Received: 22 April 2021 | Revised: 2 July 2021 | Accepted: 22 July 2021

DOI: 10.1002/osp4.546

ORIGINAL ARTICLE

Obesity Science and Practice Open Access WILEY

Breastfeeding moderates the relationship between *fat mass and obesity-associated gene* rs9939609 and body mass index among adolescents

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Funding information
Söderström König Foundation, Grant/Award Numbers: SLS-559921, SLS-655791, SLS-745221; Åke Wiberg Stiftelse, Grant/Award Number: M15-0239; Forskningsrådet om Hälsa, Arbetsliv och Välfärd, Grant/Award Number: 2015-00897; Hjärnfonden; The Swedish Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (SRA); Forskningsrådet för Arbetsliv och Socialvetenskap; Uppsala and Örebro Regional Council; Fredrik och Ingrid Thurings Stiftelse; Landstinget Västmanland; Svenska Spel Research Foundation

Abstract

Objective: Breastfeeding, which is important for early growth, is a possible moderator of genetic influence, such as the effect of the fat mass and obesity-associated gene (FTO) on body mass index (BMI). The aim of this study was to assess the moderating effect of breastfeeding duration on the relationship between *FTO* rs9939609 and BMI in a Caucasian sample.

Methods: Adolescents born in 1997 and in 1999, who were living in the Swedish county Västmanland in 2012, were invited to participate in the Survey of Adolescent Life in Västmanland. The adolescents and their parents completed self-reported questionnaires in 2012, 2015, and 2018. Genotyping of rs9939609 T > A polymorphism was conducted from saliva DNA samples. Interaction effects of parental reported breastfeeding duration in months, including regions of significance, on the relationship between rs9939609 and BMI plus overweight were assessed.

Results: Considering physical activity levels, parental reported breastfeeding duration was a moderator of the relationship between rs9939609 and BMI for the younger (regions of significance = <1.6 and >28.1 months) and older adolescents (region of significance = >19.9 months), but not for the young adults. Plots of the association between breastfeeding duration and BMI showed higher BMI for AA with short breastfeeding, but lower BMI with longer breastfeeding than AT and TT. Longer breastfeeding lowered the odds for overweight among the younger adolescents, especially among AA individuals.

Conclusion: Rs9939609 AA individuals were more susceptible than AT and TT individuals to both short and long breastfeeding durations, which is consistent with the differential susceptibility hypothesis. *FTO* rs9939609 AA might be a plasticity

variant with differential susceptibility to environmental influences. Breastfeeding duration may be one of many factors that affect the relationship between rs9939609 and BMI.

KEYWORDS
breastfeeding, *FTO*, obesity, overweight, plasticity, rs9939609

1 | INTRODUCTION

In 1975, <1% of children and adolescents (aged 5–19 years) had obesity (4% if including children and adolescents with overweigh as well) globally.[1] In 2016, approximately 40 years later, the prevalence rate of obesity had increased to 6% for girls and 8% for boys, while 18% of girls and 19% of boys were overweight.[1] Overweight and obesity are major risk factors for cardiovascular diseases, the main cause of death worldwide in 2017,[2] especially for women with insulin resistance.[3] In addition, for adolescents with higher body mass index (BMI) or obesity, the risk for several forms of cancer such as leukemia and colorectal cancer or adult diabetes type II, respectively, is also increased.[4]

Even though overweight and obesity are dependent on lifestyle factors, such as diet and physical activity, obesity is a heritable trait with known genetic influence.[5] Genome-wide association studies have identified more than 100 loci that are robustly associated with BMI.[6–8] The fat mass and obesity-associated gene (FTO; FTO alpha-ketoglutarate-dependent dioxygenase) is the gene with the strongest influence on BMI.[9] In 2007, *FTO* was determined to be one of the contributing factors to early-onset obesity and severe obesity.[10,11] Since then, knowledge regarding the association between *FTO* and overweight and obesity has increased.[12–17] *FTO*, located on chromosome 16, encodes a 2-oxoglutarate-dependent nucleic acid demethylase,[9] which is expressed in all tissues, but its highest levels are observed in brain areas that control food intake.[18,19] *FTO* also plays an important role in adipogenesis.[20] Single nucleotide polymorphisms (SNPs) in *FTO* appear to regulate the expression of other genes in a tissue-specific manner that varies depending on the developmental stage of the tissue.[21] The causal variant rs1421085 disrupts a binding site for a gene repressor, which leads to overexpression of Iroquois homeobox 3 and Iroquois homeobox 5, which in turn changes these cells to energy-storing white adipocytes and increases lipid accumulation.[22] This SNP is in high linkage disequilibrium with many other SNPs in the genetic region, including rs9939609 (distance 19,573 bp, D' = 0.98, and $R^2 = 0.92$ in the CEU population, which includes Utah residents with Northern and Western European ancestry, according to the LDlink tool[23]), which, therefore, can act as a proxy SNP.

The A allele of rs9939609 in the intron of *FTO* has been associated with higher risk for obesity[12,17] and type 2 diabetes.[12] Several studies have found that this association changed throughout the life course.[16,24] Hardy et al. found a stronger association during childhood and adolescence with a peak at 20 years, after which the association declined again during adulthood.[16] Sovio et al. found that the relationship between the A allele and the BMI was inversed for children below 2.5 years of age.[24] However, some did not find any influence by genotype,[25] while others found it only in girls.[26]

Because obesity is difficult to reverse once established,[27] it is important to identify early risk factors as well as protective factors for a healthy weight pattern. One factor that occurs during the child's earliest life and may have an impact on obesity is breastfeeding. The World Health Organization recommend exclusive breastfeeding for up to 6 months of age with continued breastfeeding for up to 2 years of age or beyond.[28] The Swedish National Board of Health and Welfare and the National Food Agency have adapted this recommendation to Swedish conditions promoting exclusive breastfeeding for up to 6 months of age and continued breastfeeding for up to 1 year or beyond.[29,30] Despite these breastfeeding promoting recommendations,[29] breastfeeding is declining in Sweden.[31] A dose–response relationship between breastfeeding duration and future overweight risk has been observed.[32] Compared with breastfeeding, formula-feeding is associated with higher levels of protein and energy intake[33] as well as higher plasma insulin levels that promote fat deposition.[34] In contrast, breastfeeding is associated with higher levels of infant control of feeding and self-regulation.[33] Altogether, formula-feeding is associated with a more rapid weight gain[34] through overnutrition.[32] This is considered as one of the main risk factors in the first 1000 days since birth for the development of childhood obesity.[35]

