Breast-Feeding Modifies the Association of *PPARγ2* Polymorphism Pro12Ala With Growth in Early Life

The Generation R Study

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OBJECTIVE—We examined whether the *PPARγ2* Ala12 allele influences growth in early life and whether this association is modified by breast-feeding.

RESEARCH DESIGN AND METHODS—This study was embedded in the Generation R Study, a prospective cohort study from early fetal life onward. *PPARγ2* was genotyped in DNA obtained from cord blood samples in 3,432 children. Information about breast-feeding was available from questionnaires. Weight, head circumference, and femur length were repeatedly measured in second and third trimesters of pregnancy, at birth, and at the ages of 1.5, 6, 11, 14, and 18 months.

RESULTS—Genotype frequency distribution was 77.6% (Pro12Pro), 20.7% (Pro12Ala), and 1.7% (Ala12Ala). Growth rates in weight from second trimester of pregnancy to 18 months were higher for Pro12Ala and Ala12Ala than for Pro12Pro carriers (differences 1.11 g/week [95% CI 0.47–1.74] and 2.65 g/week [0.45–4.87], respectively). We found an interaction between genotype and breast-feeding duration (*P* value for interaction <0.0001). In infants who were breast-fed for ≥4 months, *PPARγ2* Pro12Ala was not associated with growth rate. When breast-feeding duration was <2 months or 2–4 months, growth rate was higher in Ala12Ala than Pro12Pro carriers (differences 9.80 g/week [3.97–15.63] and 6.32 g/week [−1.04 to 13.68], respectively).

CONCLUSIONS—The *PPARγ2* Ala12 allele is associated with an increased growth rate in early life. This effect may be influenced by breast-feeding duration. Further studies should replicate these findings, identify the underlying mechanisms, and assess whether these effects persist into later life. ***Diabetes*** **58:992–999, 2009**

Previous studies have shown that common polymorphisms of peroxisome proliferator–activated receptor γ2 (*PPARγ2*) are associated with adipocyte differentiation, lipid metabolism, and insulin sensitivity (1). Recent genome-wide association (GWA) studies found consistent and robust associations of the *PPARγ2* Pro12Ala polymorphism (rs1801282) with type 2 diabetes (2,3). Furthermore, several studies have reported increased BMI in *PPARγ2* Ala12 carriers (4–6). In a meta-analysis, Masud et al. (4) found that in adults with a BMI >27 kg/m^2 carriers of the *PPARγ2* Ala12 allele had an increased BMI. Also, in subjects with normal BMI, they found a significant increase in BMI in Ala12Ala carriers versus Pro12Pro carriers. However, a recent GWA study on BMI in more than 80,000 subjects did not identify the *PPARγ2* Ala12 allele as a variant associated with BMI in the general adult population (7). Among children, it has been suggested that the *PPARγ2* Ala12 allele is associated with BMI, although evidence for this is very limited. In a small study, carriers of the *PPARγ2* Ala12 allele were shown to be heavier at age 7 years (5); in a cohort of children aged 1–6 years, an association of *PPARγ2* Ala12 allele with increased adiposity was only found in girls aged 3–4 years (6).

Common polymorphisms of *PPARγ2* may also explain previously suggested associations of growth in early fetal life and infancy with obesity (8). This association may be explained by early, modest alterations in insulin secretion and sensitivity because insulin is the most important fetal growth hormone (9). The effect of the Ala12 allele on anthropometrics and growth patterns may already be present in fetal life and infancy. In two large studies, no association was found between *PPARγ2* and birth weight, although an association with preterm birth has been suggested (10–12). Birth weight alone might be an inappropriate measure of individual growth potential because different fetal growth rates may lead to the same birth weight (13). Moreover, most growth-restricted infants catch up to their own genetically determined growth curve during the first postnatal years (14). The *PPARγ2* Ala12 allele has been found to interact with birth weight in determining further growth patterns (15). Also, the effect of *PPARγ2* genotype on metabolic phenotype appears to depend on dietary intake (16–18). No previous studies have examined the effect of breast-feeding on the association of *PPARγ2* genotype with growth in early life, while breast-feeding is well known to influence early growth and has a protective effect on the risk of obesity in childhood (19–21).

Based on previous findings, we hypothesized that the *PPARγ2* Ala12 allele is associated with increased weight gain during early life and that this association might be influenced by breast-feeding duration. We examined in a large prospective birth cohort study from fetal life onward the association of the Pro12Ala polymorphism in the *PPARγ2* gene with growth in fetal life and infancy and whether this association may be modified by breast-feeding.

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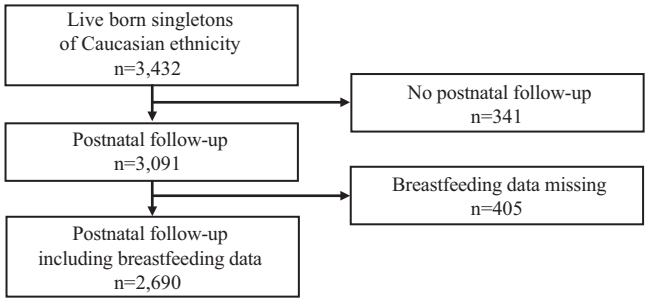

FIG. 1. Flow diagram indicating number of subjects for each analysis.

