Published in final edited form as:

Appetite. 2014 February ; 73: . doi:10.1016/j.appet.2013.10.004.

Association between the seven-repeat allele of the dopamine-4 receptor gene (DRD4) and spontaneous food intake in pre-school children

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Abstract

Background—Studies in adults show associations between the hypofunctional seven-repeat allele (7R) of the dopamine-4 receptor gene (DRD4), increased eating behaviour and/or obesity, particularly in females. We examined whether 7R is associated with total caloric intake and/or food choices in pre-schoolers.

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Methods—150 four-year-old children taking part in a birth cohort study in Canada were administered a snack test meal in a laboratory setting. Mothers also filled out a food frequency questionnaire to address childrens' habitual food consumption. Total caloric and individual macronutrient intakes during the snack meal and specific types of foods as reported in the food diaries were compared across 7R allele carriers vs. non-carriers, using current BMI as a co-variate.

Results—We found significant sex by genotype interactions for fat and protein intake during the snack test. Post-hoc testing revealed that in girls, but not boys, 7R carriers ate more fat and protein than did non-carriers. Based on the food diaries, across both sexes, 7R carriers consumed more portions of ice cream and less vegetables, eggs, nuts and whole bread, suggesting a less healthy pattern of habitual food consumption.

Conclusion—The 7R allele of DRD4 influences macronutrient intakes and specific food choices as early as four years of age. The specific pattern of results further suggests that prior associations between the 7R allele and adult overeating/obesity may originate in food choices observable in the preschool years. Longitudinal follow-up of these children will help establish the relevance of these findings for obesity risk and prevention.

Keywords

feeding behavior; dopamine; DRD4; food intake; child behavior; reward

INTRODUCTION

Food preferences are influenced by variables such as experience with food, cultural factors and perceived health benefits (Aaron et al., 1994, Sullivan and Birch, 1994, Poppitt and Prentice, 1996). Studies also suggest that genetic differences contribute to individual variation in taste perception (Kim et al., 2003), preference for specific flavors (Mennella et al., 2005), food choices (Tepper et al., 2009) and total energy intake (Cecil et al., 2008). Increased eating behaviour is a core aspect of disorders including binge eating disorder, bulimia nervosa, obesity, and mood disorders including atypical depression and seasonal affective disorder (American Psychiatric Association., 2000). One important contributor to increased food intake in these various contexts seems to be an exaggerated sensitivity to the rewarding aspects of food, a phenomenon observed in both adults (Davis et al., 2009, Appelhans et al., 2011) and children (van den Berg et al., 2011, Verbeken et al., 2012) alike.

Both animal and human studies suggest that alterations in brain dopamine activity and/or receptor sensitivity play a role in food reward processes and eating behaviour (Stice et al., 2008, Saper et al., 2002, Grigson, 2002). Food-related cues activate brain areas that are either involved in the synthesis and release of dopamine or are targets for dopamine projections (Killgore et al., 2003, Rothenmund et al., 2007). Activity in these areas is proportional to the subjective pleasure associated with food (Small et al., 2003, Demos et al., 2012). Sweet and fatty foods potentiate a greater release of dopamine, inducing more pleasurable subjective feelings than their less palatable counterparts (Grigson, 2002, Martel and Fantino, 1996). Functional magnetic resonance imaging in response to imagined intake of palatable foods shows that future increases in body mass can be predicted by weaker brain activation of specific brain areas, particularly in individuals carrying low functioning variants of dopamine receptor genes, such as the DRD2 TaqIA A1 allele or the DRD4-7R allele (Stice et al., 2008, Stice et al., 2010).

