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Genes implicated in serotonergic and dopaminergic functioning predict BMI categories

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Abstract

Objective—This study addressed the hypothesis that variation in genes associated with dopamine function (SLC6A3, DRD2, DRD4), serotonin function (SLC6A4), and regulation of monoamine levels (MAOA) may be predictive of BMI categories (obese and overweight + obese) in young adulthood and of changes in BMI as adolescents transition into young adulthood. Interactions with gender and race/ethnicity were also examined.

Research Methods and Procedures—Participants were a subsample of individuals from The National Longitudinal Study of Adolescent Health (Add Health), a nationally representative sample of adolescents followed from 1995 to 2002. The sample analyzed included a subset of 1584 unrelated individuals with genotype data. Multiple logistic regressions were conducted to evaluate associations between genotypes and obesity (BMI > 29.9) or overweight + obese combined (BMI > 25) with normal weight (BMI = 18.5–24.9) as a referent. Linear regression models were used to examine change in BMI from adolescence to young adulthood.

Results—Significant associations were found between SLC6A4 5HTTLPR and categories of BMI, and between MAOA promoter VNTR among males and categories of BMI. Stratified analyses revealed that the association between these two genes and excess BMI was significant for males overall, and for White and Hispanic males specifically. Linear regression models indicated a significant effect of SLC6A4 5HTTLPR on change in BMI from adolescence to young adulthood.

Discussion—Our findings lend further support to the involvement of genes implicated in dopamine and serotonin regulation on energy balance.

Keywords

Adolescents; Genetic Epidemiology; Serotonin; Neuro Transmitter

INTRODUCTION

Obesity increases the risk for a number of serious health conditions including cardiovascular disease, stroke, diabetes, and some cancers (1,2). Thus, the identification of biological,

psychological, environmental, social, and genetic factors that interact to influence overweight and obesity is of high importance (3). Although the causes of obesity in society are numerous, at the individual level genetics is thought to play an important role in regulating energy balance and the development of obesity (4). Both twin and family studies suggest that obesity is influenced by genetic factors (5–7) and several candidate genes have been associated with obesity-related phenotypes. The 2005 Human Obesity Gene Map reported that 127 candidate genes have been found to be significantly associated with obesity-related phenotypes across 416 studies (8). Yet, the number of genes found to be consistently associated with obesity-related phenotypes across these studies ($n = 22$) is much smaller. An emerging area of research has begun to explore the potential association between specific candidate genes regulating brain serotonin and dopamine systems with obesity. These neurotransmitter systems are hypothesized to regulate behavioral and metabolic responses associated with the development of obesity through feeding and satiety (9).

Converging evidence suggests that dopaminergic neurotransmission regulates feeding behaviors in humans (10–12) and is associated with higher body mass index (BMI) (13). Candidate gene studies of dopamine related genes have focused on the DRD2 (dopamine receptor D2), DRD4 (dopamine receptor D4), and SLC6A3 (solute carrier family 6–neurotransmitter transporter, dopamine- member 3) genes. Findings from studies of DRD2 have been mixed with some studies detecting an association with obesity-related phenotypes (14–18), whereas others report no association (19–23). At least two studies of DRD4 have shown an association between higher BMI and carriers of the 7 repeat allele found in the third exon of the gene (24,25). One of these studies suggested that the association was influenced by race, as it was present among African-Americans and Hispanics, but not American Whites (24). Finally, two studies of current smokers have reported an association between SLC6A3 and the phenotypes of obesity (20) and food reinforcement (26). Thus, there is increasing evidence that the dopaminergic system may indeed be involved in obesity-related phenotypes.

Serotonergic functioning has also been hypothesized to be important for regulating eating behaviors and body weight (27,28). For instance, administration of serotonergic agents have been shown to suppress eating behaviors (29,30) and reduce craving for high carbohydrate foods (31). Candidate gene studies of the serotonergic system have examined the amino acid transporter gene and the serotonin receptor and transporter genes. At least two studies have indicated a relationship between the solute carrier family 6 member 14 (SLC6A14) gene and obesity related phenotypes (32,33). This gene is relevant to serotonin signaling as it is associated with tryptophan availability for serotonin synthesis. An association has been found between a polymorphism of the serotonin receptor 2A locus (HTR2A -1438A>G) and higher levels of abdominal obesity and Body Mass Index (BMI) among adults (34,35) and increased energy and fat intake in children (36). There is an additional support for this polymorphism being involved in energy balance in overweight adult subjects (37). However, one subsequent study failed to detect an association with this polymorphism and obesity in children, though the study was underpowered (38). Fewer studies have examined serotonin transport gene with the exception of a recent study of Argentinean adolescents which found an association between a polymorphism of the serotonin transporter SLC6A4 and overweight (39).

