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The relationship among GNB3 rs5443, PNPLA3 rs738409, GCKR rs780094 gene polymorphisms, type of maternal gestational weight gain and neonatal outcomes (STROBE-compliant article)

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Abstract

The gestational weight gain is determined by food habits, environmental and genetic factors.

The aims of this paper were to establish relationships between maternal gene polymorphisms (patatin-like phospholipase domain-containing protein 3 rs738409 [PNPLA3 rs738409], glucokinase regulatory protein rs780094 [GCKR rs780094], and guanine nucleotide-binding protein rs5443 [GNB3 rs5443]) and mothers' gestational weight gain, but also neonatal outcomes (birth weight, length, and ponderal index [PI]).

We performed a cross-sectional study in a sample of 158 mothers and their product of conception' in an Obstetrics-Gynecology Clinic from Romania. We divided the pregnant women according to the Institute of Medicine recommendations into 3 subgroups: (1) insufficient gestational weight gain; (2) normal gestational weight gain; and (3) excessive gestational weight gain.

The gestational weight gain among pregnant women included in this study was classified as insufficient (10.1%), normal (31%), and excessive (58.9%). We found a tendency towards statistical significance for mothers that were overweight or obese before pregnancy to present an excessive gestational weight gain as compared to the normal weight ones. Similarly, we identified a tendency for statistical significance regarding the association between the variant genotype of GNB3 rs5443 and excessive gestational weight gain. We noticed differences that tended to be statistical significant concerning aspartate aminotransferase values between the 3 subgroups, mothers with excessive gestational weight gain having higher values than mothers with normal gestational weight gain (median, IQR: 22.89[17.53; 31.59] for mothers with excessive gestational weight gain versus 22.71[18.58; 27.37] for mothers with normal gestational weight gain). In mothers with excessive gestational weight gain, we found a significant association between the variant genotype of PNPLA3 rs738409 polymorphism and neonatal PI noticing a decrease of this index in case of newborns from mothers carrying the variant genotype.

Excessive gestational weight gain was noticed in pregnant women that were obese and overweight before pregnancy. We found a positive association between the variant genotype of GNB3 rs5443 polymorphism and excessive gestational weight gain. Similarly, the presence of variant genotype of PNPLA3 rs738409 in mothers was associated with a lower PI in their newborns. Our study pointed out the most important factors that influence gestational weight gain and related birth outcomes.

Abbreviations: ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, Chol = total cholesterol, DM = diabetes mellitus, GCKR rs780094 = glucokinase regulatory protein rs780094, GNB3 rs5443 = guanine nucleotide-binding protein rs5443, GWG = gestational weight gain, HDL-chol = high-density lipoprotein cholesterol, IOM = Institute of Medicine, LDL-chol = low-density lipoprotein cholesterol, MMP = matrix metalloproteinase, NAFLD = non-alcoholic liver disease, NASH = non- alcoholic steatohepatitis, PI = ponderal index, PNPLA3 rs738409 = patatin-like phospholipase domain-containing protein 3 rs738409, SNPs = single nucleotide polymorphisms, TG = triglyceride.

Keywords: PNPLA3 rs738409 gene polymorphisms, GCKR rs780094 gene polymorphisms, GNB3 rs5443 gene polymorphisms, gestational weight gain, neonatal outcomes

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1. Introduction

Obesity is a major public health problem with more than 300 million obese people worldwide.[1,2] It is estimated that by the year 2030, 1.12 billion people will be obese. In 2016, Romania was stated to be country with the lowest rates of obesity among people aged 18 years or above, 9.4%.[3] Nevertheless, a study performed on pediatric subjects from our country found that in one in 4 children was either overweight and obese underlining a likely increase in incidence in time.[4]

Obesity-related problems are severe, mostly due to its complications such as type 2 diabetes mellitus (DM), arterial hypertension, cardiovascular disorders, or dyslipidemia.[1,2] The incidence of maternal obesity increased very much lately. Moreover, 2/3 of the fertile women in the USA are overweight, and 1 in 3 is obese, while in the United Kingdom 1 in 5 is obese.[5] Pregnant woman's excessive gestational weight gain (GWG) influences birth outcomes, but it can also result in the development of other disorders during pregnancy, such as gestational diabetes, preeclampsia or eclampsia, dystocia with further negative impact on their offspring regarding birth weight resulting in macrosomia or obesity during childhood, but also neonatal distress.[5–10] Thus, the Institute of Medicine (IOM) recommended a different GWG depending on the weight at the beginning of the pregnancy. According to this recommendation, as higher the pre-pregnancy body mass index (BMI) is, the lower the weight gain during pregnancy should be, varying between 5 to 9 kg for BMI $>30 \, kg/m^2$, and 12.5 to 18 kg for BMI $<18,5 \, kg/m^2$.[7,11–12]