The exon III 7-repeat allele (7R) of DRD4 has been associated with markedly decreased affinity for dopamine and impaired intracellular signaling in comparison to other exon III alleles (Asghari et al., 1995). Our group has studied the 7R allele in several disorders characterized by increased eating that are most prevalent in females (Levitin et al., 2006,

Levitan et al., 2004, Kaplan et al., 2008). We found that among women with seasonal affective disorder, who experience both carbohydrate craving and weight gain during winter depressive episodes, the 7-repeat allele of DRD4 was associated with childhood dysphoria and both binge eating and obesity in adulthood. Similar results were found in women with bulimia nervosa (Kaplan et al., 2008). DRD4 7-repeat carriers also report significantly more craving for food in a cue-elicited food-craving test (Sobik et al., 2005).

While several associations between dopamine system genes and various aspects of overeating and weight gain are emerging in adults, no such studies have been done in children. Childhood represents a dynamic period for the development of individual differences in eating habits and food preferences. Given that childhood overweight is a strong predictor of overweight and obesity into young adulthood (Deshmukh-Taskar et al., 2006), awareness of individual vulnerability to these conditions is of great importance.

Extending from findings in adult overeating populations, this study examined whether the hypo-functional 7-repeat (7R) allele of the dopamine-4 receptor gene (DRD4) is associated with total food intake and macronutrient preferences in pre-school children. Given our prior findings in female overeater populations (Levitan et al., 2004, Kaplan et al., 2008, Levitan et al., 2006), and significant evidence for sex differences in eating behaviour and obesity (Cooke and Wardle, 2005, Galloway, 2007), as well as for brain reward processes (Hurd et al., 1999, Adinoff et al., 2003), we hypothesized that different relationships between the 7R allele and eating behaviour would emerge in girls and boys i.e. that only girls carrying the 7R allele would exhibit preference for highly palatable, highly caloric foods (rich in fat and/or sugar). We report here our findings in a cohort of preschool children taking part in a longitudinal study of neurodevelopment.

SUBJECTS AND METHODS

The study sample included 4-year old children from Montreal (Quebec) and Hamilton (Ontario), Canada. Participants were recruited from an established prospective birth cohort (Maternal Adversity, Vulnerability and Neurodevelopment - MAVAN). Eligibility criteria for mothers included age ≥ 18 years, singleton gestation, and fluency in French or English. Women with severe chronic illness, placenta previa, and history of incompetent cervix, impending delivery, or a fetus/infant affected by a major anomaly or born at a gestational age less than 37 weeks were excluded. Birth records were obtained directly from the birthing unit. At the time of the current study, there were 150 participants who had enrolled in the MAVAN study at birth, participated in the age 4y follow-up exam, and agreed to come to the study laboratory for the test meal.

The sample was comparable to the overall birth cohort in terms of maternal age at childbirth (Student's T Test, $p=0.653$), birth weight ($p=0.212$), gestational age ($p=0.993$), prevalence of the 7 repeat allele (Chi-square, $p=0.208$), income categories ($p=0.120$), and maternal education ($p=0.174$).

MAVAN is a multidisciplinary, collaborative study that includes several laboratories from across Canada. We recruited pregnant women from obstetric clinics in hospitals located in Montreal, Quebec and Hamilton, Ontario. We excluded cases of very low birth weight and include only infants born at 37 to 41 weeks gestation. The maternal age at childbirth was also comparable to the general population (study sample = 30 years, Québec =29.3 years, Ontario=30.1 years) (Statistics Canada, 2012). By design, MAVAN over-samples from low SES settings, thus the prevalence of families receiving income below the Low Income Cut Off was near 30% vs. 15% in the general population (Statistics Canada 2009). However, while education below 10 years was found in 4% of the mothers of our sample, this number

reaches 12% of adults in the provinces of Quebec and Ontario (Statistics Canada, 2011). In sum, SES status was relatively low but education relatively high relative to population norms.

Approval for the MAVAN Project was obtained from obstetricians performing deliveries at the study hospitals and by the ethics committees at hospitals and university affiliates (McGill University and l'Université de Montréal: the Royal Victoria Hospital, Jewish General Hospital, Centre Hospitalier de l'Université de Montréal, and Hôpital Maisonneuve-Rosemont.).

