Obesity susceptibility loci and dietary intake in the Look AHEAD Trial[1–3]

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ABSTRACT

Background: Genome-wide association studies (GWAS) have identified consistent associations with obesity. However, the mechanisms remain unclear.

Objective: The objective was to determine the association between obesity susceptibility loci and dietary intake.

Design: The association of GWAS-identified obesity risk alleles (*FTO, MC4R, SH2B1, BDNF, INSIG2, TNNI3K, NISCH-STAB1, MTIF3, MAP2K5, QPCTL/GIPR*, and *PPARG*) with dietary intake, measured through food-frequency questionnaires, was investigated in 2075 participants from the Look AHEAD (Action for Health in Diabetes) clinical trial. We adjusted for age, sex, population stratification, and study site.

Results: Obesity risk alleles at *FTO* rs1421085 significantly predicted more eating episodes per day ($P = 0.001$)—an effect that persisted after adjustment for body weight ($P = 0.004$). Risk variants within *BDNF* were significantly associated with more servings from the dairy product and the meat, eggs, nuts, and beans food groups ($P \leq 0.004$). The risk allele at *SH2B1* rs4788099 was significantly associated with more servings of dairy products ($P = 0.001$), whereas the risk allele at *TNNI3K* rs1514176 was significantly associated with a lower percentage of energy from protein ($P = 0.002$).

Conclusion: These findings suggest that obesity risk loci may affect the pattern and content of food consumption among overweight or obese individuals with type 2 diabetes. The Look AHEAD Genetic Ancillary Study was registered at clinicaltrials.gov as NCT01270763 and the Look AHEAD study as NCT00017953. *Am J Clin Nutr* 2012;95:1477–86.

INTRODUCTION

Obesity is a major public health problem associated with an increased risk of cardiovascular disease (1). Obesity susceptibility loci identified through genome-wide association studies (GWAS)[4] and replicated in multiple independent cohorts have provided new insights into the genetic factors that contribute to the development of obesity. The fat mass and obesity-associated gene *FTO* was one of the first genes to be identified by this approach and has been associated with obesity and body mass in numerous cohorts (2–7). Additional genes associated with obesity include *MC4R* (3), *SH2B1*, *BDNF* (7, 8), and, although less consistently replicated, *INSIG2* (9). More recent GWAS studies also implicate *TNNI3K, NISCH-STAB1, MTIF3, MAP2K5,* and *QPCTL/GIPR* (6). *INSIG2* and the Pro12Ala polymorphism in *PPARG* have also been associated with the degree of weight loss in response to behavioral interventions (10–12).

Many of these genes are expressed in the brain, particularly in the feeding centers of the hypothalamus, emphasizing the role of the central nervous system and potentially dietary intake in obesity predisposition (8). Studies in children indicated that the obesity risk allele at rs9939609 in the *FTO* gene is associated with preference for energy-dense food (13), greater consumption

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of fat and calories (14), consumption of palatable food after having eaten a meal (15), and reduced satiety (16), although a lack of association between *FTO* rs9939609 and caloric intake or the percentage of energy from fat has also been reported (17). In adult populations, *MC4R* obesity risk alleles were shown to be associated with a greater caloric intake and a greater percentage of energy from fat in one study of women from the Nurses' Health Study (18) but not in a second sample of men and women of Scottish descent (19). Obesity risk alleles at *SH2B1* were associated with greater total fat intake, SFA intake, and MUFA intake in Dutch women (20), but no associations of dietary intake with *FTO* or *MC4R* were observed.

The goal of the current study was to determine the association of obesity risk single nucleotide polymorphisms (SNPs) identified through GWAS (within or in the regions of *FTO, SH2B1, BDNF, INSIG2, TNNI3K, NISCH-STAB1, MTIF3, MAP2K5, QPCTL/GIPR, MC4R, INSIG2*, and *PPARG*) and available on the ITMAT-Broad-CARe array chip (IBC) (21) or through additional genotyping with measures of dietary intake in the Look AHEAD (Action for Health in Diabetes) cohort—a sample of ethnically diverse overweight and obese participants with type 2 diabetes.

