Pediatric RESEARCH

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The relationship between *MMP9* and *ADRA2A* gene polymorphisms and mothers–newborns' nutritional status: an exploratory path model (STROBE compliant article)

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BACKGROUND: The aim of this study was to evaluate the direct effects of matrix metalloproteinase (*MMP9* rs17577, *MMP9* rs17576) and alfa 2 adrenergic receptor (*ADRA2A* rs553668) gene polymorphisms investigated in mothers and their newborns on maternal weight gain (MWG) during pregnancy and the newborn's birth weight (BW), taking into account the presence of other related factors.

METHODS: We performed a cross-sectional study in 197 mother–newborn pairs in an Obstetrics Gynecology Clinic, in order to evaluate the demographic and anthropometric parameters, and gene polymorphism.

RESULTS: BW was positively correlated with maternal age ($p = 0.021$) and the educational level ($p = 0.002$), and negatively correlated with smoking status in pregnant women ($p < 0.001$). The *MMP9* rs17577 variant genotypes in mothers led to a lower BW ($p = 0.049$). The mothers with a variant genotype of *ADRA2A* rs553668 gene polymorphism had newborns with a higher BW ($p = 0.030$). MWG and gestational age (GesAge) influenced BW ($p < 0.05$). We noticed that newborns' variant genotype of *MMP9* rs17577 was related to a significant increase in BW ($p = 0.010$), while the newborns who carried the variant genotype of *MMP9* rs17576 expressed a negative correlation, decreasing the BW ($p = 0.032$).

CONCLUSION: Our study emphasizes the role of *MMP9* rs17577, *MMP9* rs17576, and *ADRA2A* rs553668 SNPs in BW determinism.

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INTRODUCTION

Childhood obesity is a challenge for the pediatrician due to its continuously increasing incidence, being a real public health problem, a new flagellum of the twenty-first century.[1] World Health Organization (WHO) underlined that the incidence of overweight/obesity in children is increasing, reaching alarming rates of over 40 million children, especially in developing countries.[1] Childhood obesity results in complications during adulthood, such as metabolic disorders, type 2 diabetes mellitus (DM), nonalcoholic fatty liver disease, cardiovascular diseases, arterial hypertension (AHT), and atherosclerosis, and also in social insertion problems.[2,3] Precision nutrition is a pattern of a more comprehensive and dynamic nutrition designed for the treatment and prevention of metabolic disorders associated with obesity. It is also based on the interaction with environmental and external factors. This nutritional method also includes genetic factors together with food habits, physical activity, microbiota, and metabolic factors.[4] Nevertheless, communication is an essential skill for every physician in order to determine the children and their parents to acknowledge the problem and to help them obtain the best outcome.[5]

The studies from the literature underline the fact that birth weight (BW) is influenced by gestational weight gain (GWG), pre-pregnancy body mass index (BMI), gestational diabetes, and postprandial blood glucose level, and also by the type of delivery, educational degree, smoking status, and so on.[6–9]

It was stated that obesity is accompanied by the impairment of adipogenesis, angiogenesis, and extracellular matrix remodelation.[2] The matrix metalloproteinase (*MMP*) together with plasminogen results in tissue remodeling by the destruction of the extracellular matrix.[10] *MMPs* are endopeptidases that degrade the extracellular matrix, and are zinc- and calcium-dependent. They are divided into collagenases (*MMP1*, *MMP8*, and *MMP13*), gelatinases (*MMP2* and *MMP9*), stromelysins (*MMP3*, *MMP10*, and *MMP12*), matrilysin (*MMP7*), and membrane-type MMPs.[11] *MMPs* play a role in angiogenesis, embryogenesis, inflammation, healing, and remodeling of the normal tissue.[12] In normal pregnancies, the invasion of extravillous trophoblast is essential for the transformation of maternal spiral arteries into vessels with low resistance and high capacitance, thereby providing a high amount of maternal blood for the proper development of the fetoplacental unit.[13] Thus, it seems that a high secretion of *MMP9* by decidual cells may impair this invasion.[14] An increased serum level of *MMP9* was associated with adverse pregnancy outcomes, such as intrauterine growth restriction, preeclampsia, and spontaneously early delivery.[15,16] Different studies revealed the association between certain

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MMP9 gene polymorphisms and spontaneous preterm birth,[17,18] suggesting that the alteration of gene expression might represent a risk factor for low BW or preterm delivery.[19] Nevertheless, the data from the literature regarding the role of *MMP9* gene polymorphisms in low BW genetic background are scarce. This fact further augments the value of our study. *MMP9* is a gelatinase with enzymatic activity against denaturized collagen, type IV and V, and also elastin.[20] *MMP9* is involved in the inflammatory processes associated with tumor metastases, obesity, insulin resistance, cardiac diseases, arthritis, and atherosclerosis.[11,12] *MMP9* inhibition interacts with the migration of macrophages within inflammatory conditions, while in obesity, its values are increased due to adipose tissue remodeling.[11] On the other hand, Andrade et al.[21] proved on a group of 32 obese children that plasma *MMP9* and *MMP9*/tissue inhibitor of metalloproteinase-1 (TIMP-1 ratio) is higher in obese children compared with controls, presenting a higher risk of developing atherosclerosis.[21] Nevertheless, *MMP9* proved to be inversely related to BW, which may be due to increases in gelatinase circulating levels, implying a loss of the control inhibitory effect of this gene.[15]

On the other hand, the activation of alfa 2 adrenergic receptors (ADRA2A) in adipocytes inhibits adenylate cyclase and the production of cyclic adenosine monophosphate (cAMP), while the stimulation of beta-1, -2, and -3 adrenergic ones presents reverse effects. Intracellular cAMP from adipocytes controls the protein kinases, modifying the activity of hormone-sensitive lipase, and therefore the lipolysis process.[22,23] Human fat cells are rich in b1- and b2-adrenergic receptors with a lipolytic role, whereas ADRA2A counterbalances this lipolytic effect.[24] Therefore, the blockage of human ADRA2A in vivo leads to lipolysis improvement.[23,25] Moreover, another study showed that the antilipolytic action of *ADRA2A* might be depleted in case of a mutation in the coding region.[23]

ADRA2A is involved in the inhibition of insulin secretion and lipolysis. Certain *ADRA2A* single-nucleotide gene polymorphisms (SNPs) like rs553668 and rs521674 are associated with obesity and type 2 DM. Moreover, genome-wide association studies proved that different SNPs of this gene are related to increased glucose levels at birth.[26] Based on all these facts, it is very clear that relevant defects in the *ADRA2A* gene, along with other genetic and environmental conditions, might present a dichotomous role, increasing the susceptibility of an individual to the development of obesity or underweight.[23] Even though the data in the literature assessing the effect of *ADRA2A* gene polymorphisms on BW are missing, according to the above-mentioned statements, it is reasonable to hypothesize that the presence of these variants in pregnant women might also have an impact on fetal weight, resulting in increased or low BW. Our study might be considered a pilot one due to the fact that *ADRA2A* gene polymorphisms were assessed in both mothers and newborns.

