Early Life and Genetic Associations of Adult Metabolic Dysregulation

by

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ABSTRACT

Previous research has shown an association between childhood environmental influences and metabolic dysregulation. In particular, an early family environment characterized by neglectful parenting, overt conflict, and unsupportive relationships has been associated with obesity and adiposity in adulthood. Likewise, the GABAergic (gamma aminobutyric acid) T>C single nucleotide polymorphism in the 1519 nucleotide position of the GABAAα6 receptor subunit gene has been associated with a predisposition to a higher body mass index and a larger waist circumference. Though research into gene by environment interactions on metabolic health is a developing field, allele frequency in a population's gene pool tends to remain stable across many generations, making it unlikely that single nucleotide polymorphisms are the primary cause of rising obesity rates in adulthood. Participants (n=213, M_{age} = 30.13 years, SD= 10.85; 57.7% men) from the Pittsburgh Cold Study 3 completed a demographic questionnaire, the Risky Families Questionnaire and had their height, weight and waist circumference measured during a physical exam. Participant DNA was recovered from buccal swabs and genotyped for the various allelic types of the single nucleotide polymorphisms according to published protocols. In secondary data analyses, we tested the hypothesis that early family environment, GABRA6 and their statistical interaction would be positively associated with body mass index and waist circumference. The findings provide evidence that early family environment may exert more influence than genetic predisposition when determining indices of metabolic health.

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CHAPTER 1

INTRODUCTION

Obesity and disorders related to metabolic dysregulation have been on the rise in the United States, with around 35% the adult population being classified as obese (Hursting & Dunlap, 2012). The seriousness of the health outcomes associated with metabolic dysregulation cannot be understated, as many metabolic abnormalities are risk factors for several types of cancer, type II diabetes, and heart disease (Anderson et al., 2014; Hursting & Dunlap, 2012). Additionally, these adverse health outcomes have a staggeringly negative economic effect, costing the U.S. alone billions of dollars a year on obesity-related medical care (Cawley & Meyerhoefer, 2012). Despite our current knowledge on metabolic dysregulation and its detrimental consequences, many keep succumbing to the effects of obesity. Clearly, further research needs to be conducted into the factors that influence obesity-related morbidity and mortality. There is substantial evidence in the literature supporting the assertion that heritable factors, environmental influences, and their interactions (referred to as gene-environment (G X E) interactions) contribute to a higher body mass index (BMI) and a larger waist circumference (WC), both which have been demarcated as metabolic risk outcomes (Gu et al., 2018; Van Pelt, Evans, Schechtman, Ehsani & Kohrt, 2001).

Environmental factors, including instances of severe trauma in childhood, have been linked to the development of dysregulated metabolic processes in adulthood. In

particular, physical abuse has shown a positive association with larger WC, and experiencing domestic violence has shown a positive association with both increased BMI and larger WC (Soares et al., 2018). However, less is known about whether milder, less severe forms of dysfunction also confer risk for metabolic dysregulation in adulthood. Research by Taylor, Lerner, Sage, Lehman & Seeman, (2004) states that mild to moderate levels of dysfunction, those that fall "well within normal bounds" also appear to negatively impact a child's emotional, social and biological development.

According to Repetti, Taylor & Seeman (2002), children frequently exposed to a family environment characterized by conflict, aggression and cold and unsupportive relationships can experience overactivation of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis has a central role in regulating many homeostatic systems in the body, including the metabolic system (Rutters et al., 2012). During times of acute stress, HPA axis activation serves a protective function and promotes increased cardiovascular tone, respiratory rate, and metabolism while constraining non-essential functions such as digestion, growth and immunity (Smith & Vale, 2006). However, prolonged overactivation of the HPA axis dysregulates these processes and contributes to negative health outcomes such as insulin resistance, increased propensity for type II diabetes, and abdominal obesity (McEwen & Seeman, 2009). A study conducted by Weidner, Hutt, Connor & Mendell, (1992) supports this assertion, noting that a high-conflict family environment was associated with high cholesterol in males. Even when controlling for childhood BMI and sex, children who grew up with non-supportive

parents were found to have an elevated risk of obesity in early adulthood. Thus, emerging evidence suggests that repeated exposure to stress during childhood may lead to prolonged activation of the HPA axis and in turn, contribute to poor physical health in adulthood (Miller, Chen, & Parker, 2011). Therefore, an early family environment characterized as having mild to moderate family dysfunction may predispose a child to later metabolic health adversity.

However, environmental factors are not the sole contributors of obesity-related disorders, as genetic influences also play a significant role in their development. Obesity is now widely characterized as complex disease controlled mostly by minor contributions from several genes interacting in tandem (Mutch & Clement, 2006). Therefore, a promising direction in the field of metabolic health research involves examining the contributing effects that single nucleotide polymorphisms (SNPs) have on pathogenesis. SNPs are the most common genetic differences in humans, by some estimates accounting for 90% of all genetic variability (Kao, Chong, & Lee, 2000). Due to their abundance, they are an appropriate target for assessing associations between genotype and phenotype. The GABRA6 gene is a member of the GABAergic receptor family that responds to GABA, the main inhibitory neurotransmitter of the central nervous system. A T>C polymorphism at nucleotide 1519 in the non-coding region of the GABAAα6 receptor subunit gene means that there are several allelic variants of the GABRA6 gene. GABRA6 has been denoted as a polymorphism associated with hypercortisolism and abdominal fat deposition (Rosmond, 2003), which are established risk factors for morbidity and mortality attributable to obesity

(Björntorp, 1997). Carriers of the homozygous T/T or heterozygous T/C genotypes have been shown to exhibit high waist-to-hip ratio (WHR), high abdominal sagittal diameter, and elevated diurnal cortisol secretion when compared to homozygous C/C carriers (Rosmond, Bouchard, & Björntorp, 2002). It should be noted that concerns have been raised about the validity of the association, as the polymorphism is not located in a coding region of the GABAAα6 receptor subunit gene (Rosmond et al., 2002). However, robust evidence that noncoding genomic sequences have biological utility in disease susceptibility exists for many disorders, including those related to metabolic dysfunction. Therefore, the functional role that GABRA6 plays in cortisol release, along with implications that it is directly associated with BMI and WC, should not be discounted based on its chromosomal location.