Saliva samples were collected and sent to the Centre for Addiction and Mental Health Neurogenetics Laboratory after the parents or guardians provided written informed consent and the children provided assent. Genomic deoxyribonucleic acid (DNA) was extracted using the high-salt method. All genotyping of the DNA was performed blind to the children's behavior and phenotype. 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).

Children and mothers were exposed to a test meal at approximately 10:30 a.m. including different types of foods in pre-weighed portions for 30 minutes (Frosted Flakes®, sliced apple, muffin with chocolate drops, 3.25% milk, baked beans, croissant, cooked egg, cheddar cheese, All Bran®, white bread, orange juice), table 1. Foods were chosen with the orientation of a nutritionist to represent local habitual snack items and to have similar colours (Addessi et al., 2005). Mothers were instructed to offer a light breakfast to participants at home beforehand and not to share plates or influence the children's choices. Based on the nutritional content of each food and the amount eaten, we calculated the amount of fat, carbohydrates and protein ingested (Westerterp-Plantenga et al., 1996, Zandstra et al., 2000, Vozzo et al., 2003, Boudville and Bruce, 2005). The test meal was eaten in the laboratory, in a 30m² room. A table with two sets of plates was placed in the center of the room, with chairs for mother and child on both sides (facing each other). A cushion was placed on the child's chair to facilitate accessibility of the different foods. Various efforts were made to standardize this procedure between subjects, such as booking the lab visits at mid-morning to reduce variation in satiety and hormonal levels relevant to feeding behavior; making notes on the time and content of the last meal; noting whether the child slept while driving to the laboratory or not and asking the families to avoid booking the laboratory measure the day after large "food events" such as birthdays or parties. The laboratory visit was always booked on the birthday month to ensure that children were within a few weeks of being 48 months old at the time of the Snack Test and questionnaires.

One-hundred thirty one children had complete food frequency questionnaires for analysis, (Langevin et al., 2007, Miller et al., 2008), valid for the local population (Ordre Professionnel Des Diététistes Du Québec, 2000). This is a standard procedure used to evaluate feeding behavior and habitual consumption in children (Goran, 1998, Biro et al., 2002). Mothers were asked to report their child's intake of various foods on a typical day, with the aid of a food and measures photo album to estimate the portion size of each food (Katamay et al., 2007). Based on these reports, the quantitative analysis of total caloric and macronutrient intake is derived using NutriBase® software (Version NB7 Network) [Phoenix, AZ, US]. Using this software, the quantitative measure used to assess intake of specific groups of foods for this study was daily portions (see Table 3).

Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared (kg/m²). Standing height, without shoes, was measured (to the nearest 0.1 cm) with the use of a stadiometer (Perspective Enterprises, PE-AIM-101, Portage, Michigan). 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, Illinois). These measurements were performed during the laboratory visit by trained researcher assistants.

Statistical methods

Descriptive statistics were performed for potential confounders (including sex, race/ethnicity, birth weight, gestational age, being born small for gestational age, maternal age at birth, maternal education, family income and total duration of breastfeeding) using Student t-tests (for continuous variables) and chi-squared tests (for categorical variables). In addition, we compared anthropometric variables between the groups at 48 months of age using a 2 (sex) by 2 (genotype) ANOVA. The genetic model used was based on the known hypo-functionality of the 7R allele relative to other DRD4 exon-3 VNTR alleles (Ashgari et al, 1995) i.e. 7R carriers were compared to non-carriers, as has been done in prior research (Levitan et al, 2004, 2006; Kaplan et al, 2008; Stice et al, 2008). Genotype combinations have not been shown to be relevant in the literature, therefore to assess associations between total caloric intake, as well as individual macronutrient intakes, and both sex and genotype (7R present or absent), ANCOVA was used adjusting for BMI as a covariate. ANCOVA was also used to assess consumption of groups of foods (based on food frequency questionnaires) adjusting for current BMI and total energy intake reported in the food diary. Based on our hypothesis, when significant sex x genotype interaction effects were found, *posthoc* testing was limited to 2 pair-wise comparisons i.e. comparing 7R carriers to non-carriers in females and males considered separately, continuing to use BMI as covariate. Considering that population stratification may account for the findings, a separate series of analyses using only Caucasians was also performed. Furthermore, considering that birth weight may affect food preferences (Barbieri et al., 2009, Silveira et al., 2012, Portella et al., 2012), an analysis adjusted by birth weight was also included. Finally, considering that potential cultural influences on food choices may differ between different cities, analysis after adjustment for site (Montreal vs. Hamilton) was also performed and is reported on the Results section. Statistical significance was set at $p < 0.05$. Parametric continuous data were expressed as mean $\pm$ SEM.