Earlier attempts at investigating a possible interaction effect between breastfeeding and *FTO* rs9939609 variants on future BMI have revealed inconclusive results. In a Chinese study on 1149 children ($\bar{y}$ = 10.8 years) analyzing BMI or body fat percentage, no significant interaction effect was found.[36] In contrast, a study on 959 children from Australia showed that a longer period of exclusive breastfeeding could reverse the increased BMI otherwise associated with the A allele.[37] Consistent with this, results from a study on British children revealed that at least 5 months of exclusive breastfeeding had a substantial impact on BMI growth trajectories.[38] Dedoussis et al. reported an interaction effect in two Greek samples (*FTO* rs17817449 was analyzed in one of the samples; $N = 1138$, mean age = 11.2 years and $N = 2374$, mean age = 41.4 months). However, their findings could not be replicated in a British sample of 4325 participants (mean age = 11.7 years).[39] Further studies are needed to establish a consensus regarding these relationships. To our knowledge, these associations are yet to be investigated in a Nordic sample.

Dalle Molle et al. suggested that the differential susceptibility to environment model; that individuals vary in their susceptibility to

environmental influences and plasticity,[40] is "highly applicable to the study of genetic and environmental influences on feeding behaviour and obesity risk" as well.[41] In other words, the incorporation of both positive and negative environmental influences in these studies is essential. In this case, shorter or longer breastfeeding duration could be viewed as negative or positive environmental influence, respectively. Due to the lack of studies that apply this theory on obesity research, we consider this a gap in this research field.

This study hypothesized that breastfeeding duration plays a role in weight regulation through links to feeding behavior as well as through the nutritional value and that individuals differ in their susceptibility to this influence due to genetic variants. The aim of this study was to investigate the possible effect of childhood breastfeeding duration on the association between *FTO* rs9939609 and BMI and overweight in adolescence and young adulthood in a Caucasian sample, taking the levels of physical activity into account.

2 | MATERIALS AND METHODS

2.1 | Participants

Adolescents born in 1997 and in 1999, who were living in the Swedish county Västmanland in 2012, were invited by regular mail to participate in the Survey of Adolescent Life in Västmanland cohort study.[42] Individuals who had lived in Sweden for <5 years and had language difficulties or any mental disability/severe illness were excluded. In total, 4712 adolescents were eligible for this study. The consenting adolescents and their parents completed self-reported questionnaires during three assessments, first in 2012 (W1), second in 2015 (W2), and third in 2018 (W3). At W1, adolescents were also instructed to provide a saliva sample for deoxyribonucleic acid (DNA) extraction. Response rates were 39.6% for W1, 82.7% of W1 for W2, and 57.8% of W1 for W3 for the questionnaires. Caucasian participants with available data on the variables of interest were included in this study. The study sample is presented in detail in Table 1. All participants signed an informed consent form during the first assessment (W1). For participants aged <15 years, consent was also obtained from their legal guardian. This study was approved by the Ethical Review Board of Uppsala (Dnr 2012/187) and performed in accordance with the Declaration of Helsinki.

2.2 | Genotyping

At W1, participants provided a DNA sample using a self-collection kit (Oragene•DNA, DNA Genotek). Of all the eligible study participants, 86.5% was genotyped. DNA was then extracted from 200 μL of saliva using a silica-based extraction method (Kleargene™, LGC, Biosearch Technologies). Genotyping of the *FTO* SNP rs9939609 T > A polymorphism was performed using KASP™ (LGC, Biosearch Technologies), which is specific to the targeted SNPs and uses allele-specific primers. The allele combinations TT, TA, and AA were analyzed for Hardy–Weinberg equilibrium using the χ^2 test ($p = 0.23$).

2.3 | Assessment of BMI

We calculated the adolescents' BMI from self-reported values of weight and height. Thereafter, we created binary variables for normal weight, overweight, and obesity according to international age- and sex-specific cutoffs for each wave/time point.[43] Due to a small group of adolescents with obesity in the sample, individuals with overweight and obesity were combined into an "overweight including obesity" (hereinafter referred to as "overweight") category for the binary regression model analysis to reduce the risk of overfitting. This was performed identically for all the three assessments. Continuous BMI was used as the outcome in PROCESS analyses (see Statistical analyses).

2.4 | Assessment of childhood breastfeeding duration

At W2, parents were instructed to answer two questions regarding breastfeeding. The first question was whether the child had been breastfed at all or not (Yes/No) and the second was "If yes, for how long (in months)?" For this study, a "No" response for the first question was regarded as 0 months and included in the continuous variable "breastfeeding duration in months." The breastfeeding duration was categorized into 0–3, 4–6, 7–9, 10–12, and >12 months for descriptive reasons. We also created a categorical variable using the cutoffs from the breastfeeding recommendations (0–6, 7–12, and >12 months) to be used in the binary logistic regression model analysis.

2.5 | Confounders

Age was calculated using the date of birth and the date on which the form was sent out. Sex was coded as male (1) or female (2) using information in the participants' personal identity number. For all the three time points, we used self-reported physical activity that lasted >30 min during leisure time and was accompanied by increased breathing/sweating. Responses ranged from "Never" to "Every day" with seven possible responses that were used as a continuous measurement of physical activity.

