THE JOURNAL OF CHILD PSYCHOLOGY AND PSYCHIATRY

ACAMH THE ASSOCIATION FOR CHILD AND ADOLESCENT MENTAL HEALTH

Journal of Child Psychology and Psychiatry 58:2 (2017), pp 180–188 doi:10.1111/jcpp.12646

A DRD4 gene by maternal sensitivity interaction predicts risk for overweight or obesity in two independent cohorts of preschool children

Robert D. Levitan,[1,2,3] Pauline Jansen,[4,5] Barbara Wendland,[6] Henning Tiemeier,[5] Vincent W. Jaddoe,[5] Patricia P. Silveira,[7] James L. Kennedy,[1,2,3] Leslie Atkinson,[8] Alison Fleming,[9] Marla Sokolowski,[10] Helene Gaudreau,[11] Meir Steiner,[12] Laurette Dubé,[13] Jill Hamilton,[14] Ellen Moss,[15] Ashley Wazana,[16] and Michael Meaney[7,11]

[1]Centre for Addition and Mental Health (CAMH), Toronto, ON; [2]Department of Psychiatry, University of Toronto, Toronto, ON; [3]Department of Physiology, University of Toronto, Toronto, ON, Canada; [4]Institute of Psychology, Erasmus University Rotterdam, Rotterdam; [5]Departments of Child and Adolescent Psychiatry and Psychology, and Epidemiology, and Psychiatry, Erasmus University Medical Center, Rotterdam, The Netherlands; [6]Institute of Medical Science, University of Toronto, Toronto, ON; [7]Ludmer Centre for Neuroinformatics and Mental Health, Douglas Mental Health University Institute, McGill University, Montreal, QC; [8]Ryerson University, Toronto, ON; [9]Department of Psychology, University of Toronto Mississauga, Mississauga, ON; [10]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON; [11]Department of Psychiatry and Neurology, McGill University, Montreal, QC; [12]McMaster University, Hamilton, ON; [13]Department of Management, McGill University, Montreal, QC; [14]Hospital for Sick Children, Toronto, ON; [15]Department of Psychology, Université du Québec à Montréal, Montreal, QC; [16]Centre for Child Development and Mental Health, Jewish General Hospital, McGill University, Montreal, QC, Canada

Background: Recent evidence suggests that early exposure to low maternal sensitivity is a risk factor for obesity in children and adolescents. A separate line of study shows that the seven-repeat (7R) allele of the dopamine-4 receptor gene (DRD4) increases susceptibility to environmental factors including maternal sensitivity. The current study integrates these lines of work by examining whether preschoolers carrying the 7R allele are more vulnerable to low maternal sensitivity as it relates to overweight/obesity risk. **Method:** The Maternal Adversity Vulnerability and Neurodevelopment (MAVAN) project in Canada was used as the discovery cohort ($N = 203$), while the Generation R study in the Netherlands was used as a replication sample ($N = 270$). Regression models to predict both continuous BMI *z*-scores and membership in any higher BMI category based on established World Health Organization (WHO) cutoffs for 48 months of age were completed. **Results:** In both cohorts, there was a significant maternal sensitivity by DRD4 by sex interaction predicting higher body mass indices and/or obesity risk. As hypothesized, post hoc testing revealed an inverse relationship between maternal sensitivity and body mass indices in 7R allele carriers relative to noncarriers. This finding was strongest in girls in the Canadian cohort and in boys in the Dutch cohort. **Conclusions:** Many children who carry the 7R allele of DRD4 appear to be more influenced by maternal sensitivity as it relates to overweight/obesity risk, consistent with a plasticity effect. Given the relatively small sample sizes available for these analyses, further replications will be needed to confirm and extend these results. **Keywords:** Maternal sensitivity; DRD4; obesity; sex differences.

Introduction

Early life adversity, whether biological or psychosocial in origin, can be a potent trigger for pathological weight gain over the life span (Bae, Wickrama, & O'Neal, 2014; Kaufman et al., 2007; Ong & Dunger, 2000; Suglia, Duarte, Chambers, & Boynton-Jarrett, 2012). As discussed in the *Thrifty Phenotype Hypothesis* of Hales and Barker (2001), this may reflect a basic tendency of living organisms to store energy when future food supplies may be at risk. Identifying the early environmental signals that can trigger an energy-conserving phenotype and developing interventions to limit their impact early in life might thus limit obesity rates.

While initial work on environmental adversity and weight regulation was based on human populations exposed to major environmental stressors such as war and famine (e.g. Ravelli, Stein, & Susser, 1976), more universal aspects of the early environment, including the quality of infant rearing, are also relevant to energy storage. Macaque monkeys raised in an insecure environment during early rearing have an increased likelihood of exhibiting a higher body mass index (BMI), abdominal circumference, and glucagon-like peptide-1 level in adulthood (Kaufman et al., 2007). In humans, low maternal sensitivity, defined as poorly timed or inappropriate responding to a child's emotional signals (Ainsworth, Blehar, Waters, & Wall, 1978), predicts increased risk of overweight and obesity in childhood and adolescence (Anderson, Gooze, Lemeshow, & Whitaker, 2012; Wendland et al., 2014), as does childhood adversity in general (Lissau & Sørensen, 1994; Williamson, Thompson, Anda, Dietz, & Felitti, 2002).

However, not every child exposed to low maternal sensitivity will exhibit an adverse outcome. Children differ in their vulnerability to adverse rearing

Conflict of interest statement: No conflict of interest declared.

Published by John Wiley & Sons Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK and 350 Main St, Malden, MA 02148, USA

environments (including low maternal sensitivity) and genetic factors are likely to play a role in this regard (Belsky et al., 2009; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van Ijzendoorn, 2011). The hypofunctional seven-repeat (7R) allele of the DRD4 gene moderates the effects of early rearing environment on several developmental outcomes including externalizing behavior (Windhorst et al., 2015) and inattention (Berry, Deater-Deckard, McCartney, Wang, & Petrill, 2013). Of note, 7R carriers may also be more sensitive to supportive environments (Bakermans-Kranenburg & van Ijzendoorn, 2011), consistent with differential susceptibility/differential sensitivity to context theory (Belsky et al., 2009; Ellis et al., 2011). According to this theory, carriers of 'plasticity gene' variants are expected to gain the greatest benefit from environmental support and enrichment, in addition to being the most vulnerable to adverse environmental conditions. This makes early intervention particularly important for such individuals.

