

Genome-wide meta-analysis of observational studies shows common genetic variants associated with macronutrient intake^{1–4}

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ABSTRACT

Background: Macronutrient intake varies substantially between individuals, and there is evidence that this variation is partly accounted for by genetic variants.

Objective: The objective of the study was to identify common genetic variants that are associated with macronutrient intake.

Design: We performed 2-stage genome-wide association (GWA) meta-analysis of macronutrient intake in populations of European descent. Macronutrients were assessed by using food-frequency questionnaires and analyzed as percentages of total energy consumption from total fat, protein, and carbohydrate. From the discovery GWA ($n = 38,360$), 35 independent loci associated with macronutrient intake at $P < 5 \times 10^{-6}$ were identified and taken forward to replication in 3 additional cohorts ($n = 33,533$) from the DietGen Consortium. For one locus, fat mass obesity-associated protein (*FTO*), cohorts with Illumina MetaboChip genotype data ($n = 7724$) provided additional replication data.

Results: A variant in the chromosome 19 locus (rs838145) was associated with higher carbohydrate ($\beta \pm \text{SE}$: $0.25 \pm 0.04\%$; $P = 1.68 \times 10^{-8}$) and lower fat ($\beta \pm \text{SE}$: $-0.21 \pm 0.04\%$; $P = 1.57 \times 10^{-9}$) consumption. A candidate gene in this region, fibroblast growth factor 21 (*FGF21*), encodes a fibroblast growth factor involved in glucose and lipid metabolism. The variants in this locus were associated with circulating FGF21 protein concentrations ($P < 0.05$) but not mRNA concentrations in blood or brain. The body mass index (BMI)-increasing allele of the *FTO* variant (rs1421085) was associated with higher protein intake ($\beta \pm \text{SE}$: $0.10 \pm 0.02\%$; $P = 9.96 \times 10^{-10}$), independent of BMI (after adjustment for BMI, $\beta \pm \text{SE}$: $0.08 \pm 0.02\%$; $P = 3.15 \times 10^{-7}$).

Conclusion: Our results indicate that variants in genes involved in nutrient metabolism and obesity are associated with macronutrient consumption in humans. Trials related to this study were registered at clinicaltrials.gov as NCT00005131 (Atherosclerosis Risk in Communities), NCT00005133 (Cardiovascular Health Study), NCT00005136 (Family Heart Study), NCT00005121 (Framingham Heart Study), NCT00083369 (Genetic and Environmental Determi-

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INTRODUCTION

Considerable variation in dietary choices exists across individuals. Human eating behavior is driven by many psychological and social factors, including culture, economics, and

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⁵Abbreviations used: CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; FFQ, food-frequency questionnaire; FGF21, fibroblast growth factor 21; GWA, genome-wide association; SNP, single nucleotide polymorphism.

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health beliefs. There is evidence from twin and family studies that consumption of major macronutrients has a genetic component, with estimated heritability ranging from 8% to 70% (1). Importantly, the specific genes that might underlie these associations have yet to be elucidated. The identification of genetic loci underlying macronutrient intake could provide insight into the biology of human dietary behaviors. Genome-wide linkage studies have identified several chromosomal regions for macronutrient intake (2–5). Some notable candidate genes within the linkage region include pro-opiomelanocortin (*POMC*), adiponectin (*ADIPOQ*), melanocortin receptor 4 (*MC4R*), and peroxisome proliferator-activated receptor γ (*PPAR\gamma*). Furthermore, candidate gene association studies have focused primarily on genes involved in the central control of food intake, such as dopamine receptor (*DRD2*) (6, 7) and serotonin receptor (*HTR2A*) (8, 9), and the obesity genes fat mass obesity-associated protein (*FTO*) (10–13) and *MC4R* (11, 13, 14). Many of the results are inconsistent or lack replication to confirm the initial findings. The disparate findings between the studies may be a result of the differences in the study population, dietary assessment methods, or statistical methods used.

To identify common genetic variants associated with macronutrient intake, we conducted a meta-analysis of genome-wide association (GWA)⁵ analyses of carbohydrate, protein, and total fat intake by using data from 12 discovery cohorts ($n = 38,360$) from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Nutrition Working Group (15). The top signals from this analysis were combined in a joint analysis with data from a second macronutrient meta-analysis of data from 3 cohorts ($n = 33,533$) from the DietGen Consortium (16). For single nucleotide polymorphisms (SNPs) that were directly genotyped on the Illumina MetaboChip, data from an additional 3 cohorts ($n = 7724$) were used as replication data.