2.6 | Statistical analyses

Using χ^2 tests for categorical variables and the Mann–Whitney *U* test for continuous variables, we analyzed whether there were any sex differences between rs9939609 allele variants, breastfeeding duration, age, physical activity, and overweight. In addition, we analyzed

TABLE 1 Description of the study sample

	Wave 1 (*N* = 1221)			Wave 2 (*N* = 1120)			Wave 3 (*N* = 771)		
	Female (*N* = 702)	Male (*N* = 519)	χ^2/Z (*p*)	Female (*N* = 656)	Male (*N* = 464)	χ^2/Z (*p*)	Female (*N* = 491)	Male (*N* = 280)	χ^2/Z (*p*)
Age, years (standard deviation)	14.4 (1.03)	14.4 (1.04)	−0.025 (0.80)	17.3 (1.03)	17.3 (1.05)	−0.24 (0.81)	20.4 (1.03)	20.4 (1.04)	−0.36 (0.72)
Body mass index	20.5 (3.5)	20.4 (3.3)	−0.25 (0.81)	22.2 (3.9)	22.6 (3.5)	**−2.7 (0.006)**	23.3 (4.1)	23.5 (3.4)	−1.8 (0.065)
Overweight including obesity, *N* (%)[a]	110 (15.7)	108 (20.8)	**5.38 (0.020)**	113 (17.2)	99 (21.3)	2.99 (0.084)	122 (24.8)	74 (26.4)	0.24 (0.63)
Workout > 30 min, *N* (%)			**−2.3 (0.022)**[b]			**−4.2 (<0.001)**[b]			**−4.5 (<0.001)**[b]
Never	45 (6.4)	41 (7.9)		44 (6.7)	37 (8.0)		51 (10.4)	25 (8.9)	
Less than once a month	35 (5.0)	23 (4.4)		45 (6.9)	25 (5.4)		83 (16.9)	35 (12.5)	
1–3 times a month	45 (6.4)	27 (5.2)		60 (9.1)	27 (5.8)		83 (16.9)	36 (12.9)	
Once per week	86 (12.3)	45 (8.7)		80 (12.2)	43 (9.3)		63 (12.8)	23 (8.2)	
2–3 times per week	255 (36.3)	166 (32.0)		221 (33.7)	120 (25.9)		129 (26.3)	63 (22.5)	
4–6 times per week	197 (28.1)	181 (34.9)		154 (23.5)	136 (29.3)		71 (14.5)	80 (28.6)	
Every day	39 (5.6)	36 (6.9)		52 (7.9)	76 (16.4)		11 (2.2)	18 (6.4)	
FTO rs9939609 N (%)			**7.55 (0.023)**			4.8 (0.090)			4.4 (0.11)
TT	243 (34.6)	213 (41.0)		227 (34.6)	185 (39.9)		168 (34.2)	117 (41.8)	
TA	333 (47.4)	237 (45.7)		314 (47.9)	216 (46.6)		236 (48.1)	118 (42.1)	
AA	126 (17.9)	69 (13.3)		115 (17.5)	63 (13.6)		87 (17.7)	45 (16.1)	
Breastfeeding, *N* (%)			−0.11 (0.92)[b]			−0.21 (0.84)[b]			−0.12 (0.91)[b]
0–3 months	113 (16.1)	90 (17.3)		108 (16.5)	82 (17.7)		71 (14.5)	49 (17.5)	
4–6 months	174 (24.8)	122 (23.5)		157 (23.9)	108 (23.3)		128 (26.1)	66 (23.6)	
7–9 months	201 (28.6)	143 (27.6)		192 (29.3)	127 (27.4)		142 (28.9)	70 (25.0)	
10–12 months	122 (17.4)	92 (17.7)		114 (17.4)	86 (18.5)		88 (17.9)	56 (20.0)	
>12 months	92 (13.1)	72 (13.9)		85 (13.0)	61 (13.1)		62 (12.6)	39 (13.9)	

Note: The χ^2 test was used for categorical variables and the Mann–Whitney *U*-test for continuous variables. Significant results are reported in the bold font.

[a]According to Cole et al.[43]

[b]Analyzed continuously.

rs9939609 allele variants using χ^2 tests for associations with BMI categories (normal weight and overweight) at the three time points. Rs9939609 allele variants were also analyzed for any effect of breastfeeding duration and physical activity using the Mann–Whitney *U* test.

The moderating effect of parental reported breastfeeding duration in months on the relationship between *FTO* rs9939609 variants and BMI was analyzed using the PROCESS macro tool (version 3.5[44]). We analyzed the PROCESS "Model 1" (Figure 1) separately for each time point using *FTO* rs9939609 as *X*; BMI as *Y*; breastfeeding duration in months as moderator (*W*); and sex, age, and physical activity as covariates. The confidence interval was set to 95% with 5000 bootstrap samples using no centering of the variables. We probed the interactions if $p < 0.05$ and conditioning values were set to 16th, 50th, and 84th percentiles. To determine the value(s) of the moderator where $p = 0.05$, we used the Johnson–Neyman significance output, which also defines the regions of significance.

To assess the possible interaction between rs9939609 variants and parental reported breastfeeding duration for risk of overweight, we included rs9939609 and breastfeeding duration categories (Model 1) and, additionally, an interaction term between rs9939609 and breastfeeding duration (Model 2) in three separate binary logistic regression models using overweight at each time point as the outcome. These analyses were controlled for age, sex, and physical activity at the time. Data were analyzed using IBM SPSS Statistics (version 24 for Windows; IBM SPSS).

3 | RESULTS

3.1 | Crude analyses

Female participants had rs9939609 TA and AA more frequently than male participants (Table 1). We found no sex difference regarding parental reported breastfeeding duration or age. Female participants were less likely than male participants to report physical activity levels at the low or high end of the scale (Table 1). Male participants had a higher BMI than female participants at W2 (Table 1). More male participants than can be expected were overweight at W1 in the crude analysis (Table 1). We did not find any sex difference regarding overweight at W2 or W3 (Table 1). Rs9939609 variants were not associated with overweight at any time point (W1:

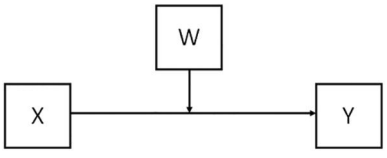

FIGURE 1 A conceptual diagram of Model 1 in the PROCESS analysis. In this case, the analysis tests breastfeeding (W) as the moderator of the association between X (rs9939609) and Y (body mass index)

TABLE 2 Breastfeeding duration in months as a moderator of the relationship between *FTO* rs9939609 variants and BMI

