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Investigating the molecular mechanisms underlying the impact of prenatal exposures to traffic-related air pollution (TRAP) on newborn metabolome and adverse birth outcomes

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2021

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An abstract of
A thesis submitted to the Faculty of the
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Master of Public Health
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Abstract

Investigating the molecular mechanisms underlying the impact of prenatal exposures to traffic-related air pollution (TRAP) on newborn metabolome and adverse birth outcomes

By Yilin Wang

Introduction: Air pollution is a significant environmental health risk that has been associated with adverse birth outcomes. However, the molecular mechanisms underlying this association remain unclear. This study aimed to investigate the impact of prenatal exposures to air pollutant on newborn metabolome and adverse birth outcomes.

Methods: In this analysis, we included 48 participants from the Atlanta African American Maternal Child cohort. We estimated individual exposure to TRAP using air pollution high-resolution metabolomics assessment. We then performed high-resolution metabolomics analysis on newborn dried blood spots collected at delivery to assess the newborn metabolome. We used statistical analysis, metabolic pathway enrichment analysis, and metabolite annotation to identify pathways and metabolites that were associated with prenatal exposure to air pollutants. We also used meet-in-the-middle analysis to identify overlapping metabolites and pathways between prenatal exposure and adverse birth outcomes.

Results: We found that prenatal exposure to air pollution was associated with significant changes in the newborn metabolome. We identified several metabolic pathways that were significantly impacted by prenatal exposure to air pollutant, including biopterin metabolism, drug metabolism - cytochrome P450, lysine metabolism, tryptophan metabolism, and several vitamin metabolism pathways. Additionally, we identified two overlapping metabolites, DMABA NHS ester and 3,4-dimethoxyphenylpropanoic acid, between maternal TRAP exposure and adverse birth outcomes.

Discussion: Our findings suggest that prenatal exposure to air pollutants may have significant impacts on the metabolome of newborns and contribute to adverse birth outcomes. The identification of specific metabolites and pathways may serve as potential biomarkers for future studies aimed at identifying high-risk populations and developing targeted interventions to mitigate the negative effects of air pollution on maternal and child health. However, this study also has some limitations, including a small sample size and lack of personal environmental monitoring data, which should be addressed in future studies.

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Introduction

Exposures to traffic-related air pollution (TRAP) have been linked to various adverse health effects previously(Boogaard H; Boogaard et al., 2022; Sears et al., 2018). Specifically, pregnant people and fetuses are particularly vulnerable, as prenatal exposures to TRAP have been associated with many pregnancy complications and adverse birth outcomes, including preeclampsia(Dadvand et al., 2013), gestational diabetes(Eze et al., 2015), preterm birth (PTB, defined as babies born alive over 22 weeks and before 37 weeks of pregnancy are completed)(Gehring et al., 2011), early-term birth (ETB, defined as babies born alive 37-39 weeks from the first date of a woman's last menstrual period). Although these studies have contributed to a growing body of evidence linking PTB / ETB with TRAP, the biological mechanisms underlying the association between prenatal exposure to TRAP and the adverse birth outcome are not clear yet. Prenatal exposures to TRAP have also been evident to affect the newborn anthropometric indexes at birth in recent studies(Gehring et al., 2011; MoghaddamHosseini et al., 2022), where higher exposures to TRAP have been associated with lower birth weight and birth length(MoghaddamHosseini et al., 2022).

Previously, some studies(Inoue et al., 2020; Kim et al., 2021; Kioumourtzoglou et al., 2019; Yan et al., 2019) have reported that prenatal exposure to TRAP is associated with perturbations in pregnant women's metabolome, however, none focus on understudied minority population. In particular, communities of color and the poor are facing higher exposures to TRAP and experiencing higher rates of adverse birth and child health outcomes.(Vogel et al., 2018) Specifically, African American (AA) people and children are exposed to higher level of TRAP and experience higher rates of adverse birth and child health outcomes. These changes might influence the metabolome of the newborns, which includes both the endogenous metabolites secreted by organism and exogenous chemicals (such as our interested chemicals – ambient air pollution) not naturally secreted by organism(Bowling & Thomas, 2014).

