Environment International 149 (2021) 106163

Contents lists available at ScienceDirect

Environment International

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

Maternal exposure to air pollutants, PCSK9 levels, fetal growth and gestational age – An Italian cohort

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ARTICLE INFO

Handling Editor: Shoji Nakayama

Keywords:
Air pollutants
Fetal growth
Gestational age
Particulate matter (PM_{10} and $PM_{2.5}$)
PCSK9

ABSTRACT

Objective: Exposure to airborne pollutants during pregnancy appears to be associated with uterine growth restriction and adverse neonatal outcome. Proprotein convertase subtilisin/kexin type (PCSK9), the key modulator of low-density lipoprotein (LDL) metabolism, increases following particulate matter (PM_{10}) exposure. Because maternal cholesterol is required for fetal growth, PCSK9 levels could be used to evaluate the potential impact of airborne pollutants on fetal growth.
Design: A cohort of 134 healthy women during early pregnancy (11–12 weeks of gestational age) was studied.
Results: A significant association between circulating PCSK9 levels and three tested air pollutants (PM_{10}, $PM_{2.5}$, nitric oxide (NO_2)) was found. Of importance, gestational age at birth was reduced by approximately 1 week for each 100 ng/mL rise in circulating PCSK9 levels, an effect that became more significant at the highest quartile of $PM_{2.5}$ (with a 1.8 week advance in delivery date for every 100 ng/mL rise in circulating PCSK9; p for interaction = 0.026). This finding was supported by an elevation of the odds ratio for urgent cesarean delivery for each 100 ng/mL rise in PCSK9 (2.99, 95% CI, 1.22–6.57), similar trends being obtained for PM_{10} and NO_2.
Conclusions: The association between exposure to air pollutants during pregnancy and elevation in PCSK9 advances our understanding of the unforeseen influences of environmental exposure in terms of pregnancy associated disorders.

1. Introduction

According to the developmental origins of the health and disease hypotheses, exposure during the intrauterine period of life modulates the risk of disease later in life. Low birth weight may be associated to an increased risk of hypertension, diabetes, and cardiovascular disease (Curhan et al., 1996) and it may also be a determinant of gestational age at delivery, with important ethnic differences (Klepac et al., 2018). Therefore, investigations on how environmental exposure, such as air pollution, affects fetal growth and the duration of pregnancy represent a crucial step in defining pathways linking prenatal exposure, intrauterine stress, and future outcomes.

The possible link between exposure to air pollutants, fetal growth and gestational age has been investigated by a growing number of studies (Clemens et al., 2017; Klepac et al., 2018). Air pollutants, in particular fine particulate matter (PM) and nitrogen dioxide (NO_2), mainly contribute towards restricting in utero growth (Aguilera et al., 2010; Carvalho et al., 2016; Hansen et al., 2008; Iniguez et al., 2012; Malmqvist et al., 2017; Ritz et al., 2014; van den Hooven et al., 2012) and adverse neonatal outcomes, such as prematurity and reduced birthweight (Dibben and Clemens 2015; Hjortebjerg et al., 2016; Malley et al., 2017; Pedersen et al., 2013; Rich et al., 2015; Stieb et al., 2016a; Stieb et al., 2016b). Despite some methodological issues, such as the variety of approaches used to determine pollutant exposure, these studies as a whole support the association between increased maternal exposure and reduced fetal growth, potentially influencing gestational

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https://doi.org/10.1016/j.envint.2020.106163
Received 20 March 2020; Received in revised form 24 August 2020; Accepted 23 September 2020
Available online 5 February 2021

age at delivery.

A key requirement of fetal growth is the maternal supply of cholesterol, essential as a structural component of membranes. Cholesterol is obtained endogenously by *de novo* synthesis and exogenously by transfer of maternal cholesterol to the fetus (Burke et al., 2009; Woollett 2005). Since the fetus does not come into direct contact with maternal cholesterol, its uptake is largely mediated by the LDL receptor (LDL-R), expressed abundantly in the placenta but at low levels, if at all, in the yolk sac (Shi et al., 1999). However, how placental endothelial cells transport cholesterol to the fetal microcirculation, regulate efflux, and deliver substantial quantities of cholesterol to the fetal circulation is still not clear (Stefulj et al., 2009).

Proprotein convertase subtilisin/kexin type 9 (PCSK9) has emerged as a major modulator of LDL metabolism, and as a marker of cardiovascular risk (Macchi et al., 2019a). Based on the assessment of plasma interferon levels, we previously reported increased levels of PCSK9 after PM exposure, a particularly significant phenomenon in individuals with a lower inflammatory burden (Macchi et al., 2019b). These individuals represent hypersusceptible subjects, more sensitive to the damaging effects of exposure to environmental PM. Although the relationship between exposure to high levels of a variety of ambient pollutants and cholesterol homeostasis have been ascribed to an altered functionality of high-density lipoproteins or to a rise in low-density lipoproteins (Li et al., 2019), PCSK9 levels in pregnant women might provide important information on the potential use of the currently available PCSK9 monoclonal antibodies in these subjects. Determination of PCSK9 levels is of particular interest since this was initially discovered as an anti-apoptotic mediator in the brain (Adorni et al., 2019) and reduced levels in rat embryos were associated with the occurrence of neural tube defects (An et al., 2015), indicating the sensitivity of this biomarker in pregnancy. Consequently, it was hypothesized that the potential reduction of neuronal inflammation and amyloid β-aggregation may be antagonized by PCSK9 (Apaijai et al., 2019).

The present study was aimed (i) to characterize the association among air pollutants (PM_{10}, $PM_{2.5}$ and NO_2) and PCSK9 levels in a cohort of Italian women during early pregnancy (11–12 weeks of gestational age) and (ii) to evaluate whether changes in PCSK9 levels may affect birth weight and gestational age at birth. Pregnant women are of particular interest since they provide a unique opportunity to evaluate the damage exerted by ambient pollution on both women and their fetuses.

