Manuscript Details

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Title	Genetically predicted gene expression of prefrontal DRD4 gene and the differential susceptibility to childhood emotional eating in response to positive environment
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Abstract

Genetic differential susceptibility states that individuals may vary both by exhibiting poor responses when exposed to adverse environments, and disproportionally benefiting from positive settings. The dopamine D4 receptor gene (DRD4) may be particularly implicated in these effects, including disturbed eating behaviors that might lead to obesity. Here, we explore differential susceptibility to positive environments according to the predicted genetically regulated gene expression of prefrontal cortex DRD4 gene. Using MAVAN as the discovery cohort (Maternal Adversity, Vulnerability and Neurodevelopment) and GUSTO as the replication cohort (Growing Up in Singapore Towards Healthy Outcomes), we analyzed the interaction between a) a Positive postnatal environmental score, that accounts for positive outcomes in the postnatal period and b) the genetically regulated gene expression of prefrontal DRD4, computed using a machine learning prediction method (PrediXcan). The outcome measures were the pro-intake domains (Emotional over-eating, Food Responsiveness, Food Enjoyment and Desire to Drink) from the Child Eating Behavior Questionnaire at 48 months of age (MAVAN) and 60 months of age (GUSTO). The interaction between the positive environment and the predicted prefrontal DRD4 gene expression was significant for emotional over-eating in MAVAN (β=-0.403, $p<0.02$), in which the high gene expression group had more or less emotional eating according to the exposure to lower or higher positive environment respectively, showing evidence of differential susceptibility criteria. In the replication cohort, a similar result was found with the pro-intake domain Desire to drink (β=-0.583, $p<0.05$). These results provide further evidence for the genetic differential susceptibility, accounting for the benefit of positive environments.

Keywords	Emotional eating; Gene expression; Differential susceptibility; DRD4.
Taxonomy	Psychological Influences on Appetite, Disordered Eating, Development of Feeding
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Suggested reviewers	Bruce Ellis, Shinobu Kitayama, Eric R Gamazon

Submission Files Included in this PDF

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The authors do not have permission to share data

Montreal, December 11th, 2019.

To:
Dr. Suzanne Higgs,
Editor-in-Chief, *Appetite*

Dear Editor-in-Chief,

I am pleased to be re-submitting, after reviewers reply, this original manuscript entitled "**Genetically predicted gene expression of prefrontal DRD4 gene and the differential susceptibility to childhood emotional eating in response to positive environment**" for consideration in your esteemed journal. We thank the Reviewers 1 and 3 for his/her comments that certainly improved our work.

In 2016, we reported that girls living under adverse socioeconomic conditions and carrying the genetic polymorphism of the dopamine D4 receptor gene (48-base-pair variable number of tandem repeats region in the third exon) 7-repeat allele consume more calories derived from fat compared to non-carriers; however, the same individuals consume less calories derived from fat when living in a privileged economic and social stratum, when compared to non-carriers[1]. This was the first evidence that the differential susceptibility framework can be applied to metabolic vulnerability. In this theory, alleles previously considered to be "risk" alleles in fact confer openness to environmental modification, a finding with important implications for disease prevention and social pediatrics. The article received an editorial from Jay Belsky[2], and considerable attention from the media and from the academic community (22 citations in 2 years).

In the current manuscript, we expand the previous work by using a sophisticated and more comprehensive genomics approach to evaluate DRD4-related differential susceptibility to obesogenic behaviors in children. We explored this theoretical framework in response to different environmental scenarios, aiming at identifying responsiveness to environmental modifications, which can help to inform the development of more cost-effective health policies.

This manuscript has not been published, posted or submitted for publication elsewhere. We have no conflicts of interest to disclose.
Thank you for your consideration.

Sincerely,

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APPETITE_2019_453

Genetically predicted gene expression of prefrontal DRD4 gene and the differential susceptibility to childhood emotional eating in response to supportive environment

Authors reply to editors and reviewers [2]

Reviewer 1

The authors have adequately responded to my earlier comments and concerns.

Reviewer 3

Thank you for the modifications brought to your article. In order to have a more informative final version, I suggest the following modifications:

1. Abstract: remove last sentence or replace by a conclusion more directly related to the present findings.

The last sentence was replaced and now reads:

These results provide further evidence for the genetic differential susceptibility, accounting for the benefit of positive environments.

2. Line 80: remove “This”

Correction was done as suggested.

3. Line 138: describe total number of participants in the MAVAN cohort and justify how the number of participants for the present study was reached (same remark for the Gusto cohort)

Sample size was defined based on which participants had all the data needed for this study available at the time of the statistical analysis procedures (genotype, variables from the environmental score, outcomes). The total sample size for both cohorts was added and now reads:

Procedure: Information collected at birth as well as at 48 months of age was used. A total of 132 out of 630 participants had data available for all the measures relevant for this study (birth records, genotype, the Child Eating Behavior Questionnaire at 4 years of age and positive postnatal environmental score).

Procedures. We used information collected at birth as well as at 5 years of age. A total of 443 participants out of 1173 had data available for all the measures relevant for this study (birth records, genotype, CBEQ at 60 months of age and positive postnatal environmental score).

4. Line 142: replace CBEQ by CEBQ

Correction was done as suggested.

5. Lines 161 and 213: Please justify the methodological choice for the calculation of the positive environment score for both cohorts (for instance using the justification used in

reply to Reviewer 2: “Phenotypes such as birth size have been extensively shown in the literature to have “programming” effects on the individual’s metabolism, altering the response to the environment and subsequently increasing the likelihood of developing non-communicable diseases such as obesity. For example, a well-known effect of poor fetal growth is the programming of food preferences, widely explored by our lab [24-27], and confirmed by others [28-31]. Therefore, we believe that these long-lasting “programming” effects work as if they were a first or immediate “layer” of the environment, dictated by the individual’s current metabolic features that result from a past exposure (poor fetal growth). We discussed extensively about these environmental “layers” in a review (Dalle Molle et al., Neuroscience and Biobehavioral Reviews, 73: 326–339) [32]. The inclusion of attachment style is aligned with the same idea. Evidence has shown its effects on development of several socioemotional characteristics [33, 34], having a programming effect on socioemotional development [35, 36].”).

