Isoleucine-to-methionine substitution at residue 148 variant of *PNPLA3* gene and metabolic outcomes in gestational diabetes[1–4]

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ABSTRACT

Background: A single nucleotide polymorphism (SNP) of the patatin-like phospholipase-3 (*PNPLA3*)/adiponutrin gene (rs738409 C>G) is strongly associated with nonalcoholic fatty liver disease; to our knowledge, no data are available on the impact of this *PNPLA3* SNP on liver and metabolic outcomes during pregnancy in patients with gestational diabetes (GD).

Objective: We evaluated the impact of the *PNPLA3* rs738409 SNP on liver enzymes, metabolic indexes, and maternal and neonatal outcomes in 200 GD patients enrolled in a lifestyle intervention.

Design: In a randomized trial with a 2 × 2 factorial design, exercise significantly improved maternal and neonatal outcomes in GD patients. Effects of the G allele on metabolic and liver indexes and maternal and neonatal outcomes were evaluated in these patients.

Results: At the end of the trial, fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR) values were significantly lower and liver enzymes significantly higher in *PNPLA3* G-allele carriers. In a multiple regression model, the G allele was associated directly with aspartate aminotransferase (β = 2.60; 95% CI: 0.99, 4.20), alanine aminotransferase (β = 3.70; 95% CI: 1.78, 5.62), and γ-glutamyl transferase (β = 3.70; 95% CI: 0.80, 6.60) and inversely with insulin (β = −2.01; 95% CI: −3.24, −0.78) and HOMA-IR (β = −0.39; −0.64, −0.14) values at the end of the trial. In a multiple logistic regression model, the G allele was associated directly with risk of developing liver enzyme elevation during pregnancy (OR: 4.21; 95% CI: 1.78, 9.97) and inversely with the birth of large-for-gestational-age newborns (OR: 0.19; 95% CI: 0.06, 0.62). No diet × genotype or exercise × genotype interaction was shown.

Conclusion: The *PNPLA3* SNP rs738409 G allele was associated with risk of mildly elevated transaminases in GD independent of a lifestyle intervention and despite a significant reduction in insulin resistance and risk of macrosomic offspring. This trial was registered at clinicaltrials.gov as NCT01506310. *Am J Clin Nutr* 2015;101:310–8.

Keywords NAFLD, *PNPLA3*, fatty liver, liver enzymes, GDM, fatty liver, transaminase, ALT, trial

INTRODUCTION

In pregnancy, metabolic changes in maternal adipose tissue ensure regular fetal growth. During the first months, an accumulation in fat depots occurs, whereas during the last trimester, the increased insulin resistance and breakdown of fat stores result in higher circulating values of free fatty acids, triglycerides, and glucose to sustain fetal metabolism (1, 2). In pregnancies complicated by gestational diabetes (GD),[5] an impaired expression of genes involved in the synthesis and accumulation of triglycerides in the adipose tissue was described (2), and the physiologic decrease in insulin sensitivity that occurs with advancing gestation is further enhanced, resulting in an excessive nutrient availability for the fetoplacentar unit, higher ambient insulin and lipid concentrations, and fetal overgrowth (3, 4).

Several population-based and genome-wide association studies connected a single nucleotide polymorphism (SNP) of the patatin-like phospholipase-3 (*PNPLA3*)/adiponutrin gene (rs738409 C>G), encoding for the isoleucine-to-methionine substitution at residue 148 (I148M) protein variant, to the presence and severity of nonalcoholic fatty liver disease (NAFLD), which is the most-common chronic liver disease and an emerging risk factor for type 2 diabetes, cardiovascular disease, and liver-related complications (5–7). Mechanisms that underlie this association are still poorly understood but appear to be independent of insulin resistance because a substantial proportion of NAFLD patients with the *PNPLA3* I148M variant do not show the hypertriglyceridemia, hyperinsulinemia, insulin resistance, or inflammation observed in the general NAFLD population (7–11).

Women with a history of GD have increased lifetime risk of developing type 2 diabetes) and NAFLD (12), but to our knowledge, mechanisms that underlie the association of GD with an increased

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[3] Supplemental Table 1 is available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[5] Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; GD, gestational diabetes; GGT, γ-glutamyl transferase; Hb A_{1c}, glycated hemoglobin; HELLP, hypertension, elevated liver enzymes and low platelets; I148M, isoleucine-to-methionine substitution at residue 148; LGA, large for gestational age; MET, metabolic equivalent of the activity; NAFLD, nonalcoholic fatty liver disease; *PNPLA3*, patatin-like phospholipase-3; SNP, single nucleotide polymorphism; SREBP-1c, sterol regulatory element binding protein 1c.