SUBJECTS AND METHODS

Participants

The design and methods of the Look AHEAD trial have been reported elsewhere (22), as have the baseline characteristics of the randomized cohort (23). Of the 5145 ethnically diverse overweight and obese Look AHEAD subjects with type 2 diabetes, aged 45–76 y at baseline, the first 2757 completed the dietary substudy (24); 2163 of the participants in the dietary substudy provided genetic consent and were in clinical centers participating in this ancillary study. Of the 2163, 65 were missing genetic data as a result of failed genotyping, and 23 were missing genetic data as a result of a high degree of missing genotype calls (>5%), yielding an effective sample size of 2075 (**Figure 1**). Participants who completed the Look AHEAD dietary substudy were somewhat younger than those who did not (57.2 ± 7.2 y compared with 60.5 ± 5.9 y; $P < 0.0001$) because of a change in the age inclusion criteria during year 2 of the recruitment period. No significant differences in sex, race, or educational attainment were found between the 2 groups. All participants included in this study provided written informed consent for participation in the Look AHEAD trial and genetic analyses in accordance with the requirements of the Institutional Review Board at their local institution. The current data analysis was approved by the Miriam Hospital Institutional Review Board, and the procedures that were followed were in accordance with The Miriam Hospital Guidelines.

Anthropometric measures

Weight and height were measured in duplicate by using a digital scale and a standard wall-mounted stadiometer. BMI was calculated as weight (in kg) divided by height (in m) squared.

Dietary assessment

The Look AHEAD semiquantitative, previously validated food-frequency questionnaire (FFQ) was selected to measure food and nutrient intakes (24–26). The FFQ is a modified version of the Diabetes Prevention Program Food-Frequency questionnaire and was designed to collect information about usual intake of food items during the preceding 6 mo. The Diabetes Prevention Program food list, developed for regional and ethnic sensitivity, was formed the basis of the Look AHEAD FFQ food list. The FFQ contained 134 line items, 20 items that can be used to adjust the 134 main items (ie, type of oil used when cooking, fat added to vegetables), and 3 quality-control questions. Meal-replacement beverages and bars were added as line items to the FFQ.

For each line item, the respondents reported their frequency of consumption and portion size consumed. The 9 frequency categories for food items ranged from "never or less than once per month" to "2 or more times per day." The 9 frequency categories for beverages ranged from "never or less than once per month" to "6 or more times per day." Portion sizes were listed as small, medium, or large. The maximum category of frequency of consumption for foods does not apply to meal replacements that could be considered foods (bars) or beverages (liquid meal replacements or reconstituted powders). The maximum category for frequency of consumption of meal replacements (either liquid, bar, or powder) was >4 times/d.

Estimates of The Food Guide Pyramid (27) food group and nutrient intake were conducted by using the Health Habits and History Questionnaire/DietSys software and Look AHEAD–specific programming written to incorporate the Look AHEAD modifications to the questionnaire. The nutrient database was modified from the Diabetes Prevention Program database to incorporate foods added for the Look AHEAD FFQ. These nutrient values were obtained primarily from the Nutrition Data System for Research (version 4.01_30, 1999; Nutrition Coordinating Center). The number of daily eating episodes was defined as the total number of meals and snacks consumed per day as reported on the FFQ.

Genotyping

The genomic DNA extraction is based on the use of the FlexiGene DNA Kit (Qiagen Inc) as described by the manufacturer, and DNA quantitation was performed by using the PicoGreen dsDNA Quantitation Reagent (Invitrogen Inc). Genotyping was carried out at the Children's Hospital of Philadelphia by using the IBC chip, a gene-centric 50,000-SNP array designed to assess potentially relevant loci across a range of cardiovascular, metabolic, and inflammatory syndromes (21). The data set with 14 SNP genotypes at or near 9 reported genes was filtered for individuals with <5% missing genotypes and SNPs with <5%

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[4]Abbreviations used: bp, base pair; CEU, US residents of European ancestry; FFQ, food-frequency questionnaire; GWAS, genome-wide association study; IBC, ITMAT-Broad-CARe array chip; Look AHEAD, Action for Health in Diabetes; MAF, minor allele frequency; PFG, pyramid food group; SNP, single nucleotide polymorphism; YRI, Yoruba people of Ibadan.