Untargeted high-resolution metabolomics (HRM) has emerged as a powerful platform detecting metabolic signals to characterize internal exposure to complex exogenous

environmental mixtures and the endogenous responses (S. Li, Dunlop, Jones, & Corwin, 2016). Previously, our group have demonstrated the feasibility and applicability of using untargeted HRM in linking TRAP exposures to internal dose and biological responses (Z. Li et al., 2022; Liang et al., 2019). To address these critical knowledge gaps, we conducted this analysis to investigate the molecular mechanisms underlying the impact of prenatal exposures to TRAP on newborn metabolome and adverse birth outcomes using untargeted HRM with meet-in-the-middle framework. (Jeong et al., 2018)

Method

Study population

In this study, we included 48 participants from the Atlanta African American (AA) Maternal and Child cohort, which is a prospective cohort study recruiting African American people receiving prenatal care in Emory Midtown Hospital (privately funded) and Grady Hospital (publicly funded).

Air pollution exposure assessment

We used spatiotemporal resolved models to generate personal exposure assessment on $PM_{2.5}$, NO_2 and O_3 concentrations for each participant based on geocoded data collected during their first visit. The TRAP concentrations were calculated to the average concentrations per day from the estimated day of conception to the delivery date of the newborn.

Measure of birth outcome

Based on the gestational age, we grouped the birth outcomes into pre-term birth (22-37 weeks), early-term birth (37-39 weeks) and full-term birth (>=39 weeks), which is based on the gestation age.

High-resolution metabolomics

Newborn dried blood spot (DBS) samples were collected at birth for medical screening and surveillance and were stored for future biomonitoring purposes. Compared to whole blood

samples, DBS samples are reliable, reproducible, and representative of the circulating metabolome in humans, and provide new insights into the intrauterine environment. For this study, DBS samples were collected within 48 hours of birth and stored under refrigeration for up to two months, then frozen at -80°C. Metabolomics profiling was conducted using established methods by the North Carolina Human Health Exposure Analysis Resource Hub. Samples were extracted with ice-cold methanol and analyzed using untargeted high-resolution metabolomics profiling via ultra-high performance liquid chromatography-high resolution mass spectrometry. Quality control samples and blanks were interspersed with study samples during the analysis sequence, and the data were processed using Progenesis QI for peak identification and alignment. Signals that differed significantly amongst the three running batches were excluded.

Statistical analysis

To investigate the association between prenatal exposure to air pollutants and newborn metabolome features, we conducted a metabolome wide association study (MWAS). Specifically, we constructed a generalized linear model to evaluate the association of prenatal exposure to air pollutants with metabolic features using the following form:

$$\log_2 Y_{ij} = \beta_0 + \beta_{1j} AirPollutant_i + \gamma_{1j} Age_i + \gamma_{2j} Education_i + \gamma_{3j} MeritalStatus_i + \gamma_{4j} Income_i + \gamma_{5j} Hospital_i + \gamma_{6j} Parity_i + \varepsilon_{ij}$$

where, $\log_2 Y_{ij}$ represents the log base 2 intensity of newborn metabolome feature j for newborn i. β_0 is the intercept, and $AirPollutant_i$ represents prenatal exposure to air pollution for participant i. We controlled for potential confounding variables in the model, including maternal age (Age_i), education level ($Education_i$), marital status ($MeritalStatus_i$), income ($Income_i$), hospital of delivery ($Hospital_i$), and parity ($Parity_i$). ε_{ij} represents residual random error.

We conducted separate analyses for each air pollutant ($PM_{2.5}$, NO_2, O_3). We also considered potential nonlinear associations by including squared terms for air pollutants in the model. To assess the goodness of fit of the model, we developed residual plots and checked for potential outliers and influential observations. The significance level we use is 0.05 and presented the

results using forest plots, with the effect estimates and confidence intervals for each metabolome feature.