SUBJECTS AND METHODS

GWA cohorts

GWA was conducted in 37,537 subjects from the following 12 cohorts from the CHARGE Consortium Nutrition Working Group: the Atherosclerosis Risk in Communities Study; the Cardiovascular Health Study; the European Prospective Investigation into Cancer and Nutrition–Norfolk (EPIC–Norfolk); the Family Heart Study; the Fenland Study; the Framingham Heart Study; the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study; the Health, Aging, and Body Composition (Health ABC) Study; the InCHIANTI Study; the Multi-Ethnic Study of Atherosclerosis (MESA); the Rotterdam Study; and the Young Finns Study. Each cohort's study protocol was reviewed and approved by their respective institutional review board (see Supplemental Table S1 under “Supplemental data” in the online issue).

Stage 2 replication MetaboChip cohorts

Data from the Gene-Lifestyle interactions And Complex traits Involved in Elevated disease Risk (GLACIER) Study from Sweden, the Malmö Diet and Cancer Study from Sweden, and The Hellenic study of Interactions between Snps and Eating in Atherosclerosis Susceptibility ($n = 7724$) were used to replicate SNPs identified by stage 1 GWA that was genotyped by the MetaboChip.

Stage 2 replication GWA cohorts

A second GWA meta-analysis of macronutrient intake was conducted in parallel by the DietGen Consortium [$n = 33,533$ (16)]. The consortium is composed of 3 US population-based cohorts: the Health Professionals Follow-Up Study (17), the Nurses' Health Study (18), and the Women's Genome Health Study (19). The DietGen Consortium performed GWA analysis by using the same methods as the CHARGE Consortium.

Assessment of macronutrient intake

Average dietary intake was assessed by using food-frequency questionnaires (FFQs). The type of FFQ used in each study varied slightly, with this variation generally designed to best capture the dietary habits of the population under study (*see* Supplemental Table S2 under "Supplemental data" in the online issue). Among these various CHARGE cohorts, 87% of the FFQs had been validated against other dietary assessment methods. On the basis of the responses to each FFQ and study-specific nutrient databases, usual nutrient consumption was calculated. The present analysis focused on the percentage of energy from total fat, protein, and carbohydrate intake.

Heritability estimates

In the Framingham Heart Study and the Family Heart Study, the heritability of macronutrient adjusted for age and sex was estimated by using the variance components method in Sequential Oligogenic Linkage Analysis Routines (SOLAR; Texas Biomedical Research Institute).

Genotyping and GWA analysis

Genome-wide genotyping was conducted by using Affymetrix or Illumina platforms. Each study performed quality control for genotyped SNPs on the basis of minor allele frequency, call rate, and departure from Hardy-Weinberg equilibrium (*see* Supplemental Table S3 under "Supplemental data" in the online issue). Phased haplotypes from HapMap CEU (build 35 or 36) were used to impute ~ 2.5 million autosomal SNPs by using a Hidden Markov model algorithm implemented in MACH (20), IMPUTE (21), or BimBam (22). Study-specific GWA analyses were conducted for each macronutrient by using genotyped and imputed SNP dosages assuming an additive genetic model. The base model included age and sex for all studies and study-specific covariates (eg, study site) and population stratification principal components when applicable (model 1). In a second model (model 2), BMI was added to the covariates in model 1. SNPs with low minor allele frequency ($<1\%$), low imputation quality (MACH: $R_{sq} < 0.3$; or IMPUTE: proper info < 0.4) were removed. The results from each study were combined in a fixed-effects meta-analysis with inverse variance weights by using METAL software (23). To account for population stratification, the association results from individual studies as well as meta-analyses were adjusted for genomic control.

