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Authors

Lemas, Dominick J
Klimentidis, Yann C
Aslibekyan, Stella
et al.

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Polymorphisms in stearoyl CoA desaturase and sterol regulatory element binding protein interact with N-3 polyunsaturated fatty acid intake to modify associations with anthropometric variables and metabolic phenotypes in Yup'ik people

Dominick J. Lemas[1,2], **Yann C. Klimentidis**[3], **Stella Aslibekyan**[4], **Howard W. Wiener**[4], **Diane M. O'Brien**[1], **Scarlett E. Hopkins**[1], **Kimber L. Stanhope**[5,6], **Peter J. Havel**[5,6], **David B. Allison**[7,8,9], **Jose R. Fernandez**[8,10], **Hemant K. Tiwari**[7], and **Bert B. Boyer**[1,*]

[1]Center for Alaska Native Health Research, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775

[2]Department of Pediatrics, Section of Neonatology, University of Colorado Denver, Aurora, CO 80045

[3]Mel and Enid Zuckerman College of Public Health, Department of Epidemiology and Biostatistics, University of Arizona, Tucson, AZ 85724

[4]Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL 35294

[5]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616

[6]Department of Nutrition, University of California, Davis, Davis, CA 95616

[7]Department of Biostatistics, Section on Statistical Genetics, University of Alabama at Birmingham, Birmingham, AL 35294

[8]Nutrition Obesity Research Center, University of Alabama at Birmingham, Birmingham, AL, 35294

[9]Office of Energetics, University of Alabama at Birmingham, Birmingham, AL, 35294

[10]Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, 35294

[*]**Corresponding author:** Bert B. Boyer, PhD, Center for Alaska Native Health Research, Institute of Arctic Biology, 311 Irving I Building, University of Alaska Fairbanks, Fairbanks, AK 99775. Phone: (907)-474-7733, Fax: (907)-474-5712, bboyer@alaska.edu.

AUTHOR CONTRIBUTIONS

DJL, HWW, SA, and HKT had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conception and design of the study: H.K.T, SHE and B.B.B. Provision of study materials or participants: S.E.H., D.M.O. and B.B.B. Collection and assembly of data: SEH, and BBB. Analysis and interpretation of the data: D.J.L, Y.C.K, S.A, H.W.W, D.M.O, S.E.H, K.L.S, P.J.H, D.B.A, J.R.F, H.K.T, and B.B.B. Statistical expertise: D.J.L, Y.C.K, S.A, H.W.W, D.M.O, D.B.A, J.R.F, and H.K.T. Drafting of the manuscript: D.J.L, Y.C.K, S.A, H.K.T, and B.B.B. Critical review of the manuscript for important intellectual content: D.J.L, Y.C.K, S.A, H.W.W, D.M.O, S.E.H, K.L.S, P.J.H, D.B.A, J.R.F, H.K.T, and B.B.B. All authors were involved in writing the paper and had final approval of the submitted and published versions.

CONFLICT OF INTEREST

Dr. Allison has received, anticipates, or has had financial interests with the Frontiers Foundation; University of Wisconsin; University of Arizona; Paul Weiss, Wharton & Garrison LLP; and Sage Publications. No other co-authors on this manuscript declare conflict of interest.

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Abstract

Scope—n-3 polyunsaturated fatty acid (n-3 PUFA) intake is associated with protection from obesity, however, the mechanisms of protection remain poorly characterized. The stearoyl CoA desaturase (*SCD*), insulin sensitive glucose transporter (*SLC2A4*), and sterol regulatory element binding protein (*SREBF1)* genes are transcriptionally regulated by n-3 PUFA intake and harbor polymorphisms associated with obesity. The present study investigated how consumption of n-3 PUFA modifies associations between *SCD*, *SLC2A4*, and *SREBF1* polymorphisms and anthropometric variables and metabolic phenotypes.

Materials and Methods—Anthropometric variables and metabolic phenotypes were measured in a cross-sectional sample of Yup'ik individuals (n=1135) and thirty-three polymorphisms were tested for main effects and interactions using linear models that account for familial correlations. n-3 PUFA intake was estimated using red blood cell nitrogen stable isotope ratios. *SCD* polymorphisms were associated with ApoA1 concentration and n-3 PUFA interactions with *SCD* polymorphisms were associated with reduced fasting cholesterol levels and waist-to-hip ratio. *SLC2A4* polymorphisms were associated with hip circumference, high-density lipoprotein and ApoA1 concentrations. *SREBF1* polymorphisms were associated with low-density lipoprotein and HOMA-IR and n-3 PUFA interactions were associated with reduced fasting insulin and HOMA-IR levels.

Conclusion—These results suggest that an individual's genotype may interact with dietary n-3 PUFAs in ways that are associated with protection from obesity-related diseases in Yup'ik people.

Keywords

$\delta^{15}N$; BMI; Alaska Native; gene-by-environment interactions; rs11190480; rs2167444; rs5415; rs5435; CANHR; n-3 PUFA

1 INTRODUCTION

Accumulation of excess body fat increases the risk of developing type-2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [1]. However, there are subgroups of "healthy obese" individuals that appear to carry excess body fat without developing adverse sequelae [2, 3]. Current understanding of the interplay of genetic and environmental factors in the "healthy obese" phenotypes remains limited despite its potential to offer valuable mechanistic insights into obesity-related diseases.

Consumption of n-3 PUFAs, namely eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), is associated with phenotypes consistent with "healthy obesity," including reduced incidence of diabetes [4], reduced adiposity [5], and improvement in fasting lipids [6]. Among Yup'ik people living in Southwest Alaska, we have previously reported that a traditional Yup'ik diet is enriched with EPA and DHA and associated with lower triglyceride levels [7, 8], reduced C-reactive protein levels [9], elevated HDL-cholesterol levels [7], reduced blood pressure, and elevated adiponectin levels, suggestive of increased insulin sensitivity [8]. Importantly, the average BMI (28.0 kg/m^2) in this Yup'ik study population is similar to the average BMI (27.0 kg/m^2) in the National Health and Nutritional Examination (NHANES) III survey [10]; however, the prevalence of T2DM in Yup'ik people is

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considerably lower (3-4%) [11, 12] than the general U.S. population (8.3%) [13]. Given the chronic high intake of n-3 PUFA observed among mostly older Yup'ik people (20 times greater than the current mean intake of the general US population [14, 15]) accumulating evidence suggests that n-3 PUFA intake may be a critical environmental exposure that contributes to the "healthy obesity" phenotype observed in Yup'ik people.