The aim of the present study was to evaluate the relationship between multiple gene polymorphisms (*MMP9* rs17577, *MMP9* rs17576, and *ADRA2A* rs553668) analyzed in mother–newborn pairs, and both maternal weight gain (MWG) during pregnancy and the newborn's BW, taking into account the presence of other related factors.

MATERIALS AND METHODS

Study sample

We performed a cross-sectional study in a group of 197 mothers and their newborns in an Obstetrics Gynecology Clinic from Romania, between March 2017 and June 2017.

The inclusion criteria were single pregnancy, maternal age (Mage) above 18 years, and the exclusion criteria were newborns' malformations, intrauterine infections, chronic diseases, intrauterine growth retardation, incomplete evaluations of the subjects, and lack of informed consent.

All mothers signed the informed consent for them and their newborns. Our study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Târgu Mureș (No. 26/2017), and it was accepted according to the principles of the Declaration of Helsinki.

Endogenous variables

BMI was assessed in all mothers and newborns. Pre-pregnancy BMI was defined as the ratio between the weight (W) at the beginning of the pregnancy and squared maternal height, being expressed in kg/m^2, and according to the Control Disease Center (CDC), we considered obese women when BMI was higher than 30.0 kg/m^2 and overweight when BMI was between 25.0 and 29.9 kg/m^2. MWG was calculated as the difference between the initial W and BW, gestational age (GesAge)—number of weeks and newborn's weight were considered as dependent variables in the data analysis. All the variables mentioned above were considered as continuous quantitative variables in the data analysis.

Exogenous variables

The social and demographic characteristics, including the maternal age, educational level, smoking status, obstetrical characteristics (parity, type of birth), and genetic polymorphisms in the mother and newborn were considered independent variables in the data analysis.

Genotyping analysis

DNA was isolated from 200 µl of fresh blood collected from newborns included in patient and control groups and from their mothers. Genotyping analyses of *MMP9* rs17577, *MMP9* rs17576, and *ADRA2A* rs553668 was performed by using TaqMan SNP Genotyping assays (assay ID: C__11655948_1; C__11655953_10, and C____996424_20) from ThermoFisher Scientific, according to the instructions recommended by the manufacturer. All the genotyping analyses were performed on a fast real-time machine, namely 7500 Fast Dx Real-Time PCR Instrument and MicroAmp Fast Optical 96-Well Reaction Plate from Applied Biosystems were used.

Statistical analysis

The newborns' and mothers' characteristics were presented as mean ± standard deviation or percentages. The univariate normality was tested by the calculation of univariate kurtosis and skewness coefficient and the associated 95% interval.

The assessment of the differences related to maternal and newborn's W gain reported to genetic polymorphisms and socio-demographic characteristics was achieved through parametric tests like Student's *t* test or univariate analysis of variance (ANOVA) (Brown–Forsythe test, respectively, as a robust test for variance inequality on groups).

Pearson's and Spearman's correlation coefficients and the associated significance tests were used to test the correlations between quantitative variables.

Structural equation modeling (SEM) (in the form of path analysis) was used to test the relationships between studied gene polymorphisms, maternal pre-pregnancy BMI, MWG during pregnancy, and BW. This modeling allowed both the investigation of simultaneously multiple dependence relationships and estimation of the error of measurement. We tested an a priori defined path model containing the above-mentioned exogenous and endogenous variables (Fig. 1). Because we had a moderate deviation from normality for GesAge, the estimation method was robust maximum likelihood. The goodness of fit for the tested model was established by the following indices:[27] (i) χ^2 test with an estimated significance level $p \geq 0.05$, (ii) $\chi^2/df < 2$, (iii) robust root mean square error of approximation (robust RMSEA) <0.05 and an upper limit of the 90% confidence interval (CI) for robust RMSEA <0.08, (iii) robust comparative fit index (robust CFI) and