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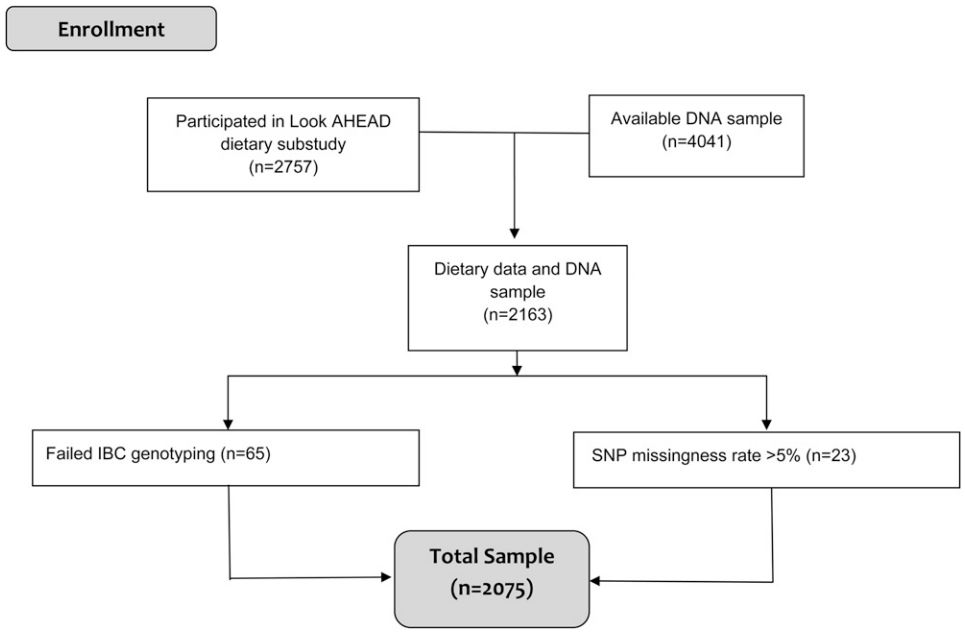

FIGURE 1. Consort flow diagram. IBC, ITMAT-Broad-CARe array chip; Look AHEAD, Look AHEAD (Action for Health in Diabetes) clinical trial; SNP, single nucleotide polymorphism.

missing data. The mean genotyping success rate for the candidate SNPs was 99.6%. Two additional SNPs in the regions of 2 additional genes were genotyped with Taqman Applied Biosystems Assays-On-Demand by using an Applied Biosystems 7900HT: the *MC4R* polymorphism rs17782313 (Applied Biosystems catalog number C__32667060_10) and the *INSIG2* rs7566605 polymorphism (Applied Biosystems catalog number C__29404113_20).

Gene and SNP selection

We searched the published literature and selected SNPs that had been associated with obesity by GWAS (2–9, 28, 29) or weight loss (10, 12) and appeared on the IBC (21) or, in the case of *MC4R* and *INSIG2*, were genotyped by Taqman. The SNP array also included Ancestry Informative SNP Markers and haplotype-tagging SNPs. GWAS obesity SNPs not on the IBC were replaced by proxies when possible by using the SNP Annotation and Proxy Search tool (30) based on haplotype maps of US residents of European ancestry (CEU) and Yoruba people of Ibadan (YRI) as follows: *FTO* rs9930506 was replaced by rs9922708 [distance 681 base pair (bp) $r^2 = 1.00$, $D' = 1.00$ in both CEU and YRI], *BDNF* rs925946 was replaced by rs1401635 (distance 26,789 bp $r^2 = 0.96$, $D' = 1.00$ in CEU; no proxy was available in YRI), *SH2B1* rs7498665 was replaced by rs4788099 (distance 27,514 bp $r^2 = 1.00$, $D' = 1.00$ in CEU and $D' = 1.00$ and $r^2 = 0.94$ in YRI), *TNNI3K* rs1514175 was replaced by rs1514176 (distance 48 bp $r^2 = 1.00$, $D' = 1.00$ in CEU and $r^2 = 1.00$, $D' = 1.00$ in YRI), *NISCH-STAB1* rs6784615 was replaced by rs4687617 (distance 2851 bp $r^2 = 1.00$, $D' = 1.00$ in CEU; no proxy was available in YRI), *MTIF3* rs4771122 was replaced by rs7988412 (distance 19898, $r^2 = 0.83$, $D' = 1.00$ in CEU; no proxy was available in YRI), *MAP2K5* rs2241423 was replaced by rs2241420 (distance 4022 bp, $r^2 = 0.91$, $D' = 1.00$ in CEU, $r^2 = 0.97$, $D' = 1.00$ in YRI), and *QPCTL/GIPR* rs2287019 was replaced by rs11672660 (distance 21988 bp, $r^2 = 0.83$, $D' = 1.00$ in CEU, $r^2 = 0.892$, $D' = 1.00$ in YRI).

