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Adiponectin and leptin gene variants and their effects on body weight trajectories in children from birth to 6 years of age: the PREDI Study

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Abstract

Excess body weight confers a high risk to human health. Body weight variation between subjects can be partially explained by genetic differences. The aim of the present study was to investigate the association of genetic variants in the *ADIPOQ* (rs2241766) and *LEP* (rs7799039) genes with body weight trajectories in children from birth to 6 years of age. This was a prospective cohort (PREDI Study). Socio-economic, biological and anthropometric data were collected at four time points: at birth in the maternity unit; 1–2, 4–5 and 6 years old at the participants' homes. Genotyping was performed by PCR-restriction fragment length polymorphism. Poisson regression and linear mixed-effect regression models were used to address the association of *ADIPOQ* and *LEP* genotypes with BMI. Excessive body weight at pre-pregnancy ($\beta = 0{\cdot}339$, $P = 0{\cdot}01$) and excessive gestational weight gain ($\beta = 0{\cdot}51$, $P < 0{\cdot}001$) were associated with children's BMI trajectory from birth to 6 years. The *ADIPOQ*-rs2241766 TG or GG genotype was associated with a higher risk of excess body weight in the first 6 years of life (both sexes relative risk 1·25, 95 % CI 1·01, 1·56; female relative risk 1·67, 95 % CI 1·20, 2·31). BMI increased over the years according to the presence of the TG or GG genotype ($\beta = 0{\cdot}01$, 95 % CI 0·01, 0·02), particularly in females ($\beta = 0{\cdot}02$, 95 % CI 0·01, 0·04). The *ADIPOQ*-rs2241766 TG and GG genotypes increased the risk of excess body weight in children from birth to 6 years of age and had a positive effect on body weight trajectories in girls. The *LEP*-rs7799039 genetic variant was not associated with body weight trajectory in children.

Key words: **Adiponectin: Leptin: Children: Overweight**

Excess body weight is the abnormal or excessive accumulation of adipose tissue and poses a high risk to human health[1,2]. The prevalence of excess body weight among children has more than doubled, with a dramatic increase from just 4 % in 1975 to over 18 % in 2016. This condition has become a global epidemic and a serious public health problem[3–5]. Children with obesity are very likely to remain obese as adults and are at risk of developing many co-morbidities, including type 2 diabetes mellitus, CVD, dyslipidaemia and other disorders[6–8].

The mechanisms of body weight regulation are complex and involve the interaction of genetic, environmental and behavioural factors that act through several physiological mediators of food intake and energy expenditure, thus affecting fat deposition[1]. Within this context, genes encoding adipokines (polypeptides secreted by adipocytes) and genes involved in energy balance play a potential role in systemic energy homoeostasis[9,10]. Adiponectin and leptin are important adipokines because of their hormonal function in energy homoeostasis, controlling food consumption and energy expenditure[1,11–14]. Furthermore, adiponectin and leptin exert endocrine functions and are particularly involved in lipogenesis and lipolysis, suggesting that the two adipokines may regulate adipose tissue metabolism[14].

Genetic variants of adiponectin (*ADIPOQ*) and leptin (*LEP*) play a role in energy balance and appetite regulation[12,13,15–17] and are associated with BMI and adiposity[17–22]. Circulating levels of adiponectin are under substantial genetic influence[23], and variants in the *ADIPOQ* gene may therefore be associated with the pathophysiology of obesity[24]. The presence of *LEP* gene variants also appears to be associated with higher levels of energy and total lipid intake, influencing the individual's weight status[25,26]. However, to the best of our knowledge, no study has evaluated the effect of the *ADIPOQ* and *LEP* genes on the weight status of children from birth to 6 years of age. Identifying genetic variants associated with excess body weight during childhood including the early infancy period will help develop strategies to prevent or reduce the prevalence of

Abbreviations: GWG, gestational weight gain; LME, linear mixed-effect.

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overweight and obesity. Within this context, the aim of the present study was to investigate the association of genetic variants in the *ADIPOQ* (rs2241766) and *LEP* (rs7799039) genes with body weight trajectories in a 6-year prospective birth cohort.

Methods

Study design and population

This prospective cohort study is part of the Predictors of Maternal and Infant Excess Body Weight (PREDI) Study, which was started in 2012 in Joinville, Brazil. The city has an estimated population of 590 000 inhabitants, a Human Development Index of 0·809 and an infant mortality rate of 8·78/1000 live births[27].

An in-depth description of the PREDI Study has been published previously[28–31]. Briefly, all women over 18 years of age who gave birth to a full-term singleton (between 37 and 42 weeks of gestation) admitted to a public maternity hospital in Joinville (Brazil) from 14 January to 16 February 2012 were invited to participate. Women with a diagnosis of pre-eclampsia, infectious disease (AIDS, hepatitis, syphilis and toxoplasmosis) and stillbirth were excluded from the study. Children with any anomalies such as ambiguous genitalia and hydrocephaly that could interfere with their weight and height measurements and those who were adopted immediately after delivery were also excluded.

The PREDI Study was approved by the Research Ethics Committee of the University of Joinville Region (protocol no. 107/2011).

