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Dopamine Regulating Genes, Negative Stressors, and Energy Balance Behaviors Among Chinese Adolescents

Rosa Ahn
Scripps College

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ABSTRACT

Context: Dopamine has been implicated as an important neurotransmitter involved in regulating appetite and food intake by modulating the reinforcement of food via the meso-limbic circuitry of the brain. Several genes have been linked with the regulation of dopamine. Monoamine oxidase A (MAOA) modulates the metabolism of serotonin and dopamine, both of which are neurotransmitters involved in the regulation of appetite and food intake. The gene coding for MAOA contains a 30-bp tandem repeat (uVNTR) polymorphism in its promoter region that has been previously identified to be associated with energy balance behaviors and body mass index (BMI). The gene coding for dopamine receptor D₄ (DRD4) contains a 16 amino acid (48-bp) repeat polymorphism that has been linked with food consumption and BMI. Lastly, the dopamine transporter gene (SLC6A3: Solute carrier family 6 – neurotransmitter transporter, dopamine – member 3) codes for a dopamine transporter protein (DAT) that mediates the active reuptake of dopamine from the synapse. The transport gene contains a 40-base variable number tandem repeat (VNTR) at the 3' untranslated region (3'-UTR) that has been previously identified to be associated with variable levels of postsynaptic dopamines.

Objective: Our goals were to investigate the population effects of the aforementioned functional polymorphisms on various types of food consumption (soda, fast food, snack, and ready to eat foods) and physical activity (exercise and TV watching), and to further explore gender differences and interaction effects with negative stressors.

Methods: The analyses were conducted with data on genotypes and self-reported behavioral characteristics among 951 Chinese adolescents 11-15 years old living in Wuhan, China.

Results: Males with the high-activity allele of MAOA had lower odds of increased soda intake (adjusted OR=0.63; 95% CI: 0.41-0.98, p=0.03) than those with low activity allele. Experience of negative stressors significantly strengthened the protective genetic effect on increasing odds of engaging in vigorous activity (adjusted OR for interaction=1.89 with 95% CI of 1.89-2.52, p-value for interaction=0.04). Additionally, combined males and females with DRD4 variant had greater odds of engaging in vigorous exercise (adjusted OR=1.39; 95% CI: 1.01-1.86, p=0.03) and of increased soda intake (adjusted OR=1.33; 95% CI: 1.01-1.76, p=0.04) than those with the wild-type allele. Among females, wild-type carriers (no 2R or 7R allele) when exposed to negative stressors were significantly more likely to engage in vigorous exercise (adjusted OR=0.14, 95% CI: 0.047-0.43, p=0.000586). Lastly, combined males and females with the DAT variant had increased odds of watching TV (adjusted OR=1.59; 95% CI: 0.61-1.77) and decreased odds of consuming fast foods (adjusted OR=0.60; 95% CI: 0.38-0.95, p=0.030654) than those with DAT wild-type. Experience of negative stressors significantly weakened the protective genetic effect on the odds for fast food consumption (adjusted OR=0.30; 95% CI: 0.13-0.66, p=0.002639).

Conclusions: Our findings confirm the genetic effects of the dopamine regulating genes polymorphisms on food consumption and physical activity, and provide new insights about interactions with negative stressors.

INTRODUCTION

Obesity has emerged as a serious public health concern, especially in developing countries undergoing rapid economic transition, such as China. An alarming increase in pediatric obesity occurred between 1985 and 2006 with the prevalence of overweight plus obesity of 15.4% in 7- to 17-year old boys and 11% in girls (1,2). Obese children and adolescents are more likely to maintain their obesity into adulthood, which may contribute to enhanced morbidity and increased likelihood of developing heart disease, type 2 diabetes, certain types of cancer, and arthritis (3-5). Obesity, a medical condition that is defined by a body mass index (BMI) – a proxy for human body fat based on an individual’s weight and height – of 30 kg/m² or above is a complex disease, which is influenced by a combination of environmental, behavioral, and genetic factors. Much attention has centered on food consumption and physical inactivity as the primary causes of obesity, and while these factors may contribute to the rise of obesity worldwide, twin and family studies have shown that genetics play a large role in determining BMI, thereby illustrating the need to place these behavioral factors in the context of genes (6).

Susceptibility to obesity is, in part, determined by joint effects of genetic and environmental factors, in which environmental factors may prompt gene-regulated phenotype expression. In previous studies, chronic stress due to either stressful life events or daily hassles has been linked to the etiology of obesity as a promoting factor by interacting with both mechanisms of energy intake (increase of appetite and energy intake) and expenditure (decrease of physical activity) (7-9). In order to develop effective prevention and treatment efforts, it is important to gain a nuanced understanding of the specific mechanisms that underlie food consumption and physical inactivity, both of which are factors that can contribute to obesity.

The underlying biophysical mechanisms may largely be attributed to the involvement of dopamine, which has been implicated as an important neurotransmitter involved in regulating food value and food intake by modulating the reinforcement of food via the meso-limbic circuitry of the brain (10, 11). Biological factors such as the density of DA receptors, the amount of DA released into the synapse, and the rapidity of its transport back into the cell by the re-uptake protein influence the sensitivity of dopamine pathways (12). There are two competing hypotheses regarding the direction of casual association between reward sensitivity and the development of obesity. One argument is the “reward deficiency syndrome” – individuals with decreased D₂ receptors (carrying the A1 allele) tend to overeat to compensate for decreased activation of reward circuits (13). The counterargument is that heightened reward sensitivity increases the risk of compulsive behavior due to increased motivation (12). Observations that dopaminergic antagonists enhance appetite, whereas dopaminergic agonists suppress appetite further provide evidence for the important role dopamine plays in appetite modulation. There are a number of genes that have been linked with the regulation of dopamine, including monoamine oxidase A (MAOA), dopamine dopamine receptor 4 (DRD4), and solute carrier family 6-neuro-transmitter transporter, dopamine-member 3 (SLC6A3). **This research was a part of a larger project directed by Dr. Bin Xie from the School of Community & Global Health at Claremont Graduate University and Dr. Dalin Li from Cedars-Sinai Medical Center. Existing genetic and behavioral phenotype data was extracted from core projects funded by the NCI Transdisciplinary Tobacco Use Research Center grants.**

Specific aims in this study are as follows:

- (1) To delineate effects of a battery of stressful life events assessed on four domains (school, family, peer and individual) on food consumption (i.e. fast food, ready to eat, snack, and soda intake) and physical activity/inactivity (i.e. exercise and TV watching).
- (2) To examine the potential impacts of several candidate genes regulating brain dopamine systems (DRD4, MAO, and SLC6A3) on food consumption and physical activity.
- (3) To investigate gene X stressful events interactions on eating and physical activity.

