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RESEARCH PAPER

Obesity polymorphisms identified in genome-wide association studies interact with n-3 polyunsaturated fatty acid intake and modify the genetic association with adiposity phenotypes in Yup'ik people

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Abstract n-3 Polyunsaturated fatty acids (n-3 PUFAs) have anti-obesity effects that may modulate risk of obesity, in part, through interactions with genetic factors. Genome-wide association studies (GWAS) have identified genetic variants associated with body mass index (BMI); however, the extent to which these variants influence adiposity through interactions with n-3 PUFAs remains unknown. We evaluated 10 highly replicated obesity GWAS single nucleotide polymorphisms (SNPs) for individual and cumulative associations with adiposity phenotypes in a cross-sectional sample of Yup'ik people ($n = 1{,}073$) and evaluated whether genetic associations with obesity were modulated by n-3 PUFA intake. A genetic risk score (GRS) was calculated by adding the BMI-increasing alleles across all 10 SNPs. Dietary intake of n-3 PUFAs was estimated using nitrogen stable isotope ratio ($\delta^{15}N$) of red blood cells, and genotype–phenotype analyses were tested in linear models accounting for familial correlations. GRS was positively associated with BMI ($p = 0.012$), PBF ($p = 0.022$), ThC ($p = 0.025$), and waist circumference ($p = 0.038$). The variance in adiposity phenotypes explained by the GRS included BMI (0.7 %), PBF (0.3 %), ThC (0.7 %), and WC (0.5 %). GRS interactions with n-3 PUFAs modified the association with adiposity and accounted for more than twice the phenotypic variation ($\sim$1–2 %), relative to GRS

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associations alone. Obesity GWAS SNPs contribute to adiposity in this study population of Yup'ik people and interactions with n-3 PUFA intake potentiated the risk of fat accumulation among individuals with high obesity GRS. These data suggest the anti-obesity effects of n-3 PUFAs among Yup'ik people may, in part, be dependent upon an individual's genetic predisposition to obesity.

Keywords BMI · Adiposity · Alaska Native · SNP · $\delta^{15}N$ · rs9939609 · rs7647305 · *FTO* · *ETV5* · Genetic risk score · CANHR · Gene-by-environment interactions

Introduction

Obesity contributes to a series of metabolic abnormalities including elevated triglycerides and inflammatory cytokines, leading to insulin resistance and the development of type 2 diabetes (T2D) and other diseases (Ogden et al. 2007). Given the increasing public health burden associated with obesity and the difficulty maintaining long-term weight loss (Curioni and Lourenço 2005; Dansinger et al. 2007; Tsai and Wadden 2005), it is important to elucidate mechanisms that influence body weight.

The heritability of obesity has been estimated to between 40 and 70 % (Maes et al. 1997; Stunkard et al. 1990). A recent meta-analysis of obesity genome-wide association studies (GWAS) conducted primarily in European samples has identified thirty-two single nucleotide polymorphisms (SNPs) associated with variation in BMI (Speliotes et al. 2010). Although a large number of SNPs are associated with excess body weight in European samples, the extent to which these loci are associated with adiposity phenotypes in other ethnic groups is unclear (Hester et al. 2012; Li et al. 2008). Furthermore, the heritability of obesity explained by the aggregate of these loci remains low (<2 %) (Speliotes et al. 2010).

Increasingly, gene-by-environment interactions have been suggested to account for the "missing heritability" in complex traits (Van IJzendoorn et al. 2011). Genetic association studies that recruit isolated populations, characterized by an environment that is less heterogeneous and reduced genetic admixture, may have a unique opportunity to delineate genetic and environmental factors that impact the heritable component of obesity (Heutink and Oostra 2002). Empirical evidence that demonstrates modifiable environmental factors interact with SNPs associated with obesity and partially account for the heritability of obesity may elucidate mechanisms that modulate the risk of obesity-related comorbidities through lifestyle interventions (Marti et al. 2008).

Dietary fat is a modifiable environmental factor that has been implicated in the development of obesity (Bray et al. 2004); however, a growing body of evidence indicates that not all dietary fatty acids are equally obesigenic (Storlien et al. 2001). Regular consumption of n-3 polyunsaturated fatty acids (n-3 PUFAs) may reduce adiposity in humans (Buckley and Howe 2010), in part, by inhibiting adipogenesis (Kopecký et al. 2009) and stimulating fat oxidation (Couet et al. 1997). Animal models have shown significant reductions in fat mass when dietary n-3 PUFAs, namely eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), are substituted for saturated fats (Hainault et al. 1993), monosaturated fats (Su and Jones 1993), and n-6 polyunsaturated fats (Jones 1989), after controlling for caloric intake. These findings have largely been confirmed by human studies (Thorsdottir et al. 2007); however, information regarding whether the anti-obesity effects attributed to n-3 PUFA is dependent upon a specific genotype remains limited (Jourdan et al. 2011). Knowledge about the interplay between genetic factors and consumption of n-3 PUFA in isolated populations with widely varying n-3 PUFA intake may facilitate the choice of more effective and specific measures of obesity prevention based upon individualized genetic makeup.