Data collection

Anthropometric measurements and clinical, biological, demographic, and socio-economic data were collected at four time points using a structured questionnaire: 2012 (baseline, at birth) at the maternity hospital; 2013–14 (first follow-up, 1–2 years old), 2016–17 (second follow-up, 4–5 years old) and 2018 (third follow-up, 6 years old) at the family home. A total of 435 mother–child pairs were enrolled in the study in 2012. After exclusions and losses, 315 pairs of women and their children continued to participate in the first follow-up of the study, 221 in the second follow-up and 187 in the third follow-up (Fig. 1).

Measurements

Maternal age (years), marital status (married/consensual union or other), education (years of schooling, ≥9 or <9), household income (minimum wage, ≥3 or <3) and parity at baseline (number of deliveries considering children born before the baseline, nulliparous or ≥1 child) were self-reported by the mother. Type of delivery (normal or Caesarean), birth weight (g), length (cm) and sex (male or female) were collected from the hospital records. Birth weight was classified considering the gestational age and sex according to INTERGROWTH-21st standards[32] and classified as follows: small for gestational age (<10th percentile), appropriate for gestational age (10th–90th percentile) or large for gestational age (>90th percentile)[33].

The pre-pregnancy BMI (weight (kg)/height (m^2)) was based on the mother's self-reported pre-pregnancy weight and

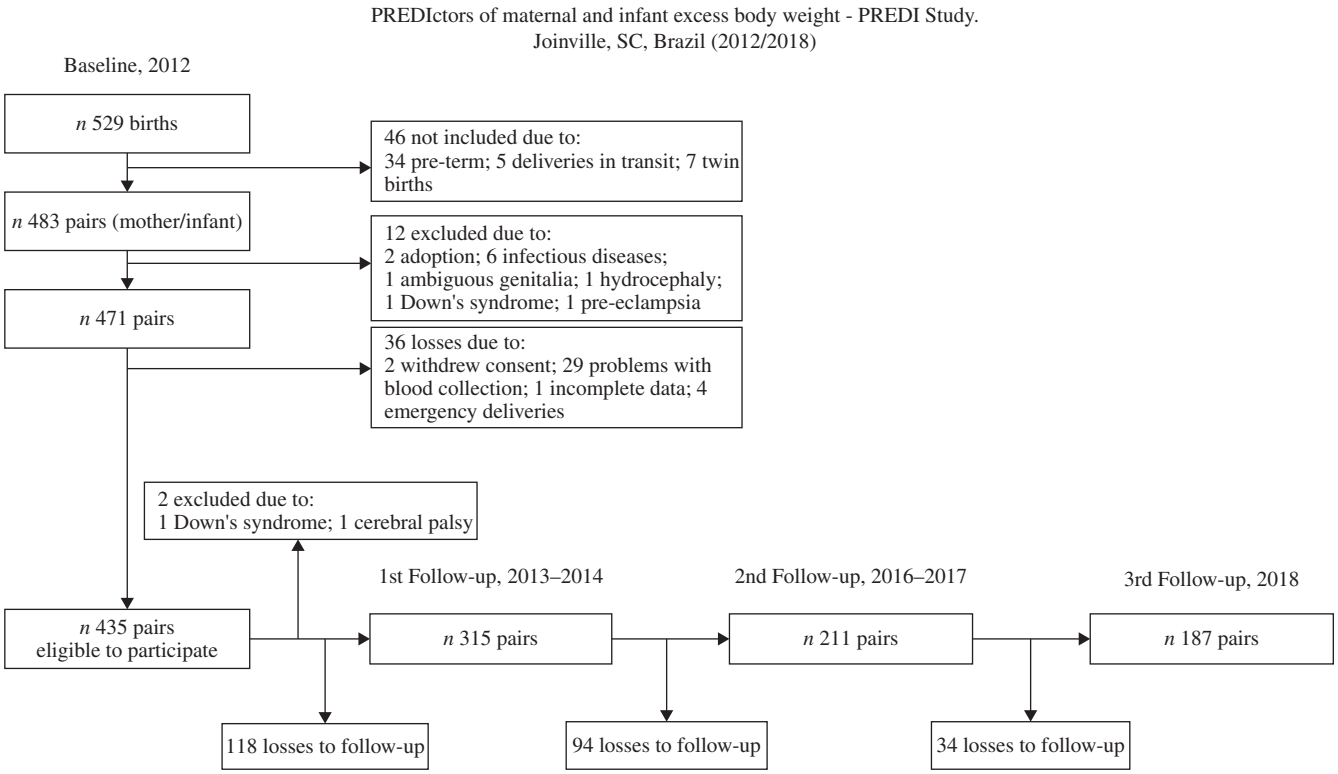

Fig. 1. Flow chart of participants through the recruitment process. PREDI Study, Brazil (2012–2018).

immediate postpartum height, which was measured to the nearest 0·1 cm with a portable stadiometer (WCS®, Compact)[34]. Pre-pregnancy BMI was classified based on the cut-offs recommended by the WHO[2]. We defined BMI ≥ 25 kg/m^2 as excess body weight. Total gestational weight gain (GWG) was obtained by subtracting self-reported pre-pregnancy weight from the weight obtained immediately before delivery. The adequacy of GWG was classified (non-excessive/excessive) according to the Institute of Medicine guidelines[35]. Excessive GWG was defined when the mother exceeded the recommended GWG[35].