Cortisol, as well as other glucocorticoids, systemically regulate the metabolism of carbohydrate, fat, and protein substrates (Christiansen et al., 2007). Normally, these regulatory processes meet the physiological demands of the body, delivering energy (in the form of triglycerides and glucose) to tissues as needed (Epel et al., 2000). However, excess concentrations of cortisol can cause the constant mobilization of triglycerides, leading to hypertriglyceridemia and increased levels of visceral fat deposition (Brindley, 1995; Sam et al., 2009). Excess concentrations of cortisol can also lead to elevated blood glucose levels, which over prolonged periods can cause insulin resistance and type II diabetes, both which have been linked to obesity (Hardy, Czech & Corvera, 2012).

Current Study

This study is a secondary data analysis of the Pittsburgh Cold Study 3, a quarantine study that examined factors for common cold susceptibility. One novel feature of the Pittsburgh Cold Study 3 was that it obtained retrospective measures of childhood experience and collected genetic samples for SNPs in genes that regulate target cell responsiveness to cortisol. In the present study, we will be examining the independent effects of early family environment and GABRA6 on BMI and WC in a community sample. We will also examine a G X E interaction consisting of GABRA6 and early family environment on BMI and WC. Given that GABRA6 (like all SNPs) remain stable across generations and that nearly half the nation's children have been found to have experienced at least one or more types of adversity, the current study provides novel insight into potential risk factors for metabolic dysregulation provided by non-severe childhood dysfunction (Sacks, Murphey & Moore, 2014; Shastry, 2002). Thus, the purpose of this study is to test the hypothesis that early family environment, GABRA6 and their statistical interaction would be positively associated with BMI and WC.

Additionally, the study will explore whether diurnal cortisol secretion mediates the association between GABRA6 and/or early family environment to BMI and WC in adulthood. Humans generally experience diurnal variation in their blood cortisol levels: the highest levels of cortisol are in the early morning and are lowest roughly 3 to 5 hours after sleep onset (Weitzman et al., 1971). Long-lasting exposure to various types of psychosocial stress in childhood can produce a prolonged or exaggerated

stress response, which may condition a sensitized physiologic stress response that could bring about cortisol dysfunction (Carpenter, Shattuck, Tyrka, Geracioto, Price, 2011; Hannibal & Bishop, 2014). Several studies analyzing cortisol activity in regard to markers of obesity have found that lower diurnal cortisol variability has been associated with higher WHR (Björntorp, Holm & Rosmond, 1999; Power, Li, & Hertzman 2006; Rosmond, Dallman & Björntorp, 1998; Wallerius, Rosmond, Ljung, Holm & Björntorp, 2003). Both higher BMI and WC are associated with neuroendocrine dysregulation, as they have been found to be negatively associated with awakening cortisol and early decline (Champaneri et al., 2013). However, there have also been contradictory findings regarding the association between diurnal cortisol secretion and markers of obesity. A study by Kumari, Chandola, Brunner & Kivimaki, (2010) reported a U-shaped curve, with the lowest and highest BMI being associated with lower diurnal cortisol variability, and Vreeburg et al., (2009) showed no association between BMI with and morning, evening, or diurnal cortisol secretion.

In addition, certain allelic variants in GABRA6 have been associated with elevated cortisol secretion; individuals possessing the T/T variant in GABRA6 had significantly higher diurnal cortisol secretion compared to individuals with the T/C variant (Rosmond et al., 2002).

As diurnal cortisol secretion is regulated through HPA activity, dysregulated HPA activity should also be studied as a potential disease mechanism linking early family environment to adult obesity. Although limited research has been conducted on the relationship between diurnal cortisol secretion and early family environment, the

existing literature suggests that maltreatment in childhood is associated with a flatter diurnal slope (Cicchetti and Rogosch, 2001; Weissbecker et al., 2006). Research by Miller, Arbel, Shapiro, Han, & Margolin, (2018) has shown that experiencing adversity in childhood is associated with the development of a flattened cortisol awakening response during adolescence, which was shown to partially mediate an association between childhood adversity and young adult BMI. Previous research on the metabolic impact of GABRA6 has only explored its direct effects on diurnal cortisol secretion and BMI (Rosmond et al., 2002). Exploring its role through a mediational pathway is important in order to gauge the true extent of its contribution to metabolic outcomes. Therefore, this study will further add to the literature by examining the incremental predictive utility of genotype and early family environment on BMI and WC.

CHAPTER 2

RESEARCH QUESTIONS AND HYPOTHESES

Research Question and Hypothesis 1

Research Question 1. To what extent do genetic contributions from an individual SNP affect metabolic dysregulation?

Hypothesis 1. Due to their link to hypercortisolism and increased risk for obesity, carriers of either the T/T or T/C GABRA6 alleles will be positively associated with BMI & WC.

Research Question and Hypothesis 2

Research Question 2. To what extent does growing up in a risky early family environment affect metabolic dysregulation?

Hypothesis 2. Growing up in a risky early family environment will be positively associated with BMI & WC.

Research Question and Hypothesis 3

Research Question 3. To what extent does the G X E interaction between an individual SNP and growing up in a risky early family environment affect metabolic dysregulation outcomes?

Hypothesis 3. Due to both hypercortisolism influences and potential dysregulation, the G X E interaction (early family environment by GABRA6) will be positively associated with BMI & WC outcomes.

Secondary Question and Hypothesis

Secondary Research Question. Does diurnal cortisol secretion mediate the relationship between GABRA6 and early family environment with metabolic dysregulation outcomes?

Secondary Hypothesis. There will be a positive relationship between GABRA6 and early family environment on BMI and WC. Diurnal cortisol secretion will mediate the relationship between GABRA6 and early family environment on BMI and WC.

CHAPTER 3

METHODS

Participants

Volunteers were recruited from the Pittsburgh, Pennsylvania greater metropolitan area through the use of newspaper advertisements. Six to eight weeks before the start of the study, volunteers were required to complete a telephone interview and undergo a physical examination to assess their health status. Volunteers were excluded from participating if they had a previous nasal or otologic surgery; tested positive for the Human Immunodeficiency Virus (HIV); presented an abnormal profile of urinalysis, complete blood count, or blood enzyme levels; were pregnant or lactating; had a history of chronic illness (such as respiratory disorders, sleep disorders, or cardiovascular disease) or take certain types of medication regularly (such as antidepressants, sleeping pills or tranquilizers). They were also excluded if they had been treated for a psychiatric illness the previous year or hospitalized for a psychiatric illness within the past five years. A total of 213 participants, 123 men and 90 women between the ages of 18 and 55 (M_{age} = 30.13 years, SD= 10.85; 57.7% men) were evaluated and judged to be in good health. Upon completion of the study, each participant was compensated for her or his participation.