Given the anti-obesigenic effects of n-3 PUFAs, the widely varying intake of n-3 PUFA in this isolated study population of Yup'ik people (nearly 50-fold range of EPA), and the availability of a precise biomarker for n-3 PUFA intake ($\delta^{15}N$; (O'Brien et al. 2009)), we evaluated whether n-3 PUFA intake modifies the association between GWAS SNPs and adiposity phenotypes. The individual and cumulative effects of 10 SNPs reproducibly associated with BMI (Frayling et al. 2007; Loos et al. 2008; Scuteri et al. 2007; Thorleifsson et al. 2009; Willer et al. 2009) at genome-wide significance ($p < 5 \times 10^{-8}$) were tested in a sample of Yup'ik people from the Center for Alaska Native Health Research (CANHR) study. We evaluated the cumulative effects of these SNPs using a genetic risk score (GRS) that estimates an individual's genetic predisposition to obesity by adding the BMI-increasing alleles across all 10 SNPs.

Methods

Participants and study design

The CANHR studies genetic, behavioral, and dietary risk factors underlying obesity and their relationship to diabetes

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and cardiovascular disease among Yup'ik people (Mohatt et al. 2007). Recruitment of Yup'ik participants was initiated in 2003 and continues in 11 Southwest Alaskan communities. All residents ≥14 years old were invited to participate, and the resulting distribution of age in our study sample reflects the age distribution among eligible participants according to 2000 US census data. Participants signed informed-consent documents before entering the study using protocols that were approved by the University of Alaska Institutional Review Board, the National and Alaska Area Indian Health Service Institutional Review Boards, and the Yukon Kuskokwim Human Studies Committee. Summary statistics regarding family data were calculated using PEDINFO in the Statistical Analysis for Genetic Epidemiology (S.A.G.E., 2009) software. The analyses in this report included 1,073 non-pregnant Yup'ik participants (41 founders, 920 non-founders, and 112 singletons) with ages ranging between 14 and 94 years at enrollment. There were 195 pedigrees in this data set with a mean size 5.52 individuals (range, 1–849) and 696 sibships with a mean size of 1.32 (range, 1–9).

Anthropometric and biochemical measurement

Anthropometric measurements were obtained by trained staff using protocols from the NHANES III Anthropometric Procedures Manual (Lohman and Roche 1988) as previously described (Boyer et al. 2007). These measurements included height, weight, and 4 circumferences (waist, hip, triceps, and thigh). Percent body fat (PBF) was measured by electrical bioimpedance using a Tanita TBF-300A bioimpedance analyzer (Tanita Corp, Arlington Heights, IL, USA).

Biomarker of n-3 PUFA intake

n-3 PUFA intake was assessed using the nitrogen stable isotope ratio ($\delta^{15}N$) of red blood cells (RBC), which has been validated as a biomarker for EPA and DHA intake as previously described (O'Brien et al. 2009). The time to 50 % turnover of RBC is approximately 45 days therefore, the mean RBC $\delta^{15}N$ values reflect a mean n-3 PUFA intake over 1.5 months. A volume of 1.8 µl aliquots of RBC were pipetted into 3.5 × 3.75 mm tin capsules, autoclaved for 20 min at 121 °C to destroy blood-borne pathogens, and dried to a constant mass of 0.2–0.4 mg. Capsules were crushed into a ball for loading into an autosampler. Samples were analyzed at the Alaska Stable Isotope Facility by continuous-flow isotope ratio mass spectrometry, using a Costech ECS4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, CA, USA) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer via the Conflo III interface (Thermo-Finnigan Inc., Breman, Germany). Isotope ratios are analyzed relative to IAEA-certified reference materials calibrated to atmospheric nitrogen, for which $^{15}N/^{14}N = 0.0036765$. By convention and for ease of interpretation, isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard})/(^{15}N/^{14}N_{standard})] \times 1{,}000$ ‰. We concurrently prepared and ran multiple laboratory standards (peptone, $\delta^{15}N = 7.00$) to assess analytical accuracy and precision; these were analyzed after every eighth sample and gave values of $\delta^{15}N = 7.01 \pm 0.24$ ‰ (mean ± SD). The range of isotopic variation in our dataset (9 ‰) was very large relative to analytical precision (0.2 ‰). We modeled the effects of n-3 PUFA intake as a categorical variable with four groups by quartile, which is hereafter referred to as $\delta^{15}N$.

SNP selection and genotyping

Due to limited resources available at the time of genotyping, we selected SNPs most reproducibly associated with BMI at genome-wide significance ($p < 5 \times 10^{-8}$) which included the 10 obesity loci in or near *FTO*, *MC4R*, *TMEM18*, *GNPDA2*, *BDNF*, *NEGR1*, *SH2B1*, *ETV5*, *MTCH2*, and *KCTD15* (Frayling et al. 2007; Loos et al. 2008; Scuteri et al. 2007; Thorleifsson et al. 2009; Willer et al. 2009) and that were found to be significantly associated with BMI in an obesity GWAS meta-analysis with 249,796 participants (Speliotes et al. 2010). In situations where more than a single SNP has been identified for a given gene, we genotyped the SNP most frequently reported in these studies. Genotyping was carried out at the Broad Institute (Cambridge, MA) by allele-specific primer extension of multiplex amplified products and detection using matrix-assisted laser desorption ionization time-of-flight spectrometry on a Sequenom iPLEX platform (Tang et al. 1999). We compared the frequency of the GWAS BMI-increasing "risk alleles" in Yup'ik people to five HapMap populations (The International HapMap Consortium 2003).