To increase the power to detect significant associations, a joint analysis with results from the DietGen Consortium ($n = 33,533$) was conducted for 35 independent genomic regions containing SNPs with $P < 10^{-6}$, a threshold that was selected on the basis of the deviation of observed P values from the expected (*see* Supplemental Figure S1 under "Supplemental data" in the online

issue). The tag SNPs for each genomic region were selected by using HapMap CEU (release rel#23a NCBI B36) linkage disequilibrium structure with a cutoff of $r^2 > 0.5$ within a 250-kb window. Of the top 35 independent loci, the top SNP in the *FTO* locus was evaluated in a second replication group ($n = 7724$) with MetaboChip data. The results from the 2 genome-wide meta-analyses and MetaboChip data were combined by using a fixed-effects inverse variance-weighted meta-analysis. For the joint analysis, genome-wide significance was considered at the Bonferroni-corrected threshold of $P < 5 \times 10^{-8}$.

Expression quantitative trait loci analysis

Proxy SNPs ($D' > 0.8$ or $r^2 > 0.8$) of the top SNPs (rs838145) on 19q13.33 were queried in expression SNP databases that included results from the following tissues: fresh lymphocytes (24, 25), fresh leukocytes (26), peripheral blood monocytes, fresh leukocytes (26), omental and subcutaneous adipose tissue (27, 28), whole-blood samples (27, 29), and liver (28, 30, 31).

Assessment of fibroblast growth factor 21 protein concentrations

In participants in the Baltimore Longitudinal Study on Aging, serum fibroblast growth factor 21 (FGF21) was measured with ELISA (Quantikine Human FGF-21 ELISA; R&D Systems) by using samples that were stored continuously at -70°C until the time of analysis of FGF21. The samples were analyzed at Johns Hopkins Hospital in the laboratory of one of the investigators (RDS). The intra- and interassay CVs were 2.8% and 7.0%, respectively. The assay has a minimum limit of detection of 4.6 pg/mL. No samples were below the lower limit of detection.

RESULTS

The mean macronutrient intake assessed through FFQs in the 12 discovery GWA studies were similar across studies and were, on average, $17 \pm 1.3\%$, $33 \pm 2.5\%$, and $48 \pm 2.9\%$ for protein, fat, and carbohydrate, respectively (*see* Supplemental Table S4 under "Supplemental data" in the online issue). Heritability estimates for protein, carbohydrate, and fat intake were similar in the 2 cohorts evaluated: $17 \pm 0.02\%$, $20 \pm 0.03\%$, and $20 \pm 0.03\%$, respectively, in the Family Heart Study; $17 \pm 0.02\%$, $20 \pm 0.02\%$, and $23 \pm 0.03\%$, respectively, in the Framingham Heart Study.

The meta-analysis of stage 1 discovery genome scans of macronutrient intake showed modest deviation from the expected distribution of P values under the null hypothesis, particularly for protein intake (*see* Supplemental Figure S1 under "Supplemental data" in the online issue). The genomic control values for the meta-analysis of carbohydrate, fat, and protein intake were 1.04, 1.04, and 1.06, respectively, for the model adjusted for age and sex (base model) and 1.04, 1.05, and 1.05, respectively, for the BMI-adjusted model. Genome-wide significant associations were observed with a locus in an ~ 7 -kb region on 4q28, near the mastermind-like 3 (*MAML3*) gene for protein intake (Table 1; *see* Supplemental Table S5 under "Supplemental data" in the online issue). The strongest association was observed for rs1350036 where the minor allele was associated with lower protein intake ($\beta_{\text{CHARGE}} \pm \text{SE}$: $-0.14 \pm 0.02\%$; $P = 1.72 \times 10^{-8}$). The signal remained significant after adjustment for BMI ($\beta_{\text{CHARGE}} \pm \text{SE}$:

