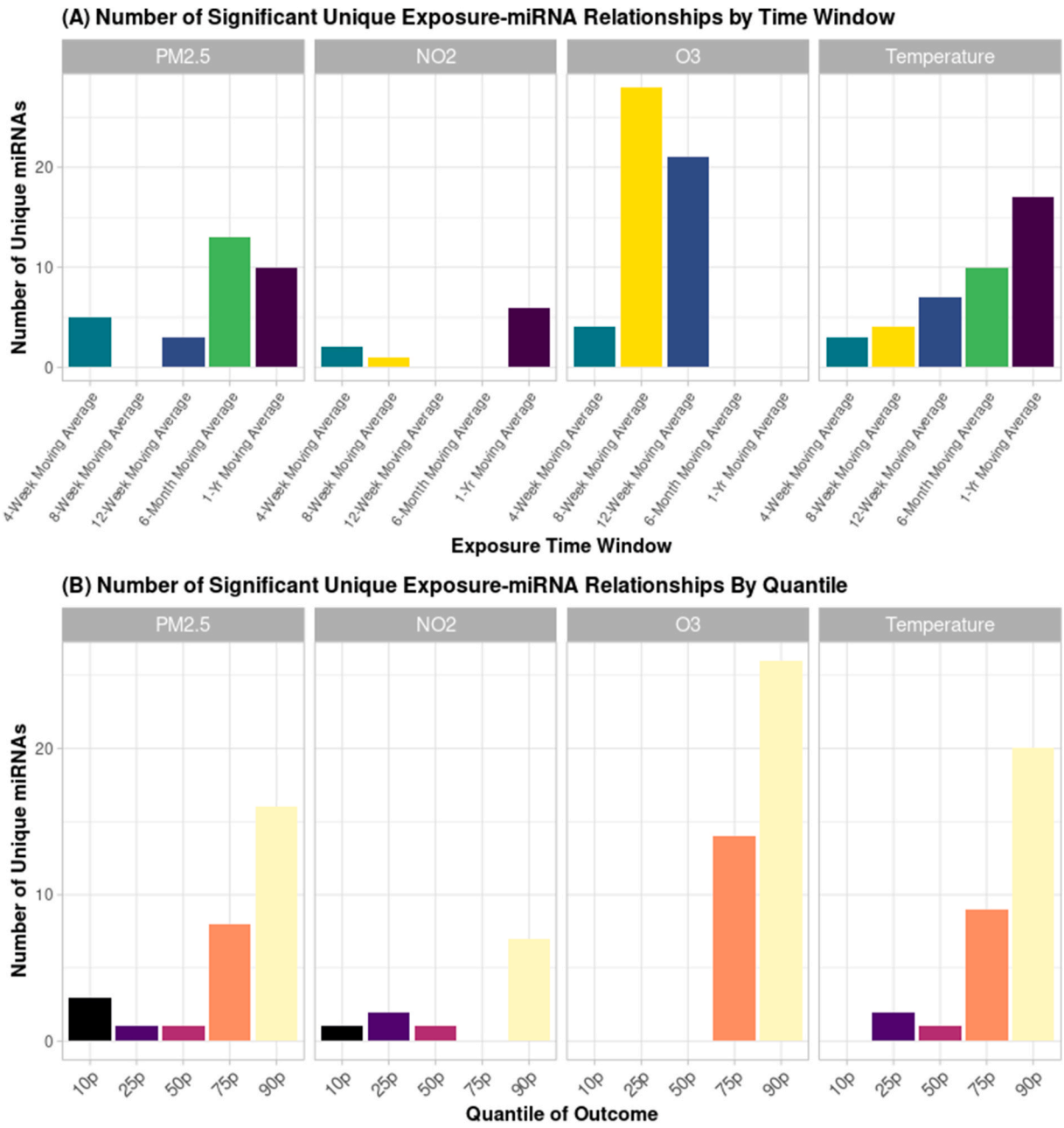

Fig. 2. Number of Unique Significant miRNAs Associated with Each Exposure by A) Time Window and B) Quantile.

were up-regulated and 56 were down-regulated. The full results can be found in Table S2.

All intermediate-term O_3 exposures were associated with significant upregulation of miR-194–5p in at least one studied quantile. All intermediate and long-term temperature exposures were associated with significant downregulation in miR-576–3p in at least one quantile. Moreover, long-term temperature exposures were also significantly associated with miR-5189–5p, miR-4306, miR-4750–5p, and miR-4504 in at least one quantile. On the other hand, long-term $PM_{2.5}$ exposures were associated with miR-15 b-3p, miR-576–5p, and miR-5187–5p in at least one quantile (see Fig. 3).