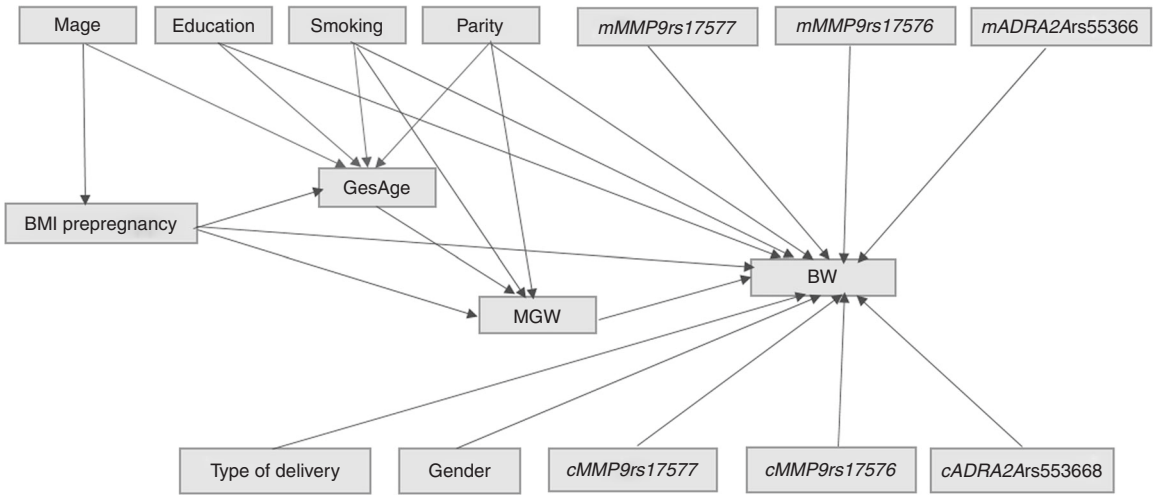

Fig. 1 The simultaneous relationships between studied gene polymorphisms, maternal and fetal characteristics, and BW. Mage = maternal age; MWG = maternal weight gain; GesAge = gestational age; BW = birth weight; *cMMP9* rs17576: matrix metalloproteinase 9 rs17576 in newborns, *mMMP9* rs17576: matrix metalloproteinase 9 rs17576 in mothers; *cMMP9* rs17577: matrix metalloproteinase 9 rs17577 in newborns, *mMMP9* rs17577: matrix metalloproteinase 9 rs17577 in mothers; *cADRA2A* rs553668: alfa 2 adrenergic receptor rs553668 in newborns, *mADRA2A* rs553668: alfa 2 adrenergic receptor rs553668 in mothers

robust Tucker–Lewis Index (robust TLI) with values ≥0.90, and (iv) standardized root mean square residual (SRMR) with a value lower than 0.10.

Direct effects in path analysis represented partial regression coefficients for each exogenous on the endogenous variable. A direct effect was considered to be significant if $p < 0.05$. The direct effects were presented in a standardized form. The modification index (MI) was also calculated in order to obtain suggestions for model modification. Values >10 are considered worthy of analyzing from a theoretical plausibility point of view.

The alfa (α) significance level was set to 0.05 in all statistical analysis. The software used in all statistical analysis was the R program, version 3.5.1, and for SEM modeling lavaan R package was used.

RESULTS

Demographic characteristics of the newborns and their mothers

In the present study, a number of 197 newborns were included, with a median BW of 3315.2 g (Table 1), of which 110 (55.8%) were males. There was a significant difference between the means of male newborns' W in comparison with those of the female newborns ($p = 0.027$), noticing an overall higher BW in male newborns (3387.4 ± 521.6 g versus 3223.8 ± 502.1 g). A number of 179 (90.9%) newborns were born at term, 12 (6.1%) were preterm, and 6 (3.0%) were delivered after term.

BW was positively correlated with maternal age ($r = 0.16$, $p = 0.021$), and also with the educational level (ANOVA test, $F(3,193) = 5.12$, $p = 0.002$); the post-test analysis using Tukey's test indicated a higher BW in newborns whose mothers had between 9 and 12 years of education or above 12 years in comparison with the mothers with no education (9–12 years of education: 3342.6 ± 559.1 g; >12 years of education: 3416.9 ± 483.6 g versus 2876.7 ± 330.3 g for no studies). Also, there were differences with a tendency toward statistical significance ($p = 0.086$) regarding newborns' W whose mothers had over 12 years of education in comparison with those whose mothers had under 8 years of studies (mean ± standard deviation: 3416.9 ± 483.6 g versus 3194.9 ± 503.2 g).

There was a significant difference between the means of newborns' BW whose mothers were smokers ($p < 0.001$), noticing overall a lower BW for these newborns, compared with those whose mothers were nonsmokers (2977.3 ± 341.3 g versus 3357.6 ± 521.7 g).

We did not find a significant association between BW and vaginal or cesarean section delivery ($p = 0.528$), the mean for BW

Table 1. Characteristics of newborns and their mothers in the studied sample

Characteristics	Mean ± SD or number of cases (%)
Newborn	
BW (g)	3315.2 ± 518.2
GesAge (weeks)	38.9 ± 1.7
BL (cm)	53.5 ± 2.9
Male (%)	110 (55.8)
Preterm birth (%)	12 (6.1)
Mothers	
W pre-pregancy (kg)	62.2 ± 12.5
W at delivery (kg)	79.1 ± 13.5
H (m)	1.6 ± 0.07
BMI pre-pregancy (kg/m^2)	23.2 ± 4.4
Maternal age (years)	28.0 ± 5.9
Educational level (%)	
No education	12 (6.1)
≤8 years	43 (21.8)
9–12 years	54 (27.4)
>12 years	88 (44.7)
Primiparous	109 (55.3)
MWG (kg)	16.9 ± 6.2
Cesarean delivery (%)	56 (28.4)
Smoking (%)	22 (11.2)
Gestational diabetes (%)	6 (3.0)
AHT during pregnancy, eclampsia (%)	12 (6.1)

AHT arterial hypertension, *BMI* body mass index, *BL* birth length, *BW* birth weight, *GesAge* gestational age, *MWG* maternal weight gain, *H* height, *SD* standard deviation, *W* weight

Table 2. Matrix of Pearson's and Spearman's correlation coefficients between maternal and neonatal anthropometric characteristics

	Mother's age (years)	BMI pre-pregnancy (kg/m^2)	MWG (kg)	GesAge (weeks)	BW (g)
Mother's age (years)	1.00	0.23 (0.001[§])	−0.04 (0.623)	0.10 (0.158)	0.16 (0.030[§])
BMI pre-pregnancy (kg/m^2)	0.22 (0.002[§])	1.00	−0.06 (0.413)	0.01 (0.921)	0.22 (0.002[§])
MWG (kg)	−0.03 (0.657)	−0.11 (0.118)	1.00	0.23 (0.001[§])	0.28 (<0.001[§])
GesAge (weeks)	0.09 (0.228)	0.04 (0.613)	0.22 (0.002)	1.00	0.46 (<0.001[§])
BW (g)	0.16 (0.021[§])	0.16 (0.028[§])	0.20 (0.005[§])	0.55 (<0.001[§])	1.00

BMI body mass index, *BW* birth weight, *GesAge* gestational age, *MWG* maternal weight gain; Pearson's correlation coefficients (lower diagonal) and Spearman's correlation coefficients (upper diagonal); significant correlations ($p < 0.05$) were denoted by [§] symbol

of newborns delivered by cesarean section being similar to that of newborns delivered vaginally (3352.2 ± 556.9 g versus 3300.4 ± 503.3 g). Similar results were obtained regarding the association with parity, with BW distribution being similar for unipara and multipara mothers (3306.7 ± 545.1 g versus 3325.6 ± 485.6 g).