BACKGROUND

Dopamine System and Obesity

Dopamine (DA) is an important neurotransmitter that is hypothesized to regulate food intake by modulating food reward via the meso-limbic circuitry of the brain (14). The activation of mesolimbic dopaminergic reward pathways in the brain will in turn interact with the HPA axis and the SAM systems (14,15). Natural rewards of food intakes, particularly of palatable sweet/high-carbohydrate or high-fat foods, stimulate the release of dopamine from neurons of the presynaptic ventral tegmental area into the nucleus accumbens, causing euphoria and reinforcement of the behavior (15). Accordingly, the “Reward Deficiency Syndrome” hypothesis has been proposed to account for this situation, in which individuals with low intrinsic dopamine activity in the brain reward pathways tend to compensate by using various reinforcing behaviors, including increased food consumption or risk-taking behaviors (16, 17). The dopaminergic system is also related to physical activity (18, 19), and blocking dopamine reuptake may increase physical activity (20, 21). Therefore reduced intrinsic dopamine activity may lead to increased food intake and/or reduced energy expenditure, both of which may contribute to the positive energy balance responsible for the development of obesity. The availability of dopamine is largely controlled by genes that regulate enzymes and receptors related to the release, transport (reuptake), metabolism and receptor binding of synaptic dopamine.

MAOA

Monoamine oxidase (MAO) is one of the major enzymes responsible for the degradation of neurotransmitters including serotonin (5-HT) and dopamine (DA), rendering them inactive in the synapses of the brain (22). MAO is classified into two forms as MAO A and MAO B (22). MAO A is the key enzyme to regulate serotonin and dopamine (DA) levels in the brain (23). Because of the important role MAO plays in regulating monoamine turnover, and thus in influencing levels of serotonin and dopamine, this gene is a logical candidate to research how individual differences affect feeding behaviors and ultimately obesity. MAO A has been identified and located on the X-chromosome at Xp11.4-11.3 (24,25). Locating polymorphisms in the MAO A gene is of particular importance because of the fact that MAO A activity levels are in large part controlled by the MAO locus and the fact that polymorphisms at this locus can be used as a marker for activity states - assessing the role of MAO in human diseases has become possible by determining the frequency of MAO A alleles in populations of interest and control groups (26).

While multiple polymorphisms in the MAO A gene have been identified such as variable number tandem repeats (VNTR) in the first intron, a G-to-T substitution at position 941, a T-to-A substitution at position 1077, and a tandem (dinucleotide) repeat in the second intron, currently only a few polymorphisms to our knowledge is known to affect expression levels of MAO A (27). Research from three studies have shown that one particular polymorphism, MAO A-uVNTR, is located 1.2 kb upstream of the MAO A coding sequences (28). This variable number tandem repeat (VNTR) on the MAO A promoter region has been shown to affect the transcriptional activity. This mutation consists of a 30-bp repeated sequence present in six allele variants containing 2, 3, 3.5, 4, or 5 copies; alleles with 3.5 or 4 repeats have been reported to be 2–10 times more efficient than those with 3 copies in transcription (28-30). A study investigating the relationship between different

VNTR alleles and MAO A mRNA expression levels and enzyme activity in post mortem human male brains has reported that there is no significant association between the aforementioned polymorphism (A-VNTR1) and expression levels of enzyme activity in the human brain. Furthermore, two other polymorphisms A-VNTR2 and A-SNP8 (VNTR located in the first intron and a SNP located in exon 8 of the MAO A gene, respectively) have been reported to have a statistically insignificant functional effect on MAO A activity; however, individuals with a particular combination of polymorphisms for A-VNTR1 and B-SNP13 (a VNTR located in intron 13 on MAOB) had significantly lower MAO A enzyme levels in the brain, thereby suggesting that an additional unknown functional polymorphism may account for variations in enzyme activity in the brain (30). However, as this was the only study in testing DNA polymorphisms at the MAO loci in post-mortem human brain (rather than in blood, fibroblasts, and cell lines), it is possible that expression levels in the post-mortem brain may not reflect activity levels in vivo. Another polymorphism, EcoRV polymorphism, has been reported to be significantly associated with MAO A activity levels (31). In a small sample of 50 obese subjects, preferential transmission of allele 1 of the EcoRV polymorphism (i.e. the allele associated with low MAO enzymatic activity) in the more obese subjects (31).

The association of monoamine oxidase A (MAOA) gene with obesity phenotypes is supported by a whole genome linkage study implicating a locus for obesity on the p arm of the X chromosome (33), as well as a study in which low activity MAOA alleles were associated with obesity in one familial data set (31). Furthermore, this association between low activity MAOA alleles and increased BMI is directionally consistent with a principle side-effect of MAOA inhibitors: weight gain (34). In a homogenous, unrelated Finnish source population, MAOA containing a length polymorphism in promoter region (MAOA-LPR) genotypes were significantly associated with variation in BMI ($p=0.005$); specifically, low activity MAOA-LPR genotype predicted higher BMI among a sample of healthy nonobese men (35). The size of MAOA-LPR genotype accounted for only 4% of total BMI variance; however, because various factors influence BMI, it is unsurprising that a locus such as MAOA explained a small percentage of the variance.

DRD4

The DRD4 gene located at chromosome 11 (11p15.5) shows considerable homology to DRD2, which maps to 11q23. It has received considerable attention because of its high affinity for the atypical antipsychotic clozapine and the unusually polymorphic nature of its gene (36). Several polymorphisms in the DRD4 gene were found, including a 48-base pair VNTR in exon 3, C-521T in the promoter, 13-base pair deletion of bases 235 to 247 in exon 1, 12-base pair repeat in exon 1, Val194Gly and a polymorphic tandem duplication of 120bp (37). A great deal of research attention has been focused on a functional VNTR polymorphism that was identified in the third exon in the DRD4 gene, the region coding for the third intracellular loop of the receptor (36). The genetic variant is a 16 amino acid (48 bp) repeat polymorphism, which is repeated two to 11 times, with two (D4.2), four (D4.4), and seven (D4.7) repeats being the most common alleles (36, 38). Mixed findings of effects of genetic variants of DRD4 were reported in the literature. A significant positive association between the 7-repeat of the DRD4 gene and obesity and binge eating was reported in a sample of women with seasonal affective disorder (SAD) with marked craving for high-carbohydrate/high-fat foods and significant weight gain during winter depressive episodes

(37,39). In contrast, the presence of the 7-repeat of the DRD4 genotype is associated with a reduction of about 12–15 in the rank of the BMI percentile score for the African-American and Hispanic samples in National Longitudinal Study of Adolescent Health (Add Health) (40). No association was detected between obesity and the 48-repeat polymorphism of the DRD4 gene in a German study (41).

