Breast-Feeding Modulates the Influence of the Peroxisome Proliferator–Activated Receptor-γ (*PPARG2*) Pro12Ala Polymorphism on Adiposity in Adolescents

The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study

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OBJECTIVE — The peroxisome proliferator–activated receptor-γ2 (*PPARG2*) Pro12Ala polymorphism has been associated with a higher BMI and a lower risk of type 2 diabetes in adulthood. The association between adiposity and *PPARG* variants can be influenced by environmental factors such as early growth, dietary fat, and (as recently shown) breast-feeding. The objectives of this study were to assess *1*) the influence of the *PPARG2* Pro12Ala polymorphism on adiposity markers in adolescents and 2) a possible modulating effect of breast-feeding on these associations.

RESEARCH DESIGN AND METHODS — Data on breast-feeding duration, BMI, and genotypes for the Pro12Ala polymorphism were available for 945 adolescents (mean age 14.7 years). The breast-feeding duration was obtained from parental records. We measured weight, height, waist circumference, and six skinfold thicknesses.

RESULTS — No significant associations between the Pro12Ala polymorphism and any of the above-mentioned anthropometric parameters were found. There were significant interactions between the *PPARG2* Pro12Ala polymorphism and breast-feeding with regard to adiposity measurements (all adjusted $P < 0.05$). Indeed, in children who had not been breast-fed, Ala12 allele carriers had higher adiposity parameters (e.g., Δ BMI +1.88 kg/m^2, adjusted for age, sex, and center, $P = 0.007$) than Pro12Pro adolescents. In contrast, in breast-fed subjects, there was no significant difference between Ala12 allele carriers and Pro12Pro children in terms of adiposity measurements, whatever the duration of breast-feeding.

CONCLUSIONS — Breast-feeding appears to counter the deleterious effect of the *PPARG2* Pro12Ala polymorphism on anthropometric parameters in adolescents.

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The peroxisome proliferator–activated receptor-γ (PPARγ) transcription factor is primarily expressed in adipocytes. It is a member of the nuclear hormone receptor family, which influences whole-body energy homeostasis via three main metabolic pathways: adipocyte differentiation, insulin sensitivity, and lipoprotein metabolism. The *PPARG* gene (located on chromosome 3) gives rise to two different proteins, PPARγ1 and PPARγ2. The PPARγ2 protein is the more abundant isoform in adipose tissue, whereas PPARγ1 is ubiquitous. Of the several variants identified in the *PPARG* gene, one of the most common (minor allele frequency of ~10% in Caucasians) is the Pro12Ala (rs1801282) substitution at codon 12 in *PPARG2*. This polymorphism has been shown to be associated with reduced ability to transactivate responsive promoters and thus with lower PPARγ2 transcriptional activity (1).

In adults, the Pro12Ala polymorphism has been associated with higher BMI, waist circumference, and obesity risk (2–4). Even though a recent meta-analysis of genome-wide association studies for BMI failed to find any association between the Pro12Ala polymorphism and childhood or adult obesity (5), another meta-analysis showed that in selected subgroups, such as Caucasians and obese subjects, the Ala12 allele was associated with greater BMI and greater insulin sen-

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sitivity (6), suggesting that if this variant does influence obesity predisposition, it may do so through context-dependent mechanisms. This finding illustrates the importance for appropriate stratification of analyses by environmental or other genetic factors when *PPARG* variants are studied. More consistently, the Pro12Ala polymorphism has been associated with a lower risk of type 2 diabetes in a meta-analysis of genome-wide association studies (7).

Data in children are scarcer. In 311 Finnish children aged 7 years, the Ala12 allele was associated with a higher ponderal index at birth and higher waist circumference in adulthood, relative to those for Pro12Pro subjects (8). In Greek girls aged 3–4 years, adiposity was higher in Ala12 allele carriers than in Pro12Pro carriers (9).

Eriksson (10) showed that the well-known association existing between low birth weight and insulin resistance later in life was seen only in Pro12Pro individuals. Moreover, Meirhaeghe et al. (11) showed that individuals carrying the Ala12 allele had lower birth weight (due to shorter gestational duration and a higher risk of preterm birth) than Pro12Pro subjects. However, this result was not confirmed in 5,652 individuals from the Northern Finland Birth Cohort of 1966 (12). Labayen et al. (13) showed that low birth weight may program a lower fat-free mass in adolescents carrying the Ala12 allele.

