Exploring the Role of BDNF and NTRK2 Genetic Polymorphisms on Eating Behaviours in Young Children

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

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2017

Abstract

**Background:** Genetic studies in obesity and eating disorders have identified brain-derived neurotrophic factor (*BDNF*) and neurotrophic receptor tyrosine kinase 2 (*NTRK2*) as candidate genes. We investigated the role of *BDNF* and *NTRK2* SNPs on eating behaviours in young children.

**Methods:** Data was obtained from a preschool-aged cohort. SNP and haplotype analysis were carried out for two phenotypes: Snack Delay Task (n=158), a behavioural measure of impulsive responding to food and Child Eating Behaviour Questionnaire (CEBQ) (n=160), a psychometric measure of eating behaviours.

**Results:** *NTRK2* rs1047896 and haplotypes containing this SNP predicted performance on Snack Delay Task. We also detected an association between *NTRK2* rs1078947 and Food Approach Score from the CEBQ that was trending towards significance. Nominal epistatic effects were observed between *BDNF* rs6265 and *NTRK2* rs1187325 on Food Approach Score.

**Conclusion:** *NTRK2* SNPs demonstrated novel associations with measures of impulsive eating and food approach behaviours. Findings should be replicated in larger samples.
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**Dr. Hélène Gaudreau** – MAVAN collaborator* from Quebec

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* MAVAN collaborators contributed to the collection of data for the cohort
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ADHD</td>
<td>Attention deficit hyperactive disorder</td>
</tr>
<tr>
<td>AgRP</td>
<td>Agouti-related peptide</td>
</tr>
<tr>
<td>ARC</td>
<td>Arcuate nucleus</td>
</tr>
<tr>
<td>ARFID</td>
<td>Avoidant/ restrictive Food Intake Disorder</td>
</tr>
<tr>
<td>AN</td>
<td>Anorexia nervosa</td>
</tr>
<tr>
<td>BED</td>
<td>Binge eating disorder</td>
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<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BN</td>
<td>Bulimia nervosa</td>
</tr>
<tr>
<td>CAMH</td>
<td>Centre for Addiction and Mental Health</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic AMP</td>
</tr>
<tr>
<td>CEBI</td>
<td>Children’s Eating Behavior Inventory</td>
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<td>CEBQ</td>
<td>Child Eating Behaviour Questionnaire</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>ChEAT</td>
<td>Children’s Eating Attitude Test</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>DEBQ</td>
<td>Dutch Eating Behaviour Questionnaire</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DRD4</td>
<td>Dopamine-4 receptor gene</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, Forth Edition</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear models</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>GTEx</td>
<td>Genotype-Tissue Expression Project Portal</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-wide association study</td>
</tr>
<tr>
<td>HWE</td>
<td>Hardy-Weinberg equilibrium</td>
</tr>
<tr>
<td>LD</td>
<td>Linkage disequilibrium</td>
</tr>
<tr>
<td>LTD</td>
<td>Long-term depression</td>
</tr>
<tr>
<td>LTP</td>
<td>Long-term potentiation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>MAF</td>
<td>Minor allele frequency</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MAVAN</td>
<td>Maternal Adversity, Vulnerability and Neurodevelopment project</td>
</tr>
<tr>
<td>mBDNF</td>
<td>Mature BDNF</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>NT3</td>
<td>Neurotrophin-3</td>
</tr>
<tr>
<td>NT4</td>
<td>Neurotrophin-4</td>
</tr>
<tr>
<td>NTRK2</td>
<td>Neurotrophic Receptor Tyrosine Kinase 2</td>
</tr>
<tr>
<td>NTS</td>
<td>Nucleus of the solitary tract</td>
</tr>
<tr>
<td>p_ACT</td>
<td>p-values adjusted for correlated tests (R program)</td>
</tr>
<tr>
<td>p75NTR</td>
<td>p75 neurotrophin receptor</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>PLCγ</td>
<td>Phospholipase Cγ</td>
</tr>
<tr>
<td>POMC</td>
<td>Proopiomelanocortin</td>
</tr>
<tr>
<td>PWS</td>
<td>Prader Willi Syndrome</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>SNPSpD</td>
<td>Single-Nucleotide Polymorphism Spectral Decomposition</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>TrkB</td>
<td>Tropomyosin receptor kinase B/ tyrosine receptor kinase B</td>
</tr>
<tr>
<td>UCP1</td>
<td>Uncoupling protein 1</td>
</tr>
<tr>
<td>UTR</td>
<td>Untranslated region</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
</tr>
<tr>
<td>WAGR</td>
<td>Wilms’ tumor, aniridina, genitourinary anomalies, and mental retardation syndrome</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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General Overview

Eating behaviours in adults and children have long been implicated in the development of weight regulation issues. Accordingly, eating behaviours are often studied in relation to body mass index (BMI), especially in clinical populations with obesity. Recent evidence suggests that eating behaviours, such as speed of eating and satiety responsiveness are heritable and likely under genetic control. In search of genetic determinants of eating behaviours, researchers have begun to refer to obesity and eating disorder literature to identify candidate genes.

Among the numerous obesity-associated genes, the brain-derived neurotrophic factor (BDNF) was of particular interest because preclinical evidence and rare mutations in humans have consistently provided support for its role as an anorexigenic factor. BDNF deficiency in animals and humans causes distinctive hyperphagic behaviours that lead to weight gain and obesity. Genetic association studies in humans have revealed that BDNF genetic polymorphisms contribute to both obesity and eating disorders. Further investigation revealed that genetic variations in the NTRK2 locus, which encodes BDNF’s receptor, TrkB, are also relevant to obesity and eating disorders. Therefore, BDNF and NTRK2 are potential candidate genes for investigation in both overeating and undereating behaviours and thus will be the focus of this thesis.

In the following review of literature, we will provide an overview of the genetics of eating behaviours. This will be followed by a section that focuses specifically on the role of BDNF and NTRK2. Due to the dearth of BDNF and NTRK2 literature on eating behaviours, references will be made to their role in obesity and eating disorder literature even though BMI and eating disorder diagnosis was not a primary outcome in the experimental section of this thesis.

The goal of the present study was to focus on the role of BDNF and NTRK2 on eating behaviours in young children (age 3-4) from the MAVAN cohort. As this was the first study of its kind to investigate the relationship between BDNF and NTRK2 genetic polymorphisms and eating behaviours in a young, non-clinical population, several important methodological choices were made. The objective of our research was to investigate a broad spectrum of eating behaviours in relation to these two genes. As
such, we included two measures of eating behaviours. The first was the Snack Delay Task – a behavioural measure of impulsive responding to a food cue. The second was the Child Eating Behaviour Questionnaire (CEBQ) – a psychometric measure that assesses a broad range of eating behaviour constructs based on parental-report. With regards to selecting genetic polymorphisms, we used a tagSNP approach to select a small number of genetic loci representative of overall variation around and within the BDNF locus. As the NTRK2 locus was too large for this approach, we employed a candidate SNP approach and selected SNP based on prior evidence in relation to BMI, obesity, or eating disorder. Results from this investigation will be hypothesis-generating and allow future research to determine associations with more specific dimensions of eating behaviour traits.
Chapter 1 Literature Review

This review will be organized into two sections. The first part will introduce the notion of eating behaviours and summarize current evidence on the genetic basis of eating behaviour traits. The second part will provide support for the role of the brain derived neurotrophic factor (BDNF) and its receptor, neurotrophic tyrosine receptor 2 (NTRK2), as candidate genes for eating behaviour research.

Part 1: Overview of childhood eating behaviours

In this section, we will provide an overview of various eating behaviours/appetitive traits, clinical implications of such behaviours, and the ways in which eating behaviours can be measured qualitatively or quantitatively in children. This will be followed by a brief review of the physiological basis of energy balance in relation to eating behaviours. Finally, we will discuss genetic influences on eating behaviours.

1.1 Clinical implications of eating behaviour traits in children

Poor eating habits are a common concern among parents of young children. Approximately 20-30% of children are perceived by their parents to have poor eating habits, though only a subgroup of those can be truly classified as having a feeding disorder (Kerzner, Milano et al. 2015). Children with severe pathological difficulties with eating can be diagnosed with “Feeding disorders of infancy and early childhood”, a type of eating disorder in the Diagnostic and Statistical Manual of Mental Disorders, Forth Edition (DSM-IV). Classification of specific types of feeding disorders from birth to consumption of solid food was developed by Chatoor in 2002 and her paper has been cited by many in the field (Chatoor 2002). Feeding disorders of infancy and early childhood has since been renamed “Avoidant/ restrictive Food Intake Disorder” (ARFID) in the recent update of DSM-5 in 2013. ARFID is characterized by selective eating or general refusal of foods (Nicely, Lane-Loney et al. 2014). This disorder is distinct from anorexia nervosa because ARFID patients do not have disturbed cognitions about weight/shape or a desire to lose weight. Based on a 11-year retrospective chart review in
Canada, the prevalence of ARFID among adolescents from eating disorders programs is 5% (Norris, Robinson et al. 2014).

At the other end of the spectrum, some children present with overeating tendencies and are at risk of overweight and obesity. Overeating tendencies are a public health concern, especially with the rising prevalence of childhood obesity. Depending on severity, children can be diagnosed with binge eating disorder (BED), which is characterized by recurrent episodes of consuming large amounts of food, often very rapidly. BED was added to the DSM-5 in 2013.

Individual differences in responsiveness to satiety and food cues are likely contributors to differential susceptibility to common obesity, which posits that specific appetitive traits at the individual level determine the risk of gaining weight in the presence of an obesogenic environment (Carnell and Wardle 2008). Since the prevalence of childhood obesity is increasing, maladaptive appetitive traits might be expected to emerge early in life. Appetitive trait assessments in children have reported correlations between eating behaviour at 3 months of age and BMI one year later (Quah, Chan et al. 2015). Children (16 months of age) with higher food responsiveness tend to eat more frequently and children with lower satiety responsiveness tend to eat larger meals (Syrad, Johnson et al. 2016). Overweight children (age 5-18) tend to eat faster (Barkeling, Ekman et al. 1992), have a greater intake of palatable food in the absence of hunger (Fisher, Cai et al. 2007), and are more vulnerable to triggers of overeating such as smell and taste of food (Jansen, Theunissen et al. 2003) as compared to leaner children. Moreover, Barkeling and colleagues (Barkeling, Ekman et al. 1992) reported that obese 11 year-old children did not display the typical eating rate declaration towards the end of a meal. These appetitive traits are likely causes of weight gain.

A longitudinal analysis by van Jaarsveld and colleagues (van Jaarsveld, Llewellyn et al. 2011) demonstrated that the association between appetitive traits and subsequent weight gain/loss is greater than the association between weight status and subsequent appetitive behaviours. This finding supports the notion that appetitive traits are susceptibility factors to weight gain/loss. The translational value of identifying these specific eating behaviours lies in the fact that modification of behaviours associated with eating such as self-control has been shown to prevent disordered behaviours such as
emotional eating (Zhu, Luo et al. 2014). Examples of intervention strategies that can correct eating behaviours include cognitive behaviour therapy, psychotherapy, pharmacological interventions, and appetite awareness (Wilfley, Vannucci et al. 2010).

In pediatric literature, the role of parents is often acknowledged as a potential determinant of children’s eating behaviour. There have been reports that bottle-feeding practices during early infancy can have long-term effects and influence child eating behaviour 6 years later (Li, Scanlon et al. 2014). Mothers who encourage bottle emptying can promote low satiety responsiveness in their child. Their child will be more likely to eat all the food on their plate, which is an indicator of poor self-regulation of food intake. Parents that exert high control over their children’s food consumption can unintentionally encourage their children to respond to external environmental cues over physiological regulated satiety cues. As a result, there is a negative correlation between parental control and a child’s ability for food intake compensation after preload consumption (Johnson and Birch 1994). Restricting children’s access to unhealthy foods also increases their preference for these foods (Faith, Scanlon et al. 2004). When these types of food become available, for example in a laboratory setting, the child tends to demonstrate greater intake and preferential selection to the food that is normally restricted (Fisher and Birch 1999, Jansen, Mulkens et al. 2007). Therefore, parental feeding practices have direct influence on the development of dysregulated eating behaviours in children. In addition, parents provide environments factors for their child that may predispose them to obesity such as food availability, portion size, and promotion of sedentary lifestyles.

Aberrant eating behaviours, even without a clinical diagnosis, contribute to the risk of developing issues with weight regulation in both adults in children. As such, research in the area of eating behaviours has also attracted attention in the field of nutrition because eating behaviours are related to diet quality. High risk eating behaviours in childhood and poor diet quality have been associated with deviations from optimal weight and an increased risk of developing chronic disease later in life as a consequence of being over or underweight.
1.2 Types of eating behaviours and how they are assessed

Eating behaviours or appetitive traits, as defined by Carnell and colleagues (Carnell, Benson et al. 2013), are enduring dispositions towards or against food. Examples of appetitive traits commonly investigated in eating behaviour literature include food-cue responsiveness (response to smell, taste, or sight of palatable food) and satiety responsiveness (response to physiological satiety signals such as gastric distension). Speed of eating, frequency of meals, and nutritional content of meals are other eating behaviours that have been investigated in prior work. In general, appetitive traits can roughly be grouped into homeostatic (e.g. satiety responsiveness) or hedonic (e.g. food responsiveness, reinforcing value of food) eating behaviours, though there is considerable behavioural and conceptual overlap between these two types of eating dispositions. Therefore, it is sometimes difficult to ascertain the underlying physiological mechanism that causes specific appetitive traits.

There are two possible means to measure appetitive traits: (1) behavioural tests in laboratory settings and (2) psychometric tests with use of questionnaires (reviewed in (Carnell, Benson et al. 2013)). Behavioural measures in a control laboratory setting generally measure three types of eating behaviours. The first type is food cue responsiveness and food approach behaviours. Children are assessed for behaviours such as eating in the absence of hunger, eating after exposure to visual or olfactory food cues, attentional bias towards food, and delay of gratification. The second type is satiety sensitivity and food avoidance behaviours. These behaviours can be quantified by measuring caloric consumption, caloric compensation after a preload meal or snack, and eating rate. The third type is food choice. These types of behavioural tests attempt to identify patterns of food preference, especially a liking towards energy-dense food. The subjective reward for consuming palatable food can also be quantified using behavioural economic principles and neuro-activation of reward-related areas such as the orbital frontal and insular cortex.

Behavioural measures in laboratory settings allow investigators to objectively observe and record eating behaviours, but such assessments arguably only capture cross-sectional behaviours. Psychometric eating behaviour questionnaires were developed to
capture stable eating behaviour traits. The two best known psychometric measures of eating behaviour are the Dutch Eating Behaviour Questionnaire (DEBQ) and the Three-factor Eating Questionnaire. These well-validated questionnaires were designed to measure external eating, emotional eating, and cognitive restraint. An alternative questionnaire specifically designed to assess normative eating behaviours as opposed to disordered behaviours in children is the Child Eating Behaviour Questionnaire (CEBQ) (Wardle, Guthrie et al. 2001). Studies using the CEBQ have shown that food approach and avoidant behaviours are not exclusively associated with obesity state; they are also good indicators of general adiposity (i.e. higher or lower weight categories) (Webber, Hill et al. 2009). Other psychometric measures of eating behaviours in children include the Children’s Eating Behaviour Inventory (CEBI) and the Children’s Eating Attitude Test (ChEAT), though the latter mainly assesses eating pathology such as dieting and bulimia. It should be noted that both behavioural and psychometric measures of appetitive traits are subjected to social desirability bias, whereby subjects or respondents are more likely to behave or respond in a way that is viewed favourably.

1.3 Physiological basis of energy balance, food intake, and eating behaviours

To appreciate the biological underpinnings of eating behaviours it is imperative to have a general understanding of the physiological basis of food intake and energy balance. Here, we discuss short- and long-term determinants of homeostatic regulation of food intake as well as mechanisms that regulate the reward value of food.

1.3.1 Short-term determinants of eating: Hunger and Satiety

The perception of hunger and the subsequent decision made to initiate a meal is poorly understood (Guyenet and Schwartz 2012). One peptide that is implicated in the stimulation of feeding and initiation of a meal is ghrelin. Ghrelin is secreted from the gastric mucosa before the onset of a meal and decreases rapidly during and after a meal. However, mice without ghrelin do not have altered eating patterns, which suggest that this peptide may not be crucial in the process of meal initiation (Wortley, Anderson et al.
As mentioned by Guyenet and colleagues (Guyenet and Schwartz 2012), hunger is likely the result of complex interactions between genetic, social, learned, environmental, circadian, and hormonal cues. The biological process underlying the perception of hunger remains to be elucidated.

Satiety is defined as the physiological state when the ingestion of food is inhibited by feelings of fullness. Satiation signals are secreted from the gastrointestinal tract after ingestion of food. After food enters the stomach, gastric distension is sensed by mechanoreceptors neurons and the signals are relayed to the hindbrain through vagal afferent and spinal sensory nerves. Satiation peptides are also released from the intestinal enteroendocrine cells through vagal afferent fibres, though some enter the brain via the circulatory system and exert their effects directly (Cummings and Overduin 2007). Cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) are examples of such satiety peptides. CCK is secreted by duodenal and jejunal mucosa in response to ingestion of fat and protein (Cummings and Overduin 2007). Its release activates vagal afferents and is able to rapidly decrease food intake. Therefore, disrupted CCK signaling impairs satiety response and can cause meal size to increase. GLP-1 and amylin, two of many satiety-inducing peptides released by the pancreas, also behave in a similar manner to CCK (Woods, Lutz et al. 2006). These afferent satiation peptides are initially processed in the nucleus of the solitary tract (NTS) of the hindbrain. Animal models with surgically disconnected hindbrain and forebrain are able to terminate meals normally but are unable to spontaneously initiate food intake (Grill and Norgren 1978, Grill and Smith 1988), suggesting that the hindbrain may be sufficient for regulating satiety. Adiposity signals (e.g. leptin) act on hypothalamic neurons and project onto the hindbrain to modulate the sensitivity of NTS neurons to satiety signals (e.g. CCK) thereby affecting food intake (Morton, Blevins et al. 2005).

1.3.2 Long-term energy balance

Long-term regulation of food intake and energy balance involves another interrelated system of brain circuits and peripheral signals. One of the most well-known hormones related to energy balance is leptin. Leptin is a hormone secreted by adipocytes and is thus an indicator of the amount of energy stored as fat in the body. It is an important homeostatic signal that relays negative feedback signals to the brain to regulate
adiposity by limiting food intake and increasing energy expenditure (Friedman 2010). Leptin receptors are found in hypothalamic areas of the brain such as the arcuate nucleus (ARC), paraventricular nucleus, and ventromedial hypothalamic nucleus (Ghamari-Langroudi, Srisai et al. 2011). The ARC integrates a number of energy balance signals including leptin, insulin and ghrelin. Leptin and insulin can stimulate proopiomelanocortin (POMC) neurons and release melanocortin peptides such as α-MSH, which acts as an anorexigenic factor to inhibit food intake (Sobrino Crespo, Perianes Cachero et al. 2014). Orexigenic neurons expressing neuropeptide Y (NPY), agouti-related peptide (AgRP) or orexin, inhibit POMC neurons to stimulate feeding behaviour and hyperphagia in response to lost fat or weight loss (Millington 2007). Together, POMC and NPY/AgRP neurons project onto the amygdala, the nucleus accumbens, and various hypothalamic brain regions to regulate energy balance (Millington 2007). Leptin also exerts effects on extrahypothalmic brain regions such as the nucleus of the solitary tract (NTS) (Kanoski, Alhadeff et al. 2014) and midbrain dopamine neurons, which are important in modulating reward and motivation (Hommel, Trinko et al. 2006).

1.3.3 Reward value of food

Another aspect that can affect food intake is the reward value of food. Olfactory, gustatory, and environmental cues from properties of food can stimulate eating and reinforce behaviours related to the ingestion of food that is perceived as rewarding (Sclafani 2004). Certain properties of food such as caloric density, texture, percentage of fat content, and carbohydrates can affect palatability of food, which is defined as the hedonic value associated with food (Sorensen, Moller et al. 2003). Palatability of food can, in turn, affect meal size and thus overall energy balance. The rewarding value of food is evaluated and reinforced by a number of brain regions as well as neural circuits within the corticolimbic, hypothalamic and midbrain areas (Kenny 2011). Dopaminergic neurons of the ventral tegmental area and substantia nigra in the midbrain are thought to be important in determining the “wanting” or motivational reward value of a food (Berridge 1996). Meanwhile, opioid peptides are implicated in the “liking” or hedonic value/palatability of a food (Berridge 1996). Some have hypothesized that dopamine and opioid signaling may interact with melanocortin signaling to influence homeostatic circuits (Olszewski, Wirth et al. 2001, He, Liu et al. 2015). Another system that may influence
reward value of food is the endocannabinoid system because agonists of cannabinoid receptors have been shown to selectively increase the consumption of palatable food (Foltin, Fischman et al. 1988), whereas antagonists suppresses palatable food consumption and causes weight loss (Christensen, Kristensen et al. 2007). As the lateral hypothalamic area participates in both food reward processing and energy homeostasis, food reward neural circuits likely interact with homeostatic circuits but the exact physiological mechanism is still under investigation.

1.4 Other factors that affect eating behaviours

In addition to hunger, satiety, long-term regulation of energy balance, and reward value of food, cognitive process and sensory factors are two other aspects can influence food intake. The conscious control of food intake is a cognitive process that can override internal homeostatic control of eating (Rolls 2007). A common example of conscious control of food intake is restraint eating and dieting; though other factors can also affect cognitive control of eating (Johnson, Pratt et al. 2012). For instance, Avoidant/Restrictive Food Intake Disorder (ARFID) can be triggered by fear of choking on food (Fisher, Rosen et al. 2014). Sensory factors including taste, smell, texture, and sight can also make food more or less palatable and thus affect eating behaviour (Rolls 2007). These factors can be affected by the portion size, variety, and availability of food.

Overall, the biological basis of short- and long-term regulation of food intake is complex. Even though substantial progress has been made to understand energy balance, the exact mechanisms underlying specific eating behaviours such as speed of eating and fussy eating are unclear. Neural circuits involved in eating behaviour are similar in humans, but individuals respond differently to environmental cues such as parental feeding habits and social influences. Some develop eating disorders and/or obesity, while others remain healthy. This suggests that there are biological factors that affect our susceptibility to these pathological disorders of eating. The following section will discuss our current understanding in the genetics of eating behaviour traits.
1.5 Genetics of eating behaviour traits

Much progress has been made to understand the physiological process of food intake and energy balance as outlined in the previous section. Another biological aspect that may help to elucidate physiological underpinnings of specific eating behaviours such as speed of eating and satiety responsiveness is genetics. This area of research has generated much interest in recent years because the heritability of such eating behaviour traits has been confirmed by a number of studies. As well, studies of pathological disorders of eating such as obesity and eating disorders have made significant headway in identifying genetic determinants of body weight. Eating behaviour is a susceptibility factor for these diseases and may be a mediator of the effects of the disease-associated genes. Here, we will discuss the heritability of eating behaviours, introduce obesity and various eating disorders, and provide an overview of current research on eating behaviour-associated genes.

1.5.1 Heritability of eating behaviours

The heritability of body weight has been long-established (Maes, Neale et al. 1997) and recent evidence has demonstrated that appetitive traits, such as speed of eating, are also under considerable genetic control (Fisher, Cai et al. 2007, Carnell, Haworth et al. 2008, Llewellyn, van Jaarsveld et al. 2008). Heritability \(h^2\) refers to extent that a trait is influenced by genetic factors and can range from \(h^2 = 0\) (no genetic influence) to \(h^2 = 1.00\) (completely influenced by genetics). A twin study in children 3 months of age \(n=4804, 2402\) pairs of twins), reported high heritability estimates for slowness in eating \(h^2 = 0.84\), and satiety responsiveness \(h^2 = 0.72\), and moderate estimates for food responsiveness \(h^2 = 0.59\), and enjoyment of food \(h^2 = 0.53\) (Llewellyn, van Jaarsveld et al. 2010). Another study in the same cohort of children estimated that approximately one-third of genetic effects that influence appetitive traits such as slowness in eating, satiety responsiveness, and appetite size also influence adiposity and weight (Llewellyn, van Jaarsveld et al. 2012). Furthermore, the concordance between monozygotic twins appears to be higher than dizygotic twins for under and overeating, eating too fast, food fussiness, and eats between meals, which means that genetics likely has an important role in eating behaviour phenotypes (Dubois, Diasparra et al. 2013). This study also reported that
appetite-related behaviours become more sensitive to environmental influences as children grow older (Dubois, Diasparra et al. 2013). Therefore, in addition to environmental factors that affect our cognitive control of eating or sensory perceptions of food, genetic factors are important determinants of eating behaviours.

1.5.2 Eating behaviour-associated genes

Since the heritability of specific eating behaviours has been established, researchers have begun to uncover genes that may contribute to eating behaviour traits. A commonly cited example is the *TAS2R38* gene, which encodes bitter taste receptors. Haplotypes of this gene influence bitter taste sensitivity thereby affecting preference for bitter tasting fruits and vegetables (Kim, Jorgenson et al. 2003). Interestingly, this gene does not appear to have a direct effect on BMI (Sausenthaler, Rzehak et al. 2009). The effect of other taste receptor associated genes on eating behaviours is under investigation.

In addition to taste perception, genes encoding neuroendocrine signals involved in hunger and satiety are likely contributors to eating behaviour traits. A common variant in the gene encoding ghrelin (*GHRL* Leu72Met), a peptide that promotes hunger, has been associated with obesity (Hinney, Hoch et al. 2002, Korbonits, Gueorguiev et al. 2002) and binge eating (Monteleone, Tortorella et al. 2007). Genetic variants encoding leptin and CCK have also been studied in relation to snacking behaviours and meal size (de Krom, van der Schouw et al. 2007).

Another approach that has been used to identify candidate genes for eating behaviour traits is to investigate obesity-associated genes. Although the biological cause of specific eating behaviours in children is unclear (Carnell and Wardle 2008), studies in the field of obesity genetics have begun to uncover genes associated with appetitive traits. (Konttinen, Llewellyn et al. 2015) recently showed that appetitive traits mediate the association between obesity-related genetic variants and anthropometric measures such as BMI and waist circumference in adults. Specifically, they demonstrated that the effect of a polygenic risk score (comprising of 90 BMI-related loci) was mediated by uncontrolled eating and emotional eating behaviours. A study with a similar design in a cohort of 10-year-old children reported that low satiety responsiveness was an intermediate
behavioural phenotype associated with genetic predisposition to obesity (Llewellyn, Trzaskowski et al. 2014).

Amongst the numerous obesity-associated gene variants, SNPs in the locus of FTO, the fat mass and obesity-associated gene, have provided the most robust evidence for its contribution to polygenic obesity. The functional mechanism for this gene on energy homeostasis is unclear but certain FTO genotypes appear to contribute to obesity risk through reducing satiety responsiveness (Wardle, Carnell et al. 2008), increasing energy intake (Cecil, Tavendale et al. 2008), and preference for energy dense foods in children (Tanofsky-Kraff, Han et al. 2009). Genes in the reward pathways such as dopamine-related genes have also been linked to increase BMI and reward seeking food intake behaviours. For instance, adolescents with the DRD2 Taq1A allele show greater emotional eating behaviours in relation to high parental psychological control (van Strien, Snoek et al. 2010). Literature on other obesity-related genetic variants involved in energy homeostasis mechanisms, such as PPARG, MC4R, and ADRB, have also demonstrated an association with eating behaviours in children. This topic has been reviewed by Cecil and colleagues (Cecil, Dalton et al. 2012).