Thank you for your comments, the following sentences were altered and now reads:

Line 142: Predictors: Positive postnatal environmental score - This score accounts for positive environmental conditions on the postnatal period of life. **Figure 1** shows which variables and cut-offs were used to compute this score. Presence of each component established by its cut-off point yield one point. The total score is represented by the summation of points. The score was built in a cumulative index manner [18], accounting for stablished predictors of child health and development [7].

Line 162: The rationale behind including these variables that represent both phenotype measures (e.g. birth size, attachment) and family environment measures (e.g. maternal mental health, marital strain) together into the same score was based on the literature of early life adversity/protection and their long-term effects on child neurodevelopment and behavior. Phenotypes such as birth size have been extensively shown in the literature to have “programming” effects on the individual’s metabolism, altering the response to the environment and subsequently increasing the likelihood of developing non-communicable diseases such as obesity. For example, a well-known effect of poor fetal growth is the programming of food preferences, widely explored by our lab (1-4), and confirmed by others (5-8). These long-lasting “programming” effects work as if they were a first or immediate “layer” of the environment, dictated by the individual’s current metabolic features that result from a past exposure. The inclusion of attachment style is aligned with the same idea. Evidence has shown its effects on development of several socioemotional characteristics (9, 10), having a programming effect on socioemotional development (11, 12). We discussed extensively about these environmental “layers” in a review (13).

Line 236: Differences can be seen on **Figure 3** that shows variables and cut-offs used in the GUSTO cohort, that were chosen to best match the score created in the discovery cohort.

6. Line 173: When you refer to the “target sample” here, what do you mean, since you don’t have a biopsy of the PFC. Do you mean the buccal cells? Please clarify. This is important to help Appetite readers not fully understand your paper.

When we say “target sample”, we mean the MAVAN cohort, and we made this clearer in the paper now. But it is important to clarify to the reviewer that the PrediXcan prediction model,

proposed by Gamazon in 2015, generates algorithms to estimate the genetically determined component of gene expression in specific brain regions from the subject's genotype from the target sample, in this case the MAVAN cohort. PrediXcan was indeed created using PFC gene expression data from human brain donors, that also had genotype data. This way, the gene expression information was translated into a model that uses only the genotype information from your sample (in this case, MAVAN or GUSTO) to estimate the gene expression of a given gene.

We made a scheme to facilitate the understanding of this portion of the manuscript. Figure was added on line 193.

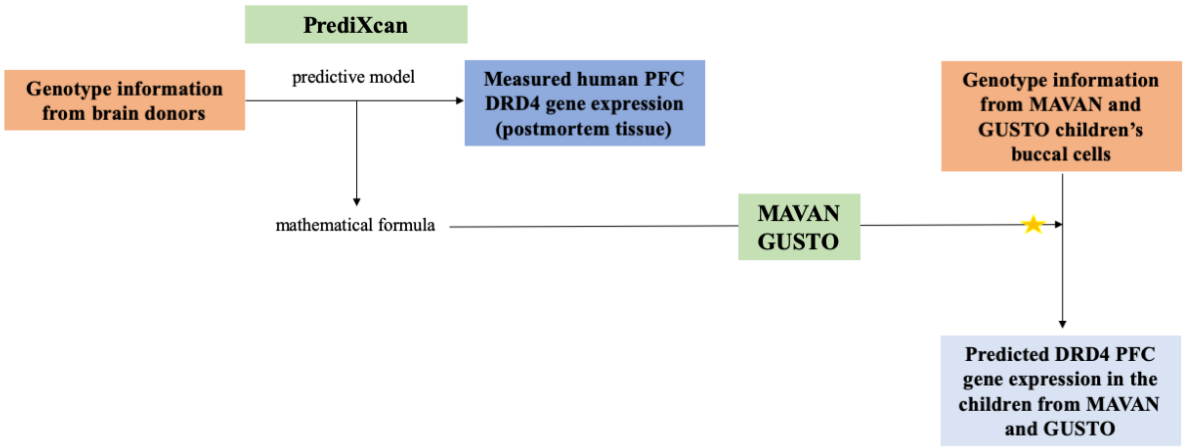

Figure 2: *Scheme for the generation of predicted DRD4 gene expression on discovery and replication cohorts. PrediXcan prediction model is applied to PFC gene expression data from human brain donors, that also had genotype data. The gene expression information was translated into a model that uses only the genotype information from our sample (in this case, MAVAN or GUSTO) to estimate the gene expression of a given gene (in this case, DRD4).*

7. Line 182 Add "or food approach" in the brackets

Correction was done as suggested.

8. Line 183 Add (or food avoidance) after "anti-intake".

Correction was done as suggested.

9. Line 217: Is 6000$ annual or monthly for the Gusto cohort?

Correction was done, figure two on the household income now reads: "Household total monthly gross income 6000$ and above"

10. Line 249: this is not clear to me why you applied a one-tailed p-value threshold for the replication study only

Thank you for pointing this out. An extended explanation was added on line 274 to 282 and now reads:

The replication analysis considered statistically significant results using one-tailed P-value thresholds. We considered the analysis done in the discovery cohort (MAVAN) to be exploratory and in this case, we used two-tailed P-value thresholds, since the direction of the forthcoming results were not anticipated. For the analysis done in the replication cohort (GUSTO) we anticipated results direction based on what we found in the discovery cohort. A one-tailed test is appropriate if the estimated value may depart from a reference value in only one direction. For that reason, the one-tailed P value thresholds were considered appropriated to confirm the results direction we saw in the discovery cohort.

11. Line 252 : Add a bracket after population structure: "(i.e. presence of a systematic difference in allele frequencies between subpopulations in a population, possibly due to different ancestry)".

Correction was done as suggested.

12. Tabled 1 and 2: Were the high DRD4 predicted expression levels of the same amplitude in the MAVAN and the GUSTO cohorts?

Thank you for pointing this out. The mean and standard deviation for the predicted DRD4 values according to Predixcan were included in the sample description table for MAVAN (table 1) and GUSTO (table 2).