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liver fat content are unknown. During normal pregnancies, and even in pregnancies complicated by GD, serum transaminase and γ-glutamyl transferase (GGT) concentrations do not change (13–15), and GGT may even slightly decline in late pregnancy as a consequence of the inhibition of hepatic GGT synthesis by sex hormones. Conversely, minor increases in alanine aminotransferase (ALT) and GGT concentrations in pregnancy may be a marker of abnormal liver fat accumulation and NAFLD (16). We hypothesized functional variants in *PNPLA3*, which is a key gene involved in lipid metabolism, could affect risk of developing NAFLD as assessed by a liver enzyme elevation and metabolic/pregnancy outcomes during GD. The knowledge of whether the *PNPLA3* polymorphism affects live, metabolic, and inflammatory variables or maternal and fetal outcomes in pregnant women with GD would have relevant preventive, therapeutic, and prognostic implications.

In a recent randomized trial that enrolled 200 GD patients, we showed that a 20-min/d brisk walking reduced maternal postprandial glucose, glycated hemoglobin (Hb A_{1c}), C-reactive protein (CRP), triglycerides, and any maternal and neonatal complications (17). We took advantage of data collected in this trial to assess the impact of *PNPLA3* SNP rs738409 on metabolic variables, liver enzymes and maternal and neonatal outcomes in GD patients.

SUBJECTS AND METHODS

Participants

All pregnant women who were attending the Sant'Anna Hospital (Torino) from July 2009 to February 2012 were enrolled. The inclusion criteria were as follows: 18–50 y of age, 24th–26th weeks of gestational age, a singleton pregnancy, and a GD diagnosis on the basis of a 75-g oral glucose tolerance test in line with the International Association of Diabetes and Pregnancy Study group deliberations based on the Hyperglycemia and Adverse Pregnancy Outcome study results (18) in accordance with subsequent guideline recommendations (19). Exclusion criteria were as follows: BMI (in kg/m^2) >40, any known diseases (included acute or chronic liver diseases of any causes), medication use before GD diagnosis, or obstetrical absolute or relative contraindications to exercise. All participants had to have normal liver enzymes and no known chronic liver disease at enrollment.

The approval of the local Ethical Committee and the written informed consent of participants were obtained. Procedures were in compliance with the Helsinki Declaration principles, as revised in 2008. This trial was registered at clinicaltrials.gov as NCT01506310.

Outcomes

The primary outcome was the difference at the end of the trial in serum ALT, aspartate aminotransferase (AST), and GGT concentrations and liver enzyme elevation across different *PNPLA3* I148M genotypes. We defined liver enzyme elevation as an ALT concentration ≥30 IU/L and AST concentration ≥30 IU/L and/or GGT concentration ≥40 IU/L according to emerging evidence suggesting a lower cutoff value for normality to detect NAFLD in the general population and pregnant women (20).

As secondary outcomes, we investigated the impact of the *PNPLA3* I148M genotype on values at the end of the trial of fasting triglycerides, insulin, HOMA-IR, CRP, Hb A_{1c}, postprandial glucose and the need for insulin therapy, percentages of maternal complications (pregnancy-induced hypertension, preeclampsia, eclampsia, acute fatty liver or hypertension, elevated liver enzymes and low platelets (HELLP) syndrome, intrahepatic cholestasis of pregnancy, postpartum hemorrhage, and placental abruption), cesarean deliveries, preterm and large-for-gestational-age (LGA) newborns, neonatal complications (hyperbilirubinemia and hypoglycemia), the newborn in-hospital stay (>4 d), and the occurrence of one or more than one of these conditions (any complications).

Intervention

An individually prescribed diet was given to each woman (carbohydrates: 48–50%; proteins: 18–20%; fats: 30–35%; fiber: 20–25 g/d; and no alcohol). The behavioral recommendations group received individual oral and written recommendations aimed at helping with healthy dietary choices. The exercise group was advised to briskly walk ≥20-min/d every day (140-min/wk; Borg's scale target rating: 12–14) (21). The behavioral recommendations plus exercise group was prescribed brisk walking ≥20-min/d every day along with the same recommendations of the behavioral recommendations group. The group diet only received no other recommendations.

Because we previously showed that exercise was effective, whereas behavioral recommendations failed to show beneficial effects on maternal and neonatal outcomes in these patients, we considered the following 2 groups: the no exercise group (i.e., diet-only group plus behavioral recommendations group) and exercise group (i.e., exercise group plus behavioral recommendations plus exercise group). Patients were evaluated at 24–26th gestational weeks at baseline and at the 38th week or before delivery (if delivery was preterm) at the end of the study.

Measurements

Participants completed a validated semiquantitative food-frequency questionnaire (22) and the Minnesota-Leisure-Time-Physical-Activity questionnaire (23) at enrollment and trial end. The leisure physical activity level of the previous month was calculated as the product of the duration and frequency of each activity (in h/wk), weighted by an estimate of the metabolic equivalent of the activity (METs), and summed for activities performed.