2. Patients and methods

2.1. Study design and participants

We recruited 134 healthy pregnant women at the "Clinica Mangiagalli", Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. They were randomly selected among individuals attending prenatal healthcare clinics during the 11–12th week of pregnancy. Exclusion criteria included a history of illicit drug use, diabetes, hypertension, previous pregnancy with pre-eclampsia/eclampsia or gestational hypertension, and current use of acetylsalicylic acid or low-molecular-weight heparin. Information about demographics and lifestyle characteristics of the mother, such as smoking habits and alcohol consumption, were collected. An informed consent form was signed by all the participants and the study was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (approval number 681/2017).

2.2. Clinical and laboratory measurements

Body weight and height were determined on a standard scale. Body mass index (BMI) was expressed as Kg/m^2. Systolic and diastolic blood pressure (SBP and DBP, respectively) were taken on the left arm using a mercury sphygmomanometer (mean of two measurements taken after 5 min of rest). Plasma lipids/lipoproteins and glucose were measured by certified enzymatic techniques on a Roche c311 autoanalyzer. Lipoprotein (a) [Lp(a)] levels were measured by immunoturbidimetry on a Roche c311 autoanalyzer. Standard evaluations for early pregnancy in Italy are serum pregnancy-associated plasma protein-A (PAPP-A), α-fetoprotein, and β-chain of human chorionic gonadotropin (hCG). We measured these parameters at 11–12 weeks of gestation. They are used as a diagnostic tool for preeclampsia which is an important cause of death and pregnancy complications for mother and newborn. The risk of such complication is considerably higher when the disease is severe and of early onset, leading to preterm birth at less than 37 weeks of gestation (Lisonkova and Joseph 2013). In the absence of clear etiology, the prevalence of preeclampsia can potentially be halved by a strategy of early identification of the high-risk group. Algorithms combining maternal demographic characteristics and medical and obstetric history with biophysical and biochemical markers at 11–13 weeks' gestation have been developed for the prediction of early or late preeclampsia. These are: uterine artery pulsatility index (PI), mean arterial pressure (MAP), PAPP-A, placental growth factor (PLGF) and hCG (Akolekar et al., 2013). These can be grouped into an algorithm, allowing clinicians and researchers to select their own gestational age cutoff to define the high-risk group potentially benefitting from therapeutic interventions starting from the first trimester of pregnancy (Akolekar et al., 2013). Gestational age was calculated from the last menstrual period and was verified by ultrasound parameters. In particular, fetal crown-rump length was used to estimate gestational age, and women were included if this parameter ranged between 45 and 84 mm.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Plasma PCSK9 concentrations were measured by a commercial ELISA kit (R&D Systems, MN). All patients fasted overnight and had blood sampled at around 09:00 a.m., thus minimizing any possible confounding effects of circadian variation in PCSK9 levels. Indeed, serum PCSK9 levels show a diurnal rhythm, with a nadir between 3 and 9 PM and a peak at 4:30 AM (Persson et al., 2009). In brief, samples were diluted 1:20 and incubated onto a microplate pre-coated with a monoclonal human PCSK9-specific antibody. Sample concentrations were obtained by a four-parameter logistic curve-fit, with a minimum detectable PCSK9 concentration of 0.219 ng/mL (Ruscica et al., 2018). Intra- and inter-assay CVs were 3.8% and 6.2%, respectively.

2.4. Air pollutant assessments

Daily air pollutant (PM_{10}, $PM_{2.5}$, and NO_2,) concentrations were derived from the archives of the Regional Environmental Protection Agency (ARPA Lombardy) an organization that collects data at a regional scale using the FARM (Flexible Air quality Regional Model) chemical-physical model of air quality (Silibello et al., 2008) - a three-dimensional Eulerian model that simulates the dispersion and chemical reactions of atmospheric pollutants. The system for the forecast of pollutant concentrations is composed of a meteorological model powered by simulation data, whereas for the initial and boundary conditions the outputs of the "Quale Aria" system are employed (http://doc.aria-net.it/QualeAria/wiki/QualeAria). Emissions are retrieved from regional, national and European inventories. The domain of the simulation with the air quality model FARM covers the territory of Lombardy, with a grid of 1×1 cm^2 cells, provided from the website with daily estimates at municipality resolution. Finally, concentration data measured from the stations of the ARPA air quality network is integrated into the simulation results by means of interpolation techniques (www.arpalombardia.it/Pages/Aria/Modellistica/I-sistemi-modellistici-in-ARPA.aspx).

The estimated levels of daily PM_{10}, $PM_{2.5}$, and NO_2 concentrations were assigned to each subject for the day of evaluation and 14 days before blood was sampled. We also calculated the average exposure from the first week before the clinic visit and 12 weeks earlier (*i.e.*,

weeks 0–1 being the mean over the first week of exposure and weeks 0–12 being the mean over the 12 weeks before the visit). All participants were assigned pollutant levels that were estimated in the Municipality of Milano, as 93% of the women lived or worked there.