The monamine oxidase A (MAOA) gene produces an enzyme that metabolizes dopamine and serotonin (in addition to noradrenaline) and it has also been examined for association with obesity phenotypes. In a large U.K. cohort ($n=1,150$) of Caucasian females, significant associations were detected between MAOA and BMI, with the low-activity genotype (3/3) being more frequent among obese females (40). This finding supports a family-based study

in which preferential transmission of the low activity related allele was observed among subjects with BMI ≥ 35 kg/m² (41).

Although the possible association between genes regulating dopamine and serotonin with obesity is promising, the relevance is uncertain with respect to population level effects. This is because many studies are derived from clinical or convenience samples making it difficult to extrapolate to general populations. Therefore, replication studies in population-based cohorts are clearly warranted (42). Using a large population-based cohort of young adults we addressed the hypothesis that variation in genes associated with dopamine function (SLC6A3, DRD2, DRD4), serotonin function (SLC6A4), and regulation of monoamine levels (MAOA) may be predictive of BMI categories (obese and overweight + obese) in young adulthood, as well as predictive of changes in BMI as adolescents transition into young adulthood. Since studies have shown that gender can affect both feeding (43) and addictive behaviors (44) and allelic frequency of these genes (45) and expression (46) vary by ethnic/racial ancestry, we further evaluated gene by gender and gene by race interactions. To examine the factors driving interaction effects, we followed significant effects with stratified analyses when sample size permitted.

METHODS

Data source

The study population was 20,745 adolescents from the National Longitudinal Study of Adolescent Health (Add Health), a nationally representative study of adolescents. The longitudinal cohort includes 15,197 eligible respondents who completed inhome surveys on three separate occasions (April to December, 1995, April to August, 1996, and August 2001 to August 2002). The mean age of survey participants in the three waves of data collection was 15.65 (SD = 1.75) years, 16.22 (SD = 1.64) years, and 22.96 (SD = 1.77) years. All survey participants at Wave III were 18 years of age or older. By design, the Add Health survey included a sample stratified by region, urbanicity, school type, ethnic mix, and size to garner a nationally representative sample. Precise details regarding the design and data collection have been described elsewhere (47,48).

Study Sample

At Wave III, a subset of individuals identified to be full siblings or twins at earlier waves ($n = 3,787$) consented to provide a saliva sample for DNA analysis. The study conformed to local institutional review board (IRB) approved procedures (further details can be obtained at www.cpc.unc.edu/projects/addhealth). For our analyses we included only unrelated individuals by randomly selecting one sibling from each sibship. Participants who were pregnant were excluded from analysis ($n=51$). For the analyses comparing normal weight individuals (defined as Body Mass Index [BMI] between 18.5 and 25) to obese individuals (BMI > 30) the total available sample included 1133 individuals. For the analyses comparing normal weight individuals to overweight and obese individuals (BMI > 25) the total available sample included 1584 individuals. Genotype was missing for one or more of the genetic markers for some individuals which resulted in variability in the total number of individuals available for each genespecific analysis.

Genotyping

Buccal samples were collected on the participants and DNA extracted using a modification of procedures previously described (49–52) (further details at www.cpc.unc.edu/projects/addhealth). Six functional polymorphisms were genotyped within six candidate genes that had been previously associated with behavioral and psychological outcomes. The following were genotyped according to previously described procedures

(53,54): a 40 basepair (bp) VNTR polymorphism in the 3' untranslated region (UTR) of the dopamine transporter (SLC6A3), a 44 bp insertion/deletion polymorphism (5HTTLPR) in the promoter of the serotonin transporter (SLC6A4), the Taq1A DRD2 polymorphism (DRD2), a 48 bp VNTR in exon 3 of the dopamine D4 receptor (DRD4), and a 30 bp VNTR in the promoter of the monoamine oxidase A (MAOA) gene. The T479A substitution in the CYP2A6 gene, associated with nicotine metabolism, was also genotyped in the same DNA samples, but this genotype data was not used in this study.