While no studies to date have examined a possible moderating effect of DRD4 related to early parenting and obesity risk, DRD4 does influence BMI and body composition in the context of other environmental factors. For example, 7R allele/season-of-birth interactions increase the risk for obesity in women with either seasonal affective disorder (Levitan et al., 2006) or bulimia nervosa (Levitan, Kaplan, Davis, Lam, & Kennedy, 2010). A study of Ariaal men in Kenya suggests that 7R carriers are better able to optimize fat-free mass in preparation for long migrations across hostile desert environments (Eisenberg, Campbell, Gray, & Sorenson, 2008).

The current study integrates these various lines of work by examining whether an interaction between the 7R allele of DRD4 and maternal sensitivity influences the risk for overweight and obesity in the preschool years. The working hypothesis based on the data reviewed above was that children who carried the 7R allele, and were exposed to low maternal sensitivity as infants, would have the greatest overweight/obesity risk as preschoolers. We tested this hypothesis in two distinct developmental cohorts from Canada and the Netherlands, using the MAVAN cohort for discovery and the Generation R cohort for replication.

Methods

Participants and methods

For this project, we included only the subsample of study participants who had full data available for each of the key study variables including DRD4 genotyping, maternal sensitivity measures, and growth measures at 48 months of age. Informed consent was obtained from all participants.

Using this approach, the final Canadian sample included 203 children recruited in either Montréal, Québec (N = 116) or Hamilton, Ontario (N = 87), Canada as part of an established prospective birth cohort, the Maternal Adversity, Vulnerability, and Neurodevelopment (MAVAN) project (O'Donnell et al., 2014). MAVAN is a multidisciplinary, collaborative study recruiting pregnant women from obstetric clinics in hospitals located in Montréal, Québec and Hamilton, Ontario, Canada. All participants were born at 37–41 weeks gestation, and had a birthweight >1,500 g.

The corresponding Dutch sample consisted of 270 children taking part in the Generation R study (Jaddoe et al., 2012). Generation R is a prospective, population-based study spanning fetal life to late childhood. It is designed to identify early environmental and genetic causes and causal pathways leading to normal and abnormal growth, development, and health during fetal life and childhood. Pregnant women living in the study area in Rotterdam were invited to participate if their expected delivery date was between April 2002 and January 2006. In the preschool years, detailed measurements were obtained in the Generation R Focus Study, a total cohort of 1,106 Generation R participants of Dutch national origin (children whose parents and grandparents were all born in the Netherlands). These measurements included mother–infant attachment and maternal sensitivity assessments where possible.

Measures

Mother–child interaction using video-taping. In the Canadian MAVAN cohort, maternal sensitivity (warm, timely, and appropriate response to infant signals) was assessed when the children were 6 months of age. Thirty minutes of nonfeeding, free-play, and mother–infant interactions were videotaped in the home when infants were 6 months of age. The recordings were later reviewed by coders who were independent of the MAVAN study and had no awareness of the primary study hypotheses. Coders used the short version (Tarabulsy et al., 2009) of the Maternal Behavior Q-set (MBQS; Pederson et al., 1990). The MBQS items were derived from and anchored in the sensitivity observations of Ainsworth and colleagues (including the Ainsworth sensitivity scales) as well as a broader sampling of the sensitivity domain (Pederson et al., 1990). The short version of the MBQS (Tarabulsy et al., 2009) involves sorting 25 items into five piles of five items each, according to whether the descriptor is like (5) or unlike (1) the mother under observation. The derived sort is then correlated with the sort of a 'prototypically predictable, coherent, and warm mother' (Tarabulsy et al., 2009, p. 133), as defined by a sample of developmental experts who were instructed to sort so as to describe the prototypically sensitive mother (Pederson et al., 1990). This correlation of the observed sort with the prototypically sensitive sort yields a score varying between −1.0 and + 1.0 (current mean = 0.42, SD = 0.42). The MBQS correlates highly with Ainsworth sensitivity ratings and child attachment security (Pederson et al., 1990). The MBQS-25 correlates positively with constructs central to infant development and attachment, including the MBQS, infant attachment security, and infant cognitive development (Pederson et al., 1990; Tarabulsy et al., 2009). In the current study, three MBQS-25 observers independently coded twenty-eight 6-month tapes to obtain intraclass correlations between 0.82 and 0.94. Again, meta-analyses using both the Ainsworth scales and the MBQS show that both are reliably related to infant attachment security (Atkinson et al., 2000; De Wolff & Van IJzendoorn, 1997).

For the Dutch Generation R cohort, maternal sensitivity was assessed when the children were 14 months of age (Tharner et al., 2012). DVD recordings were made of a 5-min unstructured play session, which were coded using the Ainsworth's 9-point maternal behavior rating scales. Coders rated two subscales – sensitivity-insensitivity and cooperation-interference. The scores on each subscale are anchored in extensive description of maternal behavior. Scores were aggregated across these two subscales into a single mean denoting

sensitivity-insensitivity. The range of unstandardized sensitivity scores was 2–9 (mean = 6.0, *SD* = 1.3), while the range of standardized sensitivity scores used for the analyses was −3.1 to 2.4 (mean = 0.06, *SD* = 1.01). The intercoder reliability [intraclass correlation coefficient (ICC)] for the current data ranged from .65 to .71 (Luijk et al., 2011).

