MULTILOCUS DOPAMINE GENE VARIATION, REWARD SENSITIVITY, AND ABERRANT EATING

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Background: Evidence suggests that aberrant eating may be partially motivated by the rewarding properties of food, in addition to caloric or metabolic need. Given the role that the neurotransmitter dopamine (DA) plays in the reinforcement of reward driven appetitive behaviors like eating, it is hypothesized that DA gene variation may contribute to the development of aberrant eating behavior by increasing sensitivity to signals of reward. **Methods:** A sample of midlife community volunteers from the Adult Health and Behavior Project (AHAB-1; $N = 921$) were genotyped for five DA gene variants, which were summed as a cumulative risk score. Participants completed the Eating Disorders Inventory (EDI) to assess aberrant eating, and the Behavioral Activation Scale (BAS) to measure reward sensitivity. **Results:** Cumulative risk scores were not associated with presence or severity of bulimic symptoms, total BAS scores, or BMI, nor were total BAS scores associated with presence or severity of bulimic symptoms. The met allele of the *COMT* val/met single nucleotide polymorphism (SNP) was associated with the presence of bulimic symptoms and drive for thinness, as well as the severity of body dissatisfaction. The insertion allele of the *DRD2* ins/del polymorphism was associated with presence of bulimic symptoms, and the severity of drive for thinness. All EDI subscales, as well as total BAS scores, predicted higher BMI. **Discussion:** Although cumulative DA risk scores were not associated with bulimic symptoms, *COMT* and *DRD2* SNPs predicted several features

of aberrant eating, suggesting that they may increase susceptibility for aberrant eating in a generally healthy population. Future studies might consider examining the relationship between these SNPs and more proximal measures of eating behavior, such as food craving, or the size, frequency, and content of dietary intake, and replicating the present study in individuals with clinically significant aberrant eating.

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INTRODUCTION

Binge eating, defined as periods of excessive eating coupled with a subjective feeling of loss of control, and other eating episodes characterized by overeating or loss of control (referred to herein as "aberrant eating") is an important clinical phenomenon. Individuals who report loss of control over eating demonstrate greater eating-related psychopathology, psychological comorbidity, and weight gain compared to those who overeat without experiencing loss of control (Tanofsky-Kraff & Yanovski. 2004), even among individuals without a diagnosable eating disorder (Vannucci, Theim, Kass, Trockel, Genkin, et al., 2013). Moreover, individuals who engage in binge eating are more likely than those who do not to be overweight or obese (Wilfley, Schwartz, Spurrell, & Fairburn, 2000; Striegel-Moore, Cachelin, Dohm, Pike, Wilfley, et al., 2001). These findings suggest that many of the mental and physical health consequences associated with clinically significant eating disorders may also be present in subclinical aberrant eating. Further, given that as many as 46% of obese individuals report binge eating (e.g. Spitzer, Yanovski, Wadden, Wing, Marcus, Stunkard, et al., 1993), it is possible that aberrant eating behaviors like binge eating and loss of control over eating may constitute a pathway to obesity for a subset of individuals. In support of this idea, prospective longitudinal research has demonstrated that aberrant eating is associated with more frequent weight-related cognitive distortions, weight gain, and weight related disease (Tanofsky-Kraff & Yanovski, 2004), highlighting the importance of exploring the etiological mechanisms influencing aberrant eating. Research has begun to reveal that motivational and reward processes, as well as the neurobiological pathways and neurotransmitter systems underlying these processes, may promote and maintain aberrant eating behavior and subsequent weight gain (Stice & Burger, 2012; Blumenthal & Gold, 2010). Therefore, it is possible that factors thought to contribute to

individual differences motivational and reward processes may confer risk for aberrant eating and weight gain.

1.1 REWARD PROCESSING, DOPAMINE, AND EATING

There is increasing evidence that the drive to overeat may be partially mediated by the rewarding properties of food, and that such hedonic eating may be influenced by dopamine (DA) signaling (Lowe & Butryn, 2007). Aberrant eating, therefore, may be understood as an appetitive behavior that is not always related to caloric need. DA may participate in reinforcement of food choices by promoting associations between palatable foods, the pleasant experience they produce, and the behavior that allowed the individual to obtain these foods (Wise, 2004). Reward learning is impaired in the presence of DA antagonists, suggesting that DA functions not to elicit the feeling of reward itself, but to pair that feeling with the eliciting stimulus (Wise & Schwartz, 1981). As such, DA may be involved in the development of taste preferences, the induction of the motivational state necessary to engage in aberrant eating, as well as the maintenance of behaviors that have been coupled with the experience of reward. Further, many of the behavioral features of eating disorders (e.g. dieting, purgative behaviors, exercise routines) are compulsive in nature (Davis & Kaptein, 2006), a feature that has been related to elevated DA processing (Bulik, Slof-Op't Landt, van Furth, & Sullivan, 2007). Therefore, DA neurotransmission may be the key to understanding many features of disordered eating.

Evidence from the animal literature has established that DA pathways such as the mesolimbic dopaminergic system (MDS; Figure 1) underlie the rewarding effects of food. In rats, MDS activation increases in response to food (Martel & Fantino, 1996). Following ablation of the DA-rich ventral tegmental area (VTA), a component of the MDS, rats no longer showed a preference for sucrose solution over water (Shimura, Kamada, &Yamamoto, 2002). These

findings suggest that the VTA is involved in developing a preference for palatable foods, as well as reinforcing consumption of these foods. Given the role of DA in supporting reward learning and appetitive motivation in animals (Nemirovsky, Avale, Brunner, & Rubinstein, 2009), altered DA transmission in MDS structures may influence the development of aberrant eating in humans by promoting stronger associations between food and its hedonic effects.

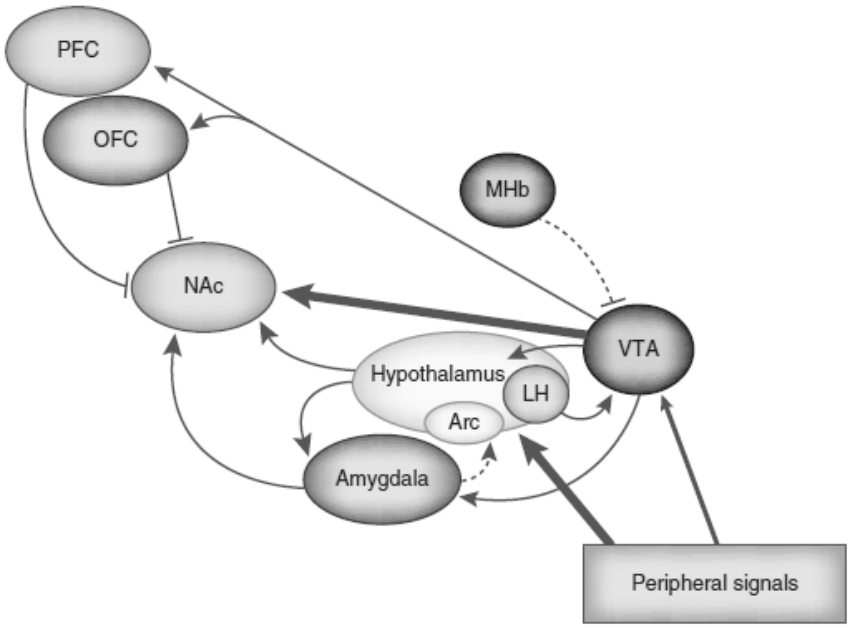

Figure 1: Components of the MDS involved in eating behavior. Figure originally published by DiLeone, Taylor, & Picciotto (2012).

1.2 DOPAMINE AND EATING: CORRESPONDANCE TO SUBSTANCE USE

There is growing evidence that excessive food consumption may induce neural and behavioral changes remarkably similar to those observed in substance abuse (e.g. Avena, Rada, & Hoebel, 2009; Stice, Spoor, Bohon, & Small, 2008; Johnson & Kenny, 2010), suggesting that factors thought to contribute to individual differences in substance use may also confer risk for aberrant

eating. For instance, rats that are intermittently given access to palatable food show binge-like consumption patterns, garnering 58% of their daily caloric intake from these discrete binge episodes despite having ad libitum access to standard chow (Avena et al., 2009). If rats exposed to a diet high in fat or sugar are food deprived for 24 hours, they begin to develop the types of withdrawal symptoms characteristically seen in both animal and human substance dependence (Avena et al., 2009). In addition, rats that binge eat sugar solutions have been shown to become more sensitive to the effects of other DA stimulants (Avena & Hoebel, 2003), providing evidence that binge eating produces changes in DA processing. Further, consumption of sucrose solutions elicits DA release in the nucleus accumbens (NAc), another region in the MDS that receives input from the VTA (Avena, Rada, & Hoeble, 2006). Interestingly, the elevation in DA occurs even during sham feeding protocols, which allow rats to ingest the sucrose solution but prevent it from reaching the stomach (Avena et al., 2006). This indicates that the taste of sugar alone can induce changes in DA transmission. Therefore, animal research demonstrating DA's role in binge eating and the correspondence between neural responses to food and to drugs may be relevant for understanding how aberrant eating develops in humans.

1.3 DOPAMINE AND EATING: EVIDENCE IN HUMANS

Food-induced DA responses similar to those described in animals have also been observed in humans. Tasting sweet food is correlated with an elevation of synaptic concentrations of DA in the dorsal striatum, a subcortical region in the MDS that is densely populated by DA receptors (Volkow, Wang, Fowler, Logan, Jayne, et al., 2002). Further, the amount of DA released is proportional to self-reported hunger and desire for food (Volkow, et al., 2002). Increased DA activation within the MDS in response to food has been observed in obese individuals who frequently binge eat (Wang, Geliebter, Volkow, Telang, Logan, et al., 2011). Using food cue

paradigms, it has also been demonstrated that, relative to non-binge eating obese subjects, obese binge eaters show greater activation in the frontal and medial orbitofrontal (OFC) regions of cortex that receive DA input (Schienle, Schafer, Hermann, & Vaitl, 2009). Additionally, activity level in these regions is correlated with self-reported reward sensitivity (Schienle, et al., 2009) and pleasure experienced while eating (Small, Jones-Gotman, & Dagher, 2003). Notably, activation in these regions, particularly in the medial OFC, is thought to be involved in encoding the reward value of reinforcing stimuli (Schienle et al., 2009). Further, these prefrontal regions are innervated by fibers originating in the VTA (Fig. 1) and are densely populated with DA receptors (Sawaguchi & Goldman-Rakic, 1991; Wang, Zhong, Gu, & Yan, 2003; Laviolette, Lipski, & Grace, 2005), suggesting that frontal region responses to food may be elicited by MDS activation (Wang et al., 2011). These findings provide evidence that DA transmission is involved in the regulation of eating behavior, and may be important for understanding aberrant eating.

Research supports the hypothesis that signaling variation within DA-rich regions contributes to individual differences in aberrant eating, with weight gain being a secondary consequence. For example, striatal DA release increases following food presentation in obese binge eaters. However, this pattern is not observed in non-binge eating obese subjects, suggesting that striatal activity may be uniquely related to binge eating (Wang et al., 2011), supporting the link between DA variation and aberrant eating. In fact, food cues only elicited excess DA release in the non-binge eating obese group after administration of methylphenidate, a compound that blocks reuptake of DA from the synapse (Wang et al., 2011). This indicates that DA processing within the striatum may participate specifically in the development of binge eating behavior. Therefore, DA signaling may only be indirectly related to weight gain through its effects on aberrant eating, a hypothesis supported by research in animals. In rat models of

binge eating, surges in extracellular DA within the NAc following a meal are evident in rats that have developed binge-like food intake patterns (Rada, Avena, & Hoebel, 2005), even in response to foods that are no longer novel. Conversely, DA release in response to familiar foods diminishes over time in rats that do not display binge eating behavior (Rada et al., 2005). This suggests that DA signaling is important to consider in the context of aberrant, rather than normative, eating behavior.