RESEARCH DESIGN AND METHODS

This study was embedded in the Generation R Study, a prospective cohort study from early fetal life onward. This study is designed to identify early environmental and genetic determinants of growth, development, and health from fetal life until young adulthood and has been described previously in detail (22,23). Fetal and postnatal growth and their main determinants were repeatedly measured by physical examinations, fetal ultrasounds, biological samples, and questionnaires. As previously reported, of all eligible children born in the study area, 61% participated in the study (22). The study has been approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents.

Analyses were restricted to children from whom DNA was available for *PPARγ2* genotyping and with Dutch or other European Caucasian ethnicity as defined by having both parents born in the Netherlands or another European country (n = 3,432). Fetal growth measurements were available in 3,331 and 3,398 children in second and third trimesters, respectively. Of these children, those living outside the study area postnatally (n = 341) were not followed up in infancy, leading to 3,091 subjects for the postnatal growth measurements (Fig. 1). Of all children followed postnatally, data on breast-feeding were missing in 405 subjects, leaving 2,690 children with complete data on postnatal growth and breast-feeding duration. Of all genotyped subjects at baseline, the mean follow-up rate per visit was 77%.

Genotyping. DNA was collected from cord blood samples at birth. Cord blood for DNA isolation was available in 59% of all live-born participating children. Missing cord blood samples were mainly due to logistical constraints at the delivery. Genotyping of the *PPARγ2* gene Pro12Ala polymorphism (rs1801282) was performed using a Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA) and Abgene QPCR ROX mix (Abgene, Hamburg, Germany). The genotyping reaction was amplified using the GeneAmp PCR system 9600 (95°C [15 min], then 40 cycles of 94°C [15 s] and 60°C [1 min]). Fluorescence was detected on the 7900HT Fast Real-Time PCR System (Applied Biosystems), and individual genotypes were determined using SDS software (version 2.3; Applied Biosystems). Genotyping was successful in 99% of the samples. To confirm the accuracy of the genotyping results, 276 randomly selected samples were genotyped for a second time with the same method. The error rate was 0%.

The genotype distribution (Pro12Pro 77.6%, Pro12Ala 20.7%, Ala12Ala 1.7%; minor allele frequency [Ala] of 22.4%) was similar to that found in previous studies, and the frequency distribution did not deviate from Hardy-Weinberg equilibrium (χ^2 = 1.82; P = 0.18) (4).

Fetal growth and birth characteristics. Fetal ultrasound examinations were carried out during visits to one of the research centers. These fetal ultrasounds were used for establishing gestational age in the first trimester and for assessing fetal growth characteristics in second and third trimesters of pregnancy (24). Fetal growth characteristic measurements used for the present study included head circumference, abdominal circumference, and femur length, measured in second and third trimesters to the nearest millimeter using standardized ultrasound procedures (25). Estimated fetal weight (EFW) was calculated using the formula by Hadlock using head circumference (HC), abdominal circumference (AC), and femur length (FL) ($\log_{10}$ EFW = 1.5662 − 0.0108 [HC] + 0.0468 [AC] + 0.171 [FL] + 0.00034 [HC]^2 − 0.003685 [AC × FL]) (26). First trimester ultrasound measures were not included as growth characteristics because these ultrasound examinations were primarily performed to establish gestational age.

Postnatal growth. Birth weight, date of birth, and sex were obtained from community midwife and hospital registries. Information on head circumference or length at birth was not available. Well-trained staff in community health centers obtained postnatal growth characteristics using standardized procedures. Weight was measured using electronic scales (SECA, Hamburg, Germany). Length was determined in supine position to the nearest millimeter until the age of 6 months using a neonatometer, after which it was measured in upright position (Holtain Limited, Dyfed, U.K.). Head circumference was measured to the nearest millimeter using a standardized tape (SECA). Based on the routine health care program, visits for measurements of these growth characteristics were grouped into five age periods: 1.5 months (range 0–3.99), 6 months (4–9.99), 11 months (10–12.99), 14 months (13–16.99), and 18 months (17–20.99). Postnatally, head circumference was only measured at 1.5, 6, and 14 months.

Breast-feeding. Information about duration of breast-feeding was obtained by postnatal questionnaires at the ages of 2, 6, and 12 months. This information was combined to form the following categories: *1*) breast-fed 0–2 months, *2*) 2–4 months, and *3*) >4 months.

Covariates. Information on maternal age, educational level, parity, and weight before pregnancy was obtained by the first questionnaire at enrollment in the study. Maternal height was measured without shoes at our research center, and BMI was calculated as weight divided by the square of height in meters. The occurrence of gestational diabetes was obtained from midwife or obstetric records.

Data analysis. We explored the differences in gestational age (weeks) and birth weight (SD) between the three genotypes with additive, dominant (*PPARγ2* Ala12Ala/Pro12Ala vs. Pro12Pro), and recessive (*PPARγ2* Pro12Pro/Pro12Ala vs. Ala12Ala) models using Mann-Whitney U tests and linear regression. To assess longitudinally measured growth characteristics from fetal life to infancy, we performed unbalanced repeated-measures regression analysis with weight, length, and head circumference in fetal life and infancy as outcomes. This regression technique takes the correlation of multiple measurements within one subject into account, assesses both the time-independent and time-dependent effect of *PPARγ2* Pro12Ala genotype on growth, and allows for incomplete outcome data (27). The best-fitting model as a function of (gestational) age was constructed using fractional polynomials (28).