Genetic factors are well-documented to contribute to obesity development. Thus, a wide spectrum of genes was proven to be involved in adipogenesis, angiogenesis and so in the determination of overweight and obesity. Among these, matrix metalloproteinase (MMP) gene polymorphisms like MMP9 rs17577, MMP9 rs17576, and alfa 2 adrenergic receptor rs553668 single nucleotide polymorphism (SNP) are involved in birth weight determinism.[13] Moreover, the studies performed on obese children and healthy controls proved that gene polymorphisms of interleukin 6, leptin receptor, tumor necrosis factor alpha own a major role in the development of childhood obesity.[14–17]

Patatin-like phospholipase domain-containing protein 3 (PNPLA3) is a protein expressed in the liver with phospholipase/transacetylase activity[18,19] that results from the replacement of isoleucine to methionine in 148 position. It was stated that the variant of patatin-like phospholipase domain-containing protein 3 rs738409 (PNPLA3 rs738409) gene polymorphism is associated with obesity-related liver impairment in both adults and children.[18,20–22] Garner underlined that homozygotes of the ancestral (C) allele of PNPLA3 rs738409 leads to a decrease of triglycerides (TG) in the liver, it's variant polymorphism increasing hepatic steatosis through the prevention of TG decrease.[23] Moreover, it seems that PNPLA3 rs738409 gene polymorphism is related to obesity and elevated levels of transaminases in teenagers suggesting that the early detection of its variant allele might be useful in the prevention of these disorders through a healthy and rigorous lifestyle.[24]

Regarding the glucokinase regulatory protein (GCKR), Hovsepian et al noticed that GA genotype of glucokinase regulatory protein rs780094 (GCKR rs780094) gene polymorphism owns a protective effect for obesity, whereas the minor allele of GCKR rs1260333 SNP might express a pathogenic role in obesity.[25] Valenti et al, in a study performed on children and teenagers, underlined that the variant alleles of both GCKR

rs780094 and PNPLA3 rs738409 gene polymorphisms increase the severity of non-alcoholic steatohepatitis (NASH) and hepatic fibrosis in obese pediatric patients.[26]

Studies regarding the role of guanine nucleotide-binding protein rs5443 (GNB3 rs5443) gene polymorphism in the development of obesity and its related comorbidities are contradictory. Thus, T allele of GNB3 rs5443 SNP was proved to be associated with obesity, arterial hypertension, and atherosclerosis.[27] Studies performed on German, Chinese and South-African people showed that T allele of this polymorphism is associated with a predisposition for obesity,[28–30] while other studies[31,32] failed in proving this association.

The aims of this paper were to establish relationships between maternal gene polymorphisms (PNPLA3 rs738409, GCKR rs780094, and GNB3 rs5443) and mothers' GWG, respective neonatal outcomes (birth weight, length and ponderal index [PI]). We also tested whether these associations differed according to GWG within, above and below IOM recommendations.

2. Material and methods

2.1. Study sample

We performed a cross-sectional study on a sample of mothers and their product of conception' admitted in an Obstetrics-Gynecology Clinic from Romania, who were consecutively selected.

The inclusion criteria consisted in single pregnancy, maternal age above 18 years, and pre-pregnancy body mass index (BMI) $\geq 18.5 \, kg/m^2$. The exclusion criteria were: gestational age below 37 weeks (a characteristic that might influence GWG), chronic maternal disorders diagnosed before the pregnancy that might influence nutritional status (e.g., hypothyroidism, diabetes mellitus, monogenic obesity syndromes, etc), and the refusal to sign the informed consent before the participation in the study. Thus, the final sample included 158 pregnant women.

Before the inclusion in the study, the mothers signed the informed consent on their behalf and their offspring. Our research was granted with the approval of Ethics Committee of the University of Medicine, Pharmacy, Sciences and Technology Târgu Mureş, (No 26/2017), and it was designed and performed in compliance to the principles of the Helsinki Declaration.

2.2. Types of measures used in this study

We assessed the body mass index (BMI) of all mothers included in this study. Thus, pre-pregnancy BMI was obtained by dividing the maternal weight (W) at the beginning of the pregnancy to squared maternal height, and expressed in kg/m^2. According to Control Disease Center, we classified women as obese for BMI $>30.0 \, kg/m^2$ and overweight for BMI was between 25.0 and 29.9 kg/m^2. Regarding GWG, it was defined as the weight gain during pregnancy (the difference between gestational weight just before the birth of the infant and the weight at the time of conception) and interpreted according to the recommendations of the IOM.[11] Thus, according to the recommendation from 2009, the pregnant women were classified into 3 subgroups:

(1) gestational weight gain below the IOM guidelines denoted by insufficient GWG;
(2) gestational weight gain within the IOM guidelines denoted by normal GWG; and
(3) gestational weight gain above the IOM guidelines denoted by excessive GWG.

