



Joint Association of Nicotinic Acetylcholine Receptor Variants with Abdominal Obesity in American Indians: The Strong Heart Family Study

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Abstract

Cigarette smoke is a strong risk factor for obesity and cardiovascular disease. The effect of genetic variants involved in nicotine metabolism on obesity or body composition has not been well studied. Though many genetic variants have previously been associated with adiposity or body fat distribution, a single variant usually confers a minimal individual risk. The goal of this study is to evaluate the joint association of multiple variants involved in cigarette smoke or nicotine dependence with obesity-related phenotypes in American Indians. To achieve this goal, we genotyped 61 tagSNPs in seven genes encoding nicotinic acetylcholine receptors (nAChRs) in 3,665 American Indians participating in the Strong Heart Family Study. Single SNP association with obesity-related traits was tested using family-based association, adjusting for traditional risk factors including smoking. Joint association of all SNPs in the seven nAChRs genes were examined by gene-family analysis based on weighted truncated product method (TPM). Multiple testing was controlled by false discovery rate (FDR). Results demonstrate that multiple SNPs showed weak individual association with one or more measures of obesity, but none survived correction for multiple testing. However, gene-family analysis revealed significant associations with waist circumference ($p = 0.0001$) and waist-to-hip ratio ($p = 0.0001$), but not body mass index ($p = 0.20$) and percent body fat ($p = 0.29$), indicating that genetic variants are jointly associated with abdominal, but not general, obesity among American Indians. The observed combined genetic effect is independent of cigarette smoking *per se*. In conclusion, multiple variants in the nAChR gene family are jointly associated with abdominal obesity in American Indians, independent of general obesity and cigarette smoking *per se*.

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Introduction

The global epidemics of overweight and obesity, and the resulting impact on diabetes, cardiovascular disease (CVD), and certain types of cancer [1–6] pose a great burden on public health. Obesity has a strong genetic predisposition, with heritability estimates ranging from 65–80%. [7] Genome-wide association studies (GWAS) and candidate gene studies have identified many genetic loci influencing body weight or body fat distribution, most of them have important role in the central nervous system. [8–13] However, inter-individual variation in body weight or body fat distribution attributable to each single variant was marginal. Collectively, the loci identified so far explain only a small proportion of disease variability, suggesting that additional genes with important roles remained to be discovered. It is well-accepted that the etiology of obesity is multifactorial involving multiple genes in biologically related pathways. A single gene with small or

weak individual effect may not cause disease individually; instead, they act jointly in the context of networks or pathways in leading to disease susceptibility. [14] Therefore, a gene-family or pathway-based approach combining information from multiple genetic variants may capture a large proportion of the causal variants, and thus should have higher power than studying single gene alone in dissecting the complex genetic architecture of obesity or its related traits.

Cigarette smoking is strongly associated with body weight, body fat distribution, obesity and insulin resistance. [15,16] However, the biological mechanisms linking smoking to body composition and obesity are incompletely understood. Cross-sectional studies indicate that cigarette smokers have lower body mass index (BMI) or body weight than nonsmokers, but heavy smokers are more likely to be overweight or obese. [17–21] In addition, smokers tend to be centrally obese and suffer from a greater risk of adverse

metabolic consequences than nonsmokers.[15,16] The differential metabolic effect of cigarette smoking on body fat distribution may imply inherent biological difference between different measures of body fat distribution. American Indians have the highest prevalence of cigarette smoking of all U. S. ethnic groups [22] and also suffer from high rates of diabetes and cardiovascular disease. It is unclear whether and how cigarette smoking contributes to body fat distribution in American Indians. Elucidation of the biological pathways underlying the association between smoking and body fat deposition has the potential to provide novel strategies for the prevention and intervention of obesity and related metabolic disorders in this ethnically important but traditionally understudied population.

Nicotine is the major bioactive component in cigarette smoke. It acts as a potent ganglionic and neural stimulant by binding to nicotinic acetylcholine receptors (*nAChRs*), a superfamily of ligand-gated ion channels that are widely present within neuronal and non-neuronal cell types.[23] Polymorphisms in genes encoding *nAChRs* have been associated with nicotine dependence [24] and lung cancer.[25] However, little research has been done to examine the potential impact of *nAChRs* variants on interindividual variability in body fat distribution. Moreover, existing studies primarily focused on single SNP analysis, which may not capture the joint contribution of multiple genes to disease risk. The goal of this study is to determine the joint association of 61 tagging SNPs in seven candidate genes involved in cigarette smoking with body fat distribution in a well-characterized population of American Indians participating in the Strong Heart Family Study.