Last, certain environmental factors (such as dietary fat and physical activity) interact with the effect of the *PPARG* polymorphism on adiposity. Mook-Kanamori et al. (14) showed that the growth rate from birth to 18 months of age was higher in Ala12Ala carriers than in Pro12Pro carriers when the duration of breast-feeding was between 0 and 4 months, whereas the Pro12Ala polymorphism was not associated with an early growth rate in infants breast-fed for longer than 4 months.

The aims of the present study were to *1*) assess the influence of the *PPARG2* Pro12Ala polymorphism on BMI, waist circumference, and the sum of six skinfold thicknesses in a sample of 945 European adolescents and *2*) test the modulating effect of breast-feeding on these associations.

RESEARCH DESIGN AND METHODS

The current report is based on data derived from the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study, the aim of which was to obtain a broad range of standardized, reliable, and comparable nutrition- and health-related data from a random sample of European adolescents aged 12.5–17.5 years. Data collection took place during 2006 and 2007 in 10 European cities. A detailed description of the HELENA study sampling has been published elsewhere (15).

All of the adolescents meeting the general HELENA inclusion criteria and having data for age, sex, and BMI were considered in the final sample (n = 3,546). To investigate biochemical assays and genetic analyses, one-third of the cohort was randomly selected for blood collection (resulting in a total of 1,155 subjects). Of the latter, the 945 adolescents with data on the *PPARG2* Pro12Ala polymorphism and BMI and breast-feeding information were included in the present study.

After receiving comprehensive information on the study's aims and methods, all adolescents and their parents or guardians signed informed consent forms. The study was performed according to the ethical guidelines of the Edinburgh revision of the 1961 Declaration of Helsinki (2000), good clinical practice, and the legislation on clinical research in each of the participating countries. The protocol was approved by the investigational review boards at the participating university medical centers.

The harmonized, standardized anthropometric measurements were strictly monitored. Participants were barefoot and in underwear, and anthropometric measurements were taken by trained researchers. Weight was measured with an electronic set of scales (Type SECA 861; precision 0.05 kg) and height was measured in the Frankfort plane with a height gauge (Type SECA 225; precision 1 mm). Waist circumference was measured with a nonelastic tape (Seca 200; precision 1 mm) to the nearest 0.1 cm. Skinfold thicknesses were measured at the left biceps, triceps, subscapular area, suprailiac area, thigh, and calf with a Holtain caliper (precision 0.2 mm), according to Lohman's anthropometric standardization reference manual. The overall score was calculated by summing the six skinfold thicknesses. Mean skinfolds and circumferences were calculated from three consecutive measurements.

Identification of sexual maturation (Tanner and Whitehouse stages I–V) was assessed by a physician. Weight and height at birth and the durations of gestation and breast-feeding were collected via a parental questionnaire. The duration of gestation was stratified into three categories: <35, between 35 and 40, and >40 weeks. The total duration of breast-feeding was recoded from six categories into four: never, <3, 3–5, and ≥6 months. The duration of exclusive breast-feeding (defined by the World Health Organization as no liquid or solid nutrition other than breast milk) was recoded in a similar manner.

A uniaxial accelerometer (ActiGraph GT1M, Pensacola, FL; http://www.theactigraph.com) was used to assess physical activity. Adolescents were instructed to place the monitor underneath clothing, at the lower back, using an elastic waistband, and to wear it for 7 consecutive days. They were also instructed to wear the accelerometer during all time awake and only to remove it during water-based activities. At least 3 days of recording with a minimum of 8 h registration/day was set as an inclusion criterion. In this study, the time-sampling interval (epoch) was set at 15 s. A measure of total volume of activity (hereafter called average physical activity) was expressed as the sum of recorded counts per epoch divided by total daily registered time expressed in minutes (counts per minute) (16).

The socioeconomic level was assessed in terms of the maternal educational level and was coded into four categories (elementary, lower secondary, higher secondary, or higher education).

Preparation of genomic DNA from whole blood and genotyping

Blood samples were drawn at school after a 10-h, overnight fast and according to a standardized collection protocol; blood for DNA extraction was collected in EDTA K3 tubes. DNA was extracted from white blood cells with the Puregene kit (Qiagen, Courtaboeuf, France) and stored at −20°C. Genotyping of the Pro12Ala polymorphism was performed on an Illumina system using GoldenGate technology (Illumina, San Diego, CA). The genotyping success rate was 99.4%.