Patients were monitored by weekly phone calls and visited every 2 wk by the physicians to monitor adverse events and protocol adherence. Patients self-monitored capillary blood glucose 4–6 times/d (preprandial and 2 h postprandial) by using the Accu-Chek glucometer (Roche Diagnostics).

Insulin treatment was prescribed by the physicians in the presence of an ultrasound finding of fetal macrosomia (abdominal circumference >70th percentile) and/or hyperglycemia. Other than insulin, hypoglycemic agents were not prescribed to the women.

Maternal complications included pregnancy-induced hypertension, preeclampsia, eclampsia, acute fatty liver, or HELLP syndrome (as defined by Italian guidelines available at http://www.sigo.it/linee-guida-sigo/linee-guida-nazionali.php), intrahepatic cholestasis of pregnancy (defined in line with guideline available at http://www.rcog.org.uk/files/rcog-corp/GTG43obstetriccholestasis.pdf), postpartum hemorrhage, and placental abruption.

Data relative to neonatal outcomes were derived from medical records. Babies were classed as LGA if their birth weights were greater than the 90th percentile, with consideration of neonatal anthropometric standards for Northern Italy (24). Births were defined as preterm if maternal gestational age at delivery was <37 wk. Neonatal hyperbilirubinemia and hypoglycemia diagnoses were collected from clinical records.

Prepregnancy weight was recorded from patient recall; weight and height were measured. Fasting glucose, Hb A_{1c}, total cholesterol, HDL cholesterol, triglycerides, insulin, and CRP values were determined before and after the study. Baseline and trial-end postprandial glucose values were the mean 2-h postprandial values reported by patients in the first and last weeks of the trial, respectively. Serum glucose concentrations were measured by using the glucose oxidase method, plasma total cholesterol, HDL cholesterol, and triglyceride values were measured by using an enzymatic colorimetric assay, and CRP values were measured by using a high-sensitivity latex agglutination assay (HITACHI 911 Analyzer; Hitachi Ltd.). The serum insulin concentration was determined by using a solid-phase ELISA kit (LDN); Hb A_{1c} was measured with direct latex-enhanced determination by Sentinel. AST, ALT, and GGT concentrations were measured by using a kinetic determination according to International Federation of Clinical Chemistry and Laboratory Medicine recommendations (HITACHI 911 Analyzer; Hitachi Ltd.).

DNA was isolated from whole blood by using an UltraClean BloodSpin DNA isolation kit according to the instructions of the manufacturer (MOBIO, distributed by CABRU).

Genotyping for the *PNPLA3* SNP rs738409 used the real-time allele-discrimination method, by using a TaqMan Allelic Discrimination Assay (Applied Biosystems). The TaqMan genotyping reaction was run on a 7300HT Fast Real-Time polymerase chain reaction system (Applied Biosystems).

The HOMA-IR was calculated according to a published algorithm (25). All laboratory measurements were centralized and blindly performed.

Random assignment

The random assignment was stratified by baseline BMI and METs and was implemented through a website (www.epiclin.it).

Blinding

Dietitians who evaluated questionnaires, obstetricians who reported maternal and neonatal complications, and laboratory personnel who performed the biochemical analyses were blinded to the group assignment.

Statistical analyses

To our knowledge, there is no data available on the prevalence of *PNPLA3* genotypes in pregnancy. Therefore, we based our analysis on data from the general population; with a *PNPLA3* rs738409 G allele frequency of 33% and a prevalence of NAFLD of 25% and assuming an effect size for the G allele ≥3 (OR for NAFLD), ≥192 subjects were needed to detect a significant ($P < 0.05$) difference in the incidence of liver enzyme elevation (see Introduction section) between *PNPLA3* genotypes with a power of 80% (7–11).

The normal distribution of data were assessed by using the Kolmogorov-Smirnov normality test; METs, triglycerides, CRP values, and before-after changes in all variables showed skewed distributions.

Women were divided according to genotype (GG, CG, or CC). Concordance of the frequency of allele distribution to the frequency predicted by the Hardy-Weinberg equilibrium was analyzed by using the chi-square test. An ANOVA and chi-square test were performed to assess differences in continuous and categorical variables, respectively. Tukey's post hoc test was used to perform comparisons between genotypes when the overall *P* value was significant. Differences in groups for not-normally distributed variables were analyzed by using the Kruskal-Wallis test. Because of the small number of homozygous GG carriers and similar baseline characteristics between homozygous GG and heterozygous CG carriers, these 2 groups were combined together in regression models. Variables that significantly differed at follow-up between groups were analyzed in a multiple regression model. Continuous variables were analyzed by using a multiple linear regression model after the log transformation of skewed variables. The impact of the *PNPLA3* genotype on NAFLD was analyzed with consideration of liver enzymes as both a continuous variable and dichotomous variable (i.e., liver enzyme elevation, as defined previously in the Outcome section). Dichotomous outcome variables were analyzed by using a standard multivariable logistic regression model. We planned a priori to adjust all analyses for the following covariates: age, prepregnancy BMI, weight change during the follow-up period, belonging to an exercise group, and baseline value of the variable.