For NO_2, there were no ex-miRNAs significantly associated with all intermediate-term or long-term exposure time windows.

3.4. KEGG pathway analysis

The unique pathways associated with ex-miRNAs found to be significantly associated with exposure levels can be found in Table 3. These pathways relate to cell functions and signaling as well as disease states such as cancer. The KEGG pathways found to be significant for each exposure time window can be found in Tables S3–S7.

3.5. Ingenuity Pathway Analysis results (IPA)

The IPA found 718 experimentally validated associations between 32 of our ex-miRNAs and mRNA targets. The full results can be found in Table S8. The miRNA-mRNA relationships were further used to identify potential pathways and clinical diseases. The ex-miRNAs we found to be significantly associated with our exposures were linked to numerous diseases including but not limited to cancer, cardiovascular disease, respiratory disease, neurological disease, and endocrine disorders.

For example, of the nine ex-miRNAs which were significantly associated with intermediate and long-term exposure time windows, two ex-miRNAs (miR-5189–5p and miR-4306) were found in the IPA to be

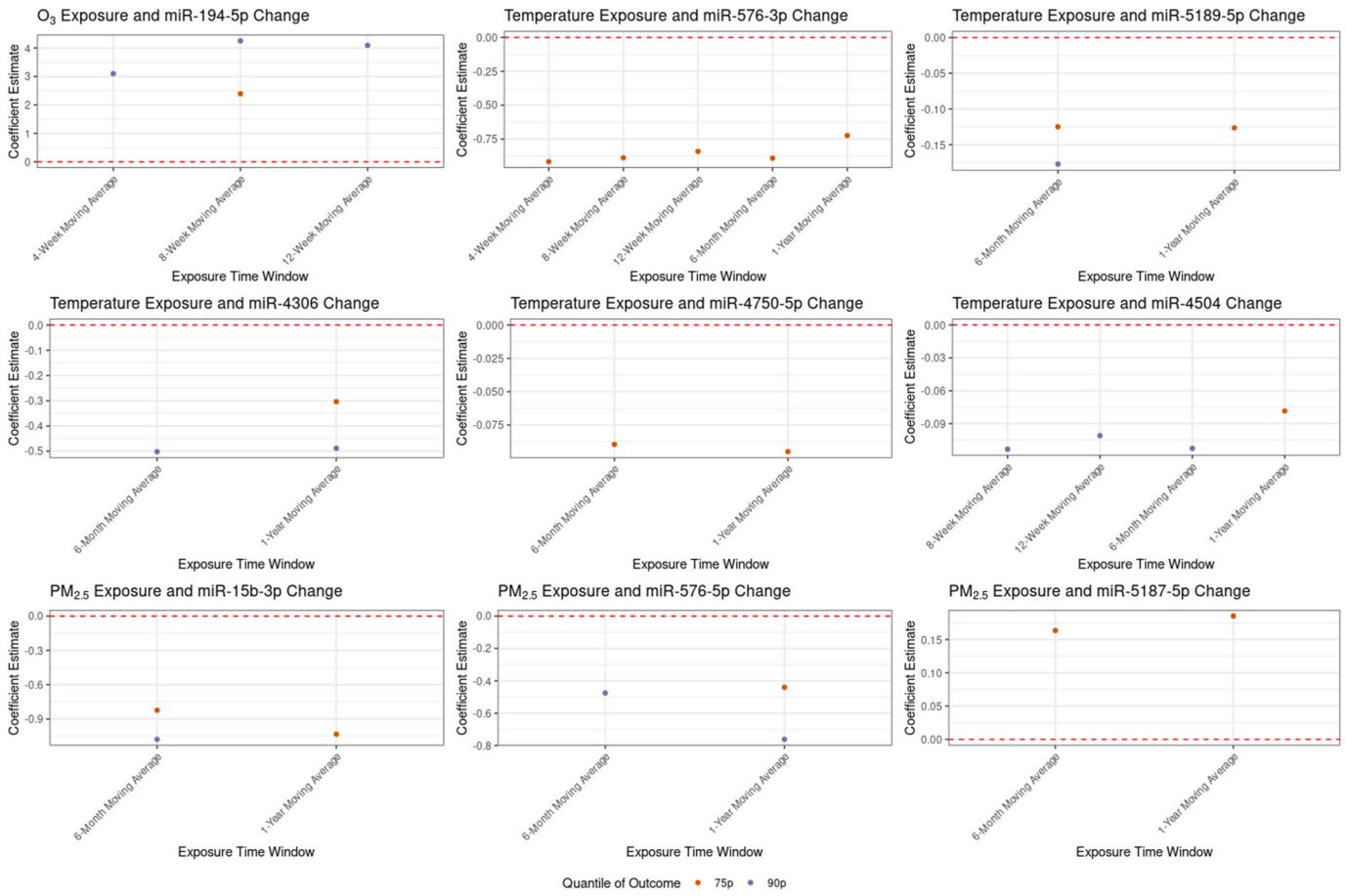

Fig. 3. Change in quantiles of ex-miRNA levels (counts per million) for each unit change in exposure for ex-miRNAs significantly associated with exposure across multiple time windows.