Statistical analyses

Statistical analyses were performed with SAS software (SAS Institute, Cary, NC). Deviation from Hardy-Weinberg equilibrium was tested using the χ^2 test (1 degree of freedom). The BMI and the sum of the

Table 1—*Descriptive characteristics of the HELENA study sample*

	n	Value
Neonatal data		
Birth weight (kg)	914	3.33 ± 0.58
Birth height (cm)	882	50.4 ± 3.2
Duration of total breast-feeding		
Never breast-fed		173 (18.3)
<3 months		279 (29.5)
3–5 months		237 (25.1)
≥6 months		256 (27.1)
Duration of pregnancy		
<35 weeks		49 (5.4)
35–40 weeks		574 (63.6)
>40 weeks		280 (31.0)
Clinical characteristics		
Boys		434 (45.9)
Girls		511 (54.1)
Pubertal status		
Tanner stage 2		12 (1.4)
Tanner stage 3/4		601 (70.3)
Tanner stage 5		242 (28.3)
Age (years)	945	14.7 ± 1.4
BMI (kg/m^2)	945	21.3 ± 3.8
Waist circumference (cm)	935	72.2 ± 9.3
Sum of 6 skinfolds (mm)	887	92.0 ± 41.4
Physical activity (cpm)	638	434 ± 151

Data are means ± SD or *n* (%).

six skinfolds were normalized by log transformation. We compared groups in terms of genotype and allele distributions by using χ^2 tests. Intergroup comparisons of quantitative variables were performed using a general linear model. Reported *P* values were systematically adjusted for confounding variables. Data on anthropometric phenotypes were adjusted for age, sex, and center. Data on weight and height at birth were adjusted for age, sex, center, and gestational duration. Study center was used as a surrogate estimate of ethnicity. The presence of interaction between polymorphism and breast-feeding for anthropometric variables was tested with a general linear model adjusted for age, sex, and center. The threshold for statistical significance was set to $P \leq 0.05$. Power calculations were performed using Quanto v1.2.4 (17).

RESULTS — Within our sample, 81.7% of adolescents had been breast-fed (Table 1). A quarter of the adolescents had finished puberty (28.3%). There were 746 (78.9%) Pro12Pro, 187 (19.8%) Pro12Ala, and 12 (1.3%) Ala12Ala subjects (Ala12 allele frequency = 0.11) in the sample. This distribution respected the Hardy-Weinberg equilibrium in the HELENA study ($P = 0.94$) and in each center separately (data not shown).

Table 2 presents the association between the *PPARG2* Pro12Ala polymorphism and the neonatal characteristics and adiposity measurements. No significant associations were found between the Pro12Ala polymorphism and BMI, waist circumference, and the sum of skinfolds. Accordingly, underweight ($n = 61$), normal-weight ($n = 663$), overweight ($n = 164$), and obese children ($n = 57$) did not differ significantly in terms of the genotype distribution of the *PPARG2* Pro12Ala polymorphism ($P = 0.82$) (data not shown). However, Ala12Ala subjects had lower weight ($P = 0.03$) and height ($P = 0.02$) at birth than subjects carrying the Pro12 allele (Table 2), independently of the duration of gestation. The genotype distribution of the polymorphism did not differ among subjects born before 35 weeks, between 35 and 40 weeks, or after 40 weeks of pregnancy ($P = 0.98$).

After checking that the distribution of the Pro12Ala polymorphism was similar in all four breast-feeding categories (never breast-fed, <3 months, 3–5 months, and ≥6 months) ($P = 0.73$), breast-feeding was introduced into the analysis. We detected significant interactions between the Pro12Ala polymorphism and breast-feeding, when considering BMI (adjusted for age, sex, and center, $P = 0.004$), waist circumference (adjusted $P = 0.03$), or skinfolds (adjusted $P = 0.03$). Indeed, in children who had not been breast-fed ($n = 173$), Ala12 allele carriers had higher BMI (+1.88 kg/m^2, adjusted $P = 0.007$) (Fig. 1*A*), higher waist circumference (+3.8 cm, adjusted $P = 0.02$) (Fig. 1*B*), and higher skinfold thicknesses (+16.3 mm, adjusted $P = 0.03$) (Fig. 1*C*) than Pro12Pro subjects. This association was not altered by further adjustment for maternal educational level, Tanner and Whitehouse stage, average physical activity level, birth weight, or duration of gestation (data not shown). In contrast, in children who had been breast-fed, there was no significant difference in adiposity measurements between Ala12 allele carriers and Pro12Pro subjects, whatever the duration of breast-