A linear regression was used to evaluate for a diet × genotype or exercise × genotype interaction on liver enzymes and metabolic variables. Genotype groups were multiplied by the dietary variable (total energy, percentages of daily macronutrients, and fiber) or log METs (h/wk), and the interaction was introduced in the model as an independent variable. A logistic regression model was used to evaluate if a diet × genotype or exercise × genotype interaction was present on maternal and neonatal complications (STATISTICA software 5.1; Statsoft Italia).

RESULTS

In our cohort, the frequency of the G allele of the SNP rs738409 was 0.28, which was similar to other Italian and European data (8, 26). The SNP was in Hardy-Weinberg equilibrium (chi-square test; $P > 0.05$). Carriers of the different *PNPLA3* genotypes were equally distributed in the intervention groups (**Table 1**). No significant difference was evident between *PNPLA3* genotypes during prepregnancy and at 24–26 wk of gestational age (**Tables 2** and **3**). At the end of the trial, fasting insulin and HOMA-IR values were significantly lower in carriers of the I148M variant (Table 3). Furthermore, G carriers showed increased values of AST, ALT, and GGT (Table 3). The prevalence of LGA newborns was significantly reduced in the presence of the G allele (**Table 4**).

In a multiple linear regression model, the SNP rs738409 G allele was associated directly and independently with AST (β = 2.60; 95% CI: 0.99, 4.20), ALT (β = 3.70; 95% CI: 1.78, 5.62), and GGT (β = 3.70; 95% CI: 0.80, 6.60) and inversely with insulin (β = −2.01; 95% CI: −3.24, −0.78) and HOMA-IR (β = −0.39; 95% CI: −0.64, −0.14) values at the end of the trial (**Table 5**). In a multiple logistic regression model, the G allele was directly and independently associated with risk of developing liver enzyme elevation during the course of pregnancy (OR: 4.21; 95%

TABLE 1
Distribution of patients in intervention groups by *PNPLA3* genotypes[1]

	No exercise			Exercise		
Groups	Diet only	Behavioral recommendations	Total	Exercise	Exercise plus behavioral recommendations	Total
n	50	49	99	51	50	101
GG, %	8.0	8.2	8.1	7.8	6.0	6.9
CG, %	40.0	36.7	38.4	43.1	42.0	42.6
CC, %	52.0	55.1	53.5	49.0	52.0	50.5

[1]*PNPLA3*, patatin-like phospholipase-3.

CI: 1.78, 9.97), and inversely associated with the birth of LGA newborns (OR: 0.19; 95% CI: 0.06, 0.62) (Table 5). No case of acute fatty liver, preeclampsia, eclampsia, or HELLP syndrome occurred; only one women (CC genotype) developed intrahepatic cholestasis of pregnancy, and 6 women had pregnancy-induced hypertension (2 women for each genotype group).

Adherence to the nutritional counseling did not differ between intervention groups and between *PNPLA3* genotypes at the end of the trial: 66.7%, 67.9%, and 65.4%, respectively, in GG, CG, and CC genotypes. Adherence to the exercise recommendations was 46.7%, 49.4%, 48.1% in the 3 genotypes, respectively. Anthropometric and laboratory values at the end of the trial by genotype (G allele compared with non–G allele) and intervention (exercise compared with no exercise) groups are presented in **Supplemental Table 1**. Fasting insulin and HOMA-IR values were significantly lower, and ALT concentrations increased, in carriers of the I148M variant independently of exercise.

No diet × genotype interaction was shown for liver enzymes, metabolic variables, and maternal and neonatal complications. Similarly, no exercise × genotype interaction was shown for liver enzymes, metabolic variables, and maternal and neonatal complications.

DISCUSSION

We showed that, in GD patients who were undergoing a lifestyle intervention, the I148M variant of the *PNPLA3* gene was associated with increased risk of developing liver enzyme elevation; furthermore, G-allele carriers significantly improved their insulin resistance and gave birth to fewer LGA newborns than did carriers of the C allele independently of the intervention, weight change, and other confounders.