Statistical analysis

Observed genotype frequencies were compared with those expected under Hardy-Weinberg equilibrium by using a chi-square test in the 2 most populous racial-ethnic groups (non-Hispanic whites and African Americans). Pearson correlations were used to examine the association between dietary variables, BMI, and weight. Multivariable linear regression analyses were carried out to examine genetic associations with the dietary variables. Additive coding for the number of copies of rare alleles was used, unless the marker minor allele frequency (MAF) fell below 20%, in which case the rare genotype was combined with the intermediate genotype. Therefore, linear regression coefficients capture the effect either of a single copy of the rare allele (MAF $\geq$0.20) or of being a rare allele carrier (MAF $<$0.20). In cases in which the risk allele differs from the rare allele, negating the regression coefficients gives the effect of each additional copy of the risk allele (MAF $\geq$0.20) or of being a risk allele homozygote (MAF $<$0.20).

All analyses were adjusted for age, sex, study site, and population stratification. For total caloric intake, we further considered whether any observed associations were attributable to weight in secondary models. For eating occasions and the Food Guide Pyramid food groups, we considered whether any observed associations were attributable to total caloric intake by including total caloric intake as a covariate in secondary models. A Huber-White sandwich estimator was used to produce SE robust to deviations from normality (31, 32). Principal component analysis of the genotypic correlation matrix of the 16 markers of interest suggested that the effective number of linearly independent markers in the data set was only 13 (33). Therefore, one can maintain the family-wise error rate at 0.05 via Sidak's adjustment

for multiplicity by declaring as statistically significant only those markers with a nominal significance level of 0.05/13 = 0.004 (34). However, because the markers were selected a priori due to their association with obesity, we also consider associations reaching a nominal threshold for statistical significance of 0.05. Analyses were performed by using Splus 8.2 for Solaris/Linux (35). The analyses were performed at Brown University.

To control for admixed study population, all IBC SNPs were examined by principal component analysis using the EIGENSTRAT algorithm (36) as implemented in Golden Helix version 7.1 (Bozeman). Results indicated that most of the variance among the Look AHEAD cohort was accounted for by the first 2 principal components, which agreed with self-reported ethnicity. Accordingly, the first 2 principal components were included as covariates in our analyses to adjust for population stratification in the multi-ethnic Look AHEAD cohort.

RESULTS

Participant characteristics of the subcohort of Look AHEAD used in these analyses are shown in **Table 1**. Participants came from an ethnically diverse background, and 56% were women. They had an average age of 57.6 y and a BMI in the obese range. Participants reported consuming ~2000 kcal/d over an average of 4.7 eating episodes. Forty percent of calories were derived from fat, 44% from carbohydrate, and 17% from protein.

SNP characteristics, including the obesity-risk allele identified in the prior literature, are presented in **Table 2**. All SNPs under study conformed to Hardy-Weinberg equilibrium, except for rs2241420 in non-Hispanic whites ($P < 0.001$) and rs1401635 in African Americans ($P = 0.02$). Given that this sample was selected for being overweight/obese and having type 2 diabetes and that the genetic markers were selected for association with obesity, we retained these markers in analyses.

The 4 *FTO* SNPs were in strong linkage disequilibrium in our white subsample ($r^2 = 0.80$–0.99), but differed in the degree of disequilibrium in our African American subsample (rs3751812 and rs1421085: $r^2 = 0.96$; rs3751812 and rs9922708: $r^2 = 0.51$; rs1421085 and rs9922708: $r^2 = 0.48$; rs9939609 with other SNPs: $r^2 < 0.13$). Two *BDNF* SNPs, rs6265 and rs10767664, were in strong linkage disequilibrium in both our white ($r^2 = 0.79$) and African American ($r^2 = 0.69$) subsamples. The third *BDNF* SNP, rs1401635, appeared unrelated to the first two ($r^2 < 0.11$ in both whites and African Americans).

Total caloric intake was positively associated with weight ($r = 0.22$, $P < 0.001$), BMI ($r = 0.14$, $P < 0.001$), and number of eating episodes ($r = 0.17$, $P < 0.001$). Number of eating episodes showed no association with BMI ($r = 0.00$, $P = 0.926$) or weight ($r = -0.04$, $P = 0.108$).