Table 2—*Association between the* PPARG2 *Pro12Ala polymorphism and body composition and neonatal characteristics in the HELENA study*

					*P**	
	Pro12Pro	Pro12Ala	Ala12Ala	*P**	X/Ala12 vs. Pro12Pro	Ala12Ala vs. X/Pro12
n	746	187	12			
BMI (kg/m²)	21.3 ± 3.6	21.4 ± 4.3	20.2 ± 2.5	0.55	0.98	0.29
Waist circumference (cm)	72.1 ± 9.2	72.8 ± 10.0	69.8 ± 7.5	0.50	0.78	0.29
Sum of 6 skinfolds (mm)	92.2 ± 41.1	92.5 ± 43.5	73.2 ± 24.0	0.52	0.79	0.31
Birth weight (kg)	3.33 ± 0.57	3.34 ± 0.57	2.90 ± 1.08	0.10†	0.43†	0.03†
n	689	177	10			
Birth height (cm)	50.4 ± 3.1	50.4 ± 2.7	47.7 ± 6.1	0.07†	0.43†	0.02†
n	666	171	10			
Duration of gestation						
<35 weeks	38 (0.78)	10 (0.20)	1 (0.02)			
35–40 weeks	454 (0.79)	113 (0.20)	7 (0.01)	0.98		
>40 weeks	219 (0.78)	58 (0.21)	3 (0.01)			

Data are means ± SD or *n* (frequency). *Adjusted for age, sex. and center. †Adjusted for age, sex, center, and gestational duration.