Maternal parameters and their role in BW determination
Regarding the mothers, we noticed a mean of weight gain of 16.96 kg (SD = 6.19 kg). Pairwise linear correlations among the BW, Mage, pre-pregnancy BMI, GesAge, and MWG are shown in Table 2. There were significant positive linear correlations between BW, MWG, and GesAge ($p < 0.05$).

The mother–newborn *MMP9* rs17577, *MMP9* rs17576, and *ADRA2A* rs553668 SNPs and BW
The maternal *MMP9* rs17577, *MMP9* rs17576, and *ADRA2A* rs553668 gene polymorphisms were in Hardy–Weinberg equilibrium ($p = 0.342$, $p = 0.216$, and $p = 0.389$). The same results concerning Hardy–Weinberg equilibrium were obtained for a newborn's studied gene polymorphisms ($p = 0.128$, $p = 0.322$, and $p = 0.333$). The *MMP9* rs17577 and *MMP9* rs17576 gene polymorphisms were in linkage disequilibrium (LD) in both mothers and newborns (mothers: $D = 0.13$, $D' = 0.94$, $r^2 = 0.66$, $\chi^2 = 171.11$, df = 1, and $p < 0.0001$; newborns: $D = 0.11$, $D'' = 0.90$, $r^2 = 0.61$, $\chi^2 = 146.68$, df = 1, and $p < 0.0001$).

In Table 3, we describe the genotype distribution for the mentioned SNPs in the study sample and the relationship between them and the newborn's BW. We found a significant difference of BW in the newborns whose mothers had the variant genotype of *MMP9* rs17577 and *ADRA2A* rs553668 in comparison with those who had the wild-type genotype of these polymorphisms, noticing a lower BW in newborns whose mothers carried the variant genotype of *MMP9* rs17577. Conversely, newborns whose mothers had the variant genotype of *ADRA2A* rs553668 polymorphism had an overall higher BW than those whose mothers had the normal genotype (Table 3). Mean BW was lower in children with the variant genotype GA+GG of *MMP9* rs17576 polymorphism, in comparison with those with a normal genotype, existing significant differences between the means of BW between the two groups ($p = 0.030$).

We did not find any association between investigated SNPs and MWG ($p > 0.05$).

Direct effects of variables under study on endogenous variables
The path model containing all variables under study with relationships of interest demonstrated a good fit to data according to the established criteria: χ^2 test (χ^2 statistics = 37.88, df = 30, $p = 0.153$), robust CFI = 0.95, robust TLI = 0.91, SRMR = 0.037, robust RMSEA = 0.036, 90% CI for robust RMSEA: [0.000; 0.068], values for MI lower than 8 (there was no suggestion for a plausible modification of the model).

The model tested showed that pre-pregnancy BMI had a direct effect on BW (Table 4). We found that for an increase of 4.4 kg/m^2 (=1 SD) in pre-pregnancy BMI, the BW increases with 0.13SD (equals 67.4 g). Each of the *MMP9* *rs*17577 and *MMP9* *rs*17576 newborn gene polymorphisms had a direct significant effect on BW. In addition, the estimations of effects showed a positive effect of *MMP9* *rs*17577 newborn gene polymorphism with an increase of 0.20 SD≈103.6 g in BW in newborns with a variant genotype, and a negative effect of *MMP9* *rs*17576 polymorphism (decrease of 0.17 SD in newborns with a variant genotype). The presence of a variant genotype of these two SNPs in mothers had a negative effect on the newborn's BW, but only with a tendency toward statistical significance ($p = 0.064$ and $p = 0.078$, respectively).

GesAge had also a positive effect on BW; an increase with 1 SD (1.7 weeks) in GesAge will conduct to 0.49 SD in BW (≈253.9 g). The smoking habit had a significant negative direct effect on BW (decrease with 0.18 SD in newborns' BW whose mothers were smokers).

Regarding MWG, the GesAge had a significant positive direct effect, while multiparous status had a negative direct effect on MWG, revealing a decrease with 0.18 SD≈93.3 g in BW multipara women.

DISCUSSION

Predictors for newborns' weight gain
Multiple studies from the literature underline the predictors for newborns' BW. The advantage of this study compared with other studies is that using path analysis, we can simultaneously measure and quantify within the same model, all the structural relationships between MWG, BW of neonates, and potential factors (genetic and clinical) that influence them.

A review from 2018 concluded that an excessive BW is triggered by excessive, pre-pregnancy BMI (≥25 kg/m^2) and gestational diabetes.[6] Moreover, Silva et al.[7] underlined that the predictors for BW were pre-gestational BMI and postprandial glucose blood levels in mothers during the third trimester of pregnancy. Thus, Farah et al.[8] showed that GWG influences BW with a value of 8.4% outcome variance, underlining that BW was correlated with smoking, parity, and GesAge.[8] On the contrary, in our study, BW was lower in newborns from smoking mothers (2977.3 ± 341.3 g versus 3357.6 ± 521.7 g). Also, we found that GesAge was a significant predictor of BW adjusting for other covariates, underlining that an increase with 1.7 weeks in GesAge will lead to an increase of BW with 253.9 g.

It is important to make the differentiation between GWG, which is a weight increase during pregnancy adjusted to the BMI, and MWG, which is the simple difference between the initial weight and the weight at the time of delivery. In a previous study of our team, an excessive GWG might be an important predictor for the newborns' BW.[28] Also, Ferrari et al.[29] underlined that mother's excessive weight gain leads to a higher BW, and Faucher et al.[30] found that obese women have a higher risk of delivering newborns with a higher BW, this fact being also associated with an increase of GesAge. The same study of our team[28] also revealed

Table 3. Bivariate associations between studied mother–newborn gene polymorphisms and BW