Growth measures at 48 months of age. For the Canadian sample, standing height without shoes was measured (to the nearest 0.1 cm) with the use of a stadiometer (Perspective Enterprises, PE-AIM-101, Portage, MI, USA). Body weight in light clothing was measured (to the nearest 0.1 kg) with the use of a digital floor scale (TANITA BF625; Arlington Heights, IL, USA). In the Dutch cohort, height was measured in standing position using a Harpenden stadiometer and weight was measured in underwear using a mechanical personal scale. Height and weight were used to calculate body mass index (BMI, kg/m^2). BMI *z*-scores were calculated based on well-established World Health Organization (WHO) growth curves for girls and boys (WHO Multicentre Growth Reference Study Group, 2006).

DRD4 genotyping. For the Canadian sample, saliva samples were collected and sent to the Centre for Addiction and Mental Health Neurogenetics Laboratory. Genomic deoxyribonucleic acid (DNA) was extracted using the high-salt method. All genotyping of the DNA was performed blind to the children's BMI. The 48-base-pair variable number of tandem repeats (VNTR) region in the third exon of DRD4 was amplified with polymerase chain reaction (PCR) techniques with primers and conditions previously described (Lichter et al., 1993).

For the Dutch sample, DNA sampling was based on cord blood. Genetic data have been generated by Taqman analyses. For genotyping, the infrastructure of the Genetic Laboratory of the Department of Internal Medicine (www.glimdna.org) was used (Luijk et al., 2011).

Statistical approach

To maximize the replication value of this study, the same general statistical approach was used for the two samples with minor differences based on the unique aspects of each cohort as described below. Analyses were conducted with IBM SPSS statistical software, version 21 (IBM SPSS Statistics for Windows, Amonk, NY, USA). The overall strategy was to use linear regression to predict continuous measures of BMI at 48 months of age, and logistic regression to predict membership in a higher BMI category at 48 months of age based on established World Health Organization (WHO) cutoffs. Each study controlled for potential confounding by birthweight, maternal BMI, exposure to breastfeeding at 3 months of age, and socioeconomic status. The main predictor variables in each case were maternal sensitivity scores, DRD4 genotype (7R present or absent), sex, and all corresponding interaction terms.

For the Canadian sample, to account for the relatedness of observations taken from the same study site (Montréal or Hamilton), linear mixed-effect models controlling for site, implementing the method of maximum likelihood were used to predict 48-month BMI *z*-scores based on maternal sensitivity as reflected by the MBQS-25 score at 6 months of age, DRD4 genotype, sex, all corresponding interaction terms, and the other covariates listed above (birthweight, maternal BMI, exposure to breastfeeding at 3 months of age, and socioeconomic status) that proved significant at $p < .05$ in the final model.

For the Dutch sample, given the relative homogeneity of the recruitment sites, a similar statistical approach as described above was used but using basic multiple regression as opposed to linear mixed-effect models as the main statistical tool.

Prediction of higher BMI categories

For both MAVAN and Generation R, to more specifically determine whether the key study variables were predictive of the higher BMI categories outlined by the WHO (i.e. at risk of overweight, overweight, or obese), a logistic regression predicting membership in any of the higher weight categories considered together (yes/no) was performed using the same predictor variables and other covariates as described above for continuous BMI measures. The goal was to determine if maternal sensitivity was relevant to childhood overweight/obesity risk based on established cutoffs.

Results

Tables 1 and 2 summarize the basic demographic characteristics of the two study samples.

Chi-square analysis revealed no significant difference in the distribution of genders, breastfeeding, small for gestational age status, or DRD4 genotype in the two samples. The mean maternal BMI in the Canadian sample was significantly higher than in the Dutch sample ($t = 5.94$, $df = 438$, $p < .001$).

Other study variables were not comparable statistically across samples as they were defined differently in Canada versus the Netherlands.

Final models predicting BMI *z*- scores at 48 months of age

In the Canadian sample, maternal BMI and birthweight percentile were identified as significant covariates, while exposure to breastfeeding at 3 months of age and socioeconomic status were not. As summarized in Table 3, there was a

Table 1 Sample characteristics for the MAVAN cohort (*N* = 203)

Variable		
Gender	Boys	Girls
	107 (52.7%)	96 (47.3%)
Socio-economic status/ maternal education blended	High/High[a]	Other
	132 (77.6%)	38 (22.4%)
Any breastfeeding at 3 months	Yes	No
	149 (74.5%)	51 (25.5%)
Small for gestational age	Yes[b]	No
	21 (10.8%)	173 (89.2%)
DRD4 seven-repeat carrier	Yes	No
	76 (37.4%)	127 (62.6%)
	Continuous variables Mean ± standard deviation	
Maternal BMI	27.6 ± 7.5	
Maternal sensitivity score[c]	0.42 ± 0.42	

Discrepancies in sample size are due to missing/incomplete data.

[a]High/High = high socio-economic status (SES) and high maternal education; Other = low SES and/or low maternal education.

[b]Small for gestational age defined as below the 10th percentile for gestational age.

[c]Based on the Maternal Behavior Q-Sort 25-item: range is −1 to + 1.

Table 2 Sample characteristics for the Generation R cohort ($N = 270$)

Variable		
Gender	Boys	Girls
	137 (50.7%)	133 (49.3%)
Socio-economic status	High[a]	Other
	154 (61.1%)	98 (38.9%)
Any breastfeeding	Yes	No
at 3 months	188 (70.9%)	77 (29.1%)
Small for gestational	Yes[b]	No
age	24 (8.9%)	246 (91.1%)
DRD4 seven-repeat	Yes	No
carrier	96 (35.6%)	174 (64.4%)
	Continuous Variables	
	Mean ± Standard Deviation	
Maternal BMI	24.3 ± 4.2	
Maternal sensitivity score[c]	0.06 ± 1.01	

Discrepancies in sample size are due to missing/incomplete data.
[a]Based on income: high is >2,000 euro net monthly income (greater than modal income).
[b]Small for gestational age defined as below the 10th percentile for gestational age.
[c]Based on Ainsworth scales (*z* standardized).