Quality control of phenotypic and genotypic data

Simple linear models were fit to each phenotype using all covariates (age, sex, community group) included in the association models, and the distributions of the residuals for each phenotype were examined for normality with the R statistical programming language (v2.10.1, R Development Core, 2009). We considered a series of transformations (square root, log, inverse, etc.) to improve normality, and the Box-Cox transformation (Box and Cox 1964) was identified as the best procedure for phenotypes whose residuals did not follow a normal distribution. Information regarding the power transformation for each adiposity

phenotypes is presented in Supplementary Table I. Familial data were stored in the Progeny database (Progeny Software LLC, South Bend, IN, USA) and merged into a single extended pedigree comprised of multiple independent families using PedMerge (Plaetke and Balbi 2010). Genotypic data were tested for Mendelian inconsistencies using PEDCHECK (O'Connell and Weeks 1998). In this sample, Illumina IV linkage panel (Illumina, Inc., San Diego, CA, USA) genotypes were available from an ongoing linkage study and were used to construct principal components using the EIGENSTRAT analysis package (Price et al. 2006). The first PC did not have an obvious interpretation to the data; however, the second PC discriminated individuals into two groups that corresponded to either coastal or inland communities. Based on this, we defined a dichotomous community group variable that indicates either coastal or inland. We assessed Hardy–Weinberg equilibrium (HWE) in the founders using PLINK (v1.07) (Purcell et al. 2007) and determined minor allele frequency (MAF) for each SNP using the FREQ module in the Statistical Analysis for Genetic Epidemiology (S.A.G.E 2009) program. The present study restricted association and interaction analysis to SNPs with MAF $\geq$5 % that did not deviate from HWE ($p \leq 0.005$). HWE multiple test correction was determined using a Bonferroni correction for 10 tests ($\alpha \leq 0.005$).

Association analysis

Each SNP was tested using as additive model for association with obesity-related phenotypes using the ASSOC module in the S.A.G.E. software package (S.A.G.E. 6.3 [2012] http://darwin.cwru.edu/sage/; George and Elston 1987). We included both demographic (age, community group, and sex) and environmental covariates (δ^{15}N) in the association analysis and calculated likelihood ratio statistics to compare 4 nested models. Model 1 included demographic covariates (age, sex and community group); Model 2 included baseline covariates and SNP to test for an additive genetic effect of SNP (defined as the number of minor alleles); Model 3 included demographic covariates, the additive genetic effect of SNP, and δ^{15}N (defined by quartiles of δ^{15}N) as an estimate of n-3 PUFA intake; Model 4 included all covariates from Model 3 and an interactions between the additive genetic effect and δ^{15}N categories. Note that the Model 4 is the only model to test gene–diet interactions. Correction for multiple testing in genetic analyses (Models 1–4) for SNPs with MAF $\geq$0.05 employed a Bonferroni correction for 10 tests ($\alpha \leq 0.005$). Effect sizes (β) were presented for association analyses as the change in transformed phenotypes according to BMI-increasing "risk allele" adjusted for demographic and environmental covariates. Statistical power that accounted for familial correlations (Nyholt 2004) was assessed using SAS version 9.1 (SAS Institute, Cary, NC). The general estimates of power in our sample using an additive genetic model at $\alpha = 0.005$ for detecting the effect sizes (β) in transformed phenotypes (i.e., BMI) between 0.1 and 1.5 were >90 % when the minor allele frequency was at least 5 %.

The obesity genetic risk score (GRS) was calculated by summing the number of risk alleles that each individual possessed. The obesity risk alleles were defined as those that were associated with higher BMI (defined as BMI $\geq$25 kg/m^2) in previous GWAS studies (Speliotes et al. 2010; Thorleifsson et al. 2009; Willer et al. 2009). To account for missing genotypes, we divided the sum of risk alleles by the number of loci included in the score. The GRS was split into three groups by tertiles (Qi et al. 2009), and a categorical variable was created to allow for non-linear effects without being unduly influenced by extreme values. We tested the GRS for association with obesity traits using two models (Models 5 and 6) run with the ASSOC module in S.A.G.E. Model 5 included the GRS as a main effect and controlled for demographic (sex, age, and community group) covariates. Model 6 tested the GRS for interaction with δ^{15}N and controlled for demographic covariates and δ^{15}N. Model 7 tested for interactions (δ^{15}N*GRS and δ^{15}N*community group) in a model that included demographic and environmental covariates. Note that Model 7 is the only model to test gene–diet interaction. In a subset of participants ($n = 488$), we evaluated 24 Hour Recalls (24HR) to estimate total energy (kcal) and total fat intake (% calories) as potential confounding dietary variables in the δ^{15}N*GRS interaction model (Model 7). The variance explained by the fixed effects in the GRS association models were calculated as the difference between the total variance of the trait and the sum of the random effects in the model (i.e., the residual variance and the family effect). The proportion of variance explained by random effects was calculated, whereby the ratio of the difference noted above was included in the numerator and the total variance of the trait was included in the denominator. We defined the proportion of the variance explained by each term in the GRS association model as the difference in proportion of variance explained by models with and without the terms of interest. Results were considered significant in the GRS analyses (Models 5–7) if the p value was $\leq$0.05 (2-tailed).

Results

Descriptive statistics

Descriptive statistics on Yup'ik men ($n = 510$) and women ($n = 563$) enrolled in this study are presented in Table 1.

Table 1 Descriptive statistics of adiposity phenotypes

Variables	Women	Men	*p* values
No. of participants	563	510	
Age (years)	38.5 ± 1.6	36.6 ± 0.6	0.0740
BMI (kg/m^2)	28.7 ± 5.6	26.3 ± 5.6	<0.0001
Percentage body fat (%)	42.8 ± 1.4	27.8 ± 1.1	<0.0001
Waist circumference (cm)	88.1 ± 21.6	87.5 ± 21.5	0.5039
Hip circumference (cm)	105.4 ± 43.0	98.7 ± 43.0	<0.0001
Thigh circumference (cm)	51.1 ± 21.4	50.3 ± 21.3	0.0110

Values are reported as mean (±SE) predicted from linear model accounting for familial correlations. *p* values for differences by gender are derived using student *t* test

Yup'ik women in this study had a mean age of 38.5 (±1.6) years and had more adiposity than men that reported a mean age of 36.6 (±0.6) years. Specifically, women had more adiposity that included greater BMI ($p < 0.0001$), percentage body fat ($p < 0.0001$), and hip circumference ($p < 0.0001$) compared to men. Interestingly, we did not detect significant differences between women and men with respect to waist circumference.