TABLE 2
Baseline values by *PNPLA3* genotypes[1]

	GG	CG	CC	*P*
n	15	81	104	
Age, y	36.1 ± 5.0[2]	35.2 ± 4.4	34.9 ± 5.0	0.63
Exercise groups, %	46.7	53.1	49.0	0.82
Nulliparous, %	30.0	35.8	35.6	0.87
Smoking, %	30.0	34.6	31.7	0.85
Pregestational weight, kg	63.9 ± 12.8	67.5 ± 11.9	66.7 ± 11.7	0.55
Pregestational BMI, kg/m^2	23.9 ± 4.2	25.0 ± 4.2	25.2 ± 4.5	0.59

[1]*P* values were evaluated by using an ANOVA or chi-square test. *PNPLA3*, patatin-like phospholipase-3.
[2]Mean ± SD (all such values).

PNPLA3 and liver fat accumulation

PNPLA3 is a protein with thioesterase, triacylglycerol hydrolase, and acylglycerol transacetylase activity (27), which can be shown in the adipose tissue and the liver. PNPLA3 expression is under nutritional and genetic control, whereby it is downregulated by fasting and upregulated by feeding through an insulin-mediated mechanism (28). Furthermore, the PNPLA3 I148M variant exhibits a loss of the lipase and increase of the acyltransferase activity with a resultant inhibition of lipolysis and enhancement of cellular triglyceride storage (8, 29–31). These properties, together with an impaired hepatic triglyceride-rich VLDL secretion (32–36), may at least partially explain the strong association of the PNPLA3 I148M variant with NAFLD (37).

We showed that, in GD patients, the *PNPLA3* G allele is significantly and independently associated with risk of developing liver enzyme elevation over the course of a pregnancy. Growing evidence suggests that liver enzyme concentrations do not change during normal or GD pregnancies, and even minor increases in ALT and GGT concentrations may reflect liver fat accumulation and NAFLD. Several potential mechanisms may underlie this association. Different from other cohorts, in whom NAFLD seems to be driven by the severity of insulin resistance and magnitude of adipose tissue accumulation (38), in our GD patients, liver enzyme elevation was unrelated to baseline or gestational changes in body weight or HOMA index. Most importantly, lifestyle interventions that effectively improved metabolic variables in GD did not prevent liver enzyme elevation in patients who were genetically predisposed to NAFLD. Therefore, other specific environmental stressors may have interacted with *PNPLA3* polymorphisms in our population. Dietary carbohydrates and omega-6 and omega-3 polyunsaturated fatty acids can modulate effects of PNPLA3 I148M on cellular triglyceride accumulation (39, 40), but we did not find any difference in dietary intake of these nutrients at baseline and throughout the trial across *PNPLA3* genotypes.

A more likely acquired factor that could interact with *PNPLA3* in the promotion of NAFLD is the increased production of sexual hormones during pregnancy. Consistently, sterol regulatory element binding protein 1c (SREBP-1c) enhances *PNPLA3* gene transcription and inhibits its degradation through the stimulation of fatty acid synthesis. SREBP-1c is a target gene of the liver X receptor/retinoid X receptor heterodimer, and both SREBP-1c and the liver X receptor/retinoid X receptor regulate the transcription of *PNPLA3* (41, 42). Because estrogens downregulate both SREBP-1c and liver X receptor expression (43), sex hormonal changes of late pregnancy may have interacted with the mutant *PNPLA3* to promote liver enzyme elevation in at-risk PNPLA3 genotypes (Table 3).

TABLE 3
Lifestyle, anthropometric, and laboratory values by *PNPLA3* genotypes[1]