	W1 BMI (N = 1221)						W2 BMI (N = 1120)						W3 BMI (N = 771)					
	Coeff	SE	*t*	*p*	LLCI	ULCI	Coeff	SE	*t*	*p*	LLCI	ULCI	Coeff	SE	*t*	*p*	LLCI	ULCI
FTO rs9939609	0.49	0.24	2.07	**0.04**	0.03	0.96	0.5	0.28	1.78	0.08	−0.05	1.04	0.45	0.34	1.33	0.19	−0.22	1.12
Breastfeeding	0.01	0.02	0.57	0.57	−0.03	0.06	0.02	0.03	0.63	0.53	−0.03	0.07	−0.02	0.03	−0.67	0.51	−0.08	0.04
Int_1a[a]	−0.05	0.02	−2.19	**0.03**	−0.1	−0.01	−0.06	0.03	−2.18	**0.03**	−0.12	−0.01	−0.04	0.03	−1.21	0.23	−0.11	0.03
Sex	0.08	0.2	0.39	0.7	−0.31	0.46	−0.34	0.23	−1.49	0.14	−0.79	0.11	−0.23	0.29	−0.79	0.43	−0.81	0.34
Age	0.69	0.09	7.37	**<0.001**	0.5	0.87	0.5	0.11	4.58	**<0.001**	0.29	0.71	0.12	0.13	0.86	0.39	−0.15	0.38
Physical activity	−0.16	0.06	−2.54	**0.01**	−0.28	−0.04	0.1	0.07	1.41	0.16	−0.04	0.23	0.003	0.08	0.04	0.97	−0.16	0.16
Constant	10.94	1.42	7.71	<0.001	8.16	13.72	13.8	1.98	6.96	<0.001	9.91	17.69	21.45	2.81	7.62	<0.001	15.93	26.98

Note: PROCESS procedure using W1 BMI (Y1), W2 BMI (Y2), and W3 BMI (Y3) as outcomes, including *FTO* rs9939609 (X) and breastfeeding (W) with sex, age at the time, and physical activity at the time as covariates. Significant results are reported in the bold font.

Abbreviations: BMI, body mass index; Coeff, unstandardized coefficient; LLCI, lower level of confidence interval; ULCI, upper level of confidence interval.

[a]Int_1a: Interaction term *FTO* rs9939609 × breastfeeding in months.

$\chi^2 = 0.092$, $p = 0.96$; W2: $\chi^2 = 0.26$, $p = 0.88$; W3: $\chi^2 = 0.24$, $p = 0.63$), age or parental reported breastfeeding duration.

3.2 | Breastfeeding as a moderator

Process analyses revealed a significant moderation effect of parental reported breastfeeding on the relationship between rs9939609 and BMI at W1 and W2 but not W3 (Table 2). R^2 for the model summary was 0.054 ($p < 0.001$) for W1 and 0.026 ($p < 0.001$) for W2. The R^2 change for the interaction term was 0.004 ($p = 0.029$) for W1 and W2. Figure 2A,B displays plots for the conditional effect of the focal predictor using data from the PROCESS output at W1 and W2. Figure 2A shows that for TT individuals, the BMI slope starts at a lower level than for AT and AA individuals, and then, it increases slightly with longer breastfeeding duration. For AT individuals, the slope starts at a level between those for TT and AA individuals, and then, it declines with longer breastfeeding duration. The slope for AA individuals starts at the highest level and has the steepest decrease as the breastfeeding duration increases. In Figure 2B, the slopes follow the same pattern as in Figure 2A, but the slopes for AT and AA overlap. The Johnson–Neyman output revealed that values <1.64 and >28.05 for the moderator were statistically significant at W1 (Figure 3A). For W2, values >19.85 for the moderator were statistically significant (Figure 3B). We did not find any statistically significant interaction effect for W3 BMI. The binary logistic regression analyses showed that parental reported breastfeeding for 7–12 months was associated with lower odds ratio (OR) for overweight at W1 compared with shorter breastfeeding (Model 1, Table 3). The combination of rs9939609 TA and breastfeeding for >12 months was associated with a lower OR than the reference combination of rs9939609 TT and breastfeeding for 0–6 months (Table 3). Using *FTO* rs9939609 as a continuous variable in the regression model for W1 overweight, we found that for each additional A allele combined with breastfeeding for >12 months, the OR decreased significantly (OR = 0.41, 95% confidence interval: 0.19–0.88). All variables in the binary logistic regression models for W2 and W3 were nonsignificant (data were not shown). The complete output from the analyses can be obtained from the main author upon reasonable request.