significant three-way interaction between maternal sensitivity, DRD4 genotype, and sex in predicting continuous BMI *z*-scores in both the uncorrected and corrected models. The maternal sensitivity by sex interaction was significant in both models. As reported previously (Wendland et al., 2014), there was a significant negative correlation between maternal sensitivity scores and BMI *z*-scores in girls, but not in boys. To explore the three-way interaction in more detail, four separate mixed-effect models to predict 48-month BMI *z*-scores with maternal sensitivity scores were completed, stratified by sex and DRD4 genotype. Table 4 indicates that in girls who carried the 7R allele of DRD4, there was a very strong negative correlation between maternal sensitivity scores and BMI *z*-scores (at $p = .001$) which remained robust in the corrected model (at $p = .002$). In boys who carried the 7R allele, there was a positive association between maternal sensitivity and BMI *z*-scores which was not significant ($B = 0.43$, $p = .19$ in the corrected model). In the Canadian sample, it thus appeared that the significant three-way interaction was largely driven by girls who carried the 7R allele.

In the Dutch sample, maternal BMI and being small for gestational age at birth were identified as significant covariates, while exposure to breastfeeding at 3 months of age and socioeconomic status were not. As summarized in Table 5, the three-way interaction between maternal sensitivity, DRD4 genotype, and sex achieved significance in the corrected model at $p = .049$, thus replicating the Canadian results. The two-way maternal sensitivity by sex interaction was significant in both models, again consistent with the Canadian sample. To determine if the direction of the maternal sensitivity by sex interaction was similar to the Canadian findings, we examined two separate models for boys and girls, correcting for significant covariates. The association between maternal sensitivity scores and BMI *z*-scores was negative but not significant in girls ($B = -0.08$, $p = .28$) and positive but not significant in boys ($B = 0.08$, $p = .23$). The general direction of this interaction was thus the same as in the Canadian sample (i.e. significantly more negative for girls than for boys), although did not achieve significance in either sex considered alone in the Generation R sample, unlike the Canadian sample as described above.

As was done for the Canadian sample, to help interpret the significant three-way interaction, separate linear regressions predicting BMI *z*-scores with maternal sensitivity scores were done in the four subgroups defined by DRD4 genotype and sex. As shown in Table 6, in contrast to the Canadian sample, there was no single subgroup that stood out as having a particularly robust association between maternal sensitivity scores and BMI *z*-scores relative to the other groups. Boys who were noncarriers of the 7R allele stood out as being the only subgroup having a positive relationship between maternal sensitivity and BMI, while the other three subgroups showed a negative relationship between these variables.

In summary, given the difficulty in replicating gene by environment interactions in general, the current results are notable in showing the same overall maternal sensitivity by sex interaction in the Generation R cohort as in the MAVAN cohort, and for the finding of a significant three-way maternal sensitivity by DRD4 by sex interaction in both cohorts, with

Table 3 Final mixed model predicting 48-month BMI *z*-scores in the MAVAN cohort ($N = 203$)

Variable	Unadjusted *B*	(95% C.I.)	*p* value	Adjusted B^1	(95% C.I.)	*p* value
Maternal sensitivity	−1.49	−2.42, −0.56	**.002**	−1.28	−2.24, −0.31	**.01**
DRD4 (absent vs. present)	−0.36	−1.04, 0.32	.30	−0.03	−0.76, 0.70	.93
Sex (male vs. female)	−0.31	−1.01, 0.40	.39	−0.33	−1.02, 0.35	.34
Maternal sensitivity by DRD4	1.38	0.25, 2.51	**.017**	0.99	−0.26, 2.24	.12
Maternal Sensitivity by sex	1.74	0.59, 2.89	**.003**	1.76	0.58, 2.93	**.004**
DRD4 by sex	0.30	−0.54, 1.15	.48	0.19	−0.71, 1.08	.68
Maternal sensitivity by DRD4 by sex	−1.68	−3.08, −0.27	**.02**	−1.84	−3.36, −0.32	**.018**

Bolded figures significant at $p \leq .05$.

some differences in the specific details as outlined above.

Prediction of higher BMI categories

In the Canadian sample, of the 203 children included in the current sample, a total of 56 (27.6%) would be considered as either 'at risk of overweight', 'overweight', or 'obese' based on WHO criteria. As shown in Table 7, consistent with the mixed model analysis of continuous BMI measures described earlier, the three-way maternal sensitivity by DRD4 by sex interaction was significant in both the uncorrected and corrected models. To explore the three-way interaction in more detail, four separate logistic regressions to predict membership in an at-risk BMI group were completed, stratified by sex and DRD4 genotype (see Table 8). This indicated that in girls who carried the 7R allele, higher maternal sensitivity was associated with a significantly lower chance of being in a higher BMI category in both the corrected and uncorrected models. No significant results were found in girls who did not carry the 7R allele or in boys independent of genotype.

In the Dutch sample, of the 270 children included in the current sample, a total of 49 (18.1%) would be considered as 'at risk of overweight', 'overweight', or 'obese' based on WHO criteria. This rate was significantly lower than in the Canadian sample (chi-square = 5.98, $p = .01$). As shown in Tables 9, there was a strong trend (at $p = .078$) for a three-way interaction between maternal sensitivity, DRD4 genotype, and sex in the corrected model, while the two-way maternal sensitivity by DRD4 interaction was significant in both the uncorrected and corrected models. Given that this was a replication sample, and to best compare these results to the Canadian data, separate logistic regressions were done in the four subgroups defined by DRD4 genotype and sex (see Table 10). This revealed that in boys who carried the 7R allele, higher maternal

Table 4 Mixed models predicting BMI *z*-scores at 48 months of age with maternal sensitivity scores, stratified by sex and DRD4 genotype [seven-repeat (7R) carrier or not] in the MAVAN cohort

	Unadjusted			Adjusted		
	B	(95% C.I.)	*p* value	*B*[a]	(95% C.I.)	*p* value
Boys						
7R carriers ($n = 42$)	0.25	−0.41, 0.92	.45			
Noncarriers ($n = 65$)	−0.05	−0.51, 0.42	.85			
Girls						
7R carriers ($n = 34$)	−1.49	−2.30, −0.68	**.001**	−1.17	−1.90, −.45	**.002**
Noncarriers ($n = 62$)	−0.11	−0.87, 0.64	.77			

Bolded figures significant at $p \leq .05$.
[a]Adjusted for maternal BMI and birthweight percentile.