To identify metabolic features associated with adverse birth outcomes, a generalized linear model was employed for each clinic visit. The model controlled for various covariates except gestational age at prenatal visits (Gestational Agei). The equation used was:

$$\log_2 Y_{ij} = \beta_0 + \beta_{1j} Birthoutcome_i + \gamma_{1j} Age_i + \gamma_{2j} Education_i + \gamma_{3j} MeritalStatus_i + \gamma_{4j} Income_i + \gamma_{5j} Hospital_i + \gamma_{6j} Parity_i + \varepsilon_{ij}$$

where, $Birthoutcome_i$ referred to preterm / early term / full term birth (categorical), with full term birth as the reference group. Two different sets of models were analyzed, one for each type of birth outcome.

All statistical analyses were performed using R (version 4.2.1).

Metabolic pathway enrichment analysis and metabolite annotation

To elucidate the functional activities of metabolic features identified from LC-HRMS output in the context of prenatal air pollution exposure and newborn metabolomics, we performed metabolic pathway enrichment analysis and metabolite annotation. Pathway enrichment was conducted using mummichog (v. 1.0.9), a novel bioinformatics platform that can predict the biological activities of metabolites without prior identification (S. Li et al., 2016). To ensure robustness, we employed two approaches to select metabolic features for pathway analysis: a raw p-value cutoff of 0.05 and a multiple testing corrected p-value cutoff of 0.2 using the Benjamini-Hochberg method for multiple comparison correction. In the first approach, to minimize the likelihood of false positive discoveries, we excluded pathways with a size smaller than the number of features matched in pathway enrichment and identified by mummichog with a p-value greater than 0.05. We also conducted a sensitivity analysis by using the 0.5th and 1st percentiles of raw p-values to perform pathway enrichment and evaluate whether the significant pathways would differ markedly under different raw p-value thresholds (Jeong et al., 2018).

Furthermore, we annotated metabolic features that were significantly associated with both prenatal exposure to air pollutants and adverse birth outcomes and were also enriched in relevant pathways. Annotation was performed by matching the mass-to-charge ratio (m/z) value of each feature to common adducts using the METLIN, Human Metabolome Database (HMDB), and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases, with a mass error threshold of 10 ppm. To reduce the likelihood of false positive matches, each annotated feature was screened based on its retention time, isotope patterns, and spectrum peak quality, as determined by examining the extracted ion chromatographs (EICs). Finally, a subset of annotated metabolites was confirmed with level one evidence(Morello-Frosch & Shenassa, 2006) by comparing their m/z, retention time, and ion dissociation patterns to analytical standards in an in-house library that contains a list of exogenous or endogenous metabolites analyzed under identical experimental conditions.

Meet-in-the-middle analysis

To examine the potential mediating role of the maternal metabolome in the association between prenatal exposure to air pollutant and adverse birth outcomes, we employed a meet-in-the-middle analysis approach (Chadeau-Hyam et al., 2011). Specifically, we first performed pathway enrichment analysis and chemical annotation separately for both the exposure and outcome variables. This involved identifying pathways and metabolites significantly associated with prenatal exposure to air pollutants and adverse birth outcomes, respectively. Next, we conducted a search to identify any common pathways or metabolites that were significantly associated with both the exposure and the outcome. This approach was chosen to investigate potential biological mechanisms underlying the observed association between prenatal exposure to air pollutants and adverse birth outcomes. The overall analytical flow of this approach is illustrated in Figure 1.

Result

Study population

Of the 48 participants included in the current analysis, 8 were preterm birth, 18 were early term

birth, and 22 were full term birth. The average age of pregnant people on their first visit was 25.9 ± 4.5 years. About 67% of study participants were married, 44% had a college degree or above, and 65% had been pregnant before. The proportion of presence of intrauterine growth restriction was 14.6%, and the proportion of occurrence of premature rupture of membranes is 2.1% (Table 1).