Overall, genetic studies on polygenic obesity have revealed numerous obesity-associated genetic variants but the functional mechanism of most genes is unknown. Studies are beginning to connect these genes to specific patterns of eating and appetitive traits. This approach will help to shed light on the role of genetic variation on behaviours that predispose to obesity and eventually uncover molecular pathways that regulate these eating behaviours.

In summary, the elucidation of genetic factors that influence eating will help to uncover the underlying biology of specific eating behaviours. This area of study will have important implications on the prevention and treatment of childhood obesity and eating disorders. Dietary interventions can be personalized based on a child’s genetic predisposition. Such interventions can normalize a child’s eating behaviour to promote healthy diets and growth.
Part 2: BDNF and NTRK2 as candidate genes for eating behaviours

The brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors, which is critical for the survival and differentiation of neurons during development. BDNF regulates neuronal plasticity mechanisms that modulate synaptic morphology, synaptic strength, and number of connections for neurotransmission (Park and Poo 2013). These effects are achieved by the binding of BDNF to the tropomyosin receptor kinase B (TrkB). TrkB, also known as neurotrophic tyrosine kinase receptor type 2 (NTRK2), is encoded by the NTRK2 gene in humans.

BDNF is also a well-known anorexigenic factor in eating behaviour literature. With the recent interest in identifying genetic determinants of eating behaviours, the goal of the second part of this literature review is to provide support for the use of BDNF and its receptor, NTRK2 in candidate gene association studies for eating behaviours. Preclinical and clinical studies on BDNF and NTRK2 in relation to eating and weight regulation will be outlined and three potential mechanisms by which BDNF can affect food intake regulation will be proposed. Due to the dearth of literature on BDNF and NTRK2 on eating behaviours in the absence of a clinical diagnosis, the role of BDNF and NTRK2 genetic polymorphisms in both adult and pediatric obesity and eating disorder literature will be reviewed. Finally, the molecular mechanisms underlying the general effects of BDNF-TrkB will be outlined.

1.6 Disrupted BDNF-TrkB signaling and its effect on eating and weight regulation

1.6.1 Animal models

Preclinical animal models consistently provide support for the role of BDNF as an anorexigenic factor. BDNF depletion via the use of BDNF+/− mice (Lyons, Mamounas et al. 1999, Kernie, Liebl et al. 2000), mice with BDNF conditional knock out (Rios, Fan et al. 2001, Tong, Ye et al. 2007), mice with BDNF knockdown in the ventromedial nucleus of the hypothalamus (Unger, Calderon et al. 2007), rats with BDNF knockdown in the
ventral tegmental area (Cordeira, Frank et al. 2010, Fanous, Terwilliger et al. 2011), and mice with a truncated long BDNF 3′UTR (Liao, An et al. 2012) all gain weight. Hyperphagia resulting from satiety deficits (Fox, Biddinger et al. 2013) appear to be the cause of weight gain as pair feeding is able to prevent excessive weight gain in these rodent models. Leptin resistance and high insulin levels have also been observed in these animals (Kernie, Liebl et al. 2000). A similar phenotype is observed in mice with hypothalamic or hindbrain TrkB deficiency (Ozek, Zimmer et al. 2015).

Interestingly, these hyperphagic and obese phenotypes in mice can be reversed. Chronic intracerebroventricular delivery of BDNF to BDNF+/- mice, which have a 50% reduction in hypothalamic expression of BDNF, can rescue the hyperhagic and obese phenotype (Kernie, Liebl et al. 2000). Moreover, peripheral administration of TrkB agonist can suppress appetite and decrease body weight in a dose-dependent manner as shown in various murine models of obesity including, diet-induced obesity, polygenic obesity, and monogenic leptin receptor deficiency (Tsao, Thomsen et al. 2008). These animals regained weight after termination of TrkB agonist administration. Therefore, there is strong support for the involvement of BDNF-TrkB signaling on food intake at the preclinical level. Specifically, reduced central BDNF signaling results in hyperphagic behaviour and weight gain.

A 2003 study by Xu and colleagues (Xu, Goulding et al. 2003) demonstrated that mice deprived of food had reduced BDNF mRNA levels in the ventromedial hypothalamus. Prior literature has shown that food deprivation increases the expression of appetite promoting orexigenic factors, while decreasing the expression of appetite suppressing anorexigenic factors (Brady, Smith et al. 1990). Together, these observations suggest that BDNF expression could pose as a susceptibility factor for binge eating behaviours.

According to a rodent study by (Monteggia, Barrot et al. 2004), loss of BDNF in early development produced more severe phenotypes than in adults, suggesting that the role of BDNF in the developing brain may be different from its role in the adult brain. Body weight was not affected in their mice with conditional BDNF knockout in forebrain regions, but more severe impairments in hippocampal-dependent learning were observed
in young mice as compared to adult. This finding supports the need to investigate the role of BDNF early in life.

### 1.6.2 Rare syndromes associated with altered BDNF/TrkB signaling in humans

The two most commonly cited case reports of rare BDNF-related mutations and non-syndromic obesity confirm that BDNF plays an important role in food intake regulation. Yeo et al. (2004) first described an 8-year-old boy with a de novo missense mutation in the gene encoding BDNF’s receptor, TrkB, who displayed severe hyperphagia and obesity (Yeo, Connie Hung et al. 2004). The loss-of-function mutation caused by a single amino acid substitution (Y722C) impaired activation of the TrkB receptor and all of its downstream signaling effects. Similarly, Gray et al. (2006) identified an 8-year-old girl, who had a de novo chromosomal inversion, 46,XX,inv(11)(p13p15.3), a region encompassing the BDNF gene (Gray, Yeo et al. 2006). The loss of one functional BDNF allele was also associated with lower levels of serum BDNF as compared to age- and weight-matched individuals. The clinical features of both of these patients include severe early-onset obesity, hyperphagia, and neurobehavioural pathology including impaired cognitive function, developmental delay and hyperactivity.

A naturally occurring model of BDNF haploinsufficiency causing syndromic obesity in humans is the Wilms’ tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome. This rare disorder, with an estimated prevalence of 1 per 500,000 to 1,000,000 people, is caused by heterozygous contiguous gene deletions on chromosome 11. The syndrome is defined by having deletions of WTI and PAX6 (in the 11p13 region), but can involve additional gene deletions nearby (Han, Liu et al. 2008). A number of case reports (Marlin, Couet et al. 1994, Tiberio, Digilio et al. 2000, Amor 2002, Gul, Ogur et al. 2002) have described severe hyperphagia and obesity in people with deletions that include the BDNF locus at 11p14.1, which is positioned approximately 4 Mb centromeric from WTI and PAX6. The most compelling evidence for BDNF’s role in overeating and obesity was reported by Han and colleagues. They discovered that 100% of WAGR patients with truncated BDNF gene develop obesity by 20 years of age (Han, Liu et al. 2008). In comparison, only 20% of WAGR patients with intact BDNF developed obesity (Han, Liu et al. 2008).
Prader Willi Syndrome (PWS) is another form of syndromic obesity. Patients with PWS have rapid onset of weight gain and severe hyperphagia early in life (Dykens, Maxwell et al. 2007). Although the pathogenic cause of PWS is on chromosome 15, researchers question the potential role of BDNF in the pathophysiology of PWS because of the distinctive energy homeostasis dysfunction in these patients. In 2010, Han and colleagues reported lower levels of circulating serum and plasma BDNF in PWS (n=13) as compared to lean healthy controls (n=13), which they believe to be reflective of cerebral BDNF output (Han, Muehlbauer et al. 2010). Their results suggest that BDNF deficiency may be the cause of altered satiety and subsequent weight gain in PWS but the connection between the pathogenic genetic region on chromosome 15 and BDNF remains unclear.

1.7 Potential effects of BDNF-TrkB on eating related circuitry

The effects of BDNF on weight regulation and food intake have been reviewed by many over the last few years (Nakazato, Hashimoto et al. 2012, Waterhouse and Xu 2013, Xu and Xie 2016). Ooi and colleagues (Ooi, Kennedy et al. 2012) previously proposed an integrated model with two pathways to classify BDNF’s role on weight and food intake regulation. The first pathway highlights BDNF’s action as a satiety factor in brain areas relevant to the homeostatic control of energy balance. The second pathway considers BDNF’s upstream effect on key monoamine pathways that influence hedonic feeding. These two pathways, as presented visually in Figure 1, will be discussed in turn. Lastly, we propose a third pathway based on BDNF’s neurotrophic effects on brain structure, connectivity, and function.
Figure 1 An integrative model for the role of BDNF in the risk of obesity, with or without psychiatric comorbidity as presented in (Ooi, Kennedy et al. 2012)

Note: The figure above was reprinted with permission. The original figure is from Ooi, C. L., Kennedy, J. L., & Levitan, R. D. (2012). A putative model of overeating and obesity based on brain-derived neurotrophic factor: Direct and indirect effects. Behavioral Neuroscience, 126(4), 505-514. http://dx.doi.org/10.1037/a0028600. Figure 2, p. 510. Publisher: American Psychological Association (APA). According to APA’s copyright and permission policy (http://www.apa.org/about/contact/copyright/index.aspx), no formal request for permission from APA or the author of the paper is required if less than three figures or tables from a journal article or book is reprinted.
1.7.1 Effect on homeostatic mechanisms

The primary function of the hypothalamus is to maintain homeostatic regulation for a number of physiological functions such as temperature, sleep, mood, and food intake. BDNF is synthesized in several key areas of the hypothalamus including the paraventricular nucleus, and the ventralmedial, dorsomedial, and lateral hypothalamus (Cordeira and Rios 2011). Likewise, BDNF’s receptor, TrkB, is widely expressed throughout the hypothalamus (Rios 2013). In animals, selective infusion of endogenous BDNF to the paraventricular nucleus, ventromedial nucleus, or dorsomedial hypothalamus in wild type rodents (Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Bariohay, Tardivel et al. 2008) or administration of TrkB agonist at the ventromedial hypothalamus (Tsao, Thomsen et al. 2008) reduced food intake and led to weight loss. In contrast, selective deletion of TrkB from the aforementioned hypothalamic nuclei induced hyperphagic obesity (Liao, Li et al. 2013). One study specifically demonstrated that deletion of TrkB from cholecystokinin (CCK)-expressing neurons in the hypothalamus caused hyperactivity of the hypothalamic–pituitary–adrenal axis and mature-onset obesity, suggesting that TrkB signaling acts as a feedback inhibitor to the hypothalamus (Geibel, Badurek et al. 2014).

In addition, BDNF has connections to extrahypothalamic regions involved in energy regulation. Infusion of BDNF to the dorsal vagal complex of the hindbrain, which integrates energy status signals from the gastrointestinal system, also resulted in decreased food intake and weight loss (Bariohay, Roux et al. 2009). BDNF has been shown to work as a downstream effector of the leptin-melanocortin system in the brainstem to regulate food intake in response to activity levels of the melanocortin receptors (Bariohay, Roux et al. 2009). Infusion of BDNF in rats counteracted the hyperphagic effect of MC4R antagonist delivery to the fourth ventricle. Interestingly, Ozek and colleagues (Ozek, Zimmer et al. 2015) showed that deletion of TrkB in the hypothalamus of mice resulted in weight gain and impaired glucose homeostasis, while reduction of TrkB in the hindbrain resulted in hyperphagic activity but no alterations in body weight. This observation may indicate that endogenous BDNF/TrkB signaling in the hypothalamus regulates central metabolic control (e.g. energy expenditure, glucose homeostasis, etc.), whereas endogenous BDNF/TrkB signaling in the hindbrain modulates food intake behaviours. However, BDNF delivery to the hindbrain has also been reported...
to elevate core body temperature, which is a correlate of energy expenditure (Spaeth, Kanoski et al. 2012).

BDNF appears to have hypoglycemic effects as BDNF-deficient obese mice develop hyperglycemia and impaired glucose tolerance (Nakagawa, Ono-Kishino et al. 2002). In several animal models of diabetes and/or obesity, peripheral administration of BDNF is able to ameliorate the diabetic and/or obese phenotype. Likewise, administration of TrkB agonist in mice induces hypoglycemia (Tsao, Thomsen et al. 2008). A reciprocal relationship has also been reported. Hypoglycemia in developing rats suppressed BDNF and TrkB expression in the prefrontal cortex (Rao, Ennis et al. 2016). Therefore, BDNF expression affects and responds to glycemic changes in the body.

In addition to food intake, energy homeostasis involves the control of energy expenditure. This component of energy balance is related to levels of BDNF as well. Thermogenesis from brown adipose tissue is a key form of energy expenditure that converts fatty acids to heat in response to stimuli such as cold exposure and overeating. The process is mediated by the expression of uncoupling protein 1 (UCP1) (Trayhurn 2016). Peripheral and intracerebroventricular injection of BDNF in food-deprived diabetic mice restores thermogenesis (Nonomura, Tsuchida et al. 2001). Direct injection of BDNF to the paraventricular hypothalamus in rats increases the expression of UCP1 in brown adipose tissue, thereby increasing energy expenditure and weight loss (Wang, Bomberg et al. 2007). In contrast, however, BDNF injection to another part of the hypothalamus, the ventralmedial hypothalamus, increases spontaneous physical activity without altering UCP1 expression (Wang, Bomberg et al. 2010). These results suggest that BDNF has very specific effects in different parts of the brain even within the hypothalamus. TrkB mRNA is also upregulated in the hippocampus, caudate, and cerebellum of rats after running (Neeper, Gomez-Pinilla et al. 1996, Widenfalk, Olson et al. 1999, Klintsova, Dickson et al. 2004), whereas deprivation of physical activity reduces BDNF and TrkB mRNA (Widenfalk, Olson et al. 1999). There is evidence that stress-induced downregulation of BDNF mRNA can be reversed with physical activity (Russo-Neustadt, Ha et al. 2001).
1.7.2 Effect on hedonic eating mechanisms

Unlike the homeostatic system, the hedonic eating system operates does not rely on metabolic signals (Yu, Vasselli et al. 2015). Rather, hedonic eating is guided by reward value of food and is modulated by factors such as emotion and stress. Mouse models with deficient BDNF-TrkB signaling develop hyperphagic behaviours, which are more pronounced when fed on high-fat diets (Fox and Byerly 2004, Cordeira, Frank et al. 2010). This led to the hypothesis that BDNF plays a role in the motivation for palatable food consumption. The dopaminergic and serotonergic systems are the two main pathways that make up the reward system of the brain. BDNF appears to affect both of these systems and this has been reviewed in detail by Ooi and colleagues (Ooi, Kennedy et al. 2012).

BDNF and its receptor TrkB are expressed in the mesolimbic dopamine system, which has been implicated in reward-seeking behaviours such as drug addiction (Pierce and Kumaresan 2006). Both hyperactivity and hypoactivity of the dopaminergic circuitry have been linked to overeating (Horstmann, Fenske et al. 2015). The mesolimbic pathway connects the ventral tegmental area (VTA) of the midbrain to the nucleus accumbens in the ventral striatum. Release of BDNF from the nucleus accumbens, promotes addictive behaviours in rats (Graham, Edwards et al. 2007). Mice with selective depletion of BDNF in the VTA but normal expression of BDNF in the hypothalamus had higher caloric intake than controls when fed high-fat diets ad libitum (Cordeira, Frank et al. 2010). No difference in food intake was observed between mutants and controls when fed standard chow ad libitum. A similar observation was noted in a conditional TrkB knockout study (Mason, Lobo et al. 2013). Under a standard chow diet, mice with complete loss of TrkB signaling in dopamine-1 receptor neurons gained more weight than wild-type controls, but not as a result of higher food intake. The authors attributed the weight gain to reduced energy expenditure, as there was no difference in locomotor activity between the two groups. In contrast, these mice dramatically increased their food intake when fed high-fat diet and gained significantly more weight than wild-type controls. Taken together, results from this study suggest that BDNF-TrkB signaling regulates food intake and body weight in response to diet. Intake of palatable food decreases BDNF expression in the VTA and creates a positive feedback loop that promotes overeating. BDNF can regulate hedonic feeding via positive modulation of the
mesolimbic dopamine system, which plays a fundamental role in food reward processes (Berridge 2009), binge eating behaviours (Naef, Pitman et al. 2015) as well as impulsivity (Cardinal, Winstanley et al. 2004). BDNF-TrkB signaling may decrease the reward of highly palatable food, thereby reducing food consumption.

BDNF is also known to affect the development of the serotonin system (Vaidya, Marek et al. 1997). Serotonin, in turn, is a neurotransmitter involved in several processes relevant to food intake such as satiety, impulsivity, general response inhibition and mood (Cunningham, Fox et al. 2011). Unlike BDNF and dopamine, there are only a few studies examining the relation between BDNF and serotonin on eating behaviours and weight status. Generally, serotonin deficiency is linked to hyperphagia and weight gain (Breisch, Zemlan et al. 1976).

In summary, BDNF appears to have an indirect role on food intake regulation by modulating neurotropic support to monoamine pathways that influence hedonic eating.

1.7.3 Modification of brain structures and functions relevant to eating

The control and regulation of food consumption is not only affected by homeostatic and hedonic mechanisms. Over the last decade, neuroimaging studies in obesity have provided insights into the neurobiological underpinnings of appetitive behaviours and body weight in humans by identifying anatomical brain changes in relation to BMI (see review by (Carnell, Gibson et al. 2012)). Cognitive neuroscientists have also joined the pursuit of uncovering brain circuits that regulate eating and have discovered neurocognitive correlates with weight status and eating behaviour (Vainik, Dagher et al. 2013, Liang, Matheson et al. 2014).

Since BDNF is widely expressed throughout the brain and CNS, its regulatory effects on food intake may not be restricted to homeostatic or hedonic eating mechanisms. As a neurotrophic factor, BDNF can affect brain structure, connectivity, and function. A neurodevelopmental hypothesis was previously proposed to account for BDNF-associated psychiatric disorders such as anorexia nervosa (Connan, Campbell et al. 2003) and schizophrenia (Nieto, Kukuljan et al. 2013). This hypothesis posits that mental illness has origins in disturbed development and maturation of the central nervous system that leads
to or predisposes to disease. The disruption is likely caused by genetic factors in combination with environmental stressors. In line with this hypothesis, BDNF may have neurotrophic effects on brain structure, connectivity, and function and these changes may be relevant to disordered eating pathophysiology.

A few studies have investigated neural correlates of eating behaviours using imaging techniques (reviewed in (Carnell, Benson et al. 2013)), but research in this area is still in its infancy. Since the design of most studies are cross-sectional the causal direction, i.e. whether these neural correlates contribute to or result from increased body weight, cannot be ascertained. Nevertheless, neurotrophic properties of the BDNF-TrkB signaling pathway may play a role in these body weight- or appetitive trait- related neural correlates.

**BDNF** genetic polymorphisms have been associated with a number of neuromorphological brain changes; most notably are volumetric changes in the hippocampus, which is an area of the brain responsible for learning, memory, and inhibitory control. A meta-analysis by (Hajek, Kopecek et al. 2012), reported that healthy carriers of the Met allele of the functional rs6265 (Val66Met) polymorphism showed bilateral hippocampal volume reduction. The Met allele of this genetic variant is associated with reduced BDNF availability. Another neuroimaging study with 111 normal healthy adults found grey matter volume deficits in the prefrontal cortex, which is responsible for planning and higher order cognitive functioning (Pezawas, Verchinski et al. 2004). Since these associations were independent of sex and age, the authors hypothesized that the changes in cortical morphology occurred before adulthood. To address this hypothesis Toro and colleagues conducted a study with 331 adolescents (age 12-19) and reported that Met carriers had lower lobar volume, lower hippocampal volume, and lower amygdalar volume (Toro, Chupin et al. 2009). In addition, grey matter in all brain lobes (i.e. frontal, parietal, occipital, and temporal) was lower in the rs6265 Met-carrier group as compared to the Val/Val group. Results from this study suggest that the rs6265 polymorphism has global effects on brain volume development early in life. Indeed, mouse models have demonstrated a critical role for BDNF during brain development as cortical neuron size and dendritic complexity is reduced in BDNF-deficient mice (Gorski, Zeiler et al. 2003, Baquet, Gorski et al. 2004). The impact of the
rs6265 polymorphism on white matter structure, however, is controversial because the Val allele has been associated with reduced fibre integrity as measured by fractional anisotropy in three independent studies (Chiang, Barysheva et al. 2011, Tost, Alam et al. 2013, Ziegler, Foret et al. 2013). In addition to rs6265, one study has reported an association between brain morphology and other BDNF polymorphisms including rs16917204 (11757G/C), rs56164415 (270C/T), and rs11030101 (−633T/A) in patients with schizophrenia and/or healthy subjects (Agartz, Sedvall et al. 2006).

Byerly and colleagues specifically investigated the role of BDNF/TrkB signaling in the formation of hypothalamic circuits during embryonic and postnatal development (Byerly, Swanson et al. 2013). Transient chemical inhibition of TrkB in mice embryos, preweaning pups, and adults all led to long-lasting effects on body weight. The magnitude of change, however, depended on the timing of the treatment. TrkB inhibition in embryos during the formation of hypothalamic nuclei and postnatally during the formation of synaptic projections between these nuclei had a more notable effect on body weight increase as compared to treatment during other periods of development. These observations suggest that TrkB signaling is especially important during hypothalamic circuitry development pre- and postnatally.

BDNF can also induce morphological changes at the level of the neuron. BDNF and its associated proneurotrophin, proBDNF, binds to TrkB and p75NTR receptors, respectively, to induced axonal growth or pruning in the central nervous system. Activity-dependent events can promote BDNF’s action on axonal branch formation and maintenance (Jeanneteau, Deinhardt et al. 2010). BDNF increases the number of primary dendrites (Suzuki, Numakawa et al. 2004), while proBDNF causes a collapse in neurite outgrowth of primary neurons (Sun, Lim et al. 2012). Therefore, the ratio of BDNF and proBDNF as well as TrkB and p75NTR can mediate neuronal growth and degeneration. At an even smaller scale, Tyler and colleagues (Tyler and Pozzo-Miller 2001) reported that BDNF induces morphological changes within synaptic structures. Treatment of neurons from the hippocampus with BDNF increases synapse numbers and this effect is attributed to the activation of the cyclic AMP (cAMP)-dependent pathway (Ji, Pang et al. 2005). In comparison, proBDNF in cultured hippocampal neurons reduced the density of dendritic spines (Koshimizu, Kiyosue et al. 2009). This means that BDNF mediates
bidirectional structural changes in the spines at excitatory synapses. Moreover, BDNF-TrkB and proBDNF-p75NTR signaling modulates the two forms of activity-dependent synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD), respectively (Woo, Teng et al. 2005). LTP and LTD, in turn, is responsible for long-term enhancement or weakening of synaptic efficiency, which is thought to underlie cognitive function, most notably learning and memory.

At the behavioural level, BDNF has the potential to affect the cognitive control of eating, which is defined as the regulation of eating behaviour by cognitive processes such as response inhibition to achieve weight loss or prevent weight gain (Wardle 1988, Westenhoefer, Broeckmann et al. 1994). In fact, BDNF polymorphisms have already been linked to several neurocognitive domains, including learning, memory, and planning abilities (Hariri, Goldberg et al. 2003, Goldberg, Iudicello et al. 2008, Carlino, De Vanna et al. 2013). Obesity is associated with a number of neurocognitive deficits and impulsivity/inhibitory control has been reported to be the most robust neurocognitive correlate of obesity/BMI in adults (Vainik, Dagher et al. 2013, Liang, Matheson et al. 2014). Several lines of study suggest that BDNF contributes to impulsive traits in various ways. Rodent studies have reported that serotonin reuptake inhibitors suppress impulsive action by increasing protein levels of BDNF in the medial prefrontal cortex (Tsutsui-Kimura, Yoshida et al. 2015). Furthermore, mice with TrkB-receptors knocked-out in the forebrain displayed greater impulsive reactions and behaviours (Zorner, Wolfer et al. 2003). Researchers have postulated that decreased BDNF mRNA in the hippocampus could weaken inhibitory control of food intake and consequently promote obesity based on the observation that that BDNF mRNA expression was negatively associated with body weight and fat mass in mice that were fed a high fat diet (Yu, Wang et al. 2009).

In humans, a number of single nucleotide polymorphisms near or within the BDNF gene have been associated with impulsivity, including rs7103411, rs10767664 (Oades, Lasky-Su et al. 2008), rs11030101, rs10835210 (Kwon, Ha et al. 2015) and rs2030324 (Su, Tao et al. 2015). One study noted that the presence of the Met allele from rs6265, which decreases BDNF availability, was paradoxically associated with better response inhibition as measured by event-related potentials during the Nogo-N2 and Nogo-P3 in 57 healthy adults (Beste, Baune et al. 2010). These two neurocognitive tasks
measures conflict monitoring and successful motor inhibition, respectively. A more recent study in 122 adults, however, observed better inhibition performance on the Go/NoGo task in Val allele carriers (Enge, Fleischhauer et al. 2016). This finding is more consistent with previous reports of cognitive deficits in Met allele carriers. (Enge, Fleischhauer et al. 2016) also reported an interaction between the BDNF Val66Met polymorphism and the 5-HTTLPR, whereby the BDNF Val/Val genotype protected against impulsive response patterns in the 5-HTTLPR l/l genotype carriers.

**BDNF** and **NTRK2** polymorphisms have been linked to attention deficit hyperactivity disorder (ADHD) (Archer and Kostrzewa 2012, Kwon, Ha et al. 2015) (Zorner, Wolfer et al. 2003), a disorder for which impulsivity is a diagnostic criterion. Impulse control defects, especially in ADHD, may cause food temptation (Davis 2010), chaotic eating patterns (Ptacek, Kuzelova et al. 2010), and loss of controlled eating (Reinblatt 2015, Reinblatt, Mahone et al. 2015), especially unhealthy foods that are high in sugar and fat. Indeed, there is evidence demonstrating overlapping neurobehavioural circuits between pediatric ADHD and conditions characterized by excessive eating such as obesity and binge eating. Seymour and colleagues (Seymour, Reinblatt et al. 2015) reported that both disorders feature functional abnormalities in brain circuits involved in reward, response inhibition, emotional professing and emotional regulation. Researchers have also suggested that a common genetic background could explain the association between increased BMI and childhood ADHD-associated behaviour traits (Albayrak, Putter et al. 2013), but the **BDNF** SNP included in this study (n=495 German children), rs10767664, did not demonstrate significance.