RESULTS

The frequency of the common 4R, 7R, and 2R alleles was 64%, 20.6% and 10.3% respectively, highly consistent with prior studies in Canadian adults (23, 24). There were 58 study subjects (38.7%; 31 boys and 27 girls) who carried at least one 7R allele. The complete distribution of genotypes was: 92 (61.3%) non-7R/non-7R, 54 (36.0 %) heterozygous for 7R, and 4 (2.7%) homozygous for 7R; this met criteria for Hardy Weinberg Equilibrium). Table 2 depicts baseline characteristics of subjects with and without at least one 7R allele. There were no statistically significant differences found for sex, ethnicity, maternal education, family income and maternal age at the baby's birth and total duration of breastfeeding at 12 months of age. Of note, there were more SGA children among the 7R non-carriers, with a lower mean birth weight in this group; gestational age was similar between 7R carriers and non-carriers. Two-way ANOVA showed no differences in anthropometrics based on DRD4 genotype, sex or the genotype x sex interaction (Table 2).

Food consumption during the test meal

Total Energy Intake—ANCOVA revealed no main effect of genotype on total caloric intake [$F(1,147)=0.968$, $p=0.327$], while the main effect of sex was significant, with boys consuming more calories than girls [$F(1,147)=8.587$, $p=0.004$]. The sex-by-genotype interaction was not significant [$F(1,147)=1.813$, $p=0.180$]. Fig 1A.

Macronutrient Intake—ANCOVA revealed a significant interaction between sex and DRD4 genotype in predicting fat consumption [$F(1,147)=6.981$, $p=0.009$]. Post-hoc testing revealed that in girls, 7R carriers show greater fat consumption than did non-carriers ($p=0.022$; Fig 1B). In boys, there was no significant difference in fat consumption based on genotype ($p=0.110$).

For carbohydrate consumption, there was no main effect of genotype [$F(1,147)=2.066$, $p=0.153$]. However, the main effect of sex was significant, with boys consuming more carbohydrates than girls [$F(1,147)=8.967$, $p=0.003$]. The sex-by-genotype interaction was also not significant [$F(1,147)=0.313$, $p=0.577$]. (Fig 1C).

For protein consumption there was a significant sex-x-genotype interaction [$F(1,147)=5.681$, $p=0.018$]. Girls with the 7R allele had increased protein intake when compared to non-carriers ($p=0.049$), while in boys there was no such difference ($p=0.111$, Fig. 1D).

The analysis adjusted by site (Montreal vs. Hamilton) yielded the same effects.

Habitual food consumption from the food frequency questionnaire

The means ($\pm$ SEM) of reported daily portions for each food are summarized in Table 3. The 7R allele was associated with an increased consumption of ice cream [95% CI 0.16–0.30 portions] when compared to non-carriers [95% CI 0.06–0.17 portions, $p=0.011$]. The main effect of sex did not reach statistical significance [$p=0.057$] and there was no interaction sex vs. genotype [$p=0.116$]. There was also a decreased consumption of eggs/nuts/whole bread in the 7R carriers [95% CI 0.99–1.75 portions] as compared to non-carriers [95% CI 1.73–2.33 portions, $p=0.008$], without main effect of sex [$p=0.156$] or interaction sex vs. genotype [$p=0.192$]. 7R carriers also consumed less vegetable portions [95% CI 1.13–1.79] than did non-carriers [95% CI 1.64–2.15, $p=0.04$], without main effect of sex [$p=0.695$] or interaction sex vs. genotype [$p=0.225$].