MWAS model

After performing data quality assurance and quality control as described in the Untargeted High-Resolution Metabolomics Analysis method, we included 6,981 metabolic features for final analyses. Table 2 displays the number of metabolites that were found to be statistically significant (raw and FDR-corrected) in association with prenatal exposure to air pollutants.

Specifically, 813 metabolic features were significantly associated with prenatal exposure to $PM_{2.5}$, 1,402 metabolic features were significantly associated with prenatal exposure to O_3, and 297 metabolic features were significantly associated with prenatal exposure to NO_2 after adjusting for covariates (p-value < 0.05). 99 metabolic features were significantly associated with prenatal exposure to $PM_{2.5}$, 1,268 metabolic features were significantly associated with prenatal exposure to O_3, and 0 metabolic feature was significantly associated with prenatal exposure to NO_2 after adjusting for covariates (FDR < 0.2).

Regarding birth outcomes, fewer significant features (N = 187, p-value < 0.05) were found to be associated with adverse birth outcome after adjusting for covariates.

Pathway enrichment analysis

We conducted pathway enrichment analysis using significant metabolic features as input. A total of 29 and 19 significant pathways were associated with prenatal exposure to air pollutants and birth outcomes, respectively. The metabolic features significantly associated with prenatal exposure to $PM_{2.5}$, NO_2, O_3 were mapped to four and 25 unique pathways. C21-steroid hormone biosynthesis and metabolism, drug metabolism – cytochrome P450, tryptophan metabolism and vitamin B3 (nicotine and nicotinamide) metabolism were the only 4 common pathways for prenatal exposure to all of three air pollutants. There are 23 significant pathways

associated with prenatal exposure to $PM_{2.5}$ or O_3, and 9 of these significant pathways overlapped.

For birth outcome-associated metabolic profiling, Biopterin metabolism and lysine metabolism are the only 2 significant pathways that are mapped for both ETB and PTB.

In the meet-in-the-middle analysis, 13 pathways were identified as overlapping pathways (Figure 2), including biopterin metabolism, drug metabolism - cytochrome P450, lysine metabolism, tryptophan metabolism, vitamin B3 (nicotinate and nicotinamide) metabolism, leukotriene metabolism, lipoate metabolism, tyrosine metabolism, limonene and pinene degradation, linoleate metabolism, vitamin B9 (folate) metabolism, vitamin D3 (cholecalciferol) metabolism, vitamin E metabolism.

Metabolite annotation and confirmation

A total of 6 metabolites were confirmed with level 1 evidence, with 4 and 4 being significantly associated with prenatal exposure to air pollutants and birth outcomes, respectively (Table 3, Table 4). DMABA NHS ester and 3,4-dimethoxyphenylpropanoic acid are the two metabolites that are identified as overlapping metabolites. In addition to the overlapping metabolites, Indole-3-methyl acetate and N-acetylleucine were also annotated when we study the association between prenatal exposure to air pollutants and newborn metabolome. Muricholic acid and tetradecenoyl-L-carnitine were the 2 additional metabolites annotated when investigated the association between adverse birth outcome and newborn metabolites.

Discussion

In our study, we utilized untargeted high-resolution metabolomics to investigate the impact of prenatal exposure to air pollutants on the metabolome of newborns. We observed significant metabolic disturbances during early and late pregnancy that were correlated with both air pollutants and adverse birth outcomes. Our findings also revealed a network of metabolites that were closely associated with various biological pathways, including inflammation, oxidative stress, placental vascularization, and insulin action. Overall, our results suggest that prenatal exposure to air pollutants may influence several biological mechanisms that contribute to

adverse birth outcomes in newborns.