The genotypes were tested for deviations from Hardy Weinberg Equilibrium (HWE). Deviations from HWE could indicate either a problem with the genotyping assay, or a true association with an outcome. To examine the second hypothesis, we tested for deviations from HWE in the overall data set, in the normal weight subset, the obese subset and the obese/overweight subset.

Body Mass Index

BMI was calculated based on height and weight ($\text{BMI} = \text{weight in kilograms}/\text{height in meters}^2$) measured by Add Health staff during the in-home interviews at Wave II and Wave III. Height and weight were self-reported at Wave I and thus, analyses of BMI were restricted to Wave II and III.

Sociodemographic variables

Covariates included indicators of socioeconomic status (e.g., parental reported education reported by parents at Wave I), chronological age of participant at Wave III, and self-identified race/ethnicity. American Indians and Asians were excluded from the analyses because they were underrepresented in the available data. Thus, our analyses only included American Whites, African-Americans, and Hispanics.

Statistical Analysis

Statistical analyses were conducted using SAS-callable SUDAAN (version 8.0) statistical software (55). SUDAAN allows for control of survey design effects of individuals clustered in sampling unit of school and stratification of geographic region. The specific genotypes were grouped for analysis according to the extant literature with these candidate genes (56,57). MAOA, which is located at chromosome Xp11.23, was stratified on gender. Two separate sets of multiple logistic regressions were conducted. In the first, regression was used to identify the variables which predicted obesity ($\text{BMI} > 29.9$) using normal weight ($\text{BMI} = 18.5\text{--}24.9$) as a referent in order to identify genetic factors associated with the highest level of risk. In the second, regression was used to predict overweight + obese combined ($\text{BMI} > 25$). This approach allowed for the identification of any risk factors associated with above normal weight. Participants who were underweight (< 18.5 ; $n = 47$) were excluded. Each polymorphism was evaluated separately. Models included the interaction between the allele and either sex or race/ethnicity. Age, race, and parental education level were included as covariates. To clarify the interpretation of significant interaction effects, stratified analyses were conducted and odds ratios were calculated when samples sizes permitted. For stratified analyses where fewer than 5 individuals were represented in a genotype category, the CLUMP program (58) was used with 10,000 simulations to further verify a significant departure of observed values from expected values.

Linear regression models were used to examine the change in BMI from Wave II to Wave III. Although Add Health did collect information regarding height and weight at Wave I (one year prior to Wave II), the information was based on self-report. Thus, to reduce potential bias, a conservative approach was taken by only looking at the change from Wave

II to Wave III where height and weight were measured by study staff. The outcome measure for these analyses was based on a raw score change in BMI (as opposed to a z-score change) as has been recommended by Cole, Faith, Pietrobelli, and Heo (59), and by Berkey and Colditz (2006) (60). Models included age, sex, self-reported race/ethnicity, and parental education as covariates.

RESULTS

Table 1 displays the overall socio-demographics by BMI category (normal weight vs. obese vs. overweight + obese). Bivariate chi square analyses revealed significant differences among BMI category for race/ethnicity, parental education and age ($p < .05$), and a trend for gender (normal vs. overweight + obese, $p = .07$). Table 2 describes genotype frequencies overall and by BMI categories included in the analyses. Genotype distributions in the normal weight BMI strata exhibited HWE (all $P_s > .05$). However, the genotype distributions in the obese BMI strata deviated from HWE for the SLC6A4 marker ($p=0.02$). Similarly, the genotype distributions in the overweight + obese BMI strata also deviated from HWE for the SLC6A4 marker ($p=0.009$). Since the genotype distributions in the larger, all inclusive data set generally met HWE, we do not believe that genotyping errors contributed to the deviations from HWE that we observed in the obese and overweight + obese strata.

