

ACU Research Bank

Short-term exposure to traffic-related air pollution reveals a compound-specific circulating miRNA profile indicating multiple disease risks

Item Type	Journal article
Authors	Krauskopf, Julian;van Veldhoven, Karin;Chadeau-Hyam, Marc;Vermeulen, Roel;Carrasco-Turigas, Glòria;Nieuwenhuijsen, Mark;Vineis, Paolo;de Kok, Theo M. C. M.;Kleinjans, Jos C.
DOI	10.1016/j.envint.2019.04.063
Publisher	Pergamon Press
Download date	2026-07-02 02:12:02
Link to Item	https://hdl.handle.net/20.500.14802/21778



Short-term exposure to traffic-related air pollution reveals a compound-specific circulating miRNA profile indicating multiple disease risks



Julian Krauskopf^{a,*}, Karin van Veldhoven^{b,c}, Marc Chadeau-Hyam^b, Roel Vermeulen^d, Glòria Carrasco-Turigas^{e,f,g}, Mark Nieuwenhuijsen^{e,f,g}, Paolo Vineis^b, Theo M. de Kok^a, Jos C. Kleinjans^a

^a Department of Toxicogenomics, GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, the Netherlands

^b MRC-PHE Centre for Environment and Health, Department of Epidemiology and Biostatistics, School of Public Health, Imperial College, London, UK

^c Department of Non-Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK

^d Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands

^e ISGlobal, Barcelona, Spain

^f Universitat Pompeu Fabra (UPF), Barcelona, Spain

^g CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain

ARTICLE INFO

Handling Editor: Xavier Querol

Keywords:

Diesel-exhaust

Air pollution

Biomarker

Environmental health

microRNAs

ABSTRACT

Traffic-related air pollution (TRAP) is a complex mixture of compounds that contributes to the pathogenesis of many diseases including several types of cancer, pulmonary, cardiovascular and neurodegenerative diseases, and more recently also diabetes mellitus. In search of an early diagnostic biomarker for improved environmental health risk assessment, recent human studies have shown that certain extracellular miRNAs are altered upon exposure to TRAP. Here, we present a global circulating miRNA analysis in a human population exposed to different levels of TRAP. The cross-over study, with sampling taking place during resting and physical activity in two different exposure scenarios, included for each subject personal exposure measurements of PM₁₀, PM_{2.5}, NO, NO₂, CO, CO₂, BC and UFP. Next-generation sequencing technology was used to identify global circulating miRNA levels across all subjects. We identified 8 miRNAs to be associated with the mixture of TRAP and 27 miRNAs that were associated with the individual pollutants NO, NO₂, CO, CO₂, BC and UFP. We did not find significant associations between miRNA levels and PM₁₀ or PM_{2.5}. Integrated network analysis revealed that these circulating miRNAs are potentially involved in processes that are implicated in the development of air pollution-induced diseases. Altogether, this study demonstrates that signatures consisting of circulating miRNAs present a potential novel biomarker to be used in health risk assessment.

1. Introduction

The WHO estimated that worldwide > 80% of the people in cities are exposed to air pollution levels that exceed the air quality limits (WHO, 2017). A major source of urban air pollution is motor vehicle emissions. These traffic-related air pollutants (TRAP) constitute a complex mixture of compounds including black carbon (BC), particulate matter (PM_{2.5}, PM₁₀), ultra-fine particles (UFP), nitrogen oxides (NO, NO₂) and carbon oxides (CO, CO₂). We still have little understanding of how exposures to these different components contribute to the development of chronic diseases (Forman and Finch, 2018).

It is well known that exposure to TRAP induces chronic respiratory tract diseases (Zhang and Batterman, 2013). However, the UFP may

also enter the circulation, and reach and accumulate in distal organs (Li et al., 2015), and even cross the blood-brain barrier (Oberdörster et al., 2004). Consequently, epidemiological studies have shown that exposure to TRAP is also associated with increased risk of cardiovascular diseases (Downward et al., 2018), nervous system diseases (Calderón-Garcidueñas and Villarreal-Ríos, 2017), and recently also diabetes mellitus (Bowe et al., 2018).

In search for a better understanding of the pathomolecular mechanisms that link TRAP exposure to the increased risk of chronic diseases, researchers have only recently started to investigate the role of microRNAs (miRNAs). These small non-coding RNAs are involved in posttranscriptional regulation by fine-tuning gene expression (Bartel, 2009). Consequently, miRNAs may impact on virtually all cellular

* Corresponding author.

E-mail address: j.krauskopf@maastrichtuniversity.nl (J. Krauskopf).

<https://doi.org/10.1016/j.envint.2019.04.063>

Received 4 February 2019; Received in revised form 4 April 2019; Accepted 25 April 2019

0160-4120/© 2019 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

processes and therefore, are to be regarded as key drivers in health and disease. Through active secretion in the course of intercellular communication, as well as through leakage upon tissue injury, miRNAs are released into the peripheral circulation. This allows for non-invasively interrogating organ pathogenesis and organ-specific mechanisms from so called 'liquid biopsies' (Krauskopf et al., 2015).

A recent review describes miRNA changes as sensitive indicators of the effects of acute and chronic environmental exposure (Vrijens et al., 2015). The first evidence of air pollution-induced changes in miRNA expression was provided through investigating the blood of healthy steel plant workers before and after a week of occupational exposure to metal-rich PM. Two miRNAs (miR-222 and miR-21) were identified to be significantly increased in post-exposure samples (Bollati et al., 2010).

Other studies have confirmed significant associations of PM exposure and the cellular levels of miR-222, which has a function in cell cycle and vascular biology, as well as miR-146a which plays an important role in inflammation, in adults (Fossati et al., 2014; Motta et al., 2013). The first study reporting extracellular miRNAs in association to air pollution identified miR-222 to positively correlate with UFP levels in the saliva of school children (Vriens et al., 2016).

We were the first to study global plasma circulating miRNAs upon moderate short-term ambient TRAP exposure in an intervention study on a small population consisting of elderly subjects being either healthy or suffering from COPD or ischemic heart disease. We showed that signatures of extracellular miRNAs are responsive to TRAP exposure which could be linked to multiple TRAP-induced health effects (Krauskopf et al., 2018).

In the present study we build on this proof of concept and investigate plasma circulating miRNAs in healthy, non-smoking adults exposed to varying levels of TRAP exposure within the 'Transportation, Air pollution and Physical Activities' (TAPAS II) study.