TABLE 1
Summary of GWA meta-analysis and joint analysis of top loci^{1/}

SNP	Chr	Allele (effect/noneffect)	Allele frequency (effect)	CHARGE		DietGen		CHARGE + DietGen		CHARGE + DietGen (BMI adjusted)	
				$\beta \pm SE$	P value	$\beta \pm SE$	P value	$\beta \pm SE$	P value	$\beta \pm SE$	P value
Carbohydrate											
rs1667320	2	G/T	0.49 (0.01)	-0.31 ± 0.06	1.25 × 10 ⁻⁷	0.02 ± 0.06	0.8147	-0.16 ± 0.04	0.0002	-0.14 ± 0.04	0.0012
rs2840445	8	A/G	0.27 (0.02)	-0.32 ± 0.06	7.55 × 10 ⁻⁷	-0.10 ± 0.07	0.1781	-0.22 ± 0.05	4.52 × 10 ⁻⁶	-0.20 ± 0.05	5.78 × 10 ⁻⁵
rs12122737	1	A/C	0.20 (0.02)	0.35 ± 0.07	1.64 × 10 ⁻⁶	-0.14 ± 0.08	0.0871	0.13 ± 0.05	0.0148	0.12 ± 0.05	0.0247
rs12356048	10	G/A	0.32 (0.02)	0.30 ± 0.06	2.44 × 10 ⁻⁶	-0.10 ± 0.07	0.1669	0.12 ± 0.05	0.0108	0.08 ± 0.05	0.1034
rs12912584	15	A/G	0.25 (0.02)	0.31 ± 0.07	3.99 × 10 ⁻⁶	-0.10 ± 0.07	0.1814	0.13 ± 0.05	0.0117	0.10 ± 0.05	0.0353
rs1542608	7	G/A	0.18 (0.02)	0.37 ± 0.08	4.23 × 10 ⁻⁶	0.00 ± 0.08	0.9857	0.19 ± 0.06	0.0008	0.17 ± 0.06	0.0035
rs10508303	10	A/C	0.07 (0.02)	0.60 ± 0.13	4.82 × 10 ⁻⁶	-0.18 ± 0.13	0.1644	0.20 ± 0.09	0.0270	0.19 ± 0.09	0.0328
rs1893113	6	T/C	0.50 (0.01)	0.27 ± 0.06	5.19 × 10 ⁻⁶	-0.01 ± 0.06	0.8810	0.14 ± 0.04	0.0011	0.12 ± 0.04	0.0045
rs8019546	14	A/G	0.30 (0.01)	0.29 ± 0.06	5.93 × 10 ⁻⁶	0.13 ± 0.07	0.0750	0.22 ± 0.05	5.01 × 10 ⁻⁶	0.19 ± 0.05	5.14 × 10 ⁻⁵
rs838145	19	G/A	0.46 (0.04)	0.27 ± 0.06	6.21 × 10 ⁻⁶	0.17 ± 0.07	0.0083	0.23 ± 0.04	3.13 × 10 ⁻⁷	0.25 ± 0.04	1.68 × 10 ⁻⁸
rs1549309	5	A/G	0.17 (0.02)	0.34 ± 0.07	9.50 × 10 ⁻⁶	0.18 ± 0.08	0.0350	0.27 ± 0.06	2.47 × 10 ⁻⁶	0.25 ± 0.06	1.59 × 10 ⁻⁵
Protein											
rs1350036	4	T/C	0.48 (0.03)	-0.14 ± 0.02	1.72 × 10 ⁻⁸	-0.01 ± 0.02	0.7601	-0.07 ± 0.02	3.49 × 10 ⁻⁵	-0.07 ± 0.02	2.19 × 10 ⁻⁵
rs1421085	16	C/T	0.42 (0.02)	0.12 ± 0.03	1.53 × 10 ⁻⁶	0.10 ± 0.03	0.0002	0.11 ± 0.02 ²	1.65 × 10 ⁻⁹	0.09 ± 0.02 ³	4.80 × 10 ⁻⁷
rs1191005	14	T/C	0.12 (0.01)	0.19 ± 0.04	1.59 × 10 ⁻⁶	-0.02 ± 0.04	0.5944	-0.08 ± 0.03	0.0042	0.08 ± 0.03	0.0042
rs9553939	13	G/A	0.43 (0.02)	-0.12 ± 0.03	3.60 × 10 ⁻⁶	-0.02 ± 0.03	0.4968	-0.07 ± 0.02	0.0002	-0.07 ± 0.02	0.0003
rs11640535	16	A/G	0.27 (0.03)	0.14 ± 0.03	3.72 × 10 ⁻⁶	0.00 ± 0.03	0.8746	0.06 ± 0.02	0.0019	0.07 ± 0.02	0.0002
rs16993715	21	T/C	0.04 (0.01)	0.32 ± 0.07	8.17 × 10 ⁻⁶	0.05 ± 0.07	0.4621	0.18 ± 0.05	0.0003	0.17 ± 0.05	0.0007
rs11699457	20	A/G	0.19 (0.01)	0.14 ± 0.03	9.08 × 10 ⁻⁶	0.03 ± 0.03	0.3966	0.08 ± 0.02	0.0002	0.08 ± 0.02	0.0003
rs7086704	10	G/T	0.25 (0.02)	-0.13 ± 0.03	9.61 × 10 ⁻⁶	-0.01 ± 0.03	0.6798	-0.07 ± 0.02	0.0008	-0.05 ± 0.02	0.0091
Fat											
rs7625360	3	C/A	0.24 (0.01)	0.30 ± 0.06	1.77 × 10 ⁻⁷	0.02 ± 0.06	0.7715	0.16 ± 0.04	9.78 × 10 ⁻⁵	0.14 ± 0.04	0.0006
rs838145	19	G/A	0.46 (0.04)	-0.25 ± 0.05	3.43 × 10 ⁻⁷	-0.19 ± 0.05	0.0002	-0.22 ± 0.04	4.48 × 10 ⁻¹⁰	-0.21 ± 0.04	1.57 × 10 ⁻⁹
rs2609186	2	A/G	0.31 (0.05)	-0.25 ± 0.05	9.93 × 10 ⁻⁷	-0.01 ± 0.05	0.8458	-0.14 ± 0.04	0.0002	-0.13 ± 0.04	0.0007
rs7997717	13	A/G	0.05 (0.01)	-0.55 ± 0.11	2.99 × 10 ⁻⁶	0.03 ± 0.12	0.8167	-0.27 ± 0.08	0.0014	-0.28 ± 0.08	0.0009
rs12156272	8	C/A	0.17 (0.01)	-0.28 ± 0.06	5.59 × 10 ⁻⁶	-0.02 ± 0.06	0.7255	-0.16 ± 0.04	0.0004	-0.16 ± 0.04	0.0003
rs3114727	7	A/G	0.40 (0.01)	0.22 ± 0.05	5.76 × 10 ⁻⁶	0.03 ± 0.05	0.5503	0.13 ± 0.03	0.0002	0.13 ± 0.03	0.0001
rs11725988	4	G/A	0.26 (0.02)	0.26 ± 0.06	6.80 × 10 ⁻⁶	0.02 ± 0.06	0.7473	0.14 ± 0.04	0.0006	0.14 ± 0.04	0.0007
rs4683038	3	T/G	0.27 (0.02)	0.25 ± 0.05	7.78 × 10 ⁻⁶	-0.02 ± 0.06	0.7704	0.12 ± 0.04	0.0026	0.11 ± 0.04	0.0052
rs10152629	15	A/G	0.02 (0.01)	0.80 ± 0.18	8.47 × 10 ⁻⁶	-0.12 ± 0.19	0.5250	0.37 ± 0.13	0.0045	0.36 ± 0.13	0.0066