Together, brain morphology changes and structural changes in the neuron have the potential to modify food intake and related behaviours. At present, this hypothesis is still speculative as literature is just beginning to link BDNF to specific parts of the complex eating circuitry, which encompasses motivation-drive, reward-saliency, inhibitory-control, and learning-memory processes among many others (Gibson, Carnell et al. 2010, Zhang, Liu et al. 2014). As a growth factor during brain development and a modifier of neuronal plasticity, BDNF has the potential to alter any part of our eating circuitry and future work should aim to elucidate specific pathways affected by this neurotrophin (Yeo and Heisler 2012).
1.8 BDNF and TrkB: A potential susceptibility factor for disordered eating

As reviewed in the previous section, animal studies have demonstrated a crucial role for BDNF and TrkB in food intake and body weight regulation. There is evidence that BDNF and TrkB influence the physiological mechanisms regulating homeostatic eating and hedonic eating. Neurotrophic effects of BDNF also have the potential to modify brain structures that are part of the eating circuitry and influence neurocognitive functions relevant to eating, such as impulsivity.

While large genetic mutations in the human BDNF gene are rare, there are many single nucleotide polymorphisms (SNPs) around and within this locus. BDNF is commonly studied in obesity literature as it has emerged in numerous BMI genome-wide association studies (GWAS). In addition, SNPs at the locus of BDNF and its receptor TrkB (encoded by NTRK2) have been identified in eating disorder literature and are, therefore, interesting candidates to study in relation to eating behaviours and appetitive traits.

Evidence is mounting that BDNF and its genetic variants may be more closely related to maladaptive appetitive behaviours such as uncontrolled eating rather than a specific eating disorder diagnosis (Cornelis, Rimm et al. 2014). In a large multiethnic study with 2163 overweight or obese subjects, carriers of the A major allele of BDNF rs10767664 and carriers of the G major allele of BDNF rs6265 consumed more calories per day (McCaffery, Papandonatos et al. 2012). As well, these alleles were associated with greater consumption of dairy product and food from the meat, eggs, nuts, and beans food groups (McCaffery, Papandonatos et al. 2012). Others research groups have reported that BDNF SNPs associate with dietary calcium and alcohol intake (Bauer, Elbers et al. 2009, Dusatkova, Zamrazilova et al. 2015). Epigenetic alterations of BDNF may also contribute to appetitive behaviours. Lower methylation of the BDNF promoter I was associated with lower satiety responsiveness among female children of African-American ancestry in a study by (Gardner, Sapienza et al. 2015).

There is also evidence that BDNF and TrkB are involved in the development of the gustatory system, which would in turn, affect food preference and consumption. Taste
bud cells in adults constantly die and are replaced, but this requires nerve fibres to locate and innervate new cells (Beidler and Smallman 1965). BDNF plays a role in the formation of new functional connections between taste receptor cells and nerve fibres. These effects are achieved by the activation and phosphorylation of TrkB in taste cells by BDNF (Nosrat, Margolskee et al. 2012, Fei and Krimm 2013). Removal of the BDNF gene in adult mice causes taste buds to lose innervation and reduce in size and number (Meng, Ohman-Gault et al. 2015).

The relationship between BDNF and specific eating behaviours deserves greater attention as clinical studies suggest that BDNF is closely linked to eating disorders and appetite related-phenotypes. At present, there is a paucity of studies on the direct effects of BDNF and/or TrkB on appetitive traits and the goal of the present thesis will be to address this gap in knowledge. In the following section, we will discuss BDNF and NTRK2 literature in the context of obesity and eating disorders, which will help to provide further support for studying these genes in relation to eating behaviours.

1.8.1 Genetic association studies in obesity and eating disorders

The study of genetic contributions to the variability of eating behaviours in non-clinical populations is relatively uncommon. BDNF and TrkB genes are usually studied in clinical populations with pathological disorders of eating such as obesity and/or eating disorders. The etiology of obesity and eating disorders is complex. Of the many risk factors identified, genetics have been implicated in the pathophysiology of both disorders. Aberrant eating behaviour, a heritable trait as discussed in section 1.5.1, is a risk factor for obesity and a diagnostic criterion for eating disorders. In this section, we will define obesity and eating disorders and discuss the role of BDNF and NTRK2 as reported in these studies. There will be a particular emphasis on BDNF rs6265 (Val66Met) when examining clinical literature, as this is the most widely studied functional variant in BDNF.

**BDNF/NTRK2 and BMI/Common Obesity**
Definition of Obesity: Obesity, as defined by the World Health Organization (WHO), is an excessive accumulation of fat that presents a risk to health. It is a complex disease associated with a number of physical, social and mental health problems. Obesity is typically defined by a body mass index (BMI) > 30 kg/m² in adults, but the definition of childhood obesity is not as consistent. WHO defines childhood obesity as 3 standard deviations above the growth standard median. The U.S Centers for Disease Control and Prevention defines childhood obesity as the 95th percentile of age sex-specific BMI, and the International Obesity Task Force provides cut-points by age and sex for children age 2 to 18.

Genome-wide association studies (GWAS) have provided valuable insights by identifying single nucleotide polymorphisms (SNPs) associated with body mass, which in turn, uncovers genes responsible for weight regulation. The largest and most recent genome-wide association study (GWAS) on adult body mass index (BMI) was conducted by Locke and colleagues (Locke, Kahali et al. 2015). In this study, Locke et al. (2005) meta-analyzed data from 125 studies (n = 339,224) and identified a total of 97 genetic loci associated with BMI increase. Most of these 97 genetic loci were located at or near genes responsible for “neuronal development processes”. A previous 2009 meta-analysis, reported that a number of BMI-associated loci were highly expressed in the hypothalamus (Willer, Speliotes et al. 2009). The study by Locke et al. (2015) extended from this finding to highlight the presence of BMI-associated loci in other areas of the brain not involved in homeostatic mechanisms. Lock et al. (2015) also performed biological pathway analysis on the 97 BMI-associated loci, and identified the neurotrophin signaling pathway as the most significant pathway associated with BMI.

Evidence from GWAS have identified a number of BDNF SNPs associated with common obesity and BMI such as rs6265 (Val66Met) (Wen, Cho et al. 2012), rs10767664 (Speliotes, Willer et al. 2010), rs4923461 (Thorleifsson, Walters et al. 2009), rs2030323 (Okada, Kubo et al. 2012), and rs988712 (Jiao, Arner et al. 2011). BDNF SNPs have also been identified in GWAS for pediatric obesity (Zhao, Bradfield et al. 2011). Additional BDNF variants associated with BMI/obesity are being discovered as new GWAS studies are published. Although these GWAS have identified numerous SNPs
that are associated with BMI, details regarding the underlying mechanism remain largely unknown (Xu, Zeng et al. 2013).

Candidate gene studies for obesity have primarily focused on the rs6265 (Val66Met) variant because the functionality of other BDNF SNPs has not been well validated. In brief, the presence of the A/Met allele is thought to decrease availability of BDNF as compared to the G/Val allele. A more detailed explanation of the molecular mechanism underlying the effects of rs6265, the most characterized BDNF SNP, will be discussed in section 1.9.4. The functional significance of rs6265 has led many to investigate its association with common obesity.

Since BDNF appears to be an anorexigenic factor, the fact that existing GWAS for BMI always report that the major Val allele of rs6265 is the “obesity-associated risk allele” and that this allele is associated with higher BMI in adults (Thorleifsson, Walters et al. 2009, Xi and Mi 2013) and in children (Zhao, Bradfield et al. 2009, Xi and Mi 2013) and in children (Zhao, Bradfield et al. 2009, Xi and Mi 2013) is a counterintuitive but well-acknowledged finding. The A/Met allele of rs6265 (Val66Met) has been reported to be associated with lower neurocognitive function (Dincheva, Glatt et al. 2012), altered brain morphology (Forde, Ronan et al. 2014), and cognitive impairments in Parkinson’s disease (Altmann, Schumacher-Schuh et al. 2016). These effects are thought to be a consequence of impaired activity-dependent BDNF secretion in the presence of the Met allele (Egan, Kojima et al. 2003). Furthermore, a mouse model with the BDNF Met/Met genotype recapitulated the phenotypic hallmarks as expected from this variant in humans, namely elevated body weight, memory impairment, and increased anxiety-related behaviours (Chen, Jing et al. 2006). Nevertheless, it appears that studies on BDNF Val66Met and neuropsychiatric disorders do not consistently report that the Met allele is selectively risk-conferring (Notaras, Hill et al. 2015). The Val allele may contribute risk to specific phenotypes.

Results from individual candidate gene studies for BDNF rs6265 and BMI, however, are not as consistent as GWAS. Even though most studies reported that the Val allele is associated with higher BMI (Gunstad, Schofield et al. 2006, Shugart, Chen et al. 2009, Timpano, Schmidt et al. 2011, Hong, Lim et al. 2012, Xiao, Russell et al. 2012), greater waist circumference (Xi, Cheng et al. 2013), and obesity (Wu, Xi et al. 2010), some studies report that the Met allele is associated with higher BMI (Beckers, Peeters et
al. 2008, Skledar, Nikolac et al. 2012), and others have found null associations between genotype and weight status (Friedel, Horro et al. 2005, Marti, Santos et al. 2009, McCaffery, Papandonatos et al. 2013, Nikolac Perkovic, Mustapic et al. 2013). Moreover, Ma and colleagues (Ma, Qiu et al. 2012) discovered a sex-specific association between the Val66Met genotype and BMI in a population of Puerto Rican adults. In their study, the Val allele was associated with higher BMI in men, but the Met allele was associated with higher BMI in women. Additional details about the aforementioned rs6265 and BMI studies can be found in Supplementary Table 1.

A recent paper by Mou and colleagues (Mou, Hyde et al. 2015) reported an association between the minor C allele of BDNF rs12291063 and common obesity in humans. According to the authors, the major T allele provides binding capacity for a transcriptional regulator. As a result, the presence of the C allele disrupts transactivation function by reducing efficiency of transcriptional factor binding. This BDNF polymorphism is common in Hispanic and African Americans and has a minor allele frequency (MAF) of 30%.

Although BDNF and the neurotrophin signaling pathway have been implicated in obesity pathophysiology, the gene encoding BDNF’s receptor, NTRK2 is not a commonly identified obesity-associated gene in GWAS. It has only been noted as a BMI-associated gene in two studies. NTRK2 rs1211166 demonstrated array-wide significance with BMI in a large (n=108,912) multiethnic gene-centric meta-analysis study (Guo, Lanktree et al. 2013) and two NTRK2 polymorphisms, rs10868204 and rs11140653, were a top hits in a BMI GWAS of 402 Australian aboriginal (Anderson, Cordell et al. 2015). NTRK2 genetic variation may have an important role early in life, as a GWAS on birth weight discovered two variants, rs7849941 and rs12340987, near the NTRK2 gene that was linked to birth weight (Metrustry, Edwards et al. 2014). The two NTRK2 SNPs in this meta-analysis were associated with birth weight in identical and non-identical females twins as well as singleton-born females, but was not associated with birth weight of singleton-born males.

In summary, the association between BMI and common BDNF genetic polymorphisms is complex and in need of further elucidation. Currently, most candidate gene studies focus on the rs6265 SNP. Future studies should examine the role of polymorphisms near or within the BDNF gene since GWAS have identified a number of
other polymorphism other than rs6265. Mou and colleagues (Mou, Hyde et al. 2015) have already provided evidence for a functional polymorphism within *BDNF* that contributes to obesity and is unrelated to rs6265. In addition, *NTRK2* should be considered a candidate gene in future studies.

**BDNF/NTRK2 and eating disorders**

*Definition of Eating Disorders:* The three major types of eating disorders as defined in The Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (American Psychiatric Association 2013) are anorexia nervosa, bulimia nervosa, and binge eating disorder. Anorexia nervosa (AN) is characterized by self-induced starvation and excessive weight loss. Two subtypes of AN, binge-eating/purging or restricting, are distinguished by the presence or absence of binge eating/ purging behaviours. In contrast, bulimia nervosa (BN) is an eating disorder associated with short but repeated episodes of overeating driven by feelings of lack of control, termed binge eating. BN also consists of two clinical subtypes, purging and nonpurging, which is classified based on the type of inappropriate compensatory behaviour to prevent weight gain. Individuals with BN-purging, engage in induced vomiting, laxatives, diuretics, or enemas, while those with BN-non-purging, chooses to fast or exercise excessively to prevent weight gain. Binge eating not followed by attempts to compensate for the food intake is characteristic of binge-eating disorder (BED), the most common eating disorder in the United States (Hudson, Hiripi et al. 2007).

Even though eating disorders have traditionally been attributed to sociocultural influences, recent genetic findings suggest that these disorders are heritable. Based on family, twin, and adoption studies, the heritability of AN, BN, and BED are estimated to be 28 to 58%, 54 to 83%, and 41 to 57%, respectively (Thornton, Mazzeo et al. 2011). To date, only two GWAS have been conducted in AN (Wang, Zhang et al. 2011, Boraska, Franklin et al. 2014). BDNF and NTRK2 were not identified in either study. GWAS have not been conducted in BN or BED. On the other hand, candidate gene studies commonly investigate the role of BDNF and NTRK2 in the pathogenesis of eating disorders.
As expected, the *BDNF* Val66Met polymorphism is often chosen as a candidate due to its functional properties. Recent publications on this association have been summarized in a 2015 review by (Notaras, Hill et al. 2015). The Met allele of rs6265 is usually reported to be the risk allele for eating disorders including AN and BN, as demonstrated in several multicenter studies with large sample sizes (Ribases, Gratacos et al. 2005, Hong, Lim et al. 2012). The Met allele has also been associated with lower caloric intake in adolescents (Arija, Ferrer-Barcala et al. 2010). Nevertheless, there are a few studies reporting no association with eating disorder diagnosis (de Krom, Bakker et al. 2005, Friedel, Horro et al. 2005, Rybakowski, Dmitrzak-Weglarz et al. 2007, Ando, Ishikawa et al. 2012). An interesting finding in eating disorder literature is that the Met allele associates with binging behaviours, which are sometimes a part the clinical picture presented by individuals with eating disorders and/or obesity (Monteleone, Zanardini et al. 2006). Akkermann and colleagues (Akkermann, Hiio et al. 2011) reported that the effect of the polymorphism on binging behaviours in females is dependent on prior food restriction. Moreover, Clarke and colleagues (Clarke, Ramoz et al. 2016) found that the Met allele mediated a higher reward value of food in females with AN. Therefore, this variant may be a moderator of dopaminergic function in the reward system of the brain. In fact, the *BDNF* Val66Met polymorphism has been shown to interact with a dopamine-4 receptor gene (*DRD4*) VNTR polymorphism on maximum BMI in BN patients (Kaplan, Levitan et al. 2008).

While the Met allele of this variant is associated with both hypophagic (i.e. AN) and hyperphagic (i.e. BN, BED) eating disorders, these findings are not entirely contradictory. Obesity and eating disorders are complex polygenic disorders whose predisposition is subjected to both physiological and environmental factors. The true biological effect of a single variant, even if proven to be functional, may be masked by other genetic or non-genetic factors that have a large effect on the symptomology of this disease associated with disorder eating. We can, however, conclude that BDNF peptide is potent anorexigenic factor, and would therefore be an ideal candidate gene for behavioural studies related to over and/or undereating behaviours.

Overall, most *BDNF* candidate gene studies investigate the role of rs6265 and there are far fewer studies on other *BDNF* polymorphisms. However, since BDNF likely
has a biological role in eating behaviour and weight regulation, other polymorphisms near or within this gene may be important to the overall genetic effects as well. For example, Mercader and colleagues (Mercader, Ribases et al. 2007) reported that the rs7124442T/rs11030102C/rs11030119G haplotype was associated with BN and rs7934165A/270T associated with AN. The former haplotype was also linked to higher BDNF levels detectable in the blood. Haplotype-based analysis has several advantages over single SNP studies. Haplotypes enhance the coverage value of polymorphisms within a gene region, thereby increasing the chances of finding the causal variant. In some cases, the haplotype themselves can be the causal variant (Stram and Seshan 2012).

*NTRK2*, which encodes BDNF’s receptor, has also been a candidate gene in eating disorder research. The entire *NTRK2* gene was screened in 91 patients with AN or BN by (Ribases, Gratacos et al. 2005). This paper identified a number of *NTRK2* polymorphisms and mutations that may contribute to eating disorder susceptibility. In particular, the rs1187325 G allele was significantly more prevalent in patients with binge eating/purging AN and the rs1047896 A allele was significantly more prevalent patients with BN. However, the large number of analysis in this study and the subsequent correction for multiple testing rendered most of the p-values to be insignificant. The SNPs identified in this study have not been shown to have functional consequences. Other candidate gene studies have identified rs1201363 and rs1439050 to be linked with lower age of eating disorder onset (Gratacos, Escaramis et al. 2010) and rs1042571 was associated with maximum BMI in patients with BN (Yilmaz, Kaplan et al. 2014).

Similar to studies in obesity, *BDNF* is a common candidate gene for eating disorders, though most studies at present focus on rs6265. *NTRK2* has also begun to generate interest in the area of eating disorders. There is a need for prospective studies to determine whether *BDNF* and *NTRK2* genetic variation predict the development of eating disorders or obesity over time.

### 1.8.2 Serum BDNF

BDNF in humans is usually quantified based on serum levels for practical reasons. An overview of peripheral BDNF circulation is provided in Section 1.9.3. Although a number of studies have investigated the association between plasma/serum level BDNF
and food intake or body mass, no systematic review or meta-analysis of these studies has been conducted thus far. With the hypothesized pathogenic role of BDNF in obesity and eating disorders, serum BDNF levels would be expected to correlate with these disorders. Indeed, patients with WAGR (Han, Liu et al. 2008) or Prader-Willi (Han, Muehlbauer et al. 2010) syndrome, have lower levels of serum BDNF and develop childhood obesity. In a study of appetitive regulatory hormones in AN, serum levels of BDNF was positively associated with dietary restraint and frequency of purging, thereby supporting its role as an anorexigenic factor (Eddy, Lawson et al. 2015).

However, the relationship between serum BDNF and body weight is ambiguous as both positive and negative correlations have been reported in literature. A positive correlation between serum BDNF levels and body mass was observed in adults (Suwa, Kishimoto et al. 2006). This relationship was also present in a study with children. In fact, according to a study by Saito and colleagues (Saito, Watanabe et al. 2009), serum BDNF levels are significantly reduced in patients with AN. Furthermore, there was a positive correlation between serum BDNF levels and BMI in patients with AN or BN patient but this correlation was not observable in healthy controls. Authors of this study also noted a correlation between BDNF levels and self-reported scores of “oral control” on the Eating Disorder inventory, which is indicative of rigid self-control. One study in overweight children, however, reported lower levels of BDNF as compared to controls and an overall negative correlation between serum BDNF levels and BMI (El-Gharbawy, Adler-Wailes et al. 2006). This finding is line with a study in adult with type 2 diabetes, who observed decreased BDNF levels in those with obesity (Li, Lang et al. 2016). A study by Lang and colleagues (Lang, Hellweg et al. 2009) has linked the functional rs6265 variant to higher levels of serum BDNF and may explain the observed association between this variant and eating disorders.

The discrepant findings in literature may be due to the multitude of factors that can affect serum levels of BDNF. Time of blood withdrawal, length of blood storage, geographical area, age, sex, smoking status and food and alcohol intake are all factors that can affect the levels of serum BDNF (Bus, Molendijk et al. 2011). Therefore, serum BDNF may not be an ideal measure of long-term BDNF levels.

It has been proposed that BDNF levels observed in eating disorders and obesity
are likely secondary adaptive mechanisms aimed at counteracting the change in energy balance that occurs in these syndromes. Monteleone and colleagues (Monteleone, Tortorella et al. 2004) proposed that BDNF reduction in AN attempts to offset the negative energy balance by promoting food intake. Decreased platelet numbers in AN patients may also account for the decrease in serum BDNF. In contrast, increased BDNF levels in obesity may be representative of adaptive mechanism to offset the positive energy balance by discouraging food consumption. Null associations between weight and BDNF levels have also been reported (Gajewksa, Sobieska et al. 2014). Moreover, there is evidence that BDNF levels do not respond to changes in weight status because levels did not change after patients with AN regained weight (Nakazato, Hashimoto et al. 2006). There is a definite need for longitudinal studies, especially in non-clinical populations, to investigate the temporal relationship between serum BDNF, appetitive behaviours, and weight regulation.

1.9 Molecular Biology of BDNF

In this section, we will discuss the synthesis and secretion of BDNF from gene to protein. BDNF’s receptor, TrkB, will also be described. We will outline the mechanism underlying the functional BDNF Val66Met polymorphism as this is the only well-characterized functional BDNF SNP.

1.9.1 From Gene to Protein

The BDNF gene at locus 11p13, in humans, extends over 70kb and is comprised of 11 exons (reviewed by (Pruunsild, Kazantseva et al. 2007)). The structure and regulation of the BDNF is complex. This complexity is mainly attributable to its use of alternative promoters, alternative splice donor and acceptor sites that produce between and within exon patterns of alternative splicing, and alternative polyadenylation sites. At least 34 different BDNF mRNA transcripts, all encoding an identical mature BDNF protein, can be synthesized from the 11 exons, and 9 functional promoters that make up the BDNF gene. Furthermore, its antisense RNA (BDNF-AS) is a non-protein coding transcript that can regulate BDNF expression (Modarresi, Faghihi et al. 2012).
Initially, BDNF mRNA is translated into a 32-kDa precursor protein known as preproBDNF in the endoplasmic reticulum. The pre-sequence of preproBDNF is then proteolytically cleaved by furin to produce proBDNF (28-kDa) (reviewed by (Rosas-Vargas, Martinez-Ezquerro et al. 2011)). ProBDNF travels away from the endoplasmic reticulum and accumulates in the Golgi apparatus where it is sorted into secretory granules. These BDNF-containing granules are trafficked to sub-cellular compartments such as dendritic spines and axonal terminals for further processing in response to extracellular or intracellular signals. ProBDNF may be proteolytically processed intracellularly or extracellularly into the 14-kDa mature form of BDNF (mBDNF).

The intracellular process involves cleaving proBDNF in the trans-Golgi network by furin or by proconvertases in secretory vesicles to form mBDNF (reviewed by (Rosas-Vargas, Martinez-Ezquerro et al. 2011)). ProBDNF is not efficiently processed by intracellular proteases so it can be secreted along with mBDNF (Mowla, Farhadi et al. 2001). Both proBDNF and mBDNF are preferentially transported into vesicles of the activity-regulated secretory pathway, but can also travel into vesicles of the constitutive pathway (reviewed by (Lessmann, Gottmann et al. 2003)). Vesicles in the constitutive pathway fuse with plasma membrane to continuously release proBDNF and mBDNF into the extracellular space, whereas vesicles in the regulated pathway accumulate and only fuse when triggered by signals from intracellular Ca\(^{2+}\) for activity-dependent secretion (reviewed by (Cunha, Brambilla et al. 2010)). The three Ca\(^{2+}\) mechanisms responsible for activity-regulated release of BDNF are dependent on the site of release. Ca\(^{2+}\) can be released from presynaptic sites (Balkowiec and Katz 2002) or intracellular stores (Griesbeck, Canossa et al. 1999). Ca\(^{2+}\) influx-dependent release can also be mediated by Ca\(^{2+}\) influx through ionotropic glutamate receptors or voltage gated Ca\(^{2+}\) channels at postsynaptic sites (Hartmann, Heumann et al. 2001).

In non-neuronal cells, such as fibroblast and Schwann cells, neurotrophins are usually secreted constitutively, but principal neurons and neuroendocrine cells primarily secrete neurotrophins in an activity-dependent manner in response to depolarization and a rise in intracellular Ca\(^{2+}\) (Mowla, Pareek et al. 1999). Unlike the other three members of the neurotrophin family, which are primarily packaged into constitutive vesicles, both proBDNF and mBDNF are preferentially packaged into vesicles of the regulated secretion
pathway (Thomas and Davies 2005). This means that majority of BDNF is released in a regulated activity-dependent manner throughout the body.

Likewise, the extracellular processing of BDNF involves proBDNF being transported in a vesicle of the regulated or constitutive pathway and released into the extracellular place where it is then cleaved by serine protease plasmin or selective matrix metalloproteinases (MMP3, MMP7, and MMP9) to release a the mBDNF peptide (Lee, Kermani et al. 2001). For many years, the fate of the prodomain that is cleaved in either the intracellular or extracellular processing process was unknown. Recently, it was discovered that the isolated prodomain functions as an independent ligand that can bind to p75^NTR and can alter neuronal morphology (Anastasia, Deinhardt et al. 2013).

The synthesis and processing of BDNF is illustrated in Figure 2.
Figure 2 The synthesis and processing of BDNF from gene to protein. Protease are italicized. The arrows are thicker in the activity-regulated pathway to indicate that both mature BDNF (mBDNF) and proBDNF preferentially sort into this pathway as opposed to the constitutive pathway. A minor truncated form of proBDNF (28kDA) can also be secreted into the extracellular space as a proteolytic product, but was not included in this figure.
BDNF can be secreted pre- or post-synaptically. The products released into the synapse bind to one of two classes of cell surface receptors that are functionally different: the pan-neurotrophin receptor (p75NTR) and the tyrosine kinase receptor B (TrkB)) (reviewed by (Rosas-Vargas, Martinez-Ezquerro et al. 2011)). ProBDNF preferentially binds to p75NTR, a tumor necrosis factor and triggers apoptosis and long-term depression (reviewed by (Cunha, Brambilla et al. 2010)). Meanwhile, mBDNF preferentially binds to the TrkB receptor to regulate neuronal growth and survival through the phosphoinositide 3-kinase (PI3K) pathway, neuronal differentiation through the mitogen-activated protein kinase (MAPK) pathway, and synaptic plasticity through the phospholipase Cγ (PLCγ) mediated pathway. It is likely that cell survival and synaptic plasticity is regulated bidirectionally by the relative availability of proBDNF and mature BDNF (Woo, Teng et al. 2005).

1.9.2 TrkB: The high-affinity receptor for BDNF

In humans, TrkB is encoded by the NTRK2 locus on chromosome 9q22 and spans more than 350kbp. The locus contains 24 exons, which generate approximately 36 different TrkB protein isoforms through alternative splicing (Luberg, Wong et al. 2010). The synthesis, regulation, and function of TrkB are highly complex as the various protein isoforms have different functional properties.

TrkB plays an indispensable role in physiological functions as evidenced by the fact that TrkB-/− mice display significant neurological deficiencies and die within a few days after birth (Klein, Smeyne et al. 1993). TrkB is widely expressed in the central nervous system and the peripheral nervous system. It is expressed in neural tissues during embryogenesis (Klein, Martin-Zanca et al. 1990) and is highly detectable in rudimentary structures of the nervous system including the developing forebrain, hindbrain, hippocampus, cerebellum, spinal cord, and dorsal root ganglion (Muragaki, Timothy et al. 1995). In adults, TrkB expression is highest in hippocampus, hypothalamus, choroid plexus and ependymal lining of the ventricles, but TrkB transcripts can also be found in most structures of the cerebrum (reviewed by (Barbacid 1994)). Similar to the role of BDNF, TrkB is implicated in the differentiation, development, maintenance, survival and synaptic plasticity of neurons.
TrkB serves as a signaling receptor for neurotrophins and displays the highest affinity to the binding of BDNF and neurotrophin-4 (NT4), though neurotrophin-3 (NT3) can also bind to TrkB with reduced affinity (reviewed by (Barbacid 1994)). Aberrant TrkB signaling has been linked to proliferative (e.g. cancers of the brain and lung), psychiatric (e.g. depression, anxiety, schizophrenia), and neurodegenerative (e.g. Alzheimer’s and Huntington’s disease) disease (reviewed by (Gupta, You et al. 2013)). Defective BDNF/TrkB signaling has specifically been implicated in promoting susceptibility to various eating disorders (e.g. anorexia nervosa and obesity).