The association between genotypes and obesity and overweight + obese can be viewed in Table 3. No associations with obesity or overweight + obese were found for polymorphisms in SLC6A3, DRD2, DRD4, or MAOA (females). Among males, the MAOA polymorphism was found to be associated with obesity and with overweight + obese. Among males, the prevalence of obesity and overweight + obese was significantly higher among carriers of the low activity variant of MAOA. Also among males a significant gene by race/ethnicity interaction was observed for MAOA for the normal vs. overweight + obese comparison (Table 4). The association between the low activity variant and the overweight + obese category was significant for Whites and Hispanics, but not for African-Americans.

A significant main effect for the 5HTTLPR polymorphism in the SLC6A4 gene was also observed (Table 3). The prevalence of obesity and overweight + obese combined was significantly lower among carriers of the l variant compared with individuals homozygous for the s variant. Significant gene by gender interaction revealed that this pattern of greater obesity and overweight + obesity among carriers of the s/s variant was significant for males, but not females (see Table 4). Significant gene by race/ethnicity interactions were also observed for White male homozygotes of either allele of the 5HTTLPR polymorphism, which were overrepresented in the obese and overweight + obese BMI categories (see Table 4). The pattern was somewhat different for Hispanic males where s/s homozygotes or s/l heterozygotes were overrepresented in the obese and overweight + obese BMI categories (see Table 4). As can be seen in Table 4, the confidence intervals for the association between the SLC6A4 gene and obesity and overweight + obesity are wide when the analysis is restricted to Hispanic males, indicating small cell counts for these comparisons. To further verify a significant departure of observed values from expected values the CLUMP (58) program was applied to this subset. The permutation analyses indicated that the genotype frequencies among obese and overweight + obese combined differed significantly in comparison to those in the normal weight group (Table 5). Also note that, as described above, the SLC6A4 polymorphism significantly deviated from HWE in the obese and overweight + obese strata, and this was confined to the non-Hispanic white ethnic group which was the largest ethnic sample.

Analyses also examined change in BMI from Wave II, when participants were on average 16.5 years of age, to Wave III, when participants were on average 22 years of age. The only polymorphism that was significantly associated with change in BMI was the 5HTTLPR polymorphism at the SLC6A4 gene (Table 6). 5HTTLPR *s/s* homozygotes demonstrated the greatest change in BMI from Wave II to Wave III, an average increase of 4.1 BMI units, compared to an increase of an average 3.5 BMI units among *l/l* homozygotes and 3.4 BMI units among *s/l* heterozygotes (Table 6 and Figure 1). A trend, although not significant, between the DRD4 Ex3 VNTR and change in BMI was observed (carriers of at least one 7 repeat allele demonstrated a mean change of 3.36, whereas carriers who did not possess any 7 repeat allele demonstrated a mean change of 3.66, $p = .07$).

DISCUSSION

The primary findings of this study are significant associations between the SLC6A4 5HTTLPR and categories of BMI, and between the MAOA promoter VNTR and categories of BMI among males. These associations were found controlling for other factors (gender, race/ethnicity, age, and parental education). A significant interaction effect was found for the association between these genes and BMI by both race/ethnicity and gender. The subsequent stratified analyses revealed that the association between SLC6A4 and excess BMI was significant for males overall, and for White and Hispanic males. SLC6A4 5HTTLPR genotypes were found to deviate from HWE in the cases (obese and overweight + obese) but not the controls (normal BMI), with the *ss* homozygote genotype found in excess of expectation. Stratified analyses for MAOA among males indicated that the promoter VNTR was significantly associated with overweight + obese in White and in Hispanic males, as well.

A unique contribution of this study is a previously unreported association between obesity and candidate gene SLC6A4 in a U.S. population-based sample. The findings of our study confirm findings from a recently published study demonstrating an association between the *s* allele of the SLC6A4 5HTTLPR polymorphism and overweight among a sample of Argentinean children and adolescents (61). They also extend these findings by providing evidence for association between overweight + obesity and the *s* allele 5HTTLPR homozygote in males of Caucasian race and Hispanic ethnicity. The present findings regarding the association between SLC6A4 and BMI categories are consistent with the putative role of serotonin on food craving and obesity (28,62) and the direction of the results are also consistent with the hypothesized expression effects of the 5HTTLPR polymorphism. The *s* allele is believed to reduce the transcriptional activity of the promoter (63) and is associated with less serotonin transporter protein (63,64). An association between anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region has been noted (63). Thus, one potential hypothesis for the association between this gene and obesity may be a mediating effect of increased dietary intake as a means of affect regulation.