Maternal age, educational level, smoking status, obstetrical characteristics (parity, type of birth), and gene polymorphisms in mothers and their product of conception defined as social and demographic characteristic were considered explanatory variables in the data analysis.

2.3. Laboratory parameters

Cholesterol (Chol), TG, high-density lipoprotein cholesterol (HDL-chol), low-density lipoprotein cholesterol (LDL-chol), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were assessed by spectrophotometry on a Cobas Integra 400 plus automated analyzer, Roche Diagnostics GmbH, Mannheim, Germany for all mothers and newborns included in our study. The upper normal limit for cholesterol level was 170 mg/dL, while for triglyceride 130 mg/dL.

2.4. Genotyping analysis

A fresh blood sample was collected from mothers and newborns in an EDTA vacutainer and it was used for extraction of genomic DNA (gDNA). The DNA was quantified by using BioSpectrometer basic (Eppendorf AG) and genotyping was performed according to the manufacturer's instructions. For genotyping of *GNB3* rs5443, *PNPLA3* rs738409 and *GCKR* rs780094 we used the 7500 Fast Dx Real-Time polymerase chain reaction (PCR) system from Thermo Fisher Scientific (USA) and the specific TaqMan assays as follow: C_2184734_10, C_7241_10, and C_2862873_10, purchased from Antisel (Romanian distributor for Thermo Fisher Scientific).

2.5. Statistical analysis

Description of the pregnant women's characteristics on the entire study sample and the 3 assessed subgroups was performed using descriptive statistics as mean ± standard deviation (SD), median with interquartile range and percentages.

Demographic characteristics (age, years) of the pregnant women with normal, excessive and insufficient gestational weight gain were compared using analysis of variance (ANOVA), while biochemical characteristics (AST, ALT, Chol, LDL-chol, HDL-chol, TG) on the 3 subgroups were assessed through Kruskal–Wallis test.

The Chi-square or Fisher exact tests were used to investigate bivariate associations between clinical/genetic factors and gestational weight gain considered as an ordinal qualitative variable.

The significance level was set to 0.05 in all statistical inferential tests. The software used for statistical analysis was the R program (version 3.5.2).

3. Results

3.1. Sample description

Characteristics of the mothers included in the study are described in Table 1. The minimum maternal age was 18 years while the maximum was 42 years. The mean pre-pregnancy BMI was 24.13 kg/m², 32.9% of the pregnant women being overweight or obese (BMI ≥25 kg/m²). According to 2009 IOM guidelines regarding GWG, weight gain among pregnant women included in this study were classified as insufficient (10.1%), normal (31%) and excessive (58.9%). The mean of GWG was 17.02 kg

while the range of gestational weight values was between 2 kg under the expected inferior limit and 24 kg over the superior limit recommended by IOM guidelines. Among our sample, more than half of the pregnant women were primipara (53.8%), and most of them had the age above 20 years (93.7%). Regarding the educational level, 50% graduated faculty, 31.6% were unem-

Table 1

Characteristics of the studied sample (n = 158).

Variables	Value
Outcomes	
GWG (kg), mean±SD	17.02 ± 6.09
GWGª, n(%)	
Normal weight gain (Normal GWG)	49 (31.0)
Excessive weight gain (Excessive GWG)	93 (58.9)
Insufficient weight gain (Insufficient GWG)	16 (10.1)
Sociodemographic factors	
Age, mean±SD	28.72 ± 5.41
Age groups, n (%)	
18–20 years	10 (6.3)
20–30 years	73 (46.2)
≥30 years	75 (47.5)
Maternal education	
No education	8 (5.1)
4 grades/8 grades	30 (19.0)
High school/post-secondary	41 (25.9)
Superior	79 (50.0)
Unemployed, n (%)	50 (31.6)
Maternal anthropometric factors	
Height (cm), mean±SD	1.63 ± 0.07
Weight (kg), mean±SD	64.35 ± 12.30
BMI_pre-pregnancy (kg/m²), mean±SD	24.13 ± 4.10
Clinical and lifestyle factors	
Parity	
Primipara	85 (53.8)
Multipara	73 (46.2)
Maternal gene polymorphisms	
GNB3 rs5443	
CC	69 (43.7)
CT	77 (48.7)
TT	12 (7.6)
PNPLA3 rs738409	
CC	87 (55.1)
GC	61 (38.6)
GG	10 (6.3)
GCKR rs780094	
TT	34 (21.5)
CT	72 (45.6)
CC	52 (32.9)
Maternal pregnancy complications	
Diabetes, n (%)	5 (3.2)
AHT, preeclampsia, n (%)	11 (7.0)
Delivery characteristics	
Caesarean section, n (%)	49 (31.0)
Gestational age (weeks), median (IQR)	39[38; 40]