Methods

Study population

The Strong Heart Family Study (SHFS) is a family-based prospective study designed to identify genetic factors for CVD, diabetes and associated risk factors in American Indians. A total of 3,665 tribal members aged 14–93 years from 94 multiplex families residing in Arizona (AZ), North and South Dakota (DK) and Oklahoma (OK) were recruited and examined between 2001 and 2003. Detailed descriptions of the SHFS protocols for the collection of phenotype data have been described previously.[26] All participants received a personal interview to collect data on demographic characteristics, medical history and lifestyle risk factors including smoking, alcohol consumption, diet and physical activity. A physical examination was given to each participant, including anthropometric and blood pressure measurements and an examination of the heart and lungs. Laboratory methods were reported previously.[26,27] All study participants provided written consent. The SHFS protocol was approved by the Institutional Review Boards from the Indian Health Service and the participating centers.

Anthropometric measurements for obesity-related traits

Anthropometric measurements included body weight, height and waist circumference (WC) measured with participants wearing light clothing and without shoes. Body mass index (BMI) was defined as body weight in kilograms divided by the square of the height in meters (kg/m^2). Waist circumference was measured at the level of the umbilicus while the participant was supine. Hip circumference was measured at the level of widest circumference over greater trochanters with the legs close together. Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Percent body fat (%BF) was estimated with an RJL impedance meter (Model B14101; RJL Equipment Company, Detroit, MI) using an equation based on total body water (M

Singer, RJL Equipment Company, personal communication, 1992).

Measurements of risk factors

Cigarette smoking was assessed *via* questionnaire. Participants were classified as current smokers, former smokers and nonsmokers. Current smokers reported smoking 100 or more cigarettes in their lifetime and were currently smoking every day or some days. Former smokers are those who had smoked 100 or more cigarettes but were no longer smoking. Nonsmokers are those who smoked fewer than 100 cigarettes or never smoked in their life time. Pack-years were calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. Participants were categorized into current drinkers, former drinkers and never drinkers based on their history of alcohol consumption. Physical activity was assessed by the mean number of steps per day calculated by averaging the total number of steps recorded each day during the 7-day period. Dietary information was collected using the Block Food Frequency Questionnaire. According to the 1997 American Diabetes Association criteria, [28] diabetes was defined as fasting plasma glucose ≥ 7.0 mmol/L or receiving insulin or oral hyperglycemic treatment. Impaired fasting glucose (IFG) was defined as a fasting glucose of 6.1–6.9 mmol/L, and fasting glucose < 6.1 mmol/L is defined as normal.

TagSNPs selection and genotyping

A total of 61 tagSNPs in seven nAChRs genes (*CHRNA3-A6*, *CHRN2-B4*) from the *nAChRs* gene family were selected and genotyped in all SHFS participants. These genes were consistently reported to be associated with cigarette smoking in previous studies. TagSNPs were selected by the computer program Haploview 4.2.[29] Other factors were also considered in selection of SNPs, including minor allele frequency, SNP location, and Illumina design score. Detailed selection criteria were reported elsewhere.[30] All genotyping was done at the Texas Biomedical Research Institute using the Illumina VeraCode technology (Illumina, Inc., San Diego, CA). The average genotyping call rates were 98% for the chosen SNPs, and sample success rate was 99.5%.

Statistical analysis

Single SNP association analysis. We tested the association of each individual SNP with BMI, WC, WHR or %BF (*all as continuous variables*) using family-based association tests by the computer program FBAT.[31] The following covariates were included as covariates: age, sex, study center, smoking status (ever vs. never smoker), alcohol consumption (current vs. former vs. never), physical activity, total daily energy intake, socioeconomic status, and history of diabetes. Continuous variables were logarithmically transformed to improve normality. Participants with missing information on smoking status ($n = 15$) or covariates ($n = 10$) were excluded from statistical analyses, resulting in a final sample size of 3,640 in the statistical analysis. Multiple testing in the individual SNP association analysis and gene-based analysis was corrected using the Storey's q-value method.[32]

Gene-based and gene-family association analysis. The association between a candidate gene (including all SNPs within that gene) and body composition was assessed by combining p-values from single SNP association analysis. This was done by weighted truncated product method (wTPM)[33] using effect size of each SNP as weight. P values of wTPM were estimated by 50,000 Monte Carlo simulations, as described previously.[33] Gene-family analysis was performed by combining p-values of

each candidate gene obtained from gene-based analysis including all seven genes in the nAChRs gene family. Detailed methods for gene-based and gene-family association analyses have been described previously.[30,34]