2.5. Statistical analysis

Descriptive statistics were performed on all variables. Continuous variables were expressed as the mean ± standard deviation (SD) or as the median with first- and third-quartile (Q1–Q3), as appropriate. Categorical data were reported as frequencies with percentages. Descriptions of each exposure variable were given by the means of boxplots, describing pollutants at each averaged time window. We applied multivariable linear regression models to evaluate the relationship between pollutant exposure (for each averaged one-week period from week 0–1 to week 0–12) and circulating PCSK9 levels. Each model was tested for normality and linearity. All potential confounders were included in the multivariate model after verifying the presence of an association in a univariate model (supplemental Table 1). Best model selection was based on the minimization of the Akaike information criterion and maximization of the explained variance of the model. The final models were adjusted for low-density lipoprotein cholesterol (LDL-C), interleukin (IL)-6, fibrinogen, season, BMI, and smoking habit. Estimated effects are reported as β and standard error (SE) associated with an increase of 1 unit in each pollutant. In order to verify the feasibility of stratifying the analyses for BMI, we tested the interaction between BMI and exposure. The same evaluation has been done in the case of smokers. We examined the association between PCSK9 and the variables measured on the newborn (gestational age at birth, weight, length, cranial circumference, APGAR score), after adjusting each model for the pollutants which best associated with PCSK9 levels in the multivariate analysis. Each model was also adjusted for birth mode (urgent caesarean, elective caesarean, and spontaneous delivery) and for the interaction between pollutant and PCSK9 concentrations. To produce the estimate and the plot, four selected values for exposure have been chosen. These have been incorporated into the equation along with the range of values for PCSK9, at a selected level of delivery mode variable. The linearity of the model with interaction has been tested as a step for defining the best model. By using a univariate logistic regression, we evaluated the odds ratio of urgent cesarean delivery associated with a 100 ng/mL increase in PCSK9.

We calculated the q-FDR values using the multiple comparison method based on Benjamini-Hochberg False Discovery Rate (FDR), which takes the high number of comparisons into account, with a threshold of 0.10 to detect significance (Benjamini and Hochberg 1995). If the association between PM_{10} and PCSK9 is considered, a sample size of 134 pregnant women is sufficient to achieve a 99% power to detect an R-Squared of 0.25 attributed to the independent variable PM_{10} using F-Test with a significance level of 0.05. The variables tested have been adjusted for 6 independent additional variables with an R-Squared of 0.20. Statistical analyses were performed with SAS software, version 9.4.

3. Results

3.1. Study population

The study population included 134 pregnant women (age 33 ± 4 years). BMI was 22.6 ± 4.2 kg/m^2. Pregnancy associated endocrine factors, such as placental growth factor (PLGF) and pregnancy associated plasma protein A (PAPP-A), were in the normal range, as was hCG (Table 1). Signs of diabetes and dyslipidemias were not found. Specifically, median glucose was 86.6 ± 14.8 mg/dL. LDL-C, non-high-density lipoprotein cholesterol (non-HDL-C), HDL-C, and triglycerides (TG) were also within the normal range (97.4 ± 22.1 mg/dL, 117.2 ± 25 mg/dL, 65.5 mg/dL and 99 ± 38.3 mg/dL, respectively). Levels of Lp(a) were

Table 1
Clinical characteristics of the pregnant women and newborns.

Characteristic	Value
Age, year	33.0 ± 4.0
Season of enrollment	
winter	33 (24.6%)
spring	34 (25.4%)
summer	26 (19.4%)
autumn	41 (30.6%)
Anthropometric and biochemical features	
BMI, Kg/m^2	22.6 ± 4.2
Categorical BMI	
Underweight (BMI < 18.5)	16 (11.9%)
Lean (18.5 ≤ BMI < 25)	87 (64.9%)
Overweight (BMI ≥ 25)	31 (23.1%)
Glucose, mg/dL	86.6 ± 14.8
TC, mg/dL	182.7 ± 30.3
LDL-C, mg/dL	97.4 ± 22.1
Lipoprotein (a), mg/dL	13.7 ± 19.0
non-HDL-C, mg/dL	117.2 ± 25.0
HDL, mg/dL	65.5 ± 13.9
TG, mg/dL	99.0 ± 38.3
PCSK9, ng/mL	193.7 ± 54.2
ICAM, pg/mL	349373 ± 66161
VCAM, pg/mL	769251 ± 190723
CRP mg/L	2.56 (1.64, 4.51)
Fibrinogen, mg/dL	146.8 ± 56.1
IL-6, pg/mL	1.7 (1.2, 2.1)
Smoking habits	
Never smoked	101 (75.4%)
Stopped during pregnancy	14 (10.5%)
Smoker	19 (14.2%)
Features related to pregnancy	
Parity	
Nulliparity	84 (62.7%)
Multiparity	50 (37.3%)
Pregnancy associated endocrine factors	
PAPP-A, IU/L	1.3 ± 0.7
PLGF, pg/mL	33.4 ± 13.6
hCG, IU/L	53.7 ± 41.3
PAPP-A MoM	1.2 (0.8, 1.6)
hCG MoM	0.9 (0.6, 1.5)
PLGF MoM	1.2 (0.8, 1.4)
Birth delivery mode	
Spontaneous	80 (70.2%)
Urgent cesarean	16 (14.0%)
Elective cesarean	18 (15.8%)
Gestational age at birth	38.7 ± 1.4
Fetal parameters	
Crown-rump length, mm	62.4 ± 5.2
Nuchal translucency thickness, mm	1.9 ± 0.4
Fetal heart rate, bpm	160.4 ± 6.1
Ductus venosus pulsatility index	1 ± 0.1
Mean blood pressure, mmHg	85.8 ± 7.1
Neonatal parameters	
Weight (gr)	3239.8 ± 467.1
Length (cm)	49.9 ± 2
Cranial circumference (cm)	34.2 ± 1.6
APGAR score	9.8 ± 0.6

BMI, body mass index; CRP, C-reactive protein; hCG, human chorionic gonadotropin; HDL-C, high-density lipoprotein cholesterol; ICAM, intercellular adhesion molecule 1; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; PAPP-A, pregnancy-associated plasma protein-A; PLGF, placental growth factor; PCSK9, proprotein convertase/subtilisin kexin type 9; TC, total cholesterol; TG, triglycerides; VCAM, vascular cell adhesion molecule 1; MoM, multiple of median. For normal distributions, continuous values are expressed as mean ± standard deviation. When not normally distributed, values are expressed as medians (Q1, Q3). Categorical values are expressed as frequencies with percentages.