TABLE 1
Baseline characteristics

Characteristic	Value (n = 2075)
Women [n (%)]	1163 (56.0)
Race [n (%)]	
African American	324 (15.6)
American Indian/Alaskan Native[1]	12 (0.6)
Asian/Pacific Islander	23 (1.1)
White	1595 (76.9)
Other (multiple)	121 (5.8)
Ethnicity [n (%)]	
Hispanic/Latino	161 (8.2)
Age (y)	57.6 ± 7.2[2]
BMI (kg/m^2)	36.3 ± 6.1
Dietary intake	
Total energy intake (kcal)	1992.1 ± 875.7
Eating occasions (no./d)	4.7 ± 1.2
Carbohydrate (% of energy)	43.6 ± 7.7
Fat (% of energy)	40.0 ± 7.0
Protein (% of energy)	17.2 ± 2.9
Bread, cereal, rice, pasta (servings/d)	3.2 ± 1.7
Vegetables (servings/d)	2.9 ± 1.5
Fruit (servings/d)	1.9 ± 1.4
Milk, yogurt, cheese (servings/d)	2.2 ± 1.6
Meat, poultry, fish, dry beans, eggs, nuts (servings/d)	2.7 ± 1.5
Fats, oils, sweets (servings/d)	2.2 ± 1.8

[1] The number of American Indian participants included in this study is less than that in the parent Look AHEAD trial.
[2] Mean ± SD (all such values).

Total calories

Obesity risk markers within *BDNF* and *FTO* were nominally associated with greater total caloric intake (**Table 3**). Risk alleles at *FTO* rs1421085, rs3751812, and rs9922708 were associated with 57–60 more calories per day per copy ($P = 0.031$–0.035). Carriers of the *AA* genotype at *BDNF* rs10767664 or the *GG* genotype at *BDNF* rs6265 consumed on average >100 kcal/d more than did carriers of the less common genotypes ($P = 0.006$–0.007). The effect of the 3 *FTO* SNPs on dietary intake was diminished by statistical adjustment for weight ($P = 0.066$–0.068). However, *BDNF* rs10767664 and rs6265 remained nominally associated with total caloric intake after statistical adjustment for weight (rs6265, $P = 0.007$; rs10767664, $P = 0.007$).

Number of eating episodes

Genetic associations with number of eating episodes are presented in **Table 4**. Obesity risk alleles at rs1421085 within *FTO* were significantly associated with eating a greater number of meals and snacks per day ($P = 0.001$), an effect that persisted after further adjustment for total caloric intake ($P = 0.004$). Obesity risk alleles at *FTO* rs3751812, rs9922708, and rs9939609 showed similar effects in the same direction but significance was nominal ($P = 0.014$–0.039). For *QPCTL/GIPR* rs11672660, *MC4R* rs17782313, and *PPARG* rs1801282, risk alleles associated with obesity or diabetes and resistance to weight loss in the prior literature were nominally associated with fewer eating occasions per day. Statistical adjustment for total caloric intake did not substantially alter these associations.

Percentage of energy from fat, carbohydrate, and protein

Genetic associations with percentage of energy from fat, carbohydrate, and protein are presented elsewhere (*see* Supplemental Table 1 under "Supplemental data" in the online issue). The obesity risk allele at rs1514176 (*TNNI3K* region) was significantly associated with a lower percentage of energy from protein ($P = 0.002$; −0.28% per copy). Each copy of the obesity risk allele at *FTO* rs1421085 was also nominally associated with a greater percentage of energy from fat ($P = 0.019$; 0.52% per

TABLE 2
SNP characteristics[1]

Chromosome	Closest gene	SNP	Major allele	Minor allele	MAF	Obesity risk allele
1	*TNNI3K*	rs1514176	A	G	0.48	G
2	*INSIG2*	rs7566605	G	C	0.31	C
3	*PPARG*	rs1801282	C	G	0.09	C[2]
3	*NISCH-STAB1*	rs4687617	A	G	0.05	A
11	*BDNF*	rs10767664	A	T	0.18	A
11	*BDNF*	rs6265	G	A	0.16	G
11	*BDNF*	rs1401635	G	C	0.29	C
13	*MTIF3*	rs7988412	G	A	0.18	A
15	*MAP2K5*	rs2241420	G	A	0.30	G
16	*SH2B1*	rs4788099	A	G	0.37	G
16	*FTO*	rs1421085	T	C	0.40	C
16	*FTO*	rs3751812	C	A	0.39	A
16	*FTO*	rs9922708	G	A	0.43	A
16	*FTO*	rs9939609	T	A	0.45	A
18	*MC4R*	rs17782313	T	C	0.25	C
19	*QPCTL/GIPR*	rs11672660	G	A	0.19	G

[1] MAF, minor allele frequency; SNP, single nucleotide polymorphism.
[2] The C allele at *PPARG* rs1801282 has been associated with diabetes and resistance to weight loss.

copy). No association with percentage of energy from carbohydrate and no other associations between the obesity risk alleles and percentage of energy from fat or carbohydrate were observed.