Procedures

Pre-Quarantine

For approximately two months before being admitted to quarantine, participants completed multiple questionnaire assessments (none which are included in these secondary analyses) and wore a wrist actigraph unit to assess rest and activity patterns. Two days before quarantine, a repeat assay of serum neutralizing antibody (RV39) titer was conducted via blood draw. This assay was used as a pre-study titer covariate. During this time, data on six additional covariates was also collected: BMI, sex/birth control use, season of exposure, how many days before the study blood for the telomere length assay (not included in these secondary analyses) was drawn, and self-reported age and race. Participants were then sequestered in a local hotel for the next six days.

Participants were interviewed in the evening over the phone consecutively over 14 days (10 weekdays and 4 weekend days) prior to viral exposure to obtain a daily assessment of their social interactions, mood, health behaviors, and physical symptoms. Multiple affective traits were assessed during the daily interviews, including depressive symptoms. The interviews lasted approximately 15 minutes, during which interviewers asked participants to rate, using a 5-point scale and a set of mood adjectives, how they had felt since awakening that day.

Genotyping. Approximately 7 to 8 weeks prior to quarantine, sample collection was done via buccal swab procedure on the inner check using a sterilized

cytobrush (Histobrush, Hardwood Products Company, USA). Buccal cells were isolated by placing the swabs in a cryovial of 500 µl of 0.9% physiological saline and agitated. Subsequently, the swabs were pressed against a wall to release liquid, and then removed from the tube (Doyle et al., 2010). This procedure was repeated three times in each cheek for each participant. The vials were stored at -80°C until they were assayed for extraction of genomic DNA. A QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) was used for the extraction, a QIAGEN Repli-g Whole Genome Amplification Kit (GIAGEN) was used for amplification, and a Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) was used for quantification (Doyle et al., 2010). Assaying was focused on the expression of the GABRA6, rs3219151 (1519 T > C, 3'-UTR) SNP.

Salivary Cortisol. Salivary cortisol samples were collected across multiple time points during the waking period of three days (Janicki-Deverts, Cohen, Turner & Doyle, 2016). Six-to-one weeks prior to quarantine there was a consecutive 2-day repeated salivary cortisol collection in the participant's natural environments (home, work, etc.). There was a 24-hour repeated salivary cortisol collection during quarantine. For collection purposes, participants were provided with a plastic collection tube containing cotton rolls (Salivettes®; Sarstedt AG & Co, Nümbrecht, Germany). They were instructed to place the cotton in their mouth to saturate it with saliva then deposit the cotton back into the tube and reseal it. Participants were provided with written instructions and a handheld computer to signal collection times. The handheld computer provided a unique code for each collection, which participants

were instructed to write on each sealed tube (along with the exact time and date of collection) and place it in their refrigerator. Participants were instructed to bring the tubes to their baseline study session to be collected by staff.

During Quarantine

Questionnaire administration. Participants were asked to complete a thirteen-item version of the Risky Families Questionnaire (RFQ) (Taylor et al., 2004) during quarantine. Items containing positive meaning were reverse scored and all items were summed such that higher scores were indicative of greater risky family environment.

Pre and Post Quarantine

Stress Reactivity. BMI and WC were obtained prior to the start of stress reactivity sessions. Participants took part in two laboratory acute stress-reactivity and recovery session to evaluate physiological responses to a challenge task; one session took place four to two weeks prior to quarantine, and the other session took place 4-6 weeks after the study. The sessions lasted around 2.25 hours and took place between 3:00 p.m. and 9:00 p.m. to control for diurnal cortisol variations. During the sessions, participants completed a modified version of the Trier Social Stress Test, a 15-minute stress protocol that involves public speaking and the completion of mental arithmetic. As the session took place, both psychological (stress, anxiety, mood, emotional response) and biological (salivary cortisol, heart rate, blood pressure, respiration rate,

heart rate variability) measures were collected. Prior to the start of both stress reactivity sessions, height, weight, WC and hip circumference were measured.

Power Analysis. A post-hoc power analysis for multiple regression was conducted in G*power to determine the detectable effect size of our results given our sample size. There was a total of seven predictors in the analyses for the first two hypotheses: early family environment and GABRA6, and the covariates: age, sex, race and depression. Hypothesis three had a total of nine predictor variables: the first order predictors and covariates, and the interaction between GABRA6 and early family environment. In order to detect a small effect, alpha was set to .05, power was set to .80, and Cohen's f^2 was set to .02 (Cohen, 1988; Faul, Erdfelder, Lang, & Buchner, 2007). The power analysis indicated that a sample size of 725 participants would be required to detect a small effect. To determine a medium effect, alpha was set to .05, the power was set to .80, and Cohen's f^2 was set to .15 (Cohen, 1988; Faul et al., 2007). The power analysis indicated that a sample size of 103 participants would be necessary to detect a medium effect. This study has a total of 213 participants, which allows us to detect a medium effect; however, the study is underpowered for detecting a small effect.

R version x64 3.4.3 was utilized to assess post-hoc power for the mediation aim (R Development Core Team, 2017). The analysis was conducted using the powerMediation.VSMc function from the powerMediation package (Qiu, 2015; Vittinghoff, Sen, & McCulloch, 2009). The sample size was designated as n and was set at 213 for all analyses. The b2 function indicates the regression coefficient for the

mediator in the regression, the sigma.m function denotes the standard deviation of the mediator, the sigma.e function specifies the standard deviation of the random error term in the regression, and the corr.xm function represents the correlation between the specified predictor and the mediator. Alpha was set at .05 for all analyses. For the mediation with GABRA6 as the predictor and BMI as the outcome, b2 was set at .0027, sigma.m was set at 215.8985, sigma.e was set at 5.9644, and corr.xm was set at -.017. The resulting power for this mediation analysis was .2971. For the mediation with GABRA6 as the predictor and WC as the outcome, b2 was set at .0082, sigma.m was set at 215.8985, sigma.e was set at 13.8464, and corr.xm was set at -.017. The resulting power for this mediation analysis was .4625. For the mediation with early family environment as the predictor and BMI as the outcome, b2 was set at .0030, sigma.m was set at 215.8985, sigma.e was set at 5.9086, and corr.xm was set at -.072. The resulting power for this mediation analysis was .3581. For the mediation with GABRA6 as the predictor and BMI as the outcome, b2 was set at .0087, sigma.m was set at 215.8985, sigma.e was set at 13.8576, and corr.xm was set at -.072. The resulting power for this mediation analysis was .5053. None of the power analyses reached a power level of .8 or greater, indicating the mediation analyses were underpowered.