13.7 ± 19 mg/dL (Table 1). Circulating levels of plasma PCSK9 were normally distributed, with a mean of 193.7 ± 54.2 ng/mL (Fig. 1). These levels were lower than the ones previously described for non-pregnant women recruited in the same geographical area (Ruscica et al., 2017). None of these healthy women was on drug treatment. The estimated levels of exposure to PM_{10}, $PM_{2.5}$, and NO_2, from the first week before the visit (week 0–1) and 12 weeks previously (week 0–12), are depicted in Fig. 2. The similarities in pollutant concentrations across the weeks of exposure were clearly observed. Mean PM_{10} and NO_2 concentrations remained below the annual regional air-quality standards of 40 μg/m^3. Mean $PM_{2.5}$ concentrations were slightly higher than the annual limits set at 25 μg/m^3.

Mean fetal crown-rump length at the time of exposure assessment was 62.4 ± 5.2 mm. Nuchal translucency thickness, an ultrasound marker for chromosomal and structural abnormalities, was within the normal range in all cases, as well as fetal heart rate and ductus venosus blood flow (Table 1). All pregnancies ended with the live birth of a phenotypically normal neonate, at a mean gestational age of 38.7 ± 1.4 weeks. Neonatal biometric parameters are presented in Table 1.

3.2. Association analyses

In order to define the exposure window to air pollutants that was most effective in modifying PCSK9 in multivariate analysis, we investigated how different averaged time lags were associated with PCSK9 levels. A positive significant effect of PM_{10} exposure on PCSK9 was found for all time lags. The effect on PCSK9 levels was maximal, when considering the mean of the lag between conception and blood drawing, *i.e.,* 0–12 weeks (Table 2). In particular, for each 1-μg/m^3 increase in PM_{10} concentration, there was a significant increase in PCSK9 levels ($\beta = 1.903$, SE = 0.733, $p = 0.011$). A similar effect was also found for NO_2 exposure, as every unit increase in NO_2 led to a 2.265 ng/mL rise in PCSK9 levels ($\beta = 2.265$, SE = 1.002, $p = 0.026$). For $PM_{2.5}$ exposure, a significant positive association was only detected for the 0–6 and 0–10-week time lags (Table 2). In order to investigate the potential interaction effect between exposure and BMI or smoking habits, sensitivity analyses have been performed. These were not significant. Moreover, the exclusion of the smoking group did not change results (data not shown).

3.3. Association of PCSK9 concentrations with gestational age for different levels of exposure to pollutants

No associations were found between PCSK9 measured at the end of the first trimester of pregnancy and features of newborns at birth, such as weight and length, crown-rump length, cranial circumference, fetal heart rate and APGAR score (supplemental Table 1). Nevertheless, when the interaction between PCSK9 concentrations and gestational age at birth was taken into account, we observed a strong modifying effect of air pollutants, particularly $PM_{2.5}$. For different $PM_{2.5}$ levels (15 μg/m^3, 24 μg/m^3, 42 μg/m^3, and 55 μg/m^3, respectively; 25th, 50th, 75th, and 95th percentile), the association was significant at the highest $PM_{2.5}$ concentrations (*i.e.,* those in the 75th and 95th percentiles; Fig. 3). For example, at a $PM_{2.5}$ concentration of 42 μg/m^3, an advance in delivery date of approximately 1 week for every 100 ng/mL rise in circulating levels of PCSK9 ($\beta = -0.810$, SE = 0.332, $p = 0.0164$) was observed. The steepness of the association was more evident when the highest $PM_{2.5}$ quartile was considered (*i.e.,* delivery by 1.28 weeks for every 100 ng/mL change in PCSK9 levels; $\beta = -1.282$, SE = 0.498, $p = 0.012$). Overall, at fixed $PM_{2.5}$ concentrations of 42 μg/m^3 and 55 μg/m^3, for every 100 ng/mL increment in PCSK9, the gestational age was anticipated by 6 to 9 days. A similar trend was found when PM_{10} and NO_2 were considered (Table 3). These findings were also supported by the observation that the odds ratio (OR) of urgent cesarean delivery associated with a 100 ng/mL rise in PCSK9 was 2.99 (95% CI 1.22–6.57).

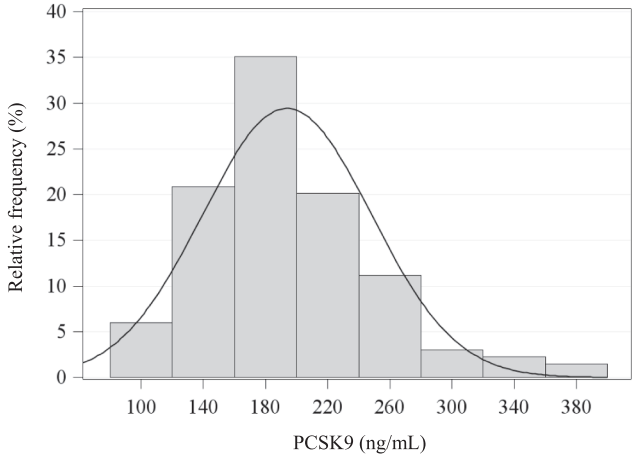

Fig. 1. *Distribution of fasting plasma concentrations of PCSK9 in 134 pregnant women.* Levels of PCSK9 were measured using a sandwich ELISA in plasma samples obtained after women fasted overnight; see Subjects and Methods for details. PCSK9, proprotein convertase subtilisin/kexin type 9.