2. Methods

2.1. Selection of the population

The TAPAS II cohort is (TAPAS II experimental study: respiratory and inflammatory health effects of air pollution and physical activity) is a controlled crossover study comprising of 30 participants (15 females and 15 males) which executed four different scenarios. The four different scenarios were completed by each participant at random order and included resting and intermittent physical activity (intervals of 15 minutes cycling and 15 minutes break for a total of 2 h) at low and high TRAP (in a pedestrian square in la Barceloneta neighborhood and on a bridge 5 m above Ronda Litoral, the city's ring road, respectively) in the city of Barcelona. Sessions were at least 5 days apart to allow sufficient time to wash-out. To avoid the influence of weather the order of the participants was fully randomized. Physical activity was modulated by cycling at such a pace that the heart rate of the participants remained within the range of 50–70% of their maximum rate. The inclusion criteria for the subjects were healthy non-smokers, in the age range of 18–60, not taking any medication (except contraceptive pills) and not suffering from any chronic disease. Also, participants were required not to take herbal supplements or vitamins for the 7 previous days and not having a chronic or acute disease i.e. infections, flu, etc.

The experiments took place within 4 month between November 2013 and February 2014 from 8:00 to 10:00 AM. In the afternoon of the day at 5.30 the subjects were transported to a nearby clinic for blood sample collection. The blood samples were stored at -80°C within 2 h after collection.

For the present study we randomly selected 24 individuals from the TAPAS cohort that completed all 4 scenarios (resting and cycling at high and low exposure), thereby balancing for gender (Table 1). These 96 samples from the 24 participants were analyzed for global circulating miRNA levels by next-generation technology. The study was

Table 1
Population.

Population		Age		BMI	
		Range	Mean	Range	Mean
Total	24	19,57	36	18.07,29.61	23.61
Males	12	20,57	35	22.11,28.47	24.43
Females	12	19,55	38	18.07,29.61	22.78

approved by the Ethic Review Committee of the Institut Municipal d'Investigació Mèdica, Barcelona and all participants gave written informed consent.

2.2. External exposure assessment

Throughout the experiments, measurements comprised of analyzing ambient air concentrations of PM_{10} and $\text{PM}_{2.5}$ (DustTrack, DRX, Model 8534, TSI, Minnesota, USA), UFP (condensation particle counter, CPC, Model 3007, TSI, Minnesota, USA), BC (portable aetholometer, Model AE-51, McAgee Scientific, California, USA) and additionally nitrogen oxide (NO), generic measurement for the nitrogen oxides ($\text{NO}_x = \text{NO} + \text{NO}_2$) (nitric oxide monitor, Model 410 Nitric Oxide Monitor, 2B Technologies, Colorado, USA, in combination with a nitrogen dioxide converter, Model 401 NO₂ Converter, 2B Technologies, Colorado, USA), carbon monoxide (CO) and carbon dioxide (CO_2) (Q-Track (Model 7565, TSI, Minnesota, USA). Pearson correlation among exposures was assessed using the 'cor' function of the R package 'stats'. The different personal exposure levels were compared between both locations (low versus high exposure level) using a paired *t*-test (R package 'stats', 't.test()' function). Per subject we calculated a Z-Score as a representative of the external exposure level of each individual pollutant $Z\text{-Score}_{(\text{compound})}$ as well as the mixture of all pollutants $Z\text{-Score}_{(\text{TRAP})}$. The $Z\text{-Score}_{(\text{TRAP})}$ was defined as the mean of the Z-Scores for each compound: $Z\text{-Score}_{(\text{compound})} = (X - \mu) / \sigma$ (where X represents the value of the subject, μ the mean and σ the standard deviation of the population (Krauskopf et al., 2017). Therefore, $Z\text{-Score}_{(\text{TRAP})} = 1/8 * (Z\text{-Score}_{(\text{NO})} + Z\text{-Score}_{(\text{NO}_x)} + Z\text{-Score}_{(\text{PM}_{10})} + Z\text{-Score}_{(\text{PM}_{2.5})} + Z\text{-Score}_{(\text{BC})} + Z\text{-Score}_{(\text{UFP})} + Z\text{-Score}_{(\text{CO})} + Z\text{-Score}_{(\text{CO}_2)})$.

2.3. Analytical procedures

Plasma samples were recovered from plasma separator tubes following centrifugation of whole blood at 1600g for 10 min at 4°C . To avoid contamination of the plasma miRNAs with blood cell-derived miRNAs, the plasma was subjected to a second centrifugation step at 16,000g before being stored at -80°C (Kroh et al., 2010). Plasma miRNAs were isolated using the miRNeasy Serum/Plasma kit (Qiagen) and the quality was evaluated on a Bioanalyzer using the small RNA Kit (Agilent). Samples were included in the analysis when showing a minimum of 1 ng RNA yield and a peak around 21 nucleotides. The sequencing libraries were prepared using the TruSeq Small RNA-Seq Preparation Kit (Illumina), and sequenced by a HiSeq 2500 (Illumina) according to the manufacture's protocol (GEO accession: GSE125647).

2.4. Statistical analysis

The quality of the sequencing data was assessed by FastQC and subsequently processed using the miRge2 pipeline and miRBase (release 21) (Kozomara and Griffiths-Jones, 2014; Lu et al., 2018). Circulating miRNAs were included in the analysis if they were detected in at least 60% of the samples. The raw data were normalized and transformed to log₂ counts per million (logCPM) using the R package 'DESeq2' (Love et al., 2014). Technical confounders (miRNA isolation batch effects, inter-day variations in library preparation and date of the sequencing run) were adjusted for by applying a linear mixed model

(LMM) using the R package ‘lme4’. After de-noising the data we used a multivariate normal model (MVN) to find associations between the Z-Score_(TRAP) and the circulating miRNA levels, and between the individual air pollutants and the circulating miRNA levels using the R package ‘nlme’. All models were adjusted for the confounders gender, age, BMI, physical activity and a variable indicating the order in which the four different scenarios were implemented. The IDs of the subjects were used as a grouping factor. Resulting p-values were declared significant based on a false discovery rate less than or equal to 0.1.

To test the stability of the model we performed leave-one-out cross validation (LOOCV). Briefly: one subject was left out at a time; MVN was performed on the remaining n – 1 subjects; until each subject was left out exactly once. At the end, we compared all resulting n coefficient vectors with the coefficient vector of the full dataset set including all subjects.

We also tested the influence of physical activity on the circulating miRNAs by applying a negative binomial test using the R package ‘DESeq2’, thereby adjusting for the exposure Z-Score_(TRAP), gender, age and BMI. We declared the association of physical activity significant based on a false discovery rate less than or equal to 0.1 and a log2 fold change > 1.