To account for differences in growth curves for weight, length, and head circumference in fetal life and infancy, growth models were constructed for three age periods: second trimester to 18 months (overall), second trimester to birth (fetal), and birth to 18 months (infancy). In the fetal and overall models, age was defined as age in weeks after conception. For the infancy model, age represented biological age in weeks, and these models were additionally adjusted for gestational age at birth. In all models, genotype was included as both intercept and interaction with age to account for differences at baseline and in growth rates. The models used are shown in the supplemental material available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db08-1311/DC1.

Finally, we examined the interaction between genotype and breast-feeding duration and the effect on growth rate. Trend tests were performed by adding both genotype and breast-feeding duration in the model as ordinal variables instead of categorical variables. All models were adjusted only for sex because population genotype distribution is assumed to be unrelated to covariates, and the effect estimates were not materially affected by adjusting for covariates such as maternal age, educational level, prepregnancy, BMI, or parity (29). The occurrence of gestational diabetes in the entire cohort was 0.6%, did not differ between the genotypes, and was, therefore, not included in the analyses. All effect estimates are presented with their 95% CI. Statistical analyses were performed using the Statistical Analysis System, Version 8.2 (SAS, Stata, College Station, TX), including the PROC MIXED module for unbalanced repeated measurements and the Statistical Package of Social Sciences, Version 15.0 for Windows (SPSS, Chicago, IL).

RESULTS

Tables 1 and 2 show the subjects characteristics. In the whole study group, mean birth weight ± SD was 3,512 ± 510 g and median gestational age was 40.3 weeks (95% range 36.7–42.4). The percentages of children born prematurely and with low birth weight were 2.9 and 2.4%, respectively. The median number of measurements (visits) per subject was seven (95% range 3–8 visits). Table 3 shows the differences in birth characteristics between the *PPARγ2* Pro12Ala genotypes. No significant differences were found in gestational age and birth weight ±SD. Pro12Ala and Ala12Ala carriers tended to have an increased risk of preterm birth, although these differences were not significant (odds ratios [95% CI] using the Pro12Pro genotype as a reference: 1.51 [0.96–2.38] for Pro12Ala and 2.08 [0.64–6.80] for Ala12Ala).

Table 4 shows the differences in growth rates between the *PPARγ2* genotypes using Pro12Pro as the reference

TABLE 1
Maternal and birth characteristics of 3,432 children

Age (years)	30.8 ± 4.8
Weight before pregnancy (kg)	66.9 ± 11.8
Height (cm)	170 ± 6.7
BMI before pregnancy (kg/m^2)	22.9 ± 3.7
Parity (% nulliparous)	60.1
Gestational diabetes (%)	0.6
Placental weight (g)	640 ± 142
Educational level (%)	
Primary school	4.5
Secondary school	39.1
Higher education	56.5
Birth characteristics	
Sex (% male)	50.8
Gestational age (weeks)	40.3 (36.7–42.4)
Birth weight (g)	3512 ± 510
Premature (gestational age <37 weeks) (%)	2.9
Birth weight <2,500 g (%)	2.4
Small for gestational age (below −2 SD) (%)	2.4
Breast-feeding duration (%)	
0–2 months	31.3
2–4 months	26.4
>4 months	42.3
Number of visits	7 (3–8)

Data are means ± SD, median (95% range), or percentages. Of the total group, data were missing on weight before pregnancy (n = 468), height (n = 5), BMI before pregnancy (n = 470), parity (n = 3), gestational diabetes (n = 39), placental weight (n = 792), educational level (n = 45), and breast-feeding duration (n = 742).

TABLE 2
Fetal and postnatal growth characteristics of 3,432 children

Second trimester	
Gestational age at visit (weeks)	20.5 (18.6–23.0)
Head circumference (cm)	18.0 ± 1.4
Femur length (mm)	33.3 ± 3.4
Estimated fetal weight (g)	380 ± 87
Third trimester	
Gestational age at visit (weeks)	30.4 (28.5–32.7)
Head circumference (cm)	28.6 ± 1.2
Femur length (mm)	57.4 ± 2.8
Estimated fetal weight (g)	1,628 ± 249
Birth	
Gestational age (weeks)	40.3 (36.7–42.4)
Weight (g)	3,512 ± 510
1.5 months	
Age at visit (months)	1.3 (0.9–3.0)
Head circumference (cm)	38.3 ± 1.5
Length (cm)	55.8 ± 2.8
Weight (g)	4,788 ± 728
6 months	
Age at visit (months)	6.1 (4.5–7.7)
Head circumference (cm)	43.5 ± 1.3
Length (cm)	67.7 ± 2.6
Weight (g)	7,798 ± 862
11 months	
Age at visit (months)	11.0 (10.1–12.5)
Length (cm)	74.4 ± 2.5
Weight (g)	9,644 ± 999
14 months	
Age at visit (months)	14.2 (13.5–15.7)
Head circumference (cm)	47.2 ± 1.3
Length (cm)	78.4 ± 2.7
Weight (g)	10,532 ± 1,089
18 months	
Age at visit (months)	18.3 (17.3–20.4)
Length (cm)	82.4 ± 3.0
Weight (g)	11,556 ± 1,214

Data are means ± SD or median (95% range).

group. In fetal life, *PPARγ2* Ala12Ala carriers tended to show an increased growth rate in weight compared with the Pro12Pro carriers. Postnatally, both the Pro12Ala and Ala12Ala carriers have an increased growth rate in weight compared with Pro12Pro carriers, although this was not significant in the latter. Furthermore, we observed an allele dose effect for each additional Ala12 allele (P = 0.0092). For weight gain over the entire period from the second trimester to 18 months, *PPARγ2* Pro12Pro carriers had a significantly lower growth rate for weight than the other genotypes, and there was a significant trend for each additional Ala12 allele ($P < 0.0001$). Figure 2 shows the estimated weight differences in grams between the genotype groups. The estimated differences at 18 months compared with the Pro12Pro group were 99 and 291 g for the Pro12Ala and Ala12Ala carriers, respectively. Prenatal head circumference growth rate was significantly lower in Ala12Ala compared with Pro12Pro carriers. No other significant differences were found in growth rate of head circumference or length.