Further, binge eating induced changes in DA signaling have been associated with enhanced responsivity to cues of impending food reward, particularly following a period of dietary restriction (Avena, 2007). Rats with restricted access to a sugar solution exhibit enhanced DA release in the NaC and altered DA receptor binding (Avena, 2007). Further, rats conditioned to lever press to obtain food show increased frequency of lever pressing following a period of abstinence, suggesting motivation to consume palatable foods is greater under conditions of restricted access (Avena, Long, & Hoebel, 2005). The increase in motivational drive to eat after a period of abstinence observed in binge eating rats is analogous to the pattern of human eating behavior in anorexia nervosa (AN), BN, and BED. Dietary restraint is considered an important predictor of aberrant eating (Fairburn, Cooper, & Shafran, 2003), and mediates the effect of drive for thinness and body dissatisfaction on subsequent episodes of aberrant eating (Stice, Shaw, & Nemeroff, 1998; Wilfley, et al., 2000). Therefore, changes in DA signaling enhance motivation to eat following dietary restraint, and may account for the relationship between weight-related cognitive distortions and aberrant eating. Together, these data suggest that variation in DA processing is involved in aberrant, rather than normative, eating, and may underlie symptomatology shared among eating disorder subtypes.

In addition to MDS signaling, DA receptor functioning is also important in the regulation of eating behavior. Repeated activation of receptors that are part of the DA D2 subtype, a family of receptors that initiate an inhibitory intracellular cascade when bound to DA, has been associated with aberrant eating and weight gain. For instance, D2 receptor antagonists precipitate hyperphagia and subsequent weight gain in animals and humans (Allison, Mentore, Heo, Chandler, Cappelleri, et al., 1999; Lee & Clifton, 2002). Further, consumption of a high fat diet is associated with decreased expression of striatal D2 autoreceptors in rats (Johnson & Kenny, 2010). Because D2 receptors reduce presynaptic synthesis of DA when DA is present in the synapse, decreased expression impairs the ability of dopaminergic cells to autoregulate DA transmission (Haubrich & Pflueger, 1982). Weakening of this negative feedback mechanism may disinhibit responses to previously learned reward cues. For example, rats that have undergone D2 receptor knockdown more quickly display binge eating behavior compared to intact rats (Johnson & Kenny, 2010), indicating that receptor downregulation may precede binge eating rather than be the consequence of it. Similarly, reduced striatal D2 receptor availability is associated with increased BMI in humans (Wang et al., 2001). Remarkably, this trend is reversed in obese patients who have undergone gastric bypass surgery. Compared to pre-surgery baseline of D2 receptor density, patients show increased D2 expression following surgery, when food intake is significantly reduced (Steele, Prokopowicz, Schweitzer, Magunsuon, Lidor, et al., 2010). These associations indicate that individual differences in DA receptor density may increase risk for weight gain by influencing food preference and intake patterns. Therefore, genetic variation associated with differential expression of DA receptors may contribute to individual differences in aberrant eating behavior.

1.4 DOPAMINE GENE VARIATION AND EATING

Research has demonstrated that DA gene variation is related to MDS function and aberrant eating, and may contribute to variation in risk for aberrant eating. Polymorphisms in genes regulating the degradation of synaptic DA as well as the expression and function of DA receptors have been linked with aberrant eating and/or MDS disturbances (see Table 1 for a summary of DA gene polymorphism studies and findings).

Table 1. Summary of DA gene polymorphism studies and findings

Polymorphism	Authors	Sample	Findings
DRD4 VNTR	Sobik et al., 2005	48 healthy multi-ethnic population	(+) 7R allele associated with elevated food craving
	Sobik et al., 2005	31 female multi-ethnic population with sub-clinical binge eating	(+) 7R associated with more drastic decline in craving over repeated presentations of food
	Kaplan et al., 2007	163 Caucasian females with BN	(+) 7R associated with BMI
	Levitan et al., 2004	131 Caucasian females with SAD	(+) 7R associated with binge eating
	Levitan et al., 2004	108 Caucasian females with SAD	(+) 7R associated with maximal lifetime BMI
	Bachner-Melman et al., 2007	620 Israeli females including those with AN and controls	(+) 7R associated with drive for thinness among healthy controls (+) 7R preferentially transmitted with other DA gene risk variants in AN participants
	Ebstein et al., 1996	107 healthy Israeli individuals	(+) 7R associated with novelty seeking
	Pogue-Geile et al., 1998	281 healthy Caucasian twins	(-) 7R not associated with novelty seeking
DRD2 Taq1A[a]	Stice et al., 2008	43 healthy Caucasian females	(+) A1 allele associated with reduced D2 receptor density and BMI
	Epstein et al., 2007	74 multi-ethnic obese and non-obese sample	(+) A1 allele associated with greater food reinforcement and carbohydrate consumption
	Noble, 2000	73 multi-ethnic obese sample	(+) A1 allele associated with carbohydrate craving and

	Davis et al., 2008	166 multi-ethnic individuals with BED and/or obesity	consumption (+) A1 allele associated with BED and reward sensitivity
	Epstein et al., 2004	88 Caucasian smokers	(+) A1 allele associated with food reinforcement and food intake
	Stice et al., 2008	43 healthy Caucasian females	(-) A1 allele not associated with increased MDS activation
	Epstein et al., 2007	74 multi-ethnic obese and non-obese individuals	(-) A1 allele not associated with binge eating
	Davis et al., 2008	230 multi-ethnic individuals with BED and/or obesity	(-) A1 allele not associated with BED
	Nikolova et al., 2011	69 healthy Caucasian individuals	(-)A1 allele not associated with ventral striatal activation
DRD2 ins/del	Lencz et al., 2010	58 multi-ethnic individuals with Schizophrenia	(+) Del allele associated with weight gain following D2 receptor blockade
	Nikolova et al., 2001	69 healthy Caucasian individuals	(+) Del allele associated with ventral striatal activation
	Davis et al., 2008	56 multi-ethnic individuals with BED and/or obesity	(-) Del allele not associated with BED
	Davis et al., 2012	230 multi-ethnic individuals with BED and/or obesity	(-) Del allele not associated with BED
DAT1 VNTR	Shinohara et al., 2009	205 Japanese individuals including those with an eating disorder and healthy controls	(+) 9R allele associated with binge eating among participants with an eating disorder
	Davis et al., 2007	78 multi-ethnic individuals including those with BED and matched controls	(+) 9R allele associated with decreased appetite in response to methylphenidate
	Dreher et al., 2009	22 healthy multi-ethnic individuals	(+) 9R associated with ventral striatal and orbitofrontal activation following food reward
	Epstein et al., 2004	88 Caucasian smokers	(-) 10R associated with greater food reinforcement and food intake

COMT Val^{158}Met	Dreher et al., 2009	27 healthy multi-ethnic individuals	(+) met allele associated with ventral striatal and orbitofrontal activation following food reward
	Frieling et al., 2006	45 female inpatients with an eating disorder (ethnicity not reported)	(+) met allele associated with higher EDI, Bulimia, and impulse regulation scores
	Yilmaz et al., 2011	75 females with BN and 148 unaffected relatives (ethnicity not reported)	(+) met allele preferentially transmitted from parents to offspring with Bulimia
	Groleau et al., 2012	166 females with Bulimia and 166 ethnicity matched control females	(-) no association between *COMT* genotype and Bulimia
	Mikolajczyk et al., 2010	216 females with threshold and subthreshold bulimia (96.7% Caucasian)	(-) met allele associated with decreased binge frequency
		104 females with an eating disorder (61 with Anorexia, 43 with Bulimia; ethnicity not reported)	(-) val allele associated with Bulimia

[a]A1 is the designation for the minor T allele for *Taq1A* genotype.

Aberrant eating has been associated with D4 receptor density. The D4 receptor is heavily expressed in the prefrontal cortex (PFC; Fig. 1; Mrzljak, Bergson, Pappy, Huff, Levenson et al., 1996), an area of the brain that participates in monitoring the delivery of an expected reward (Wantanabe, 1996; Knutson, Fong, Bennett, Adams, & Hommer, 2003). A 48 base pair functional variable number of tandem repeats (VNTR) polymorphism in the DA receptor 4 (*DRD4*) gene is related to D4 function (Van tol, Bunzow, Guan, Sunhara, Seeman, et al. 1992). The number of repeats corresponds to the relative ability of DA to bind to the D4 receptor (Van tol, et al. 1992). The 7-repeat (7R) allele has been associated with decreased affinity for DA

(Asghari, Sanyal, Buchwaldt, Paterson, Jovanovic, et al., 1995), as well as reduced signal transduction within cells expressing the D4 receptor (Chang et al., 1996). As a subtype of the D2-family of DA receptors, D4 stimulation initiates an intracellular cascade that inhibits DA release into the synapse (Asghari et al., 1995). However, this cascade is impaired in individuals with the 7R allele, resulting in an attenuated inhibitory signal in these cells. Therefore, the 7R allele may alter reward processing through reduced DA binding affinity to the D4 receptor or reduced intracellular signaling. Further, the 7R allele is associated with reduced D4 expression in the PFC (Asghari et al., 1995), which may impair top-down control of previously learned responses to rewarding stimuli (Wise et al., 2004). Individuals with the 7R allele may be more responsive to rewarding stimuli like food and have less inhibitory control over eating behavior (Wise 2004), suggesting a mechanism by which 7R carriers may be at higher risk for aberrant eating. Therefore, *DRD4* variation may be an important factor for understanding aberrant eating.

Research has demonstrated that *DRD4* variation is associated with individual differences in reward function, weight, and aberrant eating. Mice that have undergone D4 knockdown were found to display more efficient reward learning relative to their intact counterparts (Nemirovsky et al., 2009). Specifically, the knockdown mice more quickly learned to associate environmental cues with the receipt of a food relative to wildtype mice (Nemirovsky et al., 2009). In humans, the 7R allele has been associated with novelty seeking (Ebstein, Novick, Umansky, Priel, Osher, et al., 1996; Benjamin, Li, Patterson, Greenberg, Murphy, & Hamer, 1996; Munafo, Yalcin, Willis-Owen, & Flint, 2007; however, see Schinka, Letsch, & Crawford, 2002 and Pogue-Geile, Ferrell, Deka, Debski, & Manuck, 1998) and impulsivity (Munafo et al., 2007; Eisenberg, MacKillop, Modi, Beauchemin, Dang et al., 2007), personality traits that are similar to reward sensitivity. 7R allele carriers are more reactive to food cues and report more intense craving for

food following cue presentation relative to non-7R carriers (Sobik, Hutchison, & Craighead, 2005). Further, women with BN who are carriers of the 7R variant have higher BMI compared to non-7R carriers with BN (Kaplan et al., 2008). In women with seasonal affective disorder (SAD), a mood disorder characterized by seasonal binge eating and weight gain, 7R allele carriers are more likely to exhibit binge-eating behavior across their lifespan (Levitan, Masellis, Basile, Lam, Kaplan, et al., 2004). Therefore, the 7R allele may be an important factor in aberrant eating, and weight gain.

In addition to the *DRD4* VNTR, research has also identified a relationship between the *Taq1A* single nucleotide polymorphism (SNP) and individual differences in eating behavior. *Taq1A* lies within a genomic region downstream of the D2 receptor gene (*DRD2*), and may regulate *DRD2* expression (Neville, Johnstone, & Walton, 2004). The *Taq1A* minor A1 allele is associated with reduced striatal D2 density (Noble, 2000), and DA binding affinity at the D2 receptor (Jonsson et al., 1999). Decreased D2 expression associated with the A1 allele yields a weaker inhibitory signal when DA is present in the synapse, blunting the negative feedback mechanism that normally inhibits further DA release. Therefore, more DA is available to produce stronger coupling between food and the pleasure derived from eating. The A1 allele may be related to increased risk for aberrant eating by strengthening the association between food and subsequent experience of pleasure (Wise, 2004). Therefore, *Taq1A* variation may contribute to individual differences in aberrant eating behavior.