In the first follow-up, the children's weight was measured with a paediatric digital portable scale (Beurer®, BY20) to the nearest 10 g, and length was measured with a paediatric anthropometric ruler (WCS®, Wood) to the nearest 0·1 cm[36]. In the second and third follow-ups, the children's weight was measured using a portable digital scale (G-Tech, Glass-7) with a capacity of 180 kg to the nearest 0·1 kg, and height was measured with a portable stadiometer (WCS®, Compact)[34] to the nearest 0·1 cm.

The children's weight status was based on the 2006 WHO[37] and 2007 WHO[38] growth standards for BMI-for-age for children and adolescents aged 0–5 and 5–19 years, respectively. We defined BMI > 85th percentile as excess body weight in children. All anthropometric measurements were performed in duplicate and the mean values were used for analysis.

Genotyping

The genotyping method has been described previously[28]. Approximately 10 ml of blood were collected from the newborn at the time of delivery by aspiration of the umbilical vein close to the placenta up to 10 min after clamping to prevent coagulation[39]. The blood samples were transferred to an FTA CloneSaver™ card (GE Healthcare). Genomic DNA was extracted from the cards by an adaptation of the method described by Kline *et al.*[40]. The *ADIPOQ*-rs2241766 and *LEP*-rs7799039 genetic variants were analysed by the PCR followed by restriction fragment length polymorphism analysis.

DNA was amplified using the following primers: forward: 5′-TCTCTCCATGGCTGACAGTG-3′ and reverse: 5′-CCTTTCTCACCCCTTCTCACC-3′ for the *ADIPOQ* gene[41]; forward: 5′-CTTTTGTTTTGTTTTGCGACAGGGGTGC-3′ and reverse: 5′-GCTCCCTTTGCCCGACCCCG-3′ for the *LEP* gene[42]. After amplification, restriction fragment length polymorphism analysis was performed using the restriction enzymes *Sma*I for the *ADIPOQ* gene variant[41] and *Alw44*I for the *LEP* gene variant[43].

Statistical analysis

The sample size was calculated with the OpenEpi 3.02 software as described previously[39]. We performed *post hoc* tests to compute the exact power achieved considering the effect size of the polymorphism–time interaction on child BMI, an α error of 5 % and ANOVA for repeated measures with within–between interactions. Considering these parameters, the sample size achieved a power >90 % to detect a small effect size (Cohen's effects size $f = 0{\cdot}1$). The calculation was performed using the G × Power software (version 3.1.9.2)[44].

Maternal and child characteristics are expressed as mean and SD for continuous variables, and as absolute and relative frequencies for categorical variables. Student's t test and the χ^2 test were used to compare sociodemographic and biological characteristics between women and children who were lost to follow-up and those who completed the study (evaluated up to the third follow-up visit).

The agreement of genotype frequencies with Hardy–Weinberg equilibrium expectations was tested using the χ^2 test. Due to the small number of rare alleles, the genotypes were analysed using a dominant model (wild-type genotype: *ADIPOQ*-TT and *LEP*-GG *v.* genotypes with at least one risk allele: *ADIPOQ*-TG or GG and *LEP*-GA or AA).

The longitudinal association of maternal and child sociodemographic and biological characteristics with changes in children's BMI from birth to 6 years of age was assessed using longitudinal linear mixed-effect (LME) models. The longitudinal linear regression coefficient (β), mean and 95 % CI are reported. Poisson regression analysis with robust variance was used to address the association of *ADIPOQ* and *LEP* genotypes with BMI categories, with the estimation of relative risk and 95 % CI.

We further used LME models to explore the potential association between the polymorphisms and BMI trajectories (outcome) from birth to 6 years of age. Interaction terms were included in the models to examine the potential effect of differences in age and sex on the association between *ADIPOQ* and *LEP* variants and BMI trajectories: (1) child age (continuous variable) and genetic variants (genotype categories); (2) child age (continuous variable), sex (male/female) and genetic variants (genotype categories).

In all LME models, child age (months) at the follow-ups was used to model time and was fitted as a random effect with an unstructured covariance matrix in order to accommodate the wide variability of slopes among the subjects over time. All other covariates were analysed as fixed effects only. Effect plots of the longitudinal prediction and 95 % CI were constructed to illustrate the BMI trajectories from birth to 6 years of age according to *ADIPOQ*-rs2241776 gene variant, as well as the interaction between the genetic variant and sex.

The variables included in the adjusted Poisson regression models and LME analyses were chosen based on the biological plausibility of the association between *ADIPOQ* and *LEP* gene variants and the child's weight status. We also considered the small number of children carrying the risk allele, limiting the number of variables used in the models. All models were adjusted for time. The time of follow-up (baseline, first, second and third follow-up, as categorical variables) was considered for Poisson regression analysis and the child's age in months on the day of the visit (as continuous variables and defining baseline as time zero) for LME. Linear, quadratic and cubic functions of child age were included to adjust for the non-linear association between BMI and time. The models were also adjusted for education (years of schooling), GWG (non-excessive/excessive) and sex (male/female). Effect plots of the longitudinal prediction and 95 % CI were fitted to graphically illustrate the effect of the interaction between *ADIPOQ*-rs2241776 gene variant and time on the BMI trajectories from birth to 6 years of age according to child sex.

The results were considered statistically significant when $P < 0{\cdot}05$. The IBM Statistical Package for the Social Sciences (SPSS) (version 22.0, IBM Corp.) and STATA statistics software (version 12.0) were used for statistical analysis. The graphs were constructed using the R software (version 3.6.3, R Foundation for Statistical Computing, 2019).