SLC6A3

The dopamine transporter gene (SLC6A3: Solute carrier family 6 – neurotransmitter transporter, dopamine - member 3) codes for a dopamine transporter protein (DAT) that mediates the active reuptake of dopamine from the synapse by transporting released dopamine back into nerve terminals, thereby limiting the level and duration of dopamine receptor activation (42,43). The transport gene (*SLC6A3*) is located on chromosome 5p15 and has a 40-base variable number tandem repeat (VNTR) at the 3' untranslated region (3'-UTR) (44). The 40bp 3'-UTR repeat in *SLC6A3* exists commonly as 9 or 10 tandem repeats, though 3 to 11 repeats are also seen (45,46). This variable number tandem repeat (VNTR) polymorphism at 3'UTR affects *SLC6A3* gene expression and transcription and mediates concentrations of and responses to synaptic dopamine (47-49). Individuals with a single 9-repeat allele may have an approximately 20% reduction of *SLC6A3* protein expression than individuals homozygous for the 10-repeat allele, which results in a relatively higher levels of postsynaptic dopamine. Variants of the dopamine transporter gene may be related to obesity in African-American smokers (50). The likelihood of obesity in African Americans with the 10/10 *SLC6A3* genotype was 5.16 times that of African Americans with 9/9 or 9/10 *SLC6A3* genotype, and this association was not observed in non-Hispanic whites. In addition, the dopamine transporter (*SLC6A3*) genotype may interact with food reinforcement on energy consumption. Subjects high in food reinforcement scores who lacked an *SLC6A3**9 allele consumed significantly more calories (>150 kcal; $P=0.015$) than did subjects low in food reinforcement scores or those high in food reinforcement scores who carried at least one *SLC6A3**9 allele (51).

Stress

Stress, especially academic stress, family conflict and disruption of family life, among Chinese adolescents has become an increasing problem that is associated with depression, anxiety, conduct disorders, suicide attempts and drug abuse (52-55). Recent research attention has been given to link chronic stress to the development of obesity (56,57). Although a well-defined association is not described in literature, chronic stress may be a promoting factor interfering with the energy balance to maintain weight status, that is, an increase of appetite and energy intake and decrease of physical activity (58). Chronic stress is considered to influence human eating behavior by favoring the appetite for hedonic, highly palatable and energy dense foods (59). The underlying behavioral mechanisms have been considered in the Affect Regulation Model which involves an emotional coping process to ameliorate the negative emotions associated with stressors (60). It is widely accepted that the diverse threatening stimuli or stressors could have an effect on brain systems that would result in increased neuroendocrine and autonomic arousal and negative moods, leading to the use of substances like food, nicotine, and alcohol to self-medicate the resultant distress (61-62). The neuron-physiological mechanisms behind the appetite-stimulating effect of chronic stress involve activation of two other major stress pathways, the hypothalamic-pituitary-

adrenal (HPA) axis and the sympathoadrenal medullary (SAM) systems. Activation of the HPA-axis leads to the release of corticotrophin releasing factor (CRF) from the paraventricular nucleus (PVN) of the hypothalamus, which stimulates adrenocorticotrophin hormone from the anterior pituitary, which subsequently stimulates the secretion of cortisol/corticosterone from the adrenal glands (62). Increased cortisol secretion may disrupt the food intake regulation in humans by stimulating the neuropeptide Y system (food intake stimulation) and blunting the effect of the leptin system (food intake reduction), which could therefore result in a long-term increased energy intake and fat accumulation (59,63). Activation of the SAM systems leads to the release of noradrenaline and adrenaline, which are predominantly responsible for the fight-or-flight response (64). These two stress axes or systems are tightly interconnected at several levels and both are often activated simultaneously (58,62). These neurotransmitter systems could account for a neuroendocrine connection between stress and food intake regulation, and are hypothesized to regulate behavioral and metabolic responses associated with the development of obesity (65).

Psychosocial and behavioral studies have shown that with increasing levels of emotional and physiological stress, there is a decrease in behavioral control and an increase in impulsivity, and with increasing levels of distress, and chronicity of stress, the greater risk of maladaptive behaviors (66). Results from longitudinal studies indicate that chronic life stress might be causally linked to future weight gain, especially in men (59). Only a few epidemiological studies have explored the role of stress on eating behavior in the etiology of obesity among adolescents. Mellin et al. found that overweight adolescents, compared to their non-overweight peers, reported more unhealthy behaviors (reflected by extreme dieting, skipping breakfast, increased television watching) and a lower psychosocial wellbeing (reflected by their school performance, emotional distress and future educational plans) (67). Nguyen-Rodriguez et al. performed a cross-sectional survey in 11 to 15-year-old adolescents from Los Angeles and found a significant positive relationship between their perceived stress and emotional eating behavior (68). This effect was seen for both normal and overweight adolescents, indicating that BMI is not a moderator for the found association (68). Both studies indicate that stress might lead to unhealthier dietary behaviors in adolescents which might, in turn, favor the development of obesity in later life.

There is evidence for a negative association between perceived stress and levels of physical activity in adolescents (69). Physical activity is considered a protective factor against effects of stress on obesity and subjective health complaints (headache, abdominal pain, feeling nervous, sleeping difficulties) among adolescents (70,71). Physical activity is also considered to have beneficial effects on mental health and stress coping capacity (72). These findings indicate the complex interaction between stress and physical activity, which complicates the etiology of obesity, but is fundamental in the investigation. More research is necessary to unravel underlying mechanisms of the obesity-inducing effects of chronic stress, especially among adolescents (58).

Limited research has focused on the investigation of population-level gene-environmental effects of stress and genetic variants on obesity among adolescents. In this paper, we investigated the population effects of the functional polymorphisms of dopamine-regulating genes on food consumption and physical activity among Chinese adolescents 11-15 years old. In addition, we further explored gender differences and interactive effects with multiple-domains of negative stressors and hypothesized that exposure to negative stressors would prompt gene-regulated obesity.

METHODS

Sample and Data

Secondary data for this proposed study were derived from the Wuhan Smoking Prevention Trial (WSPT) in Chinese adolescents in Wuhan, China. The original main cohort at baseline in 1999 consisted of 7th grade students randomly selected from 22 middle schools in urban and rural Wuhan with four classes randomly selected from each school. Among the selected classes in each school, two classes were further randomly selected to compose a sub-cohort for measurement of body weight and height only at baseline in 1998-1999. A total of 951 healthy Chinese adolescents aged from 11 to 15 years completed weight and height measures will be included in this proposed study. The sample includes 486 boys and 465 girls. Socio-demographic, psychological and behavioral information including socioeconomic status, stressful life events, depressive symptoms, family harmony, social support availability, physical activity and inactivity were measured at baseline questionnaire surveys. Questionnaire items were translated from English to Mandarin, then back translated to English by translators fluent in both languages and trained in behavioral research. A validated Chinese version of a food frequency questionnaire with 124 food items in 22 food groups was also administered in the entire baseline cohort to assess the dietary patterns in adolescents. Genetic data were collected with DNA extracted from buccal cells in the Wuhan adolescent cohort (73).