Measures

BMI and WC. Anthropometric data were assessed prior to the start of the two reactivity sessions. Measurements of height and weight were taken without the use of shoes or overgarments. Height was recorded to the nearest half-inch or half-

centimeter, and weight was recorded to the nearest half-pound or half-kilogram. All English units were converted to metric. These data were used to calculate a participant's body BMI using the following formula: weight (kg) / height $(m)^2$. Waists were measured over a participant's garments at the level of the navel, and hip circumferences at the widest level of the buttocks. These data were used to calculate a participant's waist/hip ratio with this formula: WC (cm) / hip circumference (cm). BMI, WC and hip circumference were averaged across the two reactivity sessions to increase reliability. Average values were used in all analyses.

Risky Families Questionnaire (RFQ). Early family environment was measured by using a thirteen-item version of the RFQ, adapted from Taylor et al., (2004) and designed to retrospectively capture the respondent's family environment between ages of 5 and 15. The questionnaire employs a 5-point Likert scale, with each item ranging from 1 (not at all) to 5 (very often). Sample items include "Would you say the household you grew up in was chaotic and disorganized?" and "Would you say you were neglected while you were growing up, left on your own to fend for yourself?." The items were designed to measure to what extent the respondent felt loved, was shown affection, was verbally or physically abused, lived with a substance abuser, lived in a household that was organized and managed well, and had adults who "knew what they were up to". This measure was found to be internally consistent (Cronbach's $\alpha = .73$) and have a high inter-rater reliability (.91) (Taylor et al., 2004; Taylor et al., 2006).

GABRA6. The GABRA6 gene has an associated SNP involving a T to C substitution which results in several alleles: the homozygous C/C allele, the homozygous T/T allele, and the heterozygous T/C allele. T allele variants (either T/T or T/C alleles) have been implicated as conferring greater likelihood to be associated with cortisol secretion and/or BMI and WC.

Diurnal Cortisol. Salivary cortisol was measured seven times daily (the first sample was collected 1 hour after awakening, and the last sample was collected 11 hours after awakening) during the two consecutive pre-quarantine days. Two samples were taken on the evening of the day prior to quarantine and the remaining five measurements were collected during day 0 of quarantine. Cortisol samples were assayed at the laboratory facilities of Dr. Clemens Kirschbaum in Dresden, Germany (Janicki-Deverts et al., 2016). Cortisol concentrations were established though time-resolved fluorescence immunoassays using a cortisol-biotin conjugate as a tracer (Dressendörfer et al., 1992). Only samples that were collected within ±45 minutes of the scheduled collection time were included for analysis; any samples collected outside of this timeframe were treated as missing. The actual time rather than the expected time the participant provided for each cortisol sample was used to calculate both area under the curve (AUC) and slopes (Chin, Murphy, Janicki-Deverts, & Cohen, 2017). To calculate average diurnal cortisol levels, the AUC for each day was computed for individuals with sufficient data, which was defined as not having missed collection of any of the first three samples of the day (for steep diurnal rhythm) or missing more than two of the day's remaining samples (for flat diurnal rhythm).

Average total diurnal cortisol levels were calculated for participants who had data for a minimum of two of the three collection days by averaging total concentrations from all days with sufficient data (Chin et al., 2017). Cortisol samples across the three study days was used to demonstrate participants' typical diurnal cortisol secretion.

Covariates. Age, sex, race and depression were obtained via self-report questionnaires and interviews and included as covariates.

Depressive symptoms. Depressive symptoms were assessed using the depression subscale of the Negative Affect 2 component of the Daily Interview. Items for depressive symptoms include "sad" and "unhappy.", The scale was created by calculating a mean score across the items for each of the 14 interview days. A 5-point scale was used to score the responses (0 = you haven't felt that way at all today to 4 = you felt that way a lot today).

Data Analytic Strategy and Preparation

Evaluation of the distribution of all variables of interest was visually assessed with histograms, which indicated that none of the variables met the assumption of normality. Homoscedasticity was assessed by a visual inspection of plots of studentized residuals against unstandardized predicted values. It was determined that there was moderate heteroscedasticity in all models with BMI as the dependent variable. Linearity was assessed by partial regression plots between each predictor term and the dependent variables, and it was determined that the assumption of linearity was met for all variables. Multicollinearity was assessed by running bivariate

correlations and ensuring the correlations did not exceed .70. As none of the bivariate correlations exceeded .70, multicollinearity was not violated. There was independence of residuals, as all models produced a Durbin-Watson statistic with an approximate value of 2.00.

Logarithmic transformations were applied to the outcome variables to correct violations to normality and homoscedasticity. Analyses were performed with and without transformed outcome variables. All models displayed a medium effect size prior and subsequent to transformation (R^2= ~.20). As the direction and significance of the associations did not change, analyses were conducted with untransformed variables to aid in interpretation of the results.

Multiple linear regressions were used to test associations among early family environment, GABRA6 and their interaction on BMI and WC. The regression equations included GABRA6 and early family environment as predictors, along with age, race, sex and depression as covariates. As GABRA6 is a categorical variable, it was dummy coded to allow for inclusion in the regression analyses. The alleles for GABRA6 were dummy coded as C/C = 0, T/C and C/C= 1. C/C was chosen as the reference category based on higher incidence of hypercorisolism of the T/T and T/C carriers compared to C/C carriers (Rosmond, et al., 2002). To test an interaction effect, the dummy coded GABRA6 variable was multiplied by early family environment, and the resulting product was a variable that coded the interaction. The interaction was then tested in multiple regression model that included the GABRA6 and early family

environment first order effects, all the above listed covariates, and the early family environment X GABRA6 interaction term.