Results

Women who were lost to follow-up were younger than those who completed the study. Except for age, none of the characteristics investigated differed significantly among the follow-ups of the study (online Supplementary material 1).

The prevalence of children with excess body weight was 39·5, 42·0, 30·6 and 25·9 % at birth, 1–2, 4–5 and 6 years of age, respectively (data not shown in the tables). The study sample consisted of mothers with a mean age of 26 years (95 % CI 25·4, 26·5) and 9·4 years of schooling (95 % CI 9·1, 9·7). In addition, 45·3 % of the mothers had excessive GWG and 37·9 % were classified as having pre-pregnancy excessive body weight. Regarding weight status at birth, 23·7 % of the boys and 18·7 % of the girls were born large for gestational age (Table 1).

The prevalence of LGA newborns was 21·4 %. The frequencies of children with at least one risk allele were 23·3 and 62·3 % for *ADIPOQ*-rs2241766 and *LEP*-rs7799039, respectively (Table 1). The minor allele frequencies for *ADIPOQ*-rs2241766 and *LEP*-rs7799039 were 12·0 % (G allele) and 38·0 % (A allele) at baseline. In addition, the allele frequencies of both genetic variants were in Hardy–Weinberg equilibrium (online Supplementary material 2).

Longitudinal regression analysis showed that maternal excess body weight at pre-pregnancy ($\beta = 0{\cdot}339$, $P = 0{\cdot}010$) and excessive GWG ($\beta = 0{\cdot}510$, $P < 0{\cdot}001$) were associated with higher children's BMI from birth to 6 years of age. Girls presented lower BMI trajectories ($\beta = -0{\cdot}432$, $P = 0{\cdot}001$), while large for gestational age newborns had higher BMI trajectories ($\beta = 0{\cdot}480$, $P < 0{\cdot}001$), when compared with boys and small for gestational age/appropriate for gestational age newborns, respectively (Table 2).

Children carrying the TG or GG genotype of the *ADIPOQ*-rs2241766 variant had a higher risk of excess body weight compared with children with the TT genotype (relative risk = 1·25, 95 % CI 1·01, 1·56, $P = 0{\cdot}044$), even after adjustment for confounders. Girls carrying the TG or GG genotype were 1·67 times more likely to have excess body weight from birth to 6 years of age than those carrying the wild-type TT genotype (95 % CI 1·20, 2·31, $P = 0{\cdot}002$). However, the risk genotypes of *LEP*-rs7799039 (GA or AA) were not associated with weight status trajectory (relative risk = 0·93, 95 % CI 0·76, 1·15, $P = 0{\cdot}513$) (Table 3).

The LME models for children's BMI are shown in Table 4. The interaction between child age and *ADIPOQ*-rs2241766 genotypes was associated with children's BMI trajectory. The effect of age on increasing body weight was slightly steeper in children carrying the TG or GG genotype compared with those with the TT genotype ($\beta = 0{\cdot}12$, 95 % CI 0·01, 0·22, $P = 0{\cdot}010$) and remained significant after adjustments ($\beta = 0{\cdot}01$, 95 % CI 0·01, 0·02, $P = 0{\cdot}008$). Child sex was found to be an effect modifier of the association between *ADIPOQ*-rs2241766 and BMI during infancy. When the analysis was performed by sex, only girls with the TG or GG genotype continued to show a subtle increase in body weight trajectory over time ($\beta = 0{\cdot}02$, 95 % CI 0·01, 0·04, $P = 0{\cdot}002$) (Fig. 2). The *LEP*-rs7799039 gene variant was not associated with BMI trajectories in children ($\beta = 0{\cdot}01$, 95 % CI −0·01, 0·01, $P = 0{\cdot}586$) (Table 4).

Discussion

The present study has two main findings. First, children carrying the *ADIPOQ*-rs2241766 TG or GG genotype had a higher risk of excess body weight from birth to 6 years of age, even after adjustment for key covariates. Second, the risk genotype (TG or GG) of the *ADIPOQ* gene variant was associated with an increase in the child's body weight over the years only in girls. Additionally, there was no association between *LEP*-rs7799039 and excess body weight or weight trajectories in children. Although the evaluation of excess body weight was not the objective of the present study, it is important to highlight that it was a substantial burden in this cohort, with the observation of a high prevalence throughout the years.

The minor allele frequencies of the *ADIPOQ*-rs2241766 and *LEP*-rs7799039 genetic variants found in our study were similar to those reported in studies conducted on European and other Latin-American populations[45], suggesting similarity of these genetic predictors to other populations. Our results also agree with the findings of a meta-analysis that included 5843 adults from eighteen case–control studies[46]. The authors found that the *ADIPOQ*-rs2241766 genetic variant was associated with obesity in the overall populations (GG *v.* TT, OR = 1·39, 95 % CI 1·11, 1·73)[46]. On the other hand, some cross-sectional studies reported results different from those found in our longitudinal analysis[20,47]. In a cross-sectional study on 1469 Mexican children aged 6–12 years, the authors observed no association between the *ADIPOQ*-rs2241766 gene variant and overweight/obesity[20]. Another cross-sectional study including 453 Turkish children and adolescents aged 6–17 years also found no difference in the TT (wild type) or non-TT (TG + GG) genotype between the obese and non-obese groups[47]. However, the lack of longitudinal studies investigating children aged 0–6 years impaired direct comparison with our results.