Measures

Food Consumption. A Chinese version of youth/adolescent food frequency questionnaire (CYA-FFQ) was developed and administered in the entire baseline Wuhan adolescent cohort to get reasonably accurate and reliable picture of usual diet in adolescents. The CYA-FFQ is self-administered and contains 124 food items in 22 food groups and asks subjects to report frequency of food consumption over a 7-day period. Nutrient intakes are able to estimate according to a comprehensive nutrient database established based on predetermined standard portion size of foods and food composition tables. A validation study with the purpose to quantify the accuracy and reliability of this CYA-FFQ has been conducted in an independent representative sample of adolescents randomly selected from 8 classes of 4 schools in Wuhan, China (74,75). The energy-adjusted correlation coefficients for reliability of two FFQs were adequate, ranging from 0.34 to 0.6 with mean of 0.46 (e.g. 0.51 for protein, 0.49 for total fats, 0.45 for saturated fats, 0.48 for cholesterol, 0.47 for fiber, and 0.6 for calcium), and the coefficients were similar across different seasons. Validity coefficients between FFQ and 24-hour recalls were acceptable with the mean of correlation coefficients of 0.32 ranging from 0.15 to 0.73 and mean of Kappa coefficients of 0.48 ranging from 0.43 to 0.6. The magnitudes of coefficients for reliability and validity were similar to those reported in Harvard youth/adolescent food frequency questionnaire (YAQ) that has been widely used in the US adolescent populations. In Harvard YAQ, the reliability for nutrients ranged from 0.26 for protein and iron to 0.58 for calcium (76), and the validity between the mean energy-adjusted nutrients computed by YAQ and 24-hour recalls ranged from 0.21 for sodium to 0.58 for folate with the average coefficient of 0.54 (77). The food consumption data from this Wuhan cohort have been used to investigate determinants of invigorant consumption (78), and the consumption of dietary supplements (79). In this paper, we focused on frequency consumption of four food groups including soda, fast food, snack and ready-to-eat food.

Frequencies for food items within each food groups were coded as follow: never/rarely was coded as 0, once per week as 1, two-three times per week as 2.5, four to six times per week as 5, daily as 7, twice a day as 14, and 3 times a day as 21. Median-split for frequencies of soda intake, fast food, snack, and ready to eat were used.

Physical Activity and Inactivity. One item on vigorous physical activity adapted from the US YRBSS (80) was also included in the questionnaire for physical activity assessment, which asked subjects “How many times a week do you breathe hard and sweat for over 20 minutes while riding a bicycle, walking fast, jogging, dancing, or doing other exercise or hard physical labor?” The response scale ranges from “none” to “8 or more times” and will be dichotomized as “less than 3 times a week” and “at or greater than 3 times a week”. Sum scores of the food item frequencies were calculated within each food group and were categorized by median-split for the analysis.

TV Watching. A total of 4 items adapted from the Youth Risk Behavior Surveillance Survey (YRBSS) were used to measure time spent on watching TV/video. Participants were asked to report how much time they spent on school days and on a usual weekend or holiday, respectively. Responses to the questions ranged from none/hardly any, < 30 minutes, 30 - 59 minutes, 1 - 1.99 hours, 2 - 2.99 hours, 3 - 3.99 hours, and greater than 4 hours. The responses were converted into minutes. Minutes spent on an average day for were then calculated via the following equation: minutes spent on an average day = [(minutes spent on school days)*5 + (minutes spent on an average weekend)*2] /7. The time spent on TV watching was further dichotomized with the cut-off point set as one hour.

Stressful Life Events. Stressful life events were assessed through a checklist of 99 items, that were grouped into categories based on their domain (school, family, peers, and self) and valence (positive or negative). Positive events were those generally considered pleasant, while negative events were those generally considered unpleasant. Students were asked to indicate which of the events they had experienced in the past 6 months. For each event they had experienced, they were asked to rate the severity of the event on a 4-point Likert-type scale ranging from 0 (no effect) to 3 (severe). Descriptions of the development of the checklist and items in each scale can be found elsewhere (55). The domains of school, family, peers and individual (self) were chosen because they represent the most frequently reported sources of stress among Chinese adolescents (55, 81). This instrument measuring stressful life events was used to predict significantly depressive symptoms as they related to smoking and alcohol use in this Wuhan adolescent cohort (52, 55). In this paper, we narrowed our analyses on negative stressors (or stressful events), which included negative school stressors (e.g., too much homework, disliked school), negative family stressors (e.g., death or illness of family members, hit or scolded by parents and increased quarreling with parents, parents argued frequently), negative peer stressors (e.g., misunderstood by peers, lost face in public), negative health stressors (e.g., have taken sedative medicine, not had enough sleep), and negative violence stressors (e.g., were shocked by an event, witnessed a car accident). For each category of stressor, each student’s score was the number of events in the category that the student reported. This unit weighting method (giving each event an equal weight rather than weighting items by their severity) was used because unit weighting methods produce smoother scale distributions and tend to correlate highly with weighted scales (82). The average score of total negative stressors was calculated for the analysis.

MAOA Promoter uVNTR Polymorphism. DNA was successfully extracted from all buccal cell samples (73). The GeneScan method was used to determine the allele size of MAOA Promoter uVNTR Polymorphism. The MAOA promoter 30-bp repeat was amplified by PCR with oligonucleotide primers: VICMAOA_HermanF (5'-CAGAAACATGAGCACAAA CGCCTCA GC-3') which was labeled with VIC and MAOA_mHermanR (5'-GACCGCCACTCAGAACGGACG-3') (83). PCR reaction contained 50 ng of genomic DNA, 200nM of each primer, 1.25 unit of Amplitaq Gold polymerase from ABI with Premix Buffer E from Epicentre Biotechnologies in a total volume of 20 µl. Cycling conditions were initial denaturation at 94 °C for 10 min, followed by 32 cycles of 34s at 94 °C, 34 s at 62 °C, 1.5min at 72 °C, and a final elongation step for 7 min at 72 °C. PCR products were assayed on Applied Biosystem 3130xl Genetic Analyzer with GS500 LIZ size standard (35-500 bp, ABI). Results were analyzed using GeneMarker v1.5 (SoftGenetics) software.

DRD4 VNTR Polymorphism. DRD4 genotyping was performed based on the method of Lichter et al. Primers included 5' -AGG ACC CTC ATG GCC TTG; 5' -GCG ACT ACG TGG TCT ACT CG. 2R and 4R alleles were sequenced by separating alleles and purifying the products using Zymo Research Zymoclean gel DNA recovery kit.