Distribution of δ^{15}N in the study population

We assessed n-3 PUFA intake in 1,073 Yup'ik participants using RBC δ^{15}N as a biomarker of EPA and DHA intake. Summary statistics are grouped by gender and δ^{15}N quartiles and reported in Table 2. The mean δ^{15}N value in this study was 9.0 ‰ and ranged from 6.4 to 15.2 ‰. According to the linear relationship between RBC δ^{15}N and RBC EPA described elsewhere for this study population (O'Brien et al. 2009), the corresponding mean EPA (%RBC fatty acids) was 2.66 %. Measurement of δ^{15}N by gender yielded means of 9.1 ‰ for females and 8.7 ‰ for males. The mean RBC δ^{15}N values according to the four groups by quartile were 7.3, 8.2, 9.1, 11.0 ‰ in groups 1–4, respectively. These values correspond to EPA (% RBC fatty acids) group means of: 0.9, 1.8, 2.8, and 4.7 % (O'Brien et al. 2009). The standard deviation of δ^{15}N in this sample did not differ according to gender (1.5 ‰ for both females and males).

Genetic variation among obesity SNPs

Allele frequencies for 10 SNPs that showed significant association with obesity and/or BMI in large-scale GWAS are presented in Table 3. The frequencies for risk alleles observed in this sample of Yup'ik people were comparable to frequencies reported in other populations (Table 3). Notable exceptions include the rs10838738 (*MTCH2*) and the rs7498665 (*SH2B1*) risk alleles which are at higher frequencies among this sample of Yup'ik people. All genotyped SNPs were consistent with expected HWE ($p > 0.002$) and had MAF ≥ 0.05 with the exception of rs6265 (*BDNF*) which had MAF = 0.04.

Individual SNPs and adiposity phenotypes

Without adjustment for n-3 PUFA intake (δ^{15}N), no SNPs were significantly associated with adiposity phenotypes after Bonferroni multiple test correction (Supplementary Table II). Although not significant, 8 of the 10 SNPs tested in this study had direction-consistent association trends with BMI that were in agreement with reports from GWAS. After adjusting our analyses for n-3 PUFA intake (δ^{15}N), we found that rs9939609 (*FTO*) and rs7647305 (*ETV5*) risk alleles were associated with adiposity phenotypes (Fig. 1; Table 4). Specifically, rs9939609 (*FTO*) was positively associated with percent body fat (PBF, $p = 0.004$), and rs7647305 (*ETV5*) was negatively associated with hip and thigh circumference (HC, $p = 0.002$ and ThC, $p = 0.003$, respectively). We did not detect significant SNP interactions with n-3 PUFA intake that modified any associations with adiposity phenotypes (Supplementary Table III).

Table 2 Distribution of the RBC nitrogen stable isotope ratio (δ^{15}N)

	Sex			Quartiles of δ^{15}N**			
	Total	Women	Men	Q1	Q2	Q3	Q4
No. of participants	1,073	565	510	262	261	269	281
Mean ± SD (‰)	9.0 ± 1.5	9.1 ± 1.5	8.7 ± 1.5	7.3 ± 0.3	8.2 ± 0.2	9.1 ± 0.3	11.0 ± 1.1
Maximum	15.2	15.2	13.5	7.8	8.6	9.8	15.2
Minimum	6.4	6.4	6.4	6.4	7.8	8.6	9.8
Range (‰)	8.8	8.8	7.1	1.4	0.8	1.2	5.4

Isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}Nsample - {}^{15}N/^{14}Nstandard)/(^{15}N/^{14}Nstandard)] \times 1{,}000$ ‰. The relationship between δ^{15}N and EPA follows the linear model: EPA (%RBC fatty acid) = 1.04 × δ^{15}N − 6.7 ‰, as previously described for this population (O'Brien et al. 2009)

Table 3 Obesity GWAS SNP polymorphisms

Gene	SNP[a]	Chr	Risk allele	Frequency of risk allele	Frequency of risk allele in HAPMAP populations					HWE (p value)	References
					YRI	CEU	HCB	JPT	MEX		
NEGR1	rs2815752	1	A	0.81	0.53	0.64	0.88	0.92	0.73	0.2007	Willer et al. (2009)
TMEM18	rs7561317	2	G	0.85	0.79	0.85	0.91	0.88	0.88	0.1768	Thorleifsson et al. (2009)
ETV5	rs7647305	3	C	0.94	0.61	0.79	0.94	0.94	0.81	0.478	Thorleifsson et al. (2009)
GNPDA2	rs10938397	4	G	0.15	0.21	0.45	0.25	0.37	NA	0.2097	Willer et al. (2009)
BDNF	rs6265	11	G	0.96	0.99	0.81	0.38	0.63	0.79	1	Willer et al. (2009)
MTCH2	rs10838738	11	G	0.66	0.04	0.36	0.35	0.35	0.4	0.0037	Willer et al. (2009)
SH2B1	rs7498665	16	G	0.66	0.21	0.38	0.15	0.13	0.38	0.462	Thorleifsson et al. (2009), Willer et al. (2009)
FTO	rs9939609	16	A	0.19	0.51	0.46	0.12	0.19	NA	0.3223	Frayling et al. (2007), Scuteri et al. (2007), Willer et al. (2009)
MC4R	rs17782313	18	C	0.08	0.31	0.27	0.14	0.24	0.14	1	Loos et al. (2008), Willer et al. (2009)
KCTD15	rs29941	19	C	0.31	0.86	0.68	0.22	0.26	0.65	0.4179	Thorleifsson et al. (2009)