Data expressed as mean±standard deviation, median[percentile 25%; percentile 75%] and absolute frequencies (relative frequencies %); *P* values obtained from Student *t* test for independent samples, Mann–Whitney test or Chi-square test; *statistical significance (*P*<.05).
GCKR rs780094 T>C: glucokinase regulatory protein rs780094 (TT = reference category); CC—homozygous for C allele; CT—heterozygous; TT—*homozygous for T allele*.
GNB3 rs5443 C>T: guanine nucleotide-binding protein rs5443 (CC = reference category); CC—homozygous for C allele; CT—heterozygous; TT—*homozygous for T allele*.
PNPLA3 rs738409 G>C: patatin-like phospholipase domain-containing protein 3 rs738409 (CC = reference category); CC—homozygous for C allele; GC—heterozygous; GG—*homozygous for G allele*.
AHT = arterial hypertension, BMI = Body mass index, GWG = gestational weight gain.

ployed, 25.9% finished high school, 19% completed only 8th grade, and 5.1% had no education.

3.2. Bivariate associations between maternal anthropometric and genetic factors

We found no significant association between the mothers' educational level and GWG ($P > .05$). Nevertheless, we identified a tendency for statistical significance between GWG ($P = .05$) and overweight-obese mothers before pregnancy (Table 2), even though the median weight gain was lower in overweight or obese pregnant women when compared to those with normal weight (normal weight: 17.23 ± 6.01 kg; overweight: 17.59 ± 6.05 kg versus obese: 14.13 ± 6.32 kg).

We found no statistical significance ($P = .097$) regarding the association between the variant genotype of *GNB3* rs5443 and excessive GWG, its frequency being higher among mothers with excessive GWG in comparison to those with normal GWG (60.2% vs 57.1%). Regarding the other 2 studied

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Table 2

Comparisons of anthropometric, genetic and biochemical factors on mothers with excessive GWG, normal GWG, and insufficient GWG.

Variables	Insufficient GWG ($n_1 = 16$, 10.1%)	Normal GWG ($n_2 = 49$, 31%)	Excessive GWG ($n_3 = 93$, 58.9%)	P value
Sociodemographic factors				
Age, mean±SD	28.56±6.28	28.69±5.42	28.76±5.30	.990
Age groups, n (%)				
18–20 yrs	1 (6.3)	4 (8.2)	5 (5.4)	.814
20–30 yrs	8 (50.0)	19 (38.8)	46 (49.5)	
≥30 years	7 (43.8)	26 (53.1)	42 (45.2)	
Maternal education				.639
No education	2 (12.5)	2 (4.1)	4 (4.3)	
4 grades/8 grades	4 (25.0)	8 (16.3)	18 (19.4)	
High school/post-secondary	3 (18.8)	11 (22.4)	27 (29.0)	
Superior				
Unemployed, n (%)	7 (43.8)	13 (26.5)	30 (32.3)	.447
Maternal anthropometric factors				
Normal weight (BMI kg/m²)	14 (87.5)	38 (77.6)	54 (58.1)	.050[b]
Overweight	1 (6.3)	7 (14.3)	29 (31.2)	
Obese	1 (6.3)	4 (8.2)	10 (10.8)	
Clinical and lifestyle factors				
Parity				.080[b]
Primipara	5 (31.3)	24 (49.0)	56 (60.2)	
Multipara	11 (68.8)	25 (51.0)	37 (39.8)	
Smoking, n (%)	5 (31.3)	6 (12.2)	6 (6.5)	0.012[a]
Biochemical factors				
ALT (U/L)	9.87[8.22; 14.89]	10.60[7.42; 14.36]	11.73[7.88; 16.11]	.249
AST (U/L)	20.82[18.18; 29.98]	22.71[18.58; 27.37]	22.89[17.53; 31.59]	.094[b]
Chol (mg/dL)	205.38[179.26; 254.21]	215.37[199.33; 245.47]	212.14[190.63; 235.36]	.314
HDL (mg/dL)	59.24±15.34	57.41±10.62	59.65±16.87	.699
LDL (mg/dL)	125.92[96.63; 178.64]	136.09[117.55; 162.78]	133.62[112.30; 155.86]	.492
TG (mg/dL)	187.63[147.77; 253.24]	217.82[186.77; 262.29]	209.52[160.07; 239.03]	.433
Maternal gene polymorphisms				
GNB3 rs5443				.288
CC	11 (68.8)	21 (42.9)	37 (39.8)	
CT	4 (25.0)	25 (51.0)	48 (51.6)	
TT	1 (6.3)	3 (6.1)	8 (8.6)	
CT+TT	5 (31.3)	28 (57.1)	56 (60.2)	.097[b]
PNPLA3 rs738409				.686
CC	7 (43.8)	26 (53.1)	54 (58.1)	
GC	8 (50.0)	21 (42.9)	32 (34.4)	
GG	1 (6.3)	2 (4.1)	7 (7.5)	
GC+GG	9 (56.3)	23 (46.9)	39 (41.9)	.525
GCKR rs780094				.898
TT	4 (25.0)	12 (24.5)	18 (19.4)	
CT	8 (50.0)	22 (44.9)	42 (45.2)	
CC	4 (25.0)	15 (30.6)	33 (35.5)	
CT+CC	12 (75.0)	37 (75.5)	75 (80.6)	.741