Further, the findings of this study regarding an association between MAOA and BMI are interesting in light of one family-based study (41) and two recent large-scale association studies (40,65). Using the transmission disequilibrium test, evaluating the degree to which parents transmit this gene to their obese offspring, Camarena et al., (2004) found the low active variant to be associated with obesity (41). Extending this in a large population-based study, Need et al., (2006) (40) found that European Caucasian women (Mean age = 47 years) carriers of the low active variant were significantly more likely to be obese (BMI > 30). Ducci et al., (2006) found a significant association between BMI and MAOA genotype, with the low-activity allele associated with a higher BMI among a sample of primarily non-obese male participants with and without a history of alcohol dependence (65).

Our findings indicated some interesting potential gender effects. Gene by gender interactions indicated the relationship between gene (specifically, SLC6A4 and MAOA) and overweight or overweight + obese were significant for males. Need et al. observed an association between MAOA and categories of BMI among their female sample, whereas no association between MAOA and categories of BMI was noted in our sample of females. This may reflect an age related effect, as the mean age of women in our study was much younger (mean age of 22 years in our study vs. mean age of 48 in Need et al. study). Expression of MAOA is affected by ovarian hormones which can fluctuate with age (66). It has been shown that ovarian steroids, particularly estrogen, can decrease MAOA expression, resulting in elevated serotonin levels (66,67). The degree to which these potential age-related effects represent a true effect will need to be verified in other samples with a wider age range.

Dopamine has been associated with the reinforcement value of food (68,69) and variation in dopaminergic activity in the brain has been associated with obesity (13). The association of the MAOA low activity VNTR variant and increased BMI from this study would be consistent, in part, with these observations since MAOA is implicated in dopamine metabolism (40). However, we did not find that polymorphisms in the dopamine D2 and D4 receptors and the dopamine transporter were statistically significantly associated with BMI categories in this young adult sample. It is unlikely that these null results for the main effects models are a result of low statistical power, as many of the cell counts in these models for these dopamine related genes were of sufficient size and much larger than studies to date. Nevertheless, there are several possible explanations. First, candidate gene studies of the TaqI A polymorphism at the D2 dopamine receptor gene have not been completely consistent, with some studies reporting an association (15–17,19,70) and other reporting no association (20–22,26). Second, the difference in samples with regard to age and country of origin also make it difficult to compare our findings to other studies of dopamine candidate genes. Finally, there was only a single polymorphism within each candidate gene which was available for analysis. The authors only had access to the genotypes provided by the Add Health study, and could not perform further genotyping on the data set. It is possible that genetic variations in other parts of these (or other) genes in the dopaminergic system are associated with obesity in this population-based sample. However, it will require future studies to further examine this hypothesis.

The trends observed between DRD4 presented in our study are consistent with the findings reported by Guo et al. (24) using this same public-use data set. These investigators employed a somewhat different analytic procedure using the complete sample of related and unrelated individuals and included data from the self-report of height and weight obtained by the Add Health study at Wave I. These investigators found a significant association between DRD4 and obesity among African-Americans and Hispanics. We chose to limit our analyses to unrelated individuals only, and we further limited our analysis by using measured height and weight assessed during young adulthood to construct BMI categories. When we examined the association between change in BMI and DRD4 we did find an association approaching significance ($p = .07$).

While we are enthusiastic about the findings presented here, caution is warranted in interpretation. Initial reports from candidate gene studies, in general, may overestimate the effect (71) and modest yet significant effects are reported here. Also, we did not apply a Bonferroni correction because many of the phenotypic tests were related and this would have resulted in an over-conservative correction. However, a large number of statistical comparisons were conducted which may have potentially inflated the risk of Type I error. Thus, replication is needed before definitive conclusions can be made about the role of these genes on regulating weight and risk of obesity. Also, future studies are planned to examine