^{1/} All analyses were adjusted for age, sex and study sex and study-specific covariates (eg, study site, population stratification principal components when applicable) ($P < 10^{-6}$). CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; Chr, chromosome; GWA, genome-wide association; SNP, single nucleotide polymorphism.

^{2,3} Meta-analysis with 3 cohorts with Illumina MetaboChip data ($n = 7724$): ² $\beta \pm SE = 0.10 \pm 0.02\%$, $P = 9.96 \times 10^{-10}$; ³ $\beta \pm SE = 0.08 \pm 0.02\%$, $P = 3.15 \times 10^{-7}$.

$-0.15 \pm 0.02\%$; $P = 6.60 \times 10^{-9}$). No genome-wide significant associations were observed for carbohydrate and fat intake.

From stage 1 analysis, we took forward the SNPs that represented the 35 most significant loci ($P < 10^{-6}$) in either the base model (Table 1) or the BMI-adjusted model (see Supplemental Table S5 under "Supplemental data" in the online issue). These SNPs were analyzed jointly with data from the DietGen Consortium and 3 studies with MetaboChip data for the *FTO* (rs1421085) locus because this SNP was directly genotyped on the chip. In joint meta-analyses, genome-wide significant associations were observed for rs1421085 in the *FTO* locus on chromosome 16 in the model 1 for protein intake (Table 1), where the BMI-increasing minor allele was associated with higher protein intake ($\beta_{\text{joint}} \pm \text{SE}$: $0.10 \pm 0.02\%$; $P = 9.96 \times 10^{-10}$). In the BMI-adjusted model, a small reduction in the strength of association was observed ($\beta_{\text{joint}} \pm \text{SE}$: $0.08 \pm 0.02\%$; $P = 3.15 \times 10^{-7}$).