Taq1A has been associated with eating behavior and weight gain (e.g. Wang et al., 2001; Steele et al., 2010). It was shown that obese individuals with the A1 allele exhibited a 30-40% reduction in D2 receptor availability, as well as reduced dorsal striatal activity in response to ingestion of palatable foods (Stice et al., 2008). Further, A1 allele carriers had significantly

increased BMI at one year follow-up compared to individuals who did not possess the risk allele (Stice et al., 2008). Therefore, A1 allele carriers showed reduced striatal D2 density, reduced dorsal striatal activity in response to food cues, and higher BMI, all of which suggests that the A1 allele increases risk for aberrant eating. A1 allele carriers have also been shown to respond more strongly to behavioral reinforcement when food rewards were used as the reinforcing stimulus. Stronger reinforcement in turn predicted elevated consumption of carbohydrate-dense food when given free access (Epstein, Temple, Neiderhiser, Salis, Erbe et al., 2007), indicating that A1 carriers may eat larger portions of food more frequently, which may then produce weight gain. Additionally, those who showed a preference for palatable foods rich in carbohydrates had a higher frequency of the A1 allele compared to those who did not prefer these foods (Noble, 2000). Moreover, the A1 allele was associated with reward sensitivity among individuals with BED (Davis, Levitan, Kaplan, Carter, Reid et al., 2008). Such findings suggest that, due to low D2 receptor density, cues signaling impending food reward may be more salient for individuals carrying the A1 allele. This may then promote food preferences and patterns of eating that are more likely to result in aberrant eating and weight gain. As such, the *Taq1A* polymorphism may be a good candidate for studies measuring genetic risk factors for aberrant eating.

There is evidence to suggest that a functional insertion/deletion polymorphism (-141 ins/del) lying within the promoter region of the *DRD2* gene might also increase risk for aberrant eating. The deletion allele, which codes for the deletion of a cytosine from the DNA sequence, has been associated with reduced D2 expression (Arinami et al., 1997). Several studies have demonstrated an association between the deletion allele and activity in the ventral striatum, a component of the MDS (Forbes et al., 2009; Nikolova et al., 2011). Specifically, individuals with the deletion allele showed elevated ventral striatal reactivity relative to insertion allele carriers

during an fMRI paradigm known to engage reward circuitry, suggesting that decreased D2 density observed in the presence of the deletion allele produces deficits in inhibitory signaling in the ventral striatum (Hariri, Brown, Williamson, Flory, de Wit et al., 2006). Further, in patients with Schizophrenia who are prescribed antipsychotic medications known to induce weight gain through DA receptor blockade (Lee & Clifton, 2002), deletion allele carriers gained significantly more weight over the course of treatment relative to individuals homozygous for the insertion allele (Lencz, Robinson, Napolitano, Sevy, Kane et al., 2010). Therefore, the deletion allele may confer risk for weight gain by reducing D2 receptor density in MDS regions. Although this SNP has yet to be studied in the context of aberrant eating, its relationship to striatal receptor expression and to MDS activity supports doing so.

In addition to DA receptor gene variation, evidence suggests that genes regulating DA availability in the synapse may also have a significant impact on responsivity to food reward (e.g. Volkow et al., 2002), prompting researchers to test whether variation in DA transporter (DAT) density is associated with individual differences in eating behavior. DAT, which is highly expressed in the striatal region of the MDS, participates in reuptake of excess synaptic DA (Davis, Levitan, Kaplan, Carter, Reid et al., 2007). As such, DAT is integrally involved in regulating synaptic concentrations of DA. Functionally, reduced DAT density leads to increased synaptic concentrations of DA. Therefore, DAT may contribute to overeating and weight gain through modulation of DA availability. Consistent with this hypothesis, DAT mRNA levels and protein expression have been shown to increase in binge-eating rats (Bello, Sweigart, Lakoski, Norgren, & Hajnal, 2003). In humans, elevated DAT expression was associated with BMI healthy volunteers (Chen, Yang, Yeh, Lee, Yao, Chiu et al., 2008). Together, such findings

indicate that genetic variation associated with differential expression of the DAT protein may be related to risk for aberrant eating and weight gain.

Investigators have determined that aberrant eating behavior is associated with a VNTR located within the 3' untranslated region of the DAT gene (*DAT1*). This VNTR is associated with differential expression of DAT, with the 10 repeat (10R) allele producing an estimated 50% increase in DAT density relative to the 9 repeat (9R) allele (VanNess et al., 2005). In an experiment in which participants chose between completing tasks to receive a favorite snack and completing tasks to receive a monetary reward, individuals homozygous for the 10R allele demonstrated a greater willingness to work for food, suggesting that they found food to be a more potent reinforcer (Epstein, Wright, Paluch, Leddy, Hawk et al., 2004). Further, 10R homozygotes who were strongly reinforced by food consumed more calories when given unrestricted access to food (Epstein et al., 2004). However, the opposite effect was reported in a sample of Japanese women diagnosed with an eating disorder, with 9R carriers exhibiting more binge eating behavior than 10R carriers (Shinohara, Mizushima, Hirano, Shioe, Nakazawa et al., 2004), a finding that is more consistent with other studies examining how DAT expression relates to eating disorder risk. For instance, the promoter region of *DAT1* was shown to be hypermethylated in patients with BN, indicating that DAT expression may be lower in individuals with BN due to suppressed transcription of *DAT1* (Frieling, Romer, Scholz, Mittelbach, Wilhelm et al., 2009). It is possible that, due to elevated synaptic concentrations of DA, the reinforcement value of food is enhanced in individuals who possess the 9R allele (Davis et al., 2007). Despite inconsistencies in the literature, the 9R allele of the DAT VNTR has been more frequently associated with risk for aberrant eating, although this may reflect publication

bias. These findings support the role of DAT genetic variation in the modulation of reward system sensitivity and individual susceptibility for aberrant eating and weight gain.

In addition to DAT, other mechanisms by which synaptic DA concentrations are regulated have been linked to dopaminergic processing and eating behavior. For example, the catechol-O-methyltransferase (COMT) enzyme has been shown to play an important role in terminating the action of extracellular DA following release from presynaptic terminals (Chen, Lipska, Halim, Ma, Matsumoto et al., 2004). Unlike DAT, COMT expression is most abundant in the PFC (Dreher, Kohn, Kolachana, Weinberger, & Berman, 2009), a region strongly innervated by MDS structures that participates in response inhibition and reward-related contingency learning (Ridderinkhof, van der Wildenberger, Segalowitz, & Carter, 2004). Presumably, variation in dopaminergic activity in the PFC would contribute to variation in the ability to form associations between a stimulus like food and the positive affective response the stimulus produces. Individuals who associate food more strongly with the experience of pleasure may be at greater risk for aberrant eating, and the strength of this association appears to be strongly dependent on dopaminergic processing. Consistent with the function of COMT, research has demonstrated that cortical DA levels are significantly higher in *COMT* knockout mice relative to wild-type controls (Gogos et al., 1998). Hypothetically, COMT may contribute to variation in eating behavior through its effects on extracellular DA levels. Therefore, genetic variation in the *COMT* gene may predict individual differences in aberrant eating.

In humans, a functional SNP that codes for an amino acid substitution at codon 158 of the *COMT* gene is associated with increased risk for eating disorders (val158met; Frieling, Romer, Wilhelm, Hillemacher, Kornhuber et al., 2006). Specifically, enzymatic activity in individuals homozygous for the methionine (met) allele is reduced by 25-75% compared to valine (val)

carriers (Chen et al., 2004; Lachman, Papolos, Saito, Yu, Szumlanski et al., 1996). Lower enzymatic activity may produce elevated synaptic DA levels, enhancing DA mediated sensitivity to reward cues (Dreher et al., 2009). It is expected that carriers of the low activity met allele would be more responsive to food cues (Dreher et al., 2009), thus increasing their risk for aberrant eating. Consistent with this hypothesis, the low activity met allele has been associated with greater activation in the ventral striatum during anticipation of food reward, and in the orbitofrontal region of the PFC following delivery of a reward (Dreher et al., 2009). Importantly, the orbitofrontal region is thought to be involved both in the encoding of stimulus reward value and the regulation of food intake (Kringelbach, 2005). Met allele carriers with a diagnosed eating disorder have also been shown to score higher on self-report bulimia scales relative to val homozygotes (Frieling, et al., 2006), although it is unknown if this relationship reflects increased susceptibility to aberrant eating generally or to binge eating specifically. In addition, there is evidence to suggest that the met allele is preferentially transmitted to female probands with BN (Yilmaz, Kaplan, Zai, Levitan, & Kennedy, 2011). However, it should be noted that others have documented an association between the val allele and BN (Mikolajczyk, Grzywacz, & Samochowiec, 2010), highlighting inconsistencies in the literature. Nevertheless, the importance of the orbitofrontal cortex in the facilitation of reward learning, and the primary role that *COMT* plays in the degradation of DA in this region, suggest that variation in *COMT* activity could hypothetically influence reward processing and abnormal eating behavior. Therefore, the val158met SNP was genotyped in the present study.

With the incorporation of the *COMT* val/met SNP, five polymorphisms have been identified as candidates for increased risk of aberrant eating, and possibly higher BMI. By examining the aggregate effect of all five polymorphisms, the current proposal aims to improve

upon candidate gene studies by capturing a greater proportion of the variance in eating behavior that is attributable to DA gene variation using a cumulative genetic risk score approach.

1.5 CUMULATIVE GENETIC RISK SCORES

Although gene association studies examining the effect of a single polymorphism have provided important clues about the neurobiological processes involved in maintaining pathological eating behavior, it is clear that conditions like eating disorders are quantitative in nature and likely to be influenced by many genes (Plomin, Haworth, & Davis, 2009). Much of the available research investigating the effects of genetic variation on individual differences in complex behavior like eating have either relied on the single locus candidate gene approach (e.g. Davis et al., 2007; Levitan et a., 2004; Shinohara et al., 2004), or have examined the effects of several different polymorphisms independently of one another (e.g. Davis et al., 2008; Frieling et al., 2006). Although these strategies have suggested possible pathways through which genetic variation may produce differences in food intake and aberrant eating, they do not adequately capture the complexity of the systems involved in the regulation of eating. Given the probability that eating behavior is influenced by multiple genes, it is unlikely that a single gene variant will explain a significant proportion of phenotypic variability. A more informative approach is to examine the aggregate effect of multiple functional polymorphisms known to contribute to signaling variation within relevant pathways (Nikolova et al., 2011). Studies have demonstrated the utility of creating multilocus genetic risk scores to explain differences in MDS reactivity during anticipation of a reward. For instance, cumulative genetic risk scores representing susceptibility for elevated dopaminergic neurotransmission were shown to significantly predict ventral striatal activation above and beyond any single gene variant included in the risk scores, despite the fact that none of the individual polymorphisms were independently significant (Nikolova et al.,

2011). Therefore, creating a genetic risk score reflecting the aggregate effect of multiple genes may be more powerful for exploring neurobiological mechanisms underlying complex polygenic phenotypes than single-gene approaches. Applying the cumulative genetic risk score approach to the study of aberrant eating behavior thus has the potential to substantiate and extend upon research linking MDS activation to the regulation of eating behavior by identifying an underlying etiological mechanism, aggregate DA gene variation (Figure 2).