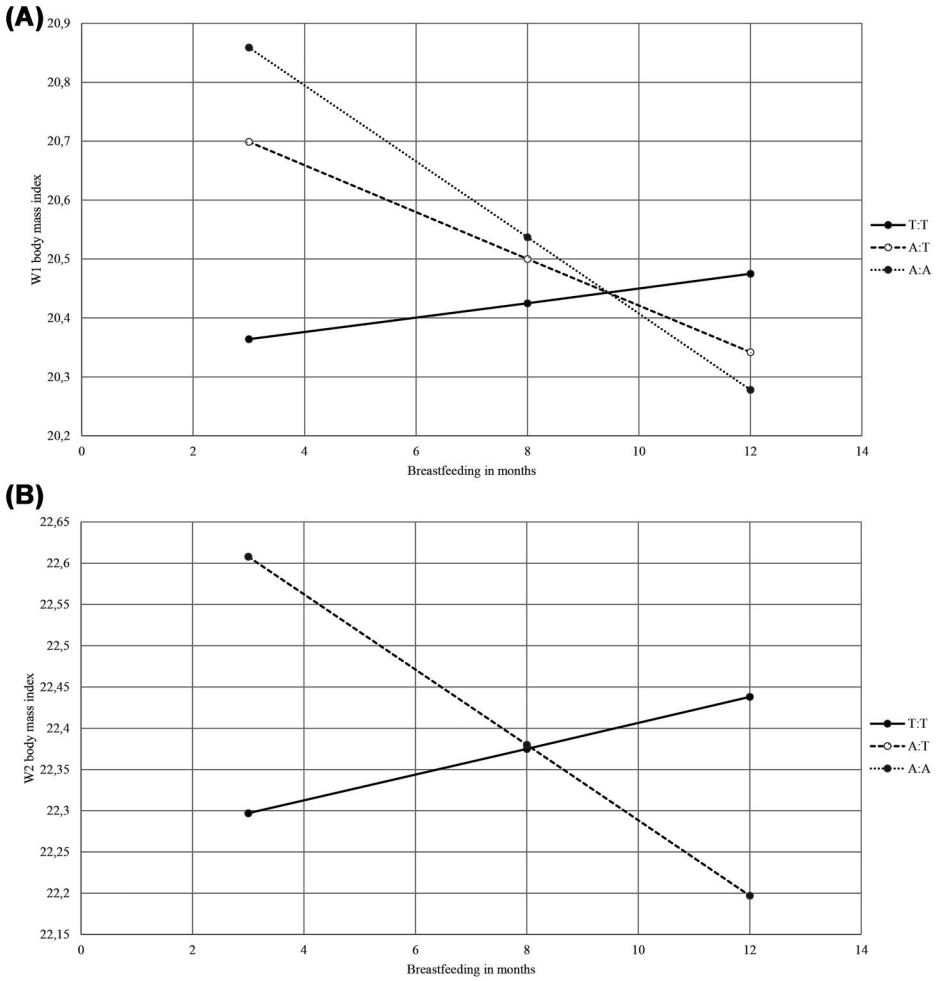

FIGURE 2 (A) Plot of the association between breastfeeding in months and W1 body mass index separately for rs9939609 allele variants AA, AT, and TT. (B) Plot of the association between breastfeeding in months and W2 body mass index separately for rs9939609 allele variants AA, AT, and TT. Lines for AT and AA are overlapping

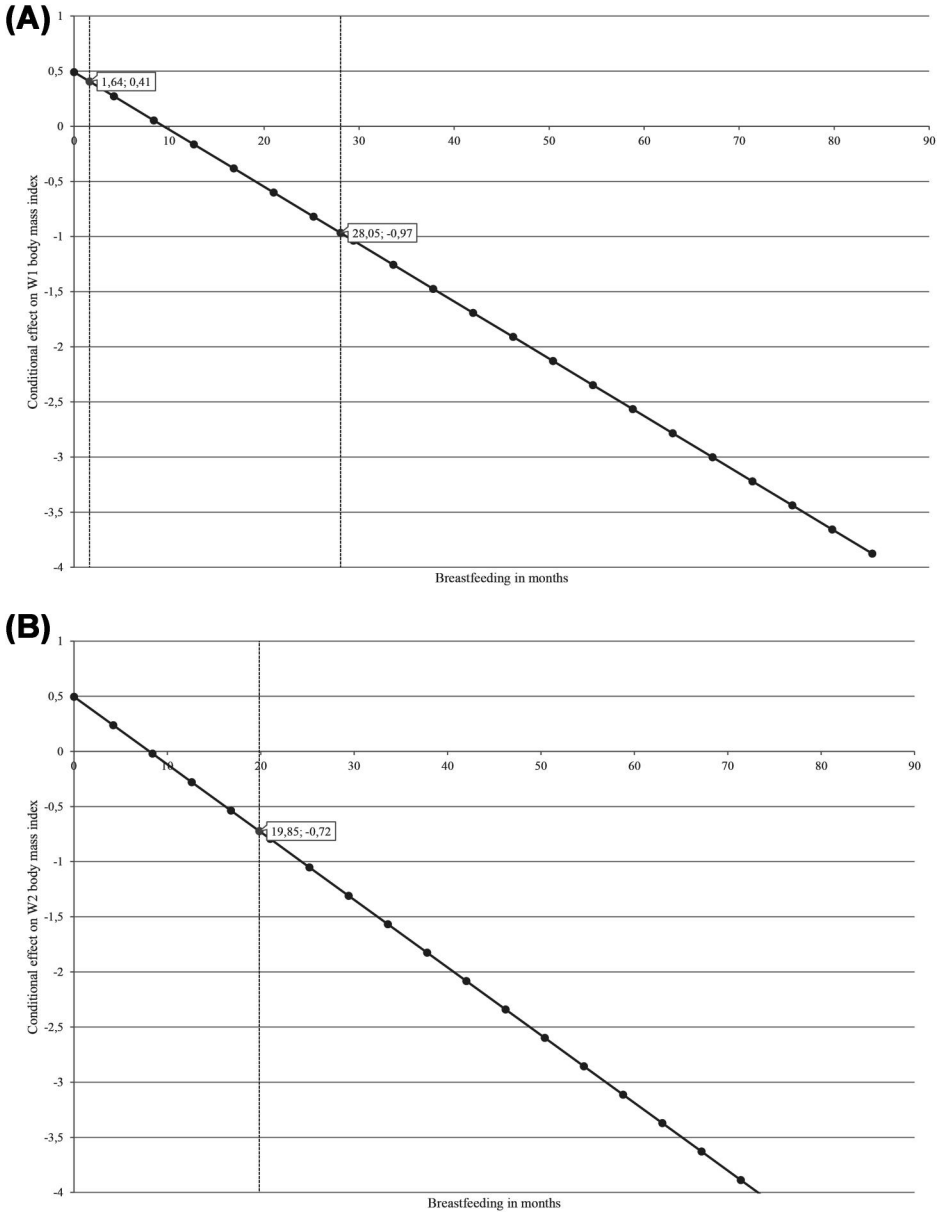

FIGURE 3 (A) Region of significance for the conditional effect of *FTO* rs9939609 on W1 body mass index at different durations of breastfeeding (in months) when controlling for sex, age, and physical activity. The dashed vertical lines indicate where $p = 0.5$. In this case, values of <1.64 and >28.05 months were statistically significant. (B) Region of significance for the conditional effect of *FTO* rs9939609 on W2 body mass index at different durations of breastfeeding (in months) when controlling for sex, age, and physical activity. The dashed vertical lines indicate where $p = 0.5$. In this case, values >19.85 months were statistically significant

4 | DISCUSSION

This study indicates that rs9939609 AA is a plasticity variant, which is receptive to both negative and positive environmental conditions in relation to BMI. In this case, its plastic nature was demonstrated by the effect of parental reported breastfeeding duration on its relationship with BMI among the adolescents in this sample. Parental reported breastfeeding was also a moderator of the relationship between rs9939609 variants and overweight among the younger adolescents.