Table 3
KEGG Pathways Associated with Significant miRNAs.

Name of KEGG Pathway	
ECM-receptor interaction	Transcriptional mis-regulation in cancer
Prion diseases	Melanoma
Fatty acid metabolism	Bacterial invasion of epithelial cells
Fatty acid biosynthesis	Renal cell carcinoma
Cell cycle	Oocyte meiosis
Viral carcinogenesis	Small cell lung cancer
Proteoglycans in cancer	Focal adhesion
Hepatitis B	RNA transport
Hippo signaling pathway	Bladder cancer
Adherens junction	Endometrial cancer
Lysine degradation	Thyroid hormone signaling pathway
p53 signaling pathway	PI3K-Akt signaling pathway
Chronic myeloid leukemia	Signaling pathways regulating pluripotency of stem cells
FoxO signaling pathway	Ubiquitin mediated proteolysis
Colorectal cancer	Non-small cell lung cancer
Glioma	Neurotrophin signaling pathway
TGF-beta signaling pathway	Shigellosis
Pathways in cancer	Spliceosome
Prostate cancer	Prolactin signaling pathway
Protein processing in endoplasmic reticulum	Arrhythmogenic right ventricular cardiomyopathy (ARVC)
Steroid biosynthesis	Endocytosis
Pancreatic cancer	

associated with mRNAs linked to several disease states including but not limited to those shown in Fig. 4 (cardiovascular disease, respiratory disease, neurological disease).

4. Discussion

Our study looked at the association between intermediate- and long-term exposure to air pollutants and ambient temperature and changes in the ex-miRNA profile of participants in the Normative Aging Study. We found all our exposures were associated with changes in the ex-miRNA profiles of our subjects. Most of the significant results were due to O_3, temperature and $PM_{2.5}$. Most significant associations were found for intermediate-term exposure to O_3, long-term exposure to $PM_{2.5}$, and both intermediate and long-term exposure to ambient temperature. Furthermore, most of the significant results were found for the 75th and 90th percentiles indicating a need to examine the full distribution of the outcome and not simply the mean or median. The ex-miRNAs we found to be significant were further examined in pathway analyses. KEGG pathway analysis indicated that our results may reflect changes in cell function and communication as well as disease states such as cancer. The IPA results revealed miRNA-mRNA relationships which may be linked to numerous disease states such as cancer, cardiovascular disease, respiratory disease, neurological disease, and endocrine disorders.

Some of our results were comparable to previous studies while others were not. A small pilot study conducted among 22 participants in NAS found long-term exposure to $PM_{2.5}$, particularly 6-month and 1-year moving averages, to be associated with alterations in several EV-miRNAs. Six-month moving averages were associated with the up-regulation of EV-miRNAs: miR-126–3p, miR-19b-3p, miR-93–5p, miR-