	GG (n = 15)	CG (n = 81)	CC (n = 104)	P
Lifestyle characteristics				
METs, h/wk				
At baseline[2]	21.0 (16.5)	19.5 (10.5)	18.8 (12.0)	0.64
At end of trial[2]	22.0 (10.0)	23.0 (13.0)	23.0 (15.5)	0.74
Total energy, kcal/d				
At baseline	2279.0 ± 193.8	2129.7 ± 310.6	2100.7 ± 348.1	0.11
At end of trial	1816.0 ± 110.2	1769.6 ± 136.3	1763.9 ± 115.3	0.32
Protein, % of kcal/d				
At baseline	15.8 ± 2.1	15.4 ± 2.4	15.6 ± 2.7	0.80
At end of trial	18.9 ± 2.3	18.8 ± 2.3	19.0 ± 1.9	0.91
Total fat, % of kcal/d				
At baseline	37.0 ± 3.6	37.5 ± 4.0	37.0 ± 4.6	0.73
At end of trial	35.4 ± 2.9	35.2 ± 3.6	34.8 ± 2.8	0.56
Saturated fat, % of kcal/d				
At baseline	12.0 ± 2.4	11.0 ± 2.3	11.3 ± 2.7	0.30
At end of trial	10.9 ± 1.0	10.5 ± 1.4	10.3 ± 1.1	0.17
Carbohydrates, % of kcal/d				
At baseline	48.2 ± 4.4	46.7 ± 5.1	47.4 ± 6.7	0.56
At end of trial	45.6 ± 2.6	46.1 ± 2.9	46.7 ± 2.5	0.18
Fiber, g/d				
At baseline	18.9 ± 1.9	19.8 ± 4.6	18.9 ± 4.6	0.30
At end of trial	23.3 ± 3.2	22.1 ± 2.3	22.8 ± 3.3	0.16
Sodium, mg/d				
At baseline	3246.1 ± 511.1	3441.8 ± 702.3	3497.9 ± 606.8	0.35
At end of trial	3014.0 ± 544.5	3300.4 ± 796.2	3323.4 ± 639.0	0.27
Alcohol users at baseline, %	26.7	22.2	24.0	0.92
Alcohol at baseline, g/d	10.2 ± 3.8	10.6 ± 6.6	11.6 ± 5.0	0.82
Alcohol users at end of trial, %	0	0	0	
Anthropometric values				
Weight, kg				
At baseline	67.2 ± 10.1	72.1 ± 11.8	72.7 ± 11.1	0.21
At end of trial	70.5 ± 11.9	73.4 ± 12.1	74.2 ± 11.3	0.50
Change[2]	2.0 (4.0)	1.0 (2.5)	1.5 (3.0)	0.56
BMI, kg/m^2				
At baseline	25.2 ± 3.5	26.7 ± 4.2	27.4 ± 4.2	0.13
At end of trial	26.5 ± 4.5	27.2 ± 4.3	28.0 ± 4.2	0.28
Change[2]	0.78 (1.57)	0.40 (1.03)	0.55 (1.19)	0.55
Laboratory values				
Fasting glucose, mg/dL				
At baseline	78.1 ± 12.3	76.5 ± 12.4	76.8 ± 11.2	0.90
At end of trial	74.3 ± 5.6	72.7 ± 9.1	73.5 ± 12.0	0.81
Change[2]	−3.0 (10.0)	−4.0 (14.0)	− 4.5 (12.0)	0.98
Postprandial glucose, mg/dL				
At baseline	123.1 ± 20.3	120.2 ± 19.9	123.2 ± 20.2	0.58
At end of trial	111.7 ± 17.4	110.6 ± 18.8	112.4 ± 18.8	0.81
Change[2]	0.0 (11.0)	0.0 (18.0)	0.0 (21.5)	0.90
Hb A_{1c}, %				
At baseline	5.0 ± 0.4	4.8 ± 0.4	4.9 ± 0.5	0.49
At end of trial	4.9 ± 0.3	4.7 ± 0.5	4.8 ± 0.4	0.56
Change[2]	0.0 (0.3)	0.0 (0.5)	0.0 (0.6)	0.91
Total cholesterol, mg/dL				
At baseline	254.9 ± 30.0	255.5 ± 42.2	256.0 ± 37.4	0.99
At end of trial	253.1 ± 42.2	253.1 ± 43.0	257.7 ± 44.0	0.76
Change[2]	0.0 (37.0)	0.0 (39.0)	3.0 (32.5)	0.98
HDL cholesterol, mg/dL				
At baseline	71.5 ± 14.2	72.5 ± 14.9	69.4 ± 11.4	0.27
At end of trial	70.6 ± 13.6	72.5 ± 14.8	71.2 ± 11.6	0.77
Change[2]	0.0 (3.0)	0.0 (5.0)	1.0 (4.5)	0.07
Triglycerides, mg/dL				
At baseline[2]	160 (68.0)	160 (61.0)	166.5 (68.0)	0.68
At end of trial[2]	165 (83.0)	157 (71.0)	157 (59.5)	0.52

(Continued)

TABLE 3 (*Continued*)