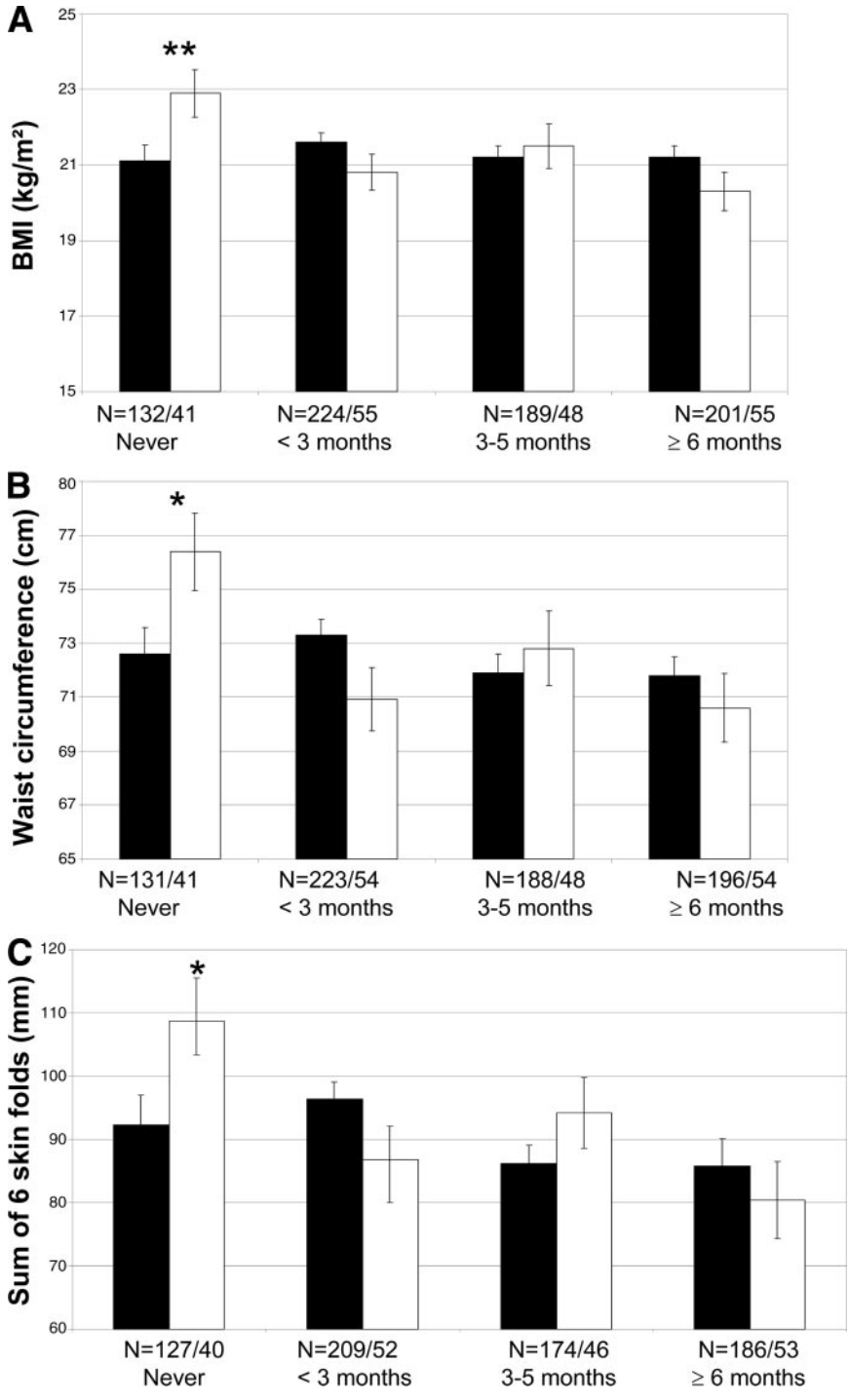

Figure 1—A*: Mean BMI as a function of the breast-feeding duration in* PPARG2 *Pro12Pro* (■) *vs. Ala12 allele carriers* (□). **P = *0.007 (adjusted for age, sex, and center).* B*: Mean waist circumference as a function of breast-feeding duration in* PPARG2 *Pro12Pro* (■) *vs. Ala12 allele carriers* (□). *P = *0.02 (adjusted for age, sex, and center).* C*: Mean sum of skinfolds as a function of breast-feeding duration in* PPARG2 *Pro12Pro* (■) *vs. Ala12 allele carriers* (□). *P = *0.03 (adjusted for age, sex, and center).*

feeding. It is noteworthy that our analyses yielded similar results when we used the duration of exclusive breast-feeding (data not shown). Furthermore, there were no significant interactions with sex ($P >$ 0.90), and the associations were similar in boys and girls (data not shown).

We performed power calculations in the whole sample ($n = 945$) using a dominant or a recessive model, and in the non–breast-fed children subsample ($n =$ 173) using a dominant model only (Table 3). As an example, the whole sample had sufficient power (>80%) to identify significant effect sizes of at least 0.75 kg/m^2 for BMI, 2.1 cm for waist circumference, 9.6 mm for skinfold thicknesses, 140 g for birth weight, and 0.7 cm for birth height using a minor allele frequency of 0.11 under a dominant model.

CONCLUSIONS — In the present study, the *PPARG2* Ala12 allele was associated with higher adiposity indexes (BMI, waist circumference, and the sum of skinfolds) in children who had not been breast-fed. However, this association was not seen in children who had been breast-fed (even for a short period).

Our results are in agreement with those of Mook-Kanamori et al. (14), who showed that the Ala12 allele was associated with increased weight gain in early infancy in non–breast-fed children (14). We observed similar findings for BMI, waist circumference, and skinfolds, even later in life (i.e., adolescence). This result supports the hypothesis whereby breast-feeding has a beneficial effect on the obesity risk later in life in a genetically predisposed group.

Our study illustrates an association between an environmental factor (breast-feeding) and the phenotypic expression of a gene (modulation of anthropometric parameters by *PPARG*) and thus suggests that phenotypes modulated by *PPARG2* polymorphisms can be influenced by gene-environment interactions early in life. Barker (18) has explained the impact of pre- and postnatal nutrition later in life by the theory of "nutritional programming": what is beneficial in utero and during the postnatal period in cases of undernutrition could become deleterious in the event of an excessive nutritional environment (i.e., metabolic diseases). The exact mechanisms involved in this type of phenomenon are still subject to speculation; they may begin to operate during fetal life and continue until the early neonatal period. A recent meta-analysis performed by the World Health Organization, including 33 studies, concluded that breast-fed individuals were less likely to be overweight and/or obese in childhood and adolescence (19). Some studies but not all showed a dose-response effect, with a more pronounced effect associated with a long duration of breast-feeding (20). The reason for the absence of a dose-response effect on the