For the mediation analyses, GABRA6 was entered as a single multicategorical variable, as the PROCESS macro automatically creates dummy variables through its multicategorical option. Two separate mediation analyses were run: a heterozygous TC alleles comparison (comparing CC compared to TC) and a homozygous TT alleles comparison (CC compared to TT). The PROCESS macro, Model 4, for SPSS (Hayes, 2013) was used to investigate four separate hypotheses: 1) diurnal cortisol secretion will statistically mediate the association between early family environment and BMI; 2) diurnal cortisol secretion will statistically mediate the association between early family environment and WC; 3) diurnal cortisol secretion will statistically mediate the association between GABRA6 and BMI; and 4) diurnal cortisol secretion will statistically mediate the association between GABRA6 and WC. . The indirect effects were estimated through bias-corrected bootstrapped confidence intervals with 5,000 iterations. This bootstrapping procedure uses the available sample and repeatedly resamples with replacements to create an empirical representation of the data (Hayes, 2013). This process is preferred over the Baron and Kenny (1986) method of mediation analysis, which requires a significant direct effect on the a pathway, the b pathway, and a reduction in significance in the c pathway when accounting for the mediator variable. Although the PROCESS macro analyzes all pathways in a mediation model, the bootstrapping procedure does not require these assumptions to be met in order to assume mediation (Hayes, 2009). Rather, this

method estimates the indirect effects from the product of the a and b pathways 5,000 times, orders them, and uses the lower 2.5% and upper 2.5% of these results as the boundaries of the 95% confidence interval. An indirect effect is achieved when the confidence intervals do not cross through zero.

CHAPTER 4

RESULTS

Regression Analyses

BMI. Early family environment was significantly positively associated with BMI, (β = .166, SE= .042, p = .012). In contrast, GABRA6 was not significantly associated with BMI, (β =. -125, SE=.936, p < .050) (Table 1).

Table 1: *Summary of multiple regression analysis with BMI as an outcome variable*

Variable	β *(SE)*	*t*	*p-value*
Early Family Environment	.166(.042)	2.532	.012
GABRA6	-.125(.936)	-1.968	.050
Age	.308(.038)	4.869	.000
Race	.142(.879)	2.223	.027
Sex	.164(.822)	2.615	.010
Depression	-.120(.401)	-1.807	.072

Note: GABRA6 = Gamma-Aminobutyric Acid Type A Receptor Alpha6 Subunit. Effect size assessed through post-hoc analyses based on sample size and parameter estimates.

The early family environment x GABRA6 interaction effect was non-significant, (β = .106, SE= .092, p = .397) (Table 2).

Table 2: *Summary of multiple regression analysis with an interaction and BMI as an outcome variable*

Variable	β *(SE)*	*t*	*p*
Early Family Environment	.077(.079)	.623	.534
GABRA6	-.125(.936)	-1.971	.050
Interaction	.106(.092)	.848	.397
Age	.315(.038)	4.936	.000
Race	. 146 (.881)	2.274	.024
Sex	.165(.823)	2.633	.009
Depression	-.125(.403)	-1.874	.062

Note: GABRA6 = Gamma-Aminobutyric Acid Type A Receptor Alpha6 Subunit. Effect size assessed through post-hoc analyses based on sample size and parameter estimates.

WC. Early family environment was significantly positively associated with WCβ = .145, SE= .099, p = .025) (Table 3).

Table 3: *Summary of multiple regression analysis with WC as an outcome variable*

Variable	β *(SE)*	*t*	*p*
Early Family Environment	.145(.099)	2.260	.025
GABRA6	-.076(2.217)	-1.215	.226
Age	.421(.090)	4.921	.000
Race	.123(2.082)	1.966	.051
Sex	-.094(1.94)	-1.539	.125
Depression	-.089(.951)	-1.368	.173

Note: GABRA6 = Gamma-Aminobutyric Acid Type A Receptor Alpha6 Subunit. Effect size assessed through post-hoc analyses based on sample size and parameter estimates.

The interaction effect was non-significant, (β = .096, SE= .217, p = .435) (Table 4).

Table 4: *Summary of multiple regression analysis with an interaction and WC as an output variable*

Variable	β *(SE)*	*t*	*p*
Early Family Environment	.065(.186)	.534	.594
GABRA6	-.076(2.219)	-1.218	.225
Interaction	.096(.217)	.783	.435
Age	.428(.091)	6.835	.000
Race	.126(2.089)	2.012	.046
Sex	-.093(1.950)	-1.517	.131
Depression	-.093(.956)	-1.430	.154

Note: GABRA6 = Gamma-Aminobutyric Acid Type A Receptor Alpha6 Subunit. Effect size assessed through post-hoc analyses based on sample size and parameter estimates.

Mediation Analyses

The results of the mediation analysis are presented in Figures 1-6. For mediation analyses including GABRA6 as a predictor variable, the reference category (the homozygous C/C allele) was compared against the homozygous T/T allele and the heterozygous T/C allele.

Early Family Environment. When BMI was entered as the outcome variable, there were no significant direct effects of early family environment on diurnal cortisol secretion (β = -1.526, SE = 1.542, p = .324) (Figure 1).

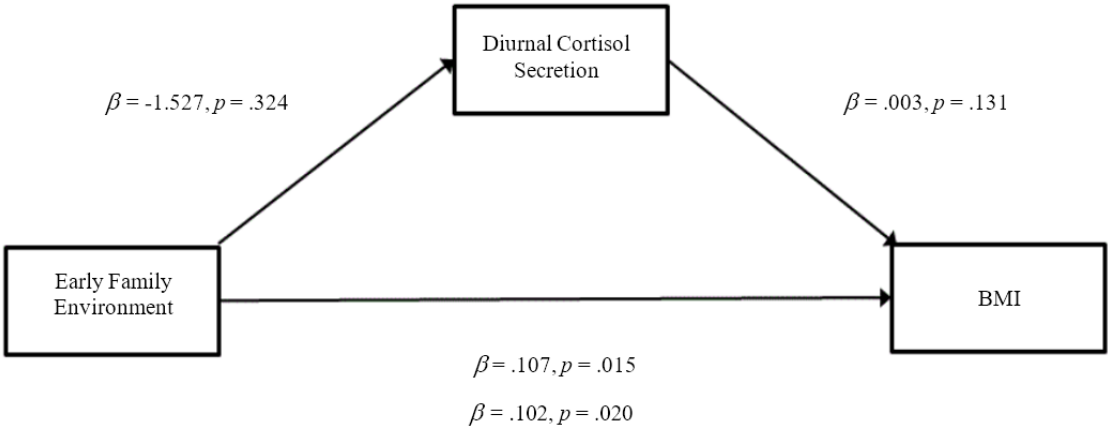

Figure 1. Mediation model for early family environment on BMI through the pathway of diurnal cortisol secretion (Model 4: Hayes, 2013).