13. Figures 3 and 4: Please add a legend to explain what the red lines mean or remove them. I don't think they are necessary

Thank you for pointing this out. An explanation of the criteria used to determine differential susceptibility on the GxE interaction was added to the statistical analysis section (line 283) and now reads:

To verify if the gene by environment interaction finding was aligned with the differential susceptibility model, we followed criteria developed by Roisman et al (2012). Three measures were considered; if regions of significance were inside the range of the environmental variation; if the markers PA (proportion affected) and PoI (proportion of interaction) were consistent with differential susceptibility; and if there was absence of nonlinear terms X2 and ZX2.

The following was added to the Figures 4 and 5 legend "The vertical lines depict the regions of significance".

14. Line 318 :Add "some" before "obesogenic behaviors".

Correction was done as suggested.

15. Line 318-20: please replace "In MAVAN, a high predicted prefrontal DRD4 gene expression decreases the risk for the development of behaviors associated with emotional over-eating in children as young as 4 years old that are raised in a more positive environment." By "In MAVAN, a high predicted prefrontal DRD4 gene expression was

associated to a decreased emotional over-eating in children as young as 4 years old that are raised in a more positive environment."

Correction was done as suggested.

16. Line 321-323: I don't understand what justifies this sentence. I don't read this in Tables 1 & 2. Please justify precisely, or remove this sentence

We agree with the suggestion and the sentence was taken out.

We thank the editors and reviewers for the careful reading of our manuscript, and for their valuable comments that contributed to improving our work.

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Abstract

Genetic differential susceptibility states that individuals may vary both by exhibiting poor responses when exposed to adverse environments, and disproportionally benefiting from positive settings. The dopamine D4 receptor gene (DRD4) may be particularly implicated in these effects, including disturbed eating behaviors that might lead to obesity. Here, we explore differential susceptibility to positive environments according to the predicted genetically regulated gene expression of prefrontal cortex DRD4 gene. Using MAVAN as the discovery cohort (Maternal Adversity, Vulnerability and Neurodevelopment) and GUSTO as the replication cohort (Growing Up in Singapore Towards Healthy Outcomes), we analyzed the interaction between a) a Positive postnatal environmental score, that accounts for positive outcomes in the postnatal period and b) the genetically regulated gene expression of prefrontal DRD4, computed using a machine learning prediction method (PrediXcan). The outcome measures were the pro-intake domains (Emotional over-eating, Food Responsiveness, Food Enjoyment and Desire to Drink) from the Child Eating Behavior Questionnaire at 48 months of age (MAVAN) and 60 months of age (GUSTO). The interaction between the positive environment and the predicted prefrontal DRD4 gene expression was significant for emotional over-eating in MAVAN (β=-0.403, $p<0.02$), in which the high gene expression group had more or less emotional eating according to the exposure to lower or higher positive environment respectively, showing evidence of differential susceptibility criteria. In the replication cohort, a similar result was found with the pro-intake domain Desire to drink (β=-0.583, $p<0.05$). These results provide further evidence for the genetic differential susceptibility, accounting for the benefit of positive environments.

Key words: Emotional eating, Gene expression, Differential susceptibility, DRD4

Genetically predicted gene expression of prefrontal DRD4 gene and the differential susceptibility to childhood emotional eating in response to positive environment

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Declarations of interest: none.

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Abstract

Genetic differential susceptibility states that individuals may vary both by exhibiting poor responses when exposed to adverse environments, and disproportionally benefiting from positive settings. The dopamine D4 receptor gene (DRD4) may be particularly implicated in these effects, including disturbed eating behaviors that might lead to obesity. Here, we explore differential susceptibility to positive environments according to the predicted genetically regulated gene expression of prefrontal cortex DRD4 gene. Using MAVAN as the discovery cohort (Maternal Adversity, Vulnerability and Neurodevelopment) and GUSTO as the replication cohort (Growing Up in Singapore Towards Healthy Outcomes), we analyzed the interaction between a) a Positive postnatal environmental score, that accounts for positive outcomes in the postnatal period and b) the genetically regulated gene expression of prefrontal DRD4, computed using a machine learning prediction method (PrediXcan). The outcome measures were the pro-intake domains (Emotional over-eating, Food Responsiveness, Food Enjoyment and Desire to Drink) from the Child Eating Behavior Questionnaire at 48 months of age (MAVAN) and 60 months of age (GUSTO). The interaction between the positive environment and the predicted prefrontal DRD4 gene expression was significant for emotional over-eating in MAVAN (β=-0.403, $p<0.02$), in which the high gene expression group had more or less emotional eating according to the exposure to lower or higher positive environment respectively, showing evidence of differential susceptibility criteria. In the replication cohort, a similar result was found with the pro-intake domain Desire to drink (β=-0.583, $p<0.05$). These results provide further evidence for the genetic differential susceptibility, accounting for the benefit of positive environments.

Key words: Emotional eating, Gene expression, Differential susceptibility, DRD4

Introduction

Genes can modulate the cellular and behavioral responses to environmental variation and some theoretical paradigms guide the understanding of these relationships. The Diathesis-Stress paradigm states that some individuals are more vulnerable than others to the negative effects of the environment[1]. However, it does not consider variations in positive aspects. The genetic differential susceptibility states that individuals may vary both by exhibiting poor responses when exposed to adverse environments, and disproportionally benefiting from positive settings (including the simple absence of adversity). These would occur to guarantee survival in different contexts. This idea is aligned with evolutionary analysis of human development, in which plasticity to environmental variations is set as a bet hedging against an uncertain future, and to avoid a costly mismatch between the individual's ability to face the environmental conditions and the actual challenges that the environment could impose [2-4]. This framework has advantages since it considers a broader spectrum of environmental influences, also shedding light on positive aspects of the environment and its consequences on development. This theoretical concept can also be seen on the proposed idea of "plasticity genes", in which dopamine seems to have a central role [2, 5]. In this sense, individuals that are highly responsive to the environment, in a differential susceptibility perspective, while being more vulnerable to the damaging effects of an exposure to environmental adversity, can also benefit more from positive environmental conditions then the nonresponsive individuals. This is equivalent to the 'orchid' children described by Boyce and Ellis [6], in a theory called biological sensitivity to context.