Table 3—*Power calculation for the* PPARG2 *Pro12Ala polymorphism effects*

	Mean Δ using a dominant model	Mean Δ using a recessive model
In the HELENA study (n = 945)		
BMI (kg/m^2)	0.75	3.15
Waist circumference (cm)	2.1	7.7
Sum of 6 skinfolds (mm)	9.6	36
Birth weight (kg)	0.14	0.50
Birth height (cm)	0.7	2.8
In non–breast-fed children (n = 173)		
BMI (kg/m^2)	2.1	NC
Waist circumference (cm)	5.0	NC
Sum of 6 skinfolds (mm)	23.1	NC

NC, not calculated.

PPARG2 Ala12 allele in our study is unclear; one possible explanation is that the programming effect of breast-feeding is more strongly influenced by gene × nutrient interactions at an early age rather than a quantitative process linked to the duration of the exposition.

A number of mechanisms can potentially explain how breast-feeding could counterbalance the deleterious effect of the Ala12 allele in adolescents. It has been shown that the association between dietary fat and BMI is influenced by *PPARG2* genotypes. Memisoglu et al. (21) found that monounsaturated fat–rich diets were inversely associated with BMI in Ala12 allele carriers, but the authors did not find any association in Pro12Pro women. Similarly, Luan et al. (22) showed that for a diet with a low polyunsaturated-to-saturated fat ratio, Ala12 allele carriers had a greater BMI than Pro12Pro carriers. Considering that breast milk constitutes a diet with specific fat intake (with a higher proportion of polyunsaturated fatty acids than formula milk [23]), our results seem to be in line with those reported by Luan et al. (22), albeit their study was conducted in adults. Moreover, one potential hypothesis is that breast milk or breast-feeding supplies factors such as prostaglandin J_2 (24), a natural PPARγ ligand. The decrease in PPARγ2 transcriptional activity observed in Ala12 allele carriers could be, therefore, compensated for by breast milk. The latter also contains a number of adipokines. It is known that PPARγ agonists (such as the thiazolidinediones) can downregulate leptin expression (25); however, the presence of this compound in breast and/or formula milk has yet to be established and would require further investigation.

We also showed in the present study that Ala12Ala subjects had a lower body weight (−430 g) and height (−2.7 cm) at birth than subjects carrying the Pro12 allele, independently of the duration of gestation. Although these results need to be considered with caution (as they concern only 12 homozygote children) and replicated, they are in line with previous data. Indeed, in two Irish population samples, we have previously shown that the *PPARG2* Ala12 allele was associated with lower birth weight (primarily caused by shorter gestational duration) (11).

The present study has certain limitations. First, the duration of gestation was coded into three categories rather than being specified in weeks and was obtained from questionnaires filled out by the parents (rather than from a national health registry). Therefore, the accuracy of the data on gestational duration needs to be considered with circumspection. Second, the "being small for gestational age" phenotype could not be assessed. However, because the duration of gestation did not influence the effect of the *PPARG2* polymorphism in the present study, we believe that this factor did not bias our results. Likewise, we lacked information on singleton or multiple pregnancies, which have different growth patterns. Other factors (such as parental weight status, food preferences, or smoking status) known to influence the effect of breast-feeding on the subject's subsequent BMI could not be assessed in our study. However, the main factors known to influence fat mass were available and did not alter the observed associations when used as confounders. Third, study center was used as a surrogate estimate of ethnicity, which is not ideal and may induce misclassification. Last, the subgroup of non–breast-fed children was relatively small (n = 173), which might make it prone to identification of false-positive associations. However, we feel confident of our data as they are in line with the data of Mook-Kanamori et al. (14).

In summary, our results suggest that breast-feeding can counterbalance the deleterious impact of the *PPARG2* Pro12Ala polymorphism on adiposity in adolescents. These findings confirm the importance of taking account of gene-environment interactions in association studies and the possible effect of early, diet-based prevention programs in population subgroups. At a time when the prevalence of obesity in children and adolescents continues to increase, our results may constitute a new argument in favor of the public health benefits of breast-feeding.

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