the interactions with other psychological states and traits which may help to better explain these associations as some of the psychological states overlap with risk of obesity. Of note, we did not find that the associations that were present for Hispanics and American Whites were also present for African-Americans in this sample. Furthermore, the association observed for Whites between SLC6A4 and obesity did not exhibit the same pattern as it did for Hispanics. These differences may represent the reduced power in the stratified model, genetic heterogeneity within the Hispanic and African-American groups, or both factors. This will need to be further studied with larger sub-samples of non-Caucasian participants. Another limitation of the current study was that other indicators of adiposity or body composition (e.g., waist circumference, skin fold measures) were not present in the Add Health study. In general, BMI is a good proxy, but examining the association between these candidate genes and other indicators of adiposity and body composition would strengthen the findings. Also of note, these analyses did not account for age of pubertal onset which is potentially important to the analyses of change in BMI; however, given that the median age at Wave II was 17 (Mean = 16.5), this may have had little affect, as most participants would likely have been post-pubertal onset. Further, as described above, it would have been ideal to have additional candidate gene data available, and multiple polymorphisms within the genes in order to test other hypothesis as well as evaluate the effects of population stratification. However, currently only a limited amount of genetic data is available for analysis using this cohort.

Our findings lend further support to of the involvement of dopamine and serotonin regulation on energy balance (9). The results underscore the need for additional research examining the role of these systems on BMI and other energy-balance behaviors (e.g., diet and physical activity). Additional work is needed to identify the potential complex gene by gene and gene by environment interactions that may further characterize these main effects.

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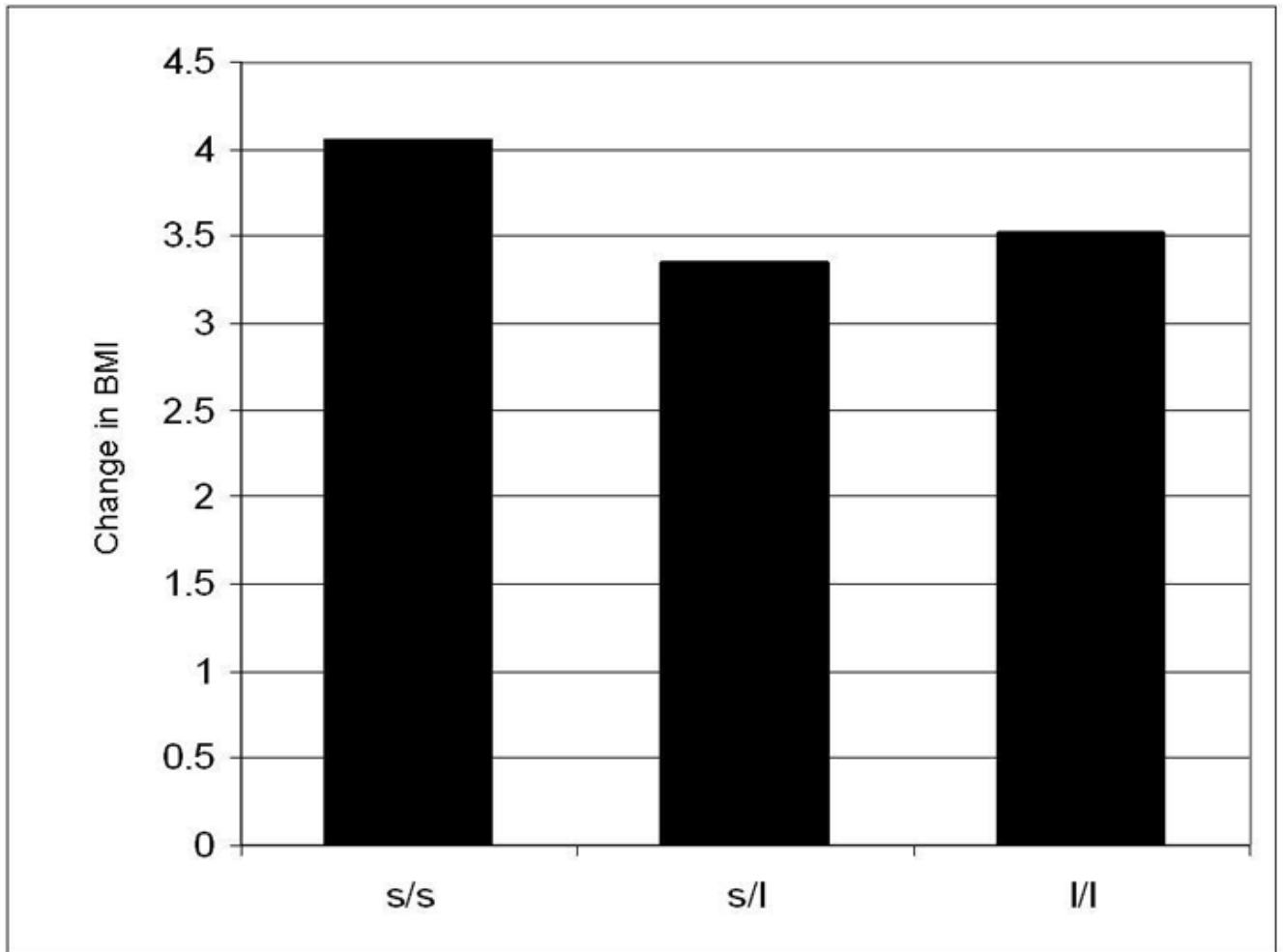