This is corroborated by evidence showing that the mesocorticolimbic pathway finishes its development later in life, compared to other neurotransmitter systems. This pathway therefore is susceptible to the influence of the environment for a much long period of time, being an obvious candidate for a biological mechanism involved in the programming by environmental conditions. This enhanced sensitivity to the environmental context, associated with specific dopamine signaling, increases the range of phenotypic possibilities, not focusing only on vulnerabilities, but also involving better outcomes in particular environmental settings [7].

Phenotypes known to be affected by these gene by environment (GxE) interactions include disturbed eating behaviors that can lead to obesity[8]. In fact, alterations on the dopaminergic pathways can lead to increased sensitivity to reward and impulsivity [9]. For example, drugs such as amphetamine and methylphenidate, known for being dopamine enhancers, improve behavioral

symptoms of most children with attention deficit hyperactivity disorder (ADHD), suggesting that dopamine signaling plays a role on the onset and maintenance of this condition related to impulsivity and other executive functions impairments [10]. Similarly dopamine function is thought to play a role in major depression symptoms, since impairments in motivation and anhedonia are all related to the disorder, and also regulated in part by the DA neurotransmission systems [11]. These dopamine signaling alterations can lead to poor decision-making processes, prompting non-adaptive behaviors such as addiction and altered eating behavior [12-14].

The dopamine D4 receptor gene (DRD4) exon III VNTR polymorphism has been particularly implicated in these effects. In 2016, Silveira et al described that variations in this specific mutation interacted with socioeconomic status (SES) according to the differential susceptibility framework, influencing fat preferences of girls at 4 years of age[15]. The same girls who are genetically more prone to develop obesogenic behaviors (increased fat intake) when raised in low SES conditions, are also less likely to develop obesogenic behaviors when raised in a positive, high SES environment. Similarly van Strien, Levitan [16] found that hypofunctional variants of the DRD4 were associated with higher emotional eating in females. However, single polymorphism approaches may not capture the whole complexity of the function of a gene. Novel genomics approaches using machine learning algorithms to predict gene expression in tissue specific regions are available[17], and these are likely able to provide a more comprehensive view of the role of a specific gene in modulating an individual response to environmental variations.

Even though the differential susceptibility hypothesis accounts for both extremes of the environmental influence (positive and negative, including the simple absence of adversity)[4], few studies have used measures that account for positive aspects of the environment[18, 19]. Work is needed to improve empirical evidence on the responsivity to positive or supporting conditions, showing that this theoretical framework is in fact relevant to understand effectiveness of interventions.

Here, we propose to expand previous work done by our laboratory [8, 19-22] by using an innovative and more comprehensive genomics approach to evaluate differential susceptibility to obesogenic behaviors in children. If the framework is indeed applicable, variations in the predicted DRD4 gene expression in the prefrontal cortex (where D4 receptors are predominantly localized) would be associated with differential responsiveness to positive circumstances, here represented by measures associated with supporting conditions in the postnatal period.

Materials and Methods

Subjects: The sample was derived from the prospective birth cohort MAVAN[23] (Maternal Adversity, Vulnerability and Neurodevelopment) which followed up children at different time points in the first years of life in Montreal (Quebec) and Hamilton (Ontario), Canada. Exclusion criteria were severe maternal 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. Ethical approvals were obtained from obstetricians performing deliveries at the study hospitals and by the ethics committees and university affiliates (McGill University and Université de Montréal, the Royal Victoria Hospital, Jewish General Hospital, Centre hospitalier de l'Université de Montréal, Hôpital Maisonneuve-Rosemont, St Joseph's Hospital and McMaster University, Hamilton, Ontario, Canada). The study was conducted in accordance with the rules and regulations of the university ethics committees and informed consent was obtained from all participants.

Procedure: Information collected at birth as well as at 48 months of age was used. A total of 132 out of 630 participants had data available for all the measures relevant for this study (birth records, genotype, the Child Eating Behavior Questionnaire at 4 years of age and positive postnatal environmental score). Children and mothers came to the laboratory for testing and to complete the scales (CEBQ, see details below). Birth records were obtained directly from the birthing units.

Predictors: Positive postnatal environmental score - This score accounts for positive environmental conditions on the postnatal period of life. **Figure 1** shows which variables and cut-offs were used to compute this score. Presence of each component established by its cut-off point yield one point. The total score is represented by the summation of points. The score was built in a cumulative index manner [19], accounting for stablished predictors of child health and development [8]. Birth weight percentiles and household gross income were calculated using the local reference[24]·[25]. Maternal mental health information was extracted from different questionnaires: Beck Depression Inventory, a 21-question multiple-choice self-report inventory[26]; Edinburgh Postnatal Depression Scale (EPDS), a 10-item self-report scale designed to screen for postpartum depression[27] and State-Trait Anxiety Inventory (STAI), a two versions 20 item each self-report scaling to measure state and trait anxiety[28]. To measure types of attachment styles in preschool-aged children the Preschool Separation – Reunion Procedure

(PSRP) was used [29, 30], having a baseline interaction followed by two separation and reunion episodes lasting 5 minutes video recorded and scored (reliability k=0.83). The Family Assessment Device (FAD), a 60-item self-report instrument, was used to assess different domains of family functioning[31]. The Marital Strain Scale of Pearlin and Schooler was used to assess chronic stress with the romantic partner [32]. Lastly, a self-report breastfeeding questionnaire[33] was used to inquire the age at which the baby (in weeks) was fed for the first time with something other than breast milk, and the age of the baby (in weeks) when mothers stopped nursing (or giving breast milk).

The rationale behind including these variables that represent both phenotype measures (e.g. birth size, attachment) and family environment measures (e.g. maternal mental health, marital strain) together into the same score was based on the literature of early life adversity/protection and their long-term effects on child neurodevelopment and behavior. Phenotypes such as birth size have been extensively shown in the literature to have "programming" effects on the individual's metabolism, altering the response to the environment and subsequently increasing the likelihood of developing non-communicable diseases such as obesity. For example, a well-known effect of poor fetal growth is the programming of food preferences, widely explored by our lab [34-37], and confirmed by others [38-41]. These long-lasting "programming" effects work as if they were a first or immediate "layer" of the environment, dictated by the individual's current metabolic features that result from a past exposure. The inclusion of attachment style is aligned with the same idea. Evidence has shown its effects on development of several socioemotional characteristics [42, 43], having a programming effect on socioemotional development [44, 45]. We discussed extensively about these environmental "layers" in a review [8].