Several of these pathways have previously been linked to adverse health outcomes. For example, alterations in biopterin metabolism have been associated with endothelial dysfunction, which can increase the risk of cardiovascular disease (Yuyun, Ng, & Ng, 2018). Similarly, dysregulation of lysine metabolism has been linked to insulin resistance and diabetes (Jozi et al., 2022). The identification of these pathways as potential biomarkers of air pollution exposure may help inform the development of targeted interventions to prevent or mitigate the negative health effects of air pollution on pregnant women and their offspring.

The identification of vitamin B9 (folate) metabolism as an overlapping pathway is particularly noteworthy, as folate is a critical nutrient for fetal development. Folate deficiency during pregnancy has been linked to neural tube defects, low birth weight, and other adverse birth outcomes (Czeizel, Dudas, Vereczkey, & Banhidy, 2013). The identification of alterations in folate metabolism in response to air pollution exposure may have important implications for public health policies aimed at reducing air pollution exposure during pregnancy.

The meet-in-the-middle analysis also identified several potential biomarkers of air pollution exposure, including metabolites involved in lysine metabolism and vitamin D3 metabolism. These biomarkers may be useful for predicting adverse health outcomes in future studies and could potentially be used to develop targeted interventions to prevent or mitigate the negative health effects of air pollution on pregnant women and their offspring.

Overall, the findings of this study highlight the complex molecular mechanisms underlying the impact of air pollution exposure on newborn metabolome and adverse birth outcomes. The identification of specific metabolic pathways and potential biomarkers associated with air pollution exposure may help inform the development of targeted interventions to prevent or mitigate the negative health effects of air pollution on pregnant women and their offspring.

The identification of DMABA NHS ester and 3,4-dimethoxyphenylpropanoic acid as overlapping metabolites in the study suggests that prenatal exposure to air pollution may lead to oxidative stress and disruption of phenylalanine and tyrosine metabolism in pregnant women and their offspring. These metabolites may serve as potential biomarkers for predicting adverse

health outcomes related to air pollution exposure.

The findings of this study support the hypothesis that prenatal exposure to air pollution may alter the newborn metabolome and lead to adverse birth outcomes. The high-resolution metabolomics analysis identified several metabolic pathways that were significantly altered by air pollution exposure, including amino acid metabolism, lipid metabolism, and energy metabolism. These metabolic alterations were associated with adverse birth outcomes such as low birth weight and preterm birth. The study also identified several potential biomarkers of air pollution exposure that may be useful for predicting adverse health outcomes in future studies.

The results of this study have implications for public health policies aimed at reducing air pollution exposure during pregnancy. The identification of specific metabolic pathways and biomarkers associated with air pollution exposure may help inform the development of targeted interventions to prevent or mitigate the negative health effects of air pollution on pregnant women and their offspring. Additionally, the findings of this study may contribute to a better understanding of the biological mechanisms underlying the impact of air pollution on pregnancy outcomes.

One of the limitations of this study is the relatively small sample size. Although the study included a diverse population of pregnant women and newborns, a larger sample size would have allowed for more robust statistical analysis and increased the generalizability of the findings. Another limitation is the lack of information on individual exposure levels to air pollution, as exposure was estimated based on the location of the participant's residence. Also, our study only focuses on the African American people, which means that the results of our study may only be applicable to the AA population. If we want to make research conclusions that are more widely applicable to different groups of people, then we need to conduct further research on related issues of different groups of people. Future studies should aim to incorporate individual exposure measurements to improve the accuracy of exposure assessment.

Conclusion

In conclusion, this study investigated the molecular mechanisms underlying the impact of prenatal exposures to air pollution on newborn metabolome and adverse birth outcomes. By using high-resolution metabolomics and statistical analysis, we identified several pathways and metabolites that were significantly associated with prenatal exposure to air pollution, including biopterin metabolism, drug metabolism - cytochrome P450, lysine metabolism, tryptophan metabolism, vitamin B3 (nicotinate and nicotinamide) metabolism, leukotriene metabolism, lipoate metabolism, tyrosine metabolism, limonene and pinene degradation, linoleate metabolism, vitamin B9 (folate) metabolism, vitamin D3 (cholecalciferol) metabolism, vitamin E metabolism, DMABA NHS ester, and 3,4-dimethoxyphenylpropanoic acid.