1.6 GREY'S BEHAVIORAL ACTIVATION SYSTEM, DOPAMINE, AND APPETITIVE MOTIVATION

Sensitivity to reward is a pertinent construct for understanding individual differences in reward-driven behavior, and may be an important factor mediating the relationship between DA gene variation and aberrant eating. Based on research conducted primarily in rodents, Gray proposed two complementary neurobiological systems thought to underlie individual differences in personality, motivation, and appetitive behavior (Gray, 1990). Within this framework, processes such as attention, arousal, and anxiety are regulated by the Behavioral Inhibition System (BIS), whereas motivated and appetitive behaviors arise from activity in the Behavioral Activation System (BAS; Gray, 1990). Gray further implicated the dopaminergic MDS as the neuroanatomical origin of the BAS (Gray, 1987), making it a particularly relevant construct for the study of the relationship between DA and eating behavior. Theoretically, DA gene variation affects sensitivity to cues signaling reward, including those related to food, thereby influencing individual risk for aberrant eating.

The Behavioral Inhibition Scale/Behavioral Activation Scale (BIS/BAS; Carver & White, 1994), developed to translate Grey's research to humans, contains a behavioral activation subscale featuring a series of self-referent statements that are thought to reflect sensitivity to

reward. The BIS/BAS has been successfully utilized to explore the relationship between eating behavior and responsivity to reward cues. Although the BIS/BAS has never been used in the context of a multilocus cumulative risk study of aberrant eating, previous studies have examined how dopaminergic signaling might be related to appetitive motivation or propensity to overeat. These studies provide a rationale for testing the relationship between mutilocus DA gene variation, BAS sensitivity, and aberrant eating behavior in the present proposal. For instance, the *Taq1A* A1 risk allele has been positively associated with BMI and sensitivity to reward as measured by the BAS in a sample of adults with BED (Davis et al., 2008). This suggests that blunted dopaminergic autoreceptor function may promote aberrant eating behavior by enhancing motivation to obtain and consume preferred foods (Davis et al., 2008).

In summary, research suggests that genetically mediated alterations in DA receptor function and DA availability may promote aberrant eating, weight gain, and obesity by enhancing sensitivity to signals of reward. Investigating the relationship between genetic variation impacting DA signaling and appetitive behavior has helped to promote a more complete understanding of the relationship between reward processing and aberrant eating. Though facets of reward processing have been repeatedly linked to excessive eating behavior and weight gain, there is a paucity of research exploring these effects in healthy individuals. It is possible that binge eating behavior is one of many risk factors for weight gain and obesity, even among individuals without a clinically significant eating disorder like BN or BED. Therefore, it is important to examine factors that influence individual risk for binge eating behavior in non-clinical samples. Establishing that DA gene variation impacts aberrant eating in a healthy sample would provide evidence that variability in the DA system influences eating and subsequent weight gain independently of other physical or mental health factors. The present study aims to

extend previous research by examining the cumulative effect of variation in multiple genes regulating DA function on aberrant eating behavior and BMI in a sample of healthy adults. Additionally, in an effort to provide evidence for a possible pathway through which DA gene variants might elevate risk for aberrant eating, the present study will test whether the association between these gene variants and aberrant eating is mediated by reward sensitivity.

2.0 RESEARCH DESIGN AND METHODS

2.1 PARTICIPANTS

Participants included 1295 healthy adults from the Adult Health and Behavior Project (AHAB-I), an extensive registry of behavioral and biological measures collected from a mid-life community sample recruited in Pittsburgh and surrounding regions between 2001 and 2005. AHAB exclusion criteria included a clinical history of neurologic illness, cardiovascular disease, Schizophrenia, or other psychoses. Volunteers were also excluded if they reported current use of use of insulin, glucocorticoid, antiarrhythmic, psychotropic, or prescription weight-loss medications. For the purposes of the present study, all non-Caucasian individuals were removed from the sample (n = 214). Of the remaining 1081 Caucasian individuals, those who had not been successfully genotyped for all five polymorphisms (n = 138; 12.4%; Table 2) or who were missing data for any of the EDI (n = 13) or BAS (n = 9) scales were removed from the sample, yielding a final sample size of 921.

Table 2. Percentage of missing data for independent variables

Variable	**Missing data (*n*, %)**
Behavioral Activation Scales	
Drive	9 (0.08)
Fun Seeking	9 (0.08)
Reward Responsiveness	9 (0.08)
Eating Disorders Inventory	
Bulimia	13 (1.2)
Drive for Thinness	13 (1.2)
Body Dissatisfaction	13 (1.2)
Polymorphisms	
Taq1A	48 (4.4)
DRD2 ins/del	58 (5.2)
COMT val/met	64 (5.9)
DAT1	33 (3.1)
DRD4	48 (4.4)

Note. Percentages are derived from the total sample of 1081 Caucasian individuals.

2.2 ASSESSMENTS

2.2.1 Eating Disorders Inventory (EDI)

The EDI is a 64-item multidimensional self-report measure of the cognitive and behavioral symptomatology typically observed in individuals diagnosed with AN and BN (Garner, Olmstead, & Polivy, 1983). Specifically, the EDI is comprised of 8 subscales assessing common dimensions of aberrant eating (Garner et al., 1983). Of these, participants completed a modified version of the EDI consisting of the Drive for Thinness, Bulimia, and Body Dissatisfaction subscales. The EDI and its subscales have been shown to possess adequate internal consistency (Cronbach's α ranging from 0.82 to 0.90; Garner et al., 1983), construct validity, and discriminant validity (Garner et al., 1983; Espelage, Mazzeo, Aggen, Quittner, Sherman, et al., 2003). Further, the EDI has been shown to be useful for identifying at-risk individuals in healthy, non-clinical samples who display symptoms of either AN or BN but who do not meet full diagnostic criteria (Klemchuk, Hutchinson, & Frank, 1990; (Schoemaker, van Strien, & van der Straak, 1994; Engelsen & Laberg, 2001). Although it is possible to utilize data from these subscales to identify individuals at risk for clinically significant aberrant eating (Garner, 2004), the primary variables of interest in the present investigation are binge eating and related bulimic symptoms. Therefore, only scores on the Bulimia subscale of the EDI will be included in the primary analyses.

For each item on the EDI, respondents indicate on a 6-point Likert scale the extent to which a given statement applies to their eating-related thoughts or behavior, ranging from "never" to "always." Depending on the content of the question, "always" or "never" may either reflect the most or the least pathological response. Typically, scores are then transformed from the original 6-point scale to a 3-point scale. The three choices reflecting the least pathological

response are scored as 0, and the remaining choices are assigned a value of 1, 2, or 3, with a 3 corresponding to the most pathological choice for a given item (Garner, Olmstead, & Polivy, 1983). However, in a study examining the validity of the EDI in a non-clinical sample, the psychometric properties of the EDI were improved when scores were not transformed to a 3-point scale (Schoemaker, van Strien, & van der Straak, 1994). As such, the present study utilized untransformed scores on the Bulimia subscale for all analyses (Table 3).

Table 3. Items on the Bulimia subscale of the Eating Disorders Inventory

Item	
3	I eat when I am upset
4	I stuff myself with food
12	I have gone on eating binges where I have felt that I could not stop
15	I think about bingeing (overeating)
17	I eat moderately in front of others and stuff myself when they are gone
19	I have the thought of trying to vomit in order to lose weight
22	I eat or drink in secrecy

2.2.2 Behavioral Inhibition and Behavioral Activation Scales (BIS/BAS)

The BIS scale was not included as one of the dependent variables, as it is not thought to be related to DA function (Gray, 1990; Avale, Falzone, Gelman, Low, Grandy et al., 2004). The 13-item BAS scale is comprised of three subscales, including Drive, Fun Seeking, and Reward Responsiveness. The Drive scale measures the degree to which respondents act towards achieving a desired goal. The Fun Seeking scale reflects the inclination to engage with novel, potentially rewarding stimuli. Finally, the Reward Responsiveness scale assesses the degree of pleasure experienced from a reward (Carver & White, 1994). For each item, participants rate on a 4-point Likert scale the extent to which a given statement applies to them, with responses ranging from 1 “strongly disagree” to 4 “strongly agree.” Scores are then summed for each of the

13 items to yield a total BAS score, reflecting the degree to which an individual is motivated by reward (Carver & White, 1994). Adequate test-retest reliability was achieved for the BAS subscales (*r*=0.59 to 0.69; Carver & White, 1994). The BAS subscales were also shown to have satisfactory convergent and discriminant validity (Carver & White, 1994), and internal consistency (Cronbach's α ranging from 0.66-0.76; Carver & White, 1994). All primary analyses were conducted utilizing total BAS scores.

2.2.3 Body Mass Index (BMI)

Participants' height and weight were measured, and used to calculate BMI ((weight[lbs]/height[in^2]) x 703).

2.3 DNA COLLECTION AND GENOTYPING

DNA was isolated from white blood cells using the PureGene kit (Gentra Systems, Minneapolis, MN, USA). All genomic regions of interest were amplified using polymerase chain reaction (PCR). *DRD4* and *DAT1* PCR products were resolved using gel electrophoresis, and visualized under UV illumination. The *COMT* val/met SNP was genotyped using florescence polarization (Chen, Levine, & Kwok, 1999). Both the *DRD2* ins/del and *Taq1A* polymorphisms were genotyped utilizing enzymatic digestion methods.

Given the close proximity of the *DRD2* ins/del and *Taq1A* polymorphisms, a preliminary analysis was conducted to test linkage disequilibrium (LD) between these two loci within the AHAB sample using the Haploview software (Barrett, Fry, Maller, & Daly, 2005). Results from the Haploview test of LD indicated that the *DRD2* ins/del and *Taq1A* polymorphisms are not in significant LD (D'=45; r^2=0; Figure 2). Therefore, both polymorphisms were included in the calculation of cumulative genetic risk scores.

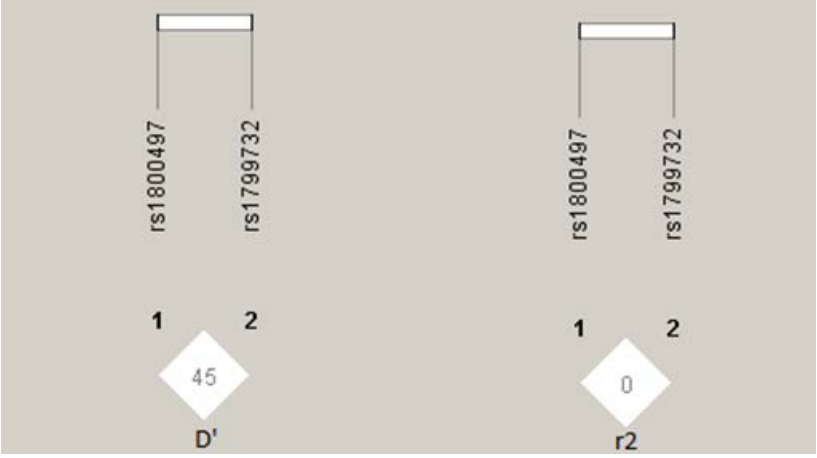

Figure 2. Linkage disequilibrium plots for DRD2 ins/del and Taq1A polymorphisms generated in HaploView (Barret et al., 2005).

All genotypes were recoded as dummy variables. Across loci, genotypes previously associated with elevated BMI, greater MDS reactivity, or eating disorder risk were assigned a score of one. In cases where there is evidence of an additive effect of genotype, heterozygous genotypes were assigned an intermediate score of 0.5. All other genotypes were coded as zero (Nikolova et al., 2011). Cumulative risk scores were then calculated for each individual by adding together the scores at each locus, with higher scores reflecting greater risk for DA-mediated aberrant eating (Table 4).