ALT = alanin aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, HDL-chol = high density lipoprotein-cholesterol, LDL-chol = low density lipoprotein-cholesterol, n_1, n_2, n_3- absolute numbers, SD = standard deviation, TG = triglycerides.

Data expressed as mean±standard deviation [percentile 25%; percentile 75%]; descriptive statistics for Apgar score were presented as median [minimum; maximum]; [a]statistical significance ($P < .05$); [b]$P < .10$.

GCKR rs780094 T>C: glucokinase regulatory protein rs780094 (TT = reference category); CC—homozygous for C allele; CT—heterozygous; TT—homozygous for T allele.

GNB3 rs5443 C>T: guanine nucleotide-binding protein rs5443 (CC = reference category); CC—homozygous for C allele; CT—heterozygous; TT—homozygous for T allele.

PNPLA3 rs738409 G>C: patatin-like phospholipase domain-containing protein 3 rs738409 (CC = reference category); CC—homozygous for C allele; GC—heterozygous; GG—homozygous for G allele.

Table 3

Associations between maternal gene polymorphisms and birth outcomes stratified by GWG.

Maternal gene polymorphisms	All sample Birth weight (g), mean±SD	Birth length (cm), mean±SD	PI (kg/m³), mean±SD	Normal GWG Birth weight (g), mean±SD	Birth length (cm), mean±SD	PI (kg/m³), mean±SD	Excessive GWG Birth weight (g), mean±SD	Birth length (cm), mean±SD	PI (kg/m³), mean±SD	Insufficient GWG Birth weight (g), mean±SD	Birth length (cm), mean±SD	PI (kg/m³), mean±SD
GNB3 rs5443												
CC	3341.9±425.4	53.8±2.3	21.5±2.0	3170.0±362.8	53.3±2.6	20.9±1.6	3496.5±400.2	54.3±2.1	21.8±2.2	3150±446.9	52.9±2.3	21.2±2.1
CT+TT	3440.8±437.8	54.2±2.5	21.6±2.2	3393.2±449.9[a]	53.9±2.6	21.6±1.7	3497.8±433.7	54.4±2.5	21.8±2.3	3066±168.8	53.6±2.7	20.1±2.5
PNPLA3 rs738409												
CC	3336.5±453.9	54.1±2.6	21.7±2.3	3355.8±483.2	53.9±2.7	21.3±1.6	3539.5±417.8	54.2±2.5	22.2±2.5	2941.4±167.8	53.1±2.4	19.7±1.8
CG+GG	3350.0±406.2	53.9±2.3	21.3±1.8	3232.6±348.9	53.3±2.4	21.4±1.8	3438.7±417.6	54.5±2.2	21.2±1.8[b]	3265.6±440.7[c]	53.1±2.4	21.8±2.2[d]
GCKR rs780094												
TT	3341.2±426.2	53.6±2.3	21.7±1.9	3281.7±448.8	53.1±2.4	21.9±1.8	3488.9±371.9	54.2±2.2	21.9±1.8	2855±125	52±1.8	20.5±2.9
CT+CC	3413.1±436.4	54.1±2.5	21.5±2.2	3303.1±424.3	53.8±2.6	21.1±1.6	3499.3±431.2	54.4±2.4	21.8±2.4	3213.3±393.6	53.5±2.4	21.0±2.1