We found a significant interaction between genotype and breast-feeding duration on growth rate in weight (P for interaction <0.0001). Figure 3 shows the differences in estimated growth rates between the genotypes in the three breast-feeding groups. When breast-feeding duration was <2 months or 2–4 months, growth rate was higher in Ala12Ala than Pro12Pro carriers (differences 9.80 g/week [95%CI 3.97–15.63] and 6.32 g/week [−1.04 to 13.68], respectively). No associations of *PPARγ2* with growth rate were found in children who were breast-fed >4 months. Table 5 shows the trends within each genotype and breast-feeding group. The maximum difference in growth rate was found between *PPARγ2* Ala12Ala carriers who were breast-fed <2 months and Pro12Pro carriers who were breast-fed >4 months (difference 12.62 g/week (6.80–18.44)). Similar effects were found in subjects who were breast-fed for 2–4 months, and we found an allele dose effect for each additional Ala12 allele in these subjects (P = 0.0264). All effect estimates did not materially change after restricting analyses to term-born subjects (>37 weeks of gestation) or after adjusting for covariates, such as maternal age, educational level, prepregnancy, BMI, or parity (data not shown). No interactions between genotype and breast-feeding on length or head circumference growth rate were observed (data not shown).

DISCUSSION

In this study, we show that the Ala allele of the *PPARγ2* Pro12Ala gene polymorphism (rs1801282) is associated with an increased growth rate in early life. Between the second trimester of pregnancy and 18 months, children with Pro12Ala and Ala12Ala had higher growth rates in weight than Pro12Pro subjects. Furthermore, our results suggest that the effect of *PPARγ2* gene polymorphism on growth in infancy depends on breast-feeding duration.

To our knowledge, this study is the first prospective cohort study that examines the association of this *PPARγ2* Pro12Ala gene polymorphism with growth from fetal life until infancy. DNA for genotyping was available in 59% of

TABLE 3
Birth characteristics according to *PPARγ2* Pro12Ala genotypes in 3,432 children

	Pro12Pro (n = 2,664)	Pro12Ala (n = 710)	Ala12Ala (n = 58)	P values		
				Additive	Dominant	Recessive
Gestational age (weeks)	40.3 (36.9–42.4)	40.3 (36.4–42.4)	40.4 (32.3–42.9)	0.35	0.69	0.91
<37 weeks	2.6 (68)	3.8 (27)	5.2 (3)			
>37 weeks	97.4 (2,596)	96.2 (683)	94.8 (55)			
Birth weight	0.04 ± 0.98	0.04 ± 1.03	0.28 ± 0.97	0.37	0.66	0.07
<−2 SD	2.2 (59)	3.4 (24)	0.0 (0)			
>−2 SD	97.8 (2,605)	96.6 (686)	100.0 (58)			

Data are means ± SD or median (95% range) for continuous variables and (%) n for dichotomous variables. Differences were tested using Mann-Whitney U-test or linear regression. Dominant model: *PPARγ2* Ala12Ala/Pro12Ala vs. Pro12Pro. Recessive model: *PPARγ2* Pro12Pro/Pro12Ala vs. Ala12Ala.

all subjects. Missing cord blood and DNA was mainly caused by logistical constraints at delivery. Children who were not genotyped had a shorter gestational age ($P <$ 0.001) and were lighter at birth ($P < 0.001$) than subjects who were genotyped. Of all genotyped subjects at baseline, the mean follow-up rate per visit was 77%. Response rates were lowest at the age of 18 months. This is mainly due to the general lower response rate at this age in the routine child care system. In our data, no differences in genotype frequency or birth characteristics were observed between children with and without postnatal growth measurements. Furthermore, similar results were observed when analyses were restricted to children aged 11 or 14 months and when breast-feeding duration was dichotomized into shorter or longer than 3 months (data not shown). Our effect estimates would be biased if the

TABLE 4
Differences in growth rate for weight by *PPARγ2* Pro12Ala genotypes using repeated measures regression analysis