Table 4. Assignment of genetic risk scores by genotype

Polymorphism	Genotypes	Score
DRD2 -141 Ins/Del	Del/Del	1
	Ins/Del	1
	Ins/Ins	0
DAT1 VNTR	9/9	1
	9/10	1
	10/10	0
DRD4 VNTR	7/7	1
	7/any	1
	All others	0

COMT Val^{158}Met	Met/Met	1
	Val/Met	0.5
	Val/Val	0
DRD2 Taq1A[a]	T/T	1
	C/T	0.5
	C/C	0

2.4 ANALYTIC APPROACH

2.4.1 Hypotheses

It is predicted that (1) individuals with higher genetic risk scores will have higher scores on the Bulimia subscale of the EDI (path A→C), (2) individuals with higher genetic risk scores will have higher total BAS scores (path A→B), and that (3) individuals with higher total BAS scores will have higher scores on the Bulimia subscale of the EDI (path B→C). In addition, it is predicted that (4) the effect of elevated genetic risk on Bulimia subscale scores will be mediated by total BAS scores. Finally, it is predicted that (5) higher scores on the Bulimia subscale will be associated with higher BMI.

2.4.2 Primary Analyses

As preliminary analyses indicate that scores on the Bulimia subscale are positively skewed and zero-inflated, application of the Ordinary Least Squares (OLS) model is not appropriate. An alternative to OLS, the two-process hurdle model, accounts for positive skew by assuming a logistic regression model for zero vs. non-zero responses and a Poisson distribution for the non-zero responses (Atkins & Gallop, 2007; McDowell, 2003). Regression coefficients were separately estimated for comparisons of zero responses to any responses (i.e. zero Bulimia symptoms vs. any) and for comparisons among all non-zero responses (i.e. severity of Bulimia symptoms among those who endorse them; Atkins & Gallop, 2007). As such, the hurdle model provides a method for determining whether the factors that contribute to the occurrence of

Bulimia symptoms (i.e. zero symptoms vs. any) differ from those that contribute to the severity of Bulimia symptoms. To test the first component of the hurdle model, Bulimia scores were dummy coded to compare zero responses to non-zero responses. Specifically, three independent regression analyses were conducted to test the following hypotheses: (1) genetic risk scores predict presence of Bulimia symptoms (i.e. A→C; Figure 3), (2) genetic risk scores predict total BAS scores (i.e. path A→B), and (3) total BAS scores predict presence of Bulimia symptoms (i.e. B→C). Logistic regression analyses were utilized to estimate path A→C and path B→C of the proposed mediation model, while a linear regression was utilized to estimate path A→B.

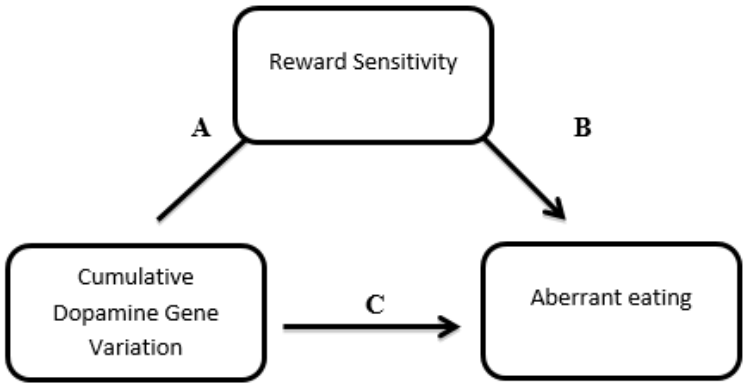

Figure 3. Model of the relationship between cumulative dopamine gene variation, reward sensitivity, and aberrant eating

To test hypothesis four, the causal steps test of mediation (Baron & Kenny, 1986) was then utilized to estimate the effect of DA cumulative risk scores on total BAS scores and the effect of total BAS scores on risk for bulimic symptomatology (e.g. zero vs. any symptoms of Bulimia on the EDI). According to the causal steps test of mediation, the following three conditions must be met in order for BAS scores to qualify as a statistical mediator of the effect of cumulative risk scores on Bulimia scores: (1) variation in cumulative risk scores must

significantly predict variation in BAS scores, (2) variation in BAS scores must significantly predict Bulimia scores, and (3) the effect of cumulative risk scores on Bulimia scores should either be reduced or disappear when controlling for BAS scores (Baron & Kenny, 1986). Unlike the product of coefficients test of mediation (Sobel, 1982), the causal steps test is limited by the fact that it does not test the statistical significance of the indirect, mediated effect. However, the causal steps test was utilized in the present study because it may not be appropriate to apply the product of coefficients test to less conventional regression analyses like hurdle modeling (A. G. C. Wright, personal communication, February 1, 2013). For example, in one of the few studies that have explored the use of the product of coefficients test of mediation with alternative regression approaches, Coxe and Mackinnon (2010) concluded that significant bias was introduced into the model when utilizing the product of coefficients method to test mediation. Therefore, the causal steps test of mediation was utilized to determine whether BAS scores mediate the relationship between cumulative risk scores and Bulimia scores rather than the product of coefficients test.

To test the second component of the hurdle model, one linear regression and two Poisson regression analyses were conducted to test the following hypotheses: (1) risk scores predict severity of Bulimia symptoms among individuals with non-zero scores on the Bulimia subscale (i.e. A→C), (2) genetic risk scores predict total BAS scores (i.e. A→B), and (3) total BAS scores predict severity of Bulimia symptoms among individuals with non-zero scores on the Bulimia subscale (i.e. B→C). Poisson regression analyses were utilized to estimate path A→C and path B→C of the proposed mediation model, while a linear regression was utilized to estimate path A→B. The causal steps test of mediation (Baron & Kenny, 1986) was performed to determine whether BAS scores mediate the effect of cumulative risk scores on non-zero Bulimia scores.

Finally, to assess the fifth hypothesis, a separate linear regression analysis was performed to determine whether Bulimia scores predicted BMI.

2.4.2.1 Bonferroni Correction

To correct for possible alpha inflation due to multiple comparisons, the significance threshold was adjusted for all 7 primary analyses utilizing the Bonferroni method, yielding an adjusted *p*-value of 0.007 (i.e. $p < 0.05/m$, where $m = 7$ comparisons).

2.4.2.2 Power Calculations

Given 80% power and α level of .05, the present study was powered to detect a small effect ($f^2 = 0.0118$) in both the A→B pathway and B→C pathway in the mediation analyses, suggesting that the study may be adequately powered to detect mediation. Although this approach may overestimate power for mediation given that it does not directly assess power to detect a mediated effect, programs designed to calculate power to detect mediation have not yet been developed (Kenny, 2012).

2.4.2.3 Covariates

In light of research demonstrating that BN prevalence rates (Rand & Kuldau, 1992) and reward sensitivity (Jorm et al., 1998) decline with age during adulthood, and that BMI increases with age (van Lenthe, Droomers, Schrijvers, & Mackenbach, 2000), all primary analyses included age as a covariate. In addition, given that the prevalence of aberrant eating is higher in females compared to males (Striegel-Moore, Rosselli, Perrin, DeBar, Terence-Wilson, et al., 2010), gender was controlled for in all primary analyses. Age and gender were only accounted for in secondary analyses if a model reached significance without covariates included.

2.4.3 Secondary Analyses

2.4.3.1 Individual Polymorphisms

To assess the amount of variance independently explained by each genetic variant, polymorphisms were added simultaneously to two additional regression analyses to predict total (1) BAS scores and (2) scores on the Bulimia subscale of the EDI. These analyses were conducted to elucidate the utility of the cumulative risk score approach by determining whether cumulative risk scores do indeed predict significantly more variance in Bulimia and total BAS scores than any individual polymorphism considered alone.

2.4.3.2 EDI Subscales

Mediation analyses were reanalyzed substituting Bulimia subscale scores with scores on the Drive for Thinness and Body Dissatisfaction subscales of the EDI to determine whether DA gene variation and/or reward sensitivity predicted features of disordered eating other than bulimic symptoms.

2.4.3.3 BAS Subscales

Research suggests that the BAS subscales significantly predict responsivity to reward cues like food independently of total BAS scores (e.g. Beaver, Lawrence, van Ditzhuijzen, Davis, Woods, et al., 2006). Therefore, mediation analyses were reanalyzed substituting total BAS scores with each of the BAS subscales.

2.4.3.4 Gender Interactions

Exploratory analyses were also conducted to test whether gender interacts with total BAS scores to predict scores on the Bulimia subscale of the EDI above and beyond the main effect of gender on Bulimia scores. It is possible that gender may moderate the relationship between BAS total scores and Bulimia scores, such that elevated BAS scores predict Bulimia scores among women

but not men. This hypothesis was tested utilizing a second stage moderation of mediation hierarchical regression model (Figure 4) with the following equation: $Y = b_0 + bX + bM + bZ + bMZ + \varepsilon$, where *X* refers to cumulative risk scores, *M* refers to total BAS scores, *Z* refers to gender, and *Y* refers to Bulimia scores (Edwards & Lambert, 2007). An additional analysis was conducted to determine whether gender moderates the association between cumulative DA gene variation and Bulimia scores. This was tested with the following equation: $Y = b_0 + bX + bM + bZ + bXZ + \varepsilon$

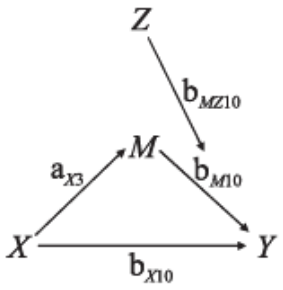

Figure 4. Second stage moderation model of mediation, whereby the mediating effect of variable M is moderated by variable Z. Figure originally published by Edwards & Lambert (2007).

2.4.3.5 Gender Stratification

To thoroughly explore the effect of gender on the relationship between cumulative risk scores and total BAS scores, between total BAS scores and Bulimia scores, and between cumulative risk scores and Bulimia scores, primary analyses were performed separately for each gender.

3.0 RESULTS

3.1 SAMPLE CHARACTERISTICS

Participants included 1295 healthy adults from the Adult Health and Behavior Project (AHAB-I), an extensive registry of behavioral and biological measures collected from a mid-life community sample recruited in Pittsburgh and surrounding regions between 2001 and 2005. AHAB exclusion criteria included a clinical history of neurologic illness, cardiovascular disease, Schizophrenia, or other psychoses. Volunteers were also excluded if they reported current use of use of insulin, glucocorticoid, antiarrhythmic, psychotropic, or prescription weight-loss medications. For the purposes of the present study, all non-Caucasian individuals were removed from the sample (n = 214). Of the remaining 1081 Caucasian individuals, those who had not been successfully genotyped for all five polymorphisms (n = 138; 12.4%; Table 2) or who were missing data for any of the EDI (n = 13) or BAS (n = 9) scales were removed from the sample, yielding a final sample size of 921.

3.2 ALLELE FREQUENCY ASSOCIATIONS

Allele distributions were in Hardy-Weinberg equilibrium (HWE) for *Taq1A* (X^2 (1, 920) = 0.46, p = 0.50), *DRD2* ins/del (X^2 (1, 920) = 1.60, p = 0.20), and *COMT* val/met (X^2 (1, 920) = 0.56, p = 0.45) polymorphisms. HWE for *DRD4* and *DAT1* VNTRs was tested using the Markov-chain method described by Guo & Thompson (1992; Table 5). Allele distributions for *DRD4* (p = 0.98) and *DAT1* (p = 0.071) VNTRs were in HWE. Cumulative risk scores (M = 1.67, SD = 0.91) were not associated with age (F (1, 920) = 0.190, p = 0.66) or gender (F (1,920) = 0.184, p = 0.67). Gender was significantly associated with *DRD2* ins/del allele frequencies (X^2 (2, 919) = 8.361, p = 0.02), with males being more likely to carry the deletion allele relative to females. Allele frequencies did not differ by gender for any of the other polymorphisms. Age was significantly

associated with *DAT1* allele frequencies (F (2, 919) = 3.119, p = 0.05), with individuals homozygous for the 9R allele being older than individuals in the other *DAT1* genotype groups. This is unlikely to be due to selective mortality attributable to the 10R allele given the age range of the sample. Age was not associated with allele frequencies for any of the other polymorphisms. Age and gender were included as covariates in all primary analyses.