Table 5 Final regression model predicting 48-month BMI *z*-scores in the Generation R cohort ($N = 270$)

	Unadjusted			Adjusted		
Variable	*B*	(95% C.I.)	*p* value	*B*[1]	(95% C.I.)	*p* value
Maternal sensitivity	0.14	−0.03, 0.30	.095	0.15	0.00, 0.31	**.05**
DRD4 (seven-repeat present vs. absent)	−0.02	−0.32, 0.29	.93	−0.11	−0.39, 0.18	.47
Sex (female vs. male)	0.08	−0.19, 0.34	.56	0.05	−0.20, 0.29	.71
Maternal sensitivity by DRD4	−0.27	−0.56, 0.03	.075	−0.22	−0.50, 0.05	.11
Maternal Sensitivity by sex	−0.32	−0.58, −0.06	**.017**	−0.30	−0.55, −0.06	**.014**
DRD4 by sex	−0.09	−0.53, 0.34	.67	−0.09	−0.50, 0.32	.67
Maternal sensitivity by DRD4 by sex	0.35	−0.09, 0.80	.12	0.42	0.001, 0.83	**.049**

Bolded figures significant at $p \leq .05$.

Table 6 Multiple regressions predicting BMI *z*-scores at 48 months of age with maternal sensitivity scores, stratified by sex and DRD4 genotype [seven-repeat (7R) carrier or not] in the Generation R cohort

	Unadjusted			Adjusted		
	B	(95% C.I.)	*p* value	*B*[a]	(95% C.I.)	*p* value
Boys						
7R carriers ($n = 48$)	−0.13	−0.35, 0.10	.28	−0.07	−0.29, 0.15	.51
Noncarriers ($n = 89$)	0.14	−0.04, 0.32	.13	0.15	−0.02, 0.32	.075
Girls						
7R carriers ($n = 48$)	−0.09	−0.35, 0.17	.49	0.01	−0.27, .28	.96
Noncarriers ($n = 85$)	−0.18	−0.38, 0.02	.07	−0.15	−0.34, .03	.099

[a]Adjusted for maternal BMI, child small for gestational age.

sensitivity was associated with a significantly lower chance of being in a higher BMI category in the uncorrected model (odds ratio = 0.40, 95% C.I. = 0.17–0.91, $p = .029$), while the corrected model was just at the cutoff for significance (adjusted odds ratio = 0.32, 95% C.I. = 0.10–1.01, $p = .051$). No significant results were found in boys who did not carry the 7R allele or in girls independent of genotype. This pattern of results suggests that exposure to high maternal sensitivity was associated with a lower risk of overweight/obesity status (and vice-versa) only in boys who carried the 7R allele.

Summary comparison of results across the two cohorts

In sum, in both the Canadian and European samples, the 7R allele appeared to accentuate the protective effect of high maternal sensitivity with respect to overweight and obesity risk as defined by established WHO categories. In the Canadian sample, this effect was clearly demonstrated in girls but not in boys, while in the Dutch sample, there was a strong trend for this effect in boys but not in girls.

Discussion

Prior research has demonstrated that young children exposed to low maternal sensitivity are at greater risk of overweight and obesity over time (Anderson et al., 2012; Wendland et al., 2014). A separate line of research has shown that the 7R allele of DRD4 can moderate the influence of maternal sensitivity exposure on developmental outcomes such as externalizing behavior and inattention (Berry et al., 2013; Windhorst et al., 2015). The current study examined whether preschoolers who carry the 7R allele are particularly vulnerable to low maternal sensitivity as it relates to overweight/obesity risk. In our discovery cohort from Canada, girls who carried the 7R allele and were exposed to low maternal sensitivity as infants had the highest risk of being in a higher WHO-defined BMI category at 48 months of age, thus supporting our overall hypothesis. In the replication cohort from Generation R, a similar though less robust effect was found in boys. The maternal sensitivity by sex interaction was significant in both cohorts, in both cases demonstrating a negative association between maternal sensitivity and continuous BMI measures in girls and the opposite pattern in boys. Though consistent with only a partial replication of results, these overall findings are of note given the inherent difficulty in replicating interaction effects overall, and gene by environment interactions in particular.

The finding of a link between low maternal sensitivity and higher risk for overweight/obesity in a subgroup of Dutch preschoolers replicates recent findings in other developmental cohorts (Anderson et al., 2012; Wendland et al., 2014). On the other hand, the finding of a significant 7R allele by maternal sensitivity interaction in establishing overweight/obesity risk in both the Canadian and Dutch cohorts is highly consistent with similar work on externalizing behavior and inattention (Berry et al., 2013; Windhorst et al., 2015). Taken as a whole, these various findings suggest that DRD4 by maternal sensitivity interactions can influence a variety of developmental processes over time. Whether this interaction also contributes to the coexpression of these same outcomes in individual probands, thus

Table 7 Final logistic regression model predicting overweight risk at 48 months of age in the MAVAN cohort ($N = 203$)

Variable	Odds ratio	(95% C.I.)	*p* value	Adjusted odds ratio[1]	(95% C.I.)	*p* value
Maternal sensitivity	0.01	0.00, 0.38	**.013**	0.002	0.00, 0.50	**.028**
DRD4 (seven-repeat)	0.47	0.09, 2.33	.35	0.52	0.07, 3.60	.51
Sex	0.44	0.08, 2.39	.35	0.38	0.06, 2.64	.33
Maternal sensitivity by DRD4	70.17	1.40, 3520.10	**.033**	378.35	0.90, 1.6E05	**.054**
Maternal Sensitivity by sex	148.31	2.84, 7748.99	**.013**	1504.79	3.81, 5.9E05	**.015**
DRD4 by sex	1.55	0.21, 11.51	.67	2.06	0.19, 21.94	.55
Maternal sensitivity by DRD4 by sex	0.12	0.00, 0.95	**.047**	0.001	0.00, 0.64	**.036**

Bolded figures significant at $p \leq .05$.