	Difference in weight gain from second trimester to birth (g/week) (n = 3,432)	Difference in weight gain from birth to 18 months (g/week) (n = 3,091)	Difference in weight gain from second trimester to 18 months (g/week) (n = 3,091)
Pro12Pro	Reference	Reference	Reference
Pro12Ala	0.50 (−0.96 to 1.98)	1.06 (0.02–2.10)	1.11 (0.47–1.74)
P	0.4988	0.0454	0.0007
Ala12Ala	4.02 (−0.59 to 8.63)	3.52 (−0.05 to 7.10)	2.65 (0.45–4.87)
P	0.0876	0.0535	0.0185
P for trend	0.1575	0.0092	<0.0001
	Difference in head circumference gain from second trimester to 1.5 months (mm × 10^{-1}/week) (n = 3,432)	Difference in head circumference gain from 1.5 to 14 months (mm × 10^{-1}/week) (n = 3,091)	Difference in head circumference gain from second trimester to 14 months (mm × 10^{-1}/week) (n = 3,091)
Pro12Pro	Reference	Reference	Reference
Pro12Ala	−0.16 (−0.51 to 0.18)	0.01 (−0.01 to 0.03)	0.05 (−0.09 to 0.19)
P	0.3617	0.1587	0.4725
Ala12Ala	−1.40 (−2.56, −0.23)	0.02 (−0.05, 0.10)	−0.48 (−1.01, 0.02)
P	0.0186	0.5104	0.0643
P for trend	0.0566	0.1293	0.8368
	Difference in length gain from second trimester to 1.5 months (mm × 10^{-1}/week) (n = N/A)	Difference in length gain from 1.5 to 18 months (mm × 10^{-1}/week) (n = 3,091)	Difference in length gain from second trimester to 14 months (mm × 10^{-1}/week) (n = N/A)
Pro12Pro	N/A	Reference	N/A
Pro12Ala	N/A	−0.11 (−0.37 to 0.14)	N/A
P		0.3864	
Ala12Ala	N/A	0.43 (−0.44 to 1.31)	N/A
P		0.3299	
P for trend		0.4429	

Data are regression coefficients (95% CI) and reflect the difference in growth rate. Models are adjusted for sex of the child. Analyses focused on growth during infancy are additionally adjusted for gestational age at birth. Estimates based on repeated-measures regression analysis. N = 3,432 for growth from second trimester to birth/1.5 months (Pro12Pro, n = 2664; Pro12Ala, n = 710; Ala12Ala, n = 58); N = 3,091 for postnatal growth and growth over the entire period (Pro12Pro, n = 2440; Pro12Ala, n = 644; Ala12Ala, n = 47).

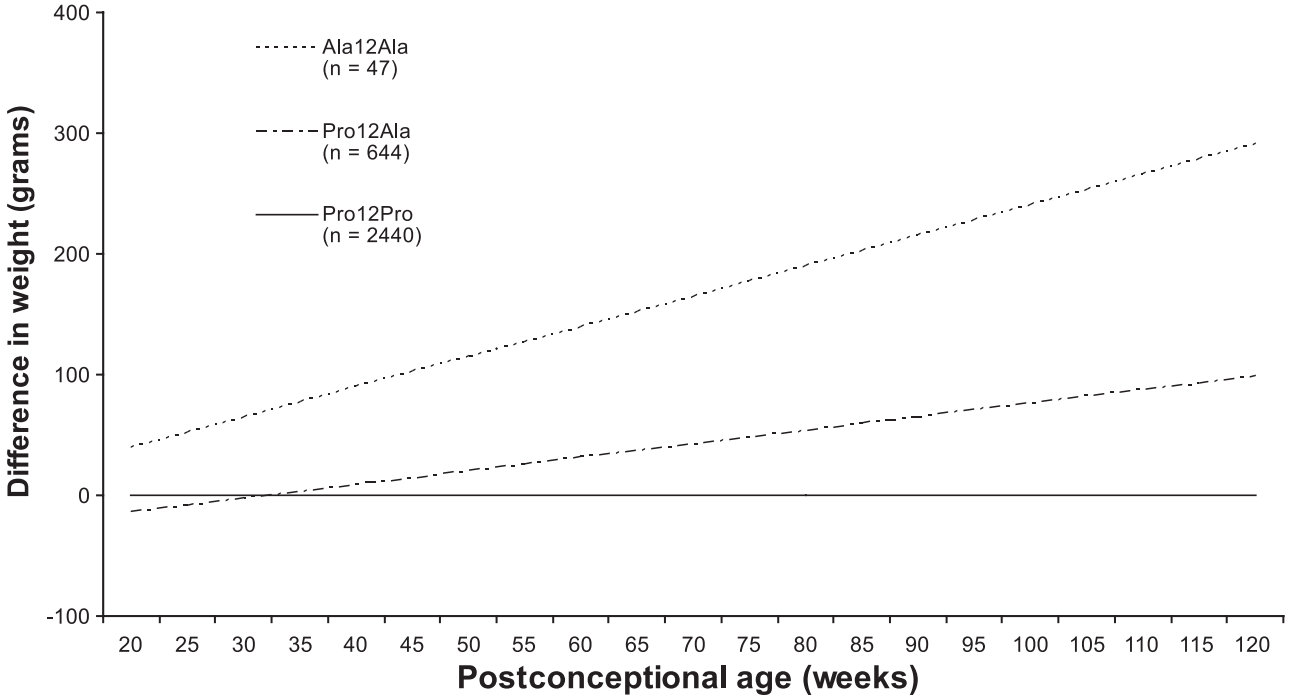

FIG. 2. Differences in weight between fetal *PPARγ2* Pro12Ala genotypes using Pro12Pro as the reference in 3,091 children. Values reflect the difference in weight and are based on repeated-measures regression analysis, using the following model: Weight (g) = $\beta_0 + \beta_1 \times$ age $+ \beta_2 \times$ age$^2 + \beta_3 \times$ genotype $+ \beta_4 \times$ genotype $\times$ age $+ \beta_5 \times$ sex.

associations between genotypes and growth characteristics differed between those with and without postnatal growth data available. This seems unlikely. Finally, similar to other studies, the number of *PPARγ2* Ala12Ala carriers was small, especially after stratifying for breast-feeding duration (4). Despite the low number of subjects in this group, we found significant effects in Ala12Ala carriers. Furthermore, we found effects in Pro12Ala carriers and significant trend effects for each additional Ala12 allele.