The mechanisms underlying the protective effects of n-3 PUFAs on obesity and cardiometabolic health include, among others, changes in expression of stearoyl CoA desaturase (*SCD*) [16, 17], transcription factor sterol regulatory element binding protein (*SREBF1)* [18] , and insulin sensitive glucose transporter (*SLC2A4,* also commonly referenced as *GLUT4* [19]. SCD is the rate-controlling enzyme catalyzing the biosynthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acid substrates [20]. In mice, *Scd1 (*mouse *SCD* homolog*)* deficiency was associated with increased energy expenditure [21], reduced adiposity [22], increased insulin sensitivity [23], protection against hypertriglyceridemia [24], and with elevated HDL-cholesterol levels [24]. On the other hand, the gene product of *SLC2A4* is the rate-limiting step in skeletal muscle glucose uptake [25] and targeted disruption of *Slc2a4* in mice- through selective knockout in muscle tissue-results in severe insulin resistance and glucose intolerance from an early age [26]. Finally, *SREBF1* is a transcription factor that plays a critical role in energy homeostasis by promoting glycolysis, lipogenesis, and adipogenesis [27]. *Srebf1* over-expression in animal models results in insulin resistance through over-accumulation of lipid/lipoprotein substrates [28].

Whole-genome linkage studies in humans have implicated *SCD*, *SLC2A4*, and *SREBF1* as obesity candidate genes [29–31] and genetic polymorphisms in these genes have been associated with metabolic syndrome [32], obesity [33, 34], T2DM [35, 36], and cholesterol metabolism [37–39]. Moreover, a clinical trial demonstrated that the effects of dietary n-3 PUFA supplementation on cardiometabolic health may vary by *SCD* [40] as well as *SREBF1* genotype [41]. Taken together, we hypothesize that genetic variation in *SCD*, *SLC2A4*, and *SREBF1* may partially explain the association between n-3 PUFA intake and "healthy obesity" observed in Yup'ik people. Therefore, the aim of the present study was to test genetic polymorphisms in *SCD*, *SLC2A4* and *SREBF1* for association with anthropometric variables, T2DM, and fasting lipid phenotypes in a study population of Yup'ik people and evaluate whether these associations are modified by a traditional diet enriched with n-3 PUFAs.

2 MATERIALS ANDMETHODS

2.1 PARTICIPANTS AND METHODS

Recruitment of Yup'ik participants was initiated in 2003 and continues in 11 Southwest Alaska communities. 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 Health Corporation Human Studies Committee. Data on familial relationships used in this study to generate pedigrees were obtained through self-report at the time participants were enrolled in the CANHR study. Summary statistics specific to

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characteristics of the Yup'ik pedigree were calculated using PEDINFO in the Statistical Analysis for Genetic Epidemiology (S.A.G.E., 2009) software.

2.2 ANTHROPOMETRIC AND BIOCHEMICAL MEASUREMENT

Trained staff obtained height, weight and 4 circumferences (waist, hip, triceps, and thigh) measurements using protocols from the NHANES III Anthropometric Procedures Manual [42] as previously described [43]. Percent body fat (PBF) was measured by electrical bioimpedance using a Tanita TBF-300A body fat analyzer (Tanita Corp, Arlington Heights, IL, U.S.A.). Blood samples were collected from participants after an overnight fast, and lipoproteins including total cholesterol, HDL-, LDL-, and VLDL- cholesterol, apolipoprotein A1, and plasma triglycerides concentrations were assayed as previously described [43]. From blood samples, we also measured fasting insulin (FI), blood glucose (FG), glycosylated hemoglobin (HbA_{1c}) and calculated HOMA-IR (homeostatic model assessment of insulin resistance) and HOMA-B (homeostatic model assessment of beta-cell function) as described previously [44]. Finally, we defined a continuous variable called 'FG & HbA_{1c}' that is described in greater detail along with information on participant medications within the **Supporting Information Methods S1.**

2.3 BIOMARKER FOR MARINE 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 [45]. The time to 50% turnover of RBC is approximately 45 days [46], therefore, the mean RBC $\delta^{15}N$ values reflect a mean n-3 PUFA intake over 1.5 months. 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: $\delta^{15}N = [(^{15}N/^{14}N\text{sample} - ^{15}N/^{14}N\text{standard})/(^{15}N/^{14}N\text{standard})] \cdot 1000‰$. The range of isotopic variation in our dataset (9‰) was very large relative to analytical precision (within 0.2‰). We hereafter refer to the nitrogen stable isotope ratio of red blood cells (RBC) as $\delta^{15}N$.

2.4 SNP GENOTYPING

Our final genotyping list included 13 SNPs in *SCD*, 13 SNPs in *SLC2A4*, and 8 SNPs in *SREBF1* that were genotyped using the Sequenom iPLEX platform at the Broad Institute [47]. Linkage disequilibrium (LD) among SNPs in each gene was calculated from haplotype frequencies estimated using the hapfreq command in the FBAT program [48]. We used pair-wise r-squared (r^2) as the metric of LD and limited presentation of haplotypes to those with >5% frequency. Information on SNP selection is presented in **Supporting Information Methods S2** and a description of the primers for SNP 12 and SNP 13 in *SLC2A4* is presented in **Supporting Information Table 1**.

2.5 ASSOCIATION ANALYSIS

Each SNP was tested for association with anthropometric variables, lipid/lipoprotein, and T2DM-related phenotypes using the program ASSOC [49] in the S.A.G.E software package.

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Each SNP included in the analysis was coded as additive by default (defined as the number of minor alleles); however when fewer than 10% of the individuals genotyped for a given SNP were homozygous for the minor allele- we coded the SNPs as dominant (defined as two copies of the major allele compared to at least one copy of the minor allele). Likelihood ratio statistics were calculated to compare 3 nested models and test the null hypothesis of no association between SNPs and outcomes after adjusting for covariates.