DAT VNTR Polymorphism. Primers and PCR conditions were used as described in previous literature (93).

Data Analysis

Descriptive statistics (mean, standard deviation and percentage) were calculated to reflect the background characteristics of the sample. Generalized mixed-effect models (for categorical phenotypes) were used to examine main effects of MAOA, DRD4, and DAT1 VNTR polymorphisms and negative stressors on various domains of food consumption (i.e. fast food, ready to eat, snacks, and soda) and physical activity and inactivity (all as dichotomized variables) with adjustment for the intra-class correlations due to the nested study sampling design (i.e. students nested within schools). Genotypes for MAOA were grouped by relative transcriptional activity into two categories, high function (3.5 or 4 repeat in males; 3.5/4 or 4/4 in females) versus low function (3 repeats in male; 3/3 or 3/other in females) according to the extant literature with this functional polymorphism of MAOA gene (11, 35, 84). As the effect of the rare alleles (2 or 5) on transcriptional activity remains inconclusive in the literature, sensitivity analyses were conducted with data excluding the 21 participants with the rare genotypes (28, 85). For genotypes of DRD4, because 2R and 7R alleles share evolutionary and biochemical similarities (i.e. blunted cAMP coupling), both alleles were grouped together. Thus DRD4 genotypes were coded as “0” for individuals lacking either 2 or 7R alleles (4R is the wild-type), “1” for those with one copy of 2R or 7R allele, and “2” for two copies of either the 2R or 7R allele. In DAT1 coding, the number of 0,1,2 represent the number of 9 repeats, i.e., 2 is 9R/9R, 1 is 9R/others and 0 is other/other. For other, most are 10R alleles.

We first analyzed the marginal effect of genotypes and negative stressors on exercise, TV watching, fast food, ready to eat, snack, and soda intake in all the subjects. Then considering the potential gender difference in genetic effects, gender-specific analyses were also conducted.

We also explored potential interactions of negative stressors with the MAOA, DRD4 and DAT genotypes on exercise, TV watching, snack, ready to eat, fast food, and soda intake. To clarify the interpretation of significant interaction effects, stratified analyses were conducted.

Covariates considered in this study included student’s self-reported age, gender, and parental education. Calories were also adjusted for food related outcomes (i.e. snack, ready to eat, fast food, and soda intake). Father and mothers’ education levels were obtained by collapsing the highest levels of education received by either parent into three categories: below high school, high school, and college. All statistical analyses were carried out using R (version 2.12.2; R foundation for Statistical Computing).

RESULTS

The general characteristics of this sample are summarized in Table 1 below. The majority of students were 13 years old and had parents with high school education attainment. On average, boys were slightly older than girls. Besides general characteristics, Table 1 also presents the information on the average frequencies of food consumption and exercise, number of hours spent on TV watching during the past week, as well as the average total number of negative stressors exposed in the past six months.

Table 1. General Characteristics of the Sample

	Overall (n=951)	Female (n=486)	Male (n=465)
	n(%)	n(%)	n(%)
Age			
11 years old	17(1.8%)	13(2.7%)	4(0.9%)
12 years old	261(27.4%)	152(31.3%)	109(23.4%)
13 years old	643(67.6%)	316(65%)	327(70.3%)
14 years old	27(2.8%)	4(0.8%)	23(4.9%)
15 years old	3(0.3%)	1(0.2%)	2(0.4%)
Parental Education			
Below High school	133(14.1%)	67(14%)	66(14.3%)
High School	547(57.9%)	275(57.1%)	272(58.7%)
College or above	265(28%)	140(29%)	125(27%)
	mean(SD)	mean(SD)	mean(SD)
Exercise	0.20(0.40)	0.1(0.3)	0.26(0.04)
TV Watching	0.45(0.5)	0.4(0.5)	0.5(0.05)
Soda Intake	3.3(3.5)	2.8(3.0)	3.9(3.9)
Fast food Intake	2.0(2.6)	2.3(2.7)	1.8(2.6)
Snack Intake	11.28(8.1)	11.57(7.83)	10.97(8.34)
Ready to Eat Intake	1.1(2.3)	0.91(1.73)	1.23(2.77)
Total Negative Stressors	0.54(0.50)	0.61(0.49)	0.47(0.50)

Table 2 presents results of marginal main effects of exposure to negative stressors on exercise, TV watching, soda, fast food, snack, and ready to eat food intake. Female subjects who were exposed to high levels of negative stressors had significantly greater odds of engaging in vigorous exercise (adjusted OR of 2.44 with 95% CI of 1.32-3.54), watching TV frequently (adjusted OR=1.97, 95% CI: 1.30-2.98, p=0.001) and consuming ready to eat foods (adjusted OR=0.65, 95% CI: 0.43-0.97; p=0.016) than counterparts with low level exposure to negative stressors.

Table 2. Marginal Main Effects of Negative Stressors on Food Consumption and Physical Activity/Inactivity						
	Female		Male		All	
Negative Stressors	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Effects on Exercise	2.44(1.32-3.54)	0.0046	1.09(0.71-1.69)	0.685	1.49(1.07-2.09)	0.019
Effects on TV Watching	1.97(1.30-2.98)	0.0014	1.19(0.81-1.75)	0.378	1.48(1.13-1.94)	0.0041
Effects on Soda	1.42(0.89-2.26)	0.136	0.87(0.57-1.33)	0.533	1.10(0.82-1.48)	0.509
Effects on Fast Food	0.87(0.57-1.32)	0.500	1.07(0.71-1.62)	0.725	0.96(0.73-1.27)	0.784
Effects on Snack	1.23(0.35-4.27)	0.749	0.84(0.19-3.70)	0.820	0.99(0.39-2.52)	0.983
Effects on Ready to Eat Foods	0.65(0.43-0.97)	0.0343	0.73(0.48-1.10)	0.136	0.71(0.53-0.94)	0.016

Age, parental education, and calories were adjusted in the models.

*Calories was not adjusted for models on Exercise and TV Watching

Median-split of average scores of negative stressors were used (0 for low and 1 for high level of stressors).

Median-split for frequencies of soda intake, fast food, snack, and ready to eat were used.

Table 3 presents results of marginal main effects of MAOA uVNTR polymorphisms on exercise, TV watching, soda, fast food, snack, and ready to eat food intake. Among males, those with the high-activity allele had lower odds of increased soda intake (adjusted OR=0.63; 95% CI: 0.41-0.98, p=0.03) than those with the low activity allele.

Note: MAOA is an X-linked gene, and thus there is no combined female and male group.