Table 5. Calculations of Hardy-Weinberg equilibrium

			Minor Allele Frequency	
Polymorphism	X^2	***p***	**Observed**	**Expected**
DRD2	1.60	0.20	0.11	0.235
Taq1A	0.46	0.50	0.20	0.296
COMT	0.56	0.45	0.46	0.390
			Risk Allele Frequency	
Polymorphism	**S.E.**	***p***	**Observed**	**Expected**
DAT1	0.0143	0.07	0.29	0.29 (9R)
DRD4	0.0034	0.98	0.18	0.21 (7R)

Note. Hardy-Weinberg Equilibrium for *DAT1* and *DRD4* variable number of tandem repeats was estimated using the Markov-chain method described in Guo & Thompson (1992).

3.3 PRIMARY ANALYSES

Cumulative risk scores were not associated with the presence of bulimic symptoms (aim 1; *OR* (1, 920) = 1.057, p = 0.49; Aim 1), or total BAS scores (B = 0.002, p = 0.99; Aim 2). Total BAS scores were not associated with presence of bulimic symptoms (*OR* (1, 920) = 1.021, p = 0.16; Aim 3). Neither cumulative risk scores (*IRR* (1, 250) = 0.928, p = 0.17; Aim 1) nor total BAS scores were associated with severity of bulimic symptoms (*IRR* (1, 250) = 0.992, p = 0.48; Aim 3). Given that the conditions for mediation were not satisfied (Baron & Kenny, 1986) in either step of the hurdle model, the causal steps test of mediation was not conducted (Aim 4).

As predicted in Aim 5, Bulimia (B = 1.004, p = <0.01), Drive for Thinness (B =

0.409, p = <0.01), and Body Dissatisfaction (B = 0.421, p = <0.01) subscales of the EDI were associated with increased BMI after controlling for the effects of age and gender (Table 6).

Table 6. Mean BMI among those with and without eating disorder symptomatology

EDI subscale	*N*	*M (SD)*	Range
Bulimia			
Present	258	29.31 (6.21)	19-49
Absent	660	26.22 (4.82)	17-52
Drive for Thinness			
Present	562	28.19 (0.24)	17-52
Absent	356	25.35 (4.34)	17-49
Body Dissatisfaction			
Present	793	27.61 (5.49)	17-52
Absent	125	23.75 (3.45)	17-41

Note. BMI was not analyzed with EDI subscale scores dichotomized as they are above.

3.4 SECONDARY ANALYSES

3.4.1 Individual Gene Effects

When analyzing the effect of each individual polymorphism on presence of bulimic symptoms in a stepwise logistic regression model, the overall model was found to be significant (X^2 (7, 914) = 13.042, p = 0.023). As expected, the overall model was improved by the inclusion of age and gender (X^2 (6, 915) = 30.907, p = <0.001; Table 7), though the five DA polymorphisms predicted a significant amount of variance in the likelihood of reporting bulimic symptoms above and beyond the effect of age and gender (block X^2 (6, 915) = 11.231, p = 0.047). Only the *COMT* val/met SNP was significantly associated with the presence of bulimic symptoms (*OR* (7, 914) = 1.819, p = 0.005) such that individuals carrying the met allele were more likely to report any bulimic symptoms compared to non-met allele carriers. Utilizing simple contrast coding to compare the relative contribution of each genotype to presence of bulimic symptoms, it was revealed that the effect size of the val/met genotype was not significantly different from the

effect size of the met/met genotype (*OR* (2, 919) = 0.821, *p* = 0.29). Met allele carriers were also significantly more likely to endorse any items on the Drive for Thinness subscale of the EDI when comparing the presence to absence of symptoms on this subscale (*OR* (1, 920) = 1.496, *p* = 0.05). Follow-up analyses utilizing simple contrasts to compare each *COMT* genotype group indicated that only individuals with the met/met genotype were more likely to report drive for thinness relative to individuals with val/val (*OR* (2, 919) = 1.594, *p* = 0.03) and val/met genotypes (*OR* (2, 919) = 0.627, *p* = 0.03). Scores on the Drive for Thinness subscale of the EDI were not significantly different for individuals with the *COMT* val/val and val/met genotypes (*OR* (2, 919) = 0.950, *p* = 0.75). Met allele carriers were not more likely to report body dissatisfaction (*OR* (1, 920) = 1.307, *p* = 0.35).

Table 7. Effects of individual dopamine loci on presence of bulimic symptoms

	Block X^2	***p***	***OR***	***p***	**95% CI**
Block 1	19.689	<0.001			
Age			0.993	0.527	0.972-1.014
Gender			1.926	<0.001	1.433-2.587
Block 2	11.218	0.047			
Taq1A			0.920	0.974	0.582-1.629
DRD2 ins/del			0.743	0.127	0.508-1.088
COMT val/met			1.819	0.005	1.193-2.773
DAT1			1.142	0.379	0.850-1.534
DRD4			1.005	0.976	0.735-1.374

When considering all individual polymorphisms together in a single Poisson regression model, the overall model was not significant (Likelihood Ratio X^2 (9, 248) = 8.223, *p* = 0.522). No individual polymorphism predicted severity of bulimic symptoms (Table 8). In contrast, *COMT* met allele carriers had more severe body dissatisfaction (Wald's X^2 (2, 791) = 11.527, *p* = 0.003), even after adjusting for the effects of age and gender (Wald's X^2 (2, 791) = 12.160, *p* =

0.002). Specifically, the incident rate for Body Dissatisfaction scores among individuals with the val/val genotype was 0.820 times lower than the incident rate among individuals with the met/met genotype. The incident rate for Body Dissatisfaction scores among individuals with the val/met genotype was 0.847 times lower than the incident rate among individuals with the met/met genotype. The *DRD2* ins/del polymorphism was significantly associated with severity of drive for thinness (Wald's X^2 (1, 561) = 4.559, p = 0.03). Contrary to the hypothesized direction of the association, the incident rate for Drive for Thinness scores among individuals homozygous for the insertion allele was 1.225 times higher than the incident rate among carriers of the presumed risk allele, the deletion allele.

Table 8. Effects of individual dopamine loci on severity of bulimic symptoms

Polymorphism	**Likelihood Ratio X^2**	***p***	**Wald X^2**	***p***	***M (SD)***
	8.123	0.522			
Age			0.242	0.623	
Gender			0.111	0.738	
Female					2.47 (1.85)
Male					2.41 (1.86)
Taq1A			1.586	0.452	
A1/A1					3.10 (2.03)
A1/A2					2.40 (1.74)
A2/A2					2.43 (1.89)
DRD2 ins/del			3.006	0.083	
del carriers					2.11 (1.85)
ins/ins					2.51 (1.96)
COMT val/met			2.227	0.328	
val/val					2.42 (1.85)
val/met					2.33 (1.80)
met/met					2.75 (1.97)
DAT1			0.126	0.722	
9R carriers					2.37 (1.56)
10R/10R					2.52 (2.09)
DRD4			0.349	0.555	
7R carriers					2.39 (1.64)
Non-7R carriers					2.47 (1.95)

3.4.2 EDI Subscales

Neither cumulative risk scores nor total BAS scores predicted presence or severity of body dissatisfaction or drive for thinness (Tables 9 & 10).

Table 9. Models predicting presence of drive for thinness and body dissatisfaction

Drive for Thinness			
Predictor	***OR***	***p***	**95% CI**
Cumulative risk scores	1.005	0.95	0.869-1.162
Total BAS scores	**1.029**	**0.04**	**1.002-1.057**
Total BAS scores (controlling for age and gender)	1.023	0.10	0.995-1.052
Body Dissatisfaction			
Predictor	***OR***	***p***	**95% CI**
Cumulative risk scores	0.919	0.42	0.748-1.129
Total BAS scores	1.011	0.57	0.974-1.050

Table 10. Models predicting severity of drive for thinness and body dissatisfaction

Drive for Thinness			
Predictor	***IRR***	***p***	**95% CI**
Cumulative risk scores	0.977	0.54	0.906-1.053
Total BAS scores	1.017	0.07	0.999-1.034
Body Dissatisfaction			
Predictor	***IRR***	***p***	**95% CI**
Cumulative risk scores	0.995	0.85	0.938-1.054
Total BAS scores	1.003	0.55	0.992-1.014

3.4.3 BAS Subscales

Cumulative risk scores were not associated with scores on the Drive (F (1, 920) = 1.467, p = 0.23), Fun Seeking (F (1, 920) = 0.176, p = 0.68), or Reward Responsiveness (F (1, 920) = 0.626, p = 0.43) subscales of the BAS. None of the subscales were significantly associated with presence or severity of bulimic symptoms (Table 11).

Table 11. Association between BAS subscale scores and presence and severity of bulimic symptoms

Presence of Bulimia Nervosa symptomatology			
Predictor	***OR***	***p***	**95% CI**
Drive	1.022	0.51	0.958-1.089
Fun Seeking	1.057	0.09	0.991-1.127
Reward Responsiveness	1.077	0.07	0.995-1.165
Severity of Bulimia Nervosa symptomatology			
Predictor	***IRR***	***p***	**95% CI**
Drive	1.003	0.90	0.956-1.053
Fun Seeking	0.970	0.13	0.932-1.009
Reward Responsiveness	0.991	0.78	0.932-1.054

3.4.4 Gender Interactions

Cumulative risk scores did not interact with gender to predict presence (*OR* (1, 920) = 1.164, p = 0.36) or severity of bulimic symptoms (*IRR* (1, 252) = 1.164, p = 0.40) after accounting for the main effect of gender on bulimic symptoms. However, cumulative risk scores interacted with gender to predict total BAS scores (B = -0.899, p = 0.01), above and beyond the main effects of both cumulative risk scores (B = 0.432, p = 0.09) and gender (B = 2.233, p = <0.01; Table 12).

Table 12. Interactive model predicting total BAS scores

Model	R^2	***SE***	ΔR^2	***F***	***B***
Additive	0.017	4.97	-----	5.20**	
Age					-0.08*
Gender					0.73**
Cumulative Risk					-0.01
Cumulative riskXgender	0.023	4.96	0.007	6.28*	
Age					-0.08**
Gender					2.23**
Cumulative Risk					0.43
Cumulative riskXgender					-0.90*

Note. * p<0.05; ** p<0.01

Specifically, increasing cumulative risk scores were associated with decreasing BAS scores among women, while increasing cumulative risk scores were associated with increasing BAS scores among men (Figure 5). However, this effect was not linear across each of the possible values of the risk score (data for each risk score value between genders not depicted). Total BAS scores did not interact with gender to predict presence (*OR* (1, 920) = 0.985, $p = 0.63$) or severity (*IRR* (1, 253) = 1.011, $p = 0.63$) of bulimic symptoms when accounting for the main effects of age, gender, and total BAS scores. Cumulative risk scores did not interact with BAS scores to predict presence (*OR* (1, 920) = 0.997, $p = 0.88$) or severity (*IRR* (1, 253) = 1.008, $p = 0.50$) of bulimic symptoms. Fully interactive models were also not significantly associated with presence or severity of bulimic symptoms.