	GG (n = 15)	CG (n = 81)	CC (n = 104)	P
Change[2]	−1.0 (29.0)	1.0 (43.0)	1.0 (40.0)	0.72
Fasting insulin, μU/mL				
At baseline[2]	12.0 ± 5.2	12.3 ± 5.0	12.4 ± 4.4	0.97
At end of trial[2]	8.6 ± 3.1[3]	10.1 ± 4.2[3]	12.1 ± 5.4	0.003
Change[2]	−2.4 (7.4)[3]	−1.4 (4.8)[3]	−0.3 (5.7)	0.015
HOMA-IR, mmol/L × μU/mL				
At baseline[2]	2.4 ± 1.2	2.4 ± 1.1	2.4 ± 0.9	0.99
At end of trial[2]	1.6 ± 0.6[3]	1.8 ± 0.9[3]	2.2 ± 1.1	0.008
Change[2]	−0.64 (1.83)[3]	−0.35 (0.91)[3]	−0.22 (1.17)	0.04
CRP, mg/L				
At baseline[2]	3.7 (5.1)	3.5 (3.6)	3.9 (4.2)	0.63
At end of trial[2]	3.2 (3.7)	3.0 (4.3)	3.2 (3.9)	0.78
Change[2]	−0.30 (1.62)	−0.31 (1.91)	−0.34 (1.45)	0.99
AST, IU/L				
At baseline	21.9 ± 2.8	21.6 ± 2.5	21.7 ± 3.2	0.93
At end of trial	33.7 ± 13.4[3,4]	23.9 ± 4.3	22.9 ± 3.6	<0.001
Change[2]	8.0 (27.0)[3,4]	1.0 (5.0)	1.0 (2.0)	0.001
ALT, IU/L				
At baseline	22.1 ± 2.5	21.2 ± 2.9	21.0 ± 3.4	0.46
At end of trial	37.2 ± 15.4[3,4]	23.1 ± 5.0	21.6 ± 3.8	<0.001
Change[2]	19.0 (33.0)[3,4]	1.0 (5.0)	0.0 (3.0)	0.005
GGT, IU/L				
At baseline	23.8 ± 5.9	22.7 ± 6.5	22.5 ± 5.8	0.75
At end of trial	35.1 ± 15.3[3,4]	25.1 ± 9.3	23.0 ± 9.9	<0.001
Change[2]	6.0 (28.0)[3,4]	0.0 (5.0)	0.0 (3.0)	0.002
Liver enzyme elevation, %				
At baseline[5]	0	0	0	
At end of trial[5]	60.0[3,4]	19.8[3]	7.7	<0.001

[1]Data are presented as means ± SDs for normally distributed variables and medians (IQRs) for nonnormally distributed variables. Change values are for differences between end-of-the-trial values minus baseline values of variables. P values were evaluated by ANOVA or chi-square test unless otherwise noted. The Tukey's post hoc test was used to perform comparisons between genotypes. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; GGT, γ-glutamyl transferase; Hb A_{1c}, glycated hemoglobin; MET, metabolic equivalent of the activity; *PNPLA3*, patatin-like phospholipase-3.

[2]P values were evaluated by Kruskal-Wallis test.

[3]$P < 0.05$ compared with CC.

[4]$P < 0.05$ compared with CG.

[5]Liver enzyme elevation: ALT concentration ≥30 IU/L and/or AST concentration ≥30 IU/L and/or GGT concentration ≥40 IU/L.

PNPLA3 and insulin resistance

The I148M mutation did not correlate with circulating concentrations of lipids, glucose, CRP and insulin sensitivity or visceral fat indexes (44–46). Rather, during the intervention, G-allele carriers showed a significant reduction of insulinemia and insulin resistance as estimated by using the HOMA-IR index, even if their weight change was not different from that of C allele carriers. In accordance with the lower insulin resistance, these G-allele carriers gave birth to an ∼4-fold lower percentage of LGA newborns. A protective effect of the G allele against insulin resistance was reported by several authors. This phenomenon was ascribed to the increased transacetylase and defective lipase activities of the mutant PNPLA3 protein, which enhance the storage of lipotoxic free fatty acids and diacylglycerol into metabolically neutral triglycerides (47). The lack of adipose tissue inflammation in NAFLD because of PNPLA3 I148M, as opposed to those observed in overweight/obesity–associated NAFLD, may, therefore, protect against insulin resistance and other features of the metabolic syndrome in these subjects. Finally, a role of PNPLA3 in hepatocyte triglyceride qualitative remodeling has been proposed. whereby the I148M mutation led to a relative depletion of long-chain and very-long-chain PUFAs coupled with an increase in MUFAs, which are less prone to oxidation and metabolic inflammation (48). Therefore, it could be hypothesized that the metabolic and/or hormonal changes of the last trimester of pregnancy may interact with the I148M

TABLE 4
Maternal and neonatal outcomes by *PNPLA3* genotypes[1]

	GG	CG	CC	P
n	15	81	104	—
Insulin treatment, %	6.7	4.9	8.7	0.62
Maternal complications, %	7.4	6.7	6.7	0.98
Cesarean deliveries, %	13.3	19.8	21.2	0.78
Preterm newborns, %	6.7	4.9	3.9	0.86
Large-for-gestational-age newborns, %	0	4.9[2]	17.3	0.01
Neonatal complications, %	6.7	6.2	2.9	0.52
Hospital stay >4 d, %	20.0	32.1	21.2	0.21
Any complications,[3] %	46.7	51.9	48.1	0.86

[1]P values were evaluated by using the chi-square test. *PNPLA3*, patatin-like phospholipase-3.

[2]$P < 0.05$ compared with CC.

[3]One or >1 of all conditions listed.