Table 3. Marginal Main Effects of MAOA VNTR Polymorphisms on Exercise, TV Watching, Soda Intake, Fast Food Intake, Snack Intake, and Ready to Eat Intake				
	Female		Male	
	OR (95% CI)	p	OR (95% CI)	p
Effects on Exercise				
MAOA VNTR	1.46(0.97-2.18)	0.0688	1.33(0.86-2.07)	0.203
Effects on TV				
MAOA VNTR	1.19(0.89-1.59)	0.2512	1.16(0.78-1.71)	0.465
Effects on Soda				
MAOA VNTR	1.02(0.73-1.44)	0.893	0.63(0.41-0.98)	0.0384
Effects on Fast Food				
MAOA VNTR	1.20(0.88-1.64)	0.244	0.96(0.64-1.46)	0.894
Effects on Snack Intake				
MAOA VNTR	1.25(0.52-3.00)	0.615	2.33(0.49-11.10)	0.289
Effects on Ready to Eat Intake				
	0.89(0.65-1.20)	0.438	0.78(0.50-1.21)	0.263

Age, parental education, and calories were adjusted in the models.

*Calories was not adjusted for models on Exercise and TV Watching

Medium-split of average scores of negative stressors were used

MAOA VNTR polymorphisms were coded as 0 for low active and 1 for active.

Median-split for frequencies of soda intake, fast food, snack, and ready to eat were used.

Table 4 presents results of marginal main effects of DRD4 genotypes on exercise, TV watching, soda, fast food, snack, and ready to eat food intake. No significant gender interaction was observed. Combined males and females with DRD4 variant had greater odds of engaging in vigorous exercise (adjusted OR=1.39; 95% CI: 1.01-1.86, p=0.03) than those counterparts with wild-type allele. Additionally, combined males and females with the variant DRD4 had greater odds of increased soda intake (adjusted OR=1.33; 95% CI: 1.01-1.76, p=0.04) than those with the wild-type allele.

Table 4. Marginal Main Effects of DRD4 VNTR Polymorphisms on Exercise, TV Watching, Soda Intake, Fast Food Intake, Snack Intake, and Ready to Eat Intake

	Female		Male		All	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Effects on Exercise						
DRD4 VNTR	1.30(0.82-2.06)	0.257	1.48(0.99-2.21)	0.0541	1.39(1.01-1.86)	0.0305
Effects on TV						
DRD4 VNTR	0.91(0.65-1.29)	0.605	1.25(0.87-1.81)	0.229	1.06(0.83-1.36)	0.618
Effects on Soda						
DRD4 VNTR	1.17(0.80-1.73)	0.424	1.51(0.99-2.29)	0.0534	1.33(1.01-1.76)	0.0420
Effects on Fast Food						
DRD4 VNTR	1.05(0.73-1.49)	0.803	0.98(0.66-1.45)	0.913	0.98(0.76-1.27)	0.889
Effects of Snack Intake						
DRD4 VNTR	1.13(0.36-3.50)	0.837	1.21(0.26-5.65)	0.785	1.09(0.48-2.47)	0.842
Effects of Ready to Eat Intake						
DRD4 VNTR	1.13(0.79-1.61)	0.492	0.87(0.58-1.31)	0.498	1.02(0.79-1.33)	0.856

Age, parental education, and calories were adjusted in the models.

*Calories was not adjusted for models on Exercise and TV Watching

DRD4 VNTR polymorphisms were coded as 0 for no copies of 2R or 7R, 1 for 1 copy of 2R or 7R, and 2 for 2 copies of 2R or 7R.

Median-split for frequencies of soda intake, fast food, snack, and ready to eat were used.

Table 5 presents results of marginal main effects of DAT UTR VNTR polymorphisms on exercise, TV watching, soda, fast food, snack, and ready to eat food intake. Combined females and males with the DAT variant had increased odds of watching TV compared to counterparts with the wild-type (adjusted OR=1.59; 95% CI: 0.61-1.77, p=0.0345). Additionally, combined females and males with the DAT variant had decreased odds of consuming fast foods (adjusted OR=0.60; 95% CI: 0.38-0.95, p=0.0307) than those with DAT wild-type. No significant gender interaction was observed.

	Female		Male		All	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Effects on Exercise						
DAT VNTR	1.28(2.77-0.59)	0.530	0.87(0.41-1.86)	0.715	1.04(0.61-1.77)	0.891
Effects on TV						
DAT VNTR	1.54(0.87-2.75)	0.141	1.60(0.81-3.14)	0.175	1.59(1.03-2.46)	0.0345
Effects on Soda						
DAT VNTR	0.56(0.27-1.15)	0.113	0.91(0.44-1.85)	0.789	0.74(0.46-1.21)	0.233
Effects on Fast Food						
DAT VNTR	0.64(0.34-1.18)	0.152	0.55(0.26-1.16)	0.116	0.60(0.38-0.95)	0.0307
Effects on Snack Intake						
DAT VNTR	1.87(0.44-7.97)	0.398	----- -	----- -	1.37(0.37-5.10)	0.643
Effects on Ready to Eat Intake						
DAT VNTR	0.99(0.55-1.80)	0.977	1.61(0.81-3.19)	0.173	1.23(0.79-1.91)	0.365

Age, parental education, and calories were adjusted in the models.

*Calories was not adjusted for models on Exercise and TV Watching

DAT1 polymorphisms were coded as 0 for no 9R alleles, 1 for one 9R allele, and 2 for 9R/9R

Median-split for frequencies of soda intake, fast food, snack, and ready to eat were used.

Estimates and p-value are unreliable for “Snack” due to model over-fitting.

In addition, a significant genotype X stressors interaction was observed between MAOA uVNTR polymorphism and exposure to negative stressors among males (Table 6). Experience of negative stressors significantly strengthened the protective genetic effect on increasing odds of engaging in vigorous activity (adjusted OR for interaction=1.89 with 95% CI of 1.89-2.52, p-value for interaction=0.04).

	Female		Male		All	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Effects on Exercise						
MAOA VNTR x negative stressor	1.74(0.81-3.71)	0.155	1.89(1.01-3.52)	0.045	1.49(0.96-2.30)	0.073
Effects on TV						
MAOA VNTR x negative stressor	1.21(0.75-1.95)	0.427	1.47(0.84-2.58)	0.179	1.15(0.82-1.62)	0.409
Effects on Soda						
MAOA VNTR negative stressor	0.92(0.53-1.57)	0.751	0.68(0.36-1.29)	0.239	0.75(0.51-1.10)	0.143
Effects on Fast Food						
MAOA VNTR x negative stressor	0.74(0.46-1.19)	0.209	0.83(0.45-1.52)	0.538	0.82(0.57-1.16)	0.256
Effects of Snack Intake						
MAOA VNTR x negative stressor	1.19(0.29-4.87)	0.809	1.55(0.19-12.9)	0.680	1.45(0.48-4.39)	0.515
Effects of Ready to Eat Intake						
MAOA VNTR x negative stressor	0.75(0.47-1.21)	0.242	0.77(0.41-1.44)	0.411	0.79(0.55-1.13)	0.192

Age, parental education, and calories were adjusted in the models.