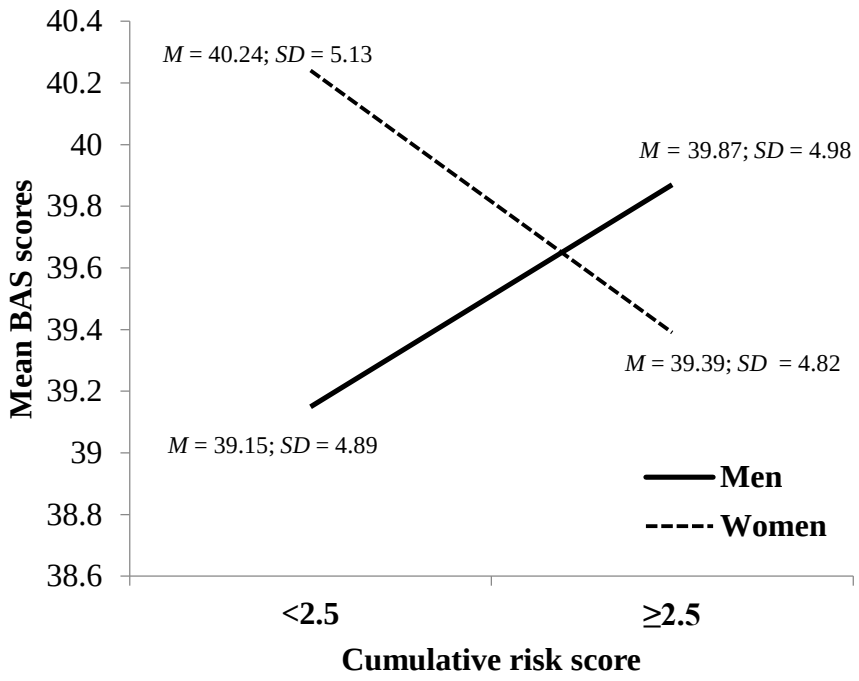

Figure 5. Interaction between cumulative risk scores and gender to predict BAS scores. Note that cumulative risk scores were dichotomized for ease of depiction, but were not analyzed dichotomously

3.4.5 Gender Stratification

Among women, cumulative risk scores were not associated with presence of bulimic symptoms (*OR* (1, 471) = 1.131, p = 0.26), or total BAS scores (B = -0.481, p = 0.07). Total BAS scores were not associated with presence of bulimic symptoms (*OR* (1, 471) = 1.015, p = 0.45). Neither cumulative risk scores (*IRR* (1, 160) = 0.889, p = 0.10) nor total BAS scores (*IRR* (1, 160) = 0.996, p = 0.81) were associated with severity of bulimic symptoms. Given that the conditions for mediation were not satisfied (Baron & Kenny, 1986) in either step of the hurdle model, the causal steps test of mediation was not conducted in women alone.

Among women, the *COMT* val/met SNP was the only individual polymorphism that was significantly associated with the presence of bulimic symptoms in the expected direction. Specifically, when coded according to the additive (*OR* (1, 471) = 2.380, p = <0.01), dominant

(*OR* (1, 471) = 1.953, p = <0.01), or recessive (*OR* (1, 471) = 1.604, p = 0.04) models of inheritance, the met allele was associated with greater likelihood of reporting bulimic symptoms relative to the val allele. The *DRD2* ins/del polymorphism was significantly associated with severity of bulimic symptoms when coded according to the additive (Wald's X^2 (1, 160) = 12.139, p = <0.01), dominant (*IRR* (1, 160) = 1.308, p = <0.01), and recessive models of inheritance (*IRR* (1, 160) = 1.868, p = <0.01). However, the association was in the direction opposite of that predicted, with the insertion allele being associated with higher BN scores relative to the deletion allele. The *DRD4* VNTR was also associated with severity of bulimic symptoms when coded according to the additive (Wald's X^2 (1, 159) = 10.539, p = <0.01) or recessive (Wald's X^2 (1, 159) = 9.718, p = <0.01) model of inheritance in women alone. However, the severity of bulimic symptoms was greater among non-7R carriers and those with one copy of the 7R allele relative to women homozygous for the 7R allele. Specifically, the incident rate for bulimic symptoms among women with one copy of the 7R allele was 1.604 times the incident rate among women homozygous for the 7R allele. The incident rate of bulimic symptoms among non-7R carriers was 1.456 times higher than the incident rate among women homozygous for the 7R allele. Significant individual gene effects did not change when adjusting for age, except that *COMT* was no longer associated with the presence of bulimic symptoms (*OR* (1, 471) = 1.604, p = 0.06) among women.

Among men, original cumulative risk scores were not associated with presence of bulimic symptoms (*OR* (1, 449) = 0.1.003, p = 0.98), or total BAS scores (B = 0.460, p = 0.07). Total BAS scores were not associated with presence of bulimic symptoms (*OR* (1, 449) = 1.033, p = 0.18). Neither cumulative risk scores (*IRR* (1, 93) = 0.982, p = 0.84) nor total BAS scores were associated with severity of bulimic symptoms (*IRR* (1, 93) = 0.986, p = 0.40).

4.0 DISCUSSION

Although prior research among clinical populations (e.g. Kaplan et al., 2007; Bachner-Melman et al., 2007; Davis et al., 2008; Epstein et al., 2007; Shinohara et al., 2009; Frieling et al., 2006; Yilmaz et al., 2011; Davis, Loxton, Levitan, Kaplan, Carter et al., 2013) and one healthy population (Nikolova et al., 2011) provided empirical justification for assessing the previously untested relationship between cumulative DA risk scores, aberrant eating, and reward sensitivity, the primary analyses of the present investigation did not find evidence to support such a relationship in a sample of healthy adults. Contrary to the primary hypotheses of this study, cumulative risk scores as originally proposed were not associated with the presence or severity of bulimic symptoms or total BAS scores. Furthermore, cumulative risk scores did not predict the presence or severity of drive for thinness or body dissatisfaction, and were not associated with scores on subscales of the BAS. Analyses also failed to detect a significant relationship between any of the BAS subscales and EDI subscales. Gender did not interact with cumulative risk scores or total BAS scores to predict the presence or severity of bulimic symptoms. BMI was not associated with cumulative risk scores, or with any individual polymorphism, contrary to previous findings demonstrating a relationship between BMI and *Taq1A* (Stice et al., 2008), and BMI and *DRD4* (Levitan et al., 2004; Kaplan et al., 2007).

On the other hand, all indices of aberrant eating, including symptoms of bulimia, drive for thinness, and body dissatisfaction, were associated with BMI, replicating previous research findings (Wilfley et al., 2000; Striegel-Moore et al., 2001). Further, total BAS scores were positively associated with BMI, supporting prior imaging research suggesting that sensitivity to signals of reward is associated with risk for obesity (Stice et al., 2008). Curiously, although EDI subscale scores and total BAS scores were associated with BMI, they were not significantly

associated with one another. This may be explained by the low base rate of aberrant eating as assessed by the EDI in the present sample. Further, the BAS does not specifically assess food-related reward processing, and instead evaluates a more general impulsivity-related personality trait that likely influences many behaviors beyond dietary patterns which could promote weight gain. For example, it has been hypothesized that preference for immediate over delayed rewards may be related to individual differences in the reinforcing value of physical activity relative to sedentary pursuits (Epstein, 1998). In contrast, the EDI measures behaviors (e.g. purging) and weight-related cognitive distortions (e.g. "I am terrified of gaining weight," "I think that my stomach is too big"), which are likely to be more directly related to BMI (Tanofsky-Kraff & Yanovski, 2004) than to reward sensitivity. Therefore, a measure that reflects food- or eating-specific reward sensitivity might have been associated with the EDI, while the BAS was not in this case.

4.1 ASSOCIATIONS BETWEEN THE *COMT* VAL/MET SNP AND ABERRANT EATING

Several ancillary analyses revealed significant relationships that are of note. As evidenced by incident rate ratios (IRR) derived from Poisson regression analyses, individuals carrying the met allele of the *COMT* val158met SNP were 87% more likely than individuals with the val/val genotype to report bulimic symptoms. Interestingly, the *COMT* val/met SNP was not predictive of the severity of bulimic symptoms, suggesting that it may be specifically related to the likelihood of endorsing bulimic symptoms, but not to the severity of these symptoms once they develop. Similarly, met carriers were 54% more likely to report drive for thinness relative to non-met carriers, but there was no relationship between *COMT* val/met genotype and severity of drive for thinness. In contrast, the met allele was associated with severity of body dissatisfaction,

but there was no association between the *COMT* val/met SNP and presence of body dissatisfaction. Specifically, the number of symptoms endorsed by individuals homozygous for the val allele was about 21% lower than the number reported by individuals homozygous for the met allele. The number of symptoms endorsed by individuals with the val/met genotype was about 17% lower than the number of symptoms reported by individuals homozygous for the met allele. This indicates that the relationship between severity of body dissatisfaction and *COMT* val/met genotype is additive, such that body dissatisfaction increases as met allele loading increases, a finding that is supported by the predominant use of the additive coding scheme in studies evaluating the effect of the *COMT* val/met SNP (Table 1). It should be noted that post-hoc power analyses demonstrated that power to detect an effect of *COMT* genotype on severity of bulimic symptoms (1- β err prob = 0.16; n = 254) and drive for thinness (1- β err prob = 0.40; n = 559) was low. This may be explained by the fact that sample sizes were reduced in Poisson analyses to include only those endorsing aberrant eating on each subscale, though the samples remained relatively large. Therefore, it is possible that these analyses may have reached significance if sample size had been increased. However, it may also be the case that the *COMT* val/met SNP is differentially related to the occurrence and severity of, various features of aberrant eating, a finding that may inform etiological models of aberrant eating.

The observations that the met allele is associated with higher likelihood of reporting bulimic symptoms and drive for thinness are consistent with previous candidate gene studies examining the relationship between the *COMT* val/met SNP and bulimic symptoms (Frieling et al., 2006; Yilmaz et al., 2011). Given that the met allele is associated with reduced enzymatic degradation of DA in the PFC (Dreher et al., 2009), it is possible that met allele carriers are more responsive to rewards like food and have a greater difficulty exerting top-down control of

behavior driven by midbrain DA activation (Carr & Sesack, 2000; Dreher et al., 2009; Yacubian, Sommer, Schroeder, Glascher, Kalisch et al., 2007). Furthermore, preliminary evidence demonstrating that impaired functional connectivity in fronto-striatal networks (Figure 1) during a reward processing task predicts persistence of binge eating following treatment for BED (Balodis, Grilo, Kober, Worhunsky, White, et al., 2013). Therefore, abnormalities in dopaminergic processing in prefrontal regions may contribute to risk for BN or BN-spectrum symptomatology through weakened coupling between prefrontal and midbrain regions, which may increase the frequency of impulsive dietary behaviors (e.g. bingeing, purging, excessive exercise).

Results indicated that the effects of *COMT* val/met genotype on bulimic symptoms and drive for thinness were not additive. Non-additive effects may explain the absence of a relationship between *COMT* val/met genotype and severity of these measures. An additive model would suggest that met allele loading would be related to the degree of eating pathology, with the severity of bulimic symptoms and drive for thinness increasing as the number of met alleles increased. However, results indicated that individuals with the met/met genotype were no more likely to report bulimic symptoms than were individuals with the val/met genotype. Similarly, Drive for Thinness scores did not differ significantly between individuals with the val/val and val/met genotypes. Therefore, based on the present data, it appears that there is no gradient in the likelihood of reporting bulimic symptoms or drive for thinness conferred on the basis of met allele loading, an observation that is consistent with the lack of an association with severity of these features of aberrant eating.