Adiponectin regulates several metabolic processes, including glucose homoeostasis and fatty acid oxidation[1]. The decrease in serum adiponectin concentration mediated by genetic variation promotes a reduction in the phosphorylation of target proteins by AMP-activated protein kinase (AMPK), which is critical for lipid and carbohydrate metabolism[13,22,48,49]. Therefore, the involvement of the metabolism of many tissues may decrease energy consumption and stimulate food intake[48]. It is reasonable to suppose that the *ADIPOQ*-rs2241766 gene variant and child age interact to change body weight trajectories over the years. Additionally, the obesogenic environment such as inadequate food habits and physical inactivity can potentiate the genetics effects over the years[50]. According to Belsky *et al.*[51], the genetic risk first manifests as a rapid growth during early childhood. After birth, children at higher genetic risk gain

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Table 1. Baseline characteristics of the study participants (PREDI Study, Brazil, 2012–2018)
(Numbers and percentages; mean values and standard deviations)

		Male				Female				Total			
Characteristic	*n*	*n*	%	Mean	SD	*n*	%	Mean	SD	*n*	%	Mean	SD
Mothers													
Age (years)	435			25·7	5·9			26·3	6·3			26·0	6·0
Marital status	435												
Married/consensual union		195	84·1			166	81·8			361	83·0		
Single		34	14·7			31	15·3			65	14·9		
Widowed/separated		3	1·3			6	3·0			9	2·1		
Education (years)	435			9·5	2·9			9·3	3·1			9·4	3·0
≥12		27	11·6			31	15·3			58	13·3		
9–12		129	55·6			94	46·3			223	51·3		
<9		76	32·8			78	38·4			154	35·4		
Monthly household income (MW)	415*			3·4	2·6			3·4	2·6			3·4	2·6
≥3		123	56·2			115	58·7			238	57·3		
<3		96	43·8			81	41·3			177	42·6		
Parity before 2012 (number of deliveries)	435			1·1	1·4			1·2	1·5			1·2	1·4
Nulliparous (<1 child)		98	42·2			81	39·9			179	41·1		
Multiparous (≥1 child)		134	57·8			122	60·1			256	58·9		
Gestational weight gain (kg)†	435			14·4	6·1			13·5	6·9			14·0	6·5
Non-excessive		125	53·9			113	55·7			238	54·7		
Excessive		107	46·1			90	44·3			197	45·3		
Pre-pregnancy BMI (kg/m²)	435			24·7	5·1			24·7	4·8			24·7	4·9
<25		152	65·5			118	58·1			270	62·1		
≥25		80	34·5			85	41·9			165	37·9		
Children													
Sex	435												
Male										232	53·3		
Female										203	46·7		
Gestational age (weeks)	435			39·1	1·1			39·1	1·0			39·1	1·0
Birth weight (kg)	435			3·5	0·5			3·3	0·4			3·4	0·5
Weight-for-age and sex (percentile)‡	435			66·1	25·5			62·0	28·4			64·2	27·0
Small for gestational age (<10th percentile)		5	2·2			7	3·4			12	2·8		
Appropriate for gestational age (10th–90th percentile)		172	74·1			158	77·8			330	75·9		
Large for gestational age (>90th percentile)		55	23·7			38	18·7			93	21·4		
Length (cm)	435			48·9	1·8			48·2	1·8			48·6	1·8
Length-for-age and sex (percentile)†	435			43·5	28·3			42·3	29·6			42·9	28·9
BMI-for-age and sex (kg/m²)§	435			14·6	1·5			14·3	1·2			14·4	1·4
Underweight (≤3rd percentile)		4	1·7			0	0·0			4	0·9		
Normal weight (>3rd-≤85th percentile)		129	55·6			130	64·0			259	59·5		
Excess body weight (>85th percentile)		99	42·7			73	36·0			172	39·5		
ADIPOQ-rs2241766 genotype	408‖												
TT		164	75·9			149	77·6			313	76·7		
TG		49	22·7			42	21·9			91	22·3		
GG		3	1·4			1	0·5			4	1·0		
LEP-rs7799039 genotype	408‖												
GG		73	33·8			81	42·2			154	37·7		
GA		105	48·6			90	46·9			195	47·8		
AA		38	17·6			21	10·9			59	14·5		

MW, minimum wage, US$ 359·54 = 1 MW in February 2012; *ADIPOQ*-T, wild-type allele; *ADIPOQ*-G, risk allele; *LEP*-G, wild-type allele; *LEP*-A, risk allele.
* Twenty mothers were unable to report the household income.
† Institute of Medicine recommendations.
‡ INTERGROWTH-21st standards.
§ WHO 2006 growth standard.
‖ Twenty-eight children without genetic material found in the sample.

weight more quickly and reach adiposity earlier and consequently a higher BMI[51]. These developmental phenotypes predict adult obesity, mediating about half the genetic effect on adult obesity risk[51]. Although the children's growth profile often contains important information about their genetic make-up and environmental exposure, BMI trajectories are difficult to model statistically because of changes in the growth rate during the childhood period[52]. As observed in our study, children tend