Frequency of BMI-increasing allele computed using FREQ module in S.A.G.E. Frequency of BMI-increasing allele among five HapMap populations: Yoruba in Ibadan, Nigeria (YRI), Utah residents with ancestry from northern and western Europe (CEU), Han Chinese in Beijing, China (CHB), Japanese in Tokyo, Japan (JPT), Mexican ancestry in Los Angeles, California. HWE p value computed using founders in PLINK (Purcell et al. 2007)

[a] Seattle SNPs Genome Variation Server on March 2008 (dbSNP build 126) Version 5.01

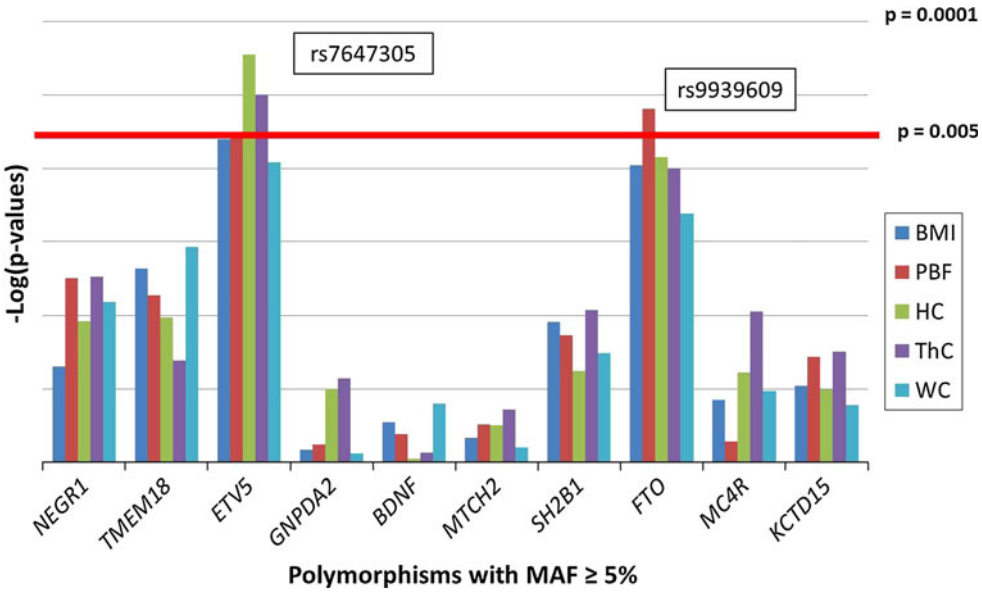

Fig. 1 Obesity GWAS polymorphisms associated with adiposity phenotypes. Association of SNPs in a linear regression model adjusted for age, sex, community membership, and n-3 PUFA intake. The *red line* represents multiple test correction that was estimated using the spectral decomposition of LD matrix (Nyholt 2004). Body mass index (*BMI*), percent body fat (*PBF*), hip circumference (*HC*), thigh circumference (*ThC*), and waist circumference (*WC*)

Genetic risk score and adiposity phenotypes

The GRS was positively associated with adiposity as measured by BMI ($p = 0.012$), PBF ($p = 0.022$), ThC ($p = 0.025$), and WC ($p = 0.038$) after adjusting for n-3 PUFA intake ($\delta^{15}N$) (Table 5). We did not observe significant interactions between the GRS and community location that modified adiposity phenotypes. The GRS interactions with n-3 PUFA intake were significant for BMI ($p = 0.011$), PBF ($p = 0.025$), and WC ($p = 0.018$). Figure 2 show that n-3 PUFA intake significantly modifies the GRS association with BMI, such that people with the highest BMI also had high levels n-3 PUFA intake (Q4) and a high GRS (T3). In contrast, our results show that people with a low GRS (T1) and high levels of n-3 PUFA intake (Q4) had a lower BMI, relative to individuals with the same GRS (T1) and low n-3 PUFA (Q1) intake (Fig. 2). The GRS interaction with n-3 PUFA explained approximately twice the phenotypic variation in adiposity phenotypes relative to GRS associations with adiposity alone

Table 4 Obesity polymorphisms associated with adiposity after $\delta^{15}N$ adjustment