Genome-wide significant associations were also observed on 19q13.33 for both fat and carbohydrate intake in the joint analysis (Table 1). The minor allele of rs838145 was associated with a higher percentage of energy intake from carbohydrate for the base model ($\beta_{\text{joint}} \pm \text{SE}$: $0.23 \pm 0.04\%$; $P = 3.13 \times 10^{-7}$) and for the BMI-adjusted model ($\beta_{\text{joint}} \pm \text{SE}$: $0.25 \pm 0.04\%$; $P = 1.7 \times 10^{-8}$) and a lower percentage of energy intake from fat for the base model ($\beta_{\text{joint}} \pm \text{SE}$: $-0.22 \pm 0.04\%$; $P = 4.48 \times 10^{-10}$) and for the BMI-adjusted model ($\beta_{\text{joint}} \pm \text{SE}$: $-0.21 \pm 0.04\%$; $P = 1.57 \times 10^{-9}$).

We identified *FGF21* as a candidate gene in the 19q13.33 region, which encodes a hormone that regulates glucose and lipid metabolism (Figure 1, A and B). To investigate whether *FGF21* could be the gene underlying this signal, we explored the association of rs838145 with the expression of the *FGF21* gene and protein. First, we used an existing expression quantitative trait loci database of *cis*-gene expression with the use of data from genome-wide expression analysis in relevant tissues that express *FGF21* including liver, adipose, and blood (see Supplemental Table S6 under "Supplemental data" in the online issue). Data show the expression of multiple genes in association with SNPs in high linkage disequilibrium with rs838145; however, the expression of *FGF21* was not supported by the data. Second, to assess the association of rs838145 with circulating concentrations of *FGF21* protein, we used plasma *FGF21* concentrations in 377 individuals in the Baltimore Longitudinal Study on Aging. The minor allele of rs838145, which was associated with a higher percentage of energy from carbohydrate and a lower percentage of energy from fat, was significantly associated with higher *FGF21* protein concentrations ($\beta \pm \text{SE}$: 36 ± 17 pg/mL; $P = 0.01$), after adjustment for age and sex. This association remained significant after further adjusting the analyses for BMI ($\beta \pm \text{SE}$: 32 ± 17 pg/mL; $P = 0.02$).

The SNP (rs1350036) in the 4q28 region that reached genome-wide significance in stage 1 for protein intake was not replicated in stage 2 and was not significant in the joint analysis for the base model ($\beta_{\text{joint}} \pm \text{SE}$: $-0.07 \pm 0.02\%$; $P = 3.49 \times 10^{-5}$; Table 1) or in the BMI-adjusted model ($\beta_{\text{joint}} \pm \text{SE}$: $-0.07 \pm 0.02\%$; $P = 2.19 \times 10^{-5}$).

DISCUSSION

In this study, we identified genome-wide significant associations of a locus on 19q13.33 for carbohydrate and fat intake, with re-

ciprocal directions of association, and with the *FTO* locus for protein intake. *FTO* was one of the first BMI loci to be identified through GWA scans and has been consistently associated with obesity in various cohorts (32, 33). The association of variants in *FTO* with protein intake in our study was largely unchanged after adjustment for BMI, suggesting that this association is not mediated by BMI. The *FTO* SNP reported in this analysis (rs1421085) is in high linkage disequilibrium ($r^2 > 0.97$) with SNPs showing the strongest association with BMI (rs993609, rs9930506) (32–34). Previous studies have investigated the association of *FTO* variants with food intake (35–37), hypothesizing an effect through the regulation of hunger and satiety (38). The focus of these studies was primarily on total energy intake rather than on specific macronutrients. Overall, the evidence was weak, but our findings highlight the importance of further investigation to clarify the interrelations of the *FTO* gene, macronutrient and energy intake, and body weight.