No studies to date have specifically explored how variation in prefrontal DA availability might differentially contribute to severity but not occurrence of body dissatisfaction. To the

extent that body dissatisfaction represents body-image specific depressogenic cognitions, it is possible that factors that predispose individuals to depression may also increase the likelihood of body dissatisfaction. Although DA has been linked to depression, it is more closely related to behavioral control and motivation rather than depressed mood or depressogenic cognitions (Neslter & Carlezon, 2006). Unlike the symptoms captured on the BN and Drive for Thinness scales (e.g. "I stuff myself with food," "I am preoccupied with the desire to be thinner"), symptoms on the Body Dissatisfaction scale are less behavioral in nature (e.g. "I think my thighs are too large"), and may therefore be more distally related to dopaminergic processing. Therefore, the effect of *COMT* genotype on presence of body dissatisfaction may not become evident unless other risk factors for depression are present, which may explain the lack of an association between *COMT* genotype and presence of body dissatisfaction in the present study. There is evidence that variation in the serotonin 2A ($5HT_{2A}$) receptor gene predicts eating disorder subtype (e.g. AN-restricting type vs. AN-binge/purge type vs. BN) as well as severity of weight concern and eating pathology among women with a diagnosed eating disorder (Ricca, Nacmias, Boldrini, Cellini, di Bernardo et al., 2004). Specifically, individuals homozygous for the $5HT_{2A}$ risk allele were more likely to meet criteria for AN-restricting type and BN, but not AN-binge/purge type. Further, individuals homozygous for the risk allele exhibited more severe eating pathology relative to other genotype groups (Ricca et al., 2004). Candidate gene studies in other clinical populations have also demonstrated that there may be unique genetic influences on susceptibility, progression, and clinical presentation of a given phenotype (e.g. Dick, Agrawal, Wang, Hinrichs, Bertelsen, Bucholz et al., 2007; Laucht, Becker, Frank, Schmidt, Esser et al., 2008; Steiger & Bruce, 2008; Steiger, Fichter, Bruce, Joober, Badawi et al., 2011; Fanous, Zhou, Aggen, Bergen, Amdur et al., 2012). For instance, variation in the gene encoding apolipoprotein

1 was found to predict progression of, but not risk for, chronic kidney disease among African Americans (Parsa, Kao, Xie, Astoer, Li et al., 2013). Although no studies to date have demonstrated a differential effect of one genetic polymorphism on risk for, and severity of, a single symptom domain, there is research to support this as a possible outcome.

It is possible that the relationship between the *COMT* val/met SNP and severity of body dissatisfaction may be related to neural mechanisms of affect regulation. In addition to the role of DA in reward learning, there is evidence that dopaminergic circuitry is also critical for establishing associations between negatively valenced emotional stimuli and environmental cues (Everitt, Cardinal, Parkinson, & Robbins, 2003; Nestler & Carlezon, 2006). Both the NAc and VTA are strongly innervated by the amygdala (Price, 2003; Jackson & Moghaddam, 2001), which in turn receives dense neural projections from regions of PFC (Jackson & Moghaddam, 2001) where COMT is most active (Dreher et al., 2009). Inhibitory projections from the PFC to the amygdala are thought to reduce amygdala activation in response to emotionally arousing stimuli, an effect that is modulated by dopaminergic input (Rosenkrantz & Grace, 2001; Rosenkrantz & Grace, 2002). It is conceivable, then, that increased DA availability observed in met allele carriers may contribute to impaired emotion regulation (e.g. eating to improve mood), which may exacerbate body dissatisfaction. Therefore, it is possible that the association between the met allele and severity of body dissatisfaction may be attributable to compromised top down regulation of behavioral responses to affectively laden environmental cues, including those related to binge eating. Post-hoc power analyses demonstrated that power to detect an effect of *COMT* genotype on presence of body dissatisfaction (1- β err prob = 0.98; n = 921) was adequate. This suggests that the failure to find an effect of *COMT* genotype on presence of body

dissatisfaction was not simply due to insufficient sample size, supporting the conclusion that *COMT* is differentially related to presence and severity of various features of aberrant eating.

Negative affect is a robust predictor of subsequent binge eating (Haedt-Matt & Keel, 2011; Crosby, Wonderlich, Engel, Simonich, Smyth et al., 2009), suggesting that individuals with BN may engage in binge eating as a way to regulate affect (Polivy & Herman, 1993). In support of this hypothesis, trajectory analyses of ecological momentary assessment (EMA) data have demonstrated that binge eating alleviates negative affect, thus reinforcing the behavior (Berg, Crosby, Cao, Peterson, Engel et al., 2013; Smyth, Wonderlich, Heron, Sliwinski, Crosby et al., 2007). The complex interactions between dopaminergic signaling, affect, and eating behavior may be one mechanism by which aberrant eating is maintained. Though participants in the present study are unlikely to develop a clinically significant eating disorder given that they are beyond the typical age of onset, these results suggest that variation in *COMT* signaling may affect aberrant eating, even among healthy individuals. On the other hand, in light of research demonstrating that certain symptoms of aberrant eating (e.g. binge eating, body dissatisfaction) are associated with weight gain, obesity (Wilfley et al., 2000; Striegel-Moore, et al., 2001) and negative affect (Stice, 2001), these findings may have important implications for determining who may be most susceptible to obesogenic eating behaviors and associated psychological distress. Future studies could use EMA techniques to determine if *COMT* val/met genotype is predictive of the trajectory of aberrant eating behaviors and their relationship to negative affect. If so, this information could be utilized to identify individuals in clinical settings who may benefit from self-monitoring and the development of alternative emotional regulation strategies.

4.2 ASSOCIATIONS BETWEEN THE *DRD2* INS/DEL POLYMORPHISM AND ABERRANT EATING

Ancillary analyses also revealed a significant association between the *DRD2* ins/del polymorphism and severity of drive for thinness. However, the relationship was in the direction opposite of that predicted. Results suggested that the number of symptoms of drive for thinness reported by individuals homozygous for the insertion allele was 23% higher than the number reported by carriers of the deletion allele. Similarly, when considering women only, the insertion allele was associated with severity of bulimic symptoms, but not with any other symptoms of aberrant eating. It should be noted that only a few studies have examined this polymorphism in the context of aberrant eating, the findings of which are inconsistent. For instance, there is evidence that deletion allele predicts weight gain among individuals being treated with D2 receptor antagonists (Lencz et al., 2010), as well as greater ventral striatal activity in response to monetary reward (Nikolova et al., 2011). However, studies have failed to support a relationship between the *DRD2* ins/del polymorphism and BED (Davis et al., 2008; Davis et al., 2012). Furthermore, there is disagreement about the functional effect of *DRD2* ins/del variation on D2 receptor expression in the brain (Arinami et al., 1997; Jonsson et al., 1999). Methodological differences in the quantification of D2 receptors may explain the conflicting results, but further research in this area is needed. Therefore, results from the present investigation should be interpreted with caution, particularly given that only 16 individuals (1.7%) in the present sample were homozygous for the deletion allele.

4.3 MODERATING EFFECT OF GENDER

Based on research demonstrating that the prevalence of aberrant eating is considerably higher among females (Striegel-Moore et al., 2010; Hudson, Hiripi, Pope, & Kessler, 2007), it was

hypothesized that gender might moderate the relationships in each path of the proposed mediation model. Though no significant main effects of cumulative risk scores or gender were detected after controlling for the effect of age, analyses revealed a significant interaction between cumulative risk scores and gender predicting total BAS scores. Specifically, increasing cumulative risk scores were associated with increasing total BAS scores among men, but with decreasing total BAS scores among women (Figure 5). This unexpected finding could be explained by the fact that men are more likely than women to engage in risk taking behaviors (e.g. gambling, reckless driving, etc.; Fischer & Smith, 2008; Byrnes, Miller, & Schafer, 1999), whereas women are more likely to engage in binge eating (Fischer & Smith, 2008). BAS items are constructed in such a way that they better capture behaviors that may be more often endorsed by men (e.g. "I have very few fears when compared to my friends," "Even if something bad is about to happen to me, I rarely experience fear or nervousness"; for a meta-analysis of gender differences in impulsivity, see Cross, Cropping, & Campbell, 2011). It is possible that men at higher genetic risk for personality traits related to impulsivity would achieve higher scores on the BAS, while women at higher genetic risk may not because they express their risk through behaviors not captured on the BAS (i.e., impulsive eating). Nevertheless, the finding that cumulative risk scores interact with gender to predict BAS scores is consistent with other studies demonstrating that the association between DA gene variation and reward-driven behavior is dependent on gender. For instance, *DRD4* and *DRD2* variation predict sensitivity to nicotine among male non-smokers, but not among women (Perkins, Lerman, Coddington, Jetton, Karelitz et al., 2008). Similarly, variation in the monoamine oxidase A gene has been associated with BMI among men, but not women (Fuemmler, Agurs-Collins, McClernon, Kollins, Kail et al., 2008), suggesting that the genetically determined rate of enzymatic degradation of DA and other

monoamines is related to risk for obesity in a gender-specific way. Future studies should further investigate the moderating effect of gender on the relationship between DA gene variation and a broader range of positively valenced appetitive drives (e.g., binge eating, sensitivity to reward cues, behavioral or decisional impulsivity) to elucidate the potential mechanisms that might underlie observed gender interactions.

4.4 LIMITATIONS AND FUTURE DIRECTIONS

There are several factors that may potentially explain the null findings, including the low incidence of eating pathology, constricted variance in the EDI scores, and method of phenotype assessment. Given that individuals in the current sample were selected on the basis of being in good health, there was minimal representation of eating pathology. Therefore, the EDI may not have been ideal to address the study questions in this sample, a hypothesis that is supported by the low variance and positive skew of the data obtained from the Bulimia subscale of the EDI. Rather than examining the relationship between cumulative DA risk scores, reward sensitivity, and aberrant eating, there may have been greater statistical power to assess normative eating behavior, such as size, frequency, and content of dietary intake as assessed by measures such as the Food Frequency Questionnaire (Rimm, Giovannucci, Stampfer, Colditz, Litin, et al., 1993). Further, changes in dietary patterns are likely to precede the onset of a diagnosable eating disorder (Fairburn & Harrison, 2003), suggesting that genetic variation may have a more proximal influence on eating behavior that may subsequently lead to the development of eating disorder symptomatology (e.g. overvaluation of weight, negative expectancies about food, binge eating). Alternatively, study questions could have been assessed utilizing a case-control design with individuals diagnosed with BN to increase statistical power (Daly & Day, 2001).

It is possible that the use of self-report measures to assess reward sensitivity and aberrant eating may have led participants to underreport the degree to which they exhibit the behaviors, symptoms, or attributes being assessed, potentially obscuring true relationships among the variables under study (Podsakoff, Mackenzie, Lee, & Podsakoff, 2003). For instance, participants may have been reluctant to endorse items that they perceived to be socially undesirable (e.g. purgative behaviors, risk taking; Podsakoff et al., 2003), or may have underestimated the extent to which the items applied to them due to recall bias (Coughlin, 1990). Future studies investigating the relationship between DA gene variation, reward sensitivity, and aberrant eating could use a clinical interview to evaluate symptoms of BN (i.e. the Eating Disorder Examination, 16th ed.; Fairburn, Cooper, & O'Connor, 2008), along with behavioral assessments of reward-driven decision-making (e.g. delay discounting tasks; Green & Meyerson, 2004).

4.5 SUMMARY

In summary, cumulative genetic risk scores were not associated with presence or severity of aberrant eating. Cumulative risk scores did not predict reward sensitivity, nor was reward sensitivity associated with any index of aberrant eating. Nevertheless, analyses revealed a significant overall effect of the five DA polymorphisms on the presence of bulimic symptoms when included in a stepwise logistic regression model above and beyond the effects of age and gender. This indicates that these polymorphisms predict a significant amount of variance in the likelihood of reporting aberrant eating. Furthermore, the current study demonstrated that gender interacts with aggregated DA gene variation to predict sensitivity to reward. Although the present study did not find evidence that sensitivity to reward was correlated with aberrant eating specifically, it is possible that the moderating effect of gender on the relationship between DA

gene variation and sensitivity to reward may have important implications for research exploring gender differences in impulsivity-related phenotypes like substance abuse, food intake, and gambling. Furthermore, results suggested that the low activity met allele of the *COMT* val/met SNP was significantly associated with various indices of aberrant eating among healthy adults, consistent with prior research in populations with a diagnosed eating disorder. Specifically, the met allele was associated with the presence, but not the severity, of bulimic symptoms and drive for thinness, while being associated only with severity of body dissatisfaction. Importantly, these results indicate that the met allele differentially predicts presence of and severity of eating disorder symptomatology, a novel finding that may enrich our understanding of the mechanisms underlying both the development and magnitude of aberrant eating.

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