Figure 1. Change in BMI from adolescence to young adulthood as a function of the 5HTTLPR polymorphisms at the SLC6A4 gene

Table 1

Gender, Ethnicity, Parental Education, and Age by Normal Weight, Obese, and Overweight + Obese.

	Normal Weight		Obese		Overweight + Obese	
	N	%	N	%	N	%
Total	751		382		833	
Gender						
Male	350	46.61	167	43.72	424	50.90
Female	401	53.39	215	56.28	409	49.10
Ethnicity						
White	523	69.64	232	60.73*	524	62.90 [†]
Black	139	18.51	67	17.53	145	17.41
Hispanic	89	11.85	83	21.72	164	19.69
Parental Education Level						
Less Than High School	59	7.85	57	14.92*	102	12.24 [†]
High School or Equivalent	146	19.44	117	30.63	232	27.85
Some College	202	26.90	87	22.77	219	26.29
College or Higher	259	34.49	76	19.90	190	22.81
Age	Mean	SD	Mean	SD	Mean	SD
	21.18	0.14	22.18 [‡]	0.13	22.05 [§]	0.13

Normal Weight = BMI 18.5–24.9; Obese = BMI >29.9; Overweight or Obese = BMI >25

* Significant χ^2 comparison between obese vs. normal weight ($p < .05$)[†] Significant χ^2 comparison between overweight + obese vs. normal weight ($p < .05$)[‡] Significant t-tests between obese vs. normal weight ($p < .05$)[§] Significant t-tests between overweight + obese vs. normal weight ($p < .05$)

Table 2

Overall Genotype Frequencies and Frequencies by Normal Weight, Obese, and Overweight + Obese.

	Total		Normal Weight		Obese		Overweight + Obese	
	N	f	N	f	N	f	N	f
DAT								
any 9	599	39.20	287	39.75	143	38.54	312	38.71
10/10	929	60.80	435	60.25	228	61.46	494	61.29
SLC6A4								
s/s	273	17.34	111	14.84	79	20.79	162	19.61
s/l	733	46.57	368	49.20	164	43.16	365	44.19
l/l	568	36.09	269	35.96	137	36.05	299	36.20
DRD2								
A1/A1 or A1/A2	700	44.44	323	43.36	179	46.98	377	45.42
A2/A2	875	55.56	422	56.64	202	53.02	453	54.58
DRD4								
At least one 7 allele	613	38.90	295	39.49	139	36.58	318	38.36
No 7 allele	963	61.10	452	60.51	241	63.42	511	61.64
MAOA (Males)								
Low Activity	314	40.94	133	38.33	76	45.78	181	43.10
Both Active	453	59.06	214	61.67	90	54.22	239	56.90
MAOA (Females)								
Low Activity	474	59.10	231	58.33	138	64.49	243	59.85
Both Active	328	40.90	165	41.67	76	35.51	163	40.15