The Johnson–Neyman output revealed that rs9939609 AA individuals were more susceptible than AT and TT individuals for both short, associated with higher BMI, and long durations, associated with lower BMI, of breastfeeding at W1, which is in line with the differential susceptibility hypothesis. However, the limits were very short and very long breastfeeding duration, where the former likely can be associated with other factors that was not evaluated, and the latter is uncommon in general. For W2 BMI, only long breastfeeding duration was important. It is noteworthy that these values depend on the range of the moderator values that are included in

TABLE 3 Binary logistic regression models investigating the association between *FTO*, breastfeeding, and their interaction for overweight including obesity at W1

	Model 1		Model 2	
	p	OR (95% CI)	*p*	OR (95% CI)
Sex (1)	**0.02**	**0.69 (0.51–0.93)**	**0.01**	**0.69 (0.51–0.93)**
Age	0.99	1 (0.87–1.15)	0.98	1 (0.86–1.15)
Physical activity	**<0.001**	**0.85 (0.78–0.93)**	**<0.001**	**0.85 (0.78–0.93)**
Breastfeeding	**0.05**		**0.04**	
Breastfeeding (1)	**0.02**	**0.67 (0.49–0.93)**	0.07	0.6 (0.35–1.04)
Breastfeeding (2)	0.28	0.78 (0.49–1.23)	0.31	1.4 (0.73–2.66)
FTO rs9939609	0.94		0.87	
FTO rs9939609 (1)	0.93	0.99 (0.71–1.37)	0.71	1.1 (0.68–1.79)
FTO rs9939609 (2)	0.78	1.07 (0.69–1.66)	0.63	1.17 (0.62–2.2)
FTO rs9939609 × breastfeeding			0.1	
FTO rs9939609 (1) by breastfeeding (1)			0.66	1.18 (0.57–2.41)
FTO rs9939609 (1) by breastfeeding (2)			**0.03**	**0.31 (0.11–0.87)**
FTO rs9939609 (2) by breastfeeding (1)			0.7	1.2 (0.47–3.07)
FTO rs9939609 (2) by breastfeeding (2)			0.14	0.28 (0.05–1.51)
Constant	0.64	0.6	0.61	0.58

Note: Binary logistic regression ($N = 1221$), method enter, using overweight including obesity at W1 as outcome, including in Model 1: sex (male = 0), age, breastfeeding 0–6 months (0)/7–12 months (1)/>12 months (2), *FTO* rs9939609 TT (0)/TA (1)/AA (2), W1 physical activity >30 min and in Model 2, additional interaction term *FTO* × breastfeeding. Significant results are reported in the bold font.
Abbreviations: CI, confidence interval; OR, odds ratio.

the analyses. In another sample, with shorter or longer breastfeeding duration, the region of significance will likely be different. In agreement with Horta et al.[45] Wu et al.[38] Abarin et al. with findings on girls,[37] and Dedoussis et al. with findings on pre-schoolers[39] long breastfeeding duration was found to counteract the effect of the rs9939609 risk allele on BMI. However, Jiang et al. could not find any significant interaction effect in their study on Chinese participants.[36] Authors investigated a binary measurement of exclusive breastfeeding duration with a cutoff at 4 months and analyzed differences between TT and A carriers, that is, combined TA and AA into one group.[36] Because any specific biological event occurring at exactly 4 months cannot be recognized, the cutoffs that represent the breastfeeding recommendations were investigated. Variations in the methods might be the main reason for the discrepancies. Four of the mentioned studies[36,38,39,45] used a binary measurement of breastfeeding, which makes it impossible to detect regions of significance for any interaction effects. Moreover, Abarin et al.[37] found a decrease in the BMI of 0.12 and 0.18 kg/m^2 per additional month of breastfeeding in the AT and AA groups, respectively, which also indicates that AA is more susceptible than AT. If rs9939609 AA indeed is a plasticity variant as suspected, the main effects would vary depending on the non-measured factors among the study participants, as discussed previously regarding serotonin transporter polymorphisms.[46] The interaction effect found in this study was statistically significant for the younger adolescents but declined in the older adolescents and was absent for the young adults. This might in part be caused by lower power for W2 and W3 analyses due to a loss of participants at the follow-up. An addition of factors such as possible injuries that affect mobility and eating habits and that influence BMI throughout a person's life will also dilute the effect of breastfeeding. Some findings suggest an age-dependent relationship between the *FTO* rs9939609 variants and BMI.[24]

Parental reports of longer breastfeeding duration were associated with lower risk for overweight among the younger adolescents. The findings indicate a protective effect from longer breastfeeding among the AA individuals, but the small sample of participants with overweight and breastfeeding for >12 months has to be considered. Wu et al.[38] showed that only AA participants with exclusive breastfeeding for <3 months had an upper quartile BMI value above the overweight line. Future studies with a larger sample size of individuals with long breastfeeding duration are warranted.

Only 13% of the participants had a breastfeeding duration >12 months. Data have shown that high-income countries have shorter breastfeeding duration than low- and middle-income countries.[47] Even in the latter, only 37% of the infants aged <6 months are exclusively breastfed as recommended.[47] Previous findings indicate that breastfeeding (partial or exclusive) is associated with a slower growth rate during infancy,[38,48] which is a major determinant of overweight and obesity later.[38]

Given the present findings, it is plausible that rs9939609 A is a plasticity allele. Breastfeeding duration might be one small piece in the large puzzle that encompasses the increasing obesity trend.

Indeed, there are many other aspects related to obesity, such as built environment, screen time, and agroalimentary environment,[49] that have not been considered in this study.

4.1 | Strengths and limitations

One major strength of this study is its longitudinal design with assessment at three time points, which makes it possible to investigate these associations at several time points during the progression from adolescence to adulthood.

One limitation of this study is that due to only few participants with obesity, such individuals were combined into a category together with participants with overweight, which could have masked an actual effect from the rs9939609 risk allele on the risk for obesity. However, international age- and sex-specific cutoffs for overweight were used, which is a strength of this study. The data were self-reported, which could be subjected to either under- or over-estimation of key variables. The response rates were low (approximately 40%) for W1. The parental reported breastfeeding duration can be subjected to recall bias, as with all retrospective research, which has to be considered in the interpretation of the findings. Rs9939609 is in close proximity to many other SNPs, which means that causality is not possible to state at this point. Information on energy intake was not assessed in the SALVe cohort and could not be incorporated in this study. However, if the hypotheses that early factors such as breastfeeding have an impact on eating behavior are correct, energy intake would be a mediator in the analyses and not have an impact on the total effect. Moreover, the risk that these findings were found by chance, as discussed in depth previously,[50] must be acknowledged.