	Study sample ($n = 197$) Cases (%)	BW (g) Mean ± SD	p value[+]
Maternal gene polymorphisms			
MMP9 rs17577			
GG	115 (58.4)	3379.4 ± 453.7	0.103
GA	75 (38.1)	3234.7 ± 610.9	
AA	7 (3.6)	3122.9 ± 228.7	
GA+AA	82 (41.7)	3225.1 ± 588.1	**0.049**
MMP9 rs17576			
AA	74 (37.6)	3343.9 ± 448.8	0.176
GA	100 (50.8)	3475.6 ± 542.2	
GG	23 (11.7)	3155.2 ± 298.8	
GA+GG	123 (62.5)	3333.7 ± 511.4	0.781
ADRA2A rs553668			
AA	9 (4.6)	2967.8 ± 634.8	0.064
GA	57 (28.9)	3310.9 ± 438.7	
GG	131 (66.5)	3374.4 ± 488.8	
GA+GG	188 (95.4)	3355.5 ± 474.2	**0.039**
Neonatal gene polymorphisms			
MMP9 rs17577			
GG	125 (63.5)	3315.9 ± 490.1	0.524
GA	68 (34.5)	3390.6 ± 489.0	
AA	4 (2.0)	3115.0 ± 345.7	
GA+AA	72 (36.5)	3375.1 ± 484.5	0.506
MMP9 rs17576			
AA	82 (41.6)	3409.8 ± 458.4	0.095
GA	95 (48.2)	3289.8 ± 501.7	
GG	20 (10.2)	3261.0 ± 523.6	
GA+GG	115 (58.4)	3284.6 ± 503.4	**0.030**
ADRA2A rs553668			
AA	11 (5.6)	3211.8 ± 386.5	
GA	61 (31.0)	3301.8 ± 535.1	0.628
GG	125 (63.5)	3366.5 ± 471.8	
GA+GG	186 (94.5)	3345.1 ± 492.9	0.497

MMP9 rs17577 G>A: matrix metalloproteinase 9 rs17577 (GG = reference category); AA—homozygous for A allele; GA—heterozygous; GG—homozygous for G allele
MMP9 rs17576 G>A: matrix metalloproteinase 9 rs17576 (GG = reference category); AA—homozygous for A allele; GA—heterozygous; GG—homozygous for G allele
ADRA2A rs553668 G>A: alfa 2 adrenergic receptor rs553668 (GG = reference category); AA—homozygous for A allele; GA—heterozygous; GG—homozygous for G allele
BW birth weight, *ANOVA* analysis of variance
The bold values are significant values (there are significant correlations between the parameters)
[+]p values obtained from one-way ANOVA or robust Brown–Forsythe test or Student's t test

Table 4. Direct effects of variables under study on endogenous variables

Pathways	Estimate of standardized coefficient	Standard error (SE)	p value
Maternal age→BMI pre-pregnancy	0.22	0.04	**<0.001**
Maternal age→GesAge	0.05	0.03	0.624
Education→GesAge	0.16	0.14	**0.045**
Multiparous→GesAge	−0.09	0.23	0.165
BMI pre-pregnancy→GesAge	0.02	0.03	0.716
Smoking→GesAge	0.05	0.32	0.348
BMI pre-pregnancy→MWG	−0.12	0.10	0.062
GesAge→MWG	0.20	0.28	**0.009**
Smoking→MWG	−0.09	1.31	0.161
Multiparous→MWG	−0.18	0.82	**0.007**
BMI pre-pregnancy→BW	0.13	0.01	**0.036**
Education→BW	0.10	0.03	0.089
MWG→BW	0.08	0.01	0.166
Gender→BW	0.09	0.06	0.120
Smoking→BW	−0.18	0.08	<**0.001**
Multiparous→BW	0.08	0.06	0.166
GesAge→BW	0.49	0.02	**<0.001**
Cesarean section→BW	0.03	0.07	0.645
cMMP9 rs17577[a]→BW	0.20	0.08	**0.010**
cMMP9 rs17576[a]→BW	−0.17	0.08	**0.032**
cADRA2A rs553668[a]→BW	−0.04	0.10	0.382
mMMP9 rs17577[b]→BW	−0.14	0.08	0.064
mMMP9 rs17576[b]→BW	0.12	0.07	0.078
mADRA2A rs553668[b]→BW	0.05	0.12	0.327

cMMP9 rs17576: matrix metalloproteinase 9 rs17576 in newborns, *mMMP9* rs17576: matrix metalloproteinase 9 rs17576 in mothers
cMMP9 rs17577: matrix metalloproteinase 9 rs17577 in newborns, *mMMP9* rs17577: matrix metalloproteinase 9 rs17577 in mothers
cADRA2A rs553668: alfa 2 adrenergic receptor rs553668 in newborns, *mADRA2A* rs553668: alfa 2 adrenergic receptor rs553668 in mothers
BMI body mass index, *BW* birth weight, *GesAge* gestational age, *MWG* maternal weight gain, *SE* standard error. Arrows indicate the direct effect of an independent variable on a dependent variable. The model explained 41.3% of BW variance and 10.5% of MWG variance
The bold values are significant values (significant correlations)
[a]Studied gene polymorphism determined at the newborn
[b]Studied gene polymorphism determined at his mother

a positive correlation between BW and GesAge, and also that maternal smoking negatively influenced the BW. Gailllard et al.[31] proved that an excessive weight gain early in pregnancy carries a higher risk for both an increased BW and further obesity in the child, and for metabolic conditions. Similarly to other studies,[32,33] we obtained a significant linear correlation between BW and MWG ($p = 0.005$), and GesAge ($p<0.001$), the mean of W increase being of 16.96 kg. Even though over 90% of all newborns were born at term, we found that male newborns had a higher BW, 3387.4 ± 521.6 g versus 3223.8 ± 502.1 g in female newborns, and those with a higher BW came from mothers with over 9 years of education. In addition, we found a tendency toward statistical significance of BW in the case of newborns from mothers with over 12 years of education, in comparison with those with an educational level of under 8 years.

Morandi et al.[34] proved that parental BMI, maternal GWG, and BW are risk factors with maternal profession and obesity. In another study of Mărginean et al.,[35] the authors proved that smoking, educational level, or AHT did not represent independent factors in newborns' obesity determining, but when these factors are associated, they present a risk of 4.07% in obesity determinism. Plachta-Danielzik et al.[32] emphasized that obesity or parental smoking and decreased physical activity are risk factors for obesity in boys, while these factors expressed in a single parent are obesity risk factors for girls.