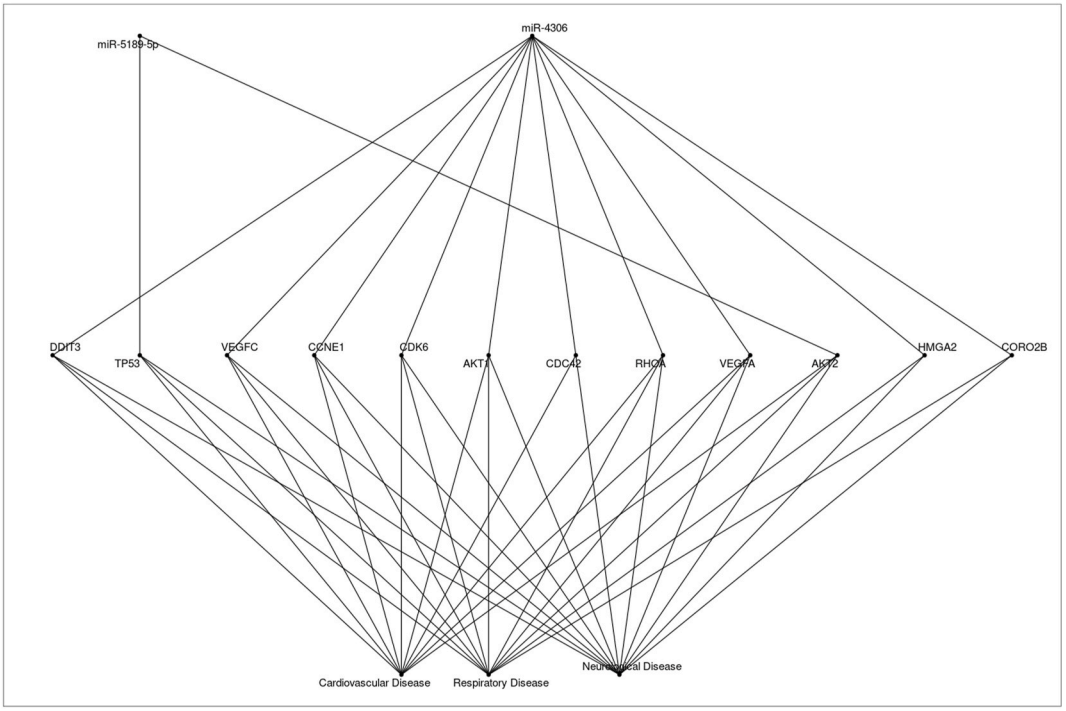

Fig. 4. IPA Analysis Results: mRNA Targets for miR-5189–5p and miR-4306.

223–3p, and miR-142–3p. One-year moving averages were associated with the up-regulation of EV-miRNAs: miR-23a-3p, miR-150–5p, miR-15a-5p, miR-191–5p, let-7a-5p (Rodosthenous et al., 2016). Meanwhile, in our study, we did not find significant changes in levels of miR-126–3p, miR-223–3p, miR-142–3p, miR-23a-3p, miR-191–5p, and let-7a-5p. We did find an up-regulation of miR-19b-3p associated with exposure to the 8-week moving and 12-week average of O_3, and an up-regulation of miR-15a-5p with exposure to the 12-week moving average of O_3 and downregulation with exposure to 4-week and 1-year moving averages of $PM_{2.5}$. Moreover, we observed a downregulation of miR-93–5p and miR-150–5p associated with exposure to the one year moving average of $PM_{2.5}$. Another pilot study conducted in the same population looking at both long-term $PM_{2.5}$ and black carbon in multi-pollutant models found significant associations for 6-month exposure to $PM_{2.5}$ and EV-miRNAs let-7g-5p, miR-1246, miR-126–3p, miR-142–3p, miR-150–5p, miR-15a-5p, miR-199a/b, and miR-223–3p. One-year exposure to $PM_{2.5}$ was significantly associated with EV-miRNAs: 130a-3p, miR-142–3p, miR-199a/b, miR-223–3p, and miR-23a-3p. None of the EV-miRNAs were associated with black carbon after correction for multiple comparisons (Rodosthenous et al., 2018). We did not find a significant association between any of our exposures and let-7g-5p, miR-1246, miR-126–3p, miR-142–3p, miR-199a-5p, miR-199b-5p, miR-199a-3p/miR-199b-3p, miR-223–3p, miR-130a-3p, miR-23a-3p. We did find a downregulation of miR-150–5p in association with one-year exposure to $PM_{2.5}$ while this study found an upregulation. We also found an upregulation of miR-15a-5p with exposure to the 12-week moving average of O_3 and down-regulation with exposure to intermediate and long-term $PM_{2.5}$ while this study found an upregulation. In a study of 55 healthy steel workers in Northern Italy, long-term exposure to PM mass and PM metals were associated with changes EV-miRNA levels. This study found PM_{10} and coarse particles (defined as PM_{10}-PM_1) to be associated with miR-21 (using adjusted p-values of <0.05). Among PM metals, aluminum was associated with changes in miR-21, cadmium was associated with miR-200c, lead and zinc were associated with miR-181b, and zinc was also associated with miR-9 (Pavanello et al., 2016). In our study we did not find significant effects for any of these EV-miRNAs. The differences seen in the results may be due to numerous factors including but not limited to: differences in population characteristics, sample size, extraction approaches, sequencing approaches, statistical methods, and model specification. Furthermore, many of the studies we compared our results to looked at only EV-miRNAs while we looked at ex-miRNAs which include EV-miRNAs as well as other extracellular miRNAs.