4. Discussion

The present study is the first one that describes circulating levels of PCSK9 in women during early pregnancy (first trimester; 134 women), demonstrating how air pollutants impact on PCSK9 and how this may be associated with birth weight and gestational age. In this study population, whose mean PCSK9 levels were lower than those previously described in non-pregnant women (Ridker et al., 2016; Ruscica et al., 2017), a definite association between exposure to PM (in particular PM_{10}) and NO_2 and increased levels of PCSK9 was observed. In addition, PCSK9 levels were associated to a lower gestational age at delivery and increased probability of urgent C-sections at delivery. A formal statistical assessment of PCSK9 as a mediator of pollutant effect on features of the newborn is not feasible, as the assumptions needed to identify the indirect and direct effects as causal effects are not satisfied and therefore conclusions from a mediation analysis would lead to invalid results.

Compared to pre-menopausal women, post-menopausal women presented with higher PCSK9 levels and the pharmacological rise of estrogen levels have been shown to lower hepatic and plasma PCSK9 (Lakoski et al., 2009; Ooi et al., 2015; Ruscica et al., 2017). In animal studies, estrogen reduced circulating LDL-C at pharmacological or supraphysiological levels by downregulating liver and plasma PCSK9 levels. Ethinylestradiol (5 mg/kg/die) treatment can reduce PCSK9 mRNA by up to 45% (Persson et al., 2009). In premenopausal women, circulating PCSK9 correlated inversely to estrogens, and PCSK9 was higher (305 ng/mL) in the follicular compared to the ovulatory (234 ng/mL) or the luteal (252 ng/mL) phases (Ghosh et al., 2015). As a possible explanation for this relationship, it seems that estradiol (E2)-associated differences in PCSK9 levels are consequent to differences in clearance rather than production of PCSK9 (Ooi et al., 2015). Thus, values reported in the present report appear to provide results in line with the physiological rise of E2 levels during pregnancy. Maternal E2 levels rise progressively from 367 pM (luteal phase) to between 11,000 and 37,000 pM at the end of pregnancy (Berkane et al., 2017). At approximately nine weeks of gestation, a hormonal ovary-to-placenta shift occurs, resulting in direct E2 placental production (Berkane et al., 2017).

In a study evaluating PCSK9 levels in pregnancies characterized by intrauterine growth restriction (IUGR), lipid/lipoprotein levels being available for 70 patients *vs* 102 controls during late gestation, the immunological expression of PCSK9 could be detected in the placenta, as well as in fetal and maternal plasma (Pecks et al., 2016). PCSK9 levels were in a higher range compared to those reported in the present study. PCSK9 levels were also evaluated in a study with a small sample size comparing normal pregnant women (n = 6), diabetics (n = 6), and

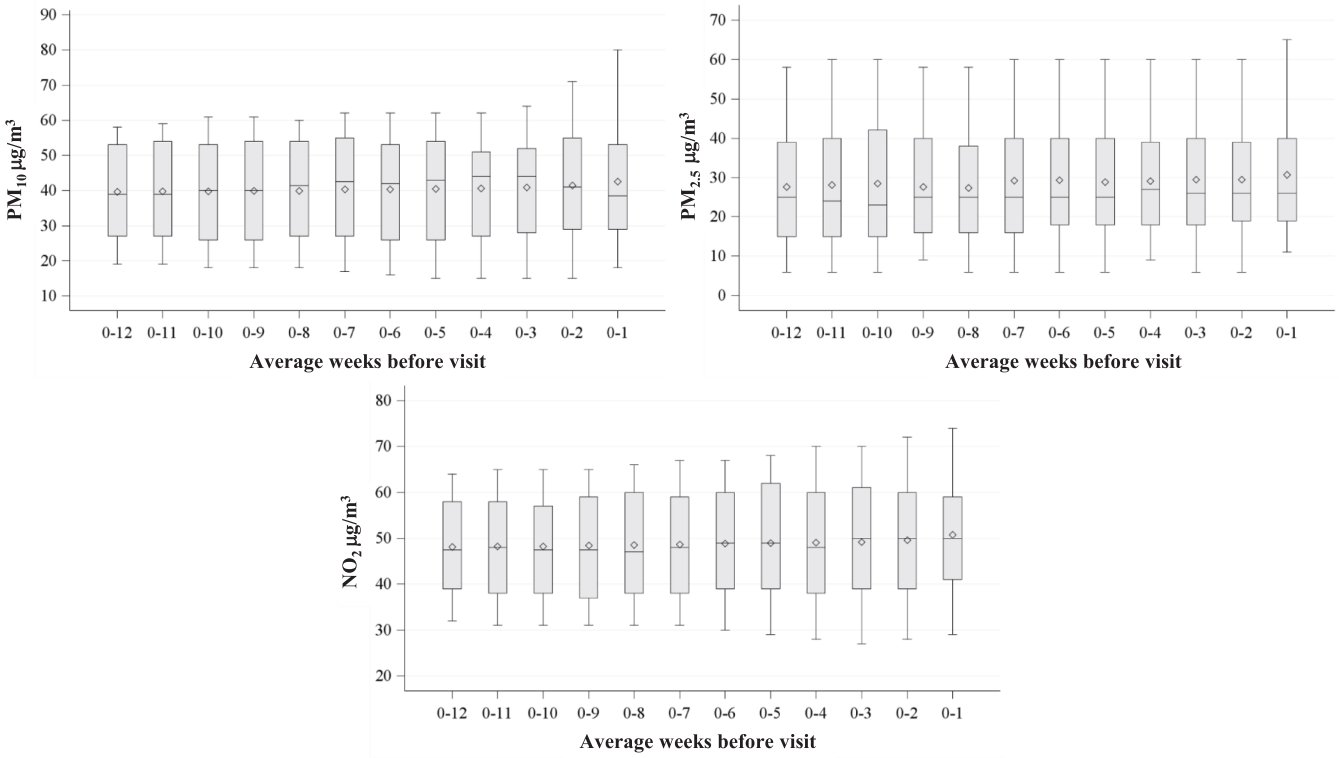

Fig. 2. *Box plot showing exposure to PM_{10}, $PM_{2.5}$, and NO_2.* Exposure was evaluated as the mean from one week before the visit (week 0–1) and 12 weeks earlier (week 0–12). PM_{10}, $PM_{2.5}$, and NO_2 were averaged over mean daily concentrations. PM, particulate matter; NO_2, nitrogen dioxide.