2.5. miRNA-gene-disease network

To identify the biological mechanisms in which the TRAP-associated miRNAs are involved we performed an integrated network analysis. First, potential gene targets for all significantly TRAP-associated miRNAs were derived from the experimentally validated miRNA-target interaction database miRTarBase (Chou et al., 2018). Only strong miRNA-gene interactions that have been validated by reporter assay, western blot or qPCR were considered. Next, the DisGeNET database (v5.0) was used to find genes associated with TRAP exposure-related diseases (Piñero et al., 2017). We manually selected cardiovascular diseases (“Cardiovascular Diseases”, “Myocardial Ischemia”, “Heart failure”, “Inflammation”, “Cardiac Arrhythmia”, “Ischemia”, “Heart Diseases”, “Coronary heart disease”, and “hypertensive disease”), respiratory tract diseases (“Chronic Obstructive Airway Disease”, “Non-Small Cell Lung Carcinoma”, “Small cell carcinoma of lung” and “Asthma”), nervous system diseases (“Parkinson’s disease”, “Dementia” and “Alzheimer’s Disease”) and Diabetes (“Diabetes Mellitus”). Only expert-curated associations were considered. Integration, network analysis and visualization was performed using cytoscape (v2.7) (Shannon et al., 2003).

3. Results

3.1. Exposure

Ambient concentrations of 8 pollutants for the low and high exposure sites (Barceloneta and Ronda respectively) are shown in Table 2. A paired t-test showed that for all pollutants the exposure was significantly higher in the high pollution site when compared to the low pollution site (Table 2). The eight exposures showed Pearson correlation coefficients (r) between 0.11 and 0.99 with CO₂ being the least correlated with the remaining exposures (r < 0.51) and BC and CO₂ (r = 0.90), PM_{2.5} and PM₁₀ (r = 0.92), and NO_x, NO and UFP (r > 0.96) showing strong correlations. The Z-Score_(TRAP) showed high correlations with NO, NO_x, PM_{2.5}, BC, UFP and CO₂ (r between 0.92 and 0.96) and moderate correlations between PM₁₀ (r = 0.74) and CO (r = 0.51) (Fig. 1).

3.2. Small RNA-sequencing

The sequencing of the 96 samples from the 24 subjects resulted on average 6.6*10⁶ (SD ± 4.5*10⁶) sequencing reads. Due to low sequencing coverage three samples were excluded from further analysis.

Table 2

TRAP data at high and low exposure sites (Barceloneta vs Ronda) and results from paired t-tests. Significant differences in exposure between high and low exposure sites for all pollutants.

Pollutant	Barceloneta		Ronda		Paired t-test P-value
	Mean	± SD	Mean	± SD	
NO (ppb)	78.27	52.93	588.70	194.00	2.20E-16
NOx (ppb)	102.84	61.56	679.39	204.76	2.20E-16
PM ₁₀ (µg/m ³)	64.77	41.72	122.87	42.48	2.20E-16
PM _{2.5} (µg/m ³)	38.75	13.40	82.71	18.01	1.15E-09
BC (µg/m ³)	6127.87	4363.18	23,408.64	5421.31	2.20E-16
UFP (particles/cm ³)	45,712.13	20,488.19	245,446.17	71,906.60	2.20E-16
CO (ppm)	0.99	1.75	2.69	4.38	1.48E-02
CO ₂ (ppm)	420.36	38.45	499.11	32.51	2.20E-16

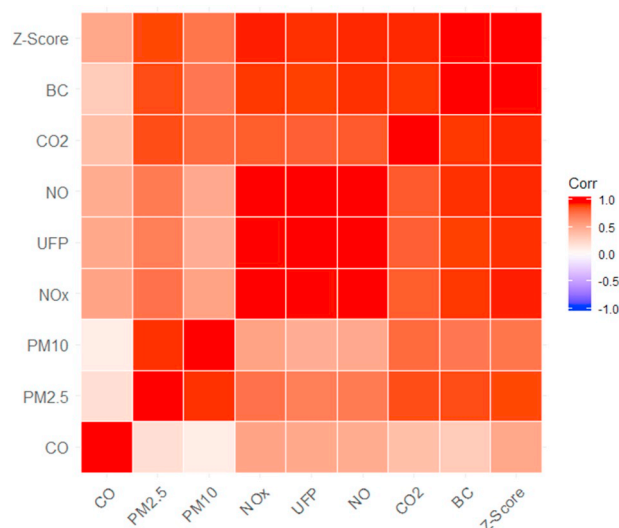


Fig. 1. Pearson correlation among the eight air contaminants. The analysis revealed that the exposures showed strong positive correlations between BC and CO₂ (r = 0.90), PM_{2.5} and PM₁₀ (r = 0.92), and NO_x, NO and UFP (r > 0.96) and a less strong positive correlation CO₂ with the remaining exposure (r < 0.51).

In total we detected 83 miRNAs with sufficient coverage across the remaining samples. The most abundant plasma miRNAs were miR-486-5p (13 logCPM), miR-92a-3p (10.80 logCPM), miR-423-5p (8.38 logCPM), miR-16-5p (8.34 logCPM) and miR-22-3p (8.05 logCPM). The mean expression of the detected miRNAs was 4.32 (± 2.01) logCPM across all plasma samples.

3.3. TRAP-associated miRNAs

We applied a MVN model in combination with the LMM to associate the detected plasma circulating miRNAs to the difference in exposure (Z-Score_(TRAP)) between Barceloneta and Ronda. The analysis showed that 9 circulating miRNAs were significantly associated with exposure. The miRNAs miR-28-3p, miR-222-3p, miR-146-5p, miR-30b-5p/30c-5p and miR-320a-3p/320b/320c/320d/320e showed a positive association with the Z-Score_(TRAP) intensity, and miR-532-5p, miR-192-5p/215-5p, miR-144-3p and miR-425-5p showed a negative association with the Z-Score_(TRAP) intensity (Fig. 2). The observed association coefficients remained subtle (range: – 0.13, 0.13) even if they appeared significant after FDR correction. Consequently, we performed a LOOCV by removing each subject from the data and performed the same model on this test dataset. After these iterations we compared the coefficients to the ones from the full dataset. The analysis showed that the beta