Food Guide Pyramid food groups

Several associations of obesity risk alleles with daily servings of breads, cereals, rice and pasta (pyramid food group 1 [PFG1]); dairy products (PFG4); meats, eggs, nuts and beans (PFG5); and fats, oils, and sweets (PFG6) were observed (*see* Supplemental Table 2 under "Supplemental data" in the online issue). For example, risk variants within *BDNF* were significantly associated with more servings from the dairy product and the meat, eggs, nuts, and beans food groups ($P \leq 0.004$), whereas the risk allele at *SH2B1* rs4788099 was also significantly associated with more servings of dairy products ($P = 0.001$). Statistical adjustment for total caloric intake largely diminished these associations, which suggests that any genetic associations with servings within these food groups were likely mediated via previously noted effects on total caloric intake. In the primary exception,

TABLE 3
Baseline association of the minor allele at each single nucleotide polymorphism with total caloric intake[1]

			Adjusted for age, sex, study site, and population stratification		Adjusted for age, sex, study site, population stratification, and weight	
Gene	Single nucleotide polymorphism	Minor allele	$\beta \pm$ SE	*P* value	$\beta \pm$ SE	*P* value
Total energy intake (kcal)						
TNNI3K	rs1514176	G	45.193 ± 26.169	0.085	42.724 ± 25.711	0.097
INSIG2	rs7566605	C	−20.850 ± 27.815	0.453	−17.082 ± 27.434	0.534
PPARG	rs1801282	G[2]	97.386 ± 52.528	0.064	94.497 ± 52.129	0.070
NISCH-STAB1	rs4687617	G	85.890 ± 61.122	0.160	78.626 ± 60.108	0.191
BDNF	rs10767664	T[2]	−104.005 ± 38.290	0.007[3]	−103.103 ± 37.993	0.007[3]
BDNF	rs6265	A[2]	−107.090 ± 38.659	0.006[3]	−103.370 ± 38.332	0.007[3]
BDNF	rs1401635	C	−25.061 ± 26.324	0.341	−23.982 ± 26.115	0.359
MTIF3	rs7988412	A	3.427 ± 40.260	0.932	0.223 ± 39.938	0.995
MAP2K5	rs2241420	A	8.400 ± 28.813	0.771	14.043 ± 28.402	0.621
SH2B1	rs4788099	G[2]	33.762 ± 27.025	0.211	22.708 ± 26.838	0.398
FTO	rs1421085	C	60.139 ± 27.833	0.031[3]	50.581 ± 27.638	0.067
FTO	rs3751812	A	58.685 ± 27.836	0.035[3]	50.746 ± 27.618	0.066
FTO	rs9922708	A	56.935 ± 26.736	0.033[3]	48.480 ± 26.540	0.068
FTO	rs9939609	A	45.604 ± 27.764	0.101	38.738 ± 27.434	0.158
MC4R	rs17782313	C	17.953 ± 30.710	0.559	12.429 ± 30.497	0.684
QPCTL/GIPR	rs11672660	A[2]	27.161 ± 40.326	0.501	34.735 ± 39.823	0.383

[1] All analyses were conducted with multivariable linear regression with the statistical covariates listed in the table ($n = 2075$). Statistical significance was determined after correction for multiple comparisons, or $P \leq 0.004$.
[2] As the marker minor allele frequency fell below 20%, the rare genotype was combined with the intermediate genotype.
[3] *P* values of nominal significance.