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Table 2. Changes in BMI (kg/m²) of children from birth to 6 years of age according to sociodemographic and biological characteristics (PREDI Study, Brazil, 2012–2018)
(Numbers; mean values and 95 % confidence intervals)

	Period of the study													
	Baseline			First follow-up			Second follow-up			Third follow-up				
Characteristic	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*β**	*P*†
Child BMI	435	14·4	14·3, 14·6	314	17·3	17·1, 17·5	216	16·1	15·8, 16·3	185	16·3	15·9, 16·6		
Mothers														
Marital status														
Married/consensual union	361	14·5	14·3, 14·6	270	17·4	17·1, 17·6	185	16·2	15·9, 16·4	157	16·3	15·9, 16·7		
Other	74	14·4	14·0, 14·7	44	17·1	16·5, 17·7	31	15·6	15·0, 16·1	28	15·8	15·3, 16·4	–0·163	0·347
Education (years)														
≥9	281	14·5	14·4, 14·7	202	17·3	17·0, 17·5	142	16·0	15·8, 16·3	125	16·2	15·7, 16·6		
<9	154	14·3	14·1, 14·5	112	17·4	17·0, 17·8	74	16·1	15·7, 16·6		16·4	15·9, 16·9	–0·150	0·260
Household income (MW)														
≥3	238	14·6	14·4, 14·7	184	17·2	17·0, 17·5	124	16·1	15·7, 16·4	110	16·3	15·8, 16·8		
<3	177	14·3	14·1, 14·5	117	17·4	17·1, 17·8	82	16·1	15·8, 16·4	67	16·3	15·9, 16·7	–0·102	0·445
Parity before 2012 (number of deliveries)														
0	179	14·5	14·2, 14·7	122	17·3	17·0, 17·7	82	16·2	15·8, 16·6	69	16·2	15·5, 16·9		
≥1	256	14·4	14·3, 14·6	192	17·3	17·1, 17·6	134	16·0	15·7, 16·3	116	16·3	15·9, 16·7	–0·066	0·610
Gestational weight gain (kg)‡														
Non-excessive	238	14·2	14·0, 14·3	174	17·2	16·9, 17·5	119	16·1	15·7, 16·4	102	16·4	15·9, 16·9		
Excessive	197	14·8	14·6, 15·0	140	17·5	17·2, 17·8	97	16·1	15·8, 16·4	83	16·1	15·6, 16·5	0·510	<0·001
Pre-pregnancy BMI (kg/m²)														
<25	270	14·3	14·1, 14·5	195	17·2	17·0, 17·5	139	15·8	15·5, 16·1	117	15·9	15·6, 16·3		
≥25	165	14·7	14·4, 14·9	119	17·5	17·1, 17·9	77	16·5	16·1, 17·0	68	16·8	16·1, 17·5	0·339	0·010
Children														
Type of delivery														
Normal	290	14·4	14·2, 14·5	211	17·3	17·0, 17·5	141	16·0	15·7, 16·3	122	16·2	15·8, 16·7		
Caesarean	145	14·6	14·3, 14·8	103	17·4	17·0, 17·8	75	16·2	15·9, 16·6	63	16·3	15·7, 16·8	0·173	0·202
Sex														
Male	232	14·6	14·4, 14·8	169	17·6	17·3, 17·9	118	16·1	15·8, 16·3	100	16·2	15·8, 16·7		
Female	203	14·3	14·1, 14·4	145	17·0	16·7, 17·3	98	16·1	15·7, 16·5	85	16·3	15·7, 16·8	–0·432	0·001
Weight status at birth§														
SGA/AGA	342	14·0	13·9, 14·2	242	17·2	16·9, 17·4	166	15·9	15·7, 16·2	143	16·2	15·8, 16·6		
LGA	93	15·9	15·7, 16·1	72	17·9	17·4, 18·4	50	16·5	15·9, 17·1	42	16·4	15·7, 17·2	0·480	<0·001
ADIPOQ-rs2241766 genotype														
TT	313	14·4	14·3, 14·6	225	17·3	17·0, 17·5	153	15·9	15·6, –16·2	132	16·0	15·7, 16·4		
TG + GG	95	14·5	14·3, 14·8	72	17·6	17·1, 18·0	50	16·6	16·0, 17·1	42	16·7	15·8017·6	0·224	0·153
LEP-rs7799039 genotype														
GG	154	14·6	14·4, 14·8	117	17·4	17·0, 17·7	78	16·2	15·8, 16·5	64	16·1	15·6, 16·5		
GA + AA	254	14·4	14·2, 14·5	180	17·3	17·0, 17·6	125	16·0	15·7, 16·3	110	16·2	15·8, 16·7	–0·142	0·302

MW, minimum wage, US$ 359·54 = 1 MW in February 2012; SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; *ADIPOQ*-T, wild-type allele; *ADIPOQ*-G, risk allele; *LEP*-G, wild-type allele; *LEP*-A, risk allele.
* β refers to the longitudinal linear regression coefficient adjusted for linear, quadratic and cubic child age.
† *P* value refers to the restricted maximum likelihood estimator.
‡ Institute of Medicine recommendations.
§ INTERGROWTH-21st fetal growth standards.