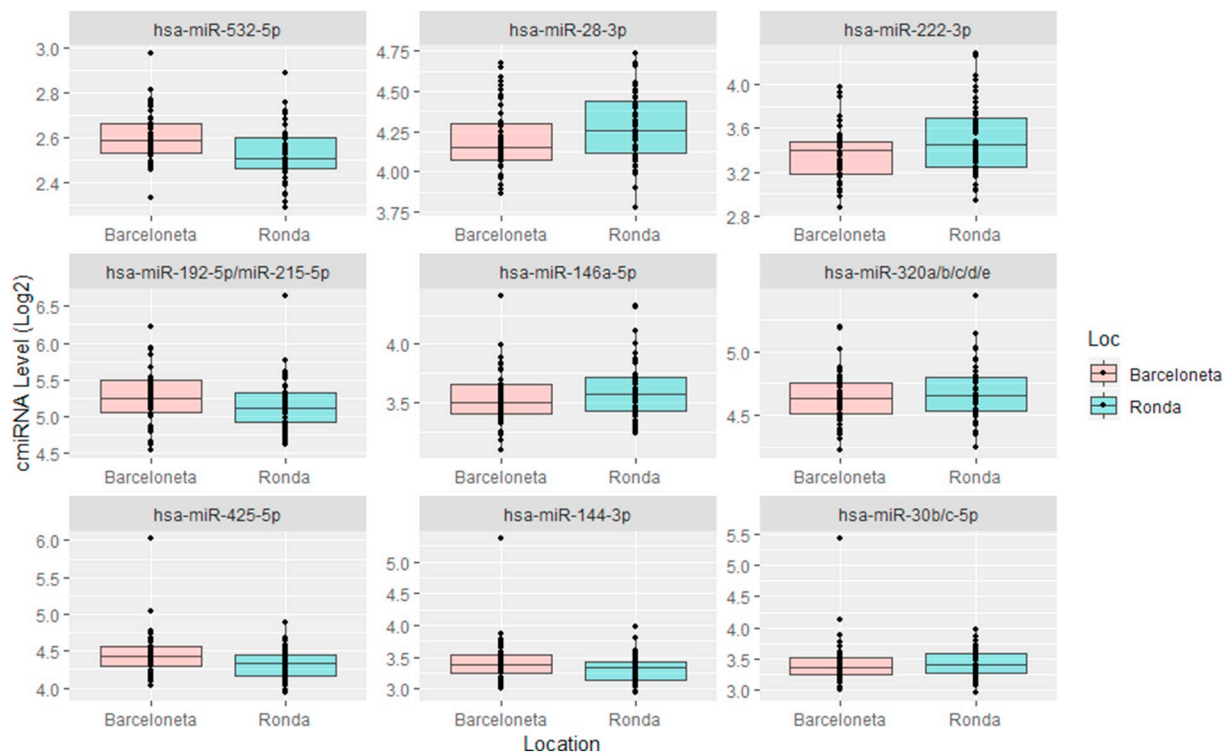


Fig. 2. Boxplot of observed significant exposure associated circulating miRNA levels (Log2) for all individuals. The figure demonstrates that expression levels of miR-28-3p, miR-222-3p, miR-146-5p, miR-30b-5p/30c-5p and miR-320a-3p/320b/320c/320d/320e was higher in Ronda compared with Barceloneta which implies a positive association with the $Z\text{-Score}_{(\text{TRAP})}$ intensity, and for miR-532-5p, miR-192-5p/215-5p, miR-144-3p and miR-425-5p a negative association with the $Z\text{-Score}_{(\text{TRAP})}$ intensity was found.

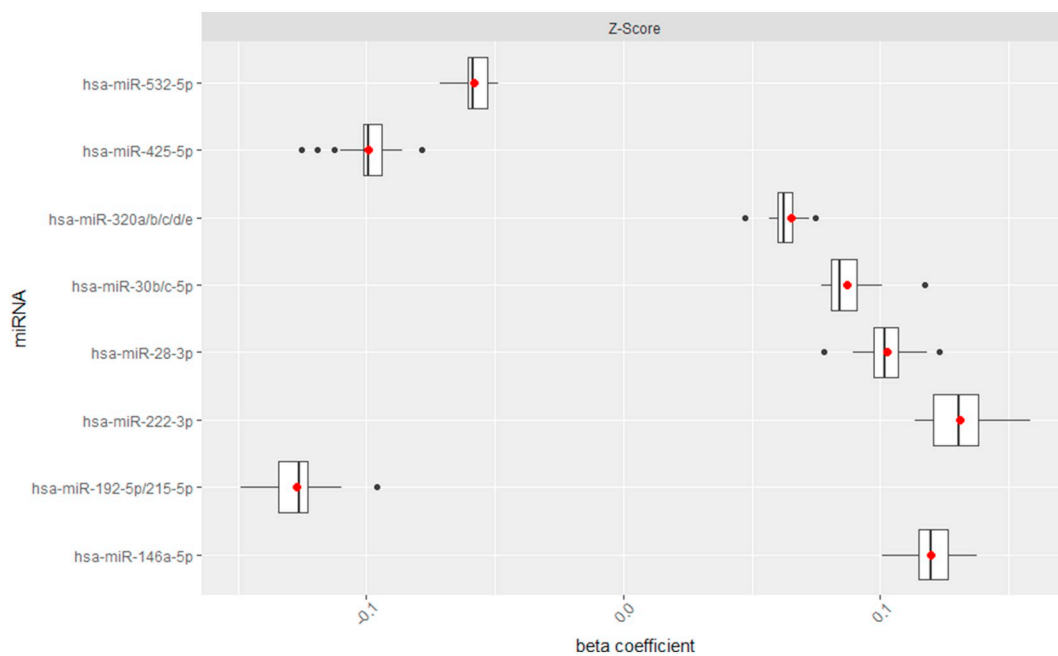


Fig. 3. Boxplot of the observed coefficients (Beta) from the cross validation step. Boxplots show the coefficients across the 24 validation steps of the LOOCV (see methods). The red dot indicates the coefficient of the model using the complete dataset. The figure shows that the subtle coefficients are robust across all miRNAs associated with the $Z\text{-Score}_{(\text{TRAP})}$ and not a result of a single or a few observations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

coefficients were robust and not depending on one or a few observations (Fig. 3).

3.4. Individual exposure-associated miRNAs

Next, we applied the same MVN model assess the association between each individual pollutant and miRNAs. We identified 29 miRNAs to be significantly associated with individual TRAP exposures across all