There were no significant direct effects of diurnal cortisol secretion on BMI (β = .003, SE = .002, p = .181). However, significant direct effects were observed from early family environment to BMI (β= .106, SE = .043, p = .015). Congruent with the regression analyses, early family environment was statistically significant for the total effect path, (β = .102, SE = .043, p = .020).

When WC was entered as the outcome variable, there were no significant direct effects of early family environment on diurnal cortisol secretion (β = -1.527, SE = 1.543, p = .324) (Figure 2).

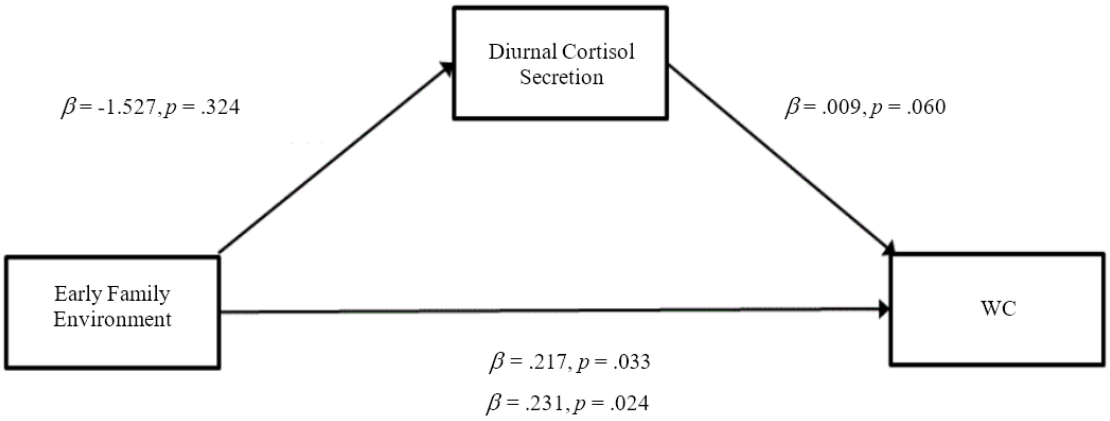

Figure 2. Mediation model for early family environment on WC through the pathway of diurnal cortisol secretion (Model 4; Hayes, 2013).

Furthermore, there were no significant direct effects of diurnal cortisol secretion on WC (β = .008, SE = .005, p = .060). There were significant direct effects between early family environment and WC (β = .231, SE = .101, p = .024). For the total effect model, early family environment was statistically significant (β = .217, SE = .101, p = .033). Again, the mediation analyses did not indicate a significant indirect effect of early family environment on WC through the pathway of diurnal cortisol secretion, indicating mediation had not occurred.

GABRA6. When BMI was entered as the outcome variable, there were no significant direct effects of the heterozygous T/C alleles comparison on diurnal cortisol secretion (β = -9.154, SE = 37.901, p = .809) (Figure 3).

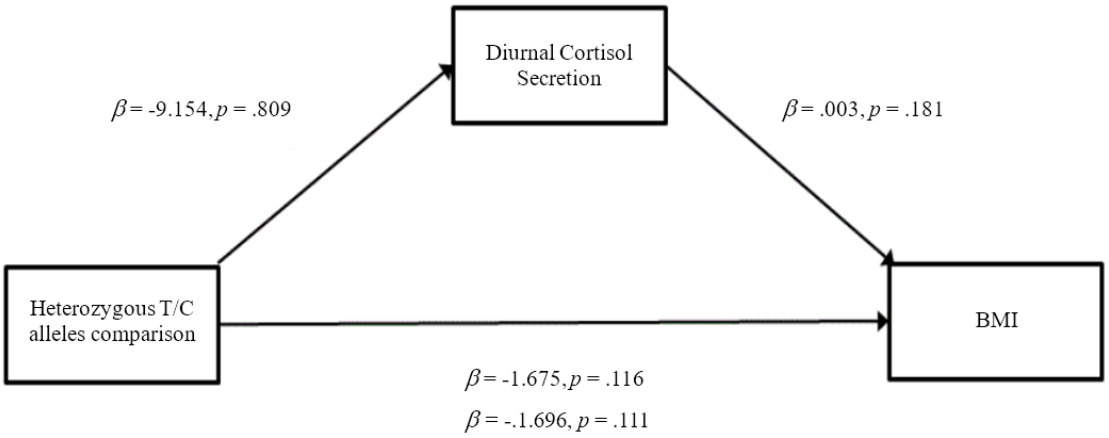

Figure 3. Mediation model for the Heterozygous T/C alleles comparison on BMI through the pathway of diurnal cortisol secretion (Model 4; Hayes, 2013).

The homozygous T/T alleles comparison was also not significantly associated with diurnal cortisol secretion (β = -.944, SE = 42.716, p = .982) (Figure 4).

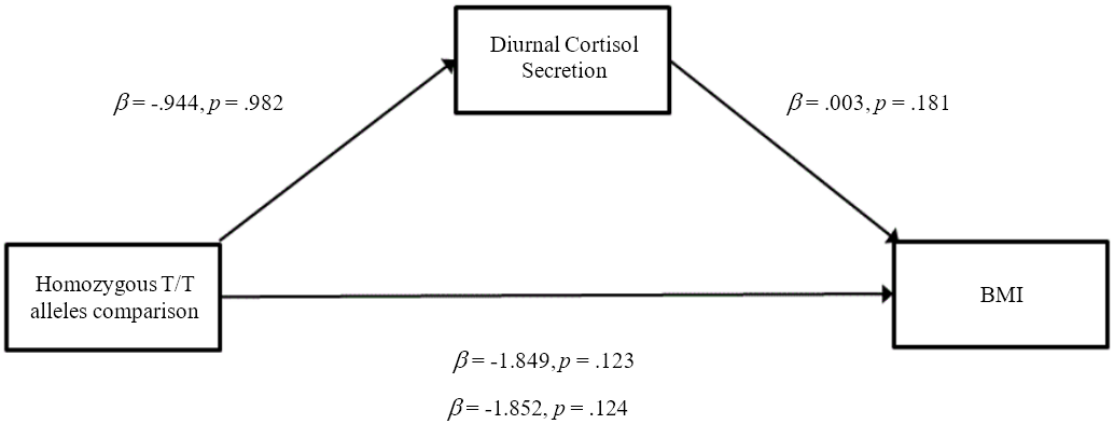

Figure 4.. Mediation model for Homozygous T/T alleles comparison on BMI through the pathway of diurnal cortisol secretion (Model 4; Hayes, 2013).