Gene	SNP	BMI	PBF	HC	ThC	WC
NEGR1	rs2815752	0.222	0.056	0.109	0.055	0.081
		($\beta = -0.3$, SE = 0.2)	($\beta = -0.4$, SE = 0.2)	($\beta = -0.4$, SE = 0.2)	($\beta = -0.6$, SE = 0.3)	($\beta = -0.5$, SE = 0.3)
TMEM18	rs7561317	0.048	0.073	0.103	0.202	0.034
		($\beta = 0.5$, SE = 0.2)	($\beta = 0.4$, SE = 0.2)	($\beta = 0.4$, SE = 0.2)	($\beta = 0.4$, SE = 0.3)	($\beta = 0.6$, SE = 0.3)
ETV5	rs7647305	0.006	0.006	**0.002**	**0.003**	0.009
		($\beta = -1.5$, SE = 0.6)	($\beta = -1.4$, SE = 0.5)	($\beta = -1.8$, SE = 0.6)	($\beta = -2.0$, SE = 0.7)	($\beta = -1.9$, SE = 0.7)
GNPDA2	rs10938397	0.821	0.761	0.315	0.267	0.867
		($\beta = 0.1$, SE = 0.2)	($\beta = 0.1$, SE = 0.2)	($\beta = 0.2$, SE = 0.2)	($\beta = 0.3$, SE = 0.3)	($\beta = 0.1$, SE = 0.3)
BDNF	rs6265	0.531	0.646	0.943	0.859	0.396
		($\beta = 0.4$, SE = 0.6)	($\beta = 0.2$, SE = 0.5)	($\beta = 0.0$, SE = 0.6)	($\beta = 0.1$, SE = 0.7)	($\beta = 0.6$, SE = 0.7)
MTCH2	rs10838738	0.682	0.550	0.556	0.440	0.797
		($\beta = 0.1$ SE = 0.2)	($\beta = 0.1$, SE = 0.2)	($\beta = 0.1$, SE = 0.2)	($\beta = 0.2$, SE = 0.2)	($\beta = 0.1$, SE = 0.2)
SH2B1	rs7498665	0.111	0.136	0.239	0.092	0.179
		($\beta = 0.3$, SE = 0.2)	($\beta = 0.2$, SE = 0.2)	($\beta = 0.2$, SE = 0.2)	($\beta = 0.4$, SE = 0.2)	($\beta = 0.3$, SE = 0.2)
FTO	rs9939609	0.010	**0.004**	0.008	0.010	0.020
		($\beta = 0.6$, SE = 0.2)	($\beta = 0.6$, SE = 0.2)	($\beta = 0.6$, SE = 0.2)	($\beta = 0.7$, SE = 0.3)	($\beta = 0.7$, SE = 0.3)
MC4R	rs17782313	0.377	0.726	0.243	0.094	0.328
		($\beta = 0.3$, SE = 0.3)	($\beta = 0.1$, SE = 0.3)	($\beta = 0.4$, SE = 0.3)	($\beta = 0.6$, SE = 0.4)	($\beta = 0.4$, SE = 0.4)
KCTD15	rs29941	0.301	0.192	0.319	0.177	0.405
		($\beta = 0.2$, SE = 0.2)	($\beta = 0.2$, SE = 0.2)	($\beta = 0.2$, SE = 0.2)	($\beta = 0.3$, SE = 0.2)	($\beta = 0.2$, SE = 0.3)

p values for association of obesity SNPs adjusted for age, sex, community group, and n-3 PUFA intake. Estimates of effect size (β) are reported for additive model and BMI-increasing alleles using transformed phenotypes. Results significant at $p \leq 0.005$ are highlighted in bold. Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC), and waist circumference (WC)

Table 5 Interaction with n-3 PUFA intake modifies the GRS association with adiposity phenotypes

Outcome	Main effect[a]	GRS*n-3 PUFA intake[b]	GRS*community group[c]
BMI (kg/m^2)	**0.012**	**0.011**	0.480
Percentage body fat (%)	**0.022**	**0.025**	0.469
Hip circumference (cm)	0.08	0.068	0.916
Thigh circumference (cm)	**0.025**	0.084	0.555
Waist circumference (cm)	**0.038**	**0.018**	0.517

Results significant at $p \leq 0.05$ are highlighted in bold

[a] *p* values for GRS analysis adjusted for sex, age, community group, and n-3 PUFA intake

[b] *p* values for interaction analysis (GRS*n-3 PUFA) adjusted for sex, age, community group, and n-3 PUFA intake

[c] *p* values for interaction analysis (GRS*community group) adjusted for sex, age, community group, and n-3 PUFA intake

(Table 6). Figure 3 demonstrates the $\delta^{15}N$*GRS interaction corrected for either (A) total energy (kcal) or (B) total fat intake (% calories) in a subset of participants ($n = 488$) did not significantly change the trend or strength of the $\delta^{15}N$*GRS interaction (Supplementary Table IV).

Discussion

In this study, we evaluated 10 of the 32 loci identified in a previous meta-analysis of obesity GWAS (Speliotes et al. 2010) for individual and cumulative associations with adiposity phenotypes in a cohort of Yup'ik people and assessed whether exposure to n-3 PUFAs modified these associations. After corrections for n-3 PUFA consumption, we found rs9939609 (*FTO*) was positively associated PBF and rs7647305 (*ETV5*) was negatively associated with ThC and HC. Interestingly, controlled feeding studies in rodents have shown that n-3 PUFAs decrease adiposity by reducing central fat mass (Tiryaki-Sönmez et al. 2011); however, epidemiological evidence in humans supporting the anti-obesity effects of n-3 PUFAs remains controversial and is often limited to n-3 PUFA supplementation studies (Buckley and Howe 2010). Our analyses indicate that genetic predisposition to obesity, as measured by the GRS, was positively associated with adiposity and interactions

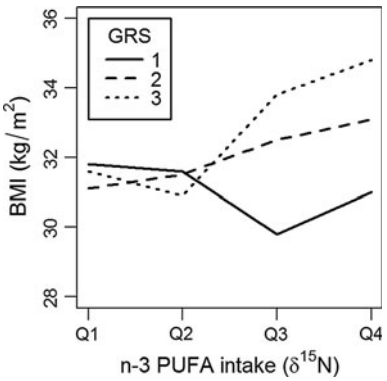

Fig. 2 n-3 PUFA intake modifies the GRS association with BMI ($p = 0.011$). n-3 PUFA intake (δ^{15}N) is represented as a categorical variable with four groups by quartile (*Q1–Q4*) where *Q1* is lowest level of n-3 PUFA intake and *Q4* is the highest level of n-3 PUFA intake. The GRS is represented as a categorical variable with three groups by tertiles (*T1–T3*) where *T1* is lowest obesity GRS and *T3* is highest obesity GRS