NB: in table were mentioned only the values of P<.05 or P between .05 and .10, for other comparisons obtaining insignificant results;[a] t(47)=1.87, P=.068; [b]t(91)=2.21, P=.030; [c]t(10.8)=2.03, P=.068 [d]t(14) = 2.03, P = .062.
GCKR rs780094 T>C: glucokinase regulatory protein rs780094 (TT = reference category); CC—homozygous for C allele; CT—heterozygous; TT—homozygous for T allele.
GNB3 rs5443 C>T: guanine nucleotide-binding protein rs5443 (CC = reference category); CC—homozygous for C allele; CT—heterozygous; TT—homozygous for T allele.
PNPLA3 rs738409 G>C: patatin-like phospholipase domain-containing protein 3 rs738409 (CC = reference category); CC—homozygous for C allele; GC—heterozygous; GG—homozygous for G allele.
PI=ponderal index, SD=standard deviation.

polymorphisms, we found no significant differences between the 3 subgroups included in our study.

We noticed differences that tended to be significantly different concerning the values of AST in our 3 subgroups. Thus, mothers with excessive GWG presented higher values of this parameter in comparison to those with normal GWG, (median, IQR: 22.89 [17.53; 31.59] for mothers with excessive GWG versus 22.71 [18.58; 27.37] for mothers with normal GWG).

3.3. Bivariate associations between maternal variant genotypes and birth outcomes by gestational weight gain above and below IOM recommendations

None of the 3 single nucleotide polymorphisms (SNPs) was significantly associated with the assessed neonatal outcomes: birth weight, birth length or PI (P >.05). GWG stratified results for PI were significantly different in pregnant women with excessive GWG, normal GWG, and insufficient GWG. Thus, in normal GWG subgroup, we found no association between the variant genotype of the PNPLA3rs738409 maternal gene polymorphism and neonatal PI. Contrariwise, in pregnant women with excessive GWG, we identified a significant association between the variant genotype of the same polymorphism and PI. Thus, we noticed a decrease of PI in case of pregnant women carrying the variant (mutant) genotype versus those with wild (normal) genotype (Table 3). In pregnant women with GWG below IOM recommendations, PI was generally higher in those with variant genotype in comparison to those with wild genotype, with tendency towards statistical significance (P=.068)

4. Discussions

4.1. The role of GWG in obesity or overweight

Some studies from the literature established that GWG owns a special role in the determinism of birth weight, being defined as a potential risk factor for child's obesity, along with genetic factors, lifestyle and family particularities.[33,34] According to Lau et al,[34] the risk for obesity/overweight increases in pre-school aged children with up to 73% if GWG is higher than the IOM

recommended limits.[11] This fact might be a result of the accumulation of fetal adipose tissue especially during the 2nd trimester of pregnancy, or of the synthesis of fatty free acids from maternal glucose. Also, genetic and environmental factors seem to be more important than the intrauterine ones.[35,36] Spencer et al, in a review from 2015, pointed out that almost 50% of fertile women (25–34 years of age) are obese or overweight, expressing a substantial risk for weight gain in comparison to age-matched males.[37] In our study, 32.9% of women were overweight or obese at the beginning of the pregnancy, and 60% of them had a GWG above the IOM recommended limits. The same review underlined that both GWG and weight gain persistence after pregnancy express a long-term impact in predicting obesity for this group of women.[37] Moreover, excessive GWG increases the risk for preeclampsia, gestational diabetes, stillbirth, and cesarean section need.[37] In addition, an increase of GWG above the recommended limits might result in childhood obesity that can persist during adulthood. Also, it was proven that up to 20% of the women that gain more than 5 kg above the recommended limit develop DM, metabolic syndrome and cardiovascular disorders.[37] Effective communication strategies are mandatory for the prevention of this continuous increase in obesity incidence worldwide.[38] Farah et al proved that the impact of GWG on birth weight was of 5%,[7] while Ferrari et al pointed out a total outcome variance de 8.4%.[39] Faucher et al[40] and a previous study of our team,[33] showed that obese women carry a high risk for increased gestational age and newborns with higher birth weight.

4.2. GNB3 rs5443 SNP and GWG

Several studies underlined the role of genetic determinism in the development of obesity or DM, pre-pregnancy weight, GWG, and postpartum persistence weight gain.[13,41–43] It was proved that the T allele of GNB3 rs5443 SNP is associated with obesity, atherosclerosis, hypertension, or DM,[27–29] while other alleles were not found to be related with this disorders.[31,32,44] The contradictory results were most likely due to the small number of cases, ethnicity or assessed genes. Dishy et al proved that in pregnant women, TT allele of GNB3 gene is associated in Caucasians, Afro-Americans and Hispanics with GWG,[45] while

Gutersohn et al[46] showed in the same group that GWG is related also with the persistence of weight gain after birth. A meta-analysis including *GNB3* gene studies on non-pregnant women proved that TT homozygotes are more susceptible for developing overweight or obesity.[47] The *GNB3* gene seems to be associated with mood and hunger, influencing food intake behavior.[48] Chandrasekaran et al[44] proved that C825T polymorphism [rs5443] of the *GNB3* gene is not related to type 2 DM in Indian population, while Faramarzyian[49] et al proved a positive association between TT genotype of *GNB3* rs5443 gene polymorphism and obesity risk.