These findings suggest that prenatal exposure to air pollution may have significant impacts on the metabolome of newborns and contribute to adverse birth outcomes. The identification of specific metabolites and pathways may serve as potential biomarkers for future studies aimed at identifying high-risk populations and developing targeted interventions to mitigate the negative effects of air pollution on maternal and child health.

Tables and Figures

Table1.
Baseline demographic data of the 48 study participants

	Preterm (N = 8)	Early Term (N = 18)	Full Term (N = 22)
Age, years, mean (SD)	24.8 (4.13)	26.2 (4.66)	26.1 (4.59)
Sex of infant			
Male	7 (87.5%)	7 (38.9%)	11 (50.0%)
Female	1 (12.5%)	11 (61.1%)	11 (50.0%)
Highest education			
Less than high school	2 (25.0%)	2 (11.1%)	2 (9.1%)
High school	5 (62.5%)	10 (55.6%)	6 (27.3%)
Some college or more	1 (12.5%)	6 (33.3%)	14 (63.6%)
Marital status			
Married or cohabiting	2 (25.0%)	7 (38.9%)	7 (31.8%)
Not married or cohabiting	6 (75.0%)	11 (61.1%)	15 (68.2%)
Hospital of prenatal care			
Emory	1 (12.5%)	9 (50.0%)	14 (63.6%)
Grady	7 (87.5%)	9 (50.0%)	8 (36.4%)
Prior births			
No prior birth	1 (12.5%)	7 (38.9%)	9 (40.9%)
1 prior birth	2 (25.0%)	7 (38.9%)	6 (27.3%)
2 or more prior births	5 (62.5%)	4 (22.2%)	7 (31.8%)
Occurrence of premature rupture of membranes			
No PROM[a]	8 (100%)	17 (94.4%)	22 (100%)
PROM[a]	0 (0%)	1 (5.6%)	0 (0%)
Presence of intrauterine growth restriction			
No	6 (75.0%)	14 (77.8%)	21 (95.5%)
Yes	2 (25.0%)	4 (22.2%)	1 (4.5%)

[a] premature rupture of membranes

Table2.

Number of significant features for each set of the Metabolomics-Wide Association Study (MWAS) models

Predictor	FDR[a] q < 0.2	RAW p < 0.05
Air Pollutant Exposure		
$PM_{2.5}$[b]	99	813
NO_2[c]	0	297
O_3[d]	1268	1402
Birth Outcome		
PTB	3	545
ETB	0	245

[a] A multiple testing corrected p-value cutoff of 0.2 using the Benjamini-Hochberg method for multiple comparison correction

[b] Fine particulate matter

[c] Nitrogen Dioxide

[d] Trioxygen

Table3.

Chemical identify of metabolites for air pollutant exposure (raw $p < 0.05$).

m / z	RT (s)	Identified Metabolite	$\boldsymbol{\beta_1}$	$\boldsymbol{\beta_2}$	$\boldsymbol{\beta_3}$	Pathways
263.1024	4.45	DMABA NHS ester	0.103	0.002	0.071	-
249.0538	2.74	3,4-dimethoxyphenylpropanoic acid	0.046	0.061	0.062	-
207.1127	9.58	INDOLE-3-METHYL ACETATE	-0.531	-0.078	0.024	Indoles metabolite
173.1052	6.81	N-ACETYLLEUCINE	-0.102	-0.005	-0.008	Amino acid metabolite

Note: Chemical identity of metabolic features was confirmed by matching peaks via accurate mass to charge ratio and retention time to authentic reference standards under the same conditions using tandem mass spectrometry.