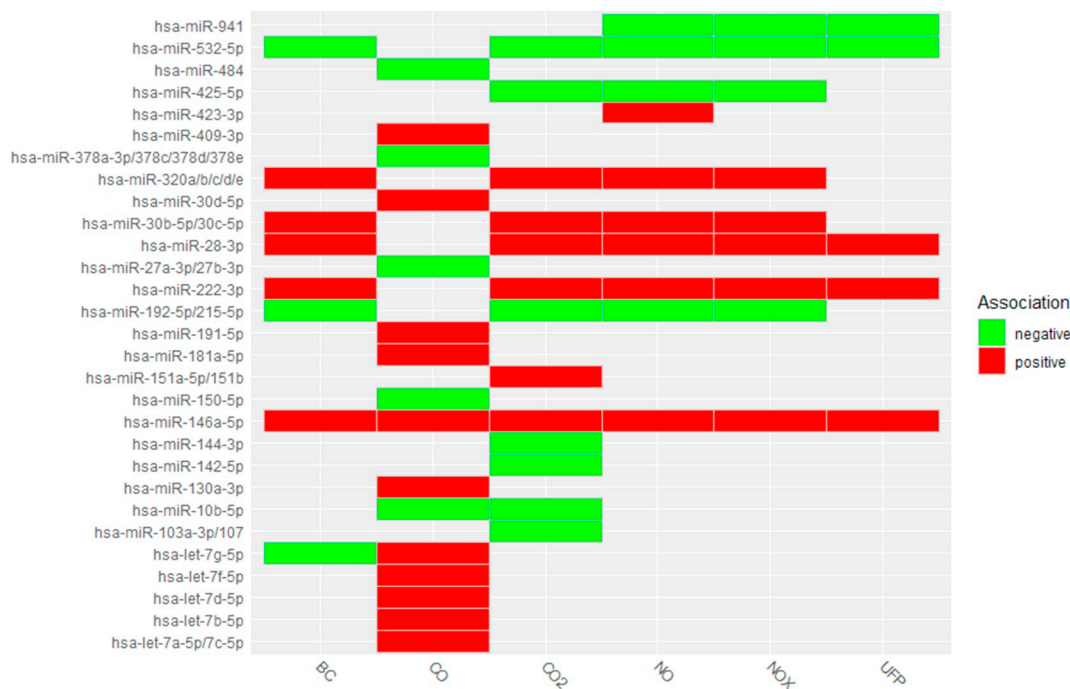


Fig. 4. Significant associations of the individual TRAP pollutants and circulating miRNAs. The figure shows the significant TRAP associated circulating miRNAs based on a false discovery rate of less than or equal to 0.1. Green indicates a significant negative correlation, and red a significant positive correlation with the individual pollutants. Grey indicates that no significant association was observed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

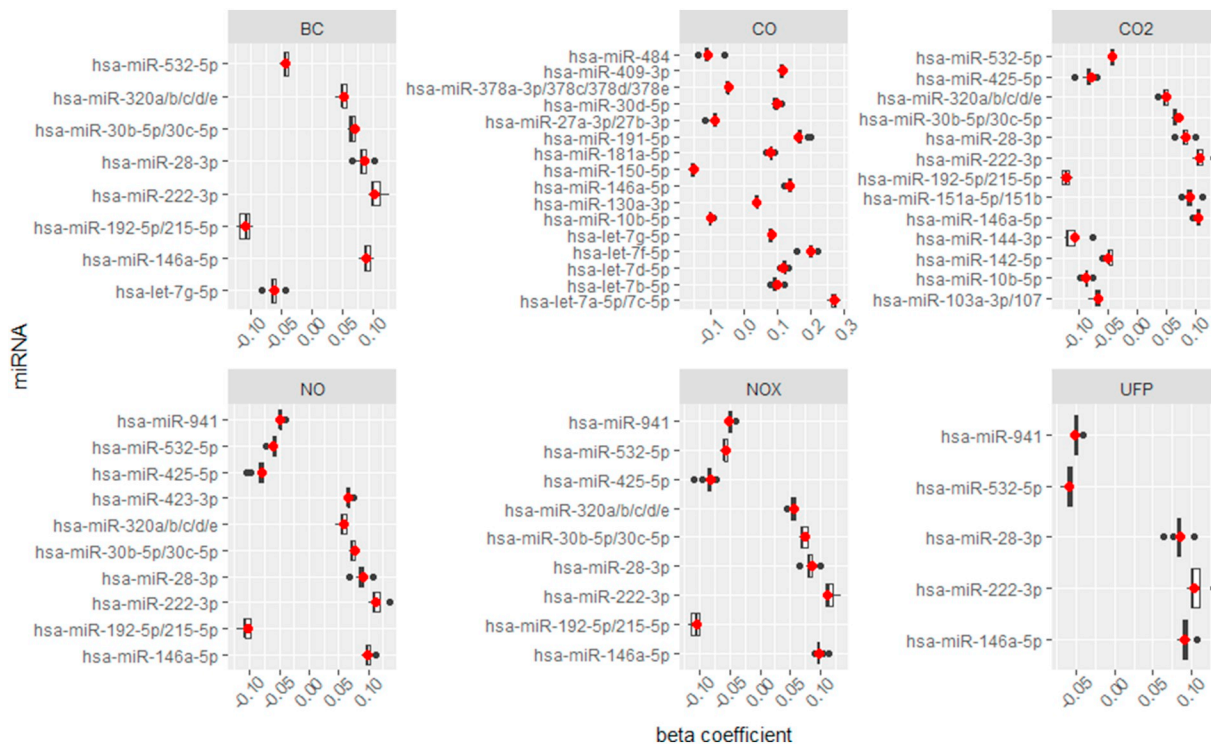


Fig. 5. Boxplot of the observed coefficients (Beta) from the cross validation step. Boxplots show the coefficient across all models of the validation step. The red dot indicates the coefficient of the model using the complete dataset. The figure shows that the subtle coefficients are robust across all miRNAs associated with the individual pollutants, and not a result of a single or a few observations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

subjects. These miRNAs showed in total 61 significant associations with 6 individual pollutants, namely 8 associations with BC, 16 associations with CO, 13 associations with CO₂, 10 associations with NO, 9 associations with NO_x and 5 associations with UFP (Fig. 4). We did not find

any significant associations with PM₁₀ and PM_{2.5}. Also here we performed a LOOCV and received robust coefficients (Fig. 5).

We also analyzed the data without adjustment for confounders but while correcting for technical variation. While we identified 9 miRNAs

significantly associated with the combined Z-Score when adjusting for physiological confounders and technical variation, 8 of these miRNAs remained significant when only adjusting for the technical variation. The analysis regarding the individual pollutants identified 29 significant miRNA associations when adjusting for physiological confounders and technical variation. While only adjusting for technical variation 24 of these miRNAs remained significant.

3.5. TRAP-associated miRNA-gene-disease network

To identify potentially perturbed pathways related to the TRAP-associated miRNAs we created a miRNA target disease interaction network. For this analysis we included the 29 miRNAs that were associated with the individual TRAP pollutants. The miRNA target interaction database 'mirTarBase' identified 616 strong gene targets for all TRAP-associated miRNAs. The identified targets were then used to explore the gene-disease interaction database 'DisGeNET' for potentially perturbed disease interactions for each miRNA. Integration of the miRNA-gene target and the gene-disease interactions networks resulted in a miRNA-disease interaction network consisting of 936 interactions for 27 miRNAs, 136 genes and 15 TRAP associated diseases. Only miR-941 and miR-423-3p did not return any interaction. Subsequently, Cytoscape (v2.7) was used for network analysis and network visualization. For visualization purposes only gene targets were included that were targeted by at least 3 miRNAs (Fig. 6). This analysis revealed four hub genes that appeared to dominate the network. *TP53* is potentially targeted by 8 miRNAs (hsa-miR-10b-5p, hsa-miR-150-5p, hsa-miR-151a-5p/151ba-5p, hsa-miR-222-3p, hsa-miR-27a-3p/27b-3p, hsa-miR-28-3p, hsa-miR-30b-5p/30c-5p-5p and hsa-miR-30d-5p) and implicated in the development of 3 diseases (Lung Cancer, Ischemia and COPD). Four miRNAs (hsa-miR-103a-3p/107, hsa-miR-150-5p, hsa-miR-181a-5p and hsa-miR-378a/c/d/e-3p) potentially target *VEGFA*, which is implicated in the pathogenesis of 7 diseases (Alzheimer's disease, Lung Cancer, Asthma, Inflammation, Myocardial Ischemia, Heart failure and Heart Diseases). *IL6* is potentially targeted by 4 miRNAs (hsa-let-7a-5p/7c-5p, hsa-let-7f-5p, hsa-miR-103a-3p/107 and hsa-miR-146a-5p) and implicated in 5 diseases (Inflammation, Parkinson's disease, Myocardial Ischemia, Heart failure and Ischemia). *PTEN* is targeted by 8 miRNAs (hsa-miR-103a-3p/107, hsa-miR-10b-5p, hsa-miR-130a-3p, hsa-miR-142-5p, hsa-miR-144-3p, hsa-miR-181a-5p, hsa-miR-222-3p and hsa-miR-425-5p) and implicated in 3 diseases (Lung Cancer, Asthma and Cardiovascular Diseases).