*Calories was not adjusted for models on Exercise and TV Watching

Medium-split of average scores of negative stressors were used (0 for low and 1 for high level of stressors).

MAOA VNTR polymorphisms were coded as 0 for low active and 1 for active.

Median-split for frequencies of soda intake, fast food, snack, and ready to eat were used.

A significant genotype X stressors interaction was observed between DRD4 genotypes and exposure to negative stressors among females (Table 7). The effect of negative stressors on the odds of engaging in vigorous exercise was significantly moderated by DRD4 genotypes (adjusted OR for interaction=0.14 with 95% CI of 0.04-0.43, p-value for interaction= 5.36×10^{-4}).

	Female		Male		All	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Effects on Exercise						
DRD4 VNTR x negative stressor	0.14(0.04-0.43)	0.0005	0.94(1.70-0.52)	0.837	1.46(0.91-2.34)	0.113
Effects on TV						
DRD4 VNTR x negative stressor	0.84(0.44-1.59)	0.585	1.31(0.77-2.21)	0.314	1.08(0.74-1.60)	0.682
Effects on Soda						
DRD4 VNTR negative stressor	1.19(0.60-2.36)	0.620	1.47(0.81-2.67)	0.207	1.30(0.85-1.99)	0.232
Effects on Fast Food						
DRD4 VNTR x negative stressor	0.96(0.53-1.74)	0.881	0.92(0.53-1.62)	0.784	0.90(0.60-1.34)	0.610
Effects of Snack Intake						
DRD4 VNTR x negative stressor	1.16(0.21-6.58)	0.863	0.54(0.06-5.05)	0.587	0.86(0.24-3.05)	0.812
Effects of Ready to Eat Intake						
DRD4 VNTR x negative stressor	0.87(0.48-1.60)	0.658	0.92(0.52-1.62)	0.762	0.91(0.60-1.37)	0.649

Age, parental education, and calories were adjusted in the models.

*Calories was not adjusted for models on Exercise and TV Watching

DRD4 VNTR polymorphisms were coded as 0 for no copies of 2R or 7R, 1 for 1 copy of 2R or 7R, and 2 for 2 copies of 2R or 7R.

Median-split of average scores of negative stressors were used (0 for low and 1 for high level of stressors).

Median-split for frequencies of soda intake, fast food, snack, and ready to eat were used.

To better understand this interaction effect, the association between negative stressors and physical activity in females were analyzed in different stratifications of DRD4 genotypes (Table 8). Females with the wild-type carrier when exposed to negative stressors were significantly more likely to engage in vigorous exercise (adjusted OR=9.36, 95% CI: 2.72-32.23, p=0.000392). This significant effect was not observed among females with DRD4 variant carriers (adjusted OR 0.47, 95% CI: 0.18-1.24, p-value 0.127).

Table 8: Details of the DRD4 x negative stressor interaction on exercise in females

		Exposure to Negative Stressors			
		Yes (1)	No (0)	OR (95%CI)	p-value
DRD4>=1 (Variants)	Exercise =1	14 (0.54)	12 (0.46)	0.47(0.18-1.24)	0.127
	Exercise =0	105(0.67)	51 (0.33)		
DRD4=0 (Wild-type)	Exercise =1	35 (0.90)	4 (0.10)	9.36(2.72-32.2)	0.000392
	Exercise =0	146 (0.54)	122 (0.46)		

A significant genotype X stressors interaction was observed between DAT genotypes and exposure to negative stressors (Table 9). The effect of negative stressors on the odds of consuming fast food was significantly moderated by DAT (adjusted OR=0.30; 95%: 0.13-0.66, p-value=0.002). And the results are consistent in males and females (in females, adjusted OR=0.29, 95% CI: 0.10-0.85, p-value for interaction=0.0247; in males, adjusted OR=0.20, 95% CI: 0.09-0.94, p-value=0.039). Experience of negative stressors significantly weakened the protective genetic effect on decreasing odds of consuming fast food.

Table 9. Interaction Between Negative Stressors and DAT UTR VNTR Polymorphisms on Exercise, TV Watching, Soda Intake, and Fast Food Intake						
	Female		Male		All	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Effects on Exercise						
DAT VNTR x negative stressor	-----	-----	1.13(0.40-3.15)	0.818	0.81(0.32-2.05)	0.652
Effects on TV						
DAT VNTR x negative stressor	0.75(0.25-2.27)	0.611	1.50(0.59-3.79)	0.392	1.11(0.56-2.19)	0.760
Effects on Soda						
DAT VNTR negative stressor	0.82(0.26-2.53)	0.725	0.67(0.23-1.97)	0.468	0.73(0.10-1.54)	0.407
Effects on Fast Food						
DAT VNTR x negative stressor	0.29(0.10-0.85)	0.024	0.29(0.09-0.94)	0.039	0.30(0.13-0.66)	0.002
Effects of Snack Intake						
DAT VNTR x negative stressor	4.33(0.47- 40.21)	0.197	-----	-----	2.13(0.36-12.4)	0.404
Effects of Ready to Eat Intake						
DAT VNTR x negative stressor	0.82(0.29-2.28)	0.699	1.40(0.52-3.78)	0.511	1.02(0.51-2.06)	0.949

Age, parental education, and calories were adjusted in the models.

*Calories was not adjusted for models on Exercise and TV Watching

DAT1 polymorphisms were coded as 0 for no 9R alleles, 1 for one 9R allele, and 2 for 9R/9R

Median-split for frequencies of soda intake, fast food, snack, and ready to eat were used.

Estimates and p-value are unreliable for “Snack” due to model over-fitting.

DISCUSSION

The first major findings of the study were the significant effects of negative stressors on exercise, TV watching, and ready to eat food consumption. Exposure to negative stressors resulted in significantly greater odds in engaging in exercise and decreased odds of consuming ready to eat food for females only. These two effects are directionally consistent with reduced risk for obesity. However, exposure to negative stressors also increased the odds of watching TV, a sedentary activity that has been positively correlated with obesity (86).

Although a well-defined association is not described in the literature, chronic stress may be a promoting factor interfering with energy balance to maintain weight status, that is, an increase of appetite and energy intake and decrease of physical activity (87-89). It is widely acknowledged that diverse threatening stimuli or stressors could have an effect on the activation of major stress pathways or brain systems, such as the hypothalamic-pituitary-adrenal (HPA) axis and the sympathoadrenal medullary (SAM) systems, that would result in increased neuroendocrine and autonomic arousal and negative moods, leading to the use of substances like food, nicotine, and alcohol to self-medicate the resultant distress (15,90). Our findings that negative stressors decrease the likelihood of consuming ready to eat food do not fit in the aforementioned paradigm, most likely because ready to eat foods are not representative of overall consumption of various types of food items. For instance, females exposed to negative stress had greater odds of drinking soda and consuming snacks than those with no exposure to negative stressors. Although we did not find significant results in all food categories, the mix of increased and decreased odds of consuming certain types of food items may provide insight on food preferences when exposed to stress.