Model 1 included baseline covariates (age, sex, community membership) and δ^{15}N; Model 2 included baseline covariates, δ^{15}N, and SNP to test for main genetic effect; Model 3 included baseline covariates, the genetic effect of SNPs, δ^{15}N, and interactions between the genetic effect and δ^{15}N. Note that model 3 is the only model to directly test gene-diet interactions under the null hypothesis between continuous δ^{15}N and ordinal SNP genotypes. Additionally, lipid/lipoprotein and T2DM-related phenotypes included BMI and medication use as baseline covariates in Model 1, 2 and 3. We treated each phenotype being tested as representing a separate family of null hypotheses and correct for the number of tests within each family [50]. P-values were compared to the conventional significance threshold ($p<0.05$, 2-tailed), as well as significance thresholds adjusted for multiple test correction. Multiple test correction in this study was calculated according to the number of non-redundant SNPs with MAF 0.05 for *SCD* (7 tests; α 0.007), *SLC2A4* (6 tests; α 0.008), and *SREBF1* (4 tests; α 0.013) using spectral decomposition of LD matrices [51].

Estimates of effect size (β) were extracted from linear models adjusted for demographic and environmental covariates. Statistical power that accounted for familial correlations [51] 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%. Additional information on data management, statistical analyses and multiple test correction can be found in **Supporting Information Methods S3 and S4**.

3 RESULTS

3.1 CHARACTERISTICS OF YUP'IK PARTICIPANTS

Our study population of 1135 non-pregnant Yup'ik participants represents 195 pedigrees with a mean pedigree size of 5.82 individuals (**Table 1**). In general, Yup'ik women had significantly greater levels of adiposity (BMI, PBF, and HC) and higher fasting total cholesterol, HDL-cholesterol and ApoA1 concentrations relative to Yup'ik men ($p<0.0001$). Additionally, Yup'ik women had significantly higher FI, HOMA-IR, and HOMA-B, and significantly lower waist-to-hip ratio and FG. Thirteen individuals reported taking T2DM-related medication and 55 individuals reported taking lipid/lipoprotein medications.

3.2 DISTRIBUTION OF δ^{15}N IN STUDY POPULATION

Summary statistics for δ^{15}N, our biomarker of n-3 PUFA intake, are grouped by gender and reported in **Table 2**. The overall mean δ^{15}N value in this study was 9.0‰, and ranged from 6.2‰ to 15.2‰. According to the linear relationship between RBC δ^{15}N and RBC EPA described elsewhere for this cohort [45], the corresponding mean EPA (%RBC fatty acids)

was 2.6%. The standard deviation of δ^{15}N in this sample did not differ according to gender (1.5‰ for both females and males).

3.3 GENETIC VARIATION IN *SCD*, *SLC2A4*, AND *SREBF1*

A comprehensive list of thirty-four SNPs in *SCD*, *SLC2A4* and *SREBF1* were genotyped in 1135 Yup'ik participants with a mean genotyping success rate of 93.2% (range 81.2-99.4%). The rs41290540 SNP in *SCD* (MAF=0.12) was the only polymorphism with MAF 0.05 that deviated significantly from HWE proportions (p=0.0002) and was therefore excluded from the analysis. The genetic analyses in this study included the 23 SNPs with MAF 0.05 that did not deviate from HWE proportions (**Table 3**). Two SNPs (rs11557927 and 2282180) in the analysis were coded as dominant. We have limited the presentation and discussion of the results below to those tests that were statistically significant; however, all tabular results for SNP association, gene-diet interactions, and pairwise linkage disequilibrium (LD) results are presented in **Supporting Information Tables 2-8.**

3.4 ANALYSIS OF LIPID/LIPOPROTEIN PHENOTYPES

Figure 1 presents associations between SNPs in *SCD*, *SLC2A4,* and *SREBF1* with MAF 0.05 and lipid/lipoprotein phenotypes. The solid red line represents statistical significance according to multiple test correction for each gene. Notably, rs2167444 and rs7849 in *SCD* were in weak LD (r^2=0.11) and significantly associated with fasting plasma ApoA1 concentrations. The rs5415 and rs5435 polymorphisms in *SLC2A4* were in moderate LD (r^2=0.48) and significantly associated with both fasting plasma ApoA1 concentrations and HDL-cholesterol. An *SREBF1* SNP (rs8066560) was significantly associated with fasting LDL-cholesterol.

Figure 2 illustrates how n-3 PUFA intake modifies the association between rs11190480 in *SCD* and fasting cholesterol. Specifically, n-3 PUFA intake is positively associated with fasting cholesterol levels among all rs11190480 genotypes and this association is significantly attenuated among participants carrying both copies of the rs11190480 major allele (A/A). The mean δ^{15}N by rs11190480 genotype are as follows: A/A—9.0‰ (95% CI: 9.0, 9.1), A/G—9.0‰ (95% CI: 8.9, 9.2), G/G—9.1‰ (95% CI: 8.6, 9.7). We did not detect any significant interactions between SNPs in *SLC2A4* and *SREBF1* and n-3 PUFA intake with lipid/lipoprotein phenotypes.

3.5 ANALYSIS OF ANTHROPOMETRIC VARIABLES

We did not detect significant associations between *SCD or SREBF1* polymorphisms and anthropometric variables upon adjusting for multiple testing (**Figure 3**). However, three polymorphisms (rs2654185, rs5415 and rs5417) in *SLC2A4* were positively correlated with hip circumference and the rs2654185 locus was also positively associated with thigh circumference. **Figure 4a** shows that n-3 PUFA intake is positively correlated with waist-to-hip ratio (WHR) among all rs599961 genotypes and this association is attenuated among individuals carrying at least one copy of the minor rs599961 allele (T/G and G/G). Similarly, **Figure 4b** shows that n-3 PUFA intake was positively correlated with WHR ratio among all rs7849 genotypes and this association was significantly attenuated among heterozygous

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individuals (T/C). We did not detect significant interactions between SNPs in *SLC2A4* or *SREBF1* and n-3 PUFA intake on anthropometric variables.