Normal Weight = BMI 18.5–24.9; Obese = BMI >29.9; Overweight or Obese = BMI >25

Table 3

OR, CI, and p-values for Main Effects Models* and Sex and Ethnicity Interactions

	Normal vs. Obese			Normal vs. Overweight + Obese		
	OR	CI	P	OR	CI	P
DAT						
any 9 vs 10/10	0.99	0.73–1.35	ns	0.96	0.74–1.23	ns
DAT × Sex			ns			ns
DAT × Ethnicity			ns			ns
DRD2						
any A1 vs A2/A2	1.06	0.81–1.36	ns	1.00	0.81–1.25	ns
DRD2 × Sex			ns			ns
DRD2 × Ethnicity			ns			ns
DRD4						
one 7 vs. no 7	0.79	0.58–1.07	ns	0.91	0.72–1.14	ns
DRD4 × Sex			ns			ns
DRD4 × Ethnicity			ns			ns
MAOA						
Females						
Low Activity vs. Both Active	1.00	0.69–1.41	ns	0.93	0.68–1.27	ns
MAOA × Ethnicity			ns			ns
Males						
Low Activity vs. Both Active	1.85	1.18–2.94	0.008	1.41	1.01–1.92	0.04
MAOA × Ethnicity			ns			0.04
SLC6A4						
Main Effect of SLC6A4 [†]			0.036			0.04
s/s vs. l/l	1.31	0.84–2.04	ns	1.21	0.85–1.71	ns
l/l vs s/l	1.32	0.93–1.85	ns	1.23	0.96–1.59	ns
s/s vs. s/l	1.72	1.10–2.70	0.02	1.49	1.05–2.13	0.02
SLC6A4 × Sex			0.048			0.0149
SLC6A4 × Ethnicity			0.005			0.008

* All main effects models adjusted for sex, race, age, parental education level.

[†] Indicates p-value for main effect of gene in model not including interaction terms.

Table 4
OR, CI, and p-values for Stratified Analyses Based on Significant Interaction Effects

	Normal vs. Obese			Normal vs. Overweight + Obese		
	OR	CI	p	OR	CI	p
SLC6A4						
Males						
s/s vs. l/l	1.84	0.98-3.48	ns*	1.69	1.03-2.78	0.04
l/l vs. s/l	1.16	0.68-1.97	ns	1.00	0.71-1.42	ns
s/s vs. s/l	2.14	1.21-3.80	0.01	1.69	1.08-2.66	0.02
White Males						
s/s vs. l/l	1.14	0.57-2.29	ns	1.37	0.76-2.45	ns
l/l vs. s/l	2.64	1.62-4.29	0.0002	1.47	1.05-2.05	0.02
s/s vs. s/l	3.00	1.49-6.05	0.006	2.01	1.16-3.49	0.01
Hispanic Males						
s/s vs. l/l	21.35	1.32-144.5	0.0001	5.76	1.85-17.87	0.0007
l/l vs. s/l	0.08	0.01-0.48	0.0006	0.24	0.10-0.60	0.002
s/s vs. s/l	1.66	0.50-5.52	ns	1.41	0.47-4.16	ns
MAOA (males)						
White						
Low Activity vs. Both Active	ns [†]			1.42	1.00-1.99	0.04
Hispanic						
Low Activity vs. Both Active	ns			3.68	1.47-9.18	0.002

* p-value approached significance p=0.06

[†] nonsignificant interaction effect, thus stratified analyses not computed.

Table 5

SLC6A4 genotype frequencies differed between BMI categories for Hispanic males, with the obese and overweight group showing an excess of the s/s and s/l genotypes.

Gene	Genotype	Normal	Obese	X ² value *	p	Overweight + Obese	X ² value *	p
SLC6A4	s/s	9	15	10.47	0.0028	30	6.47	0.023
	s/l	19	26			50		
	l/l	13	2			12		
Total		41	43			92		

* Calculated using CLUMP (Sham & Curtis, 1995)

Table 6

Results of Multiple Regression Model* Examining the Association between SLC6A4 and Change in BMI from Wave II to Wave III[†] and Least Square Means for Genotype

	beta	se (beta)	CI (beta)	Genotype	LS Means	LS Mean Contrasts	significance
intercept	4.03	0.32	3.39, 4.68				
s/s vs. l/l	0.54	0.27	0.01, 1.07	s/s	4.06	s/s vs. l/l	0.047
s/l vs. l/l	0.17	0.22	-0.61, 0.27	s/l	3.35	s/l vs. l/l	ns
s/s vs. s/l	referent			l/l	3.52	s/s vs. s/l	0.02

* Model adjusted for race/ethnicity, age, sex, and parental education level.

[†] Change based on differences score; Gene × sex and gene × ethnicity interactions not significant