Czarnobay et al.[6] proved that BW is higher in the case of cesarean section versus vaginal delivery. Contrary to the findings of these authors, in our study, we did not find associations between BW and type of delivery. Regarding parity, most of the studies did not prove a correlation between type of delivery and BW.[6] Similarly, we did not find any association between BW and parity. Nevertheless, GesAge has a positive effect on MGW, while multiparity and smoking showed a negative effect on BW.

The role of *MMP9* rs17577, *MMP9* rs17576, and *ADRA2A* rs553668 SNPs on BW

It is well documented that *MMPs* have a role in adipogenesis, especially by remodeling the extracellular matrix.[2] *MMP9* rs17576 and *MMP9* rs17577 are functional polymorphisms of the *MMP9* gene that were found to alter both the expression and activity of *MMP9*.[33] Therefore, *MMP9* rs17576 was associated with lower *MMP9* plasma levels in obese subjects, proving that it alters the activity of *MMP9*.[36] On the other hand, *MMP9* rs17577 proved to alter the expression of *MMP9*, being associated with increased levels of plasma *MMP9* in obese patients.[33] Nevertheless, these associations depend on the presence or absence of different genotypes in these groups of patients.

The adequate development of the fetoplacental unit depends on the invasion of an extravillous trophoblast, which plays a vital role in the transformation of maternal spiral arteries into vessels with low resistance and high capacitance.[13] It was proven that an elevated secretion of *MMP9* by decidual cells may impair this invasion.[14] An increased serum level of *MMP9* was proven to be associated with adverse pregnancy outcomes. Therefore, a large case–control study proved a positive association between the serum level of *MMP9* and both preeclampsia and spontaneous early delivery, but failed to identify the same relationship in the case of small newborns for GesAge.[16] On the contrary, another study underlined elevated levels of *MMP9* in newborns that were small for GesAge, being an independent predictor of vascular dysfunction, leading to its potential role as a predictor for cardiovascular risk.[15] Even though, in our study, we did not assess the serum levels of *MMP9*, we noticed that the newborns from mothers that carried the variant genotype of *MMP9* rs17577 had a lower BW in comparison with those from mothers with the normal genotype. Moreover, we noticed that mean BW was lower in children with the variant genotype GA+GG of *MMP9* rs17576 polymorphism, in comparison with those carrying the normal genotype. Therefore, we may state that a possible explanation for the differences regarding the association between MMP9 serum levels and BW identified in the studies mentioned above could be related to the expression of either variant or normal genotypes in mothers or newborns. In addition, using the path model containing all variables, we noticed that pre-pregnancy BMI had a direct effect on BW, and an increase of 4.4 kg/m^2 leads to higher BW with 67.4 g. The newborns that carried the variant genotype of *MMP9 rs*17577 had an increase of BW with 103.6g, while the presence of a variant genotype of *MMP9 rs*17576 in newborns proved to have a negative effect on BW. Therefore, both *MMP9* rs17576 and *MMP9* rs17577 SNPs were significantly related with newborns' BW, with a negative slope of relationship for *MMP9* rs17576 and a positive slope for *MMP9* rs17577. We did not find associations between the studied *MMP9* SNPs and MWG. Other genetic studies revealed that different MMP9 gene polymorphisms are related to spontaneous preterm birth in African American and Chinese women,[17,18] but not in European Caucasian ones.[19] Therefore, it is very clear that an increase in both MMP9 serum levels and gene expression will favor the extracellular matrix degradation in the fetal membranes and placenta, representing a potential cause for small-for-GesAge newborns or preterm delivery.[19]

Later on in life, the studies reported opposite results, proving that MMP9 may be related to obesity development. Moreover, multiple studies focused on the role of *MMP9* polymorphisms and haplotypes in children's obesity determinism. Luizon et al.[33] proved that three functional polymorphisms, *MMP9* rs3918242, *MMP9* rs17576, and *MMP9* rs17577, modify the activity of serum *MMP9*, being associated with obesity and cardiovascular risk.[33] Also, the authors noticed that obese individuals with over three metabolic risk factors carrying the variant of *MMP9* rs17577 have higher serum levels of *MMP9* in comparison with those with a wild-type homozygous genotype, but they did not find any correlations with *MMP9* rs17576.[33]

The activation of ADRA2A in adipocytes inhibits the production of cAMP by inhibiting the adenylate cyclase.[22,23] It is well-documented that the sympathetic nervous system owns an essential role in the regulation of glucose and lipid metabolism.[37] Therefore, adrenaline will lead to increased glucose levels by stimulating glycogenolysis and by lowering glucose clearance through a beta-adrenergic mechanism, and also by inhibiting insulin release and enhancing glucagon secretion by alpha-adrenergic ones.[37] Moreover, these two receptors are also involved in the regulation of lipolysis in human adipocytes.[38] While the stimulation of beta-adrenergic receptors stimulates lipolysis, the stimulation of the alpha-adrenergic ones inhibits this process.[38] Therefore, human adipocytes express adrenergic receptors, such as β1, β2, and β3 that can stimulate lipolysis, and also α2 receptors with the role in lipolysis inhibition, resulting in the statement that the adrenergic receptor gene may represent reasonable candidate genes for obesity development.[39] Moreover, variants in the *ADRA2A* receptor can result in leanness by altering the lypolytic activity in fat tissue. Thus, a mutation in the coding region might result in a depletion of its antilipolytic action, leading to reduced lipid storage.[36] Based on all these facts, the well-known physiological ability of *ADRA2A* to counteract lipolytic activity in adipose tissue could be genetically modulated by altering the function of this receptor.

There are *ADRA2A* polymorphisms that are associated with obesity and type 2 DM, such as *ADRA2A* rs553668 and *ADRA2A* rs521674.[40] Thus, Långberg et al.[40] proved that homozygote women for normal AA and TT genotypes of *ADRA2A* rs553668 and *ADRA2A* rs521674 SNPs have a higher incidence of obesity and type 2 DM. Hamann et al.[23] in exchange proved in a study performed on obese children and teenagers that the gene encoding the human *ADRA2A* is less likely to predispose to obesity. Similarly, in our study, the variant genotype of *ADRA2A* rs553668 had no effect on newborns' BW, and therefore it does not influence its nutritional status. Moreover, the newborns from mothers that carried the variant genotype of *ADRA2A* rs553668 polymorphism had a lower BW versus the control group. Therefore, our findings sustain the mechanism mentioned above that variants of *ADRA2A* might be associated with reduced lipid storage, and subsequently lower BW. Thus, our findings suggest that the presence of a variant genotype of *ADRA2A* rs553668 polymorphism in mothers may modulate the weight of their fetuses, being associated with a lower BW.