3.6 ANALYSIS OF TYPE-2 DIABETES-RELATED PHENOTYPES

We did not detect *SCD* or *SLCA24* polymorphisms significantly associated with T2D-related phenotypes upon adjustment for multiple testing (**Figure 5**). Importantly, we observed a *SREBF1* SNP (rs2297508) that was significantly associated with HOMA-IR and detected a significant interaction between n-3 PUFA intake and rs2282180 in *SREBF1* on HOMA-IR. **Figure 6** shows the positive correlation between n-3 PUFA intake and HOMA-IR is notably stronger in those with at least one copy of the rs2282180 minor allele (G/A and A/A). We did not detect significant interactions between SNPs in *SCD* and *SLC2A4* and n-3 PUFA intake on T2DM-related phenotypes.

4 DISCUSSIONS

Our primary findings in a cross-sectional sample of Yup'ik people demonstrate genetic variations in *SCD, SLC2A4,* and *SREBF1* were associated with fasting lipid/lipoprotein phenotypes and a traditional dietary pattern rich in n-3 PUFA intake modified the association between *SCD* and *SREBF1* polymorphisms with anthropometric variables, circulating lipid/lipoprotein and T2DM-related phenotypes. People adhering to a traditional Yup'ik dietary pattern rich in marine mammals and fish have consume n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at levels up to 20 times higher than in the general US population [8, 52]. Using the nitrogen stable isotope ratio (d^{15}N) as a validated, objective measure of the traditional dietary pattern, we have shown that high levels of traditional food intake are associated with high adiponectin and a low leptin:adiponectin ratio, suggestive of increased insulin sensitivity [8]. Published findings have shown that the transition away from the Yup'ik diet is associated with an increase in the prevalence of overweight and obesity, and that the prevalence of overweight and obesity in the population now mirrors that of the general U.S. population [11, 53]. At the same time, however, the prevalence of metabolic syndrome (15%)[43] and T2D (3%) among Yup'ik people remains lower than in the U.S. general population [14, 15], suggesting that consumption of a traditional diet enriched with n-3 PUFAs may contribute to "healthy obesity". The results of this study extend what is known the traditional diet enriched inn-3 PUFA and the "healthy obese" phenotype observed in Yup'ik people by demonstrating that the traditional diet rich in n-3 PUFAs is associated with anthropometric variables, circulating lipid/lipoprotein and T2DM-related phenotypes in Yup'ik people through gene-diet interactions with *SCD, SLC2A4,* and *SREBF1* genotypes.

Previous studies that have examined *SCD* polymorphisms associated with obesity-related phenotypes have produced conflicting results [32, 36, 54]. Liew *et al.* failed to detect *SCD* polymorphisms associated with T2DM-related traits, BMI, or waist-hip-ratio in a large European case-control study [54]. Gong *et al.* reported that *SCD* genetic variation was positively associated with metabolic syndrome (MetS) in a Costa Rican cohort [32] and Rudkowska *et al.* reported that *SCD* genotypes in a Canadian cohort were associated with cardiometabolic risk factors alone or in combination with n-3 PUFA supplementation [40].

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In contrast, Warensjö *et al.* reported *SCD* polymorphisms were associated with increased insulin sensitivity, reduced BMI and smaller waist circumference in Swedish men [33]. Our analyses did not evaluate the contribution of *SCD* polymorphisms to T2DM status and MetS status directly, given the historically low prevalence of both T2DM (<3%) [11] and MetS (8.6 % in men and 19.8% in women) [43] observed among Yup'ik people. Nevertheless, we investigated the association between *SCD* polymorphisms with four of the five traits associated with MetS (FG, HDL-cholesterol, triglycerides and WC) as well as additional T2DM-related phenotypes and observed the minor allele of several *SCD* polymorphisms were nominally associated with a modest increase in FG (rs599961) and FG & HbA_{1c} (rs599961 and rs7849). Collectively, our results are consistent with Warensjö *et al.,* demonstrating that *SCD* genetic variation was associated with reduced adiposity and suggest that SCD activity may be an important mechanism that contributes to "healthy obesity" in this population.

More recently, the rs5435 minor allele in *SLC2A4* was positively associated with T2DM and fasting insulin levels in a South Indian cohort [35] and the rs2654185 minor allele was positively associated with fasting HbA_{1c} levels in a cohort of Japanese men [36]. Our results demonstrate the *SLC2A4* rs2654185 polymorphism was significantly and positively associated with hip circumference (p=0.005) and thigh circumference (p=0.008). Additionally, we found the rs5435 minor allele, previously linked to risk of T2DM [35], was significantly associated with lower fasting HDL-cholesterol (p=0.006) and ApoA1 concentrations (p=0.005). The rs5415 polymorphism in *SLC2A4* was also inversely associated with fasting HDL-cholesterol (p=0.001) and ApoA1 levels (p=0.004) and positively associated with hip circumference (p=0.004). Taken together, we extend the findings of Bodhini *et al.* [35] and Xi *et al.* [36] by demonstrating *SLC2A4* polymorphisms are associated with reduced fasting HDL-cholesterol levels and provide novel evidence that *SLC2A4* is associated with anthropometric variables by increasing hip and thigh circumference.

In our study, we found the *SREBF1* non-synonymous G952G (rs2297508) variant was significantly associated with elevated HOMA-IR levels (p=0.009) and nominally associated with elevated LDL-cholesterol levels (p=0.028) and fasting insulin levels (p=0.014). Consistent with these results, *SREBF1* polymorphisms that were correlated with G952G (rs2297508) were associated with increased LDL-cholesterol (rs2282180: p=0.024 and rs8066560: p=0.012), elevated total cholesterol (rs8066560: p=0.031) and variation in fasting insulin levels (rs9899634: p=0.046, rs8066560: p=0.024 and rs9902941: p=0.027). Collectively, our results are consistent with the findings of Bouchard-Mercier *et al.* [41] by demonstrating that *SREBF1* polymorphisms are associated with phenotypes that may impact insulin sensitivity, potentially through mechanisms that alter insulin secretion and increase lipid/lipoprotein synthesis.