TABLE 5
Association of variables at the end of the trial with the G allele in a multiple regression model and logistic regression model[1]

	Values	*P*
Multiple linear regression model[2]		
Insulin, μU/mL	−2.01 (−3.24, −0.78)	0.002
HOMA-IR, mmol/L × μU/mL	−0.39 (−0.64, −0.14)	0.004
AST, IU/L	2.60 (0.99, 4.20)	0.002
ALT, IU/L	3.70 (1.78, 5.62)	<0.001
GGT, IU/L	3.70 (0.80, 6.60)	0.013
Multiple logistic regression model[3]		
Liver enzyme elevation[4]	4.21 (1.78, 9.97)	0.001
Large-for-gestational-age newborns	0.19 (0.06, 0.62)	0.006

[1]One model for each variable, adjusted for age, prepregnancy BMI, change in weight during the follow-up period, belonging to an exercise group, and the baseline value of the variable. Continuous variables were analyzed by using a multiple linear regression model after log transformation of skewed variables. Dichotomous outcome variables were analyzed by using a standard multivariable logistic regression model. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase.

[2]Values are βs (95% CIs).

[3]Values are ORs (95% CIs).

[4]ALT concentration ≥30 IU/L and/or AST concentration ≥30 IU/L and/or GGT concentration ≥40 IU/L.

mutation in G-allele carriers to upregulate *PNPLA3* activity, eventually promoting neutral lipid storage and attenuating insulin resistance.

The inverse relation between the I148M variant and insulin resistance was consistent with part but not all available literature (49–51); differences in age, race, obesity status, and other clinical characteristics of enrolled populations might justify at least in part these discrepancies.

Implications for pregnancy

To date, very few human studies have assessed the role of the *PNPLA3* gene polymorphism in modulating a response to lifestyle intervention. In a small study, the G allele in *PNPLA3* enhanced the susceptibility to weight-loss–induced liver fat reduction (52). We previously showed that exercise effectively reduced maternal postprandial glucose, Hb A_{1c}, CRP, triglycerides, and any maternal and neonatal complications. In the current study, we report that the percentage of LGA newborns was significantly lower in women carrying the G allele. Insulin resistance in GD patients is accompanied by increased circulating fatty acids and triglycerides, which are available for fetal growth and fat-mass enlargement. In the last trimester, a physiologically increased breakdown of fat depots leads to an enhanced flux of free fatty acids and glycerol to the maternal liver where fatty acids are converted to ketone bodies and glycerol to glucose, which can cross the placenta and sustain fetal growth (53). In women with the I148M mutation, these processes might be impaired, and the possible increased maternal liver fat accumulation might reflect a lower nutrient transport toward the fetus. Alternatively, the reduced insulin resistance of G-allele carriers may explain their reduced prevalence of LGA babies in our GD patients. From a practical stand point, lifestyle interventions that effectively improved metabolic variables in GD failed to prevent the development of liver disease in genetically predisposed GD patients who developed liver enzyme elevation at the end of pregnancy. Because NAFLD is an emerging risk factor for liver-related and cardiometabolic complications, additional follow-up studies in women with the at-risk *PNPLA3* genotype and a history of GD will determine their risk of developing these complications.

Limitations and strengths

Several limitations of the current study need to be mentioned. The number of patients was fairly small, and our results should be confirmed in larger cohorts. We did not directly measure the liver fat content by suing imaging techniques but used liver enzymes as markers of liver fat accumulation. Finally, despite extensive validation, the HOMA-IR index is not the gold standard to evaluate insulin sensitivity because it is strongly dependent on the precision of the insulin assay. This study was not powered to detect differences in the incidence of specific maternal and neonatal complications. Recall errors might have occurred during the compilation of questionnaires.

However, to our knowledge, these are the first data that evaluate the role of rs738409 *PNPLA3* variants on pregnancy outcomes in GD patients. Other strengths include the thorough assessment of diet and physical activity throughout the trial and multiple variables blindly and centrally measured.

In conclusion, the *PNPLA3* SNP rs738409 was associated during the course of pregnancies complicated by GD with liver enzyme elevation and reduced insulin resistance and prevalence of LGA newborns. These associations, if confirmed in larger cohorts, might have potential implications not only for the mothers but also for the offspring and subsequent generations.

The authors' responsibilities were as follows—SB: participated in the conception and design of the study, supervision of data collection, data analysis, interpretation of findings of the study, and manuscript writing and revision; RG: participated in the data analysis, interpretation of findings of the study, and manuscript writing and revision; G Menato: participated in the data analysis, interpretation of the findings of the study, and manuscript revision; SC, VP, and SP: participated in the data collection, interpretation of findings of the study, and manuscript revision; MD, EG, and MC: participated in the interpretation of findings of the study and manuscript writing and revision; G Musso: participated in the conception and design of the study, interpretation of findings of the study, and manuscript writing and revision; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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