TABLE 4
Baseline association of the minor allele at each single nucleotide polymorphism with the number of eating occasions[1]

Gene	Single nucleotide polymorphism	Minor allele	Adjusted for age, sex, study site, and population stratification		Adjusted for age, sex, study site, population stratification, and total energy intake	
			$\beta \pm$ SE	*P* value	$\beta \pm$ SE	*P* value
Eating occasions (no./d)						
TNNI3K	rs1514176	G	−0.030 ± 0.037	0.414	−0.041 ± 0.036	0.255
INSIG2	rs7566605	C	−0.042 ± 0.038	0.263	−0.037 ± 0.037	0.317
PPARG	rs1801282	G[2]	0.153 ± 0.074	0.038[3]	0.128 ± 0.073	0.080
NISCH-STAB1	rs4687617	G	0.027 ± 0.082	0.742	0.006 ± 0.081	0.945
BDNF	rs10767664	T[2]	−0.024 ± 0.056	0.661	0.003 ± 0.055	0.951
BDNF	rs6265	A[2]	−0.005 ± 0.058	0.927	0.023 ± 0.057	0.682
BDNF	rs1401635	C	−0.054 ± 0.039	0.160	−0.048 ± 0.038	0.205
MTIF3	rs7988412	A	0.028 ± 0.054	0.601	0.026 ± 0.053	0.623
MAP2K5	rs2241420	A	−0.023 ± 0.037	0.524	−0.026 ± 0.036	0.470
SH2B1	rs4788099	G[2]	−0.003 ± 0.038	0.936	−0.011 ± 0.037	0.758
FTO	rs1421085	C	0.125 ± 0.039	0.001[4]	0.109 ± 0.038	0.004[4]
FTO	rs3751812	A	0.096 ± 0.039	0.014[3]	0.081 ± 0.038	0.034[3]
FTO	rs9922708	A	0.077 ± 0.037	0.039[3]	0.063 ± 0.037	0.087
FTO	rs9939609	A	0.087 ± 0.037	0.018[3]	0.075 ± 0.036	0.037[3]
MC4R	rs17782313	C	−0.113 ± 0.041	0.006[3]	−0.112 ± 0.041	0.006[3]
QPCTL/GIPR	rs11672660	A[2]	0.154 ± 0.054	0.005[3]	0.146 ± 0.053	0.006[3]

[1] All analyses were conducted with multivariable linear regression with the statistical covariates listed in the table (n = 2075).
[2] Because the marker minor allele frequency fell below 20%, the rare genotype was combined with the intermediate genotype.
[3] *P* values of nominal significance.
[4] Statistical significance after correction for multiple comparisons, or $P \leq 0.004$.

statistical adjustment for total caloric intake did not substantially alter the association of *SH2B1* rs4788099 with more servings per day of dairy products (P = 0.002). No associations were observed with servings of vegetables (PFG2) or fruit (PFG3) before or after statistical adjustment for total caloric intake.

DISCUSSION

GWAS have been successful in identifying common genetic variants that are associated with obesity. However, the mechanisms through which these polymorphisms affect obesity remain unclear. Here we report that select obesity genetic risk markers may affect meal patterning and servings per day within specific Food Guide Pyramid food groups, such as dairy products, as measured by an FFQ.

Obesity risk alleles at *FTO* rs1421085 were significantly associated with a greater number of meals and snacks per day, with nominal associations with greater total caloric intake, greater percentage of energy from fat, and more servings of fats, oils, and sweets. The association of *FTO* obesity risk SNPs with a greater number of eating occasions was not substantially diminished by adjustment for total caloric intake. The overall picture suggests that variation in *FTO* may bias meal patterning and perhaps total caloric intake and consumption of sweet or high-fat foods.

Because rs1421085 is in high linkage disequilibrium with rs9939609, our results are largely consistent with prior research in children, which indicates that *FTO* rs9939609 is associated with a preference for energy-dense food (13), greater consumption of fat and calories (14), consumption of palatable food after having eaten a meal (37), and reduced satiety (15). However, we tested rs9939609 directly in our analyses and found more consistent associations with rs1421085. Previously in adults, no association between *FTO* rs9939609 and caloric intake or percentage of energy from fat intake has been found (17, 20). Although this suggests that rs1421085 may better capture the effect of this region on dietary intake, the functional significance of this locus in obesity and the role of both rs1421085 and rs9939609 remain to be determined (38, 39).

Risk alleles at *BDNF* rs6265 and rs10767664 predicted a pattern of dietary variables, including servings of meats, eggs, nuts, and beans and servings of dairy products with nominal associations with total caloric intake; servings of breads, cereals, rice, and pasta; and servings of sweets and fats. *BDNF* and its primary receptor TrkB are expressed key brain regions of the hypothalamus and dorsal vagal complex related to body weight and energy homeostasis (40–44). Targeted disruption of *BDNF* in transgenic models results in hyperphagia and obesity (45–49). *BDNF* rs6265 leads to a valine-to-methionine substitution at position 66 (Val66Met) in the prodomain of the gene. Moreover, at least one case study also links rare mutations in *BDNF* to severe obesity in an 8-y-old girl (50). The associations with servings within the various food groups were diminished on statistical adjustment for total caloric intake, which suggests that the primary effect was on caloric intake but that the total was achieved through servings with the associated food groups.