Our previous work demonstrates that a traditional Yup'ik diet rich in n-3 PUFA intake is positively associated with total cholesterol, LDL, HDL and ApoA1 and inversely associated with TG in this study population having a 50-fold variation in consumption of EPA [8, 45]. Although n-3 PUFA supplementation is not always linked to a change in total plasma cholesterol levels [55], previous reports have demonstrated a significant decrease in total

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cholesterol and a significant increase in HDL cholesterol after fish oil supplementation [56]. In this study, we found that *SCD* polymorphisms were positively associated with ApoA1 and that *SCD* genotypes attenuated the positive associations between the traditional dietary pattern and fasting cholesterol levels (**Figure 2**). Furthermore, the observed positive correlation between intake of the traditional diet and waist-to-hip ratio (WHR) was attenuated among individuals carrying the most common rs599961 (**Figure 4a**) and rs7849 (**Figure 4b**) genotypes in *SCD*. An important observation made by our study demonstrates the positive correlation between intake of the traditional diet and HOMA-IR was attenuated among individuals carrying the dominant *SREBF1* rs2282180 (G/G) (**Figure 6**). This result is supported by previous observations in a Canadian cohort that reported *SREBF1* genetic variation mediates associations between fish oil supplementation and insulin sensitivity [41]. Collectively, our results suggest that relatively common genetic variants in *SCD* and *SREBF1* (0.05 MAF) attenuated the positive associations between intake of a traditional diet rich in n-3 PUFAs and increases in fasting cholesterol and HbA1c levels, as well as the WHR among Yup'ik participants.

The strengths of this study include a Yup‘ik study population was ideally suited for investigations of the impact of n-3 PUFA [7, 9] and genetic factors [57, 58] on anthropometric variables, lipid/lipoprotein, and T2DM-related phenotypes given the 50-fold range of EPA consumption in this study population [45]. Our analyses also included a precise and objective measure of n-3 PUFA intake using nitrogen stable isotope ratios from red blood cells [59] and a sample size large enough to detect SNP associations and a statistical approach that accounts for familial relationships among participants [49]. Differences in results reported in the present study in comparison to other investigations may include, but are not limited to, small sample size, population stratification and differences in statistical analysis [60, 61]. The main limitation of this study was a cross-sectional experimental design that prevents the temporal order of exposures and outcomes to be determined and thus it is important to point out we are reporting only associations and causality is unclear. Additional limitations include potentially confounding factors (e.g. alcohol intake, exercise, nutrients enriched in the traditional diet in additional to n-3 PUFAs, and smoking status) that were not measured. Some of these factors have been shown to influence lipid, insulin and glucose metabolism.

In summary, the primary findings of this study demonstrate that polymorphisms within or near *SCD*, *SLC2A4, SREBF1* are associated with anthropometric variables, lipid/lipoprotein, and T2DM-related phenotypes in a study population with widely varying n-3 PUFA intake. Moreover, our analyses revealed beneficial gene-diet interactions with *SCD* polymorphisms that attenuated the positive n-3 PUFA associations with fasting cholesterol levels and waist-to-hip ratio among individuals carrying the most common genotypes at those loci. Consistent with these results, we also found that n-3 PUFA intake attenuated the positive associations between *SREBF1* polymorphisms with fasting HOMA-IR among individuals carrying the dominant rs2282180 genotype. Taken together, these results support our hypothesis that interactions between common *SCD, SLC2A4,* and *SREBF1* genotypes and n-3 PUFA intake are associated with cardiometabolic phenotypes consistent with "healthy obesity". Additional comprehensive genomic studies in longitudinal cohorts of

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diverse populations having variable intake of n-3 PUFAs will be necessary to replicate these findings and determine the generalizability and public health implications of our findings.

Supplementary Material

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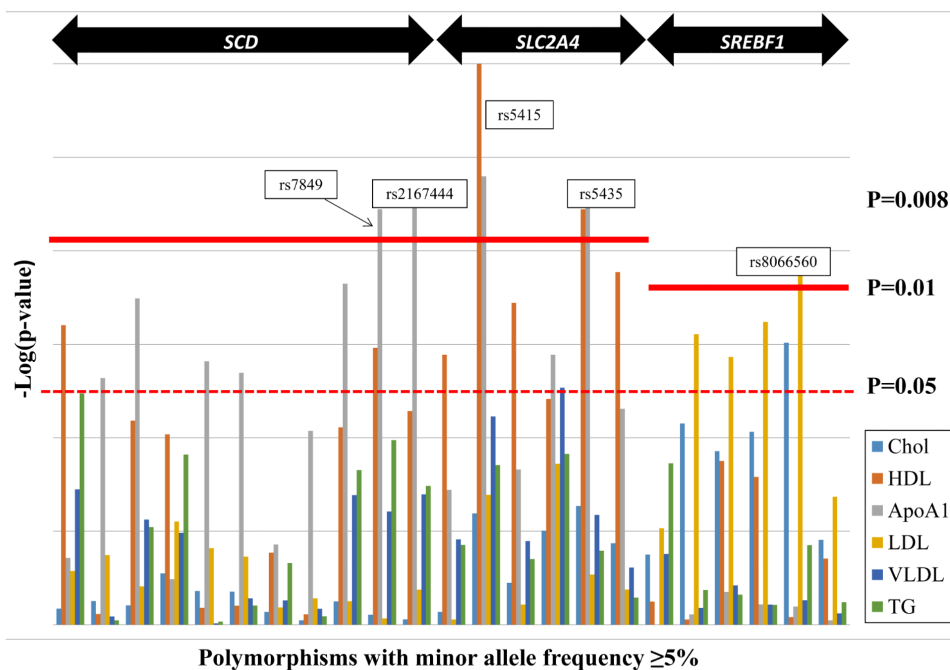

Figure 1. *SCD*, *SLC2A4* and *SREBF1* polymorphisms that are associated with lipid/lipoprotein phenotypes

Association of SNPs in a linear model adjusted for age, sex, lipid/lipoprotein medications, community membership, BMI, and n-3 PUFA intake. The solid red line represents statistical significance according to multiple test correction for each gene, estimated using the spectral decomposition of LD matrix [51]. The dotted red line represents nominal significance (p −640.05). Total cholesterol (Chol), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL) and triglycerides (TG).