An interesting observation in TrkB /-/ mice is that they do not display any feeding behaviour and die 1 day after birth (Klein, Smeyne et al. 1993). This suggests that TrkB and its signaling pathway is an essential regulator of feeding behaviour. The exact mechanism for this effect is unclear but several additional observations from this study offer some insight. When manually fed milk-formula using a tube TrkB /-/ mice inhaled the milk instead of swallowing. This raises the possibility that there are abnormalities in their motor system involved in the process of swallowing. A number of lesion and cellular deficiencies in the neuronal systems involved in feeding, such as the trigeminal ganglion and facial motor nucleus, were also noted.

1.9.3 Peripheral Circulation

A substantial part of circulating BDNF concentrations in the body originates from neurons and glia cells in the CNS since BDNF readily crosses the blood-brain barrier in both directions (Poduslo and Curran 1996, Pan, Banks et al. 1998). Indeed, a positive correlation between levels of BDNF in the brain and in the circulatory system is observed in a number of mammalian species including rat, pig and mouse (Klein, Williamson et al. 2011). According to (Rasmussen, Brassard et al. 2009), the brain contributes approximately 72% of circulating BDNF at rest and 84% during exercise. The remaining source of BDNF is synthesized by peripheral cells including endothelial cells (Nakahashi, Fujimura et al. 2000), smooth muscle cells (Matthews, Astrom et al. 2009), endocrine cells, and immune cells (Linker, Gold et al. 2009). In the circulatory system, BDNF is initially stored in platelets but can be released into the plasma by granulation of coagulation factors (Nakazato, Hashimoto et al. 2012). As a result, average serum levels
of BDNF are about 50 -100 times higher than plasma levels (Fujimura, Altar et al. 2002). In the periphery, BDNF has immunotrophic, epitheliotrophic and metabotrophic effects (Chaldakov, Tonchev et al. 2009). However, preclinical evidence has demonstrated that peripheral BDNF can have direct effects on the brain because administration of peripheral BDNF affects molecular signaling substrates, neurogenesis, and behaviour (Schmidt and Duman 2010).

1.9.4 The Val66Met Polymorphism

The most widely studied SNP in the human BDNF gene is Val66Met (dbSNP number rs6265). This functional BDNF SNP only exist in humans and encodes a nucleotide change from a guanine (G) to an adenine (A) at position 196 (G196A) of the BDNF gene, which leads to a valine (Val) to methionine (Met) substitution at codon 66 (Val66Met) of the BDNF protein. Depolarization-dependent secretion of BDNF in hippocampal neurons is significantly impaired when val66 in the proBDNF sequence is replaced with a met (Chen, Patel et al. 2004). The prevalence of this non-synonymous missense variant in the human population is approximately 20-25%.

Since this SNP is located in the 5’ pro-BDNF sequence, Egan and colleagues hypothesized that the effects of the amino acid substitution would likely not affect the structural or functional properties of the mature protein. With use of in vitro hippocampal neurons, they observed that localization of BDNF after post-translational modification in the Golgi apparatus was dependent on whether the neuron expressed BDNF_{Val} or BDNF_{Met}. BDNF_{Val} localized in dendrites and synapses, whereas BDNF_{Met} localized in cell bodies. Later studies clarified that BDNF_{Met} mRNA failed to interact with translin, a DNA-binding protein that directed BDNF mRNA to dendrites for local synthesis and secretion (Chiaruttini, Vicario et al. 2009). Quantitative analysis revealed that the Val66Met polymorphism significantly attenuated the activity-dependent secretion of BDNF protein (Egan, Kojima et al. 2003). In contrast, the constitutive secretion of BDNF protein, in the absence of neuronal depolarization, was unaffected by this polymorphism.

In 2005, Chen et al., identified a key chaperone protein in the Golgi apparatus called sortilin, which directed intracellular trafficking of newly synthesized BDNF into
the regulated secretory pathway by interacting with the prodomain of BDNF (Chen, Ieraci et al. 2005). Sortilin, interacted with a discrete region of the 5’ prodomain, which encompassed the BDNF Val66Met SNP (Chen, Ieraci et al. 2005). Accordingly, mice expressing BDNF_{Met} displayed impaired sorting, localization, and activity-dependent secretion of BDNF granules, resulting in low levels of extracellular BDNF despite normal intracellular expression of BDNF (Chen, Patel et al. 2004).

In summary, based on in vitro cell culture experiments in neurosecretory cells and primary cultured neurons, the Met substitution in the prodomain appears to cause two trafficking defects: (1) decrease BDNF localization into neuronal dendrites, and (2) decrease localization of BDNF to secretory granules, thus impairing activity-dependent secretion of BDNF. Although BDNF can be secreted constitutively, BDNF is primarily released through the activity-dependent secretory pathway. As a result, BDNF_{Met} represents a significant decrease in available BDNF.

More recently, (Anastasia, Deinhardt et al. 2013) proposed that the effects of this polymorphism may not strictly arise from deficient BDNF translocation and secretion. They discovered that the isolated 66Met domain induced neuronal morphological changes. Specifically, the presence of this SNP induced acute growth cone retraction. The physiological consequence of the 66Val domain is unknown. (Anastasia, Deinhardt et al. 2013) suggested that the prodomain may act as a ligand to proneurotropic receptor targets involved in dopaminergic wiring such as SorCS2, a member of the VPS10 domain containing receptor family. The question remains as to whether these in vitro observations and effects translate to in vivo impairments in humans.

A summary of the physiological mechanism underlying BDNF Val66Met is presented in Figure 3 below.
Interestingly, Lang and colleagues (Lang, Hellweg et al. 2009) reported that the Met allele of BDNF Val66Met was associated with increased serum BDNF concentrations. Although the authors were did not explore the exact mechanism causing the serum BDNF increase, they hypothesized that carriers of the Met allele constitutively upregulate peripheral BDNF concentrations to compensate for the defective intracellular protein signaling. Based on this finding, we must interpret BDNF genetic studies with caution. The Val66Met polymorphism may lead to decreased BDNF levels in the brain, but peripheral levels of BDNF seem to be increased.

1.10 Summary and Conclusion

A compelling body of evidence has implied a pivotal role for BDNF in pathological processes that lead to abnormal food intake and excessive weight gain. BDNF deficiency in animals and humans leads to distinctive hyperphagic behaviours and weight gain. This suggests that BDNF plays a critical role in the control of eating by acting as an anorexigenic factor. Furthermore, SNPs near or within the BDNF and NTRK2 loci have been implicated in promoting genetic susceptibility to obesity as well as eating
disorders but their mechanism of action has yet to be elucidated. The BDNF-TrkB signaling pathway appears to have (1) direct effects on homeostatic mechanisms, (2) indirect effects on the reward system responsible for hedonic feeding, and (3) a neurotrophic role on overall brain structure, connectivity, and function, which may be relevant to the cognitive control of eating. Alterations and/or modifications to neuro-circuitry that controls eating may give rise to over or undereating tendencies, which leads to weight gain/loss.

Currently, our understanding of BDNF’s effect on BMI in humans is limited. The anorexigenic role of BDNF based on preclinical evidence does not seem to be directly translatable in the clinical setting. Although decreased secretion of BDNF, as in the case of a Val to Met substitution at rs6265, should lead to greater food intake and weight gain, the opposite effect is usually observed in human genetic epidemiological studies. This may be due to the large amount of literature solely focused on the role of BDNF Val66Met, which may or may not completely encompass the role of the entire BDNF protein on eating behaviours. It is necessary for future studies to expand the scope of investigation to other BDNF genetic variants. NTRK2 have also been suggested as a candidate gene in obesity and eating disorders research due to its biological relevance to BDNF, but there remains a paucity of research on this gene.

The heritability of weight and appetitive traits has been established and several obesity-associated genes (e.g. FTO) have been linked to distinct eating behaviours that pose a risk for weight gain. Associations between appetitive traits and subsequent weight gain are detectable in early childhood, suggesting that food intake mechanisms begin to take effect early in life. It is therefore, important to investigate genetic predisposition to eating behaviours in young populations as most literature to date focuses on adult eating behaviours or a large age range of <18 year olds. These factors were taken into account when designing the subsequent studies for this thesis.
Chapter 2 Research Aims and Hypotheses

2.1 Research Aims

BDNF acts as an anorexigenic factor in animals and humans to decrease food intake, thereby leading to weight gain. Genetic polymorphisms in the \textit{BDNF} or \textit{NTRK2} loci, the gene encoding BDNF’s high-affinity receptor TrkB, are associated with obesity and eating disorders. Since aberrant eating behaviours is a risk factor for both diseases, \textit{BDNF} and \textit{NTRK2} genetic polymorphisms may play a role in the development of certain eating behaviours.

As previously alluded to in the “General Overview”, the overall research aim of this thesis was to investigate the role of \textit{BDNF} and \textit{NTRK2} genetic polymorphisms on eating behaviours in young children (aged 3-4). Analysis was conducted on the Maternal Adversity, Vulnerability, and Neurodevelopment (MAVAN) cohort. Details about this cohort are provided in the subsequent Methods chapter (Chapter 3).

This study is novel because genetic associations with eating behaviours have never been investigated in such a young non-clinical population; though others have reported that eating habits are predictive of BMI and weight gain in children as young as 3 months of age (Quah, Chan et al. 2015). Since this is the first study of its kind to investigate the relationship between \textit{BDNF} and \textit{NTRK2} genetic variation and eating behaviours in a young, non-clinical population, we could not identify a specific type of eating behaviour to investigate. Instead, our goal was to generate data for more specific hypothesis to be made in the future. We decided to investigate a broad spectrum of eating behaviours, using a behavioural measure (Snack Delay Task) and a psychometric measure (Child Eating Behaviour Questionnaire (CEBQ)) of eating behaviour as our phenotypes of interest. With regards to selecting genetic variants, we used a tagSNP approach to select a small number of genetic loci representative of overall variation near or within the \textit{BDNF} locus. As the \textit{NTRK2} locus was too large for this approach, we employed a candidate SNP approach and selected SNPs based on prior evidence in relation to BMI,
obesity, or eating disorder. The following section will describe the rationale for the selection of genetic and phenotypic variables in greater detail.

Studying appetitive traits in young children can limit various confounding factors more relevant for adolescents and adults such as body image concerns, chronic metabolic changes, and medications among others. This will allow us to determine biological causes of such behaviours. Furthermore, investigating early signs of predispositions towards food are more useful than evaluating these behaviours after the development of obesity. Results from this study can directly inform genetic screening for eating behaviours that pose a risk for over or undereating practices and identify children that would benefit from dietary intervention early in life.

2.2 Study Design Strategy

In designing the study, we had to take into account a major limitation of sample size. The MAVAN cohort was rich with phenotypic information. Behavioural assessments in laboratory settings and questionnaire data were collected from the subjects, allowing for a wide selection of phenotypes over critical periods of child development (i.e. from birth to 6 years of age). As a trade-off, the sample size is modest and relatively small for genetic analysis, especially since genetic data was not available for all subjects. In addition, there were missing covariate data for some subjects. Due to the low sample size constraint, it was important for us to limit the number of tests of association.

The following section will detail how we attempted to examine the association between BDNF and NTRK2 polymorphisms and a broad range of eating behaviours, while attempting to minimize the number of comparisons performed.
2.2.1 Selection of Genetic Variables

(1) **BDNF**
Numerous **BDNF** SNPs have been identified in previous BMI, obesity, or eating disorder literature, suggesting that the entire **BDNF** locus should be studied in relation to eating behaviours. We chose to use a tagSNP approach to select **BDNF** SNPs for analysis. This technique considers the linkage disequilibrium (LD) between SNPs and identifies an optimal selection of SNPs that are representative of overall variation within a region of interest. We identified **BDNF** SNPs relevant to BMI/obesity in adults or children based on prior literature and ensured that the tagSNP selection process captured these specific SNPs. Gene expression databases were also consulted to find **BDNF** SNPs that affect its expression in the brain. Overall, the tagSNP approach allowed us to select a small number of SNPs that are representative of variation near or within the **BDNF** locus. We also investigated structured patterns of **BDNF** polymorphisms by studying haplotypes constructed by the tagSNPs. Haplotypes are a group of genetic polymorphisms that are inherited together as a group (Clark 2004).

(2) **NTRK2**
The **NTRK2** gene region was too large for tagging, especially since we had a sample size constraint. As well, no functional **NTRK2** SNP have been reported in literature to date. Therefore, we chose to use a candidate SNP approach to select **NTRK2** variants identified from previous research in relation to BMI, obesity, or eating disorders. It should be noted that there are far fewer reports of **NTRK2** SNPs and these phenotypes as compared to **BDNF**. Gene expression databases were also consulted to find **NTRK2** SNPs that affect its expression in the brain. We investigated structured patterns of **NTRK2** polymorphisms by studying haplotypes constructed by the candidate **NTRK2** SNPs.
2.2.2 Selection of Phenotypes from MAVAN

In order to capture a broad spectrum of eating behaviours, we included two measures of eating behaviours. The first was the Snack Delay Task – a behavioural measure of impulsive responding to a food cue. The second was the Child Eating Behaviour Questionnaire (CEBQ) – a psychometric measure that assesses a broad range of eating behaviour constructs based on parental-report.

(1) Snack Delay Task

The Snack Delay Task is a laboratory-based behavioural measure of impulsive responding in the presence of an appetitive stimulus. This behavioural test was originally designed as part of a battery of tasks to measure inhibitory control in toddlers and preschoolers (Kochanska, Murray et al. 1996). It has since been used as a measure of delay of gratification (Spinrad, Eisenberg et al. 2007), self-regulation (Denham, Warren-Khot et al. 2012), response inhibition (Pauli-Pott, Roller et al. 2014), and impulsive eating (Silveira, Agranonik et al. 2012). Spinrad and colleagues (Spinrad, Eisenberg et al. 2007) reported that Snack Delay Task performance at 18 and 30 months of age was significantly, though modestly, correlated with mothers’ and caregivers’ report of attention shifting and inhibitory control. Recently, Miller and colleagues (Miller, Rosenblum et al. 2016) utilized the Snack Delay Task as a measure of behavioural self-regulation and found that better performance associated with lower child BMI and odds of being overweight/obese. In contrast, child’s performance on a non-food related delay task was not associated with BMI or odds of being overweight/obese. This suggests that the Snack Delay Task measures a very specific form of impulsivity related to food cues and body weight. The fact that performance on the Snack Delay Task at 36 months of age predicted increase consumption of palatable fat one year later further supports this hypothesis (Silveira, Agranonik et al. 2012). For the purposes of this study we used the Snack Delay Task as a measure of impulsive responding to food. Children from the MAVAN cohort completed this task in front of research assistants, who then provided an objective score of the child’s performance and behaviour. Performance on the Snack
Delay Task may be indicative of a child’s ability to inhibit response in an obesogenic environment.

(2) *Child Eating Behaviour Questionnaire (CEBQ)*
As the Snack Delay Task only assesses one aspect of eating behaviour, i.e. impulsive/restraint eating, we chose a second phenotype that would provide more information on a range of appetitive traits. The second phenotype of interest was the Child Eating Behaviour Questionnaire (CEBQ), a parental-report questionnaire that was designed to measure eating behaviours in a general non-clinical population. Both over and undereating extremes of appetitive traits are assessed by questions in the CEBQ. The CEBQ measures eight domains of appetitive traits (further details are provided in the Methods chapter, Section 3.4.2). In order to reduce the number of analysis, we subgrouped the eight domains into food approach and food avoidant behaviours.

**Note on BMI**
Based on the well-known role of BDNF and the BDNF-TrkB signaling pathway on weight regulation, BMI was initially a potential phenotype of interest. We, however, decided not to include BMI as a primary outcome in this study. This is because many genetic association studies on BDNF and BMI have already been published. The novelty of our research aim lies in the fact that we are investigating the role of *BDNF* and *NTRK2* genetic variation on eating behaviours, which should precede weight gain or loss. In addition, we had to limit our number of association analyses.

Nevertheless, due to the inseparable bidirectional relationship between eating behaviours and BMI, we decided to examine BMI as a covariate pending significant results to determine potential mediation effects of BMI or pleotropic effects of the gene. Children’s height and weight were recorded at 36 and 48 months. BMI was calculated using the following formula: weight (kg) divided by (height (m))^2. Z-scores for children’s BMI were calculated based on standards from World Health Organization (WHO) growth curves for girls and boys (WHO Multicentre Growth Reference Study Group 2006).
2.3 Research Questions & Hypotheses

Based on the key considerations outlined in the previous section, we limited our investigation a priori to three key questions in order to reduce multiple comparisons and false positive results. The three key questions are as follows:

**Research Question # 1:**
Are BDNF and NTRK2 genetic polymorphisms associated with performance on an objective measure of impulsive responding to a food cue?

*Hypothesis #1:* We hypothesize that genotypes from BDNF and NTRK2 polymorphisms will be associated with performance on an objective assessment of impulsivity towards a food cue as measured by the Snack Delay Task. Behavioural impulsivity is the most robust neurocognitive correlate of obesity/ body weight in adults and in children (Vainik, Dagher et al. 2013, Liang, Matheson et al. 2014). Furthermore, impulsivity has been linked to BDNF and NTRK2 polymorphisms in prior literature (Oades, Lasky-Su et al. 2008, Ribases, Hervas et al. 2008, Su, Tao et al. 2014, Kwon, Ha et al. 2015, Su, Tao et al. 2015). As genetic contributors to obesity susceptibility, BDNF and its receptor NTRK2 may contribute to an impulsive eating phenotype.

**Research Question # 2:**
Are BDNF and NTRK2 genetic polymorphisms associated with dimensions of eating styles that are implicated in the development of weight problems as measured by a parental-reported questionnaire?

*Hypothesis #2:* We hypothesize that genotypes from BDNF and NTRK2 polymorphisms will be associated with higher scores for the eating behaviours assessed by the CEBQ. However, since BDNF and NTRK2 are both obesity- and
eating disorder- associated genes, their polymorphisms may contribute to high scores for behaviours implicated in both weight gain and weight loss.

**Research Question #3:**
Does the BDNF rs6265 functional variant interact with NTRK2 genetic polymorphisms to predict impulsive responding to a food cue in a controlled setting and/or parental-reported eating behaviours implicated in the development of weight problems?

**Hypothesis #3:** Since BDNF and its receptor are biologically linked, BDNF and NTRK2 polymorphisms may have epistatic effects on appetitive behaviours. In fact, epistatic interactions between BDNF and NTRK2 variants have been reported in other contexts, such as schizophrenia and depression (Lin, Hong et al. 2009, Lin, Su et al. 2013). We specifically tested for BDNF rs6265 because this is the only well-characterized functional BDNF polymorphism. No definitively functional NTRK2 polymorphism has been identified to date.

**2.4 Correction for Multiple Comparisons**

Since our selection of SNPs is essentially from two genes, association analysis on individual SNPs should not be considered independent tests of association. Therefore, the commonly used Bonferroni correction for multiple comparisons, which assumes complete independence of tests, would be too conservative. For Snack Delay analysis, we determined an adjusted experiment-wide p-value significance threshold using the method proposed by D.R. Nyholt called the Single-Nucleotide Polymorphism Spectral Decomposition (SNPSpD) (Nyholt 2004). This method uses spectral decomposition (SpD) of matrices of pairwise LD between SNPs to calculate the experiment wide threshold. In addition, we were analyzing multiple phenotypes that may also have some residual correlation. For CEBQ scores (i.e. Food Approach/Avoidant are derived from the same questionnaire), we determined the multiple testing adjusted p-value using
p_ACT program (Conneely and Boehnke 2007). This program takes into account correlated genotypes and phenotypes. A Bonferroni correction was used to determine the p-value threshold of significance for haplotype analysis and gene-gene interactions as neither of the programs are able to correct for haplotypes or interaction analyses.

2.5 Summary of Study Design

The overall goal was to determine whether BDNF and NTRK2 genetic polymorphisms predict eating behaviours in children that may lead to pathological eating practices and significant weight gain/loss in future. Considering the small sample size (n = approx. 160, depending on phenotype), we limited our genetic and phenotypic variables in our study design. Therefore, we only assessed for three broad types of eating behaviours: impulsive responding to food, food approach, and food avoidant behaviours.

The following figure (Figure 4) summarizes the study design that were used to address the four research questions:

**Figure 4** Summary of study design
Chapter 3 Methods

3.1 The Maternal Adversity, Vulnerability, and Neurodevelopment (MAVAN) cohort

We obtained data from an established prospective birth cohort – (the Maternal Adversity, Vulnerability and Neurodevelopment project-MAVAN) (O'Donnell, Gaudreau et al. 2014). Pregnant women were recruited from obstetric clinics in Hamilton (Ontario) and Montreal (Quebec), Canada and their children were followed from birth. All mothers were ≥ 18 years, fluent in English or French, and had a singleton gestation. Those with complications such as severe chronic illness, placenta previa, history of incompetent cervix, impending delivery, or a fetus/infant affected by a major anomaly or born at a gestational age less than 37 weeks were excluded. Written informed consent was obtained from all participant mothers.

Approval for the MAVAN project was obtained from obstetricians performing deliveries at the study hospitals and by the ethics committees and university affiliates (McGill University and l’Université de Montréal: the Royal Victoria Hospital, Jewish General Hospital, Centre hospitalier de l’Université de Montréal, and Hôpital Maisonneuve-Rosemount), and St. Joseph’s Hospital and McMaster University, Hamilton, Ontario, Canada.

3.2 Genetic Variable Selection

3.2.1 BDNF tagSNPs

$BDNF$ SNPs for this study were selected based on functional evidence and/or relevance to adult or child BMI literature. The Genotype-Tissue Expression project (GTEx) Portal (http://www.gtexportal.org/home/), was used to identify functional $BDNF$ SNPs, but the database did not reveal any SNP that affected BDNF expression in any particular brain region. This may be due to the fact that gene expression databases have relatively small sample sizes and are unable to capture SNPs with smaller effects. The literature that guided our selection of BDNF SNPs is listed in Table 1.
Haploview version 4.2 was used to select TagSNPs \( (r^2 \geq 0.8) \) with MAF = 0.05 and Hardy-Weinberg equilibrium = 0.001 using the CEU population in HapMap. We only selected BDNF SNPs in that were polymorphic in European populations. The final 5 tagSNPs (Table 1) captures 74% of common genetic variation between 27,645,655 and 27,723,312 on chromosome 11 (~77kb region).

### Table 1 BDNF tagSNPs

<table>
<thead>
<tr>
<th>tagSNPs</th>
<th>Chromosomal location (hg18)</th>
<th>Alleles Captured</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6265</td>
<td>Chr.11: 27,636,492</td>
<td>rs6265, rs10501087, rs2049045, rs11030104, rs10767664, rs16917237, rs7103411, rs925947, rs2030323, rs988748, rs6484320</td>
<td>(Mizui, Ishikawa et al. 2015), (Wen, Cho et al. 2012), (Thorleifsson, Walters et al. 2009), (Okada, Kubo et al. 2012, Locke, Kahali et al. 2015), (Speliotes, Willer et al. 2010)</td>
</tr>
<tr>
<td>rs11030102</td>
<td>Chr.11: 27,63,8172</td>
<td>rs11030102, rs11030107, rs10835211, rs17309874</td>
<td>(Mercader, Ribases et al. 2007)</td>
</tr>
<tr>
<td>rs11030108</td>
<td>Chr.11: 27,652,040</td>
<td>rs11030108, rs962369, rs10767658, rs1401635, rs1519480, rs925946, rs7127507, rs11030121, rs1013402, rs7124442, rs11030119</td>
<td>(Thorleifsson, Walters et al. 2009)</td>
</tr>
<tr>
<td>rs7103873</td>
<td>Chr.11: 27,656,893</td>
<td>rs7103873, rs7931247, rs11030101, rs2049046, rs2030324, rs1519479, rs2203877, rs10767665, rs10835210, rs7934165</td>
<td></td>
</tr>
<tr>
<td>rs56164415</td>
<td>Chr.11: 27,678,311</td>
<td>rs56164415</td>
<td>(Kunugi, Ueki et al. 2001)</td>
</tr>
</tbody>
</table>

#### 3.2.2 NTRK2 SNPs

Since no NTRK2 SNP has been reported to have definitive functional properties, candidate SNPs were selected based on previous reports of association with obesity, BMI, or eating disorders. GTEx portal did not identify any SNP that affected NTRK2 expression in the brain. The following candidate SNPs from the NTRK2 locus were selected for genetic analysis (Table 2).
### Table 2 NTRK2 candidate SNPs

<table>
<thead>
<tr>
<th>NTRK2 candidate SNPs</th>
<th>Chromosomal location (hg18)</th>
<th>Relevance to obesity, BMI, or eating disorders</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1187325</td>
<td>Chr.9: 86,475,415</td>
<td>C allele associated with binge/eating purging Anorexia Nervosa</td>
<td>(Ribases, Gratacos et al. 2005)</td>
</tr>
<tr>
<td>rs1211166</td>
<td>Chr.9: 86,475,812</td>
<td>A allele associated with higher BMI</td>
<td>(Guo, Lanktree et al. 2013)</td>
</tr>
<tr>
<td>rs1439050</td>
<td>Chr.9: 86,478,013</td>
<td>T allele associated with a lower age of eating disorder onset</td>
<td>(Gratacos, Escaramis et al. 2010)</td>
</tr>
<tr>
<td>rs1047896</td>
<td>Chr.9: 86,615,793</td>
<td>G allele associated with Bulimia Nervosa</td>
<td>(Ribases, Gratacos et al. 2005)</td>
</tr>
<tr>
<td>rs1078947</td>
<td>Chr.9: 86,753,072</td>
<td>T allele associated with highest BMI in patients with Bulimia Nervosa</td>
<td>(Yilmaz, Kaplan et al. 2014)</td>
</tr>
</tbody>
</table>

We assessed for linkage disequilibrium (LD) between each of the NTRK2 SNPs using the SNP Annotation and Proxy Search (SNAP Version 2.2) from the Broad Institute ([http://archive.broadinstitute.org/mpg/snap/ldsearchpw.php](http://archive.broadinstitute.org/mpg/snap/ldsearchpw.php)). These five SNPs were not in LD with one another \((r^2 < 0.8)\) and are polymorphic in the European population \((\text{MAF} > 0.05)\).

### Table 3 Pairwise LD (D’ and \(r^2\)) for the five candidate NTRK2 SNPs from SNAP

<table>
<thead>
<tr>
<th></th>
<th>rs1187325</th>
<th>rs1211166</th>
<th>rs1439050</th>
<th>rs1047896</th>
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<td>0.010</td>
<td>0.041</td>
<td>0.083</td>
<td>0.011</td>
<td></td>
</tr>
</tbody>
</table>
3.3 Sources of Genetic Data

Genotyping was performed on this cohort prior to the conception of this project. All deoxyribonucleic acid (DNA) genotyping was performed by research assistants who were blinded from the child’s phenotypic information. Genotypes were determined using one of two methods: Illumina PsychArray and/or TaqMan SNP genotyping assay.