Table 3. Poisson regression models for BMI of children according to the *ADIPOQ*-rs2241766 and *LEP-rs7799039* genotypes (PREDI Study, Brazil, 2012, 2018 (*n* 174))
(Relative risks (RR) and 95 % confidence intervals)

	Model 1			Model 2		
Genotype	RR*	95 % CI	*P*†	RR*	95 % CI	*P*†
ADIPOQ-rs2241766						
TT *v.* TG or GG	1·27	1·02, 1·58	0·035	1·25	1·01, 1·56	0·044
Log likelihood	–782·46			–777·776		
Male (*n* 96)						
TT *v.* TG or GG	1·03	0·76, 1·39	0·861	1·01	0·74, 1·36	0·966
Log likelihood	–440·40			–438·47		
Female (*n* 78)						
TT *v.* TG or GG	1·65	1·19, 2·27	0·002	1·66	1·20, 2·31	0·002
Log likelihood	–338·38			–336·68		
LEP-rs7799039						
GG *v.* GA or AA	0·95	0·77, 1·16	0·590	0·93	0·76, 1·15	0·513
Log likelihood	–784·46			–779·517		

ADIPOQ-T, wild-type allele; *ADIPOQ*-G, risk allele; *LEP*-G, wild-type allele; *LEP*-A, risk allele.
* Poisson regression.
† *P* value refers to Wald test. Model 1 was only adjusted for follow-up period as time variable. Model 2 was further adjusted for follow-up period, education (years), gestational weight gain (non-excessive/excessive) and sex (male/female).

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Table 4. Linear mixed-effect regression models for BMI of children according to the *ADIPOQ*-rs2241766 and *LEP-rs7799039* genotypes (PREDI Study, Brazil, 2012–2018 (*n* 408))
(*β* Coefficients and 95 % confidence intervals)

	Model 1			Model 2		
Genotype	*β**	95 % CI	*P*†	*β**	95 % CI	*P*†
ADIPOQ-rs2241766						
TT *v.* TG or GG	0·22	−0·08, 0·53	0·153	0·19	−0·11, 0·49	0·224
Log likelihood	−2046·69			−2034·27		
Interaction term						
TT *v.* TG or GG × age	0·12	0·01, 0·22	0·010	0·01	0·01, 0·02	0·008
Log likelihood	−2043·38			−2030·76		
TT *v.* TG or GG × sex (male/female)	0·02	−0·01, 0·04	0·066	0·02	−0·01, 0·04	0·062
Log likelihood	−2032·65			−2025·32		
Male (*n* 216)						
TT *v.* TG or GG × age	0·01	−0·01, 0·02	0·522	0·01	−0·01, 0·02	0·518
Log likelihood	−1116·66			−1112·71		
Female (*n* 192)						
TT *v.* TG or GG × age	0·02	0·01, 0·04	0·002	0·02	0·01, 0·04	0·002
Log likelihood	−908·74			−904·79		
LEP-rs7799039						
GG *v.* GA or AA	−0·14	−0·41, 0·13	0·302	0·01	−0·01, 0·01	0·586
Log likelihood	−2047·16			−2034·162		
Interaction term						
GG *v.* GA or AA × age	0·01	−0·01, 0·01	0·560	0·01	−0·01, 0·01	0·586
Log likelihood	−2046·99			−2034·16		
GG *v.* GA or AA × sex (male/female)	−0·01	−0·02, 0·01	0·666	−0·01	−0·02, 0·01	0·700
Log likelihood	−2037·27			−2030·42		

ADIPOQ-T, wild-type allele; *ADIPOQ*-G, risk allele; *LEP*-G, wild-type allele; *LEP*-A, risk allele.
* *β* refers to the linear mixed-effect regression coefficient.
† *P* value refers to the maximum likelihood estimator. Model 1 was only adjusted for linear, quadratic and cubic child age (months). Model 2 was further adjusted for maternal education years, gestational weight gain (non-excessive/excessive) and sex (male/female).