Furthermore, there were no significant direct effects of diurnal cortisol secretion on BMI (β = .003, SE = .002, p = .181). The heterozygous T/C alleles

comparison did not have any significant direct effects on BMI (β = -1.675, SE = 1.060, p = .116) nor did the homozygous T/T alleles comparison (β = -1.849, SE = 1.195, p = .123). For the total effect model, the heterozygous T/C alleles comparison did not show significant effects (β= -1.696, SE = 1.062, p = .111), nor did the homozygous T/T alleles comparison (β = -1.852, SE = 1.197, p = .124).

When WC was entered as the outcome variable, there were no significant direct effects of the heterozygous T/C alleles comparison on diurnal cortisol secretion (β = -9.154, SE = 37.901, p = .809) (Figure 5).

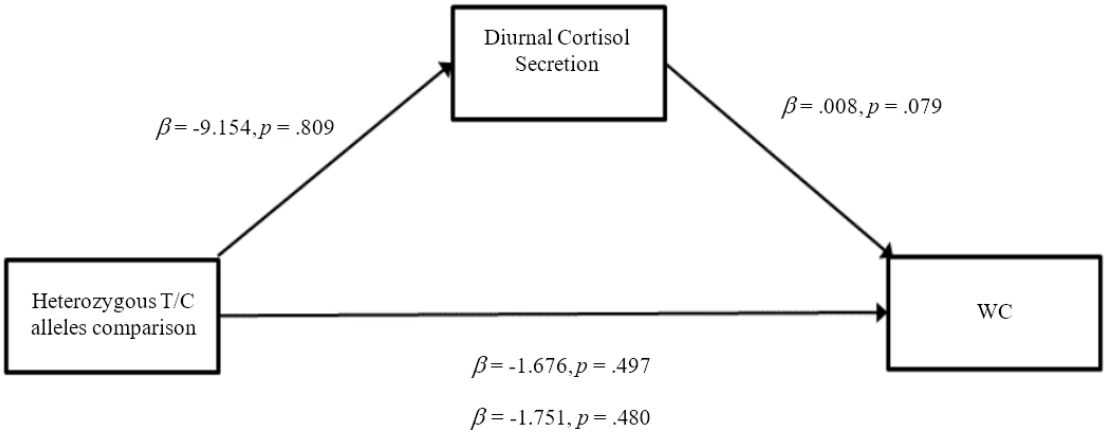

Figure 5. Mediation model for Heterozygous T/C alleles comparison on WC through the pathway of diurnal cortisol secretion (Model 4; Hayes, 2013).

Similar outcomes were observed for the homozygous T/T alleles comparison on diurnal cortisol secretion (β = -9.154, SE = 42.716, p = .982) (Figure 6).

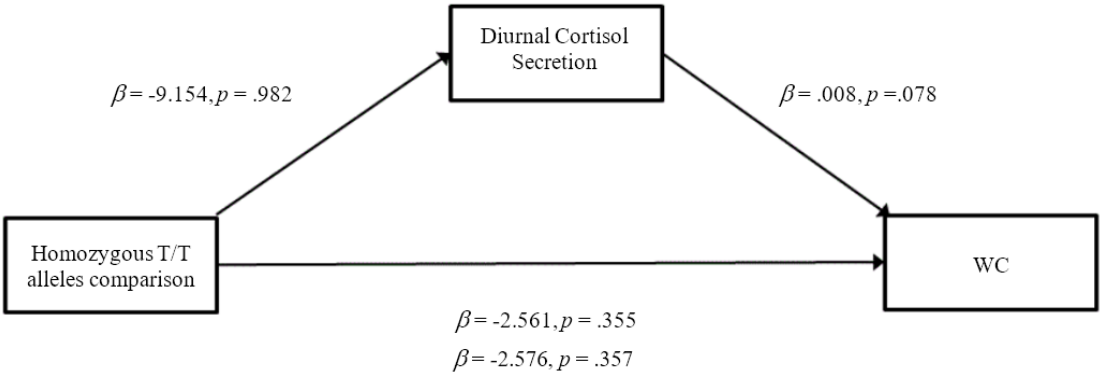

Figure 6. Mediation model for Homozygous T/T alleles comparison on WC through the pathway of diurnal cortisol secretion (Model 4; Hayes, 2013).

Furthermore, there were no significant direct effects of diurnal cortisol secretion on WC (β = .008, SE = .005, p = .079). The heterozygous T/C alleles comparison did not have any significant direct effects on WC (β = -1.676, SE = 2.461, p = .497) nor did the homozygous T/T alleles comparison (β = -2.568, SE = 2.774, p = .355). For the total effect model, the heterozygous T/C alleles comparison did not show significant effects (β = -1.751, SE = 2.475, p = .480), nor did the homozygous T/T alleles comparison (β = -2.576, SE = 2.789, p = .357). The mediation analyses did not indicate a significant indirect effect of GABRA6 on BMI and WC through the pathway of diurnal cortisol secretion, suggesting a lack of mediation had occurred.

CHAPTER 5

DISCUSSION

The aims of the current study were to (1) separately assess the contributions of GABRA6 and an early family environment on BMI and WC in a community sample, (2) examine a G X E interaction consisting of GABRA6 and an early family environment on BMI and WC, (3) explore diurnal cortisol secretion as a pathway linking the association between GABRA6 and an early family environment on BMI and WC.

Based on previous findings, it was hypothesized that early family environment would be predictive of BMI & WC (McEwen & Seeman, 2009; Soares et al., 2018, Weidner et al., 1992). When controlling for age, sex, race, and depression, the hypothesis was supported, as early family environment was positively associated with both BMI and WC. Although prior work delineated the health consequences of severe family dysfunction, this study provided evidence that even milder forms of negative childhood exposures, including being raised in an unsupportive family environment that lacks parental warmth can be associated with adverse metabolic outcomes in adulthood.