Because the path analysis technique requires a large number of cases relative to the estimated parameters, the limitations of our study consist of the relatively small ratio of subjects to free parameters that might lead to a decrease of the statistical power of this study. The lack of assessment of other factors that might be involved in BW determinism, mothers and newborns, such as maternal diet (classic, Mediterranean, vegetarian, etc.) might be an essential factor for MWG, and the fact that we did not assess also the serum levels of *MMP9* and *ADRA2A*.

We must also mention the strengths of this study, among which there is the accuracy of all measurements in mothers and newborns or the well-documented role of the three studied SNPs in the development of obesity and the prediction of the fetuses' BW or MWG. Path analysis as a form of structural equation modeling has allowed the testing of a conceptual graphical model containing all variables under study. Moreover, for the assessment

of BMI, we did not use self-reported weight and height, but instead we measured them ourselves. Even though the role of *MMP9* rs17577, *MMP9* rs17576, and *ADRA2A* rs553668 SNPs in obesity determinism is well known, this is among the few studies that established correlations between these SNPs and other maternal and neonatal parameters. Thus, it might be considered a pilot study that should be extended on a higher number of subjects requiring the assessment of newborns further in life. It is also the first cross-sectional study performed in Romania on mothers and their newborns that uses a model with multiple variables establishing important correlations between MWG and three gene polymorphisms, and also their influence on the newborns' BW. Nevertheless, further studies on larger samples are required from different geographic areas in order to establish clearly the interactions between these genetic factors and BW or their correlation with other maternal and neonatal parameters.

CONCLUSIONS

In our study, the results of bivariate analysis showed a positive correlation between BW, MWG, and GesAge, noticing a higher BW for newborns from mothers with higher educational levels and a lower BW in case of those from smoking mothers. The newborns from mothers carrying the variant genotypes of *MMP9* rs17577 SNP had a significantly lower BW, in comparison with those that expressed the variant genotype of *ADRA2A* rs553668 polymorphism, who had a higher BW. The path analysis results showed that the significant predictors of MWG were GesAge and multiparity, while the predictors for BW were mother's smoking habit, maternal pre-pregnancy BMI, GesAge, and newborns' variant genotypes of *MMP9* rs17577 and *MMP9* rs17576 SNPs. The presence of newborns' variant genotype from *MMP9* rs17577 SNP had a direct positive effect on BW, so it produced an increase in BW, while the newborns who carried the variant genotype of *MMP9* rs17576 polymorphism had a negative direct effect, decreasing the BW. We may underline the role of *MMP9* rs17577, *MMP9* rs17576, and *ADRA2A* rs553668 in BW determinism that can be useful in establishing the risk factors related to delivery.

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AUTHOR CONTRIBUTIONS

C.O.M., C.M., C.B. and M.I. conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the paper. L.E.M., C.O.M. designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the paper. F.T. and C.B. performed the genetic tests. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ADDITIONAL INFORMATION