**Illumina PsychArray:** Buccal swabs were collected from the two study sites (Montreal, Quebec and Hamilton, Ontario) and processed at the Douglas Mental Health University Institute in Montreal, Quebec. An Infinium Illumina PsychArray chip was run on these subjects using standard protocol to obtain genotype data for variants associated with common psychiatric disorders. The \( BDNF \) and \( NTRK2 \) gene region was part of this array and SNPs not captured by the PsychArray chip were imputed by our collaborators at the Douglas Mental Health University Institute. Data from this genome-wide genotyping assay has not yet been published at the time this thesis was written. As a result, we were unable to obtain details on quality control or imputation methods from our collaborators.

**TaqMan genotyping:** In addition, some buccal swabs were processed at the Centre for Addiction and Mental Health (CAMH) Neurogenetics Laboratory in Toronto, Ontario. DNA was extracted from the swabs and genotypes for the \( BDNF \) polymorphisms were determined using standard protocol for the TaqMan® SNP genotyping assay on the ABI Prism 7000 (Applied Biosystems, Foster City, California, USA). Two \( BDNF \) SNPs were genotyped using this method – rs6265 and rs56164415. The on-demand assay for rs6265 (C__11592758_10) and a custom-made assay for rs56164415 was used for genotyping.

The TaqMan data for these two SNPs were compared to genotypes obtained using the PsychArray chip for quality control. Discrepent \( BDNF \) rs6265 genotypes were detected in 2% of individuals (\( n=3 \)). Individuals with discrepant genotype were excluded from further analysis. The genotypes for \( BDNF \) rs56164415 were consistent between data obtained from PsychArray and TaqMan genotyping.
3.4 Eating Behaviour phenotypes

3.4.1 Snack Delay Task

Children from the MAVAN cohort completed the Snack Delay Task (Kochanska, Murray et al. 1996) at 3 years of age (36 months). The children sat in front of a table where a single M&M candy was placed under a glass cup. They were instructed to place their hands flat on a table and refrain from eating the candy until the research assistant rang a bell. This trial was repeated four times, each with a different delay time before the bell was rang (10s, 20s, 15s, and 30s). Halfway between each delay time, the experimenter lifted the bell but did not ring it. After each trial, the children were given a behavioural score ranging from 1 to 7 based on their attempt to eat the candy before the bell rang. The coding was as follows:

1 = eats the candy before the bell is lifted  
2 = eats the candy after the bell is lifted  
3 = touches the candy before the bell is lifted  
4 = touches the candy after the bell is lifted  
5 = touches the bell or cup before the bell is lifted  
6 = touches the bell or cup after the bell is lifted  
7 = waits for the bell to ring before touching cup or bell

In addition, the children were evaluated on their ability to wait for the M&M (i.e. snack delay latency to eat) and were given a score of 1 or 2. The coding was as follows:

1 = child keeps hands on mat during the entire time either before OR after the bell is lifted  
2 = child keeps hands on mat during the entire time before AND after the bell is lifted

The behavioural score (ranging from 1 to 7) was added to the snack delay latency to eat score (either 1 or 2) to create an overall performance score (ranging from 2 to 9) for each of the four snack delay trials. Therefore, the possible range of score for any one of the trials ranges from 2 (highly impulsive) to 9 (highly restrained).
Finally, a “global cooperation score” was given to each child to rate the child’s ability to engage and complete the task. The coding was as follows:

- **0** = the child is unwilling or unable to engage in the task
- **1** = the child is unwilling or unable to complete the task because of feeling tired, angry, irritable, or sick, or does not have the capacity to understand the instructions
- **2** = the child does all the trials but has comprehensional or motivational difficulties, or is passive or inhibited
- **3** = the child understands the task well and participates

Those with a global cooperation score less than 2 were excluded from the analysis (n=6).

An “Average Snack Delay performance score” was computed by summing the individual scores in the four Snack Delay trials and dividing the sum by 4. The variability of Average Snack Delay Scores in our sample was relatively low (see Figure 5). A large proportion of children (approx. 33%) had a perfect score of 9 and were able to inhibit response without any difficulty. We, therefore, created a binary variable with two categories: (1) Passed the Snack Delay Task (i.e. had score of 9) or (2) Failed the Snack Delay Task (i.e. had a score <9). This method of dichotomizing Snack Delay Task scores was previously used by Tonnsen and colleagues (Tonnsen, Grefer et al. 2014) because low variability of scores was also noted in their sample.
3.4.2 Child Eating Behaviour Questionnaire (CEBQ)

Parents from the MAVAN cohort filled out the Child Eating Behaviour Questionnaire (CEBQ) when the child was 4 years of age (48 months). The CEBQ was developed as a means to identify early behavioural precursors of obesity and eating disorders in children (Wardle, Guthrie et al. 2001). It is a well-validated psychometric tool with high internal validity and good test-retest reliability (Wardle, Guthrie et al. 2001). The CEBQ has also been validated against behavioural measures of eating (Carnell and Wardle 2007). This parental-reported questionnaire is comprised of 35 questions, each rated on a five-point Likert scale ranging from never (1) to always (5) and assesses eight dimensions of eating behaviours. Further details on each of the eight eating dimensions are provided in the Table 4 below. The eight dimensions can be subgrouped into “food approach behaviours” (Food responsiveness, Enjoyment of food, Desire to drink, Emotional overeating) and “food avoidant behaviours” (Satiety responsiveness, Slowness in eating, Food fussiness, Emotional undereating). Food approach behaviours are positively associated with BMI, while food avoidant behaviours are negatively associated with BMI (Webber, Hill et al. 2009, Santos, Ho-Urriola et al. 2011).

![Figure 5 Distribution of Average Snack Delay Scores](image)

Figure 5 Distribution of Average Snack Delay Scores
Table 4 Description of CEBQ subscales

<table>
<thead>
<tr>
<th>Food approach behaviours</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food responsiveness (FR)</td>
<td>Measures the inclination to eat when given the choice</td>
</tr>
<tr>
<td>Enjoyment of food (EF)</td>
<td>Measures the love of eating and interest in food</td>
</tr>
<tr>
<td>Desire to drink (DD)</td>
<td>Measures the inclination to drink when given the choice</td>
</tr>
<tr>
<td></td>
<td>DD scores reflects preference for and frequency of consuming sweetened drinks</td>
</tr>
<tr>
<td></td>
<td>(e.g. soft drinks) rather than a general thirst as DD scores are not linked</td>
</tr>
<tr>
<td></td>
<td>to intake of water or fruit juice (Sweetman, Wardle et al. 2008). Consumption</td>
</tr>
<tr>
<td></td>
<td>of sugar-sweetened drinks is associated with childhood obesity (Ludwig, Petet</td>
</tr>
<tr>
<td></td>
<td>e et al. 2001). Consumption of sugar-sweetened drinks is associated with child</td>
</tr>
<tr>
<td></td>
<td>hood obesity (Ludwig, Peterson et al. 2001).</td>
</tr>
<tr>
<td>Emotional overeating (EOE)</td>
<td>Measures the tendency to consume more when stressed, anxious, annoyed, or wor</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Food avoidant behaviours</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satiety responsiveness (SR)</td>
<td>Measures how easily a child gets full and stops eating</td>
</tr>
<tr>
<td>Slowness in eating (SE)</td>
<td>Measures how quickly/slowly a child finishes a meal</td>
</tr>
<tr>
<td>Food Fussiness (FF)</td>
<td>Measures willingness/unwillingness to try new foods and flavors</td>
</tr>
<tr>
<td>Emotional undereating (EUE)</td>
<td>Measures the tendency to consume less when upset, angry, tired, or happy</td>
</tr>
</tbody>
</table>

For the MAVAN cohort, we summed the scores for Food responsiveness, Enjoyment of food, Desire to drink, and Emotional overeating to create a rough combined index of “food approach” behaviours. This method was previously used by Santos and colleagues (Santos, Ho-Urriola et al. 2011). As well, we summed the scores for Satiety responsiveness, Slowness in eating, Food fussiness, and Emotional undereating to create a composite “food avoidant” score. This effectively reduces the number of statistical tests, which is especially important when there are multiple predictor variables (i.e. genetic variants) as in our case. Both Food Approach and Food Avoidant composite scores were normally distributed (Kolmogorov-Smirnov test p-value > 0.05). A z-score was calculated for the Food Approach and Food Avoidant scores so that results could be interpreted based on deviation from norm.

Cronbach’s alpha is a measure of how well a set of variables belong to a group and a Cronbach’s alpha of 0.7 or higher is considered to be indicative of good internal consistency. The Cronbach’s alpha for the Food Approach score was 0.563, while the Cronbach’s alpha for the Food Avoidant score was 0.709. Although Cronbach’s alpha was <0.7 for the Food Approach score, we decided to include this score in our analysis.
The eating behaviour construct measured by this score is valid as all of the individual questions making up this score measure a child’s tendency towards food. The low Cronbach’s alpha in our cohort maybe due to small sample size. We attempted to re-categorize the eight eating behaviour domains using factor analysis, but this also resulted in one component with a Cronbach’s alpha <0.7.

3.5 Covariates

The following covariates were considered based on prior literature on eating behaviours:

- Sex
- Birth weight (z-score)
- Mother’s BMI

Sex:
Sex-specific differences in food intake and eating behaviours are commonly reported in the literature (Leblanc, Begin et al. 2014). Females tend to show greater dietary restraint and inhibition as compared to males (Provencher, Drapeau et al. 2003).

Birth weight:
Children born small for gestational age often present with feeding difficulties and poor eating patterns such as eating smaller quantities (Oliveira, de Lauzon-Guillain et al. 2015), less motivation to eat, and a narrower food repertoire (Migraine, Nicklaus et al. 2013). Birth weight was measured in grams.

Mother’s BMI:
Appetitive restraint in children has been shown to negatively correlate with maternal BMI (Albuquerque, Severo et al. 2016). It will also be important to control for this factor because the CEBQ is a parental-reported questionnaire. An overweight or underweight mother might provide biased ratings for their child’s eating behaviour. Mother’s BMI was assessed when the child was 48 or 72 months of age. If a measurement was taken at both
time points, an average BMI was calculated. BMI was calculated using the following formula: weight (kg) divided by [height (m)]².

It should be noted that parental eating behaviours have been reported to affect children’s eating behaviour. However, this was not included as a covariate because, for the purposes of this study, we hypothesized that genetics contribute to these behaviours in both mothers and their children.

We assessed for correlations between these three potential covariates and our outcomes of interest (i.e. Snack Delay and CEBQ scores), irrespective of genetic data. Covariates were included in analysis only if they had a statistically significant correlation with a phenotype.

3.6 Statistical Analysis

Since the MAVAN cohort was predominately Caucasian, we restricted our analysis to subjects with Caucasian ancestry to control for population stratification in our genetic analysis. As well, all siblings were excluded from analysis. Due to the design of MAVAN, measures of the outcome variables were conducted at the same age for every participant. Therefore, all analysis will have inherently controlled for age and ethnicity. Other than haplotype analysis, all statistical analyses were performed on IBM Statistical Package for the Social Sciences (SPSS) Statistics software, version 21. Hardy-Weinberg equilibrium (HWE) was assessed for each SNP and those that deviated from HWE (i.e. \( p_{\text{HWE}} < 0.05 \)) were excluded from subsequent analysis. Pairwise LD analyses were calculated for pairs of SNPs within a locus using Haploview version 4.2.
3.6.1 Are \textit{BDNF} and \textit{NTRK2} genetic polymorphisms associated with performance on an objective measure of impulsive responding to a food cue?

\textit{Single-marker SNP analysis:}

The association between SNPs and the two categories of Snack Delay Task performance (i.e. Pass or Fail) was tested using binary logistic regression. Covariates were included in the analysis if shown to correlate with Snack Delay Task performance raw scores. Each of the 5 \textit{BDNF} and 5 \textit{NTRK2} SNPs were analyzed independently using an additive model of genetic inheritance. SNPs were coded as follows: 0= homozygous for major allele, 1= heterozygous, and 2= homozygous for the minor allele. For SNPs with low genotype frequency (i.e. a genotype group with n <5), individuals with homozygous minor allele genotype were combined with the heterozygotes according to a dominant model of genetic inheritance. We performed power calculations using Quanto version 1.2.4. With a sample size of 158 and assuming a minor allele frequency of 0.15, we had more than 80% power to detect an odds ratio of 2.90 and 3.15 under an additive model or dominant model of genetic inheritance, respectively.

\textit{Haplotype analysis:}

Unphased version 3.1.7 (Dudbridge 2008) was used to construct three-marker haplotypes based on the 5 \textit{BDNF} tagSNPs and the 5 \textit{NTRK2} SNPs. Haplotypes with frequencies less than 5% were excluded from the association analysis. Based on the constructed haplotypes, association analyses were carried out on Unphased to determine haplotypic associations in \textit{BDNF} or \textit{NTRK2} genes and performance on the Snack Delay Task. A binary Snack Delay Score (i.e. Pass or Fail) was used in haplotype analysis.
3.6.2 Are BDNF and NTRK2 genetic polymorphisms associated with dimensions of eating styles that are implicated in the development of weight problems as measured by a parental-reported questionnaire?

**Single-marker SNP analysis:**

Preliminary analysis indicated that the Food Approach score and the Food Avoidant score from the CEBQ were normally distributed (Kolmogorov–Smirnov test p-value > 0.05). We used linear regression to analyze the association between BDNF tagSNPs and NTRK2 SNPs on the scores derived from the CEBQ (i.e. Food Approach and Food Avoidant). Covariates were included in the analysis if shown to correlate with Food Approach or Food Avoidant CEBQ score. Each of the 5 BDNF tagSNPs and 5 NTRK2 candidate SNPs were analyzed independently under an additive model of genetic inheritance. For SNPs with low genotype frequency, individuals with homozygous minor allele genotype were combined with the heterozygotes according to a dominant model of genetic inheritance. As a post hoc test, if any of the SNPs demonstrated significant association with Food Approach or Food Avoidant score, we further investigated for its association with the four corresponding CEBQ subscales that made up the score. A p-value <0.05 was considered significant for exploratory post hoc tests. Power calculations were performed on Quanto version 1.2.4. Assuming a minor allele frequency of 0.15, we had more than 80% power to explain 5% of the variance in the phenotype in our sample of 160 children using either an additive or dominant model of genetic inheritance.

**Haplotype analysis:**

Unphased version 3.1.7 (Dudbridge 2008) was used to construct three-marker haplotypes based on the 5 BDNF tagSNPs and the 5 NTRK2 SNPs. Haplotypes with frequencies less than 5% were excluded from the association analysis. Based on the constructed haplotypes, association analyses were carried out on Unphased to determine haplotypic associations in BDNF or NTRK2 genes and Food Approach/ Food Avoidant score. No covariates were included in haplotype analysis as Unphased is not well-suited for analysis with covariates.
3.6.3 Does the $BDNF$ rs6265 functional variant interact with $NTRK2$ genetic polymorphisms to predict impulsive responding to a food cue in a controlled setting and/or parental-reported eating behaviours implicated in the development of weight problems?

**Gene-Gene interaction analysis:**

General linear models (GLM) were used to test for the interaction between the functional $BDNF$ Val66Met variant (rs6265) and 5 candidate $NTRK2$ SNPs on Snack Delay Performance (i.e. Pass or Fail). Only PsychArray genetic data was used in this analysis. GLM was also used to test for the same interaction on the Food Approach/ Food Avoidant scores from the CEBQ. Covariates were included in the analysis if they correlated with one of the phenotypes. For $NTRK2$ SNPs with low genotype frequency, individuals with homozygous minor allele genotypes were combined with the heterozygotes. According to power calculations using Quanto version 1.2.4, our sample was not well-powered to detect gene-gene interaction effects on the Snack Delay Task. For the CEBQ, however, assuming that each of the two genes account for 5% of the variance, we had 80% power to detect an interaction that explains an additional 5% of the variance in the phenotype.

3.6.4 Testing BMI as a mediator/moderator of effect

Although BMI was not a main outcome of our study, we will examine the potential effects of BMI as a mediator pending significant associations with the $BDNF$ and/or $NTRK2$ SNPs. This is because our genes and SNPs of interested were selected from obesity and/or eating disorder literature, which have close ties with body weight. For the purposes of this study we wanted to study eating behaviours independent of the effects of BMI as their bidirectional relationship may pose a risk for false positive results. The analysis will be performed by examining the effect of including the child’s BMI corresponding to the time of phenotype assessment in our statistical models.
3.6.5 Multiple testing corrections
A two-sided p < 0.05 before multiple testing corrections was considered nominally significant for all analysis.

**Single SNP association analysis for Snack Delay Task**
Single-Nucleotide Polymorphism Spectral Decomposition (SNPSpD) (Nyhold 2004) was used to correct for multiple comparisons in the analysis for Snack Delay Task. Adjusted p-values were derived from a modified version of SNPSpD as proposed by Li and Ji (Li and Ji 2005). The Li and Ji method more accurately estimates the effective number of independent marker loci ($M_{eff,Li}$) to keep the Type I Error Rate at 5% without lowering statistical power. Gene-wide adjusted p-values were calculated by multiplying unadjusted p-values by the $M_{eff,Li}$ for the corresponding gene. Hypothesis-wide adjusted p-values were calculated by multiplying unadjusted p-values by the summation of $M_{eff,Li}$ values for BDNF and NTRK2.

**Single SNP association analysis for CEBQ Food Approach/Avoidant Score**
The Food Approach and Food Avoidant score from the CEBQ were somewhat correlated (Pearson’s $R = -0.134$, $p = 0.048$). We employed the method proposed by Conneely and Boehnke (Conneely and Boehnke 2007) to calculate adjusted p-values for association analysis using $p_{ACT}$. This program is implemented in R and adjusts p-values for multiple association tests between correlated traits and SNPs. If the adjusted p-value remained <0.05 after $p_{ACT}$ correction, the association was considered to be statistically significant.

**BDNF and NTRK2 haplotypes with Snack Delay Task or CEBQ Food Approach/Avoidant Score**
The haplotypic association p-value was corrected for multiple testing using Bonferroni correction, i.e. dividing 0.05 by the number of haplotype groups created.
Gene x Gene interactions with Snack Delay Task or CEBQ Food Approach/Avoidant Score

A Bonferroni p-value was used to correct for multiple comparisons by dividing 0.05 by the number of independent tests.

3.6.6 Summary of Statistical Methods

Table 5 Summary of Statistical Methods

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Analysis</th>
<th>Statistical Test</th>
<th>Multiple testing correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snack Delay Task</td>
<td>Single SNP association analysis</td>
<td>Binary logistic regression</td>
<td>SNPSpD</td>
</tr>
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<td>Haplotypic analysis</td>
<td>Chi-square</td>
<td>Bonferroni</td>
</tr>
<tr>
<td></td>
<td>Gene x Gene interaction</td>
<td>General linear model</td>
<td>Bonferroni</td>
</tr>
<tr>
<td>CEBQ: Food Approach &amp; Food Avoidant Score</td>
<td>Single SNP association analysis</td>
<td>Linear Regression</td>
<td>p_ACT</td>
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<tr>
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<td>Haplotypic analysis</td>
<td>Chi-square</td>
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<tr>
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<td>Gene x Gene interaction</td>
<td>General linear model</td>
<td>Bonferroni</td>
</tr>
</tbody>
</table>
Chapter 4 Results

4.1 MAVAN Cohort Demographics

In total, 183 unrelated children from the MAVAN cohort with Caucasian ancestry provided samples for genetic analysis (Males =49.7%). These 183 children were genotyped using the PsychArray. An additional 62 subjects were genotyped using TaqMan for a subset of the SNPs. These individuals only had data for two SNPs of interest, namely $BDNF$ rs6265 and $BDNF$ rs56164415. All genotyping was performed prior to designing this study. Since we combined PsychArray and TaqMan data for rs6265 and rs56164415, the sample size for these two variants was larger than the other SNPs of interest.

The collection of phenotypic data from the MAVAN cohort was performed at age appropriate time points. The Snack Delay Task was administered when the child was 36 months of age and the CEBQ was completed by parents when the child was 48 months of age. The following table (Table 6) displays the mean values for birth weight, BMI and mother’s BMI as these are potentially relevant covariates or mediators in our analysis.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (grams)</td>
<td>3338.4 ± 467.7</td>
</tr>
<tr>
<td>Child’s BMI at 36 months (kg/m²)</td>
<td>16.2 ± 1.5</td>
</tr>
<tr>
<td>Child’s BMI at 48 months (kg/m²)</td>
<td>16.0 ± 1.5</td>
</tr>
<tr>
<td>Mother’s BMI (kg/m²)</td>
<td>27.4 ± 6.64</td>
</tr>
</tbody>
</table>

These values do not differ significantly even if accounting for the additional 62 subjects with TaqMan data for $BDNF$ rs6265 and $BDNF$ rs56164415. Further demographic details of the MAVAN cohort can be found in the published methodology (O'Donnell, Gaudreau et al. 2014).
4.2 Hardy-Weinberg Equilibrium and Linkage Disequilibrium

We assessed the Hardy-Weinberg equilibrium of each of the BDNF and NTRK2 SNPs stratified by ethnicity (i.e. Caucasian or non-Caucasian) (Table 7). NTRK2 rs1211166 was excluded from further analysis because it had a HWE p-value of <0.05 in our subgroup of Caucasians.

**Table 7** Analysis of Hardy-Weinberg equilibrium in BDNF and NTRK2 SNPs

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype</th>
<th>Caucasians Frequency</th>
<th>HWE</th>
<th>Non-Caucasians Frequency</th>
<th>HWE</th>
<th>Total Frequency</th>
<th>HWE</th>
</tr>
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<tr>
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<tr>
<td></td>
<td>rs6265</td>
<td>CC</td>
<td>119</td>
<td>0.668</td>
<td>22</td>
<td>0.797</td>
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<td>CT</td>
<td>56</td>
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HWE = Hardy-Weinberg Equilibrium p-value; * means PsychArray genetic data combined with additional TaqMan genotypes; Bold means p-value <0.05
Linkage disequilibrium (LD) analyses were performed for the 5 BDNF and 4 NTRK2 SNPs. Pairwise $r^2$ values are shown in Figure 6.1 and 6.2. These $r^2$ values are in accordance with what has been reported for a reference European ancestry population in the 1000 genomes project (accessed via SNAP tool).

**Figure 6.1** BDNF gene structure and linkage disequilibrium among the SNPs investigated. Multiple transcripts can be produced for this gene through alternative splicing. Arrows represent direction of transcription and grey boxes represent exon regions. Study SNPs are indicated along the BDNF gene structure. LD plot was created in Haploview version 4.2 (Barrett, Fry et al. 2005). Values in the LD plot are $r^2$ statistics. Standard Haploview colour scheme was used: bright red: D’=1 and logarithm of the odds (LOD) $\geq 2$; shades of red: D’<1 and LOD $\geq 2$; blue: D’=1 and LOD <2.
Figure 6.2 NTRK2 gene structure and linkage disequilibrium among the SNPs investigated. Multiple transcripts can be produced for this gene through alternative splicing. Arrows represent direction of transcription and grey boxes represent exon regions. Study SNPs are indicated along the NTRK2 gene structure. LD plot was created in Haploview version 4.2. Values in the LD plot are $r^2$ statistics. Standard Haploview colour scheme was used: bright red: $D'=1$ and logarithm of the odds (LOD) $\geq2$; shades of pink: $D'<1$ and LOD $\geq2$; white: $D'<1$ and LOD $<2$. 
4.3 Assessment of Covariates

Mother’s BMI was positively correlated with Food Approach Score from the CEBQ (Pearson R = 0.148, p = 0.041) and was included in analysis for this phenotype. No covariates were included in analysis for Snack Delay Task or Food Avoidant score as none of the three covariates correlated with these phenotypes (Table 8).

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<th>Sex</th>
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<th>Mother’s BMI</th>
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4.4 BDNF and NTRK2 genetic-association analysis: Snack Delay Task

164 children with Caucasian ancestry completed the Snack Delay Task at 36 months of age. Six children were excluded from analysis because they had a Global Cooperation Score less than 2. Therefore, the final sample size used in analysis was 158.

Single-marker SNP analysis:

Each of the five BDNF SNPs and four NTRK2 SNPs were tested for association with performance on the Snack Delay Task (Pass or Fail) using a binary logistic regression model (Table 9). Although no covariates were included in the statistical model, we chose to use regression instead of chi-square in order to perform analysis using an additive model of genetic inheritance. None of the five BDNF SNPs included in this study demonstrated significant association. One of the NTRK2 SNPs, rs1047896, was significantly associated with Snack Delay Task performance using an additive (OR [95%CI] = 2.885 [1.284-6.485], p = 0.010) or dominant (OR [95%CI] = 2.925 [1.245-6.872], p-value = 0.014) model of genetic inheritance. Carriers of the minor G allele were more likely to “fail” the Snack Delay Task (Figure 7), which is indicative of the inability to inhibit response in the presence of an appetite stimulus. The $M_{\text{eff}}$ for the 5 BDNF SNPs was 4 and the $M_{\text{eff}}$ for the 4 NTRK2 SNPs was 3. These values were used to calculate the gene-wide and hypothesis-wide adjusted p-values. The association for NTRK2 rs1047896 remained significant after correction for gene-wide multiple comparisons, but did not withstand hypothesis-wide multiple testing correction (Table 9).
Table 9 Association analysis of BDNF and NTRK2 SNPs with Snack Delay Task performance (Pass or Fail)

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<th>Fail (N)</th>
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* means PsychArray genetic data combined with additional TaqMan genotypes; a Genotypes were regrouped according to the dominant model of inheritance by combining the minor allele homozygotes with the heterozygotes
Haplotype analysis:

Three-marker haplotypes were created for *BDNF* and *NTRK2* SNPs and were tested for association with performance on the Snack Delay Task. Haplotype analyses were performed using a three SNP sliding window. This created three sliding windows for the five *BDNF* SNPs and two sliding windows for the four *NTRK2* SNPs (Table 10).

No associations were observed between *BDNF* haplotypes and Snack Delay Task performance. Both groups of *NTRK2* haplotypes demonstrated significant association with Snack Delay performance (p = 0.044 and p = 0.006). Specifically, the C-T-G haplotype formed by rs1187325-rs1439050-rs1047896 (p = 0.027) and the T-G-C haplotype formed by rs1439050-rs1047896-rs1078947 (p = 0.013) associated with failure of the Snack Delay Task. The G-G-A haplotype formed by rs1187325-rs1439050-rs1047896 (p = 0.009) and the G-A-C haplotype formed by rs1439050-rs1047896-rs1078947 (p = 0.004) associated with successfully passing the Snack Delay Task.

