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Linkage and association analysis of obesity traits reveals novel loci and interactions with dietary n-3 fatty acids in an Alaska Native (Yup'ik) population

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ABSTRACT

Objective. To identify novel genetic markers of obesity-related traits and to identify gene-diet interactions with n-3 polyunsaturated fatty acid (n-3 PUFA) intake in Yup'ik people.

Material and methods. We measured body composition, plasma adipokines and ghrelin in 982 participants enrolled in the Center for Alaska Native Health Research (CANHR) Study. We conducted a genome-wide SNP linkage scan and targeted association analysis, fitting additional models to investigate putative gene-diet interactions. Finally, we performed bioinformatic analysis to uncover likely candidate genes within the identified linkage peaks.

Results. We observed evidence of linkage for all obesity-related traits, replicating previous results and identifying novel regions of interest for adiponectin (10q26.13-2) and thigh circumference (8q21.11-13). Bioinformatic analysis revealed *DOCK1*, *PTPRE* (10q26.13-2) and *FABP4* (8q21.11-13) as putative candidate genes in the newly identified regions. Targeted SNP analysis under the linkage peaks identified associations between three SNPs and obesity-related traits: rs1007750 on chromosome 8 and thigh circumference (P = 0.0005), rs878953 on chromosome 5 and thigh skinfold (P = 0.0004), and rs1596854 on chromosome 11 for waist circumference (P = 0.0003). Finally, we showed that n-3 PUFA modified the association

Abbreviations: BMI, body mass index; GWAS, genome-wide association study; n-3 PUFA, omega-3 polyunsaturated fatty acids; CANHR, Center for Alaska Native Health Research; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; $\delta^{15}N$, nitrogen stable isotope ratio; RBC, red blood cell; SNP, single nucleotide polymorphism; LOD, logarithm of the odds.

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between obesity related traits and two additional variants (rs2048417 on chromosome 3 for adiponectin, P for interaction = 0.0006 and rs730414 on chromosome 11 for percentage body fat, P for interaction = 0.0004).

Conclusions. This study presents evidence of novel genomic regions and gene-diet interactions that may contribute to the pathophysiology of obesity-related traits among Yup'ik people.

1. Introduction

Obesity is a complex disorder arising from multiple interactions of genes, behavior and environment. Family-based heritability estimates provide strong evidence of genetic contributions to obesity-related phenotypes [1–3]. Although more than 40 genome-wide linkage scans and association studies, as well as several hundred candidate gene studies, have yielded numerous obesity-related loci, a large proportion of the genetic risk for obesity remains unexplained [3–5]. While a few genomic regions, e.g., 1p36, 2p21, 3q27, 10p12, and 11q23-24 or genes such as *FTO* and *MC4R* have been widely replicated across various studies, other findings remain largely inconsistent [3,4,6]. Moreover, body mass index (BMI) is an imperfect proxy for body fat with differential validity among populations, which highlights the importance of studying additional phenotypes such as skinfold thickness or circumference measures [2].

Meta-analyses of genome wide association studies (GWAS) and linkage scans suggest that obesity-related phenotypes may be influenced by many genes with small effects [7]. Such genes can be fruitfully studied in geographically remote populations, such as the Yup'ik people, due to their extended family structure and reduced genetic variation [8]. Yup'ik people living in Southwest Alaska have a high prevalence of the 'healthy obese' phenotype, where obesity is not closely tied with other metabolic complications as is commonly seen in other populations [9]. Specifically, Yup'ik people have a historically low prevalence of insulin resistance, metabolic syndrome and type 2 diabetes despite obesity prevalence similar to those of the US and Canadian general populations [10–15]. Such unique separation of obesity with other metabolic complications may be related to the traditional Yup'ik diet, which is high in marine-derived omega-3 polyunsaturated fatty acids (n-3 PUFA) [9,16]. Although prior studies from our group have found associations between candidate SNPs in *FTO*, *ETV5*, *HHEX*, *CDKAL1* and metabolic traits [17,18], the genetic contributions to obesity in the Yup'ik have not been comprehensively examined at the genome-wide level.

In this study, we examined the genetic architecture of obesity-related phenotypes, including BMI, multiple measures of body composition, and plasma adipokine concentrations, in Yup'ik people. We conducted a whole genome linkage scan and targeted association testing within observed linkage peaks, supplemented by a bioinformatic analysis. In addition, we investigated whether a diet rich in marine n-3 PUFA intake modifies the associations between genetic variants and obesity traits.

2. Material and Methods

2.1. Participants

The Center for Alaska Native Health Research (CANHR) studies genetic, behavioral, and dietary risk factors underlying obesity and their relationship to diabetes and cardiovascular disease among Yup'ik people [15]. Recruitment of participants from 11 Southwest Alaska communities began in 2003 and is currently ongoing. This study sample consists of 982 non-pregnant Yup'ik individuals that were ≥14 years old at the time of enrollment and reflects the age distribution of all eligible participants according to the 2000 U.S. census.

2.2. Ethics

Participants provided written informed consent. The Institutional Review Boards of the University of Alaska and the National and Alaska Area Indian Health Service, as well as the Yukon-Kuskokwim Human Studies Committee, approved the study protocols.

2.3. Measurements of Anthropometric, Clinical, and Dietary Characteristics

Anthropometric measurements, including height, weight, four circumferences (waist, hip, triceps, and thigh), and four skinfolds (abdominal, subscapular, triceps and thigh) were collected using protocols from the National Health and Nutrition Examination Survey III Anthropometric Procedures Manual [19] as previously described [14] and percentage body fat was measured by electrical bioimpedance using a Tanita TBF-300A body composition analyzer (Tanita, Arlington Heights, IL). Blood samples were collected from participants after an overnight fast, and lipoproteins including total cholesterol, high density lipoprotein (HDL-) cholesterol, low density lipoprotein (LDL-) cholesterol, very low density lipoprotein (VLDL-) cholesterol, apolipoprotein A1, and plasma triglycerides were assayed as previously described [14]. Adiponectin and leptin were measured by radioimmunoassay (Linco Research, St Charles, MO for adiponectin and leptin; and Phoenix Pharmaceuticals, Burlingame, CA for ghrelin). Intra- and inter-assay coefficients of variation were respectively 7.1% and 12.1% for adiponectin, 6.7% and 11.1% for leptin, and 3.4% and 25.4% for ghrelin [20]. Long-term intake of n-3 PUFA was estimated using the nitrogen stable isotope ratio ($\delta^{15}N$) of red blood cells (RBC), as previously described [21–23].