Several studies have found associations between this *PPARγ2* Pro12Ala gene polymorphism and body composition (4,30,31). A meta-analysis demonstrated that the Ala12Ala is associated with higher BMI in adulthood (4). However, in a recent very large GWA study, the *PPARγ2* Ala12 allele was not identified as a BMI variant in adulthood (7). A number of studies have suggested that the *PPARγ2* Ala12 allele may be associated with adiposity during childhood, although evidence of this is limited (5,6,15). In a small study, Pihlajamäki et al. (5) showed that children with the Ala12 allele were heavier at age 7 years. Also, in a cohort of children from 1–6 years of age, an association of the *PPARγ2* Ala12 allele with increased adiposity was only found in girls aged 3–4 years (6). Two large birth cohort studies found no association between this *PPARγ2* polymorphism and birth weight (10,11). Eriksson et al. (32) described a gene–birth weight interaction in adults, where individuals with the Ala12 allele and a lower birth weight were at risk of increased lipid levels.

No other studies have examined the effect of this polymorphism on growth in fetal life and infancy in a large

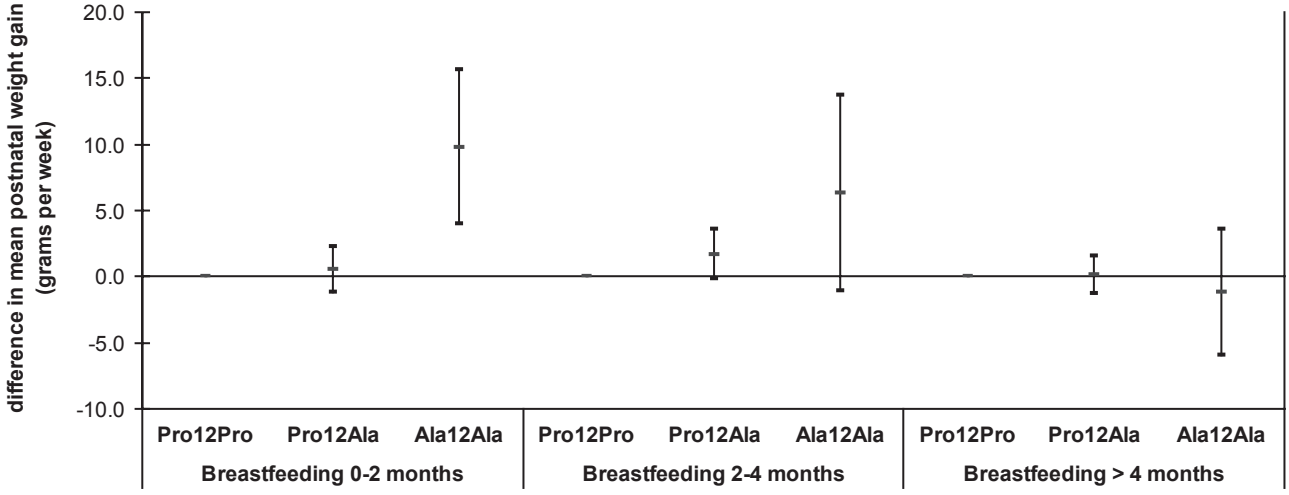

FIG. 3. Differences in postnatal weight between fetal *PPARγ2* Pro12Ala genotypes stratified by duration of breast-feeding in 2,690 children. Values are regression coefficients (95% CI) and reflect the difference in weight gain (g/week). Model is adjusted for gestational age at birth and sex of the child. Estimates based on repeated-measures regression analysis.

TABLE 5
Interaction between fetal *PPARγ2* Pro12Ala genotype and breast-feeding in relation to growth rate (g/week) between birth and 18 months in 2,690 children

	Breast-feeding duration			
	0–2 months (n = 843)	2–4 months (n = 710)	>4 months (n = 1,137)	P for trend
Pro12Pro (N = 2,079)	2.82 (1.78–3.85)	1.37 (0.28–2.46)	0 (ref.)	<0.0001
n	663	547	869	
P	<0.0001	0.0135		
Pro12Ala (N = 570)	3.34 (1.64–5.05)	3.05 (1.27–4.84)	0.17 (−1.26 to 1.59)	0.0013
n	166	155	249	
P	0.0001	0.0008	0.8170	
Ala12Ala (N = 41)	12.62 (6.80–18.44)	7.69 (0.35–15.03)	−1.21 (−5.99 to 3.56)	0.0003
	14	8	19	
	<0.0001	0.0399	0.6183	
P for trend	0.0432	0.0264	0.9555	interaction <0.0001

Data are regression coefficients (95% CI) unless otherwise indicated and reflect the difference in weight gain from birth to 18 months (g/week). Model is adjusted for gestational age at birth and sex of the child. Estimates based on repeated-measures regression analysis.

prospective cohort. Most studies on weight and body composition were performed retrospectively on cross-sectional data. We believe that if this *PPARγ2* polymorphism is truly associated with growth in early life, associations with longitudinally measured growth patterns might be expected to be stronger than those with only one or two growth measurements. Our study showed that carriers of at least one *PPARγ2* Ala12 allele had an increased growth rate in weight until the age of 18 months. The postnatal effect on growth rate appeared to be dependent on duration of breast-feeding. No association was found between genotype and growth rate in children who had received at ≤4 months of breast-feeding. Among children who were breast-fed for <4 months, a significant positive effect on growth rate of up to almost 10 g/week was found in the *PPARγ2* Ala12 allele carriers. The size of the effect was inversely related to breast-feeding duration. These findings may indicate a possible gene-nutrition interaction with regards to growth rate.