Table 6 Variation (%) in adiposity phenotypes explained by GRS association and GRS interactions with n-3 PUFA intake

Variable	BMI	PBF	HC	ThC	WC
GRS	**0.7**	**0.3**	0.5	**0.7**	**0.5**
GRS*δ^{15}N	**1.7**	**0.8**	1.4	1.5	**1.4**

Results significant at $p \leq 0.05$ are highlighted in bold. Variation attributed to GRS after adjustment for age, sex, community group, and n-3 PUFA intake. Variation attributed to GRS*n-3 PUFA interaction after adjustment for age, sex, community group, and n-3 PUFA intake

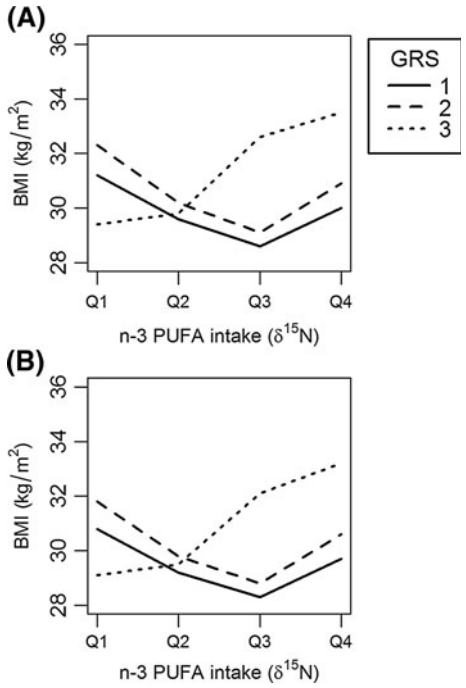

Fig. 3 n-3 PUFA intake modifies the GRS association with BMI after correction for either **a** total fat intake ($p = 0.034$) or **b** total energy intake ($p = 0.033$). In a subset of participants ($n = 488$), we evaluated whether total fat intake (kcal) or total energy intake (% calories) confounded the GRS*n-3 PUFA intake interaction with BMI by including these variables, derived from 24 Hour Recalls (24HR), as an additional covariate in the δ^{15}N*GRS interaction model. n-3 PUFA intake (δ^{15}N) is represented as a categorical variable with four groups by quartile (*Q1–Q4*) where *Q1* is lowest level of n-3 PUFA intake and *Q4* is the highest level of n-3 PUFA intake. The GRS is represented as a categorical variable with three groups by tertiles (*T1–T3*) where *T1* is lowest obesity GRS and *T3* is highest obesity GRS

with n-3 PUFA intake modified these associations whereby among individuals with a low GRS, n-3 PUFA intake have either no effect on BMI or they are anti-obesigenic. However, individuals with moderate (2) or high (3) GRS, n-3 PUFAs intake is obesigenic. Taken together, these results suggest the anti-obesity effects of n-3 PUFA in humans may be dependent upon an individual's genetic predisposition to obesity.

GWAS have consistently identified SNPs associated with BMI in European populations that are located within the first intron of *FTO,* such as rs9939609 (Frayling et al. 2007; Speliotes et al. 2010; Willer et al. 2009). To date, most *FTO* candidate gene studies have been conducted on the rs9939609 SNP mainly because this variant has the strongest known association of any SNP with BMI (Tung and Yeo 2011). Subsequent to the discovery of the rs9939609 SNP, studies on the association of *FTO* with BMI in European (Cauchi et al. 2009), African (Adeyemo et al. 2010), and Asian (Cha et al. 2008) study populations have largely confirmed the importance of *FTO* as a candidate gene for obesity, although this is not without exception (Karns et al. 2011; Li et al. 2008). Our data indicate the rs9939609 (*FTO*) risk allele was positively associated with PBF in Yup'ik people and supports other studies indicating that variation in *FTO* contributes to fat accumulation (Moore et al. 2011).

In contrast to rs9939609 (*FTO*), candidate gene studies that have replicated the rs7647305 (*ETV5*) association with obesity remain limited (Elks et al. 2010; Li et al. 2011; Sandholt et al. 2011). A prospective sample of 7,146 European children showed the *ETV5* (rs7647305) SNP was associated with increased BMI and body weight (Elks et al. 2010), and a cohort of 18,014 middle-aged Danish adults demonstrated the *ETV5* (rs7647305) SNP was associated with increased odds of being overweight and/or obese (Sandholt et al. 2011). Finally, a prospective study among 20,428 European adults with an average follow-up of 12.9 years demonstrated the *ETV5* (rs7647305) SNP was associated with protection from developing T2D, and this association was stronger after correcting for BMI (Li et al. 2011).

Our study was not able to evaluate whether the *ETV5* (rs7647305) SNP was associated with protection from

developing T2D in Yup'ik people due to our cross-sectional study design. Interestingly, we found the *ETV5* (rs7647305) associations with adiposity phenotypes in Yup'ik people were "opposite" to those reported in participants of European ancestry (Elks et al. 2010; Sandholt et al. 2011). Although this lack of consistency can be attributed to type I error, theoretical modeling has argued that "flip-flop" association can be attributed to population differences that occur when the SNP of interest is correlated with the causal variant through linkage disequilibrium (Lin et al. 2007). Specifically, our results demonstrate the *ETV5* (rs7647305) C allele was associated with reduced HC and ThC and near associations for reduced BMI, PBF, and WC. Moreover, despite the fact that overweight and obesity prevalence among Yup'ik people resembles the prevalence of overweight and obesity in the general US population, T2D prevalence remains low ($\sim$3 %) (Mohatt et al. 2007). These results are largely consistent with the finding of Li et al. (2011) and together suggest the *ETV5* (rs7647305) loci may protect against the development of T2D by reducing obesity.