It is notable that, with the exception of *FTO*, other loci identified in previous linkage analyses (2–5) and candidate gene association studies (6–9, 11, 13, 14) of macronutrient intake were not among the top signals in our study. There have been 4 linkage studies of macronutrient intake, and although each study identified significant linkage regions, there were none that were consistent across the studies (2–5). It is possible that there are macronutrient loci that are specific to the families in each study. However, discrepancies may also be a result of differences in study population, methods for dietary assessment, or statistical methods used.

We report novel associations of a locus on 19q13.33 with higher carbohydrate and lower fat consumption, independent of BMI. One gene in this region with potential links to macronutrient intake was *FGF21*, which encodes a hormone produced primarily in the liver that is an important regulator of glucose and lipid metabolism (39, 40). In vitro, *FGF21* promotes insulin-independent glucose uptake through the transcription of *GLUT1* in rodent and human adipocytes (41). Pharmacologic doses of *FGF21* improve glucose clearance and insulin sensitivity and lower plasma triglycerides and free fatty acids in diabetic and obese animal models (41–44). *FGF21* also plays a critical role in regulating metabolic adaptation in nutritional states in which the primary source of energy is derived from fatty acids. During prolonged starvation or consumption of a ketogenic diet (high fat, low carbohydrate), *FGF21* increases lipolysis in adipose tissue and increases ketogenesis and β -oxidation in the liver (45, 46). In humans, the role of *FGF21* is not well defined. Some reports have linked *FGF21* with BMI and other metabolic variables, but results are not consistent (47–49). In our study, we observed a significant association between the 19q13.33 variant with circulating protein concentrations of *FGF21* but not with gene expression levels in liver, fat, and blood tissues. If *FGF21* is the gene underlying the signal on 19q13.33, it is possible that there is a metabolic shift in glucose and fat metabolism, such that carriers of the minor allele preferentially consume more energy from carbohydrate and less from fat.

This study, along with the DietGen Consortium meta-analysis (16), is the largest GWA study of macronutrient intake in European ancestry cohorts. Interestingly, both studies identified associations with the locus on 19q13.33 but for different macronutrients. The DietGen Consortium observed a genome-wide significant association of 19q13.33 with protein intake, whereas we found associations with fat and carbohydrate intake. Although the