The analysis adjusted by site (Montreal vs. Hamilton) regarding macronutrient intake yielded the same results.

Analysis of Caucasian Probands

To determine whether population stratification might have accounted for the current findings, we repeated our main analyses in the subgroup of probands of Caucasian ancestry ($N=115$). In this sub-sample, the overall statistical model remained highly significant in the genotype vs. sex interaction for fat [$F(1,114)=4.410$, $p=0.034$] and met a trend level of significance for protein consumption [$F(1,114)=3.643$, $p=0.059$] during the test meal. The results were also comparable for the habitual food consumption of ice cream [95% CI 0.14–0.23 portions in 7R carriers compared to 0.06–0.12 portions in non-carriers, $P=0.001$] and eggs/nuts/whole bread [95% CI 0.84–1.73 portions in 7R carriers compared to 1.78–2.47 portions in non-carriers, $P=0.004$]; vegetable consumption was similar between the genotype groups [95% CI 1.09–1.86 portions in 7R carriers compared to 1.60–2.18 portions in non-carriers, $P=0.100$]. Thus, limiting the analysis to Caucasian probands only did not have a major effect on the overall pattern of results.

Adjustment for birth weight, gestational age and BMI z scores

To determine whether the group differences in the prevalence of SGA shown in Table 2 might have accounted for the current findings, we repeated our main analyses adjusting the results for the birth weight ratio (the ratio between the infant birth weight and the sex-specific mean birth weight for each gestational age for the local population) (Kramer et al., 2001). The overall statistical model remained significant with respect to the genotype vs. sex

interactions for fat [$F(1,145)=6.891$, $p=0.010$] and protein consumption [$F(1,145)=5.609$, $p=0.019$] during the test meal. The results were also comparable for the habitual food consumption of ice cream [95% CI 0.17–0.31 portions in 7R carriers compared to 0.06–0.17 in non-carriers, $P=0.007$] and eggs/nuts/whole bread [95% CI 0.97–1.73 portions in 7R carriers compared to 1.75–2.35 portions in non-carriers, $P=0.005$]; vegetables consumption met a trend level of significance [95% CI 1.17–1.82 portions in 7R carriers compared to 1.62–2.13 portions in non-carriers, $P=0.07$]. Thus, adjusting the analysis for the birth size/gestational age did not have a major effect on the overall pattern of results.

Adjusting the analysis for BMI z scores (WHO, 2006) instead of raw BMI lead to the same results in all analysis.

DISCUSSION

To our knowledge, this is the first study to report an association between a dopamine gene variant and eating behavior in a pediatric sample, and among the first to show a specific genetic effect on macronutrient selection as early as 48 months of age (Cecil et al., 2008). We found interactions between the hypofunctional 7R allele of the DRD4 exon-3 VNTR polymorphism and sex on spontaneous macronutrient consumption in 4-year old children. Specifically, we demonstrated that girls who carried the 7R allele ate significantly more fat and proteins than did non-carriers. We also found effects of the 7R allele on the daily consumption of specific foods, decreasing the ingestion of vegetables and eggs, nuts and whole bread and increasing the consumption of ice cream, suggesting a less healthy pattern of habitual food consumption. It was recently shown that among adolescent males high in depressive symptoms, 7R carriers reported consuming less low-calorie foods than non-carriers (Agurs-Collins and Fuemmeler, 2011); although contradicting our gender specific findings, the study supports the genetic evidence demonstrated here.

The 7R allele has previously been linked to binge eating and obesity in women with winter seasonal affective disorder (Levitan et al., 2006, Levitan et al., 2004) and to maximal lifetime BMI in women with bulimia nervosa (Kaplan et al., 2008). This same allele has also been associated with increased fat free mass in a migrating population of males in Africa (Eisenberg et al., 2008). Our data extend these findings by demonstrating that the 7R allele is related to feeding behavior during early childhood. Further follow-up will be needed to determine the relevance of these findings in predicting eating behaviour and/or obesity as the cohort ages.