A very recent study of Groth et al performed on Afro-American pregnant women showed that CC homozygotes of *GNB3* rs5443 polymorphism presented a weight gain of more than 6 kg when compared to CT heterozygotes.[50] Thus, obese or overweight Afro-American pregnant women that carry the variant allele of this gene have a higher risk for excessive GWG.[50] Similarly, our study found a tendency towards statistical significance between the variant genotype of *GNB3* rs5443 and excessive GWG (60.2% vs 57.1%, $P = .097$). Contrariwise, Groth and Morrison-Beedy found no association between pre-pregnancy BMI or GWG.[41] Ishikawa et al[51] pointed out an association between the same SNP and the levels of total cholesterol. Nevertheless, we found no association between *GNB3* rs5443 SNP and total cholesterol, birth weight or PI.

4.3. PNPLA3 rs738409 SNP and GWG

PNPLA3 gene is related to the development of chronic liver disease and hepatic cirrhosis.[18,52–56] The variant genotype of *PNPLA3* rs738409 is associated with obesity in both adults and children.[18,20–22] Certain studies pointed out the fact that *PNPLA3* gene increases hepatic steatosis by hindering TG decrease.[23] The variant allele of *PNPLA3* rs738409 gene polymorphisms might contribute to the increase of AST and ALT, being correlated with age, gender, BMI and type 2 DM[57,58] in GG homozygotes of this SNP. Moreover, the same gene polymorphism seems to be involved in the development of non-alcoholic steatohepatitis (NASH) or type 1 DM.[57,58] Another study proved that *PNPLA3* rs738409 polymorphism might own a role in obesity determinism and elevated liver transaminases in teenagers, but their long-term evolution might be influenced by a healthy and rigorous lifestyle.[24] Moreover, in patients with complicated obesity, the GG homozygotes of the same gene polymorphisms were positively correlated with glucose, fibrinogen, insulin resistance or NASH.[58] According to Nishioji et al[59] allele G of *PNPLA3* rs738409 gene polymorphism is a risk factor for both non-alcoholic liver disease (NAFLD) and weight gain of over 10 kg in adult Japanese people above the age of 20 years. Similarly, Oniki et al underlined that homozygotes for *PNPLA3* rs738409 gene polymorphisms must be long-term monitored in order to detect the occurrence of NAFLD[60] as a result of long-term obesity.

Bo et al a pointed out on 200 pregnant women with gestational diabetes that G allele of *PNPLA3* SNP rs738409 is associated with elevated transaminases and risk for macrosomic offspring, independent of lifestyle intervention and despite a reduction of insulin resistance.[61] Forbes et al underlined the fact that women with a history of gestational diabetes own a higher risk for type 2 DM and NAFLD, with an unknown mechanism[62] and Kanth et al emphasized that variant alleles of *PNPLA3* SNPs were associated with high levels of ALT and TG.[63] Tai et al also

demonstrated that the GG genotype of *PNPLA3* rs738409 increased the susceptibility for NASH in individuals with severe obesity and NAFLD, being correlated with the degree of NAFLD.[64] In our study, we noticed a tendency towards statistical significance regarding AST values in case of mothers with excessive GWG versus those with normal GWG. We found only 5 cases (3.2%) with gestational diabetes in our study sample, thus we were not able to statistically analyze this pathology. In another study of Tai et al (2016), the authors showed that GG genotype *PNPLA3* rs738409 had an additional negative impact on NASH in Taiwanese obese patients.[65] Contrariwise, in our study, regarding the *PNPLA3* rs738409 SNP, we noticed that in pregnant women with GWG below the IOM recommendations, the PI was generally higher in those with variant genotype as compared to those carrying the wild-type homozygous genotype, with a tendency towards statistical significance.