3.6. Circulating miRNAs and physical activity

Since it is known that circulating miRNAs participate in the physiological response we speculated that physical activity might confound the effect of TRAP exposure on the circulating miRNAs. Therefore we tested if the identified miRNAs are responsive to physical activity, and found 2 miRNAs to be upregulated (hsa-miR-146b-5p and hsa-miR-19b-3p) and 6 miRNAs are downregulated (hsa-miR-99b-5p, hsa-miR-125a-5p, hsa-miR-484, hsa-miR-151a-5p/151b, hsa-let-7b-5p and hsa-miR-443b-5p) upon physical activity. Of these only hsa-miR-151a-5p/151b and hsa-miR-484 has been identified to be associated with the exposure.

4. Discussion

We performed a global analysis of circulating miRNA in plasma of subjects exposed to different levels of ambient TRAP. The cross-over study design with sampling taking place after resting and physical activity cycles in two different exposure scenarios, combined with personal air pollution exposure measurements allowed us to identify 29 miRNAs that are perturbed in correlation to the TRAP exposure. Of these, 9 miRNAs were associated with the mixture of TRAP (Z-Score_(TRAP)) and 29 miRNAs were associated with 6 individual

compounds of TRAP: NO, NO₂, CO, CO₂, BC and UFP. We did not find significant associations between PM₁₀ or PM_{2.5} and circulating miRNAs.

We validated the response of 2 miRNAs, namely hsa-miR-222 and hsa-miR-146a, that have been extensively studied in humans upon long-term exposure to PM (Fossati et al., 2014; Motta et al., 2013; Vriens et al., 2016). Both have been positively correlated with the mixture of TRAP and the individual compounds UFP, NO, NO₂ and CO in the present study. Furthermore, we identified multiple miRNAs responding to higher TRAP levels that have not been identified earlier (Fig. 4). Interestingly, multiple of these miRNAs have already been investigated in relation to diseases that are induced by TRAP exposure. For instance the roles of hsa-miR-103a-3p/107, hsa-miR-146a and hsa-miR-181 in Alzheimer's diseases, and their potential as therapeutic targets and diagnostic biomarkers has been recently reviewed (Gupta et al., 2017). The miRNAs hsa-miR-222, hsa-miR-146a, hsa-miR-27a-3p/27b-3p and hsa-miR-320 have been confirmed in patients suffering from cardiovascular disease, vascular inflammation, cardiomyocyte apoptosis and cardiac hypertrophy (de Lucia et al., 2017; Ding et al., 2017). Furthermore, hsa-let-7, hsa-miR-130a-3p, hsa-miR-222-3p, hsa-miR-30b-5p/30c-5p, hsa-miR-150-5p have been found in patients with multiple respiratory diseases (Brown et al., 2014).

Results of an integrated network analysis highlighted the putative role of these miRNAs in respiratory, cardiovascular and neurodegenerative diseases as well as diabetes mellitus. This network suggests through which miRNA-gene interactions the TRAP-responsive miRNAs may act as drivers of disease. The hub genes *TP53*, *VEGFA*, *IL6* and *PTEN* were identified, which are involved in the pathogenesis of all major TRAP-induced diseases (Fig. 6).

We found 3 miRNAs overlapping with an earlier study (Krauskopf et al., 2018) we performed on TRAP-associated circulating miRNAs (hsa-miR-103a-3p/107, hsa-miR-150-5p, hsa-miR-30d-5p). However, in that study hsa-miR-103a-3p/107 showed a positive, and hsa-miR-150-a and hsa-miR-30d-5p a negative association with PM₁₀ and BC, while in the present study we identified hsa-miR-103a-3p/107 and hsa-miR-150-5p were positively and hsa-miR-30d-5p negatively associated with the carbon oxides. This incoherence in findings may be due to the fact that in the present study not only the exposure intensities were drastically higher but the pollution has also originated from different sources (diesel power versus general urban pollution). Furthermore, the study designs (physical activity vs resting) and population (disease endpoints) and blood handling were different. Further, as a consequence of lower sequencing depth in the present study we did not detect the cardiovascular disease-associated miRNAs hsa-miR-499 and hsa-miR-133 that have been identified as TRAP-responsive in our previous study (Krauskopf et al., 2018). This limited overlap in results between the two cohorts was reported in earlier studies on transcriptomics and miRNA analysis in peripheral blood cells (Espín-Pérez et al., 2018).

It has previously been reported that miRNAs are responsive to physical activity (Xu et al., 2015). Since these miRNAs could potentially confound the results of this study we performed a test to identify these miRNAs. Only 2 miRNAs were altered upon physical activity, which were also declared significantly associated with the exposure. While hsa-miR-484 is negatively associated with exposure and physical activity, hsa-miR-151a-5p/151b is downregulated upon physical activity but positively associated with TRAP exposure.

5. Conclusions

The study identified a compound-specific circulating miRNA profile associated with short-term exposure to ambient TRAP exposure. We confirmed TRAP-induced perturbations of previously identified miRNAs, but also discovered multiple novel potentially TRAP-responsive miRNAs. Integrated network analysis showed that these miRNAs possibly contribute to the chain of events connecting TRAP exposure to increased risk of respiratory, cardiovascular as well as