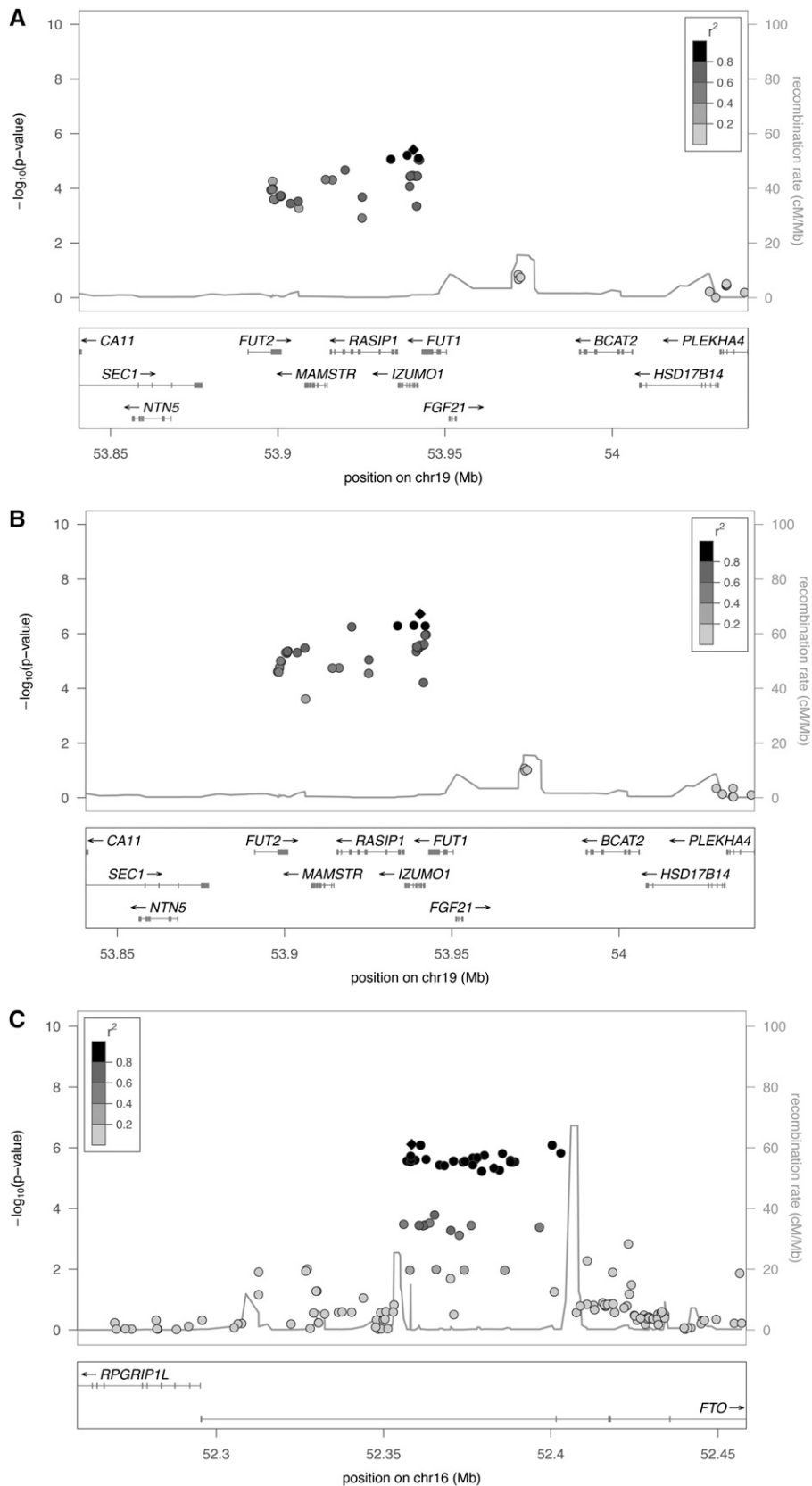


FIGURE 1. Regional association plot of 19q13.33 and the *FTO* locus region. The panels show $-\log_{10} P$ values for SNPs that passed quality control from the stage 1 meta-analysis of intakes of carbohydrate (A), fat (B), and protein (C) as percentages of total energy adjusted for age, sex, and study-specific covariates (eg, study site, population-stratification principal components when applicable). The SNPs shown are those within 1 Mb of the index SNP: rs8183145 on chromosome 19 (A, B) and rs1421085 on chromosome 16 (C). The degree of linkage disequilibrium (r^2) is shown from low (<0.2 ; light gray) to high (≥ 0.8 ; black). chr, chromosome; cM, centimorgan; Mb, mega base pair; SNP, single nucleotide polymorphism.

reasons for these differences are unclear, it is possible that this locus is not specific to intake of any single macronutrient. Total dietary energy is derived from different macronutrients; therefore, when there is a change in the percentage of energy from one macronutrient, a change in one or both of the other macronutrients may occur in the opposite direction. Thus, the results of the 2 studies suggest that this locus is associated with dietary macronutrient composition rather than with the consumption of a specific macronutrient.

Although the associations reported in this study are significant, the effects are small in magnitude. These estimates may be underestimated because of measurement errors of macronutrient intake from FFQs. The FFQ is a commonly used dietary assessment tool in epidemiologic studies because it is feasible to use in large studies and accurately rank-orders individuals across the spectra of foods and nutrients; however, the validity of estimates varies across nutrients and populations. Of the cohorts involved in the CHARGE discovery meta-analysis, 10 of 12 used validated FFQs. Nevertheless, the clinical significance of differences in macronutrient intake of $\sim 0.2\%$, even when extended over a lifetime, is not clear. In this light, the size and scope of our study are important strengths to detect such a modest impact of genetics, and our findings are most relevant to elucidate the potential underlying biology of very complex human dietary behaviors. Given the complexity of the investigated trait, it is likely that other as yet unidentified loci, as well as other genetic differences such as copy number variants, contribute to macronutrient intake. Furthermore, replication of these findings in non-European cohorts will be needed to determine whether the identified loci are associated with macronutrient intake in other populations.

In conclusion, we report associations of a locus on chromosome 19 with carbohydrate and fat intake and of the *FTO* locus with protein intake. We also propose *FGF21* as one strong candidate for the former association, and our findings support the need for further functional assessment and fine mapping of 19q13.33. Our results support a role of common genetic variants on macronutrient consumption in humans.

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