Table 2
Multivariate analyses reporting the associations among exposures to PM_{10}, $PM_{2.5}$, NO_2, and PCSK9 levels.

Average exposure	PM_{10}				$PM_{2.5}$				NO_2			
	β	SE	P-value	R^2	β	SE	P-value	R^2	β	SE	P-value	R^2
0–1 week	0.398	0.481	0.410	0.20	0.361	0.503	0.474	0.20	0.543	0.801	0.500	0.20
0–2 weeks	1.253	0.581	**0.034**	0.24	0.978	0.513	0.060	0.24	1.909	0.898	**0.036**	0.26
0–3 weeks	1.892	0.639	**0.004**	0.27	0.920	0.469	0.053	0.23	2.050	0.868	**0.020**	0.26
0–4 weeks	1.766	0.710	**0.015**	0.25	0.217	0.562	0.700	0.19	2.173	0.962	**0.026**	0.25
0–5 weeks	1.585	0.700	**0.026**	0.24	0.467	0.508	0.360	0.22	2.206	0.979	**0.027**	0.25
0–6 weeks	1.609	0.636	**0.013**	0.25	1.016	0.390	**0.011**	0.29	2.221	0.888	**0.014**	0.27
0–7 weeks	1.554	0.633	**0.016**	0.25	0.523	0.449	0.248	0.20	2.176	0.854	**0.013**	0.27
0–8 weeks	1.490	0.668	**0.028**	0.24	−0.157	0.524	0.765	0.19	2.038	0.868	**0.021**	0.26
0–9 weeks	1.512	0.675	**0.028**	0.24	0.584	0.528	0.271	0.23	2.022	0.896	**0.026**	0.26
0–10 weeks	1.695	0.672	**0.013**	0.25	1.156	0.492	**0.021**	0.24	2.197	0.926	**0.020**	0.26
0–11 weeks	1.857	0.695	**0.009**	0.26	0.866	0.482	0.076	0.22	2.333	0.943	**0.015**	0.27
0–12 weeks	1.903	0.733	**0.011**	0.25	−0.088	0.599	0.884	0.19	2.265	1.002	**0.026**	0.26

Exposure is evaluated as the average from the first week before the visit (0–1 weeks) and 12 weeks earlier (0–12). Significant P-values ($P \leq 0.05$) are reported in bold when the p-FDR is < 0.10. β regression coefficients are reported for 1 µg/m^3 increase in the concentration of each pollutant. Estimates were calculated from multivariate models adjusted for low-density lipoprotein cholesterol, interleukin-6, fibrinogen, season, body mass index, and smoking habits. PCSK9, proprotein convertase subtilisin/kexin type 9; PM, particulate matter; NO_2, nitrogen dioxide; SE, standard error. R^2 defines the proportion of the total variance explained by the model.

overweight/obese women that had diabetes (n = 10) at term (Dube et al., 2013). The last two groups had significantly reduced PCSK9 levels, with raised LDL-receptor activity and reduced LDL-C levels. The authors attributed these findings to the maternal inflammatory status with raised placental cytokines, as widely demonstrated in diabetic pregnancies (Heitritter et al., 2005). Dyslipidemia early in pregnancy is independently associated with an increased risk of pre-term birth (Adank et al., 2019). Recently, the ABCD (Amsterdam Born Children and Their Development) study showed that atherogenic lipid profiles during the first trimester confer an increased risk of adverse pregnancy outcomes, including maternal morbidity, mortality, and preterm delivery. Thus, considering that none of our child-bearing women was dyslipidemic, the present report presents a novel finding on the association between plasma PCKS9 elevation and time of delivery. Overall, time of delivery was estimated to be anticipated by between 0.8 and 1.8 weeks for every 100 ng/mL increment in PCSK9 levels. Moreover, the preliminary observation that the risk of experiencing an urgent cesarean delivery is significantly higher (OR = 2.99) in women with higher PCSK9 levels suggests a general condition not perceived during pregnancy, but related to peripartum risk. Notwithstanding the low number of subjects on which this observation is based, a possible explanation may be related to the pleiotropic pro-inflammatory effect driven by PCSK9. Our group has extensively reported of a feed-forward-loop between PCSK9 and inflammation (Ricci et al., 2018; Ruscica et al., 2016). In a gene-expression analysis of pro-inflammatory cytokine levels in the placenta among delivery modes (vaginal deliveries, caesarean delivery