The risk allele at rs1514176, an intronic SNP located within *TNNI3K*, was significantly associated with a lower percentage of energy from protein and nominally associated with more daily servings of fats, oils, and sweets. *TNNI3K* is a cardiac troponin-interacting kinase expressed primarily in the heart (51). Little is currently known about the mechanisms through which this gene

region influences obesity. Because protein is considered to be the most satiating macronutrient (52), diets lower in protein intake may actually contribute to increased overall energy intake as a result of reduced overall feelings of fullness and thereby to a greater degree of positive energy balance.

The risk allele at rs4788099 in the *SH2B1* region was significantly associated with more daily servings of dairy products. *SH2B1* is involved in leptin signaling. Deletions in this region, including *SH2B1*, have been associated with early-onset obesity (53). Whereas consumption of more daily servings of dairy products, particularly lower-fat dairy products, has been associated with lower adiposity (54), the food group in this investigation contains both low- and high-fat products. Thus, this relation may be driven by greater intake of high-fat dairy product servings.

It was interesting to note that the number of eating episodes was among the dietary phenotypes most strongly associated with genetic polymorphisms. The relation of number of eating occasions and risk of overweight has been debated. In laboratory studies, it has been shown in some but not all (55, 56) studies that spreading the same total caloric intake over a greater number of meals per day may better control appetite. In free-living populations, it is quite plausible that extra eating occasions could promote greater caloric intake and weight gain; however, the data appear mixed. Certain studies find that a greater number of meals per day is associated with lower body weight (57), whereas others find the opposite effect or no association (58, 59).

The strengths of this study included a large ethnically diverse sample inclusive of men and women, control for population stratifications, use of a well-validated FFQ, and genotyping of many genetic markers previously associated with obesity.

Limitations include basing conclusions on a select cohort of individuals (overweight or obese with type 2 diabetes) and, therefore, conclusions may not be generalizable. Similar to other genetic association studies, the size of the cohort may have prevented us from detecting more modest effects. Although we observed consistent patterns of association, many of the associations were of nominal significance and replication in an independent cohort should be conducted to support or refute these results.

Our study was also cross-sectional in design, which prevented us from determining the time precedence of genetic associations with diet and obesity (eg, whether genetic associations with diet preceded genetic associations with obesity, or vice versa). For example, if diet is a mechanism through which these SNPs increase risk of obesity, it would be expected that associations with diet would precede associations with obesity. It is also possible that people with larger body sizes eat more to maintain their weight, in which case genetic associations with obesity would be expected to precede those with diet. These are important questions for future research with longitudinal modeling.

The measurement of dietary intake also has limitations. Whereas the USDA's 5-pass method, 24-h dietary recall is currently considered to be the gold standard of dietary assessment (60, 61), FFQs are usually used in large samples to assess dietary intake over a specified time period because of their low cost, ease of administration (62), and prior validation against the 24-h dietary recall (26). Because FFQs are conducted by self-report, it is recognized that underreporting of dietary intake is common, particularly in individuals who are overweight (63–66), have diabetes (66), and are wanting to reduce their weight (64). It is plausible that a tendency to underreport dietary intake may have influenced our results. Given the variability of body weight in the sample, it is also possible that systematic underreporting may have occurred, such that greater underreporting occurred among those with the highest BMI. Moreover, physical activity and other factors that determine energy expenditure and thereby energy intake may influence the correlation between weight and caloric intake. Nonetheless, underreporting should have restricted the range of self-reported dietary intake in our sample, rendering the detection of significant associations more difficult. Thus, the identification of genetic associations with dietary intake via FFQs suggests that the strength of these associations may improve with more precise measurement.

In summary, our results suggest that select obesity genetic risk markers—particularly markers within *FTO, BDNF, TNNI3K*, and *SH2B1*—predict a pattern of obesogenic dietary intake, including a higher number of eating occasions per day and more servings from calorically dense food groups. If replicated, these results could inform the mechanisms through which these genetic markers are associated with adiposity.

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