After applying the Bonferroni correction for multiple testing, p< 0.01 (5 three-marker haplotype groups were examined; 0.05/5 = 0.01) was considered statistically significant for the overall p-value in *NTRK2* haplotypes. Since analyses of individual allele combinations that made up a haplotype group were *post hoc* analyses, a p-value of 0.05 was considered significant. Therefore, the G-A-C and T-G-C haplotypes formed by rs1439050-rs1047896-rs1078947 remained significant after multiple testing corrections.
Table 10 Haplotype analysis of *BDNF* and *NTRK2* SNPs with Snack Delay Task performance (Pass or Fail)

### **BDNF Haplotypes**

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Overall p-value</th>
<th>Haplotype</th>
<th>Pass Count (Frequency)</th>
<th>Fail Count (Frequency)</th>
<th>Chi-square</th>
<th>Haplotype p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6265</td>
<td>0.891</td>
<td>C-C-A</td>
<td>6 (6.5%)</td>
<td>10 (4.5%)</td>
<td>0.547</td>
<td>0.459</td>
</tr>
<tr>
<td>rs11030102</td>
<td></td>
<td>C-C-G</td>
<td>48 (52.2%)</td>
<td>121 (54.5%)</td>
<td>0.142</td>
<td>0.706</td>
</tr>
<tr>
<td>rs11030108</td>
<td></td>
<td>C-G-A</td>
<td>23 (25%)</td>
<td>53 (23.9%)</td>
<td>0.045</td>
<td>0.832</td>
</tr>
<tr>
<td>rs7103873</td>
<td></td>
<td>T-C-G</td>
<td>15 (16.3%)</td>
<td>38 (17.1%)</td>
<td>0.031</td>
<td>0.861</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Overall p-value</th>
<th>Haplotype</th>
<th>Pass Count (Frequency)</th>
<th>Fail Count (Frequency)</th>
<th>Chi-square</th>
<th>Haplotype p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7103873</td>
<td>0.900</td>
<td>C-A-G</td>
<td>6 (6.7%)</td>
<td>10 (4.6%)</td>
<td>0.532</td>
<td>0.466</td>
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<tr>
<td>rs11030102</td>
<td></td>
<td>C-G-G</td>
<td>18 (20.0%)</td>
<td>43 (19.9%)</td>
<td>0.0003</td>
<td>0.985</td>
</tr>
<tr>
<td>rs1047896</td>
<td></td>
<td>C-G-C</td>
<td>43 (47.8%)</td>
<td>109 (50.4%)</td>
<td>0.183</td>
<td>0.669</td>
</tr>
<tr>
<td>rs56164415</td>
<td></td>
<td>G-A-G</td>
<td>23 (25.6%)</td>
<td>54 (25.0%)</td>
<td>0.010</td>
<td>0.919</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Overall p-value</th>
<th>Haplotype</th>
<th>Pass Count (Frequency)</th>
<th>Fail Count (Frequency)</th>
<th>Chi-square</th>
<th>Haplotype p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11030108</td>
<td>0.938</td>
<td>A-G-G</td>
<td>22 (25.6%)</td>
<td>50 (24.0%)</td>
<td>0.078</td>
<td>0.780</td>
</tr>
<tr>
<td>rs7103873</td>
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<td>A-G-A</td>
<td>5 (5.8%)</td>
<td>9 (4.3%)</td>
<td>0.297</td>
<td>0.586</td>
</tr>
<tr>
<td>rs56164415</td>
<td></td>
<td>G-G-G</td>
<td>17 (19.8%)</td>
<td>43 (20.6%)</td>
<td>0.031</td>
<td>0.861</td>
</tr>
<tr>
<td>rs56164415</td>
<td></td>
<td>G-C-G</td>
<td>42 (48.8%)</td>
<td>106 (51.0%)</td>
<td>0.110</td>
<td>0.740</td>
</tr>
</tbody>
</table>

### **NTRK2 Haplotypes**

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Overall p-value</th>
<th>Haplotype</th>
<th>Pass Count (Frequency)</th>
<th>Fail Count (Frequency)</th>
<th>Chi-square</th>
<th>Haplotype p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1187325</td>
<td><strong>0.044</strong></td>
<td>C-G-A</td>
<td>7 (7.9%)</td>
<td>21 (9.9%)</td>
<td>0.279</td>
<td>0.597</td>
</tr>
<tr>
<td>rs1439050</td>
<td></td>
<td>C-T-G</td>
<td>5 (5.3%)</td>
<td>29 (13.8%)</td>
<td>4.912</td>
<td><strong>0.027</strong></td>
</tr>
<tr>
<td>rs1047896</td>
<td></td>
<td>C-T-A</td>
<td>17 (19.7%)</td>
<td>46 (21.6%)</td>
<td>0.114</td>
<td>0.737</td>
</tr>
<tr>
<td>rs1047896</td>
<td></td>
<td>G-G-G</td>
<td>3 (3.8%)</td>
<td>17 (7.9%)</td>
<td>1.708</td>
<td>0.191</td>
</tr>
<tr>
<td>rs1047896</td>
<td></td>
<td>G-G-A</td>
<td>56 (63.2%)</td>
<td>99 (46.8%)</td>
<td>6.733</td>
<td><strong>0.009</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Overall p-value</th>
<th>Haplotype</th>
<th>Pass Count (Frequency)</th>
<th>Fail Count (Frequency)</th>
<th>Chi-square</th>
<th>Haplotype p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1439050</td>
<td><strong>0.006</strong></td>
<td>G-G-C</td>
<td>1 (1.9%)</td>
<td>10 (6.8%)</td>
<td>2.362</td>
<td>0.124</td>
</tr>
<tr>
<td>rs1047896</td>
<td></td>
<td>G-A-C</td>
<td>53 (77.5%)</td>
<td>89 (57.5%)</td>
<td>8.205</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>rs1047896</td>
<td></td>
<td>T-G-C</td>
<td>2 (2.5%)</td>
<td>20 (12.7%)</td>
<td>6.120</td>
<td><strong>0.013</strong></td>
</tr>
<tr>
<td>rs1078947</td>
<td></td>
<td>T-A-C</td>
<td>12 (18.1%)</td>
<td>35 (23.0%)</td>
<td>0.658</td>
<td>0.417</td>
</tr>
</tbody>
</table>
We created the following regional LD plot for \textit{NTRK2} rs1047896 (Figure 8) using SNAP (http://archive.broadinstitute.org/mpg/snap/ldplot.php#). The rs1047896 SNP appears to be in LD with multiple SNPs in the 3' region of the \textit{NTRK2} gene.

Figure 8 Regional Linkage Disequilibrium plot for rs1047896. This plot displays SNPs within 250kb of rs1047896 using SNP information from the CEU population in the 1000 Genomes Project.
4.5 \textit{BDNF} and \textit{NTRK2} genetic-association analysis: Food Approach and Food Avoidant Score on the CEBQ

Child Eating Behaviour Questionnaire (CEBQ) data was collected for 160 children with Caucasian ancestry at 48 months of age.

\textit{Single-marker SNP analysis:}
Each of the five \textit{BDNF} SNPs and four \textit{NTRK2} SNPs were tested for association with Food Approach and Food Avoidant z-score using linear regression (Table 1). Mother’s BMI was used as a covariate in the statistical models for Food Approach score analysis. None of the five \textit{BDNF} SNPs included in this study demonstrated significant association with either Food Approach or Food Avoidant z-score. One of the \textit{NTRK2} SNPs, rs1078947, was associated with Food Approach score using an additive (B=0.548±0.192, \(p = 0.005\)) or dominant (B=0.567±0.213, \(p\)-value = 0.009) model of genetic inheritance. Carriers of the minor T allele had a slightly higher Food Approach score (Figure 9). Multiple testing corrected \(p\)-values were calculated using the p\_ACT program. The \(p\)-values for \textit{NTRK2} rs1078947 on Food Approach did not remain significant after the correction for multiple testing (adjusted \(p\)-values for additive and dominant model = 0.101 and 0.169, respectively). None of the four \textit{NTRK2} SNPs associated with Food Avoidant scores.

\textbf{Table 1} Association analysis of \textit{BDNF} and \textit{NTRK2} SNPs with Food Approach and Food Avoidant Scores from the CEBQ

<table>
<thead>
<tr>
<th>Food Approach Score</th>
<th>Gene</th>
<th>SNP</th>
<th>Genotype</th>
<th>Frequency</th>
<th>(\beta)</th>
<th>SE</th>
<th>(p)-value</th>
<th>Regrouped genotype*</th>
<th>(\beta)</th>
<th>SE</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>rs6265</td>
<td>CC</td>
<td>94</td>
<td>-0.082</td>
<td>0.164</td>
<td>0.619</td>
<td>94</td>
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<tr>
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<td>rs6265*</td>
<td>CC</td>
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<td>-0.196</td>
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<td>SNP</td>
<td>Genotype</td>
<td>Frequency</td>
<td>β</td>
<td>SE</td>
<td>p-value</td>
<td>Regrouped genotype</td>
<td>β</td>
<td>SE</td>
<td>p-value</td>
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</tr>
<tr>
<td>NTRK2</td>
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<td>GG GC CC</td>
<td>50 68 22</td>
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<td>GG GT TT</td>
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<td>93 43 5</td>
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<td>0.192</td>
<td><strong>0.005</strong></td>
<td>106 27 2</td>
<td>0.567</td>
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</tr>
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<td>BDNF</td>
<td>rs6265</td>
<td>CC CT TT</td>
<td>105 50 5</td>
<td>-0.069</td>
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<td>rs6265*</td>
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<td>146 64 7</td>
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<td>0.667</td>
<td>146 64 7</td>
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<td>0.643</td>
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<tr>
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<td>93 56 10</td>
<td>0.096</td>
<td>0.124</td>
<td>0.437</td>
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<td>79 65 15</td>
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<td>0.116</td>
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<td>0.237</td>
<td>0.507</td>
<td>138 14 1</td>
<td>0.170</td>
<td>0.260</td>
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<tr>
<td></td>
<td>rs56164415*</td>
<td>GG GA AA</td>
<td>189 24 1</td>
<td>0.251</td>
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<td>189 24 1</td>
<td>0.268</td>
<td>0.213</td>
<td>0.210</td>
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<tr>
<td></td>
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<td>GG GC CC</td>
<td>55 81 22</td>
<td>0.102</td>
<td>0.114</td>
<td>0.396</td>
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</tr>
<tr>
<td></td>
<td>rs1439050</td>
<td>GG GT TT</td>
<td>68 71 17</td>
<td>0.007</td>
<td>0.115</td>
<td>0.925</td>
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</tr>
<tr>
<td></td>
<td>rs1047896</td>
<td>AA AG GG</td>
<td>105 48 5</td>
<td>-0.018</td>
<td>0.142</td>
<td>0.896</td>
<td>105 48 5</td>
<td>-0.032</td>
<td>0.163</td>
<td>0.844</td>
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<tr>
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<td>rs1078947</td>
<td>CC CT TT</td>
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<td>-0.005</td>
<td>0.173</td>
<td>0.977</td>
<td>119 31 2</td>
<td>-0.011</td>
<td>0.189</td>
<td>0.956</td>
<td></td>
</tr>
</tbody>
</table>

SE = Standard error; * means PsychArray genetic data combined with additional TaqMan genotypes; ** Genotypes were regrouped according to the dominant model of inheritance by combining the minor allele homozygotes with the heterozygotes.
Post hoc analysis for rs1078947 and Food Approach subscales

Even though the adjusted p-value for rs1078947 and Food Approach score did not cross the threshold for statistical significance after multiple testing corrections, we decided to perform post hoc tests because the goal of the overall project was to generate testable hypotheses for future investigation. The Food Approach Score encompasses several aspects of eating behaviours and we were interested in potential associations between this NTRK2 SNP and specific eating behaviours.

To further understand the association between NTRK2 rs1078947 and “Food Approach” eating behaviours, we examined this SNP in relation to the four individual subscales of the CEBQ that made up the composite Food Approach score (Table 12). Non-parametric tests were used in analysis because scores for Food responsiveness, Enjoyment of food, Desire to drink, and Emotional overeating were not normally distributed (Kolmogorov–Smirnov test p-value <0.05).

Tests of associations using three (CC vs CT vs TT) or two (CC vs CT+TT) genotype groups revealed significant associations between the rs1078947 SNP and Food responsiveness (p-value_{3groups}=0.024, p-value_{2groups}=0.018), Desire to drink (p-value_{3groups}=0.022, p-value_{2groups}=0.006), and Emotional overeating behaviours (p-
value_{3\text{groups}}=0.044, p-value_{2\text{groups}}=0.030). As this was a post hoc exploratory analysis, the p-value threshold of significance was set at 0.05.

**Table 12** Association between NTRK2 rs1078947 and CEBQ Food Approach Subscales

<table>
<thead>
<tr>
<th>Food Approach subscales</th>
<th>(CC vs CT vs TT)</th>
<th>(CC vs CT+TT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value (Kruskal-Wallis)</td>
<td>p-value (Mann-Whitney U)</td>
</tr>
<tr>
<td>Food responsiveness</td>
<td>0.024</td>
<td>0.018</td>
</tr>
<tr>
<td>Enjoyment of food</td>
<td>0.626</td>
<td>0.986</td>
</tr>
<tr>
<td>Desire to drink</td>
<td>0.022</td>
<td>0.006</td>
</tr>
<tr>
<td>Emotional overeating</td>
<td>0.044</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Significant genotypic associations are shown in the following graphs (Figure 10.1, 10.2, and 10.3).

**Figure 10.1** NTRK2 rs1078947 genotype and Food Responsiveness z-Score
**Figure 10.2** *NTRK2* rs1078947 genotype and Desire to Drink z-Score

**Figure 10.3** *NTRK2* rs1078947 genotype and Emotional Overeating z-Score

**Haplotype analysis:**

Three-marker haplotypes were constructed for *BDNF* and *NTRK2* SNPs and haplotype groups were tested for association with a composite Food Approach and Food Avoidant scores from the CEBQ. No associations were observed between *BDNF* or *NTRK2* haplotypes and Food Approach or Food Avoidant scores (Table 13).
Table 13 Haplotype analysis of *BDNF* and *NTRK2* SNPs with Food Approach and Food Avoidant Scores from the CEBQ

### CEBQ Food Approach Score

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNPs</th>
<th>Overall p-value</th>
<th>Haplotype</th>
<th>Count (Frequency)</th>
<th>Chi-square</th>
<th>Haplotype p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF</td>
<td>rs6265 rs11030102 rs11030108</td>
<td>0.936</td>
<td>C-C-A</td>
<td>19 (5.9%)</td>
<td>0.017</td>
<td>0.897</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-C-G</td>
<td>164 (51.6%)</td>
<td>0.116</td>
<td>0.734</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-G-A</td>
<td>76 (23.9%)</td>
<td>0.423</td>
<td>0.516</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T-C-G</td>
<td>59 (18.6%)</td>
<td>0.039</td>
<td>0.844</td>
</tr>
<tr>
<td></td>
<td>rs11030102 rs11030108 rs7103873</td>
<td>0.951</td>
<td>C-A-G</td>
<td>19 (6.2%)</td>
<td>0.036</td>
<td>0.850</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-G-G</td>
<td>64 (20.9%)</td>
<td>0.138</td>
<td>0.710</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-G-C</td>
<td>147 (48.0%)</td>
<td>0.003</td>
<td>0.957</td>
</tr>
<tr>
<td></td>
<td>rs11030108 rs7103873 rs56164415</td>
<td>0.934</td>
<td>G-A-G</td>
<td>76 (24.8%)</td>
<td>0.268</td>
<td>0.605</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A-G-G</td>
<td>71 (24.3%)</td>
<td>0.034</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A-G-A</td>
<td>16 (5.5%)</td>
<td>0.221</td>
<td>0.639</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G-G-G</td>
<td>62 (21.2%)</td>
<td>0.152</td>
<td>0.696</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G-C-G</td>
<td>143 (48.9%)</td>
<td>0.140</td>
<td>0.708</td>
</tr>
<tr>
<td>NTRK2</td>
<td>rs1187325 rs1439050 rs1047896</td>
<td>0.572</td>
<td>C-G-A</td>
<td>19 (7.0%)</td>
<td>0.003</td>
<td>0.959</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-T-G</td>
<td>39 (14.3%)</td>
<td>1.287</td>
<td>0.257</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-T-A</td>
<td>58 (21.3%)</td>
<td>1.140</td>
<td>0.286</td>
</tr>
<tr>
<td></td>
<td>rs1439050 rs1047896 rs1078947</td>
<td>0.866</td>
<td>G-G-A</td>
<td>156 (57.3%)</td>
<td>0.003</td>
<td>0.957</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G-A-C</td>
<td>139 (66.8%)</td>
<td>0.180</td>
<td>0.671</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T-G-C</td>
<td>23 (11.1%)</td>
<td>0.004</td>
<td>0.947</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T-A-C</td>
<td>46 (22.1%)</td>
<td>0.283</td>
<td>0.595</td>
</tr>
</tbody>
</table>

### CEBQ Food Avoidant Score

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNPs</th>
<th>Overall p-value</th>
<th>Haplotype</th>
<th>Count (Frequency)</th>
<th>Chi-square</th>
<th>Haplotype p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF</td>
<td>rs6265 rs11030102 rs11030108</td>
<td>0.936</td>
<td>C-C-A</td>
<td>19 (5.9%)</td>
<td>0.296</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-C-G</td>
<td>164 (51.6%)</td>
<td>0.450</td>
<td>0.503</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-G-A</td>
<td>76 (23.9%)</td>
<td>0.632</td>
<td>0.427</td>
</tr>
<tr>
<td></td>
<td>rs11030102 rs11030108 rs7103873</td>
<td>0.951</td>
<td>C-A-G</td>
<td>19 (6.2%)</td>
<td>0.211</td>
<td>0.646</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-G-G</td>
<td>64 (20.9%)</td>
<td>1.998</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-G-C</td>
<td>147 (48.0%)</td>
<td>0.161</td>
<td>0.688</td>
</tr>
<tr>
<td></td>
<td>rs11030108 rs7103873</td>
<td>0.934</td>
<td>G-A-G</td>
<td>76 (24.8%)</td>
<td>0.372</td>
<td>0.542</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A-G-G</td>
<td>71 (24.3%)</td>
<td>1.272</td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A-G-A</td>
<td>16 (5.5%)</td>
<td>0.319</td>
<td>0.572</td>
</tr>
<tr>
<td>Haplotype</td>
<td>rs56164415</td>
<td>G-G-G</td>
<td>62 (21.2%)</td>
<td>2.130</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>-----------</td>
<td>------------</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G-C-G</td>
<td>143 (48.9%)</td>
<td>0.001</td>
<td>0.975</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-G-A</td>
<td>19 (7.0%)</td>
<td>0.063</td>
<td>0.801</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-T-G</td>
<td>39 (14.3%)</td>
<td>0.734</td>
<td>0.392</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-T-A</td>
<td>58 (21.3%)</td>
<td>1.195</td>
<td>0.274</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G-G-A</td>
<td>156 (57.3%)</td>
<td>0.028</td>
<td>0.866</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G-A-C</td>
<td>139 (66.8%)</td>
<td>0.008</td>
<td>0.930</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-G-C</td>
<td>23 (11.1%)</td>
<td>0.013</td>
<td>0.908</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-A-C</td>
<td>46 (22.1%)</td>
<td>0.0001</td>
<td>0.990</td>
<td></td>
</tr>
</tbody>
</table>

We created the following regional LD plot for NTRK2 rs1078947 using SNAP (Figure 11). It should be noted that the rs1078947 SNP is located within in the NTRK2 gene (Figure 6.2), but the green line in Figure 10 likely represents the shortest NTRK2 transcript. rs1078947 appears to be in LD with some SNPs near the 3’ end of the gene.

Figure 11 Regional Linkage Disequilibrium plot for rs1078947. This plot displays SNPs within 250kb of rs1078947 using SNP information from the CEU population in the 1000 Genomes Project.
4.6 $BDNF$ rs6265 x $NTRK2$ SNPs on Snack Delay Task Performance and CEBQ Scores

**Gene-Gene interaction analysis:**

We accessed for gene-gene interactions between the functional $BDNF$ rs6265 variant and the four $NTRK2$ SNPs using GLM. $BDNF$ rs6265 did not interact with any of the four $NTRK2$ SNPs on Snack Delay Task performance (n=157) (Table 14). We also assessed the association between $BDNF$ and $NTRK2$ SNPs on the composite Food Approach (n=140) and Food Avoidant (n=158) scores from the CEBQ (Table 15). Mother’s BMI was included as a covariate for Food Approach Score analyses. We detected an epistatic effect between $BDNF$ rs6265 and $NTRK2$ rs1187325 (F=4.934, p=0.009), but the main effects of the individual SNPs in the model were not significant ($BDNF$ rs6265: F=1.701, p=0.187; $NTRK2$ rs1187325: F=0.530, p=0.590). A Bonferroni correction was applied for multiple testing in the gene-gene interaction analyses and was calculated to be 0.05/12=0.004 (there were 12 independent statistical tests in total). Therefore, the epistatic effect did not cross this threshold and can only be considered nominally significant.

**Table 14** Interaction between $BDNF$ rs6265 and $NTRK2$ SNPs on Snack Delay Task performance (Pass or Fail)

<table>
<thead>
<tr>
<th></th>
<th>$BDNF$ rs6265 x SNP Interaction</th>
<th>F</th>
<th>p-value</th>
<th>Eta Square</th>
<th>Observed Power $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$BDNF$ rs6265 x $NTRK2$ rs1187325</td>
<td></td>
<td>0.545</td>
<td>0.652</td>
<td>0.011</td>
<td>0.160</td>
</tr>
<tr>
<td>$BDNF$ Val/Met x $NTRK2$ rs1187325</td>
<td></td>
<td>0.263</td>
<td>0.769</td>
<td>0.003</td>
<td>0.091</td>
</tr>
<tr>
<td>$BDNF$ rs6265 x $NTRK2$ rs1439050</td>
<td></td>
<td>0.756</td>
<td>0.520</td>
<td>0.015</td>
<td>0.209</td>
</tr>
<tr>
<td>$BDNF$ Val/Met x $NTRK2$ rs1439050</td>
<td></td>
<td>0.138</td>
<td>0.871</td>
<td>0.002</td>
<td>0.071</td>
</tr>
<tr>
<td>$BDNF$ rs6265 x $NTRK2$ rs1047896 $^a$</td>
<td></td>
<td>0.607</td>
<td>0.546</td>
<td>0.008</td>
<td>0.150</td>
</tr>
<tr>
<td>$BDNF$ Val/Met x $NTRK2$ rs1047896 $^a$</td>
<td></td>
<td>0.129</td>
<td>0.720</td>
<td>0.001</td>
<td>0.065</td>
</tr>
<tr>
<td>$BDNF$ rs6265 x $NTRK2$ rs1078947 $^a$</td>
<td></td>
<td>0.080</td>
<td>0.923</td>
<td>0.001</td>
<td>0.062</td>
</tr>
<tr>
<td>$BDNF$ Val/Met x $NTRK2$ rs1078947 $^a$</td>
<td></td>
<td>0.005</td>
<td>0.945</td>
<td>0.000</td>
<td>0.051</td>
</tr>
</tbody>
</table>

$^a$ Due to low genotype counts, genotypes were regroup by combining the minor allele homozygotes with the heterozygotes; $^b$ Observed power is the probability of correctly rejecting the null hypothesis
**Table 15** Interaction between BDNF rs6265 and NTRK2 SNPs on Food Approach and Food Avoidant Scores from the CEBQ

<table>
<thead>
<tr>
<th>BDNF rs6265 x SNP Interaction</th>
<th>BDNF rs6265 x NTRK2 rs1187325</th>
<th>BDNF Val/Met x NTRK2 rs1187325</th>
<th>BDNF rs6265 x NTRK2 rs1439050</th>
<th>BDNF Val/Met x NTRK2 rs1439050</th>
<th>BDNF rs6265 x NTRK2 rs1047896</th>
<th>BDNF Val/Met x NTRK2 rs1047896</th>
<th>BDNF rs6265 x NTRK2 rs1078947</th>
<th>BDNF Val/Met x NTRK2 rs1078947</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food Approach Score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF rs6265 x NTRK2 rs1187325</td>
<td>4.934</td>
<td>0.009</td>
<td>0.070</td>
<td>0.800</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF Val/Met x NTRK2 rs1187325</td>
<td>5.025</td>
<td>0.008</td>
<td>0.070</td>
<td>0.808</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF rs6265 x NTRK2 rs1439050</td>
<td>0.012</td>
<td>0.912</td>
<td>0.000</td>
<td>0.051</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF Val/Met x NTRK2 rs1439050</td>
<td>0.019</td>
<td>0.951</td>
<td>0.000</td>
<td>0.050</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF rs6265 x NTRK2 rs1047896</td>
<td>1.795</td>
<td>0.170</td>
<td>0.026</td>
<td>0.370</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF Val/Met x NTRK2 rs1047896</td>
<td>3.183</td>
<td>0.077</td>
<td>0.023</td>
<td>0.425</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF rs6265 x NTRK2 rs1078947</td>
<td>1.544</td>
<td>0.218</td>
<td>0.024</td>
<td>0.323</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF Val/Met x NTRK2 rs1078947</td>
<td>0.464</td>
<td>0.497</td>
<td>0.004</td>
<td>0.104</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Food Avoidant Score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF rs6265 x NTRK2 rs1187325</td>
<td>0.569</td>
<td>0.636</td>
<td>0.011</td>
<td>0.166</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF Val/Met x NTRK2 rs1187325</td>
<td>0.267</td>
<td>0.766</td>
<td>0.004</td>
<td>0.092</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF rs6265 x NTRK2 rs1439050</td>
<td>0.836</td>
<td>0.476</td>
<td>0.017</td>
<td>0.228</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF Val/Met x NTRK2 rs1439050</td>
<td>0.397</td>
<td>0.673</td>
<td>0.005</td>
<td>0.113</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF rs6265 x NTRK2 rs1047896</td>
<td>0.151</td>
<td>0.860</td>
<td>0.002</td>
<td>0.073</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF Val/Met x NTRK2 rs1047896</td>
<td>0.063</td>
<td>0.802</td>
<td>0.000</td>
<td>0.057</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF rs6265 x NTRK2 rs1078947</td>
<td>1.557</td>
<td>0.214</td>
<td>0.021</td>
<td>0.326</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF Val/Met x NTRK2 rs1078947</td>
<td>1.093</td>
<td>0.297</td>
<td>0.007</td>
<td>0.180</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Due to low genotype counts, genotypes were regrouped by combining the minor allele homozygotes with the heterozygotes;
* Observed power is the probability of correctly rejecting the null hypothesis
4.7 Potential mediation or moderation effects of child’s BMI on our results

*NTRK2* rs1047896 and Snack Delay Task Performance

*NTRK2* rs1047896 was analyzed using the dominant model only. The p-values for the associations between the three factors are shown in Figure 12 below. *NTRK2* rs1047896 was not associated with the child’s BMI at 36 months of age and BMI was not associated with Snack Delay Task Performance. The main effect of z-BMI (36 months) was not significant when added into the association model between *NTRK2* rs1047896 and Snack Delay Task (OR [95% CI] = 1.142 [0.819-1.594], p = 0.434), but the main effect of *NTRK2* rs1047896 remained significant in this model (OR [95% CI] = 3.411 [1.383-8.416], p = 0.008) (data not shown in figure). Since BMI was not a significant covariate and did not independently predict the outcome, BMI does not appear to be a mediator or moderator of Snack Delay Task Performance.