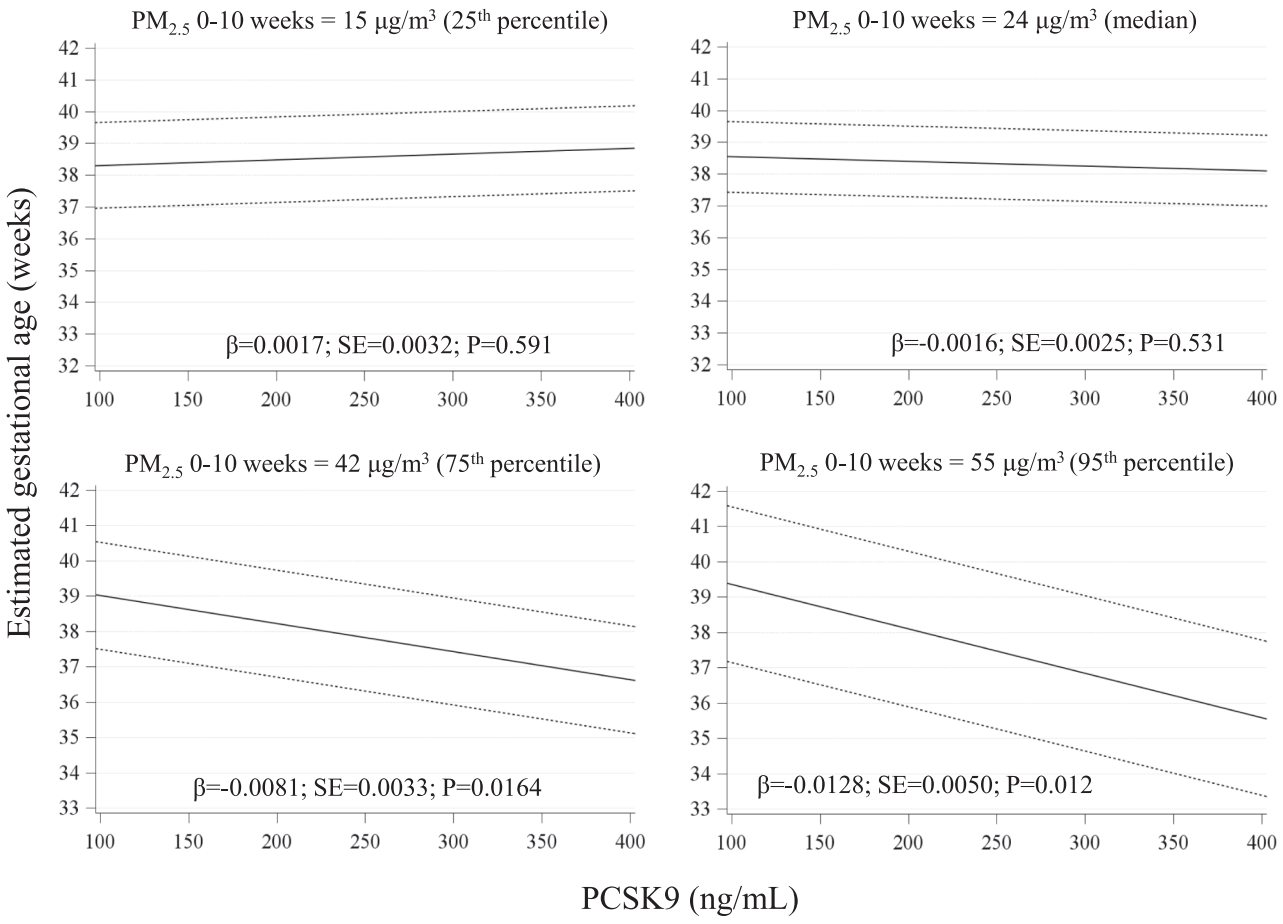

Fig. 3. *Interaction effect of $PM_{2.5}$ and PCSK9 levels on gestational age*. Strength of association between PCSK9 and gestational age at four selected levels of $PM_{2.5}$ (25^{th}, 50^{th}, 75^{th}, and 95^{th} percentiles). Estimates were calculated from multivariate models adjusted for birth mode (spontaneous, urgent, and elective cesarean) and interaction between PCSK9 and $PM_{2.5}$ at 0–10 weeks; the P-value for the interaction term was = 0.0258. To produce the plot, the levels of $PM_{2.5}$ were included into the equation along with the range of values for PCSK9, at a selected level of delivery mode variable (*i.e.* spontaneous). Adjusted β regression coefficients are reported for 1 μg/m^3 increase in PCSK9 concentration. PCSK9, proprotein convertase subtilisin/kexin type 9; PM, particulate matter.

Table 3
Association between PCSK9 and gestational age at selected values of $PM_{2.5}$, PM_{10}, and NO_2.

Dependent variable	Independent variable	Interaction term (p-value)	Pollutant cut off for β_{PCSK9} estimate	$\beta_{x1\ ng/mL\ PCSK9}$ (SE)	% change for 100 ng/mL PCSK9 (SE)		P-value
PCSK9	Gestational age	PCSK9*$PM_{2.5}$ (P = 0.0258)	15 μg/m^3	0.0017 (0.0032)	0.5%	(0.9%)	0.591
			24 μg/m^3	−0.0015 (0.0025)	−0.4%	(0.7%)	0.531
			42 μg/m^3	−0.0081 (0.0033)	−2.3%	(0.9%)	**0.016**
			55 μg/m^3	−0.0128 (0.0050)	−3.7%	(1.4%)	**0.012**
PCSK9	Gestational age	PCSK9*PM_{10} (P = 0.0051)	25 μg/m^3	0.0037 (0.0033)	1.1%	(0.9%)	0.266
			50 μg/m^3	−0.0030 (0.0024)	−0.9%	(0.7%)	0.212
			75 μg/m^3	−0.0102 (0.0034)	−2.9%	(1.0%)	**0.004**
			95 μg/m^3	−0.0117 (0.0038)	−3.3%	(1.1%)	**0.003**
PCSK9	Gestational age	PCSK9*NO_2 (P = 0.0031)	38 μg/m^3	0.0034 (0.0032)	1.0%	(0.9%)	0.291
			49 μg/m^3	−0.0036 (0.0024)	−1.0%	(0.7%)	0.131
			58 μg/m^3	−0.0093 (0.0031)	−2.7%	(0.9%)	**0.004**
			64 μg/m^3	−0.0131 (0.0041)	−3.7%	(1.2%)	**0.002**

Multivariable models adjusted for birth mode, PCSK9, pollutant concentration, and the interaction term PCSK9*pollutant concentration. Each model was run separately with one interaction term. The cut-offs selected for each pollutant were: 25^{th} percentile, median, 75^{th} percentile and 95^{th} percentile. PCSK9, proprotein convertase subtilisin/kexin type 9; SE, standard error. Significant P-values ($P \leq 0.05$) are reported in bold.

with medical indications, caesarean delivery on maternal request and urgent cesarean delivery), the highest levels were found in women undergoing urgent cesarean delivery (Hu et al., 2017).