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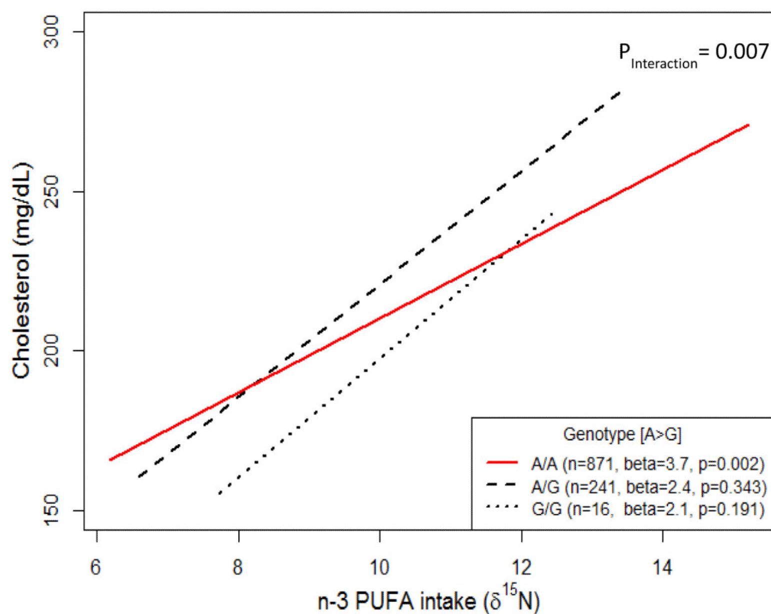

Figure 2. n-3 PUFA intake (δ^{15}15N) modifies a *SCD* SNP associated with fasting cholesterol levels (rs11190480: p=0.007)

n-3 PUFA intake was positively associated (p=0.007) with fasting cholesterol levels among all rs11190480 genotypes and this association was significantly attenuated among participants carrying both copies of the rs11190480 major allele (A/A). Gene-diet interaction between continuous n-3 PUFA intake (δ^{15}N) and categorical rs11190480 genotypes were adjusted for participant age, sex, lipid medications, community membership, BMI, and n-3 PUFA intake. The figure legend notes the SNP [major>minor] allele, the sample size, beta and p-value for each genotype stratification.

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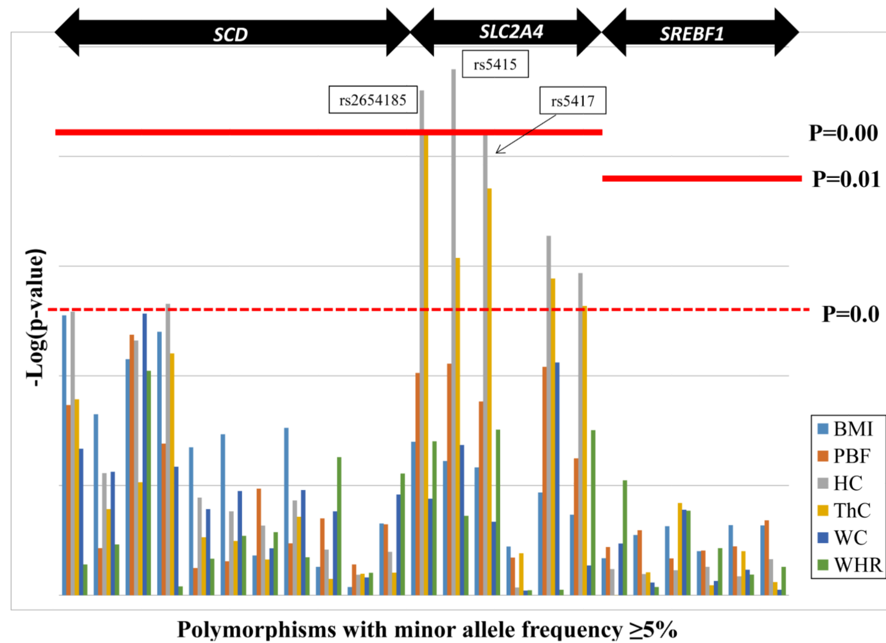

Figure 3. *SCD*, *SLC2A4* and *SREBF1* polymorphisms that are associated with anthropometric variables

Association of SNPs in a linear model adjusted for age, sex, community membership, and n-3 PUFA intake. The solid red line represents statistical significance according to multiple test correction for each gene, estimated using the spectral decomposition of LD matrix [51]. The dotted red line represents nominal significance (p 0.05). Body mass index (BMI), percent body fat (PBF), hip circumference (HC), thigh circumference (ThC), waist circumference (WC), and waist-to-hip ratio (WHR).

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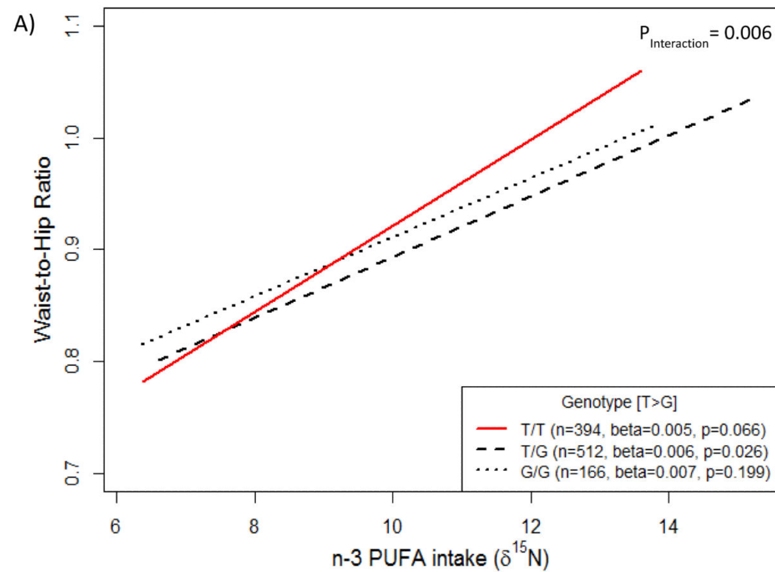

Figure 4. n-3 PUFA intake (δ^{15}N) modifies *SCD* SNPs associated with waist-to-hip ratio (rs599961:p=0.006 and rs7849:p=0.007)

(A) n-3 PUFA intake is positively correlated with waist-to-hip ratio (WHR) among all rs599961 genotypes and this association is attenuated among individuals carrying at least one copy of the minor rs599961 allele (T/G and G/G). **(B)** n-3 PUFA intake was positively correlated with WHR ratio among all rs7849 genotypes and this association was significantly attenuated among heterozygous individuals (T/C). Gene-diet interaction between continuous n-3 PUFA intake (δ^{15}N) and categorical *SCD* SNP (rs599961 and rs7849) genotypes were adjusted for participant age, sex, lipid medications, community membership, BMI, and n-3 PUFA intake. The figure legend notes the SNP [major>minor] allele, the sample size, beta and p-value for each genotype stratification.