5 | CONCLUSION

FTO rs9939609 AA might be a plasticity variant, with differential susceptibility to environmental influences. Breastfeeding duration may act as one of many moderators of the relationship between *FTO* rs9939609 and BMI among Caucasian adolescents. Future studies on this research topic should consider the differential susceptibility hypothesis.

ACKNOWLEDGMENTS

The authors would like to thank Mattias Rehn for providing help with the data files and syntax. They would also like to thank the laboratory at the Department of Neuroscience, Biomedical Center, Uppsala University led by assistant professor Erika Comasco for assistance with the genotype analyses. Finally, they also thank all participants for their contribution to this study. This research was supported by grants from the Söderström König Foundation (SLS-559921, SLS-655791, and SLS-745221), Åke Wiberg Foundation (M15-0239), and Swedish Research Council for Health, Working Life and Welfare (FORTE) (2015-00897) to Cecilia Åslund and grants from the Swedish Brain Foundation (Hjärnfonden), The Swedish Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (SRA), Swedish Council for Working Life and Social Research (FAS), Uppsala and Örebro Regional Council, Fredrik and Ingrid Thurings Foundation, County Council of Västmanland, Söderström König Foundation, and Svenska Spel Research Foundation to Kent W. Nilsson.

CONFLICT OF INTEREST

Sofia H. Kanders, Kent W. Nilsson, and Cecilia Åslund have no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the study design. Kent W. Nilsson and Cecilia Åslund provided the data. Sofia H. Kanders analyzed the data. All authors interpreted the data. Sofia H. Kanders drafted the manuscript. All authors contributed to critical revisions and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data are not openly available due to confidentiality.

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REFERENCES

1. World Health Organization. *Obesity and overweight 2018*; 2020. Accessed March 3, 2020. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight
2. Global Burden of Disease Collaborative Network. *Global Burden of Disease Study 2017 (GBD 2017) Results*. Institute for Health Metrics and Evaluation (IHME); 2018.
3. Manrique-Acevedo C, Chinnakotla B, Padilla J, Martinez-Lemus LA, Gozal D. Obesity and cardiovascular disease in women. *Int J Obes*. 2020;44:1210–1226. https://doi.org/10.1038/s41366-020-0548-0
4. Weihrauch-Bluher S, Schwarz P, Klusmann JH. Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. *Metabolism Clin Exp*. 2019;92:147-152. https://doi.org/10.1016/j.metabol.2018.12.001
5. Khera AV, Chaffin M, Wade KH, et al. Polygenic prediction of weight and obesity trajectories from birth to adulthood. *Cell*. 2019;177:587-596e9. https://doi.org/10.1016/j.cell.2019.03.028
6. Locke AE, Kahali B, Berndt SI, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518:197-206.
7. Yengo L, Sidorenko J, Kemper KE, et al. Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. *Hum Mol Genet*. 2018;27:3641-3649. https://doi.org/10.1093/hmg/ddy271
8. Speakman JR, Loos RJF, O'Rahilly S, Hirschhorn JN, Allison DB. GWAS for BMI: a treasure trove of fundamental insights into the genetic basis of obesity. *Int J Obes*. 2018;42:1524-1531. https://doi.org/10.1038/s41366-018-0147-5
9. Goodarzi MO. Genetics of obesity: what genetic association studies have taught us about the biology of obesity and its complications. *Lancet Diabetes Endo*. 2018;6:223-236. https://doi.org/10.1016/S2213-8587(17)30200-0