Considering that 7R allele carriers report increased craving for both food (Sobik et al., 2005) and drugs (Hutchison et al., 2002) in cue-elicited tasks, as well as demonstrate a biased attention for contextually cued emotion stimuli (Wells et al., 2013), it is possible that the current results related to 7R were mediated by reward processes, with the particular macronutrient preference depending on sex differences. In other words, as the Snack Test provides the real food cues to the children in order to measure consumption (as opposed to the information assessed by the food diaries), the increased fat and protein intakes seen in girls carrying the 7R allele could be a result of increased contextual salience of these macronutrients during this task. One of the strengths of the present study is that food choices were assessed using actual food consumption under controlled conditions, as well as using a mother-report of habitual food intake, and the results between the two tools were consistent. Any apparent discrepancy between the two measurements could be explain by the fact that, by design, the two measurements (Snack Test and Food Frequency questionnaire) are very different. While the Snack Test was performed to elicit spontaneous food preferences (in this study, for specific macronutrients), the second represents the habitual consumption of the children, which is more influenced by other variables such as availability (not

necessarily preference), maternal induction, maternal perception of what is consumed, etc. Therefore, in our point of view, the comparison between the two tools should be more general (healthy choices vs. non-healthy, palatable foods vs. regular foods, etc), and not specific for macronutrients, because the two tools measure different aspects of feeding behavior in different ways.

There are several potential mechanisms that could explain emerging links between the 7R allele and increased food intake (Levitan et al., 2004, Kaplan et al., 2008, Levitan et al., 2006). For example, studies in animals have proposed a possible role for DRD4 in satiety processes mediated at the level of the hypothalamus (Huang et al., 2005). The DRD4 receptor is predominantly localized in areas that are innervated by meso-cortical projections from the ventral tegmental area (VTA), including the prefrontal cortex, cingulate gyrus, and insula (Schoots and Van Tol, 2003). Studies link the 7-repeat allele to reduced dopamine functioning (Asghari et al., 1995), suggesting it may affect reward sensitivity. Various lines of work demonstrate that 7-repeat carriers have an increased risk for several reward-based psychopathologies including substance abuse (Vandenbergh et al., 2000, Ellis et al., 2011, Brody et al., 2012), pathological gambling (Comings et al., 2001), opiate dependence (Kotler et al., 1997), binge drinking (Vaughn et al., 2009), promiscuous sexual behavior and sexual infidelity (Garcia et al., 2010), increased sensitivity to alcohol's effects on social bonding (Creswell et al., 2012), increased consumption of cigarettes per day in smokers (Das et al., 2011) as well as novelty seeking (Benjamin et al., 1996, Ebstein et al., 1997, Ebstein et al., 1996). It is well established that food *per se* is a potent activator of the brain's reward circuitry (Grigson, 2002, Holden, 2001, Volkow and Wise, 2005).

Understanding the genetic basis of food intake and food preferences in school aged children and adolescents is extraordinarily difficult due to the major confounding effects of hormonal changes, body image concerns, and dieting (Hill, 2002). As the current sample was studied at just 48 months of age, our results are likely to be independent of these factors, and provide an excellent starting point to understand genetic contributions to eating behaviour over the lifespan.

This study was limited by a small sample size and resulting statistical power, especially when considering multiple interactions (such as gene x sex x environment), which need further exploration in future studies for which a larger sample size is needed. In this sample, there were more SGA children in the 7R non-carrier group (7R-), which could be a confounding factor, as we have previously shown that intrauterine growth alters the behavior towards a food reward and increases the consumption of palatable foods (Barbieri et al., 2009, Silveira et al., 2012, Portella et al., 2012); therefore, having more SGA children in the 7R- group might have limited our ability to show the group differences that were found here. The adjusted analysis demonstrated that the genotype effects and interactions with sex on macronutrient spontaneous macronutrient intake in the test meal and habitual food consumption are independent of fetal growth.