**Figure 12** Mediation model for *NTRK2* rs1047896 and Snack Delay Task

*a* Linear regression was used  
*b* Binary logistic regression was used
**NTRK2 rs1078947 and Food Approach Score**

Even though the association between *NTRK2* rs1078947 on Food Approach score did not remain significant after multiple testing corrections, we decided examine the potential mediation effects of BMI because the overall goal of this study was to generate testable hypotheses for future studies. *NTRK2* rs1078947 was analyzed using the dominant model only. The p-values for the associations between the three variables are shown in Figure 13 below.

*NTRK2* rs1078947 was not associated with the child’s BMI at 48 months of age, but z-BMI independently associated with Food Approach Score (B = 0.229±0.070, p = 0.001). The main effect of z-BMI (48 months) was not significant when added into the association model between *NTRK2* rs1078947 and Food Approach score (B = 0.129±0.094, p = 0.175), but the main effect of *NTRK2* rs1078947 remained significant in this model (B = 0.540±0.215, p = 0.013) (data not shown in figure). Since BMI was not a significant covariate and was not associated with the SNP, BMI does not appear to be a mediator or moderator for the effect of *NTRK2* rs1078947 on Snack Delay Task Performance. Due to the bidirectional relationship between eating behaviours and weight, it was not surprising that a significant association between BMI and Food Approach Score was observed.

![Figure 13 Mediation model for NTRK2 rs1078947 and Food Approach score from the CEBQ](image)

* Linear Regression was used
* Mother’s BMI was used as a covariate
**BDNF rs6265 x NTRK2 rs1187325 and Food Approach Score**

The interaction between *BDNF* rs6265 and *NTRK2* rs1187325 did not remain significant after correction for multiple comparisons, but we decided to investigate BMI as a potential covariate nonetheless. Genotypes for *BDNF* rs6265 were analyzed using a dominant model and dichotomized into Val/Val or Met-carriers. *NTRK2* rs1187325 was analyzed as three genotype groups. The p-values for the associations between the three factors are shown in Figure 14 below.

The interaction between *BDNF* rs6265 x *NTRK2* rs1187325 does not appear to predict BMI at 48 months of age. The association between BMI and Food Approach Score has been acknowledge in the previous results section for *NTRK2* rs1078947 and Food Approach Score. When z-BMI (48 months) was added to the association model for *BDNF* rs6265 x *NTRK2* rs1187325 and Food Approach score, the main effect of the interaction term remained significant (F_{interaction} = 5.135, p = 0.007), but z-BMI was not significant covariate (F = 2.866, p = 0.093). Therefore, z-BMI does not appear to be a mediator or moderator for the effect between *BDNF* rs6265 x *NTRK2* rs1187325 and Food Approach score.

![Diagram](image_url)

**Figure 14** Mediation and moderation model for *BDNF* rs6265 x *NTRK2* rs1187325 and z-BMI on Food Approach score from the CEBQ

\(^a\) General linear model was used  
\(^b\) Linear Regression was used  
\(^c\) Mother’s BMI was used as a covariate
Chapter 5 Discussion

Previous epidemiological studies in twins have established that eating behaviour traits are under strong genetic influence. Our overall research objective was to investigate the role of genetic polymorphisms in the BDNF and NTRK2 loci on eating behaviours in young children. There was a particular focus on children because their eating behaviours are less likely to be affected by confounding factors such as body image concerns, chronic metabolic changes, and medications. Results from the present study extended from current literature, which explored the association between BDNF and NTRK2 SNPs on BMI and eating disorder diagnosis. We examined the role of these genetic variants on a potential intermediate phenotype, eating behaviours.

To address the research objective, we conducted genetic association analysis using genetic and phenotypic data from the MAVAN cohort. We examined BDNF tagSNPs and selected candidate NTRK2 SNPs as this gene was too large for tagging (~350kbp). In order to minimize the number of analyses, we limited our phenotypes to a behavioural measure of impulsive responding to food (i.e. the Snack Delay Task) and a psychometric measure of various eating behaviours (i.e. Child Eating Behaviour Questionnaire). Individual SNP analyses, haplotype based analyses, and gene-gene interaction analyses were performed.

We hypothesized that BDNF and NTRK2 genetic polymorphisms and their haplotypes would be associated with impulsive responding to food as measured by performance on the Snack Delay Task. We also expected these SNPs to contribute to dimensions of eating styles that are implicated in the development of weight problems as measured by composite Food Approach and Food Avoidant scores from the Child Eating Behaviour Questionnaire (CEBQ). Due to the biological relationship between BDNF and its receptor TrkB, encoded by NTRK2, we expected gene-gene interactions between their SNPs.

There were 4 main findings from this study and each will be discussed in turn. This will be followed by a general discussion, strengths and limitations, and clinical implications of the present study.
5.1 Main Findings

Finding #1: *NTRK2* rs1047896 was associated with performance on the Snack Delay Task

Our analysis revealed that rs1047896, a variant located in the tyrosine kinase B neurotrophin receptor gene (*NTRK2*), may contribute to susceptibility to impulsive eating as measured by the Snack Delay Task in 3 year old children. Children with the homozygous major allele (A) genotype were more likely to pass the Snack Delay Task as compared to those who carried the minor G allele (*p* unadjusted = 0.014). The *p*-value remained significant after gene-wide multiple testing corrections (*p* adjusted = 0.042), but not hypothesis-wide correction (*p* adjusted = 0.098).

We also identified two *NTRK2* haplotype groups associated with Snack Delay Task performance. The frequency of the C-T-G haplotype formed by rs1187325-rs1439050-rs1047896 and the T-G-C haplotype formed by rs1439050-rs1047896-rs1078947 is higher in children who failed the Snack Delay Task. Failure of the Snack Delay task is indicative of poorer response inhibition to food. The frequency of the G-G-A haplotype of rs1187325-rs1439050-rs1047896 and the G-A-C haplotype of rs1439050-rs1047896-rs1078947 was higher in children who successfully passed the Snack Delay Task. This implies that these two haplotypes may protect against impulsive responding to food. However, only the two haplotypes formed by rs1439050-rs1047896-rs1078947 remained significant after multiple testing corrections. As haplotype analysis, especially *post hoc* analysis within a haplotype group, creates groups with low sample size, our results should be interpreted with caution until replicated. Nevertheless, findings from haplotype analyses further support the results from individual SNP analyses because haplotypes that contained the G allele of *NTRK2* rs1047896 are more common in the group that failed the Snack Delay Task.

It is usually difficult to recruit a large number of subjects for the assessment of eating behaviour traits in laboratory settings. Therefore, given our constraints, we were only able to establish a sample size of 158 children. Prior studies on the Snack Delay Task had a similar number of subjects, though genetic contributions to performance on this task have never been investigated.
A previous study in the MAVAN cohort reported that impulsive responding to food as measured by performance on the Snack Delay Task at 36 months of age was predictive of higher consumption of palatable fat during a structured lab meal test and higher BMI at 48 months of age (Silveira, Agranonik et al. 2012). Impulse control deficits may predispose to or be indicative of binge eating tendencies. Others studies have observed negative correlations between self-regulation during childhood and weight status or subsequent weight gain (Seeyave, Coleman et al. 2009, Schlam, Wilson et al. 2013, Miller, Rosenblum et al. 2016). Furthermore, there is evidence in adult subjects that greater food reward sensitivity and diminished inhibitory control are associated with increased palatable food intake (Appelhans, Woolf et al. 2011). Better performance on the Snack Delay Task has demonstrated an association with lower BMI and lower odds of being overweight/obese, but no such correlation was observed for a non-food delay task (Miller, Rosenblum et al. 2016). The association between weight and food-specific impulsivity, but not with general tendency to react impulsively has also been reported in adults (Houben, Nederkoorn et al. 2014).

The rs1047896 SNP was originally identified by Ribases and colleagues (Ribases, Gratacos et al. 2005) after screening the entire NTRK2 gene in 91 patients with eating disorders. To date no other study has investigated this particular NTRK2 variant in any other disease context. Ribases and colleagues reported that the genotype frequency of this SNP significantly differed between BN and controls (Ribases, Gratacos et al. 2005). The minor G allele of this SNP was more frequent in the BN group. In addition, a haplotype identified in their study consisting of the G and the A allele of rs1187325 and rs1047896 was less frequent in the BN group, suggesting that these two alleles are protective against the disorder. Both of these finding appear to be consistent with our results if impulsive responding to food as measured by the Snack Delay Task is indicative of binge eating tendencies, a clinical characteristic of BN. Based on our current data, however, we cannot determine whether NTRK2 genotypes contribute specifically to impulsivity in the presence of a food cue or to an overall impulsive trait.

rs1047896 is located at exon 16 of the NTRK2 gene and no evidence for its functional consequence has been described to date. Even though rs1047896 is located in a non-coding region for the full-length TrkB receptor, it acts as a 3’ untranslated region
(UTR) for a C-terminal truncated receptor, TrkB-T1. Truncated TrkB receptors compete for ligand binding with the full-length receptor, but do not produce the same downstream effects (Brodeur, Minturn et al. 2009). It has also been suggested that TrkB-T1 activates different signaling pathways than TrkB as exome regions beyond exon 15 encode the intracellular domain of the receptor (Rose, Blum et al. 2003, Ohira, Kumanogoh et al. 2005).

RegulomeDB (http://www.regulomedb.org/) was consulted to examine the potential functional consequences of this variant. This database provides scores that represent confidence of a variant’s functionality based on its location and potential to affect gene regulation (Boyle, Hong et al. 2012). No score was assigned to rs1047896, suggesting that this SNP is unlikely to affect transcription factor binding. According to Haploreg (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php), there are 20 SNPs in LD ($r^2 >= 0.8$) with rs1047896, but no particular variant had notable overlap with enhancer or promoter marks.

We investigated the correlations between rs1047896 genotype and brain-tissue gene-expression levels using the open access Braineac database—the Brain eQTL Almanac (http://www.braineac.org/). The minor G allele of this SNP appears to lower the expression of NTRK2 in the medulla as compared to the A allele ($p=0.014$) (Supplementary Table 2B and Supplementary Figure 1H). A recent paper in Cell Metabolism reported that GABAergic neurons in the medulla oblongata are activated by NPY-triggered signaling from the hypothalamus to inhibit metabolic thermogenesis, while promoting feeding and mastication in murine models (Nakamura, Yanagawa et al. 2017). Expression of NTRK2 was not affected by rs1047896 in other brain regions. The eQTL data for the other three NTRK2 SNPs that made up the significant haplotypes is included in Supplementary Table 2B and Supplementary Figures 1F, 1G, and 1I. The functionality of these NTRK2 SNPs has not been described in prior work.

None of the five BDNF tagSNP included in this study associated with performance on the Snack Delay Task. The role of BDNF polymorphisms on impulsive responding to a food cue has never been investigated but prior literature has found an association with general impulsive traits (Oades, Lasky-Su et al. 2008, Kwon, Ha et al. 2015, Su, Tao et al. 2015).
Finding #2: The association between NTRK2 rs1078947 and Food Approach Score from the CEBQ was trending towards significance

Among the four NTRK2 SNPs included in this study, rs1078947 demonstrated an association with the composite Food Approach Score from the CEBQ that was trending towards significance. Carriers of the minor T allele had a higher Food Approach z-score, which is indicative of frequently demonstrating behaviours that pose a risk for overeating. Post hoc analysis of individual sub-scales of the CEBQ that made up the composite Food Approach Score revealed that the T allele associated with higher scores on Food responsiveness, Desire to drink, and Emotional overeating. The subscales that made up the Food Approach composite score have previously demonstrated a positive association with weights of children (Webber, Hill et al. 2009, Santos, Ho-Urriola et al. 2011).

Prior studies have described clinical implications of individual subscales from the CEBQ. Scores from the Food responsiveness scale are not only relevant to weight status, may also be part of a “fussy” eating behaviour profile as described by Tharner and colleagues (Tharner, Jansen et al. 2014). Fussy eaters are more likely to have lower intake of vegetables and higher intake of sweet foods. The Desire to drink score reflects a preference for and frequency of consuming sweetened drinks (e.g. soft drinks) rather than a general thirst as the scores are not linked to intake of water or fruit juice (Sweetman, Wardle et al. 2008). The consumption of sugar-sweetened drinks is associated with childhood obesity (Ludwig, Peterson et al. 2001). With respect to emotional overeating as measured by the CEBQ, studies to date have only correlated this scale with BMI. However, emotional eating behaviour is a phenotype studied in eating disorder psychopathology literature, particularly with binge eating disorder (Masheb and Grilo 2006). Previously, Ashcroft and colleagues (Ashcroft, Semmler et al. 2008) reported a high level of continuity for eating behaviours as measured by the CEBQ. This was evidenced by significant correlations between CEBQ scores collected at 4 and 11 years of age and suggests that the CEBQ may be able to capture stable appetitive traits.

The rs1078947 SNP was identified as a potential contributor to eating disorders in prior literature, but the risk allele is unclear. Ribases and colleagues (Ribases, Gratacos et al. 2005) reported that the C allele of rs2253891 (a proxy of r²=1.0 for rs1078947) was linked to higher maximal BMI in patients with anorexia nervosa, while Yilmaz and
colleagues (Yilmaz, Kaplan et al. 2014) reported that the T allele of rs1078947 was associated with higher maximal BMI in patients with bulimia nervosa. Our results are consistent with the latter study as a greater Food Approach score can lead to weight gain. This SNP may be a susceptibility factor for BN with effects on eating behaviours as early as 4 years of age.

rs1078947 is located at an intron of NTRK2 and no evidence for its functional consequence has been described. RegulomeDB assigns a score of 5 to rs1078947, which means that SNP is not likely to affect transcription factor binding. According to Haploreg there are 9 SNPs in LD (r^2 >= 0.8) with rs1078947, but no particular variant had notable overlap with enhancer or promoter marks.

We investigated the correlations between rs1078947 genotype and brain-tissue gene-expression levels using the open access Braineac database—the Brain eQTL Almanac (http://www.braineac.org/). The minor T allele of this SNP appears to lower the expression of NTRK2 in the thalamus (p=0.0025), medulla (p=0.016), and occipital cortex (p=0.048) as compared to the C allele (Supplementary Table 2B and Supplementary Figure 1I). Both the thalamus (Piech, Lewis et al. 2009) and the medulla (Nakamura, Yanagawa et al. 2017) have previously been implicated in food intake regulation. Individual BDNF SNP analysis on Food Approach Score did not reveal any significant findings. We also did not detect any significant haplotypes with our selected BDNF or NTRK2 polymorphisms.

**Finding #3: BDNF and NTRK2 genetic variants were not associated with Food Avoidant Score from the CEBQ**

While prior studies have examined BDNF and NTRK2 in relation to eating disorders such as AN, to our knowledge, this is the first attempt to evaluate the effects of common genetic variation across the BDNF and NTRK2 loci on undereating behaviours. We did not find any significant associations between BDNF or NTRK2 SNPs and the composite Food Avoidant score from the CEBQ. Haplotypes created by these SNPs also did not reveal any notable associations.
The negative findings may be due to a number of reasons. AN is a complex disorder that is not solely driven by undereating behaviours. The role of BDNF and NTRK2 on AN may be more related to the development of comorbid conditions such as affective and anxiety disorders (Berrettini 2004). One particular study by Gamero-Villarroel and colleagues (Gamero-Villarroel, Gordillo et al. 2014) found no differences in the distribution of BDNF haplotypes in AN/BN and control subjects. Rather, the BDNF haplotypes in their study associated with psychopathological symptoms in eating disorders as measured by the SCL-90R inventory. Another reason may be due to the fact that there exist moderators and mediators of BDNF and NTRK2’s effect on eating behaviours that which we have not examined. For example, BDNF and NTRK2 are well-established regulators of neuronal development and synaptic plasticity. It is, therefore, not surprising that BDNF also affects cognitive functions such as learning and memory. A separate line of research has correlated weight status with neurocognitive function (Vainik, Dagher et al. 2013, Liang, Matheson et al. 2014). It is unknown whether the effects of BDNF/NTRK2 on cognition and food intake/weight status are pleiotropic or interrelated. Since MAVAN is a pediatric cohort, cognitive development among the children may not be uniform and could be a mediator or moderator for the development of eating behaviours implicated in the development of weight regulation issues. This possibility could be examined in future investigation. Lastly, the lack of association may be due to the age of the MAVAN cohort. Our study has a cross-sectional design and we have only investigated the association between the candidate genes and our selected eating behaviours at 3 or 4 years of age. The effects of the gene may present later in life, especially since BDNF is a known contributor to neuronal plasticity with effects throughout life.

It should be noted that a high Food Avoidant score is theoretically a proxy for a low Food Approach score. However, rs1078947, the NTRK2 SNP that associated with a higher Food Approach score, did not associate with a lower Food Avoidant score in the present study. The subscales in the CEBQ were originally designed to represent conceptually distinct traits, but the extent that which each scale reflects independent dimension of eating behaviours has yet to be elucidated (Carnell and Wardle 2007). To date, the eight subscales of the CEBQ are usually examined as unique dimensions of eating, but in attempts to limit the number of analysis in our study with multiple genetic
variables, we created two composite scores. The eating behaviour dimensions in the composite Food Approach score may truly be distinct from the dimensions in the Food Avoidant score, at least in our cohort. The Food Approach and Food Avoidant scores from the CEBQ from our study were somewhat correlated (p=0.048), but had a very low Pearson correlation of -0.134.

Finding #4: Gene-gene interaction between \textit{BDNF} rs6265 and \textit{NTRK2} rs1187325 on Food Approach Score from the CEBQ trended towards significance

Eating behaviours is an umbrella term, encompassing a number of appetitive behaviours that can either contribute to weight gain or loss, such as speed of eating, food responsiveness, and satiety responsiveness. Though the biological mechanisms underlying these behaviours are unclear, they are likely the result of a number of interacting factors including social, hormonal, and genetic. Individual genetic variants may only have minor marginal effects on eating behaviours. It is possible that epistatic interactions between genes that contribute to food intake regulation play a role in eating behaviours and should be considered.

\textit{BDNF} and its receptor \textit{NTRK2} likely contribute to the development of appetitive traits because these two genes have been associated with obesity and eating disorders. Furthermore, the interaction effect between \textit{BDNF} and \textit{NTRK2} has been described in other disease context such as schizophrenia and depression (Lin, Hong et al. 2009, Lin, Su et al. 2013). In the present study, we examined the potential gene-gene interaction effects on impulsive responding to a food cue, as measured by the Snack Delay Task and food approach/avoidant behaviours, as measured by the CEBQ. Specifically, we examined the functional \textit{BDNF} rs6265 polymorphism and its interaction with four candidate \textit{NTRK2} SNPs that have been reported in prior obesity or eating disorder studies.

An association between \textit{BDNF} rs6265 x \textit{NTRK2} rs1187325 and Food Approach z-score from the CEBQ was observed in our study. The p-value, however, did not withstand multiple testing corrections. Nevertheless, these results offered the first evidence that there may be an interaction between \textit{BDNF} and \textit{NTRK2} genetic variants on eating behaviour phenotypes that should be explored in future studies. Only one \textit{BDNF} SNP and
four *NTRK2* SNPs were included so as to limit the number of analysis, but there are potentially other interactions between these two genes.

rs6265 is a missense genetic variant at the BDNF locus. According to the open access Braineac database—the Brain eQTL Almanac (http://www.braineac.org/), the minor T allele of this SNP affects the expression of BDNF in the frontal cortex (p=0.0079) (Supplementary Figure 1A), which potentially influences the cognitive control of eating behaviours. Based on the well-documented functional consequence of this missense SNP, rs6265 has a greater effect on intracellular transport rather than expression of *BDNF*. Therefore, gene expression databases may not capture the true functional consequence of this genetic variant.

rs1187325 is genetic variant located at the 5’ UTR of *NTRK2* and its functional consequence has not been described in prior literature. We investigated the correlations between rs1187325 genotype and brain-tissue gene-expression levels using Braineac (Supplementary Figure 1F). This SNP does not appear to affect the expression of *NTRK2* in brain regions with a definitive connection to appetitive traits or food intake regulation. RegulomeDB assigns a score of 4 to rs1187325, which means that there is minimal evidence that this SNP affects transcription factor binding. According to Haploreg, rs1187325 overlaps with a few DNA hypersensitive sites, promoter marks, and enhancer marks for several cells lines, including brain tissues. This SNP is located at a transcription start site with several promoter histone marks around the region.

### 5.2 General Discussion

Previous research has established that BDNF is a central molecule involved in the regulation of food intake and weight. Animal models with conditional BDNF knockout or BDNF haploinsufficiency develop an obese phenotype characterized by hyperphagia. Weight gain and hyperphagia in these animals can be reversed by exogenous infusion of BDNF. Research in humans has primarily focused on the role of BDNF on body mass, obesity, and eating disorders. Numerous *BDNF* genetic variants have been identified in BMI GWAS and candidate gene studies for BMI, obesity, and eating disorders. Genetic
variants in the NTRK2 locus, have also been studied in relation to these phenotypes, but there are comparatively fewer studies on the NTRK2 gene.

In the present study, we chose to focus on the relationship between BDNF and NTRK2 genetic polymorphisms and eating behaviours, a potential intermediate phenotype that predisposes individuals to diet-induced obesity and other eating disorders. Aberrant eating behaviours, such as binge eating, are characteristic of eating disorders and may lead to weight gain and obesity. In recent years, neuroimaging studies have started to link these behaviours with changes in brain activity and morphology. Hence, BDNF and NTRK2 have become interesting candidates as they are ubiquitously expressed in the central nervous system. BDNF acts on appetitive centers in the brain such as the hindbrain (Bariohay, Roux et al. 2009) and its expression in the hypothalamus is affected by levels of leptin, a satiety hormone (Komori, Morikawa et al. 2006). According to the Genotype-Tissue Expression project (GTEx) Portal (http://www.gtexportal.org/home/), BDNF and NTRK2 show high expression in brain regions related to food intake behaviour (Supplementary Figure 2A and 2B). In particular, NTRK2 expression is high in areas of the brain associated with emotional processing (i.e. amygdala) (Zhang, Li et al. 2011), anticipatory reward processing, and impulse control (i.e. anterior cingulate cortex and nucleus accumbens) (De Silva, Salem et al. 2012). All of these brain regions are involved in the hedonic control of eating behaviour and many other processes/functions.

The design of the present study brings together two theories from obesity literature. Carnell and Wardle (Carnell and Wardle 2008) proposed a behavioural susceptibility model which posits that specific appetitive traits at the individual level determine the risk of gaining weight in the presence of an obesogenic environment. In a separate line of research, investigators are discovering key connections between neurocognitive functions, neuroanatomical connections, and body weight Val-Laillet and colleagues (Val-Laillet, Aarts et al. 2015). This prompted the use of a brain-based approach to studying weight regulation, whereby investigators are beginning to shift their focus from metabolic causes of weight gain/loss to a focus on brain circuitry and mechanisms that regulate appetite traits. The study of BDNF and NTRK2 effectively makes use of two obesity- and eating disorder- associated genes known to function
primarily in the brain and examines their effects on eating behaviours, which may pose as susceptibility factors to weight gain/loss.

Overall, our results suggest that single BDNF SNPs and their haplotypes do not play a major role in appetitive traits or susceptibility to high risk eating behaviours such as impulsive eating in pre-school aged children; even the functional BDNF rs6265 variant did not associate with any of the eating behaviour phenotypes included in this study. This is somewhat inconsistent with our initial hypothesis as we expected to find significant associations with BDNF polymorphisms. Prior literature has provided extensive evidence that BDNF acts at several centers of the brain to regulate eating behaviour such as the hypothalamus and the mesolimbic dopamine system. Nevertheless, current studies on the rs6265 variant have provided an inconsistent direction of association with body weight. Whether the Val or Met allele confers susceptibility to obesity or eating disorders is still unclear. In fact, some studies are unable to confirm a role between the most widely studied BDNF rs6265 polymorphism and body weight, obesity, or eating disorders (see Section 1.8.1).

Although BDNF genetic variants did not appear to be relevant to the eating behaviours that we examined in this study, future investigation should not exclude this gene. In animals, BDNF deficiency causes diet-induced obesity and levels of BDNF responds to diet (Molteni, Barnard et al. 2002). It is highly plausible that BDNF SNPs contribute to eating behaviours and associations may be found in cohorts with a different demographic than MAVAN e.g. different age group, non-Caucasian populations, or in male/female subgroups. Previously, Robiou-du-Pont and colleagues (Robiou-du-Pont, Yengo et al. 2013) reported a nominal association between BDNF rs925946 and self-reported snacking behaviour in three adult European population. Snacking behaviour, in turn, has been positively associated with risk of obesity and weight gain (Bes-Rastrollo, Sanchez-Villegas et al. 2010). rs925946 is in perfect LD (r²=1) with rs11030108, one of the BDNF tagSNPs included in our study, but we did not find any associations with eating behaviour traits examined in the present study. In a larger sample, additional BDNF polymorphisms or mutations can be examined. In addition, eating behaviour is a polygenic phenotype and single gene studies may only be able to reveal correlations with very specific forms of eating behaviours. A summary of eQTL p-values from Braineac
In contrast, our results with NTRK2 SNPs suggest that genetic variants within this locus are relevant to eating behaviours. We identified a NTRK2 SNP (rs1047896) and NTRK2 haplotypes that demonstrated a significant association performance on the Snack Delay Task, which measures impulsive responding to an appetitive stimulus. This was a promising finding because the association remained significant after gene-wide correction for multiple testing. We also reported a nominal association between a NTRK2 SNP (rs1078947) and Food Approach behaviours as measured by the CEBQ as well as a nominal epistatic effect between BDNF rs6265 x NTRK2 rs1187325 and Food Approach behaviours.

NTRK2 alterations may lead to more severe phenotypes than variations at the BDNF locus. This theory is supported by the fact that in addition to BDNF, NTRK2 receptors binds to other neurotrophins, including NT-4 and NT-3. In animal studies, NT-4 deficient mice have reduced vagal sensory innovation in the small intestine, leading to blunted satiety response and hyperphagia (Byerly and Fox 2006). Similar effects are observed in NT-3 knock-out mice (Fox, Biddinger et al. 2013). The binding of other neurotrophins to the TrkB receptor may activate other downstream signaling pathways.

No prior study has investigated the role of NTRK2 polymorphisms on appetitive traits. Therefore, our findings should be considered preliminary until replicated in another cohort. A summary of eQTL p-values from Braineac for the 4 NTRK2 SNPs included in this study is provided in Supplementary Table 2B. It should be noted that since the functionality of the 4 candidate NTRK2 SNPs has not been reported, these SNPs might be in LD with an actual causative variant.

Even with the associations between gene polymorphisms and eating behaviour traits, it is difficult to determine specific brain regions and neural circuits that contribute to food intake behaviours because BDNF and NTRK2 are widely expressed in the brain. Furthermore, appetitive behaviours are likely controlled by complex networks in the brain that integrate homoeostatic signals, hedonic motivation/drive, and cognitive behaviours such as response inhibition. This makes BDNF and NTRK2 interesting candidates, but
also suggests that it is difficult to discern specific neurocircuitry based on association with information from these two genes.