10. Dina C, Meyre D, Gallina S, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet*. 2007;39:724-726. https://doi.org/10.1038/ng2048
11. Hinney A, Nguyen TT, Scherag A, et al. Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants. *PLOS One*. 2007;2:e1361. https://doi.org/10.1371/journal.pone.0001361
12. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*. 2007;316:889-894.
13. Oyeyemi BF, Ologunde CA, Olaoye AB, Alamukii NA. FTO gene associates and interacts with obesity risk, physical activity, energy intake, and time spent sitting: pilot study in a Nigerian population. *J Obes*. 2017;2017:3245270. https://doi.org/10.1155/2017/3245270
14. Warrington NM, Howe LD, Paternoster L, et al. A genome-wide association study of body mass index across early life and childhood. *Int J Epidemiol*. 2015;44:700-712. https://doi.org/10.1093/ije/dyv077
15. Hallman DM, Friedel VC, Eissa MA, et al. The association of variants in the FTO gene with longitudinal body mass index profiles in non-Hispanic white children and adolescents. *Int J Obes*. 2012;36:61-68. https://doi.org/10.1038/ijo.2011.190
16. Hardy R, Wills AK, Wong A, et al. Life course variations in the associations between FTO and MC4R gene variants and body size. *Hum Mol Genet*. 2010;19:545-552. https://doi.org/10.1093/hmg/ddp504
17. Reuter CP, de Mello ED, da Silva PT, et al. Overweight and obesity in schoolchildren: hierarchical analysis of associated demographic, behavioral, and biological factors. *J Obes*. 2018;2018:6128034. https://doi.org/10.1155/2018/6128034
18. Gerken T, Girard CA, Tung YC, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science*. 2007;318:1469-1472. https://doi.org/10.1126/science.1151710
19. Zhao X, Yang Y, Sun BF, Zhao YL, Yang YG. FTO and obesity: mechanisms of association. *Curr Diabetes Rep*. 2014;14:486. https://doi.org/10.1007/s11892-014-0486-0
20. Merkestein M, Sellayah D. Role of FTO in adipocyte development and function: recent insights. *Int J Endocrinol*. 2015;2015:521381. https://doi.org/10.1155/2015/521381
21. Speakman JR. The "fat mass and obesity related" (*FTO*) gene: mechanisms of impact on obesity and energy balance. *Curr Obes Rep*. 2015;4:73-91. https://doi.org/10.1007/s13679-015-0143-1
22. Claussnitzer M, Dankel SN, Kim KH, et al. FTO obesity variant circuitry and adipocyte browning in humans. *N Engl J Med*. 2015;373:895-907. https://doi.org/10.1056/NEJMoa1502214
23. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics*. 2015;31:3555-3557. https://doi.org/10.1093/bioinformatics/btv402
24. Sovio U, Mook-Kanamori DO, Warrington NM, et al. Association between common variation at the FTO locus and changes in body mass index from infancy to late childhood: the complex nature of genetic association through growth and development. *PLOS Genet*. 2011;7:e1001307. https://doi.org/10.1371/journal.pgen.1001307
25. Rodrigues LDS, Santos AMD, Lima MIS, Simoes VMF, Pereira SR. Association between the FTO gene polymorphism and obesity in Brazilian adolescents from the Northeast region. *J Pediatr*. 2020;96(5):630-637. https://doi.org/10.1016/j.jped.2019.05.006
26. Gonzalez-Herrera L, Zavala-Castro J, Ayala-Caceres C, et al. Genetic variation of *FTO*: *rs1421085 T>C*, *rs8057044 G>A*, *rs9939609 T>A*, and copy number (CNV) in Mexican Mayan school-aged children with obesity/overweight and with normal weight. *Am J Hum Biol*. 2019;31:e23192. https://doi.org/10.1002/ajhb.23192
27. The NS, Suchindran C, North KE, Popkin BM, Gordon-Larsen P. Association of adolescent obesity with risk of severe obesity in adulthood. *Jama*. 2010;304(18):2042-2047. https://doi.org/10.1001/jama.2010.1635
28. Saadeh MR. A new global strategy for infant and young child feeding. *Forum Nutr*. 2003;56:236-238.
29. The National Board of Health and Welfare, National Food Agency. *Tio steg som främjar amning*. 2014.
30. Lenanders Grafiska AB. *Bra mat för spädbarn under ett år*. 2011.
31. The National Board of Health and Welfare. *Breast-Feeding and Smoking Habits Among Parents of Infants Born in 2012*. 2014. Accessed January 19, 2021. www.socialstyrelsen.se
32. Plagemann A, Harder T, Schellong K, Schulz S, Stupin J.H. Early postnatal life as a critical time window for determination of long-term metabolic health. *Best Pract Res Clin Endocrinol Metab*. 2012;26:641-653. https://doi.org/10.1016/j.beem.2012.03.008
33. Thompson AL. Developmental origins of obesity: early feeding environments, infant growth, and the intestinal microbiome. *Am J Hum Biol*. 2012;24:350-360. https://doi.org/10.1002/ajhb.22254
34. Brands B, Demmelmair H, Koletzko B, Project E. How growth due to infant nutrition influences obesity and later disease risk. *Acta Paediatr*. 2014;103:578-585. https://doi.org/10.1111/apa.12593
35. Mameli C, Mazzantini S, Zuccotti GV. Nutrition in the first 1000 days: the origin of childhood obesity. *Int J Environ Res Public Health*. 2016;13(9):838. https://doi.org/10.3390/ijerph13090838
36. Jiang YR, Mei H, Lin QM, et al. Interaction effects of FTO rs9939609 polymorphism and lifestyle factors on obesity indices in early adolescence. *Obes Res Clin Pract*. 2019;13:352-357. https://doi.org/10.1016/j.orcp.2019.06.004
37. Abarin T, Wu YY, Warrington N, Lye S, Pennell C, Briollais L. The impact of breastfeeding on FTO-related BMI growth trajectories: an application to the Raine pregnancy cohort study. *Int J Epidemiol*. 2012;41(6):1650-1660. https://doi.org/10.1093/ije/dys171
38. Wu VY, Lye S, Briollais L. The role of early life growth development, the FTO gene and exclusive breastfeeding on child BMI trajectories. *Int J Epidemiol*. 2017;46:1512-1522. https://doi.org/10.1093/ije/dyx081
39. Dedoussis GV, Yannakoulia M, Timpson NJ, et al. Does a short breastfeeding period protect from FTO-induced adiposity in children? *Int J Pediatr Obes*. 2011;6:e326-e335. https://doi.org/10.3109/17477166.2010.490269
40. Belsky J, Jonassaint C, Pluess M, Stanton M, Brummett B, Williams R. Vulnerability genes or plasticity genes? *Mol Psychiatry*. 2009;14:746-754. https://doi.org/10.1038/mp.2009.44
41. Dalle Molle R, Fatemi H, Dagher A, Levitan RD, Silveira PP, Dube L. Gene and environment interaction: is the differential susceptibility hypothesis relevant for obesity? *Neurosci Biobehav Rev*. 2017;73:326-339. https://doi.org/10.1016/j.neubiorev.2016.12.028
42. Vadlin S, Aslund C, Nilsson KW. A longitudinal study of the individual- and group-level problematic gaming and associations with problem gambling among Swedish adolescents. *Brain Behav*. 2018;8:e00949.
43. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. 2000;320:1240-1243.
44. Hayes AF. *Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach (Methodology in the Social Sciences)*. 2nd ed. Guilford Press; 2018.
45. Horta BL, Victora CG, Franca GVA, et al. Breastfeeding moderates *FTO* related adiposity: a birth cohort study with 30 years of follow-up. *Sci Rep*. 2018;8:2530. https://doi.org/10.1038/S41598-018-20939-4
46. Aslund C, Nilsson KW. Individual biological sensitivity to environmental influences: testing the differential susceptibility properties of

the 5HTTLPR polymorphism in relation to depressive symptoms and delinquency in two adolescent general samples. *Journal of Neural Transmission*. 2018;125(6):977-993. https://doi.org/10.1007/s00702-018-1854-8

47. Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet*. 2016;387:475-490. https://doi.org/10.1016/S0140-6736(15)01024-7
48. Patro-Golab B, Zalewski BM, Polaczek A, Szajewska H. Duration of breastfeeding and early growth: a systematic Review of current evidence. *Breastfeed Med*. 2019;14:218-229. https://doi.org/10.1089/bfm.2018.0187
49. Nicolaidis S. Environment and obesity. *Metabolism Clin Exp*. 2019;100S:153942. https://doi.org/10.1016/j.metabol.2019.07.006
50. Dick DM. Gene-environment interaction in psychological traits and disorders. *Annu Rev Clin Psychol*. 2011;7:383-409. https://doi.org/10.1146/annurev-clinpsy-032210-104518

How to cite this article: Kanders SH, Nilsson KW, Åslund C. Breastfeeding moderates the relationship between *fat mass and obesity-associated gene* rs9939609 and body mass index among adolescents. *Obes Sci Pract*. 2022;8(1):66-76. https://doi.org/10.1002/osp4.546