There have been a limited number of previous studies that also have suggested a gene-nutrition interaction concerning this *PPARγ2* polymorphism. In a large cohort, Memisoglu et al. (33) found that the relationship between dietary fat intake and BMI was dependent on *PPARγ2* genotype. In this study, dietary fat intake was strongly associated with an increased risk of obesity among Pro12Pro carriers, although no association was reported among carriers of the Ala12 allele. Luan et al. (17) found that dietary fat intake (expressed as polyunsaturated-to-saturated fat ratio) was not associated with BMI or fasting insulin levels in Pro12Pro carriers but was inversely related in these outcomes in Ala12 allele carriers. Other studies, however, were not able to replicate these results (18,33). Our results would be in line with the findings of Luan et al. (34) based on the assumption that breast milk contains more polyunsaturated fatty acids than formula. Longer breast-feeding duration could lead to a higher polyunsaturated fat intake and, subsequently, to a relatively lower growth rate among Ala12Ala carriers who were breast-fed compared with those who were formula fed. Breast-feeding has also been indicated to have a protective effect on the risk of obesity, although the effect appears to be limited (19–21). Our results suggest that this protective effect might be modified by *PPARγ2*, because we found the highest growth rates in children who were never breast-fed or were breast-fed for <4 months and were Ala12 allele carriers. Furthermore, rapid weight gain in the first months of life is associated with increased risk of obesity in childhood (35). Based on the current study, it could be hypothesized that the interaction between *PPARγ2* and breast-feeding plays an important role in this association.

From the current data, however, it remains unclear whether breast-feeding reduces the risk of an increased growth rate in *PPARγ2* Ala12 allele carriers or formula feeding increases that same risk. The associations may be explained by either growth-stimulating or metabolism-inhibiting activities among Ala12 allele carriers. A previous study has shown that the *PPARγ2* Ala12 allele is associated with a moderate reduction in *PPARγ2* transcriptional activity (36). However, this study also demonstrated decreased BMI in Ala12 allele carriers, a finding that has been not confirmed by a large meta-analysis (4,36). Mouse models showed that mice with reduced *PPARγ2* activity seem to be resistant to high-fat diet–induced obesity, but not high-carbohydrate diet–induced obesity (37). The current study did not allow us to measure calorie intake of breast-fed or formula-fed children. Calorie intake in breast-feeding has been shown to be dependent on maternal factors, such as body composition and nutritional status (38). All these factors indicate that the underlying mechanism in which *PPARγ2* Pro12Ala interacts with early dietary intake is highly complex and emphasizes the necessity for further genetic association and functional studies.

In summary, our results suggest that this *PPARγ2* polymorphism influences growth rate from early fetal life to infancy. This effect on growth rate is restricted to infants who were breast-fed for <4 months. Studies in larger cohorts with a longer follow-up period will allow us to examine whether these effects persist throughout childhood. Additionally, systematic searches for common genetic variants by means of GWA studies may enable us to obtain a more complete understanding of what genes are involved in growth in fetal life and infancy and how they interact with the environment.