Table 8 Logistic regressions predicting overweight risk at 48 months of age with maternal sensitivity scores, stratified by sex and DRD4 genotype [seven-repeat (7R) carrier or not] in the MAVAN cohort

	Odds ratio	(95% C.I.)	*p* value	Adjusted odds ratio[a]	(95% C.I.)	*p* value
Boys						
7R carriers ($n = 42$)	1.48	0.31, 7.05	.62			
Noncarriers ($n = 65$)	1.24	0.38, 4.06	.72			
Girls						
7R carriers ($n = 34$)	0.01	0.00, 0.38	**.013**	0.00	0.00–0.64	**.038**
Noncarriers ($n = 62$)	0.70	0.16, 3.00	.63			

Bolded figures significant at $p \leq .05$.
[a]Adjusted for maternal BMI.

Table 9 Final logistic regression model predicting overweight risk at 48 months of age in the Generation R cohort ($N = 270$)

Variable	Odds ratio	(95% C.I.)	*p* value	Adjusted odds ratio[1]	(95% C.I.)	*p* value
Maternal sensitivity	1.12	0.71, 1.77	.64	1.17	0.73, 1.90	.51
DRD4 (seven-repeat present vs. absent)	1.82	0.65, 5.05	.25	2.25	0.76, 6.64	.14
Sex (female vs. male)	1.70	0.77, 3.78	.19	1.85	0.81, 4.22	.15
Maternal sensitivity by DRD4	0.36	0.14, 0.92	**.033**	0.34	0.12, 0.94	**.038**
Maternal Sensitivity by sex	0.56	0.26, 1.21	.14	0.57	0.26, 1.23	.15
DRD4 by sex	2.11	0.51, 8.78	.30	2.16	0.50, 9.45	.31
Maternal sensitivity by DRD4 by sex	3.14	0.77, 12.74	.11	3.93	0.86, 18.00	.078

Bolded figures significant at $p \leq .05$.

Table 10 Logistic regressions predicting overweight risk at 48 months of age with maternal sensitivity scores, stratified by sex and DRD4 genotype [seven-repeat (7R) carrier or not] in the Generation R cohort

	Odds ratio	(95% C.I.)	*p* value	Adjusted odds ratio[a]	(95% C.I.)	*p* value
Boys						
7R carriers ($n = 48$)	0.40	0.17, 0.91	**.029**	0.32	0.10, 1.01	.051
Noncarriers ($n = 89$)	1.12	0.71, 1.77	.64	1.19	0.72, 1.95	.50
Girls						
7R carriers ($n = 48$)	0.70	0.31, 1.60	.39	0.83	0.32, 2.14	.70
Noncarriers ($n = 85$)	0.62	0.34, 1.16	.14	0.64	0.35, 1.18	.15

Bolded figures significant at $p \leq .05$.
[a]Adjusted for maternal BMI and small for gestational age.

establishing their comorbidity, is an important question for future research.

Prior research in adults has shown that the 7R allele interacts with other early environmental signals to influence BMI. For example, in women with either seasonal affective disorder or bulimia nervosa, the 7R allele interacts with season-of-birth in establishing later obesity risk (Levitan et al., 2006, 2010). Taken together with the current findings, this suggests that very early in development, the 7R allele may increase sensitivity to multiple environmental signals relevant to lifetime weight regulation. There is evidence that the 7R allele has been positively selected in recent human evolution (Ding et al., 2002), although the basis for this selection is unknown. We speculate that the ability of 7R to promote developmental plasticity in response to a variety of environmental cues, particularly as it relates to energy conserving processes in the face of early adversity, may be one basis of this positive selection effect.

Limitations and future directions

While the current findings are highly consistent with prior studies looking at 7R allele by maternal sensitivity interactions for externalizing behavior (Windhorst et al., 2015) and inattention (Berry et al., 2013), further replications of this interaction as it relates to overweight/obesity risk are needed. Pending replication in other samples, it will be of great interest to study the translational potential of this work in terms of population health. There is now significant evidence that simple video-based interventions to optimize specific maternal sensitivity behaviors during infancy can have protective effects for various developmental outcomes (Kalinauskiene et al., 2009; Moss et al., 2011). Whether this is true of obesity prevention, and whether the 7R allele moderates this putative effect, are natural extensions of this work. It can be argued that obesity is a natural focus for such interventional approaches given that energy regulation is highly susceptible to early environmental influences, and given the challenges in implementing obesity interventions at later stages of development.

A number of limitations of the current findings merit consideration. While the Dutch sample replicated the general finding of an association between low maternal sensitivity and higher risk for overweight/obesity in the Canadian sample, moderated by the 7R allele and sex, the specific pattern of results was somewhat different in the two cohorts. In the Dutch sample only, the 7R allele appeared to influence the relationship between maternal sensitivity and BMI in boys more so than in girls – in this case, boys who carried the 7R allele showed the negative association between low maternal sensitivity and higher BMIs seen in girls in the Canadian cohort, while noncarriers did not. In other words, the Dutch boys behaved like the Canadian girls with respect to the 7R allele-maternal sensitivity interaction. In trying to explain this difference in the findings, it should be noted that the two-way interactions between maternal sensitivity and sex were very similar in the two cohorts, that is in both cases, there was a significantly greater negative association between maternal sensitivity and BMI *z*-scores in girls than in boys. This suggests that the different way that maternal sensitivity was measured across

the two cohorts is unlikely to explain the three-way interaction differences in Canada versus Europe, that is if this were the case, the corresponding two-way interactions should also have differed. It is only when the DRD4 gene was added to the statistical modeling that the sex differences in the two cohorts emerged. There is a dearth of previous literature linking sex differences, adversity, and DRD4 in the extant literature, although Reiner and Spangler (2011) did show an interaction between sex, the DRD4-7R allele, and adversity in predicting personality traits in a German sample. Pending more research of this type, it is difficult to speculate on the mechanisms that might explain the specific pattern of sex differences related to the DRD4 gene across the two sites in the current study. More research examining the interaction of sex, DRD4, and specific parental feeding practices on child eating behavior would be of particular help in this regard.