Discovery cohort - Score: Positive / Time: Postnatal

- Birth size percentile greater or equal to 40% and below or equal to 70%
- Gestational age between 39-40 weeks
- Maternal mental health - presence of either BDI (Beck Depression Inventory) below 2, EPDS (Edinburgh Postnatal Depression Scale) below 3 or STAI (State-Trait Anxiety Inventory) below 53
- Household total gross income 80,000$ and above
- Secure attachment (as measured by The Preschool Separation – Reunion Procedure - PSRP)
- Good family function (as measured by Family Assessment Device – FAD. Score below 1.15)
- The Marital Strain Scale score below 1.45
- Still breastfeeding at 3 months

Figure 1: *Variables and cut-offs used to create the Positive postnatal environmental score in MAVAN. Presence of each component (described in each bullet) yielded 1 point, and the scores represent the summation of points.*

This figure is intended to be a single fitting image

Genetically regulated expression of prefrontal DRD4 gene – The genetically regulated expression of prefrontal DRD4 gene is computed using a machine learning prediction method (PrediXcan)[17]. This algorithm was built using a reference dataset from human brain donors (postmortem), being therefore tissue-specific. This reference dataset is composed by data from GTEx project [46], GEUVADIS [47] and DGN [48] containing both genotype and gene expression levels. The PrediXcan prediction model, proposed by Gamazon in 2015, uses a machine learning approach to generate algorithms to estimate the genetically determined component of gene expression in specific brain regions from the subject's genotype in the target sample, in this case MAVAN cohort. For the genetic score used in this study, we applied this algorithm to our two samples, and were able to calculate a predicted DRD4 PFC gene expression using the genotype information available in the children from our birth cohorts (**Figure 2**).

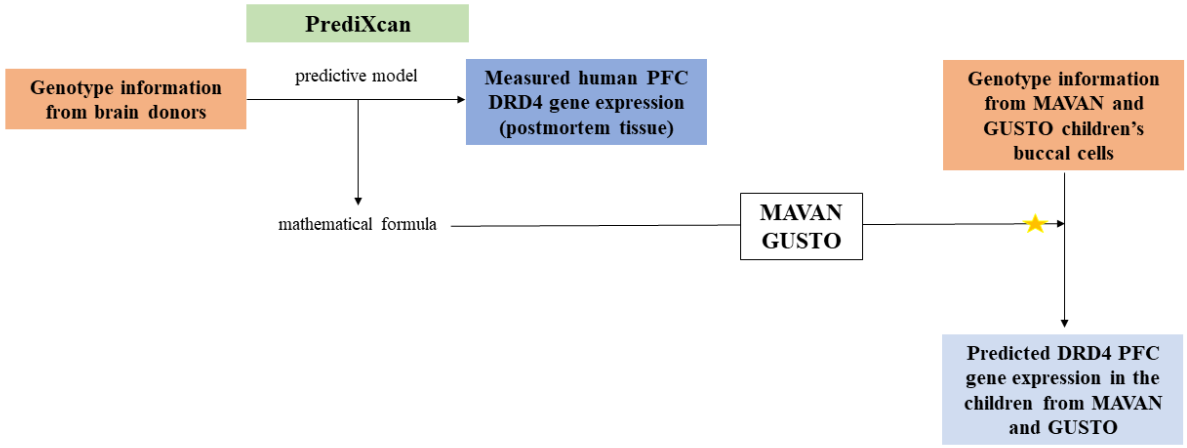

Figure 2: *Scheme for the generation of predicted DRD4 gene expression on discovery and replication cohorts. PrediXcan prediction model is applied to PFC gene expression data from human brain donors, that also had genotype data. The gene expression information was translated into a model that uses only the genotype information from our sample (in this case, MAVAN or GUSTO) to estimate the gene expression of a given gene (in this case, DRD4).*

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In MAVAN, we genotyped 242,211 autosomal SNPs using genome-wide platforms (PsychArray/PsychChip, Illumina) from 200ng of genomic DNA derived from the buccal epithelial cells. After quality control procedures and imputation, 20,790,893 SNPs with an info score >0.80 and posterior genotype probabilities >0.90 were available to be used in PrediXcan.

Outcome: The Child Eating Behavior Questionnaire[49] is designed to assess children's eating styles that have been hypothesized to contribute both to underweight and overweight. Having domains that reflect behaviors of food pro-intake (positive inclinations for eating or food approach) and anti-intake (or food avoidance). It is a parent-report measure comprised of 35 items, each rated on a five-point Likert scale that ranges from never to always. The instrument is ideal for use in research investigating the early precursors of eating disorders or obesity. The psychometric properties of the instrument have been evaluated and show robust factor structure, good internal and test-retest reliability[49]. A more recent study also shows validity of the questionnaire against behavioral measures of eating [50]. The outcome measures used were the four domains from the questionnaire that reflect pro-intake behaviors [51]: Enjoyment of Food, Food Responsiveness, Desire to Drink and Emotional over-eating. Overall these items describe

pro-intake behaviors either by enjoyment of food, being responsive to food, having a high desire to drink or over-eating in response to negative emotions.

Replication cohort: *Subjects.* The sample included children from the prospective birth cohort GUSTO (Growing Up in Singapore Towards Healthy Outcomes)[52]. Pregnant women aged 18 years and above were recruited at the National University Hospital (NUH) and KK Women's and Children's Hospital (KKH) in Singapore, being of Chinese, Malay or Indian ethnicity with homogeneous parental ethnic background. Mothers receiving chemotherapy, psychotropic drugs or who had type I diabetes mellitus were excluded. Besides that, for the sake of comparison with the MAVAN cohort, only non-preterm children (born above 37 weeks of gestation) were considered. The study was approved by the National Healthcare Group Domain Specific Review Board (NHG DSRB) and the Sing Health Centralized Institutional Review Board (CIRB). Informed written consent was obtained from each participant. A descriptive paper details other aspects of the cohort [52].