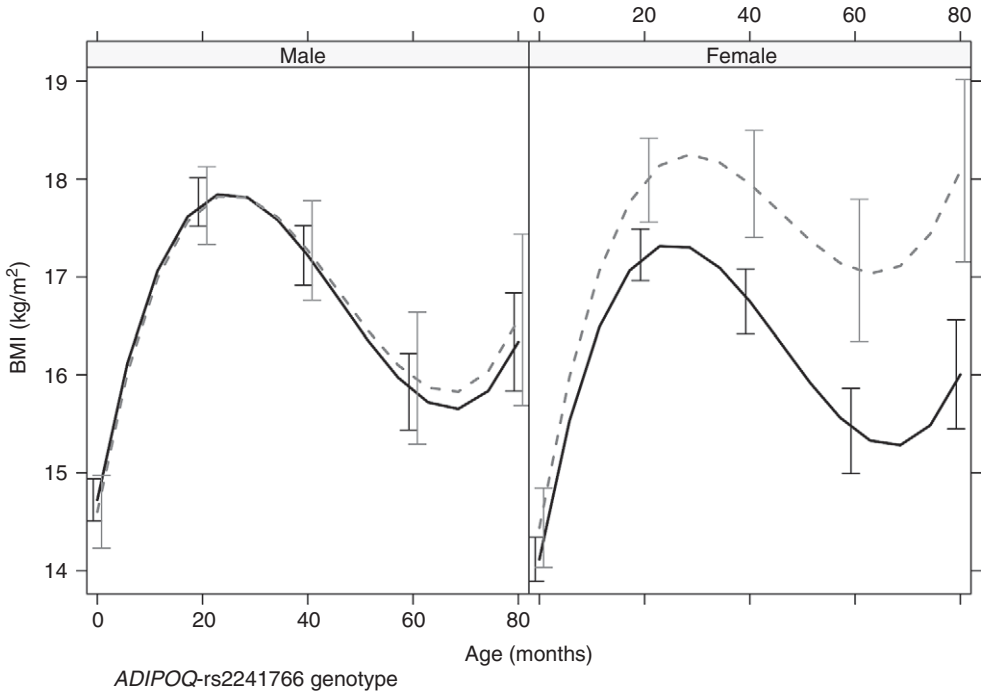

Fig. 2. BMI (kg/m²) trajectories in children from birth to 6 years of age according to *ADIPOQ*-rs2241766 genotype and sex. Number of observations = 1082; number of children = 408 (male *n* 216; female *n* 192); average observations per children = 2·7. The linear mixed-effect models were adjusted for linear, quadratic and cubic child age (months), maternal education (years), maternal gestational weight gain (non-excessive/excessive) and child sex (male/female). Data are presented as the model coefficients (*β*) and 95 % confidence intervals, stratified by child sex. *ADIPOQ*-T, wild-type allele; *ADIPOQ*-G, risk allele. ——, TT; - - -, TG or GG.

to have a fast increase in BMI from birth to the first 2 years of age. Warrington *et al.*[52] suggest that this increase in BMI can be explained by the fact that they reach peak adiposity at this age[52]. After this period, the BMI decreases until 6 years, probably at adiposity rebound, and then progressively increases[52]. In addition, we also demonstrated that girls start the BMI trajectory with lower values than boys but have an earlier rebound[52]. Indeed, age and sex exert different effects on the *ADIPOQ* genetic variant in excess body weight, reinforcing its genetic effect on the child's weight status.

Literature data on the life cycle effect of the *LEP*-rs7799039 genetic variant are contradictory. Some studies involving different adult populations revealed an association with excess body weight[18,42,53]. In contrast, other studies on children found no association between *LEP*-rs7799039 and obesity[54–56], in agreement with our findings. Within this context, Cieslak *et al.*[56] screened a cohort of Polish obese and non-obese children and adolescents for polymorphisms in the 5′-flanking regions of the genes encoding adiponectin, leptin and resistin and found no consistent evidence of an association between obesity and the *LEP* gene variant. It is important to note that, in polygenic diseases such as obesity, the environment exerts a strong influence on the phenotype and genetic variations are only one factor in the obesogenic pathway[57]. In addition to external environmental factors, the interaction with other genetic variants that may lead to different outcomes needs to be considered, which in turn also depends on the effect of the group of genes acting together.

To our knowledge, this is the first study to prospectively investigate the association of *ADIPOQ*-rs2241766 and *LEP*-rs7799039 genetic variants with excess body weight and body weight trajectories in children from birth to 6 years of age. Considering the complexity of various cumulative effects and their direction (including environmental, genetic and other biological factors), we believe that screening for a genetic predictor such as *ADIPOQ*-rs2241766 may contribute to the understanding of the etiopathogenesis of obesity. Identifying genetic predictors that affect the susceptibility to changes in body weight can provide insight into the pathophysiological mechanisms underlying body weight regulation and fat distribution and may thus indicate new approaches for the treatment and prevention of obesity[1].

The present study has several strengths. First, the study allowed longitudinal analysis of the data and fitting models in children from birth to 6 years of age. Furthermore, the data were collected by a trained research group and most of the researchers remained on the team at baseline and follow-ups, thus reducing possible biases. However, some limitations of the present study should be highlighted. First, there were losses over the follow-ups; however, the sample has been similar since baseline and the losses were almost random. In addition, it is important to highlight that the LME models consider individuals with at least one measure of exposure and outcome. Thus, the real sample included in the models considered 408 children with 1082 observations and an average of 2·7 repeated measures per children. We observed small effects of *ADIPOQ*-rs2241766 (effect size $f = 0{\cdot}11$) and *LEP*-rs7799039 (effect size $f = 0{\cdot}06$) on child BMI trajectories. We further conducted *post hoc* power tests to compute the power achieved in the statistical analysis. Our results indicated that, for a small effect size (Cohen's effects size $f = 0{\cdot}1$) in the longitudinal associations, the power achieved was higher than 80 %. Finally, the self-reported variables such as pre-pregnancy weight, household income and schooling are vulnerable to reporter bias.

Conclusion

The *ADIPOQ*-rs2241766 TG and GG genotypes increased the risk of excess body weight in children from birth to 6 years of age. In addition, the risk of a child having higher BMI values from birth to 6 years of age and carrying the risk genotype (TG or GG) was associated with female sex. Finally, the *LEP*-rs7799039 genetic variant was not associated with body weight trajectory in children. Our results may encourage further studies exploring weight trajectories in order to clarify the genetic basis of sex differences in plasma adiponectin levels.

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C. K. formulated the research question, collected and analysed the data, and wrote the manuscript. D. R. F. analysed the data and revised the manuscript. G. K. suggested analyses and critically revised the manuscript. P. H. C. F. revised the manuscript. M. F. M. organized and designed the study, formulated the research question, and revised the manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary materials referred to in this article, please visit https://doi.org/10.1017/S0007114520002780

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