To our knowledge, this was the first study to investigate the relationship between BDNF and NTRK2 gene polymorphisms and eating behaviours in young children. At present, there are very few studies in pre-school aged populations, but prior studies have reported that parents notice unhealthy eating habits at very early stages of child development (Kerzner, Milano et al. 2015). We have expanded the scope of BDNF literature by examining polymorphisms other than the functional rs6265 variant and included NTRK2 polymorphisms in our investigation. Based on the results of this study, we propose that BDNF’s high-affinity receptor, TrkB, may be involved in the pathogenesis of high risk eating behaviours.

5.3 Strengths and Limitations

As mentioned previously, the study of eating behaviours in a cohort of children offered the advantage of minimizing confounding factors that are more relevant to adolescents and adults such as body image concerns, chronic metabolic changes, and medications. These factors can confound the relationship between genetic predisposition and eating behaviour traits observed or measured.

The MAVAN cohort offered extensive and detailed phenotypic data. Accordingly, behavioural and psychometric measures of eating behaviour were recorded for these children. These two measures offered distinct information about a child’s appetitive traits. The Snack Delay Task objectively measures impulsive responding to a food cue in a controlled laboratory setting. This behavioural task has never been studied in relation to genetics. Therefore, the present study offered the first indication of genetic contributions to impulsive responding as measured by this task. The CEBQ is a well-validated questionnaire that has only been studied in relation to genetics in four prior studies. Specifically, these studies investigated genetic polymorphism of leptin, FTO, and melanocortin-4 receptor. The present study extended the investigation to additional genes implicated in food intake and weight regulation. Together, these two tasks offered an objective measure of impulsive responding to food and a parental-reported measure of a
broad range of stable appetitive traits. Nevertheless, both behavioural and psychometric measures are subjected to social desirability bias whereby subjects or respondents are more likely to behave or respond in a way that is viewed favourably.

There are several limitations to our study that should be acknowledged. While the MAVAN sample provided detailed phenotypic information, our sample sizes were relatively small. Since the overall goal of the present study was to investigate the role of BDNF and NTRK2 on a variety of eating behaviours that encompasses both ends of the spectrum (i.e. overeating and undereating tendencies), we had to be mindful of the number of analysis performed.

When selecting genetic variables, we limited our investigation to 5 BDNF tagSNPs and 4 candidate NTRK2 SNPs. As a result, our selection of genetic factors did not account for all genetic variation within BDNF or NTRK2. We also did not explore remote regulator elements, which can control the expression of these genes but reside at loci far away from the actual gene region. Our small sample size resulted in low MAF (n<5) for some of the SNPs. Low genotype frequency may have affected our ability to identify outliers, but we counteracted this limitation by analyzing these SNPs using a dominant rather than additive model.

We also attempted to limit our selection of phenotypic variables. The eating behaviour subscales in CEBQ were regrouped into Food Approach and Food Avoidant composite scores so as not to perform separate analysis on each subscale. However, the grouping of CEBQ subscales into composite scores may have masked the genetic effect on specific subscales. As previously mentioned in Section 3.4.2, Cronbach’s alpha for the food approach scores in our sample was relatively low. Each subscale may be representative of unique dimensions of eating behaviours.

Other than sample size, our study was limited by the fact that we only evaluated eating behaviours at one time point. Repeated measures of these behaviours are necessary to confirm whether genotypes are predictive of stable eating behaviour traits. As well, since eating behaviours were evaluated at such a young age, we do not know whether performance on the Snack Delay Task and responses on the Food Approach subscales of the CEBQ confers a long-term risk to the development of obesity or other hyperphagic
eating disorders such as bulimia nervosa or binge eating disorder. Usually, the CEBQ scores are assessed as a continuous variable as no clinically relevant cut-points have been established.

Another limitation was that additional covariates could be considered when examining eating behaviour as a phenotype. For example, childhood obesity literature has suggested that weight status may be affected by parenting. Negative reactivity in early childhood was linked to weight gain only when their mothers had a lower parenting self-efficacy belief that they can adequately care for their child (Anzman-Frasca, Stifter et al. 2013). Likewise, parenting styles may also have effects on child’s eating behaviour. As this was the first study to investigate genetic contributions to appetitive traits in young children, we did not want to introduce too many mediation or moderation factors in our analysis.

Our selection of genetic variables can also be seen as a limitation. We employed a tagSNP approach for the BDNF locus and a candidate SNP approach for the NTRK2 locus based on prior clinical studies. As a result, the functionality of most of the SNPs included in this study is unclear so we were unable to discern specific biological pathways that led to the phenotypes.

Finally, it should be acknowledged that although candidate gene studies have long been used to identify disease-associated genetic variant, these types of studies have fallen out of favour in the past decade. One of the major criticisms about candidate gene studies is that it is often difficult to correctly specify candidate genes based on current biological knowledge. As a result, these studies are prone to type II errors. Existing candidate gene studies are also subjected to publication bias and confirmation bias. In contrast, hypothesis-free GWAS, which investigate the entire genome, are considered a more comprehensive and unbiased approach. Despite the limitations of candidate gene studies, this approach is not completely obsolete, as these types of studies are still being published. Candidate gene studies may generate new leads on specific biological pathways that contribute to the phenotype of interest and are arguably less costly than conducting a GWAS. The reason why we chose this method for the present study is because our phenotype of interest, i.e. eating behaviours, was broad and not as well-defined as a disease state. It was, therefore, necessary to select specific genetic variables
to assess for association with a spectrum of eating behaviours in order to form testable hypotheses. We chose two candidate genes with strong evidence for their role in regulating eating behaviour \textit{a priori}. The role of BDNF and NTRK2 in promoting hyperphagic behaviours has been demonstrated in animal models and both genes have previously been identified in obesity and eating disorders literature. Therefore, eating behaviours may be an intermediate phenotype for the effects of BDNF and NTRK2 on these diseases. Undoubtedly, future studies of eating behaviours should be conducted as large genome-wide studies. Replication of results from candidate gene studies in a GWAS context will lend credibility to the preliminary findings.

5.4 Clinical implications

The investigation of genetic contributions to appetitive traits has implications in at least three populations: 1) those with obesity, 2) those with eating disorders, and 3) those with high risk eating behaviours that predispose to these diseases. Individuals with high genetic susceptibility to aberrant eating behaviours can be candidates for pharmacological therapies, lifestyle changes specifically targeting the deficient or excessive biological factor, or interventions to normalize the eating behaviour.

Over the past three decades, the prevalence of obesity has increased in many parts of the world and is a growing public health concern. The prevalence of childhood obesity increased by 47.1% from 1980 to 2013 and obesity is developing earlier in childhood (Lee, Pilli et al. 2010). Although there is a general understanding of the risk factors for obesity, current treatment for obesity has been met with limited success and its prevalence continues to rise. Treatment of obesity is complicated by the multitude of etiological factors including metabolism, level of physical activity, taste receptor sensitivity, and etc. It is therefore crucial to identify causal factors and understand the biological processes underlying overeating tendencies and weight gain. Obesity GWAS has identified numerous SNPs that are associated with BMI, but details regarding the underlying mechanism remain largely unknown (Xu, Zeng et al. 2013).
Eating disorders are another group of disorders that have been suggested to have genetic origins. Though the prevalence of eating disorders is less than that of obesity, it is also a public health concern. Similar to obesity, eating disorders can cause comorbid diseases such as heart problems, cancer, and kidney disease. AN has the highest mortality rate among psychiatric disorders (Arcelus, Mitchell et al. 2011) and binge-eating disorder can lead to obesity (de Zwaan 2001).

A common pathophysiological cause and symptom of obesity and eating disorder is abnormal eating behaviours, which include tendencies to overeat or undereat. The identification of genetic susceptibility to these tendencies would help elucidate the biological underpinnings of such behaviours and to identify individuals that would benefit from prevention or treatment programs that could address the specific genetic risk factor. This is especially timely because the obesogenic environment created by availability and ease of access to high caloric foods in the last few decades has been shown to have a powerful and pervasive effect on body weight (Gauthier and Krajicek 2013). A better understanding of the associations between BDNF and NTRK2 genetic variants and eating behaviours will facilitate the development of novel treatment and prevention strategies.

Pharmacological treatment is a potential treatment option for those with disrupted BDNF/TrkB signaling. Administration of exogenous BDNF may be a potential therapy. BDNF crosses the blood-brain barrier (Pan, Banks et al. 1998) and there is a correlation between levels of BDNF in the brain and blood (Klein, Williamson et al. 2011). There are a few caveats to this approach that should be acknowledged. Firstly, it would be necessary to develop a delivery system to promote the delivery of the neurotrophic factor from the periphery to the CNS. Several research groups are working towards this goal by testing the utility of a nanoparticulate drug delivery system (Pilakka-Kanthikeel, Atluri et al. 2013, Khalin, Alyautdin et al. 2015). More importantly, however, is that BDNF levels are usually maintained within an optimal range in neurons. Brain-region-specific alterations in BDNF levels outside of the optimal range, and the corresponding up or down regulation of TrkB signaling can lead to various disease conditions as discussed by Gupta and colleagues (Gupta, You et al. 2013). Another potential complication of exogenous neurotrophin administration is the unintended activation of the p75NTR (reviewed by (Lu, Pang et al. 2005)). The binding of mature neurotrophins and proneurotrophins to
p75NTR induces cell death. However, p75NTR can form multimermic receptor complex with Trk receptors and the binding of mature neurotrophins to these receptors have pro-survival effects. These effects alter the natural balance of mature and proneurotrophins that regulate cell survival and synaptic plasticity.

Since the TrkB signaling pathway has been implicated in a number of neuropsychiatric and neurodegenerative disorders, pharmacological modulation of this pathway has been considered a potential treatment option. The therapeutic potential of TrkB pathway modulation was previously discussed in a review by Gupta and colleagues (Gupta, You et al. 2013). Through the use of synthetic or natural compounds, BDNF peptide mimetics or customized antibodies, various agonists and antagonists of TrkB are being tested for their therapeutic value in stimulating or inhibiting TrkB receptors. Most of this work is done in experimental models of neurodegenerative disease, glaucoma, and depression, but modulation of TrkB may have a therapeutic role on eating behaviour regulation as well. Tsao and colleagues (Tsao, Thomsen et al. 2008) demonstrated that peripheral administration of TrkB agonist can suppress appetite and decrease body weight in a dose-dependent manner in mice.

Over time, as we elucidate the genetic markers of eating behaviours traits, we will be able to identify high priority families for early dietary intervention. BDNF levels can be manipulated through diet and exercise. A randomized controlled trial reported that 30mg of zinc for 12 weeks can increase serum levels of BDNF in overweight or obese subjects (Solati, Jazayeri et al. 2015). Another trial demonstrated that a Mediterranean diet increased plasma BDNF after 3 years of intervention (Sanchez-Villegas, Galbete et al. 2011). Meanwhile, a recent meta-analysis confirmed that exercise had an enhancing effect on BDNF levels, even after a single session of exercise (Szuhany, Bugatti et al. 2015). There is also some pre-clinical evidence that exercise can increase TrkB mRNA levels in the brain (Kim, Bang et al. 2005). Knowing an individual’s genetic risk for over or underactive of BDNF/TrkB signaling can help to inform their dietary and physical activity choices. In addition, individuals at risk should be made aware that acute and chronic stress reduces the expression of BDNF and NTRK2 (Shi, Shao et al. 2010).

Interventions can also target the pathological eating behaviour(s). For example, impulse control training using the go/no-go task with food image stimuli is effective in
reducing the consumption of high calorie food and can facilitate weight loss (Veling, van Koningsbruggen et al. 2014). In time, prevention and treatment of aberrant eating behaviours will not only help to address obesity, it will also help to improve the nutritional value of diets as children and adults are made aware of their genetic predispositions and begin to make conscious choices about their food consumption patterns.

Lastly, *BDNF* and *NTRK2* are widely implicated in psychiatric disease. Given the high comorbidity of obesity with psychiatric disorders such as depression and anxiety, understanding genetic contributions from *BDNF* and *NTRK2* polymorphisms can help shed light on the pathophysiology of weight gain in psychiatric disorders as well.
Chapter 6 Conclusions

The aim of the present study was to investigate the role of \textit{BDNF} and \textit{NTRK2} genetic polymorphisms on eating behaviours in early childhood. At present, both of these genes are common candidates in relation to obesity and eating disorders. It is, however, unknown whether genetic polymorphisms near or within these two loci associate with eating behaviours traits.

The following summarizes our findings based on the three research questions formulated to address the research aim.

1. Are \textit{BDNF} and \textit{NTRK2} genetic polymorphisms associated with performance on an objective measure of impulsive responding to a food cue?

   \textit{NTRK2} rs1047896 and haplotypes containing this SNP were associated with performance on the Snack Delay Task. No associations were observed with \textit{BDNF} genetic variants.

2. Are \textit{BDNF} and \textit{NTRK2} genetic polymorphisms associated with dimensions of eating styles that are implicated in the development of weight problems as measured by a parental-reported questionnaire?

   The association between \textit{NTRK2} rs1078947 and Food Approach Score from the CEBQ was trending towards significance but no associations were observed with \textit{BDNF} SNPs. \textit{BDNF} and \textit{NTRK2} genetic variants were not associated with Food Avoidant Score from the CEBQ.

3. Does the \textit{BDNF} rs6265 functional variant interact with \textit{NTRK2} genetic polymorphisms to predict impulsive responding to a food cue in a controlled setting and/or parental-reported eating behaviours implicated in the development of weight problems?

   The interaction between \textit{BDNF} rs6265 and \textit{NTRK2} rs1187325 on Food Approach Score from the CEBQ trended towards significance.
These findings are somewhat supportive of our initial hypothesis. Our data extends from current NTRK2 literature by suggesting that eating behaviours may be an intermediate phenotype for its effect on obesity and eating disorders. NTRK2 genetic polymorphisms associated with Snack Delay Task performance and Food approach behaviours as quantified using the CEBQ. We initially expected BDNF polymorphisms to associate with these phenotypes but no such relationship was observed. Since BDNF has biological links to appetitive and satiety centers of the brain, our lack of finding may be due to our modest sample size. Nevertheless, the fact that NTRK2 SNPs demonstrated statistically significant associations even with the small sample suggests that SNPs within NTRK2 merit further investigation.

Even though BDNF and NTRK2 have been studied in the context of obesity and eating disorders, to our knowledge, this is the first study to investigate the role of BDNF and NTRK2 genetic variation on eating behaviour traits. We chose to investigate this phenotype in a developmental cohort as behaviour of this age group is more likely to be driven by innate biological factors. As we age, various confounding factors such as body image concerns and medication use can confound the true relationship between genes and appetitive traits.

Our finding does not appear to support the hypothesis that BDNF genetic variation contributes to eating behaviour traits, but this should not discourage the investigation of BDNF as a candidate gene. A number of limitations were noted in the present study, most notably our modest sample size. Furthermore, we discovered a nominal gene-gene interaction effect between the functional BDNF rs6265 and a NTRK2 SNP. Other interactions between BDNF and NTRK2 SNPs on appetitive traits may exist. Due to the well-established putative role of BDNF and TrkB on food intake and weight regulation, future studies should continue to elucidate associations between genetic variants that affect their expression and eating behaviour traits.
Chapter 7 Future Directions

Prior research has confirmed that eating behaviour traits are heritable and genetics likely contribute to the susceptibility of certain appetitive traits. Currently, there has only been a few studies investigating links between genetic variants and eating behaviours. Results from our study extended from this area of research and have revealed several interesting signals between NTRK2 genetic variants and eating behaviours in young children. This finding should be confirmed in other pediatric cohorts with larger sample sizes.

With the intention to examine the association between BDNF and NTRK2 genetic polymorphisms and a broad range of eating behaviour traits, some behaviours in this study had were grouped to create a composite score to minimize the number of comparisons. This method achieved our objective of creating hypothesis-generating data for future studies. Based on our results, future investigation of NTRK2 should focus on behaviours that lead to overeating since we only observed associations between NTRK2 SNPs and behaviours that promote eating. Specific subscales of the CEBQ could be examined. As well, it would be beneficial to have longitudinal measures of appetitive behaviours to establish its relevance to BMI and the development of obesity or eating disorder.

Future studies should not only aim to replicate our finding in NTRK2; the selection of genetic variables can also be expanded as eating behaviours are likely under polygenic influence. The largest and most recent genome-wide association study (GWAS) on adult body mass index (BMI) identified the neurotrophin signaling pathway as the most significant pathway associated with BMI (Locke, Kahali et al. 2015). Genes in the neurotrophin signaling pathway such as FOXO3, NFkB1, MAP2K5, and SH2B1, can be investigated in relation to eating behaviours, especially impulsive eating behaviours and overeating tendencies.

As previously mentioned, our results should not discourage future investigation of BDNF genetic variants. BDNF SNPs still merit further investigation because of BDNF’s putative role in food intake, BMI, and eating disorders, as demonstrated in pre-clinical and clinical studies. In the present study, we detected an interaction between the
functional BDNF rs6265 polymorphism and a NTRK2 SNP. There are potentially other interactions between these two genes and this possibility could be explored. Several obesity susceptibility genes such as FTO and MC4R have been shown to interact with dietary fats from calorie-dense foods and dietary carbohydrates from sugar-sweetened beverages to promote weight gain (reviewed in (Garver, Newman et al. 2013)). Likewise, BDNF and NTRK2 genes may interact with these dietary macronutrients to affect eating behaviours and thus promote weight gain/loss. Future investigation could consider gene-environment interactions.

As BDNF and NTRK2 are genes involved in neurodevelopment, it would be interesting to examine associations between these genes and functional or anatomical changes in the brain using neuroimaging techniques. Neuroimaging studies in obese and non-obese populations have already revealed areas of the brain that are relevant to food intake such as the amygdala, medial frontal/orbital cortex, insula, and the reward circuitry (Holsen, Zarcone et al. 2005, Bruce, Holsen et al. 2010, Stice, Yokum et al. 2010). Functional magnetic resonance imaging (fMRI) studies have also reported associations between neuroactivation of certain brain regions and eating behaviours such as external eating (Passamonti, Rowe et al. 2009), emotional eating (Wood, Schembre et al. 2016), binge eating (Geliebter, Ladell et al. 2006), and food addiction (Gearhardt, Yokum et al. 2011). BDNF and NTRK2 genetic variants may contribute to the functional or anatomical changes as reported in these studies and this would provide a physiological mechanism to explain the effects of BDNF/NTRK2 on appetitive traits.

Lastly, functional studies on the NTRK2 variants identified in this study would help to elucidate the mechanism by which alterations in the BDNF/NTRK2 signaling pathway contribute to the development of appetitive traits. Nevertheless, studies on genetic variation only provide information about baseline risk. Transcriptomic and proteonomic analyses can help clarify what affects the expression of these genes, where they are expressed, what are their functions, and how exactly they can affect the phenotype of interest.
## Appendix

**Supplementary Table 1:** Association of the *BDNF* gene Val66Met with BMI and obesity

<table>
<thead>
<tr>
<th>Reference (year)</th>
<th>Population studied</th>
<th>Major Outcome (Met vs Val)</th>
<th>Total Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckers et al., 2008 (Beckers, Peeters et al. 2008)</td>
<td>Belgian Women: Obese vs healthy controls</td>
<td>↑ BMI associated with Met-Met genotype as compared with Val-Met or Val-Val genotypes</td>
<td>Obese = 532 Controls = 197</td>
</tr>
<tr>
<td>Skledar et al., 2012 (Skledar, Nikolac et al. 2012)</td>
<td>Healthy Caucasian children and adolescents of the same Croatian background: Obese vs Overweight vs Normal weight vs Underweight</td>
<td>66Met allele carriers associated with obesity Val-Met genotype increased risk of obesity</td>
<td>Croatian children = 300</td>
</tr>
<tr>
<td>Gunstad et al., 2006 (Gunstad, Schofield et al. 2006)</td>
<td>Healthy adults: General population</td>
<td>↓ BMI associated with Met-Met genotype as compared with Val-Met or Val-Val genotypes in women only</td>
<td>Adults = 481</td>
</tr>
<tr>
<td>Shugart et al., 2009 (Shugart, Chen et al. 2009)</td>
<td>British women: general population</td>
<td>↓ BMI associated with Met-Met genotype as compared with Val-Met or Val-Val genotypes</td>
<td>Healthy women = 10,109</td>
</tr>
<tr>
<td>Wu et al., 2010 (Wu, Xi et al. 2010)</td>
<td>Children: Obese vs Overweight vs Control</td>
<td>66Val allele associated with obesity</td>
<td>Obese = 1,229 Overweight = 655 Control = 1,619</td>
</tr>
<tr>
<td>Timpano, Schmidt, Wheaton, Wendland, &amp; Murphy, 2011 (Timpano, Schmidt et al. 2011)</td>
<td>Obsessive compulsive disorder - Hoarding and non-hoarding</td>
<td>Val-Val associated with hoarding and greater BMI</td>
<td>Probands with OCD = 301</td>
</tr>
<tr>
<td>Xiao, Russell, &amp; Liu, 2012 (Xiao, Russell et al. 2012)</td>
<td>Fibromyalgia syndrome (FMS)</td>
<td>In adults with FMS, Val-Val associated with higher BMI as compared with Val-Met (Met/Met was not analyzed due to low frequency of distribution) No association between genotype and BMI in healthy controls</td>
<td>Fibromyalgia syndrome = 95 Healthy control = 58</td>
</tr>
<tr>
<td>Hong et al., 2012 (Hong, Lim et al. 2012)</td>
<td>Korean adults: healthy population</td>
<td>↓ body fat percentage in 66Met allele carriers compared with 66Val carriers Val66met genotype correlates with BMI</td>
<td>Korean adults = 20,270</td>
</tr>
<tr>
<td>Xi et al., 2013 (Xi, Cheng et al. 2013)</td>
<td>Children: Obese vs Normal weight</td>
<td>↓ waist circumference in 66Met carriers as compared with 66Val carriers</td>
<td>Obese = 1196 Normal weight = 2306</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Findings</td>
<td>Sample Size</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>-------------</td>
</tr>
</tbody>
</table>
| Ma et al., 2012 (Ma, Qiu et al. 2012) | Puerto Rican adults: general population   | **Men:** Val-Val associated with higher BMI, waist circumference, hip, and weight than Val-Met or Met-Met. Men with Val-Val had twice the risk of being overweight than the other two genotypes.  
**Women:** Val-Val associated with lower BMI, hip and weight than Val-Met or Met-Met. Women with the Val-Val were 50% less likely to be overweight compared to the other two genotypes. | Men = 552  
Women = 595 |
| Friedel et al., 2005 (Friedel, Horro et al. 2005) | German Children and adolescents: Obese vs Healthy underweight | No association between Val66Met and obesity                                  | Obese = 183  
Healthy underweight = 187 |
| Marti et al., 2009 (Marti, Santos et al. 2009) | Adults: Obese vs healthy controls          | No association between Val66Met and obesity                                  | Obese = 159  
Controls = 154 |
| Nikolac Perkovic et al., 2013 (Nikolac Perkovic, Mustapic et al. 2013) | BMI in healthy adults                      | No association between Val66Met and overweight or obesity  
No association with BMI change over time | Caucasians of Croatian origin = 339 |
| McCaffery et al., 2013 (McCaffery, Papandonatos et al. 2013) | Multiethnic adult population: Overweight or Obese with type 2 diabetes | No association between Val66Met and baseline weight or BMI                  | Adults = 3,899 |

Abbreviations: ↑ = higher, ↓= lower
Supplementary Table 2 Summary of eQTL data from Braineac for the (A) BDNF and (B) NTRK2 SNPs in this study

A) BDNF

<table>
<thead>
<tr>
<th>SNP</th>
<th>exprID</th>
<th>CRBL</th>
<th>FCTX</th>
<th>HIPP</th>
<th>MEDU</th>
<th>OCTX</th>
<th>PUTM</th>
<th>SNIG</th>
<th>TCTX</th>
<th>THAL</th>
<th>WHMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6265</td>
<td>3367249</td>
<td>0.34</td>
<td>0.42</td>
<td>0.6</td>
<td>0.32</td>
<td>0.4</td>
<td>0.059</td>
<td>0.98</td>
<td>0.24</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>rs11030102</td>
<td>3367234</td>
<td>0.024</td>
<td>0.25</td>
<td>0.16</td>
<td>0.3</td>
<td>0.24</td>
<td>0.0076</td>
<td>0.99</td>
<td>0.41</td>
<td>0.42</td>
<td>0.43</td>
</tr>
<tr>
<td>rs11030108</td>
<td>3367234</td>
<td>0.21</td>
<td>0.46</td>
<td>0.55</td>
<td>0.0064</td>
<td>0.11</td>
<td>0.016</td>
<td>0.23</td>
<td>0.12</td>
<td>0.36</td>
<td>0.78</td>
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<td>rs7103873</td>
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<td>0.55</td>
<td>0.16</td>
<td>0.15</td>
<td>0.0048</td>
<td>0.82</td>
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<td>0.55</td>
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<td>0.29</td>
<td>0.29</td>
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<tr>
<td>rs56164415</td>
<td>3367236</td>
<td>0.9</td>
<td>0.72</td>
<td>0.17</td>
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<td>0.038</td>
<td>0.097</td>
<td>0.46</td>
<td>0.85</td>
<td>0.86</td>
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</table>

B) NTRK2

<table>
<thead>
<tr>
<th>SNP</th>
<th>exprID</th>
<th>CRBL</th>
<th>FCTX</th>
<th>HIPP</th>
<th>MEDU</th>
<th>OCTX</th>
<th>PUTM</th>
<th>SNIG</th>
<th>TCTX</th>
<th>THAL</th>
<th>WHMT</th>
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<tbody>
<tr>
<td>rs1187325</td>
<td>3177122</td>
<td>0.95</td>
<td>0.074</td>
<td>0.38</td>
<td>0.051</td>
<td>0.029</td>
<td>0.68</td>
<td>0.08</td>
<td>0.32</td>
<td>0.22</td>
<td>0.2</td>
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<tr>
<td>rs1439050</td>
<td>3177118</td>
<td>0.6</td>
<td>0.59</td>
<td>0.94</td>
<td>0.99</td>
<td>0.26</td>
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<td>0.0074</td>
<td>0.92</td>
<td>0.51</td>
<td>0.71</td>
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<td>rs1047896</td>
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<td>0.92</td>
<td>0.56</td>
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<td>rs1078947</td>
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<td>0.96</td>
<td>0.74</td>
<td>0.85</td>
<td>0.0025</td>
<td>0.24</td>
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</table>

CRBL = cerebral cortex  
FCTX = frontal cortex  
HIPP = hippocampus  
MEDU = medulla  
OCTX = occipital cortex  
PUTM = putamen  
SNIG = substantia nigra  
TCTX = temporal cortex  
THAL = thalamus  
WHMT = white matter  

Note: Bold means p-value <0.05, but none of the above p-values are statistically significant after considering multiple testing corrections

Supplementary Figure 1 eQTL results from Braineac for BDNF SNPs (A to E) and NTRK2 SNPs (F to I)
Supplementary Figure 2 Results from the GTex Portal for (A) BDNF and (B) NTRK2
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