Procedures. We used information collected at birth as well as at 5 years of age. A total of 443 participants out of 1173 had data available for all the measures relevant for this study (birth records, genotype, CBEQ at 60 months of age and positive postnatal environmental score). Children and mothers came to the laboratory for testing and to complete scales. Birth records were obtained directly from the birthing units.

Predictors. Positive postnatal environmental score - Was defined and calculated as described in the MAVAN cohort above, except attachment style and marital relationship quality that were not available in this cohort. Differences can be seen on **Figure 3** that shows variables and cut-offs used in the GUSTO cohort, that were chosen to best match the score created in the discovery cohort.

Replication cohort - Score: Positive / Time: Postnatal
• Birth size percentile greater or equal to 40% and below or equal to 70% • Gestational age between 39-40 weeks • Household total monthly gross income 6000$ and above • Family function greater or above 85th percentile (FAD lower or equal to 1.35) • Maternal mental health at 3 months (presence of either BDI lower or equal to 1, EPDS lower or equal to 1, or STAI lower or equal to 49) Still breastfeeding at 3 months

Figure 3: *Variables and cut-offs used to create the positive postnatal environmental score in GUSTO. Presence of each component (described in each bullet) yielded 1 point, and the scores represent the summation of points.*

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Genetically regulated expression of prefrontal DRD4 gene – It was computed using the same machine learning prediction method (PrediXcan)[17] and brain region as described in the MAVAN cohort. Genomic DNA in GUSTO was extracted from frozen umbilical cord specimens. Samples were genotyped on Illumina Omni express arrays and on Illumina Exome arrays, following the manufacturer's instructions (Expression Analysis Inc). Further quality control on the genotyping calls were previously described[53]. SNPs were verified for a genotyping rate ≥95% and no deviation from Hardy–Weinberg equilibrium ($P < 0.001$), and minor allele frequency ≥0.05, using PLINK[54, 55].

Outcome. The outcome measures were the same used in the MAVAN cohort from the Child Eating Behavior Questionnaire[49], with the four domains that reflect pro-intake behaviors: Enjoyment of Food, Food Responsiveness, Desire to Drink and Emotional over-eating.

Statistical analysis

Statistical analysis of the participants' baseline characteristics was performed using Student's T test for continuous data and chi-square tests for categorical variables (Table 1 and Table 2). For the baseline comparisons, a median split was used to define the high and low DRD4 predicted gene expression groups. For the main analysis, linear regression models using continuous DRD4 predicted gene expression values on the PFC, positive postnatal environmental and the interaction term between these two variables were performed for the four domains of the CBEQ considered in this study (Enjoyment of Food, Food Responsiveness, Desire to Drink and Emotional Over-Eating). Regression analysis were corrected for multiple comparisons. The replication analysis considered statistically significant results using one-tailed P-value thresholds. We considered the analysis done in the discovery cohort (MAVAN) to be exploratory and in this case, we used two-tailed P-value thresholds, since the direction of the forthcoming results were not anticipated. For the analysis done in the replication cohort (GUSTO) we anticipated results direction based on what we found in the discovery cohort. A one-tailed test is appropriate if the

estimated value may depart from a reference value in only one direction. For that reason, the one-tailed P value thresholds were considered appropriated to confirm the results direction we saw in the discovery cohort. Preliminary analysis adjusted by sex showed no main effect or interaction with sex, therefore in the main analysis boys and girls were analyzed together. To verify if the gene by environment interaction finding was aligned with the differential susceptibility model, we followed criteria developed by Roisman et al (2012). Three measures were considered; if regions of significance were inside the range of the environmental variation; if the markers PA (proportion affected) and PoI (proportion of interaction) were consistent with differential susceptibility; and if there was absence of nonlinear terms X2 and ZX2.

We examined population structure (i.e. presence of a systematic difference in allele frequencies between subpopulations in a population, possibly due to different ancestry) and the models were adjusted by principal components that reflect population stratification [56, 57]. By adding the principal components, we aim to adjust for false results due to ancestry differences. For that, first we pruned our datasets to common variants (MAF>0.05) that were not in linkage disequilibrium (r2<0.20) with a sliding window (50 kilobases) approach that examined linkage disequilibrium in increments of 5 SNPs using PLINK 1.9 [58]. We performed a principal component analysis using SMARTPCA on this pruned dataset and generated a scree plot (see Hari Dass, McCracken [59] for scree plot for the MAVAN cohort). Based on the inspection of the scree plot, the first three principal components were the most informative of population structure in both cohorts and were included in all analyses. No other co-variates were used in the regression analysis. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (SPSS Inc., Chicago, IL, USA) and R software[60-62]. Significance levels for all measures were set at $p < 0.05$.

Table 1: *Sample description and differences between High and Low DRD4 predicted gene expression groups in MAVAN.*

Sample Description

	Total sample (n=132)		Low DRD4 (n=67)		High DRD4 (n=65)		
Variable	**Mean or n**	**SD or %**	**Mean or n**	**SD or %**	**Mean or n**	**SD or %**	***p***

Birth weight (g)	3320.95	455.38	3322.79	450.15	3318.93	464.66	0.96
Gestational age (weeks)	39.18	1.21	39.15	1.06	39.2	1.36	0.82
Maternal age at birth (years)	30.81	4.75	31.13	4.04	30.46	5.09	0.4
Montreal site	76	57%	42	31.8%	27	20.4%	0.42
Female sex	68	51%	37	28%	31	23.4%	0.61
Income below Can$80,000	56	44%	45	34%	22	16.6%	0.36
Maternal education high school or less	2	1.5%	2	1.5%	0	0.0%	0.47
Positive postnatal environmental score	3.4	1.52	3.57	1.51	3.34	1.52	0.38
Food Responsiveness	2.27	0.8	2.14	0.81	2.39	0.77	0.09
Food enjoyment	3.58	0.75	3.46	0.8	3.72	0.68	0.06
Desire to drink	3	1.07	3.02	1.11	2.98	1.04	0.82
Emotional over-eating	1.61	0.6	1.62	0.6	1.61	0.6	0.9
PrediXCan DRD4 PFC	-0.13	0.22	-0.32	0.15	0.05	0.06	-

MAVAN participants' characteristics by prefrontal DRD4 predicted gene expression group. Data are expressed as means (standard deviations) or number of participants (percentages).