While the extensive phenotyping and assessment of possible covariates, mediators, and moderators are key strengths of the MAVAN and Generation R cohorts, observational studies such as these cannot establish true causality. Despite this important limitation, descriptive longitudinal cohort studies such as these help to inform translational work by identifying the highest risk cases or those most likely to benefit from early intervention.

Acknowledgements

The MAVAN study has been supported by multiple operating grants from the Canadian Institutes of Health Research (CIHR), as well as private support from the Faculty of Medicine, McGill University and the Norlien Foundation (Calgary, Alberta) and the WOCO Foundation (London, Ontario). R.L. has received support for this project from the CAMH Foundation and the Cameron Wilson Chair in Depression Studies, University of Toronto Department of Psychiatry. The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the School of Law and Faculty of Social Sciences at the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR-MDC), Rotterdam. The general design of the Generation R Study is made possible by financial support from Erasmus Medical Center, Erasmus University Rotterdam, the Dutch Ministry of Health, Welfare and Sport, and the Netherlands Organization for Health Research and Development (ZonMw). V.J. received an additional grant from the Netherlands Organization for Scientific Research (VIDI 016.136.361). P.J. received a grant from the Dutch Diabetes Foundation (grant no. 2013.81.1664). The authors have declared that they have no competing or potential conflicts of interest. The authors gratefully acknowledge the contribution of general practitioners, hospitals, midwives, and pharmacies in Rotterdam. They also wish to thank Jessica Grummitt, Patricia Szymkow, and Carmen McPherson for their extensive help with MAVAN study organization and data management; Marcos Sanches and Tamara Arenovich for their generous statistical help.

Correspondence

Robert D. Levitan, c/o CAMH, 100 Stokes Street, room 4289, Toronto, ON M6J 1H4, Canada; Email: robert.levitan@camh.ca

Key points

- Early exposure to low maternal sensitivity is a risk factor for obesity in children and adolescents. However, not all children are equally affected by low maternal sensitivity. For example, the seven-repeat (7R) allele of the dopamine-4 receptor gene (DRD4) increases susceptibility to maternal sensitivity as it relates to inattention.
- We examined whether preschoolers carrying the 7R allele are more vulnerable to low maternal sensitivity as it relates to overweight/obesity risk.
- Consistent with our hypothesis, 7R carriers showed a greater association between low maternal sensitivity and higher body mass indices in two developmental cohorts (the MAVAN study and Generation-Rotterdam). Different moderating effects of gender were found in these two cohorts, with girls being more affected in the Canadian cohort and boys being more affected in the Dutch cohort.
- 7R carriers may be an ideal target for novel early obesity prevention programs based on improving sensitive maternal behaviors.

References

Ainsworth, M.D., Blehar, M.C., Waters, E., & Wall, S. (1978). *Patterns of attachment: A psychological study of the strange situation.* Hillside, NJ: Erlbaum.

Anderson, S.E., Gooze, R.A., Lemeshow, S., & Whitaker, R.C. (2012). Quality of early maternal-child relationship and risk of adolescent obesity. *Pediatrics*, *129*, 132–140.

Atkinson, L., Niccols, G.A., Paglia, A., Coolbear, J., Parker, K.C.H., Poulton, L., … & Sitareneos, G. (2000). A meta-analysis of time between maternal sensitivity and attachment assessments: Implications for internal working models in infancy/toddlerhood. *Journal of Social and Personal Relationships*, *17*, 791–810.

Bae, D., Wickrama, K.A., & O'Neal, C.W. (2014). Social consequences of early socioeconomic adversity and youth BMI trajectories: Gender and race/ethnicity differences. *Journal of Adolescence*, *37*, 883–892.

Bakermans-Kranenburg, M.J., & van Ijzendoorn, M.H. (2011). Differential susceptibility to rearing environment depending on dopamine-related genes: New evidence and a meta-analysis. *Development and Psychopathology*, *23*, 39–52.

Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., & Williams, R. (2009). Vulnerability genes or plasticity genes? *Molecular Psychiatry*, *14*, 746–754.

Berry, D., Deater-Deckard, K., McCartney, K., Wang, Z., & Petrill, S.A. (2013). Gene-environment interaction between dopamine receptor D4 7-repeat polymorphism and early maternal sensitivity predicts inattention trajectories across middle childhood. *Development and Psychopathology*, *25*, 291–306.

De Wolff, M.S., & Van IJzendoorn, M.H. (1997). Sensitivity and attachment: A meta-analysis on parental antecedents of infant attachment. *Child Development*, *68*, 571–591.

Ding, Y.C., Chi, H.C., Grady, D.L., Morishima, A., Kidd, J.R., Kidd, K.K., … & Moyzis, R.K. (2002). Evidence of positive selection acting at the human dopamine receptor D4 gene locus. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 309–314.

Eisenberg, D.T., Campbell, B., Gray, P.B., & Sorenson, M.D. (2008). Dopamine receptor genetic polymorphisms and body composition in undernourished pastoralists: An exploration of nutrition indices among nomadic and recently settled Ariaal men of northern Kenya. *BMC Evolutionary Biology*, *8*, 173.

Ellis, B.J., Boyce, W.T., Belsky, J., Bakermans-Kranenburg, M.J., & van Ijzendoorn, M.H. (2011). Differential susceptibility to the environment: An evolutionary–neurodevelopmental theory. *Development and Psychopathology*, *23*, 7–28.

Hales, C.N., & Barker, D.J. (2001). The thrifty phenotype hypothesis. *British Medical Bulletin*, *60*, 5–20.