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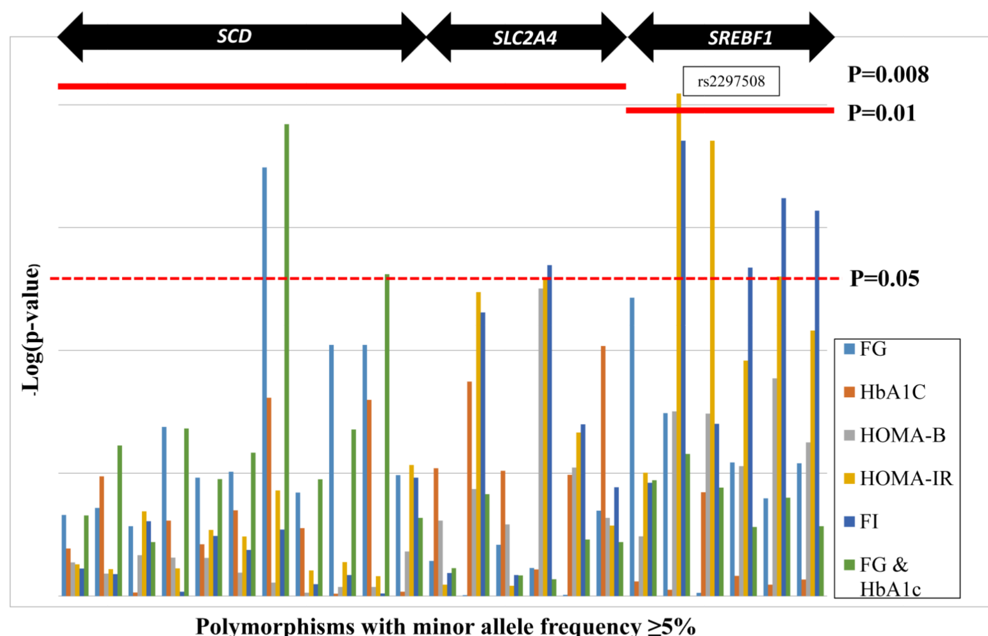

Figure 5. *SCD*, *SLC2A4* and *SREBF1* polymorphisms that are associated with type-2 diabetes mellitus related phenotypes

Association of SNPs in a linear model adjusted for age, sex, diabetes medications, community membership, BMI and n-3 PUFA intake. The solid red line represents statistical significance according to multiple test correction for each gene, estimated using the spectral decomposition of LD matrix [51]. The dotted red line represents nominal significance (p 0.05). Fasting blood glucose (FG), glycosylated hemoglobin (HbA_{1c}), homeostatic model assessment of beta-cell function (HOMA-B), homeostatic model assessment of insulin resistance (HOMA-IR), fasting insulin (FI), and fasting blood glucose & glycosylated hemoglobin (FG & HbA_{1c}). (*) denotes SNPs that were analyzed using a dominant model of inheritance.

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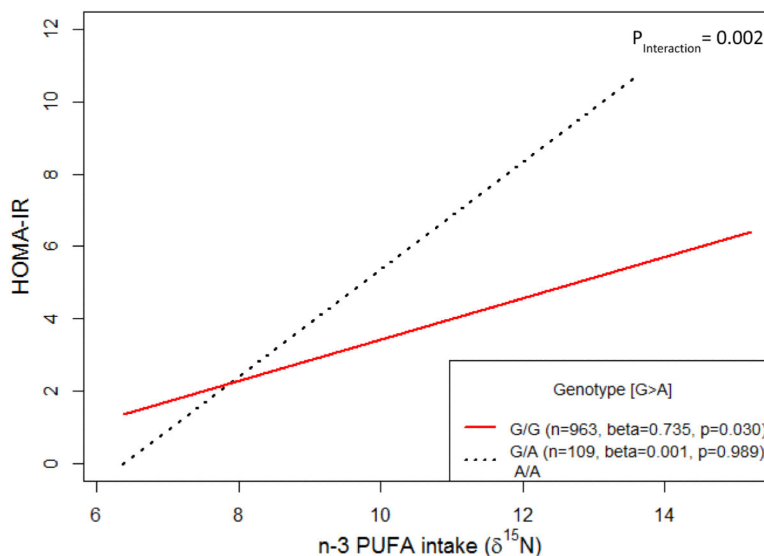

Figure 6. n-3 PUFA intake (δ^{15}N) modifies *SREBF1* SNP association with HOMA-IR (rs2282180*: p=0.002)

The positive association between n-3 PUFA intake and HOMA-IR is attenuated among individuals carrying two copies of the rs2282180 major allele (G/G). Gene-diet interaction between continuous n-3 PUFA intake (δ^{15}N) and categorical rs2282180 genotypes were adjusted for participant age, sex, community membership, BMI, and n-3 PUFA intake. The figure legend notes the SNP [major>minor] allele, the sample size, beta and p-value for each genotype stratification. (*) denotes that SNP was analyzed using dominant model of inheritance.