The lack of an association between PCSK9 levels and newborn features (*e.g.,* crown-rump length, nuchal translucency, fetal heart rate, and ductus venosus pulsatility index) might be of interest since PCSK9 was previously described to regulate LDL-C metabolism at least in the fetal compartment (Pecks et al., 2016). Moreover, PCSK9, initially identified as a factor implicated in the differentiation of cortical neurons (Seidah et al., 2003), has been recently described as a possible modulator of brain cholesterol homeostasis (Adorni et al., 2019).

Cardiovascular (CV) risk factors in pregnancy have been evaluated by many investigators, indicating that a large number of pregnancies are associated with preexisting CV risk (Adank et al., 2019; Benschop et al., 2019a; Hauspurg et al., 2019; Lim and Mahmood 2015). Thus, in addition to evaluating lipoprotein cholesterol concentrations, it was of interest to evaluate a key regulator of cholesterolemia (*i.e.,* PCSK9 levels) in the current study. This protein fosters the catabolism of the LDL receptor, thus increasing cholesterolemia. The highly significant correlation between PCSK9 levels and LDL-cholesterolemia is relevant since lipid levels during early pregnancy could be used to identify women at risk for future cardiovascular diseases (CVD) (Adank et al., 2019). Indeed, complications occurring during pregnancy are indicative of future increased risk of atherosclerotic disease (Jasper and Skelding 2018). Epidemiological data consistently show an early onset of CVD in women who experienced pregnancy loss, preterm pregnancy, or pregnancy complicated by intrauterine growth restrictions (Benschop et al., 2019). Based on these observations, the American Heart Association recognizes pregnancy complications as independent risk factors for future CVD (Benschop et al., 2019).

PM inhalation is an established trigger of CV events (Cicoira 2018). Such events may occur within hours or days of exposure. Short-term exposure to PM pollution contributes to acute CV morbidity and mortality and a long-term exposure to elevated PM levels is associated with reduced life expectancy (Brook et al., 2004). A previous report on an obese population showed that 12- and 6-month exposure to PM_{10} is associated with a significant rise in circulating PCSK9 levels, positively associated with the Framingham Risk Score (Macchi et al., 2019b). However, the association between PCSK9 levels and coronary risk has provided contrasting findings (Leander et al., 2016; Macchi et al., 2019b; Ridker et al., 2016). The results of the current study on pregnant women support the finding that ambient pollutants (*i.e.* PM_{10}, $PM_{2.5}$, and NO_2) are associated with raised PCSK9 levels. Overall, some of the differences we have found may rely on the fact that in the region of Lombardy PM_{10} is mainly constituted by fine particles, and $PM_{2.5}$ represents 58–94% of the PM_{10} (Bigi and Ghermandi 2014). Therefore, it is possible that PM_{10} measures, being more accurate than $PM_{2.5}$, perform better in catching the effects of ambient exposure on PCSK9. NO_2 exposure is also well characterized in Lombardy and the main source is the vehicular traffic (Krzyzanowski, 2005) accounting for the large majority of emissions of this pollutant (around 70% in the studied area). Consequently, the current study provides a further insight into the potential association of CV risk variables with pollutants. A meta-analysis of 21.09 million participants showed that each 10 $\mu g/m^3$ rise in $PM_{2.5}$ corresponds to an increased relative risk (RR) of total CVD events (RR 1.12, 95% CI 1.05–1.19), CVD incidence (RR 1.12, 95% CI 1.05–1.19), and CVD mortality (RR 1.11, 95% CI 1.08–1.14). A more robust association was obtained with NO_2, whereby every 10 $\mu g/m^3$ increase in NO_2 led to a higher risk of total CVD events (RR 1.36, 95% CI 1.09–1.64) and CVD mortality (RR 1.46, 95% CI 1.13–1.79) (Yang et al., 2019).

Finally, the following limitations have to be acknowledged: 1) the lack of serial measurements of PCSK9 during pregnancy; 2) the need to confirm this evidence in a larger number of pregnant women, in particular to support the data showing an association between PCSK9 levels and the occurrence of an urgent C-section; 3) the application of distributed lag models, since our results were based on average exposure to each pollutant. A larger study population could help clarifying how the events are distributed over the time period with models suitable to investigate the role of single lag exposure such as distributed lag models.

5. Conclusions

This study demonstrated that, in healthy pregnant women, PCSK9 levels are associated to the outcomes of pregnancy occurring at delivery (approximately 28–30 weeks after PCSK9 was quantified). These findings are of special interest because they suggest that PCSK9 regulation is modified early (possibly induced by air pollutants), with potential consequences on the entire pregnancy. Therefore, results of this study advance current knowledge on how PCSK9 contributes to pregnancy, and how it is associated with a number of variables potentially linked to pregnancy diseases.

Declaration and verification

The work described has not been published previously nor under consideration for publication elsewhere.

Contribution to authorship

VB, MR, NP conceived the study and wrote the manuscript; CM measured PCSK9 and all the biochemical variables and wrote the manuscript; SI performed all the statistical analyses; AC and CRS critically edited the manuscript; LF, LC, BI, MFG, ED collected all the data and helped in biochemical analyses.

Details of ethics approval

Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (approval number 681/2017).

Fundings

Cariplo Foundation (2015-0552 and 2018-0511 to MR) and Grants from MIUR Progetto Eccellenza (to AC) and Fondazione Carlo Sirtori (CRS).

Declaration of Competing Interest

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

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106163.

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