Jaddoe, V.W.V., van Duijn, C.M., Franco, O.H., van der Heijden, A.J., van IJzendoorn, M.H., de Jongste, J.C., … & Hofman, A. (2012). The Generation R Study: Design and cohort update 2012. *European Journal of Epidemiology*, *27*, 739–756.

Kalinauskiene, L., Cekuoliene, D., Van Ijzendoorn, M.H., Bakermans-Kranenburg, M.J., Juffer, F., & Kusakovskaja, I. (2009). Supporting insensitive mothers: The Vilnius randomized control trial of video-feedback intervention to promote maternal sensitivity and infant attachment security. *Child: Care, Health and Development*, *35*, 613–623.

Kaufman, D., Banerji, M.A., Shorman, I., Smith, E.L., Coplan, J.D., Rosenblum, L.A., & Kral, J.G. (2007). Early-life stress and the development of obesity and insulin resistance in juvenile bonnet macaques. *Diabetes*, *56*, 1382–1386.

Levitan, R.D., Kaplan, A.S., Davis, C., Lam, R.W., & Kennedy, J.L. (2010). A season-of-birth/DRD4 interaction predicts maximal body mass index in women with bulimia nervosa. *Neuropsychopharmacology*, *35*, 1729–1733.

Levitan, R.D., Masellis, M., Lam, R.W., Kaplan, A.S., Davis, C., Tharmalingam, S., … & Kennedy, J.L. (2006). A birth-season/DRD4 gene interaction predicts weight gain and obesity in women with seasonal affective disorder: A seasonal thrifty phenotype hypothesis. *Neuropsychopharmacology*, *31*, 2498–2503.

Lichter, J.B., Barr, C.L., Kennedy, J.L., Van Tol, H.H., Kidd, K.K., & Livak, K.J. (1993). A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. *Human Molecular Genetics*, *2*, 767–773.

Lissau, I., & Sørensen, T.I. (1994). Parental neglect during childhood and increased risk of obesity in young adulthood. *Lancet*, *343*, 324–327.

Luijk, M.P., Roisman, G.I., Haltigan, J.D., Tiemeier, H., Booth-Laforce, C., van Ijzendoorn, M.H., … & Bakermans-Kranenburg, M.J. (2011). Dopaminergic, serotonergic, and oxytonergic candidate genes associated with infant attachment security and disorganization? In search of main and interaction effects *Journal of Child Psychology and Psychiatry*, *52*, 1295–1307.

Moss, E., Dubois-Comtois, K., Cyr, C., Tarabulsy, G.M., St-Laurent, D., & Bernier, A. (2011). Efficacy of a home-visiting intervention aimed at improving maternal sensitivity, child attachment, and behavioral outcomes for maltreated children: A randomized control trial. *Development and Psychopathology*, *23*, 195–210.

O'Donnell, K.A., Gaudreau, H., Colalillo, S., Steiner, M., Atkinson, L., Moss, E., … & MAVAN Research Team (2014). The maternal adversity, vulnerability and neurodevelopment project: Theory and methodology. *Canadian Journal of Psychiatry*, *59*, 497–508.

Ong, K.K., & Dunger, D.B. (2000). Thrifty genotypes and phenotypes in the pathogenesis of type 2 diabetes mellitus. *Journal of Pediatric Endocrinology and Metabolism*, *13*(Suppl 6), 1419–1424.

Pederson, D.R., Moran, G., Sitko, C., Campbell, K., Ghesquire, K., & Acton, H. (1990). Maternal sensitivity and the security of infant–mother attachment: A q-sort study. *Child Development*, *61*, 1974–1983.

Ravelli, G.P., Stein, Z.A., & Susser, M.W. (1976). Obesity in young men after famine exposure in utero and early infancy. *New England Journal of Medicine*, *295*, 349–353.

Reiner, I., & Spangler, G. (2011). Dopamine D4 receptor exon III polymorphism, adverse life events and personality traits in a nonclinical German adult sample. *Neuropsychobiology*, *63*, 52–58.

Suglia, S.F., Duarte, C.S., Chambers, E.C., & Boynton-Jarrett, R. (2012). Cumulative social risk and obesity in early childhood. *Pediatrics*, *129*, e1173–e1179.

Tarabulsy, G.M., Provost, M.A., Bordeleau, S., Trudel-Fitzgerald, C., Moran, G., Pederson, D.R., … & Pierce, T. (2009). Validation of a short version of the maternal behavior Q-set applied to a brief video record of mother–infant interaction. *Infant Behavior and Development*, *32*, 132–136.

Tharner, A., Luijk, M.P., Raat, H., Ijzendoorn, M.H., Bakermans-Kranenburg, M.J., Moll, H.A., … & Tiemeier, H. (2012). Breastfeeding and its relation to maternal sensitivity and infant attachment. *Journal of Developmental and Behavioral Pediatrics*, *33*, 396–404.

WHO Multicentre Growth Reference Study Group (2006). *WHO child growth standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development*. Geneva: World Health Organization.

Wendland, B.E., Atkinson, L., Steiner, M., Fleming, A.S., Pencharz, P., Moss, E., … & MAVAN Study Team (2014). Low maternal sensitivity at 6 months of age predicts higher BMI in 48 month old girls but not boys. *Appetite*, *82*, 97–102.

Williamson, D.F., Thompson, T.J., Anda, R.F., Dietz, W.H., & Felitti, V. (2002). Body weight and obesity in adults and self-reported abuse in childhood. *International Journal of Obesity and Related Metabolic Disorders*, *26*, 1075–1082.

Windhorst, D.A., Mileva-Seitz, V.R., Linting, M., Hofman, A., Jaddoe, V.W., Verhulst, F.C., … & Bakermans-Kranenburg, M.J. (2015). Differential susceptibility in a developmental perspective: DRD4 and maternal sensitivity predicting externalizing behavior. *Developmental Psychobiology*, *57*, 35–49.

Accepted for publication: 13 July 2016
First published online: 11 October 2016