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Table 1

Descriptive statistics of Yup'ik participants[a],[b]

Variables	Women	Men	p-values
No. of participants	597	538	
Age (yrs)	40.5 (38.1 - 42.6)	38.8 (37.8 - 39.8)	0.1271
Anthropometric Variables			
BMI (kg/m^2)	28.9 (28.1 - 29.8)	26.4 (26.0 - 26.8)	<0.0001
Percentage Body Fat (%)	43.1 (41.8 - 44.4)	28.0 (27.4 - 28.6)	<0.0001
Waist Circumference (cm)	88.4 (86.5 - 90.4)	87.8 (86.8 - 88.8)	0.4456
Hip Circumference (cm)	105.8 (104.3 - 107.4)	98.9 (98.2 - 98.7)	<0.0001
Thigh Circumference (cm)	51.2 (50.5 - 51.9)	50.4 (50.0 - 50.8)	0.0101
Waist-to-Hip Ratio	0.86 (0.86 – 0.87)	0.92 (0.91 – 0.92)	<0.0001
Lipid/lipoprotein Measures			
Cholesterol (mg/dL)	215.4 (209.1 - 221.9)	205.8 (202.6 - 209.1)	<0.0001
HDL (mg/dL)	66.2 (63.8 - 68.8)	56.8 (55.7 - 58.0)	<0.0001
Apolipoprotein A-I (mg/dL)	175.9 (172.1 - 179.9)	162.9 (168.0 - 165.0)	<0.0001
LDL (mg/dL)	130.2 (125.2 - 135.3)	130 (127.2 - 132.8)	0.9090
VLDL (mg/dL)	15.2 (14.3 - 16.1)	15.2 (14.7 - 15.7)	0.9720
Triglyceride (mg/dL)	75.6 (71.4 - 80.1)	74.3 (71.9 - 76.9)	0.5029
Diabetes-Related Measures			
Fasting glucose (mg/dL)	90.2 (88.9 -91.6)	92.2 (91.5 - 93.0)	0.0007
HbA_{1c} (%)	5.36 (5.31 - 5.41)	5.42 (5.40 - 5.45)	0.0049
HOMA-B	232.6 (215.5 - 249.6)	189.2 (180.9 - 197.5)	<0.0001
HOMA-IR	3.9 (3.5 - 4.2)	3.4 (3.3 - 3.6)	0.0049
Fasting insulin (μU/ml)	15.3 (14.3 - 16.3)	13.4 (13.0 - 14.0)	<0.0001

[a]Values are reported as mean (95% CI) predicted from linear model accounting for familial correlations among 195 pedigrees with a mean pedigree size of 5.82 individuals (range, 1-893).

[b]p-values for differences by gender are derived using student t-test.

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Table 2

Distribution of the RBC nitrogen stable isotope ratio (δ^{15}N) in Yup'ik people[a,b] Total Women Men

	Total	Women	Men
No. of participants	1135	597	538
Mean ± SD (‰)	9.0 ± 1.5	9.1 ± 1.5	8.8 ± 1.5
Maximum	15.2	15.2	13.5
Minimum	6.2	6.4	6.2
Range (‰)	9	8.8	7.3

[a]Isotope ratios are presented as delta values in "permil" relative to atmospheric nitrogen: $\delta^{15}N = [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard})/({}^{15}N/^{14}N_{standard})] \cdot 1000‰$.

[b]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 [45].

Table 3

SCD, SLC2A4, and *SREBF1* polymorphisms with MAF 0.05

Gene	Chr[a]	Position (bp)	SNP #[b]	SNP[c]	Allele[d]	MAF[e]	Genotype[g]			N Genotyped	HWE p-value[f]
							Homozygous Major	Heterozygous	Homozygous Minor		
SCD	10	102099192	1	rs1502593	G>A	0.21	747	266	26	1039	0.725
SCD	10	102100891	2	rs522951	C>G	0.34	482	470	113	1065	0.926
SCD	10	102102065	3	rs11190480	A>G	0.12	871	241	16	1128	0.897
SCD	10	102104453	4	rs3071	A>C	0.19	764	271	17	1052	0.256
SCD	10	102104997	5	rs3829160	G>A	0.34	487	473	110	1070	0.781
SCD	10	102106301	6	rs2234970	A>C	0.34	487	461	111	1059	0.917
SCD	10	102107197	7	rs599961	T>G	0.32	394	512	166	1072	0.983
SCD	10	102111552	8	rs41290540	A>C	0.12	820	255	1	1076	0.000
SCD	10	102111569	9	rs3978768	A>G	0.33	496	433	113	1042	0.263
SCD	10	102111806	10	rs11557927*	T>G	0.09	769	146	7	922	0.923
SCD	10	102112593	11	rs7849	T>C	0.44	279	557	238	1074	0.257
SCD	10	102114734	12	rs2167444	T>A	0.13	799	232	17	1048	0.976
SLC2A4	17	7124086	13	rs2654185	A>C	0.31	615	386	71	1072	0.384
SLC2A4	17	7125205	14	rs5415	T>C	0.50	309	479	215	1003	0.305
SLC2A4	17	7125786	15	rs5417	C>A	0.31	616	382	69	1067	0.407
SLC2A4	17	7126746	16	rs16956647	C>T	0.15	702	326	44	1072	0.487
SLC2A4	17	7127847	17	rs5435	T>C	0.48	310	555	209	1074	0.211
SLC2A4	17	7133979	18	rs3744405	G>A	0.30	621	389	72	1082	0.356
SREBF1	17	17651995	19	rs4925114	G>A	0.37	432	497	148	1077	0.816
SREBF1	17	17656042	20	rs2297508	G>C	0.46	294	498	212	1004	0.817
SREBF1	17	17661563	21	rs2282180*	G>A	0.05	963	107	2	1072	0.975
SREBF1	17	17668668	22	rs9899634	T>A	0.49	266	554	245	1065	0.629
SREBF1	17	17668768	23	rs8066560	C>A	0.44	320	554	197	1071	0.234
SREBF1	17	17674485	24	rs9902941	C>T	0.49	265	561	246	1072	0.157

[a]Chromosome;

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[b]SNP# as displayed in linkage disequilibrium plot (Suppl. Figure 1);

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

[d]Major > Minor;

[e]MAF computed using FREQ in S.A.G.E.;

[f]HWE p-values computed using GCC HW test that accounts for relationships among individuals [62];

[g]Genotype.

*denotes SNPs that were analyzed using a dominant model of inheritance.

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