It is possible that maternal attitudes and behaviours, differentially expressed in boys and girls, contributed in some way to our findings. However, several other sex differences that have more direct relevance to the DRD4 gene, merit further consideration (Reiner and Spangler, 2011, Dmitrieva et al., 2011). One unique feature of the hypo-functional 7R allele - the main focus of the current analyses - is its positive selection over the past 45,000 years of human evolution (Ding et al., 2002). Based on findings from various genetic association studies linking the 7R allele to different phenotypes, prior authors have suggested that the basis for this positive selection may have included a nutritional and/or reproductive advantage in men in the context of migration (Eisenberg et al., 2008, Harpending and Cochran, 2002), and/or a similar advantage in women related to seasonal conservation of

body mass at northern latitudes (Levitan et al., 2004, Levitan et al., 2006). It is difficult to speculate on how the current findings might relate to the positive selection of the 7R allele, however when combined with these prior studies, the current results suggest that an ability of 7R to optimize food intake in the face of predictably adverse environments, modified based on the unique long term survival and/or reproductive needs of the two sexes, is of some interest.

In conclusion, this study demonstrates that the DRD4 7-repeat allele affects habitual food consumption and interacts with sex to influence macronutrient preferences early in childhood. Considering that some 7R carriers are prone to binge eating and overweight in adulthood (Levitan et al., 2004, Levitan et al., 2006), it is possible that altered feeding preferences established early in life contribute to this vulnerability. Knowledge about these specific behavioral characteristics may be important for obesity prevention and primary care, and to understand the origins of various disorders of eating behavior and mood.

Acknowledgments

This work was funded by the Canadian Institute of Health Research (CIHR). Silveira PP was funded by a CIHR fellowship grant (200610CFE-170826-164844). The authors thank Tamara Arenovich for her help with statistical analyses and Jessica Grummitt for her help with data management.

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Highlights

We found that girls who were 7R-carriers ate more fat and protein in a snack-test paradigm.

7R-carriers from both sexes consumed more ice cream in a food frequency questionnaire.

7R-carriers ate fewer portions of vegetables, eggs, nuts and whole bread.

These pre-school children were not overweight or obese.

The 7R allele of the DRD4 gene appears to influence macronutrient intake and food choice early in childhood.

Figure 1.

Food consumption during the test meal. Data are expressed as means $\pm$ SEM. For the analysis, ANCOVA was performed using genotype and sex as independent variables and current BMI as co-variables. 1A – Total caloric intake; 1B – Fat consumption; 1C – Carbohydrate consumption; 1D – Protein consumption. * $P < 0.05$ for pairwise comparisons of genotype within sex.

Table 1

Portion sizes and content of the Snack test

Food	Portion size	Kcal/portion	Fat (g/portion)	Protein (g/portion)	Carbohydrates (g/portion)
Froasted Flakes	30g	110	0.2	1.0	27
All bran	30g	97	0.4	3.5	24
Milk 3.25%	200 ml	128	6.4	7.2	10
Orange juice	200 ml	96	0	0.8	23
Croissant	2 units (35g)	130	6.6	4.4	15
Egg	1 unit	77	5.3	6.3	0.5
White bread	1 slice	100	1.2	3.5	18.5
Apple	100g	52	0.2	0.3	14
Maple syrup beans	200g	272	1.2	12	54
Cheese	50g	200	16.7	12	0
Muffin with chocolate drops	1 unit (60g)	210	5.0	3.0	36

Children had 30 minutes to freely choose and eat; portions were weighed before and after the exposure.

Table 2

Study participants' baseline characteristics according to genotype (DRD4 7 repeat positive vs. Negative). Data are expressed as mean±SEM or proportions (percentages).