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Table 2: *Sample description and differences between High and Low DRD4 predicted gene expression groups in GUSTO*

	Sample Description						
	Total sample (n=428)		**Low DRD4 (n=223)**		**High DRD4 (n=205)**		
Variable	**Mean or n**	**SD or %**	**Mean or n**	**SD or %**	**Mean or n**	**SD or %**	**p**
Birth weight (g)	3122.42	427.06	3151.09	422.83	3091.24	430.48	0.15
Gestational age (weeks)	38.46	1.28	38.56	1.2	38.35	1.36	0.1
Maternal age at birth (years)	31.31	5.08	31.18	4.91	31.45	5.26	0.58
Female sex	203	47.4%	114	51.1%	89	43.4%	0.11

Sample Description							
Income below $6,000	302	70,6%	156	70%	146	71.2%	0.77
Maternal education high school or less	277	64.7%	144	64.9%	133	65.5%	0.89
Positive postnatal environmental score	2.11	1.24	2.09	1.27	2.14	1.2	0.67
Food responsiveness	2.4	0.69	2.41	0.69	2.39	0.69	0.83
Food enjoyment	3.5	0.79	3.51	0.82	3.5	0.76	0.89
Desire to drink	2.74	0.9	2.84	0.94	2.62	0.84	0.01*
Emotional over-eating	2.79	0.79	2.76	0.77	2.82	0.82	0.44
PrediXCan DRD4 PFC	-0.01	0.11	-0.10	0.11	0.06	0.04	-

GUSTO participants' characteristics by prefrontal DRD4 predicted gene expression group. Data are expressed as means (standard deviations) or number of participants (percentages).

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Results

Baseline comparisons between predicted gene expression groups can be seen in **Table 1** and **Table 2**. No differences were found between the two groups (high and low predicted prefrontal DRD4 gene expression) in relation to the main confounding variables in both cohorts.

In MAVAN, we observed a statistically significant interaction effect between the positive environment score and the predicted prefrontal DRD4 gene expression on emotional over-eating (β =-0.403, p=0.0159). A simple slope analysis revealed that a more positive environment is associated with lower emotional over-eating as the DRD4 predicted gene expression increases (Figure 3). On **Figure 4,** groups are divided by plus and minus one standard deviation for the sake of visualization. We confirmed that the interaction is aligned with the differential susceptibility model according to Roisman et al (2012) method [63], since the regions of significance were inside the range of the environmental variation; moreover, the markers PA= 0.54 and PoI=0.52 were consistent with differential susceptibility, as well as the absence of nonlinear terms X^2 and ZX^2). This means that the same genetic profile associated with increased benefit from a more positive environment, is also more affected by a less positive environment, showing more emotional over-eating. After adjusting by multiple comparison this result remains significant.

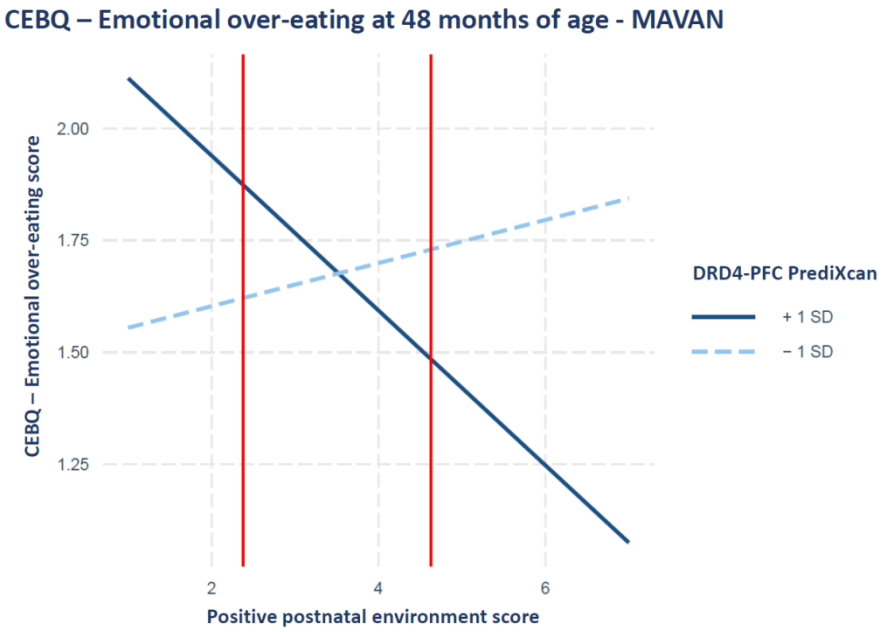

Figure 4: *Evidence of differential susceptibility - Interaction between positive postnatal environmental score and predicted DRD4 gene expression on Emotional over-eating at 48 months of age. MAVAN Cohort. The vertical lines depict the regions of significance*

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On the same regression model, the predicted prefrontal DRD4 expression had an independent effect on emotional over-eating (β=1.388. p=0.0240) as well as the positive postnatal environmental score (β= -0.098, p=0.0129). The same association was not found for the other domains in the CEBQ: Desire to drink (β=-0.142, p=0.62051); Food Enjoyment (β =-0.088, p=0.660) and Food Responsiveness (β =-0.047, p=0.968).

In the replication cohort, similar results were found with another pro-intake domain from the CEBQ. The interaction between the positive environment and the predicted prefrontal DRD4 gene expression was statistically significant on the domain desire to drink (β=-0.579, p= 0.01455). Simple slope analysis revealed that as the score for the positive environment increases and the gene expression score also increases, there is a decrease in the desire to drink score. For the sake of visualization of the results, on **Figure 5** the participants are divided in plus and minus one standard deviation from the mean. After adjusting for multiple comparisons this result was found marginally significant (p=0.0582). No association was not found for the other CEBQ pro intake domains: Emotional over-eating (β= -0.046, p=0.3903), Food Enjoyment (β = -0.357, p=0.08866), Food Responsiveness (β =-0.375, p=0.0660); no evidence for differential susceptibility was detected in this cohort.