4.4. GCKR rs780094 SNP and GWG

GCKR is involved in the regulation of glucokinase (GCK) activity, with a role in glucose homeostasis, being associated with both diabetes mellitus and gestational diabetes.[66] The *GCKR* rs780094 SNP leads to the decrease of blood glucose levels and increase of TG ones through the stimulation of lipogenic effect genes.[66]

The study of Lin et al found that carriers of variant genes of both *GCKR* rs780094 and *PNPLA3* rs738409 gene polymorphisms in Taiwanese children present an increased risk for NAFLD.[67,68] Also, Sliz et al proved that T allele of *GCKR* rs780094 SNP owns an increased risk for developing NAFLD in adult people.[69]

Angheben-Oliviera et al proved in a study performed on 252 pregnant women carrying the C allele of *GCKR* rs780094 gene polymorphism have 1.41 folds higher risk for developing gestational diabetes versus control group.[70] Even though Garner et al pointed out that variant allele of *GCKR* rs780094 gene polymorphism is associated with high levels of ALT and AST, in our study, we found no similar associations. Other studies performed on adult populations proved that the carriers of variant allele of *GCKR* rs780094 SNP have an increased risk for NAFLD.[67–69] Moreover, the same allele was also related to NASH and cystic fibrosis in children and teenagers.[26] Contrariwise, Hovsepian proved that CT genotype of *GCKR* rs780094 SNP has a protective effect regarding obesity development.[25] The study of Murata-Mori performed on Japanese, showed that TT homozygotes of *GCKR* rs780094 gene polymorphism present lower levels of glycated hemoglobin in community-dwelling men, but not in women.[71] Contrariwise, carriers of C allele of the same SNP have a higher risk for developing gestational DM.[70] Similarly, Huopio et al underlined on 533 Finish pregnant women[72] along with Stuebe et al[73] in Caucasian and Afro-American women that C allele of this polymorphism is associated with gestational diabetes. In our study, we found no associations between *GCKR* rs780094 gene polymorphism and liver transaminases, TG, lipid profile, type 2 DM, birth weight or PI.

Our limitations regarding this research comprise the relatively small number of cases assessed in the present study since not 200 couples included initially fulfilled the inclusions criteria, and we were forced to exclude those with the gestational age below 37 weeks and twin gestation, remaining 158 cases. This fact might have lowered the statistical power of our study. Another

limitation consists in the fact that the population included in our study originates from a single area of Romania, and a single area of Europe. Moreover, our research lacks the assessment of maternal food habits, maternal complications, environmental factors and the maternal postpartum fat mass that could represent important factors in birth weight determinism. The *PNPLA3* rs738409, *GCKR* rs780094, and *GNB3* rs5443 gene polymorphisms should be assessed also in the newborns' father, and not only in their mother. In addition, it would be of major clinical and scientific value to extend the study on a bigger geographic area from Europe.

The strengths of our study consist in the accuracy of data collection at the time of birth for all mothers and their offspring admitted in a tertiary Obstetrics-Gynecology Clinic that was consecutively included in this study independent of any conveniences. This fact is of major interest because it really improved knowledge on the role of these studied SNPs regarding the neonatal risk for obesity and their impact on PI and fetuses' birth weight. The BMI was calculated taking into account the measured weight and height, and not using self-reported weight and height. All clinical, laboratory, anthropometric and genetic parameters were assessed in both mothers and newborns. We must underline that this is the first cross-sectional study in Romania that assessed the associations between *PNPLA3* rs738409, *GCKR* rs780094, and *GNB3* rs5443 gene polymorphisms and GWG. Moreover, we established the relationship between these 3 gene polymorphisms and the newborn's PI and birthweight.

Besides all the aforementioned facts, to the best of our knowledge, no similar studies were reported in the literature. Thus, this study might be considered a pilot one that must be extended on a bigger population. Moreover, these parameters should be assessed also transversally on a longer period of time in order to evaluate supplementary maternal risk factors and the newborns' nutritional status further in life. Thus, our intention is to follow-up the mothers and their infants for at least 2 years in order to assess the evolution of their nutritional status. Nevertheless, it would be ideal if we could monitor the pediatric subjects until adolescence in order to assess their weight during the critical periods.

5. Conclusions

Excessive GWG was noticed in women that were overweight or obese before pregnancy. We found a significant association between the variant genotype of *GNB3* rs5443 and excessive GWG. Moreover, we identified a tendency towards statistical significance between AST levels on the 3 subgroups, encountering higher values for pregnant women with excessive GWG. The variant genotype of *PNPLA3* rs738409 was significantly associated with a smaller neonatal PI in comparison to the wild genotype. In the subgroup of pregnant women with GWG below IOM recommendations, their offspring's PI was generally higher in those carrying the variant genotype of *PNPLA3* rs738409 SNP as compared to the wild-type genotype carriers. Our study pointed out the most important factors that influence GWG, PI, and related birth outcomes, but further studies on bigger groups and extended geographic areas are necessary.

Author contributions

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