KEGG pathway analyses revealed several biological pathways related to our ex-miRNAs that affect disease states such as cancer as well as important biological functions such as cell function and communication. Changes in fatty acid metabolism have been linked to cardiomyopathies and changes in cardiac function (Fillmore et al., 2014; Lopaschuk et al., 2010). Alterations in fatty acid metabolism have also been linked to neuroinflammation, neurodegeneration, and demyelination (Bogie et al., 2020). Similarly, changes in fatty acid synthesis may play a role in neuroinflammation and neurogenesis (Bogie et al., 2020). Adherens junctions are junctions connecting cells to one another and are found throughout the body. Dysfunction in adherens junctions may lead to arrythmias in the heart and have been associated with inflammatory bowel disease (Mehta et al., 2015; Nesterova et al., 2020).

Our study had several limitations. Our exposure assignment relied on predictive models. While these models had high validation metrics, there is still potential for measurement error. We expect this error to be non-differential. Recent studies suggest the direction of bias is likely downward (Wei et al., 2022). There was some missing air pollutant data for 1999, particularly for the long-term moving averages. The lack of significant results may be due to the smaller sample size for those analyses. Furthermore, our cohort is fairly homogenous and composed mainly of elderly white men. As such, the generalizability of our results may be limited, though our results can be more easily compared within this group. Moreover, we only looked at ex-miRNAs that were detectable in at least forty percent of our samples, restricting the number of ex-miRNAs we were able to study. As with any epidemiological study, there is also a risk of residual confounding by unmeasured confounders.

Our study also possessed several strengths. Our cohort included individuals who have been extensively followed-up, and the multiple observations for each person is a plus as within person variability is less than between person variability, providing more power. We used a

longitudinal quantile regression which accounted for both repeated observations from some individuals and the potential non-normal distribution of the outcomes. We used multiple types of pathway analyses to further examine the biomedical implications of our results. We assessed numerous exposure time windows to better capture the full impact of exposure to each pollutant and temperature. Our results require further examination in larger populations with greater demographic variability for more definitive conclusions.

Our study demonstrated that exposure to environmental risk factors, particularly O_3, $PM_{2.5}$ and ambient temperature, are associated with changes in the ex-miRNA profile of participants in the Normative Aging Study. Further epidemiological studies in larger, more diverse populations are needed to better understand the relationship between these exposures and ex-miRNAs.

Credit author statement

Mahdieh Danesh Yazdi: Conceptualization, Data curation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Feiby L Nassan**: Conceptualization, Data curation, Writing – review & editing. **Anna Kosheleva**: Data curation, Writing – review & editing. **Cuicui Wang**: Methodology, Writing – review & editing. **Zongli Xu**: Software, Formal analysis. **Qian Di**: Data curation, Writing – review & editing. **Weebrb J Requia**: Data curation, Writing – review & editing. **Nicole T Comfort**: Data curation, Writing – review & editing. **Haotian Wu**: Data curation, Writing – review & editing. **Louise C Laurent**: Data curation, Writing – review & editing. **Peter DeHoff**: Data curation. **Pantel Vokonas**: Data curation, Writing-Review &Editing. **Andrea A Baccarelli**: Data curation, Writing – review & editing, Funding acquisition. **Joel D Schwartz**: Conceptualization, Methodology, Writing – review & editing, Supervision, Resources, Funding acquisition.

Funding

This work was supported by grant R01ES027747 from the National Institute of Environmental Health Sciences and resources and use of facilities at the Veterans Affairs Boston Healthcare System. The views expressed in this article are those of the authors and do not reflect the position or policy of the Department of Veterans Affairs or the US Government.

The VA Boston Health Care System and Harvard TH Chan School of Public Health Institutional Review Boards approved this study. All participants provided written consent for inclusion in the cohort.

Data sharing

The authors are not permitted to publicly share this data.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Joel D Schwartz reports financial support was provided by National Institute of Environmental Health Sciences by grant R01ES027747. Joel D Schwartz reports a relationship with US Department of Justice that includes: paid expert testimony. Feiby L. Nassan is a current employee and a shareholder of Biogen, Cambridge, MA. The original work on this study at Harvard T. H. Chan School of Public Health (HSPH), however, pre-dated the current employment. This manuscript does not mention any Biogen products or any of the disease states that Biogen is actively doing research in (to the coauthor's knowledge).

Data availability

The authors do not have permission to share data.

Acknowledgements

This work was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences. The VA Normative Aging Study is supported by the Cooperative Studies Program/Epidemiology Research and Information Center of the US Department of Veterans Affairs and is a component of the Massachusetts Veterans Epidemiology Research and Information Center, Boston, MA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.115949.

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