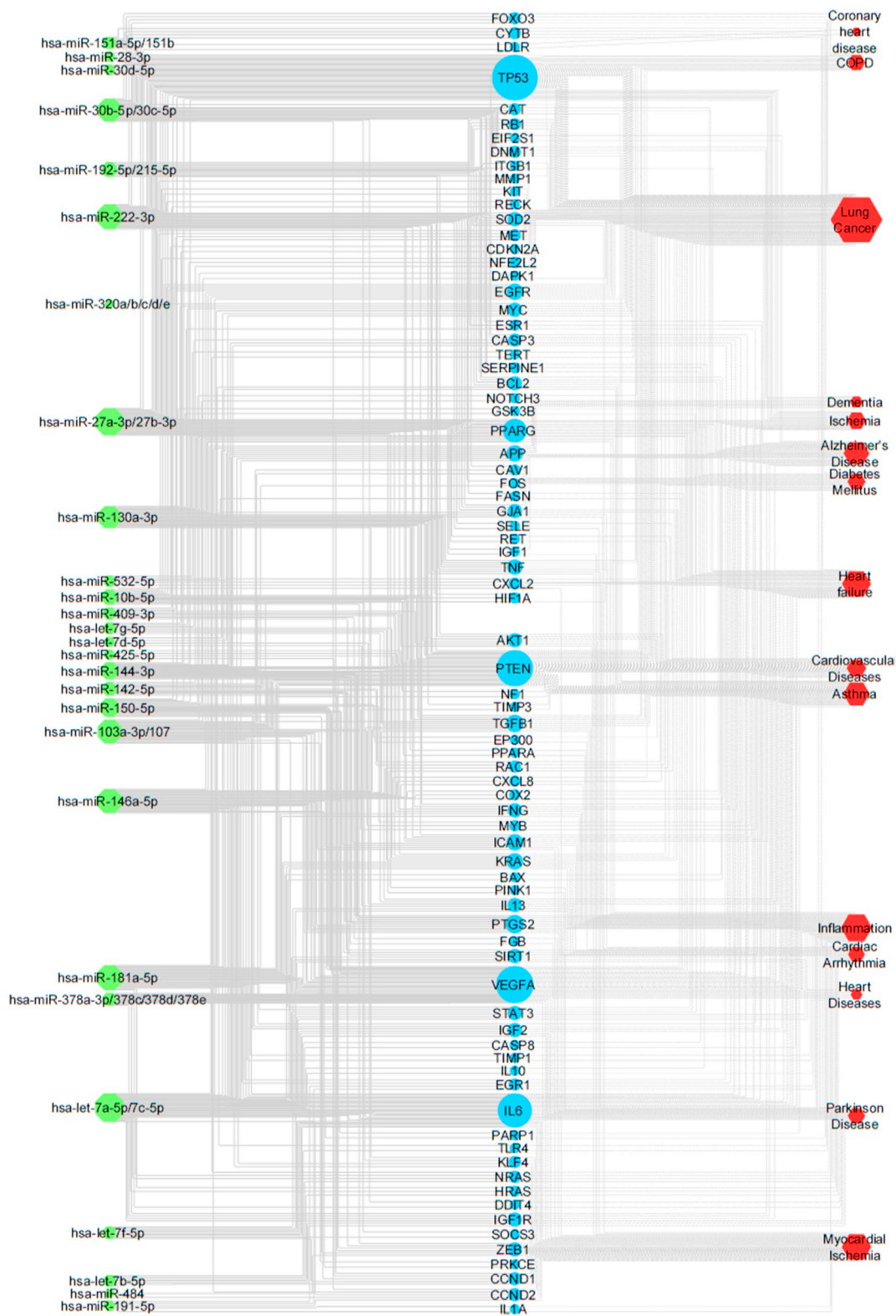


Fig. 6. miRNA-gene-disease network. The network shows the TRAP-associated miRNAs, gene targets and expert curated disease interactions. Only gene targets are included that had a minimum of 3 incoming interactions with miRNAs. The edges between miRNAs and genes are strong validated interactions from ‘miRtarBase’. The edges between gene targets and disease outcomes are indicating expert curated associations derived from ‘DisGenet’. The amount of edges corresponds to the number of evidence found in either ‘miRtarBase’ or ‘DisGenet’. Node size corresponds to the number of interactions.

neurodegenerative diseases and diabetes mellitus. Despite the small sample size, and the necessity of studies with a more dedicated design to the individual miRNAs identified in this study the proposed circulating miRNAs may present promising biomarker candidates to be used

in health risk assessment in relation to short-term TRAP exposure and TRAP-induced health effects.

Acknowledgments

This work has been supported by the European Union within the frame of the Exposomics (226756) project.