The GRS, which evaluates the cumulative association of 10 obesity GWAS loci, was positively associated with several measures of obesity (BMI, PBF, ThC, and WC) in our study population. Consistent with previous studies, our analysis demonstrated the GRS predicts <1 % of the BMI variation in this sample of Yup'ik people (0.7 % for BMI). Li et al. (2010) included 12 SNPs that explained 0.9 % of BMI variation in Europeans, Takeuchi et al. (2011) calculated a GRS with 14 SNPs that explained 0.65 % among Japanese, and Peterson et al. (2011) included 56 SNPs explained 0.66 % of the variation in BMI among a mixed sample of European- and African-Americans. We calculated a GRS in this study using 5 obesity GWAS SNPs that overlap with Li et al. (2010), 7 obesity GWAS SNPs that overlap with Takeuchi et al. (2011), and 9 obesity GWAS SNPs that overlap with Peterson et al. (2011). Taken together, these results suggest that while considerable progress has been made in gene discovery, obesity SNPs highly replicated in GWAS do not yet predict a significant proportion of the heritability attributed to adiposity phenotypes.

Increasingly, experimental designs are considering gene–environment interactions to account for the "missing heritability" associated with complex phenotypes like obesity (Van IJzendoorn et al. 2011). Given the established contribution of n-3 PUFAs to changes in adiposity phenotypes and the availability of a precise biomarker for n-3 PUFA intake (δ^{15}N) in Yup'ik people (O'Brien et al. 2009), we tested whether individuals SNPs and the GRS associations with adiposity phenotypes were modified by consumption of n-3 PUFA. Our analysis demonstrated that elevated n-3 PUFA intake with an increased GRS strengthened the association with adiposity phenotypes. Although n-3 PUFA intake (δ^{15}N) differs according to community group (Supplementary Table V), we did not detect significant GRS*community group interactions that modified adiposity phenotypes. Interestingly, the interaction of GRS with n-3 PUFA intake accounted for approximately twice the phenotypic variation as the genetic risk score alone. These results are consistent with gene-by-environment analysis conducted by Li et al. (2010) that demonstrated physical activity interactions with a GRS, calculated from 12 obesity risk alleles, accounted for twice as much phenotypic variation in obesity-related traits relative to the genetic risk score alone.

The strengths of this study include a sample size with adequate statistical power (>90 %) to detect SNPS associated with adiposity phenotypes, and a statistical approach that accounts for relationships among participants while also allowing for covariates. Our isolated study population of Yup'ik people were ideally suited to test the contribution of interactions between n-3 PUFA intake (Makhoul et al. 2010, 2011) and genetic factors (Lemas et al. 2012) to changes in adiposity phenotypes. Given the 50-fold range of n-3 PUFA consumption from naturally harvested foods in the region that this study population resides in (Bersamin et al. 2008), an additional strength of this study includes a biomarker of EPA and DHA intake (δ^{15}N) that can be precisely estimated in large epidemiological studies (O'Brien et al. 2009). A potential limitation of our study was that n-3 PUFA intake may be confounded by total fat and total energy intake due to a traditional diet pattern that is high in fat. Given this limitation, it is possible that the δ^{15}N*GRS interaction with adiposity phenotypes may have been influenced by total fat or total energy intake. We evaluated this limitation in a subset of participants ($n = 488$) by including total energy (kcal) and total fat intake (% calories), derived from 24 Hour Recalls (24HR), as an additional covariate in the δ^{15}N*GRS interaction model (Model 7). Our follow-up analysis did not find that including total energy and total fat or intake in the δ^{15}N*GRS interaction model significantly changed the results. These data indicate the GRS*n-3 PUFA interactions were not appreciably confounded by total fat and total energy intake and suggest that n-3 PUFA intake may modulate the risk associated with obesity susceptibility genes.

In conclusion, cross-sectional studies of this nature have potential to elucidate novel gene–diet interactions that may ultimately help account for some of the "missing heritability" associated with obesity (Manolio et al. 2009). To the extent that genetic predisposition to obesity is estimated by 10 SNPs reproducibly identified through GWAS, our results suggest that including n-3 PUFA interactions increased the ability to detect the contribution of these

SNPs to changes in BMI. In contrast, individuals with no genetic predisposition to obesity, as measured by GRS, had less adiposity in the presence of high n-3 PUFA intake. Although animal and human studies have reported that consumption of n-3 PUFAs may reduce adiposity (Buckley and Howe 2010), our results indicate the anti-obesity effects of n-3 PUFAs may, in part, be dependent upon an individual's genetic predisposition to obesity. Characterizing gene–diet interactions in with high intakes of n-3 PUFAs may help identify individuals that are likely to benefit from specific dietary interventions. Interestingly, these analyses do suggest anti-obesity effects of n-3 PUFA intake are, in part, dependent upon an individual's genotype and may play an essential role in determining the risk associated with obesity susceptibility genes among Yup'ik people. Additional genomic studies will be required to replicate these results in large circumpolar populations with widely varying intake of n-3 PUFA in order to determine the validity and public health implication.

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