While negative stressors can induce physiological changes that enhance certain types of food consumption, current literature also suggests that exercise and TV watching can be used as coping mechanisms. Specifically, physical activity may enhance response habituation to stress, thereby reducing the negative physiological impact of stress (91). Similarly, motivation from TV watching may primarily derive from the desire to elevate the mood – relieving stress, escaping from stressors, and entering an alternate reality (92). While these are plausible explanations for our findings of significantly higher odds observed for females engaging in vigorous exercise and TV watching when exposed to negative stressors, further research on biomarkers may help elucidate the pathways of negative stressors to weight-regulated behaviors.

The second major findings are significant associations of MAOA genotype with exercise in males. Specifically, males with the high activity allele had significantly decreased odds of soda intake. This finding is directionally consistent with lower risk for obesity, and consistent with studies that have reported associations of the high-activity MAOA uVNTR polymorphism with lower risks of obesity and decreased BMI even though findings varied across populations (31,35,93). Ducci et al reported a significant association between BMI and the MAOA uVNTR polymorphism, with the low-activity allele associated with a higher BMI among a sample of primarily non-obese men participants with and without a history of alcohol dependence (35). Xie et al also recently confirmed the genetic effect of MAOA uVNTR polymorphisms on BMI among Chinese adolescents (94). In subsamples of the National Longitudinal Study of Adolescent Health (Add Health), significant associations were found between the MAOA promoter uVNTR and categories of BMI (obese and

overweight + obese) among White and Hispanic, but not African-American, men in a US cohort of young adolescents and adults (84). Carrying the high-activity allele could be related to higher levels of dopamine metabolism, as higher levels of homovanillic acid (HVA), cerebrospinal fluid (CSF) concentrations of one major metabolite of dopamine, were found among women with the high-activity MAOA allele (93, 95). Additionally, an age-related effect of ovarian hormones (such as estrogen) on MAOA gene expression may explain the observed findings in the literature. Specifically, ovarian steroids can decrease MAOA expression, which in turn may lead to an elevated level of serotonin in circulation (84, 96, 97).

Significant associations of DRD4 genotype with exercise were also found in combined females and males. These DRD4 variant allele carriers (i.e. 2 repeat or 7 repeat carriers) had significantly greater odds of engaging in vigorous exercise, as well as consuming soda. Our findings with respect to exercise are consistent with Guo's study, which reported a significant association between 7R/7R genotype of the DRD4 gene and decreased BMI for African-Americans and Hispanic-Americans (40). However, the 7R allele has also been shown to result in increased reward-seeking behaviors, namely increased food cravings (98). Stice et al also reported that European Americans with weaker activation of brain reward circuitry in response to imagined food intake were at increased risk for future weight gain if they were 7-repeat allele DRD4 carriers (99). The discrepancy in current literature on DRD4 may be explained by ethnic differences; however, further research is needed to confirm this. Both the 2 repeat and 7 repeat allele of DRD4 have been linked to compromised signal transduction ability and reduced cAMP levels, thereby resulting in decreased receptor sensibility (100).

Significant associations of DAT genotype with fast food consumption were observed for combined females and males. Specifically, combined males and females with the DAT variant (9-repeat carriers) had significantly decreased odds of consuming fast foods. This finding is consistent with Fuemmeler's report, in which 10-repeat carriers were associated with increased consumption of high-calorie sweet foods (84). Changes in SLC6A3 activity may reflect changes in dopamine function: the 10 repeat allele may have higher concentrations of DAT protein, and thus, lower postsynaptic concentrations of dopamine. This in turn may lead SLC6A3 10/10 individuals to consume higher amounts of energy-dense foods as a way of compensating for reduced levels of dopamine in the postsynaptic cleft.

Finally, the findings indicate new insight about potential genotype interactions with negative stressors. The protective effects of the high-activity MAOA genotype on physical activity in males were significantly strengthened by the exposure to negative stressors. This is an interesting result given the report by Xie et al that exposure to negative familial stressors significantly weakened the protective genetic effect of the high activity allele (94). The potential mechanism of the observed gender difference is unclear. Very limited efforts in the past have been made to explore potential gene x environment interaction. Fuemmeler et al reported a significant MAOA genotype x depressive symptoms interaction from their analyses with the Add Health data (11). In their study, males with a MAOA high-active allele and high depressive symptoms were at decreased risk of obesity (OR 0.22; 95% CI 0.06–0.78) and overweight plus obesity (OR 0.48; 95% CI 0.26–0.89) (11). Other reports on gene-environment interaction were mainly from the research on the role of MAOA in the development of antisocial behaviors (101-103). Carriers of low-activity MAOA alleles with

adverse experiences early in life, such as childhood maltreatment, were more likely to develop antisocial problems in their later lives (101-103).

Additionally, the protective effects of 2R and 7R alleles of DRD4 on engaging in exercise in females were significantly weakened by exposure to negative stressors. This is the first study to our knowledge to report such a finding. Further replication studies are needed to confirm our results. Before definitive conclusion can be made about the role that negative stressors and this gene have on food consumption and physical activity, we have to acknowledge certain limitations of the study. Due to the use of self-reporting we cannot discount the possibility that respondents altered their answers to achieve social desirability. Additionally, the participant's feelings at the time of completing questionnaires may heavily influence the way in which they report their behaviors. Our analyses relied on a fairly large and homo-ethnic sample (i.e. Chinese Han ethnicity only), but we were still limited by lack of replication. In addition, we did not have objective measures for physical activity and stress. Although checklists of stressful life events have been widely used to characterize chronic stress among adolescents, it would have been ideal to have had biological markers of stress, like saliva or hair cortisol assessment, to quantify the status of stress. Also, results may not necessarily be generalizable to persons of other ancestries.

Other than these concerns, our findings demonstrate the genetic effects MAOA uVNTR polymorphism and DRD4 genotype on the odds of food consumption and physical activity and convey the potential to derive new sights about the interactions with negative stressors. With the overwhelming references in media and within literature on food intake and exercise as two simple causes of obesity, we hope that our findings will dispel myths surrounding obesity and productively add to current discussion by acknowledging that these energy-balance behaviors are not necessarily causes but markers of the biochemical processes of obesity. The results highlight the need for future research to examine the role of genes and stressful environmental interactions on the development of specific energy-balance behaviors.

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DECLARATION OF INTERESTS

None declared.

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