References

- Bartel, D.P., 2009. MicroRNAs: target recognition and regulatory functions. *Cell* 136, 215–233. <https://doi.org/10.1016/J.CELL.2009.01.002>.
- Bollati, V., Marinelli, B., Apostoli, P., Bonzini, M., Nordio, F., Hoxha, M., Pegoraro, V., Motta, V., Tarantini, L., Cantone, L., Schwartz, J., Bertazzi, P.A., Baccarelli, A., 2010. Exposure to metal-rich particulate matter modifies the expression of candidate MicroRNAs in peripheral blood leukocytes. *Environ. Health Perspect.* 118, 763–768. <https://doi.org/10.1289/ehp.0901300>.
- Bowe, B., Xie, Y., Li, T., Yan, Y., Xian, H., Al-Aly, Z., 2018. The 2016 global and national burden of diabetes mellitus attributable to PM2.5 air pollution. *Lancet Planet. Heal.* 2, e301–e312. [https://doi.org/10.1016/S2542-5196\(18\)30140-2](https://doi.org/10.1016/S2542-5196(18)30140-2).
- Brown, D., Rahman, M., Nana-Sinkam, S.P., 2014. MicroRNAs in respiratory disease. A clinician's overview. *Ann. Am. Thorac. Soc.* 11, 1277–1285. <https://doi.org/10.1513/AnnalsATS.201404-179FR>.
- Calderón-Garcidueñas, L., Villarreal-Ríos, R., 2017. Living close to heavy traffic roads, air pollution, and dementia. *Lancet* 389, 675–677. [https://doi.org/10.1016/S0140-6736\(16\)32596-X](https://doi.org/10.1016/S0140-6736(16)32596-X).
- Chou, C.-H., Shrestha, S., Yang, C.-D., Chang, N.-W., Lin, Y.-L., Liao, K.-W., Huang, W.-C., Sun, T.-H., Tu, S.-J., Lee, W.-H., Chiew, M.-Y., Tai, C.-S., Wei, T.-Y., Tsai, T.-R., Huang, H.-T., Wang, C.-Y., Wu, H.-Y., Ho, S.-Y., Chen, P.-R., Chuang, C.-H., Hsieh, P.-J., Wu, Y.-S., Chen, W.-L., Li, M.-J., Wu, Y.-C., Huang, X.-Y., Ng, F.L., Buddhakosai, W., Huang, P.-C., Lan, K.-C., Huang, C.-Y., Weng, S.-L., Cheng, Y.-N., Liang, C., Hsu, W.-L., Huang, H.-D., 2018. miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res.* 46, D296–D302. <https://doi.org/10.1093/nar/gkx1067>.
- de Lucia, C., Komici, K., Borghetti, G., Femminella, G.D., Bencivenga, L., Cannavo, A., Corbi, G., Ferrara, N., Houser, S.R., Koch, W.J., Rengo, G., 2017. microRNA in cardiovascular aging and age-related cardiovascular diseases. *Front. Med.* 4, 74. <https://doi.org/10.3389/fmed.2017.00074>.
- Ding, S., Huang, H., Xu, Y., Zhu, H., Zhong, C., 2017. MiR-222 in cardiovascular diseases: physiology and pathology. *Biomed. Res. Int.* 2017, 4962426. <https://doi.org/10.1155/2017/4962426>.
- Downward, G.S., van Nunen, E.J.H.M., Kerckhoffs, J., Vineis, P., Brunekreef, B., Boer, J.M.A., Messier, K.P., Roy, A., Verschuren, W.M.M., van der Schouw, Y.T., Sluijs, I., Gulliver, J., Hoek, G., Vermeulen, R., 2018. Long-term exposure to ultrafine particles and incidence of cardiovascular and cerebrovascular disease in a prospective study of a Dutch cohort. *Environ. Health Perspect.* 126, 127007. <https://doi.org/10.1289/EHP3047>.
- Espín-Pérez, A., Krauskopf, J., Chadeau-Hyam, M., van Veldhoven, K., Chung, F., Cullinan, P., Piepers, J., van Herwijnen, M., Kubesch, N., Carrasco-Turigas, G., Nieuwenhuijsen, M., Vineis, P., Kleinjans, J.C.S., de Kok, T.M.C.M., 2018. Short-term transcriptome and microRNAs responses to exposure to different air pollutants in two population studies. *Environ. Pollut.* 242, 182–190. <https://doi.org/10.1016/J.ENVPOL.2018.06.051>.
- Forman, H.J., Finch, C.E., 2018. A critical review of assays for hazardous components of air pollution. *Free Radic. Biol. Med.* 117, 202–217. <https://doi.org/10.1016/j.freeradbiomed.2018.01.030>.
- Fossati, S., Baccarelli, A., Zanobetti, A., Hoxha, M., Vokonas, P.S., Wright, R.O., Schwartz, J., 2014. Ambient particulate air pollution and MicroRNAs in elderly men. *Epidemiology* 25, 68–78. <https://doi.org/10.1097/EDE.000000000000026>.
- Gupta, P., Bhattacharjee, S., Sharma, A.R., Sharma, G., Lee, S.-S., Chakraborty, C., 2017. miRNAs in Alzheimer disease - a therapeutic perspective. *Curr. Alzheimer Res.* 14, 1198–1206. <https://doi.org/10.2174/1567205014666170829101016>.
- Kozomara, A., Griffiths-Jones, S., 2014. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res.* 42, D68–D73. <https://doi.org/10.1093/nar/gkt1181>.
- Krauskopf, J., Verheijen, M., Kleinjans, J.C., de Kok, T.M., Caiment, F., 2015. Development and regulatory application of microRNA biomarkers. *Biomark. Med.* 9, 1137–1151. <https://doi.org/10.2217/bmm.15.50>.
- Krauskopf, J., de Kok, T.M., Hebels, D.G., Bergdahl, I.A., Johansson, A., Spaeth, F., Kiviranta, H., Rantakokko, P., Kyrtopoulos, S.A., Kleinjans, J.C., 2017. MicroRNA profile for health risk assessment: environmental exposure to persistent organic pollutants strongly affects the human blood microRNA machinery. *Sci. Rep.* 7, 9262. <https://doi.org/10.1038/s41598-017-10167-7>.
- Krauskopf, J., Caiment, F., van Veldhoven, K., Chadeau-Hyam, M., Sinharay, R., Chung, K.F., Cullinan, P., Collins, P., Barratt, B., Kelly, F.J., Vermeulen, R., Vineis, P., de Kok, T.M., Kleinjans, J.C., 2018. The human circulating miRNome reflects multiple organ disease risks in association with short-term exposure to traffic-related air pollution. *Environ. Int.* 113, 26–34. <https://doi.org/10.1016/j.envint.2018.01.014>.
- Kroh, E.M., Parkin, R.K., Mitchell, P.S., Tewari, M., 2010. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). *Methods* 50, 298–301. <https://doi.org/10.1016/j.ymeth.2010.01.032>.
- Li, Q., Liu, H., Alattar, M., Jiang, S., Han, J., Ma, Y., Jiang, C., 2015. The preferential accumulation of heavy metals in different tissues following frequent respiratory exposure to PM2.5 in rats. *Sci. Rep.* 5, 16936. <https://doi.org/10.1038/srep16936>.
- Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550. <https://doi.org/10.1186/s13059-014-0550-8>.
- Lu, Y., Baras, A.S., Halushka, M.K., 2018. miRge 2.0 for comprehensive analysis of microRNA sequencing data. *BMC Bioinformatics* 19, 275. <https://doi.org/10.1186/s12859-018-2287-y>.
- Motta, V., Angelici, L., Nordio, F., Bollati, V., Fossati, S., Frascati, F., Tinaglia, V., Bertazzi, P.A., Battaglia, C., Baccarelli, A.A., 2013. Integrative analysis of miRNA and inflammatory gene expression after acute particulate matter exposure. *Toxicol. Sci.* 132, 307–316. <https://doi.org/10.1093/toxsci/kft013>.
- Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., Cox, C., 2004. Translocation of inhaled ultrafine particles to the brain. *Inhal. Toxicol.* 16, 437–445. <https://doi.org/10.1080/08958370490439597>.
- Piñero, J., Bravo, Á., Queralt-Rosinach, N., Gutiérrez-Sacristán, A., Deu-Pons, J., Centeno, E., García-García, J., Sanz, F., Furlong, L.I., 2017. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res.* 45, D833–D839. <https://doi.org/10.1093/nar/gkw943>.
- Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504. <https://doi.org/10.1101/gr.1239303>.
- Vriens, A., Nawrot, T.S., Saenen, N.D., Provost, E.B., Kicinski, M., Lefebvre, W., Vanpoucke, C., Van Deun, J., De Wever, O., Vrijens, K., De Boever, P., Plusquin, M., 2016. Recent exposure to ultrafine particles in school children alters miR-222 expression in the extracellular fraction of saliva. *Environ. Health* 15, 80. <https://doi.org/10.1186/s12940-016-0162-8>.
- Vrijens, K., Bollati, V., Nawrot, T.S., 2015. MicroRNAs as potential signatures of environmental exposure or effect: a systematic review. *Environ. Health Perspect.* 123, 399–411. <https://doi.org/10.1289/ehp.1408459>.
- WHO, 2017. WHO | WHO Global Urban Ambient Air Pollution Database (Update 2016). WHO.
- Xu, T., Liu, Q., Yao, J., Dai, Y., Wang, H., Xiao, J., 2015. Circulating microRNAs in response to exercise. *Scand. J. Med. Sci. Sports* 25, e149–e154. <https://doi.org/10.1111/sms.12421>.
- Zhang, K., Batterman, S., 2013. Air pollution and health risks due to vehicle traffic. *Sci. Total Environ.* 450–451, 307–316. <https://doi.org/10.1016/j.scitotenv.2013.01.074>.