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REFERENCES

1. WHO. *World Health Statistics* (2014). http://www.who.int/gho/publications/world_health_statistics/2014/en/. Accessed 14 July 2018.
2. Crandall, D. L., Hausman, G. J. & Kral, J. G. A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. *Microcirculation* **4**, 211–232 (1997).
3. Wu, P. et al. Interactions of central obesity with rs3918242 on risk of non-alcoholic fat liver disease: a preliminary case-control study. *Int. J. Clin. Exp. Pathol.* **8**, 4165–4170 (2015).
4. Mărginean, C. O., Bănescu, C., Duicu, C., Voidăzan, S. & Mărginean, C. Angiotensin-converting enzyme gene insertion/deletion polymorphism in nutritional disorders in children. *Eur. J. Nutr.* **54**, 1245–1254 (2015).
5. Mărginean, C. O. et al. Communication skills in pediatrics—the relationship between pediatrician and child. *Medicine (Baltim.)* **96**, e8399 (2017).
6. Czarnobay, S. A., et al. Predictors of excess birth weight in Brazil: a systematic review. *J. Pediatr (Rio J)* (2018). https://doi.org/10.1016/j.jped.2018.04.006
7. Silva, S. et al. Predictive factors for birth weight of newborns of mothers with gestational diabetes mellitus. *Diabetes Res. Clin. Pract.* **138**, 262–270 (2018).
8. Farah, N., Stuart, B., Donnelly, V., Kennelly, M. M. & Turner, M. J. The influence of maternal body composition on birth weight. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **157**, 14–17 (2011).
9. Mărginean, C. et al. The FTO rs9939609 and LEPR rs1137101 mothers–newborns gene polymorphisms and maternal fat mass index effects on anthropometric characteristics in newborns: a cross-sectional study on mothers–newborns gene polymorphisms—the FTO-LEPR Study (STROBE-compliant article). *Medicine (Baltimore)* **95**, e5551 (2016).
10. Dollery, C. M., McEwan, J. R. & Henney, A. M. Matrix metalloproteinases and cardiovascular disease. *Circ. Res.* **77**, 863–868 (1995).
11. Sindhu, S., Al-Roub, A., Koshy, M., Thomas, R. & Ahmad, R. Palmitate-induced MMP-9 expression in the human monocytic cells is mediated through the TLR4-MyD88 dependent mechanism. *Cell. Physiol. Biochem.* **39**, 889–900 (2016).
12. Hijova, E. Matrix metalloproteinases: their biological functions and clinical implications. *Bratisl. Lek. Listy* **106**, 127–132 (2005).
13. Pijnenborg, R., Vercruysse, L. & Hanssens, M. The uterine spiral arteries in human pregnancy: facts and controversies. *Placenta* **27**, 939–958 (2006).
14. Lockwood, C. J. et al. Matrix metalloproteinase 9 (MMP9) expression in pre-eclamptic decidua and MMP9 induction by tumor necrosis factor alpha and interleukin 1 beta in human first trimester decidual cells. *Biol. Reprod.* **78**, 1064–1072 (2008).
15. Sesso, R. & Franco, M. C. P. Abnormalities in metalloproteinase pathways and IGF-I axis: a link between birth weight, hypertension, and vascular damage in childhood. *Am. J. Hypertens.* **23**, 6–11 (2010).
16. Poon, L. C. Y., Nekrasova, E., Anastassopoulos, P., Livanos, P. & Nicolaides, K. H. First-trimester maternal serum matrix metalloproteinase-9 (MMP-9) and adverse pregnancy outcome. *Prenat. Diagn.* **29**, 553–559 (2009).
17. Ferrand, P. E. et al. A polymorphism in the matrix metalloproteinase-9 promoter is associated with increased risk of preterm premature rupture of membranes in African Americans. *Mol. Hum. Reprod.* **8**, 494–501 (2002).
18. Fang, X. et al. Polymorphisms in the MTHRF, VDR, MMP-9 and IL-β genes and the risk of premature rupture of membranes. *Gynecol. Obstet. Invest.* **70**, 206–214 (2010).
19. Pereza, N. et al. Functional polymorphisms of matrix metalloproteinases 1 and 9 genes in women with spontaneous preterm birth. *Dis. Markers* **2014**, 171036 (2014).
20. Vu, T. H. et al. MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* **93**, 411–422 (1998).
21. Andrade, C., Bosco, A., Sandrim, V. & Silva, F. MMP-9 levels and IMT of carotid arteries are elevated in obese children and adolescents compared to non-obese. *Arq. Bras. Cardiol.* **108**, 198–203 (2017).
22. Lowell, B. B. & Spiegelman, B. M. Towards a molecular understanding of adaptive thermogenesis. *Nature* **404**, 652–660 (2000).
23. Hamann, A. et al. Identification of a deletion variant in the gene encoding the human alpha(2A)-adrenergic receptor. *Eur. J. Endocrinol.* **144**, 291–295 (2001).
24. Lafontan, M. & Berlan, M. Evidence for the alpha 2 nature of the alpha-adrenergic receptor inhibiting lipolysis in human fat cells. *Eur. J. Pharmacol.* **66**, 87–93 (1980).
25. Lafontan, M. et al. Adrenergic regulation of adipocyte metabolism. *Hum. Reprod.* **12**(Suppl. 1), 6–20 (1997).
26. Sohani, Z. N. et al. Risk alleles in/near ADCY5, ADRA2A, CDKAL1, CDKN2A/B, GRB10, and TCF7L2 elevate plasma glucose levels at birth and in early childhood: results from the FAMILY Study. *PLoS ONE* **11**, e0152107 (2016).
27. Kline, R. *Principles and Practice of Structural Equation Modelling* (eds.) David A. Kenny, Todd D. Little, Founding Editor 23–139 (Guilford Press, New York, 2015).
28. Mărginean, C. et al. Impact of demographic, genetic, and bioimpedance factors on gestational weight gain and birth weight in a Romanian population: A cross-sectional study in mothers and their newborns: the Monebo study (STROBE-compliant article). *Medicine (Baltim.)* **95**, e4098 (2016).
29. Ferrari, N., Mallmann, P., Brockmeier, K., Strüder, H. K. & Graf, C. Secular trends in pregnancy weight gain in German women and their influences on foetal outcome: a hospital-based study. *BMC Pregnancy Childbirth* **14**, 228 (2014).

30. Faucher, M. A. & Barger, M. K. Gestational weight gain in obese women by class of obesity and select maternal/newborn outcomes: a systematic review. *Women Birth*. **28**, e70–e79 (2015).
31. Gaillard, R., Steegers, Ea. P., Franco, O. H., Hofman, A. & Jaddoe, V. W. V. Maternal weight gain in different periods of pregnancy and childhood cardio-metabolic outcomes. The Generation R Study. *Int. J. Obes. (Lond.)*. **39**, 677–685 (2015).
32. Plachta-Danielzik, S., Landsberg, B., Johannsen, M., Lange, D. & Müller, M. J. Determinants of the prevalence and incidence of overweight in children and adolescents. *Public Health Nutr*. **13**, 1870–1881 (2010).
33. Luizon, M. R. et al. Plasma matrix metalloproteinase-9 levels, MMP-9 gene haplotypes, and cardiovascular risk in obese subjects. *Mol. Biol. Rep*. **43**, 463–471 (2016).
34. Morandi, A. et al. Estimation of newborn risk for child or adolescent obesity: lessons from longitudinal birth cohorts. *PLoS ONE* **7**, e49919 (2012).
35. Mărginean, C. et al. The role of TGF-β1 869 T>C and PPAR γ2 34 C>G polymorphisms, fat mass, and anthropometric characteristics in predicting childhood obesity at birth: a cross-sectional study according the parental characteristics and newborn's risk for child obesity (the newborns obesity's risk) NOR study. *Medicine (Baltim.)* **95**, e4265 (2016).
36. Belo, V. A. et al. Matrix metalloproteinase-9 genetic variations affect MMP-9 levels in obese children. *Int. J. Obes. (Lond.)*. **36**, 69–75 (2012).
37. Bratusch-Marrain, P. R. Insulin-counteracting hormones: their impact on glucose metabolism. *Diabetologia* **24**, 74–79 (1983).
38. Efendić, S. Catecholamines and metabolism of human adipose tissue. 3. Comparison between the regulation of lipolysis in omental and subcutaneous adipose tissue. *Acta Med. Scand*. **187**, 477–483 (1970).
39. Ukkola, O. et al. Interactions among the alpha2-, beta2-, and beta3-adrenergic receptor genes and obesity-related phenotypes in the Quebec Family Study. *Metab. Clin. Exp*. **49**, 1063–1070 (2000).
40. Långberg, E.-C., Seed Ahmed, M., Efendic, S., Gu, H. F. & Östenson, C.-G. Genetic association of adrenergic receptor alpha 2A with obesity and type 2 diabetes. *Obesity (Silver Spring)*. **21**, 1720–1725 (2013).