SNPs are often studied through the candidate gene approach (CGA), which allows for a selective exploration of a gene or genomic regions of interest for a trait or disease risk based on *a priori* hypotheses. An integral advantage of this technique when compared with studies with untargeted screening (such as genome-wide

association studies or GWAS) is their relative inexpensiveness and rapidity, and their emphasis on genes that have previously related to the disease. CGA studies are particularly useful in situations where allele frequencies are low, effect sizes are small, or the study population of interest is limited or unique (Jorgensen et al., 2009). CGA studies are also valuable for validating previous reports of genetic associations with disease in different populations (Jorgensen et al., 2009; Patnala, Clements & Batra, 2013). Nonetheless, this approach does have some limitations, including obstructing the discovery of new biological pathways, reliance of previous knowledge about a gene, and its limited ability to include all possible causative genes (Tabor et al., 2002; Wang et al., 2013; Zhu & Zhao, 2007).

It was hypothesized that T allele carriers of GABRA6 would be predictive of BMI & WC. When controlling for age, sex, race and depression, the hypothesis was not supported, as no significant association was found with any allele variant of GABRA6. The lack of support for the contribution of a single SNP for metabolic dysregulation is congruent with the commonly accepted credence in genetic research that individual susceptibility to many diseases -including metabolic dysregulation - is a cumulative consequence derived from numerous low-penetrating genetic variables. Additionally, although GABRA6 has previously been linked with risk for metabolic dysregulation (making it an ideal gene to study under the candidate gene approach (CGA)), the lack of significant associations with both BMI and WC could be explained by limitations inherent in the CGA, such as small sample size, low power and low replicability (Tabor, Risch, & Myers, 2002; Vimaleswaran et al., 2012). As

outlined by the power analyses, the study's mediation analyses were underpowered and the regression analyses were unable to detect small effects, indicating that a larger sample size may be needed to detect significant effects. This is congruent with previous research, which has generally found that candidate-gene studies investigating different traits have been wanting. An exhaustive review by Alghamdi & Padmanabhan (2014) found that only 6 out of 166 assumed associations were reliably replicated. This approach has also been criticized for its inability to recognize additional functional variants due to perplexity caused by phenotypic and locus heterogeneity and population stratification (differences in allele frequencies in a homogenous population) (Alghamdi & Padmanabhan, 2014). An unfortunate consequence of such a limitation is obtaining a potentially incomplete picture of disease pathology and precluding the discovery of new biological pathways. (Tabor et al., 2002; Wang et al., 2013). In addition, the choice of coding scheme for the GABRA6 allelic variants may have influenced the non-significant outcome, as the polymorphism was dummy coded functionally, according to the cortisol activity of the alleles. Had the alleles been dummy coded based on prevalence (making the homozygous and heterozygous t alleles the refence category), different results might have been obtained.

As G X E interactions can heighten the accuracy of epidemiological risk models by clarifying pathogenic biological processes leading to disease, an additional aim of the study was to examine a putative interaction between GABRA6 variants and early family environment. When controlling for age, race, sex and depression, the

hypothesis was not supported, suggesting that the GABRA6 polymorphism does not interact with early family environment to predict BMI and WC in a community sample. It is possible that the method employed to calculate the interaction could have contributed to the lack of significance, as different methods for quantifying interactions can produce different results. This study employed a multiplicative interaction, which uses the product term of the two risk factors in a logistic-regression model. Utilizing an additive interaction, which compares the outcome of interest of those exposed to both factors with the sum of the effects ascribed to each of the two factors independently, might have yielded a different outcome. Therefore, different methods should be considered when analyzing interactions in complex diseases.

Lastly, it was hypothesized that diurnal cortisol secretion would mediate the relationship between GABRA6 alleles/early family environment and BMI/WC. When controlling for age, race, sex, depression and educational attainment, the hypothesis was not supported. Mediation analyses indicated non-significant indirect effects of both GABRA6 and early family environment on BMI and WC through the pathway of diurnal cortisol secretion. Although previous work has found an association between diurnal cortisol secretion and markers of obesity (Björntorp et al., 1999; Miller et al., 2018; Rosmond et al., 1998), the overall literature still shows mixed results between the link in diurnal cortisol profile and anthropometry measures of adiposity (Champaneri et al., 2013; Kumari et al., 2010). A possible explanation for these findings would be limitations of small sample size, single gender, and/or single

race/ethnicity (Champaneri et al., 2013). Clearly, more research is needed in this area to clarify the association.

Limitations and Future Research

The results of this study must be viewed in the context of limitations that may inform future research. The sample size, although sufficiently large to detect psychosocial effects such as childhood maltreatment, is likely underpowered to detect genetic contributions from single SNPs, particularly those with low penetrance. Future studies seeking to assess genetic contributions from single SNPs may wish to employ a larger participant pool. As only one buccal swab was obtained from the participants, we are limited to analyzing only a single instance in time of the participant's health. This study was based on self-report, which potentially leads to underreporting on sensitive issues, such as parental abuse and lack of warmth. Due to retrospective reporting on the RFQ, there are inherent memory-related biases which call into question the reliability and validity of the participant's long-term recall. In the future, researchers should consider replicating this study utilizing a longitudinal design in order to address this limitation. Additionally, the generalizability of these findings is limited as the sample had relatively high education levels and lacked geographical diversity. Moving forward, it should be determined if these findings are similar in other samples, including clinical populations.

The current study has several strengths. This is the first study to examine a G X E interaction between GABRA6 and an early family environment as characterized by growing up in an adverse family environment. Previous studies have also not

examined the potential mediating role of diurnal cortisol secretion between genotype and early family environment to BMI and WC. In addition, apart from a few studies (Rosmond et al., 2002; Rosmond & Bouchard & Björntorp, 2002; Rosmond, 2003), the exact role of GABRA6 in metabolic processes has not been extensively studied. Given the high rate of false positive associations when performing candidate gene studies, the results of this study may have implications for understanding the true extent GABRA6 is involved in hypercortisolism and obesity, as we were unable to replicate previous findings linking it to central obesity.

Lastly, although no significant interaction was seen with GABRA6, it is worthwhile to note that perhaps other polymorphisms of candidate genes associated with central obesity, such as the β2-Adrenergic receptor (ADRB2), Glucagon receptor (GCGR), or Jeptin receptor (LEPR), may show interactive effects with early family environment (Rosmond, 2003).

Clinical Implications

The current findings suggest that developing an effective health improvement program requires a protracted, multilayered approach which includes interventions designed to help people change, implement, and maintain behaviors aimed at promoting a healthy family environment. Such interventions would ideally help parents identify the kinds of behaviors that have detrimental effects on a child's behavioral and self-regulatory skills, or that are known to cause repeated incidences of stress. These findings further suggest that when working with clients in weight-

management settings, clinicians should consider assessing early family environment as a risk factor for metabolic dysregulation.

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