Sample characteristics	DRD4 7 repeat negative (n= 92)	DRD4 7 repeat positive (n=58)	7 repeat negative vs. positive (p)*
Males (%)	47/92 (31.3%)	31/58 (20.7%)	0.45
Caucasian (%)	69/79 (53.1%)	46/51 (35.4%)	0.42
Birth weight (g)	3230.40±57.17	3418.52±60.52	0.02
Gestational age (weeks)	38.91±0.14	39.28±0.16	0.09
SGA (%)	26/90 (17.6%)	7/58 (7.4%)	0.012
Maternal age at birth (y)	29.49±0.55	30.78±0.60	0.12
Maternal education below 10 years (%)	2/76 (1.6%)	4/51 (3.1%)	0.17
Family income below LICO (%)	23/90 (15.6%)	13/57 (8.8%)	0.43
Total duration of breastfeeding (weeks)	25.77±2.06	26.07±2.52	0.93

Anthropometric measurements at 48 months of age	Males (n=47)	Females (n=45)	Males (n=31)	Females (n=27)	Sex vs. Genotype comparison
Body weight (kg)	17.71±0.40	17.30±0.59	17.23±0.35	16.53±0.50	0.77
Height (cm)	103.59±0.88	101.78±0.85	102.59±0.96	103.45±1.07	0.19
BMI (kg/m ²)	16.20±0.23	16.46±0.40	16.14±0.24	15.47±0.28	0.19

For the analysis of the study sample characteristics, Student's T Test and Chi-square test were used. For the anthropometric measurements analysis, Two-Way-ANOVA showed no differences between the groups (see Results section). The same results are found if using height, weight and BMI z scores (WHO, 2006). Totals may differ for some variables because of missing data. LICO=Low Income Cut Off (Statistics Canada, 2005). SGA=Small for gestational age (birth weight below 10th percentile, 46).

Table 3

Habitual consumption of specific foods – Food frequency questionnaire

	Girls		Boys		ANCOVA		
	7R- (n=44)	7R+ (n=22)	7R- (n=39)	7R+ (n=28)	Sex	DRD4	Int
Rice, pasta, cereal	0.83±0.09	0.87±0.10	0.89±0.06	0.97±0.13			
Fruits <i>Fruits, citric juice, other juices</i>	3.16±0.75	3.01±0.39	2.92±0.35	2.38±0.26			
Milk products <i>Milk, yogurt, cheese, cream soup</i>	3.43±0.29	3.44±0.41	4.03±0.29	3.54±0.46			
Take outs <i>Hot dog, pizza, chinese food, hamburger</i>	0.21±0.03	0.27±0.04	0.32±0.04	0.25±0.03			
Meats <i>Chicken, beef, liver sausage</i>	1.25±0.11	1.26±0.18	1.51±0.41	1.22±0.10			
Fat toppings <i>Bacon lard, sauces, mayonese, margarine, butter</i>	1.59±0.25	1.55±0.28	1.54±0.21	1.85±0.26			
Ice cream	0.13±0.05	0.30±0.09	0.12±0.03	0.14±0.03	#		*
Sweets <i>Chocolate, honey, maple syrup, jelly, Jam, sugar, desserts</i>	1.03±0.15	0.81±0.13	0.95±0.11	1.09±0.16			
Eggs, nuts, whole bread <i>Eggs, peanut butter, nuts, whole bread</i>	1.90±0.22	1.24±0.19	2.06±0.30	1.70±0.18			*
Beans	0.11±0.03	0.22±0.06	0.15±0.03	0.14±0.03			
Vegetables <i>Cooked and uncooked vegetables, potatoes</i>	1.93±0.22	1.55±0.26	1.71±0.20	1.55±0.18			*
Cookies and biscuits	1.62±0.29	3.29±1.26	1.79±0.24	1.81±0.38			
White bread, cake	0.35±0.06	0.53±0.15	0.45±0.07	0.46±0.15			

Data are expressed as mean±SEM of portions/day. For the analysis, Two-Way-ANOVA adjusted for BMI and total energy intake was performed.

* $P<0.05$

$P<0.06$