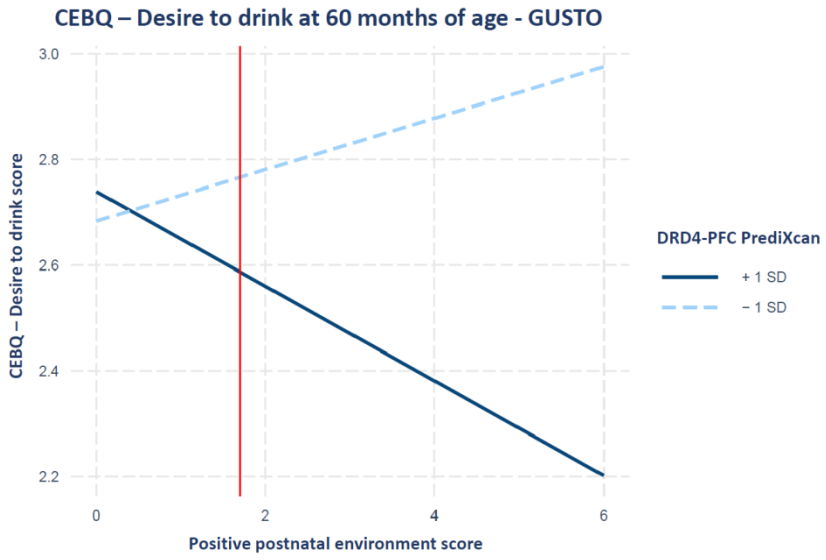

Figure 5: *Interaction between positive postnatal environmental score and predicted DRD4 gene expression on Desire to Drink at 60 months of age. Gusto Cohort. The vertical lines depict the regions of significance*

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Discussion and conclusion

In this study, we demonstrated on both cohorts that environment and genetics were associated with some obesogenic behaviors in children. In MAVAN, a high predicted prefrontal DRD4 gene expression decreases the risk for the development of behaviors associated with emotional over-eating in children as young as 4 years old that are raised in a more positive environment. Since we found evidence of differential susceptibility, the opposite relationship is also true, in which these same children, if raised in a less positive environment are in a higher risk to develop obesogenic behaviors as measured by the CEBQ instrument. In fact, emotional over-eating has been linked to difficulties in weight loss among adults that underwent treatment for obesity [64] being a stronger predictor of weight gain than life style factors such as little physical activity and consumption of fruits and vegetables [65]. Emotional over-eating seems to be a risk factor not only for the development of obesity but for its maintenance as well.

In the GUSTO cohort, a high predicted prefrontal DRD4 gene expression decreases the risk for the development of behaviors associated with the domain desire to drink in children as young as 5 years old that are raised in a more positive environment. Although the domains desire to drink and emotional over-eating are known to be weekly correlated [66], it is also known that both have a relationship with onset of obesogenic behaviors [64, 65, 67]. Besides that the domain desire to drink is also considered pro-intake, and is associated with the consumption of high sugar-sweetened beverages [68]. In fact, the overconsumption of high sugary drinks [69] and the desire

to drink domain have been related to obesity and overweight in children [67]. Although this result did not survive correction for multiple comparisons, it could be seen as valid since it emerged from an a priori hypothesis and previous published results [15, 21, 70] characterizing this analysis as non-exploratory.

Despite the difference in the significant domains between the two cohorts, we were able to demonstrate the effect of the interaction between positive environmental conditions and the predicted prefrontal DRD4 gene expression on eating behaviors associated with obesity and overweight. Explanations for the dissimilar results between the cohorts may involve cultural or behavioral aspects associated with eating styles. The lack of evidence for differential susceptibility in GUSTO could be explained by the fact that the positive environment score in this cohort does not include an evaluation of attachment styles as does MAVAN, due to the lack of this data in GUSTO.

Evidence from the literature showing the relationship between pro intake behaviors and the function of the DRD4 gene variants [15, 16, 71, 72] and also between dopamine related genes and susceptibility for environment influences [2], corroborates the relationship seen on this work. It is important to emphasize that we used a novel genomic approach to predict gene expression in a tissue specific manner[17], being able to provide a more comprehensive view of the role of a specific gene in modulating an individual response to environmental variations. It seems that individual variation on the function of dopaminergic pathways, here represented by the variations of the predicted prefrontal DRD4 gene expression, could be one of the underlying biological process that explain the relationship between variations in a positive environment and reduced probability to develop obesogenic behaviors. This could be happening by altering the subjects' reward sensitivity and decision-making behaviors at critical time points during development.

Insights from neuroscience and GxE studies are crucial to understand the biological processes underlying children's behavior and susceptibility to negative/positive outcomes. This has implications for understanding the development of several important health outcomes, including growth and its deviations, as well as metabolic alterations.

These results provide further evidence for the genetic differential susceptibility[2], that accounts not only for how vulnerable an individual is to adversity, but also how much they will benefit from positive environments. It is known that children vary according to their susceptibility to the environmental variations, but this framework brings a biological explanation for this

observed phenomenon, and accounts for a better characterization of the adverse as well as the positive environment. Indeed, this is demonstrated here, being the characterization of the environment in terms of positive circumstances one of the innovative aspects of this study. It gives strong support for the theoretical framework used, since most of the studies in the area focus on measures characterizing the environment in terms of adversity only [18, 19]. Here we show that even when the starting point is a positive characterization of the environment, a moderation effect in agreement with the genetic differential susceptibility framework can be detected, in this case in relation to eating behavior. Applying this novel approach to the developmental neuropsychology and developmental origins of health and disease agenda guides the elaboration of more efficacious and cost-effective interventions, targeting individuals that would benefit the most from interventions. Furthermore, this broadens the scope of scientific evidence for interventions that focus on promotion of health rather than preventing diseases.

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