Abbreviations: m/z: mass to charge ratio; RT: retention time.

$\boldsymbol{\beta_1, \beta_2, \beta_3}$ refer to beta coefficients of $PM_{2.5}$, NO_2, and O_3

Table4.

Chemical identify of metabolites for adverse birth outcome (raw $p < 0.05$).

m / z	RT (s)	Identified Metabolite	$\boldsymbol{\beta_1}$	$\boldsymbol{\beta_2}$	Pathways
263.1024	4.45	DMABA NHS ester	-0.236	-0.133	-
249.0538	2.74	3,4-dimethoxyphenylpropanoic acid	0.126	0.092	-
373.2734	12.94	Muricholic acid	0.473	-0.225	Bile acid
387.3214	12.86	Tetradecenoyl-L-carnitine	0.978	-0.227	Acylcarnitine

Note: Chemical identity of metabolic features was confirmed by matching peaks via accurate mass to charge ratio and retention time to authentic reference standards under the same conditions using tandem mass spectrometry.

Abbreviations: m/z: mass to charge ratio; RT: retention time.

$\boldsymbol{\beta_1}, \boldsymbol{\beta_2}$ refer to beta coefficients of PTB and ETB

Figure1.

The flow chart of meet-in-the-middle approach in our study.

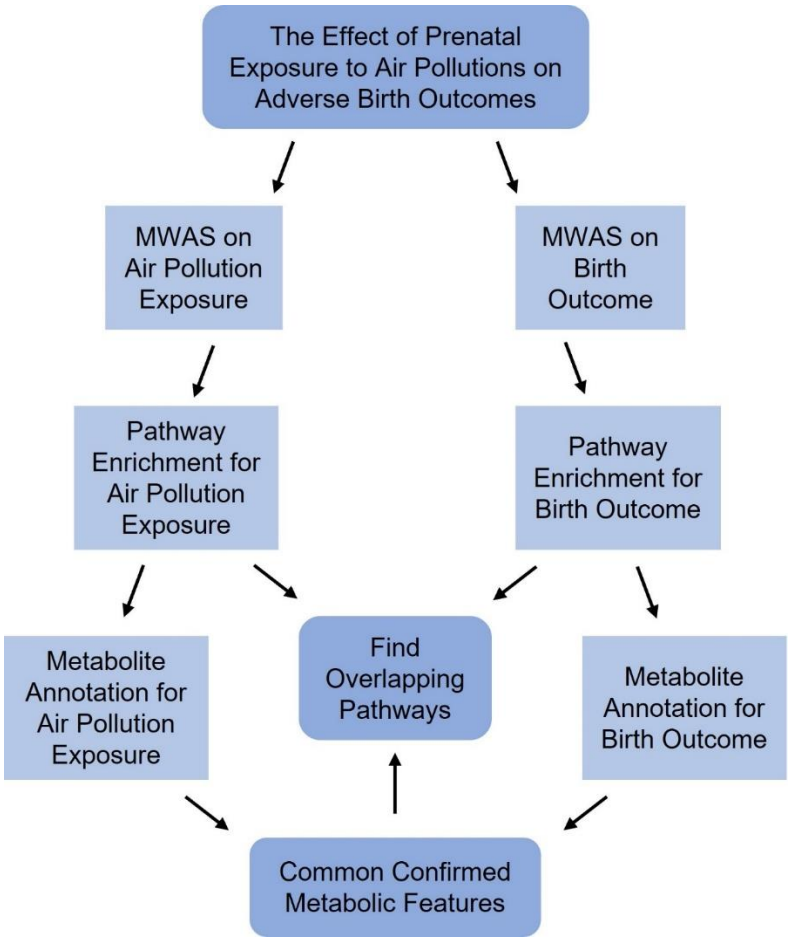
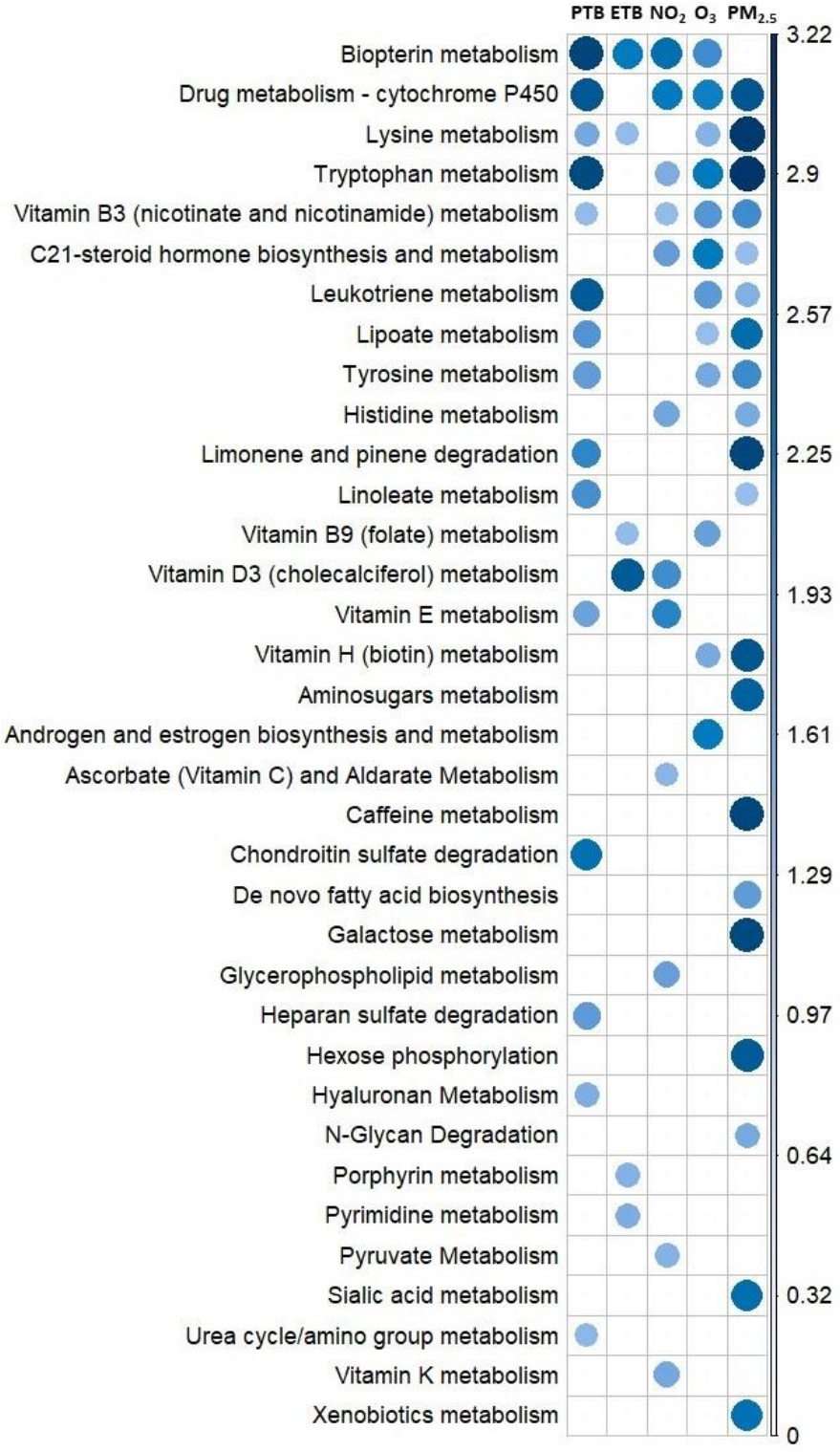


Figure2.

The bubble plot for the association between each metabolic pathway and significant features that were associated with air pollutants and birth outcome.



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