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REFERENCES

1. Auwerx J: PPARgamma, the ultimate thrifty gene. *Diabetologia* 42:1033–1049, 1999
2. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341–1345, 2007
3. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS, Wellcome Trust Case Control C, McCarthy MI, Hattersley AT: Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316:1336–1341, 2007
4. Masud S, Ye S, Group SAS: Effect of the peroxisome proliferator activated receptor-gamma gene Pro12Ala variant on body mass index: a meta-analysis. *J Med Genet* 40:773–780, 2003
5. Pihlajamaki J, Vanhala M, Vanhala P, Laakso M: The Pro12Ala polymorphism of the PPAR gamma 2 gene regulates weight from birth to adulthood. *Obes Res* 12:187–190, 2004
6. Lagou V, Scott RA, Manios Y, Chen TL, Wang G, Grammatikaki E, Kortsalioudaki C, Liarigkovinos T, Moschonis G, Roma-Giannikou E, Pitsiladis YP: Impact of peroxisome proliferator-activated receptors gamma and delta on adiposity in toddlers and preschoolers in the GENESIS study. *Obesity (Silver Spring)* 16:913–918, 2008
7. Willer CJ, Speliotes EK, Loos RJ, et al.: Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 41:25–34, 2009
8. Whitaker RC, Dietz WH: Role of the prenatal environment in the development of obesity. *J Pediatr* 132:768–776, 1998
9. Fowden AL: Endocrine regulation of fetal growth. *Reprod Fertil Dev* 7:351–363, 1995
10. Bennett AJ, Sovio U, Ruokonen A, Martikainen H, Pouta A, Hartikainen AL, Franks S, Elliott P, Jarvelin MR, McCarthy MI: No evidence that established type 2 diabetes susceptibility variants in the PPARG and KCNJ11 genes have pleiotropic effects on early growth. *Diabetologia* 51:82–85, 2008
11. Pfab T, Poralla C, Richter CM, Godes M, Slowinski T, Priem F, Halle H, Hocher B: Fetal and maternal peroxisome proliferator-activated receptor gamma2 Pro12Ala does not influence birth weight. *Obesity (Silver Spring)* 14:1880–1885, 2006
12. Meirhaeghe A, Boreham CA, Murray LJ, Richard F, Davey Smith G, Young IS, Amouyel P: A possible role for the PPARG Pro12Ala polymorphism in preterm birth. *Diabetes* 56:494–498, 2007
13. Geelhoed JJ, Mook-Kanamori DO, Witteman JC, Hofman A, van Duijn CM, Moll HA, Steegers EA, Hokken-Koelega AC, Jaddoe VW: Variation in the IGF1 gene and growth in foetal life and infancy. The Generation R Study. *Clin Endocrinol (Oxf)* 68:382–389, 2008
14. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL: Children born small for gestational age: do they catch up? *Pediatr Res* 38:267–271, 1995
15. Labayen I, Moreno LA, Marti A, Gonzalez-Lamuno D, Warnberg J, Ortega FB, Bueno G, Nova E, Ruiz JR, Garagorri JM, Martinez JA, Garcia-Fuentes M, Bueno M, Group AS: Effect of the Ala12 allele in the PPARgamma-2 gene on the relationship between birth weight and body composition in adolescents: the AVENA study. *Pediatr Res* 62:615–619, 2007
16. Lindi VI, Uusitupa MI, Lindstrom J, Louheranta A, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Tuomilehto J, Finnish Diabetes Prevention Study: Association of the Pro12Ala polymorphism in the PPAR-γ2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. *Diabetes* 51:2581–2586, 2002
17. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ: Evidence for gene-nutrient interaction at the PPARγ locus. *Diabetes* 50:686–689, 2001
18. Robitaille J, Despres JP, Perusse L, Vohl MC: The PPAR-gamma P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: results from the Quebec Family Study. *Clin Genet* 63:109–116, 2003
19. Dietz WH: Breast-feeding may help prevent childhood overweight. *JAMA* 285:2506–2507, 2001
20. Gillman MW, Rifas-Shiman SL, Camargo CA Jr, Berkey CS, Frazier AL, Rockett HR, Field AE, Colditz GA: Risk of overweight among adolescents who were breast-fed as infants. *JAMA* 285:2461–2467, 2001
21. Owen CG, Martin RM, Whincup PH, Davey-Smith G, Gillman MW, Cook DG: The effect of breast-feeding on mean body mass index throughout life: a quantitative review of published and unpublished observational evidence. *Am J Clin Nutr* 82:1298–1307, 2005
22. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, Witteman JC, Hofman A: The Generation R Study: design and cohort profile. *Eur J Epidemiol* 21:475–484, 2006
23. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A: The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. *Eur J Epidemiol* 22:917–923, 2007
24. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, Moll HA, Jaddoe VW, Witteman JC: New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. *Ultrasound Obstet Gynecol* 31:388–396, 2008
25. Royal College of Obstetricians and Gynaecologists: Routine ultrasound screening in pregnancy: protocol RCOG. RCOG Press London, U.K., 2000
26. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK: Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. *Radiology* 150:535–540, 1984
27. SAS Institute: *SAS/STAT User's Guide*. Cary, NC, STATS Publishing, 1998
28. Royston P, Ambler G, Sauerbrei W: The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol* 28:964–974, 1999
29. Davey Smith G, Ebrahim S: What can mendelian randomisation tell us about modifiable behavioural and environmental exposures? *BMJ* 330:1076–1079, 2005
30. Kim KS, Choi SM, Shin SU, Yang HS, Yoon Y: Effects of peroxisome proliferator-activated receptor-gamma 2 Pro12Ala polymorphism on body fat distribution in female Korean subjects. *Metabolism* 53:1538–1543, 2004
31. Beamer BA, Yen CJ, Andersen RE, Muller D, Elahi D, Cheskin LJ, Andres R, Roth J, Shuldiner AR: Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor-γ2 gene with obesity in two Caucasian populations. *Diabetes* 47:1806–1808, 1998
32. Eriksson J, Lindi V, Uusitupa M, Forsen T, Laakso M, Osmond C, Barker D: The effects of the Pro12Ala polymorphism of the PPARgamma-2 gene on lipid metabolism interact with body size at birth. *Clin Genet* 64:366–370, 2003
33. Memisoglu A, Hu FB, Hankinson SE, Manson JE, De Vivo I, Willett WC, Hunter DJ: Interaction between a peroxisome proliferator-activated receptor gamma gene polymorphism and dietary fat intake in relation to body mass. *Hum Mol Genet* 12:2923–2929, 2003
34. Carver JD: Advances in nutritional modifications of infant formulas. *Am J Clin Nutr* 77:1550S–1554S, 2003
35. Stettler N, Zemel BS, Kumanyika S, Stallings VA: Infant weight gain and childhood overweight status in a multicenter, cohort study. *Pediatrics* 109:194–199, 2002
36. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J: A Pro12Ala substitution in PPAR-gamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20:284–287, 1998
37. Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura T, Fujita T, Ezaki O, Aizawa S, Kadowaki T, et al.: PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell* 4:597–609, 1999
38. Butte NF, Garza C, Stuff JE, Smith EO, Nichols BL: Effect of maternal diet and body composition on lactational performance. *Am J Clin Nutr* 39:296–306, 1984