Sensitivity analyses. To examine the impact of diabetes on the association between genetic variants and body fat distribution, we conducted additional analysis by excluding participants with diabetes status. To determine whether cigarette smoking influences the association between genetic variants and body composition, we compared results before and after adjustment for smoking status. In addition, we conducted analysis to examine whether the association of genetic variants with central obesity is independent of general obesity by additional adjusting for BMI. To investigate whether results from gene-based or gene-family analysis are primarily driven by the most significant SNPs, we conducted additional analysis by removing the most significant SNP from statistical analyses. All analyses were done using R 2.15.2 and SAS 9.2 (SAS Institute Inc., Cary, NC).

Results

Baseline characteristics of the study participants

Table 1 presents the baseline characteristics of the study participants according to smoking status. Compared to never smokers, ever smokers (current plus former smoker) were older, more likely to be males, and had higher levels of total cholesterol and triglyceride. In addition, ever smokers had significantly larger WHR than never smokers, suggesting that they are more likely to be centrally obese. No significant difference in BMI, WC or %BF was observed between ever smokers and never smokers.

Association of individual SNP with body composition

The pattern of linkage disequilibrium (LD) and allele frequencies of the studied SNPs stratified by study center (AZ, OK and DK) were described previously.[30] Results for single SNP association were listed in **Table 2**, which shows that multiple SNPs were marginally associated with one or more body composition measures, but none survived correction for multiple testing.

Gene-based and gene-family association with obesity-related traits

Table 3 presents the results for gene-based and gene-family association analyses. For gene-based analysis, genetic variants in *CHRNA5* were significantly associated with waist circumference and marginally associated with WHR. Another three genes, *CHRNA3*, *CHRNA3*, *CHRNA3* and *CHRNA4*, showed weak association with waist circumference and/or WHR. The gene-family comprising all seven nAChRs genes showed significant associations with both waist circumference and WHR. However, no association, either gene-based or gene-family analysis, was observed for BMI or percent body fat.

Results for sensitivity analysis

Given the strong correlation between obesity and diabetes, we examined the potential impact of diabetes on the association of the nAChR gene family with obesity measures by excluding participants with diabetes (**Tables S1**). It shows that the nAChRs gene family remained to be significantly associated with abdominal (central) obesity among normoglycemic subjects, suggesting that the observed genetic association was not driven by diabetes. Similar results for gene-family analysis were observed before and after adjustments for smoking status or pack-years, suggesting that smoking *per se* did not affect the observed genetic associations with

Table 1. Characteristics of study participants according to smoking status (n = 3,640).

	Ever smoker (n = 2116) (mean ± SD or %)	Never smoker (n = 1524) (mean ± SD or %)	P [‡]
Age (years)			
Mean	41.2±15.6	37.9±18.5	<0.0001
Range	14.09–93.25	15.45–90.84	
Male sex (%)	44.5	33.9	<0.0001
Type 2 Diabetes (%)	24.2	21.0	0.49
Body mass index (kg/m ²)	32.2±7.9	32.3±7.9	0.72
Waist circumference (cm)	105.3±18.2	103.6±19.1	0.20
Waist-to-hip ratio	0.92±0.08	0.90±0.08	0.002
Percent body fat	36.8±10.0	38.0±10.4	0.62
Systolic blood pressure (mmHg)	123.4±17.0	121.7±17.3	0.39
Diastolic blood pressure(mmHg)	76.8±10.9	75.5±11.5	0.17
High-density lipoprotein (mg/dL)	50.5±14.5	51.3±14.7	0.44
Low-density lipoprotein (mg/dL)	99.4±29.7	96.4±28.9	0.30
Plasma hsCRP (mg/L)	7.0±9.2	6.8±9.9	0.45
eGFR (ml/min/1.73 m ²)	99.1±27.2	101.8±30.1	0.96
Total cholesterol (mg/dL)	183.4±38.6	177.2±34.8	0.006
Total triglyceride (mg/dL)	177.2±197.3	155.4±123.8	0.01
Fasting glucose (mg/dL)	115.8±52.7	112.1±52.8	0.52
Insulin (uU/mL)	18.7±20.2	18.8±20.4	0.74

[‡]P values were obtained by GEE, adjusting for age and sex when appropriate; [‡] Former plus current smokers. doi:10.1371/journal.pone.0102220.t001

Table 2. P-values of single SNP association analysis for the 61 SNPs with obesity measures by FBAT (n = 3,640).

SNP	Gene	BMI	WC	WHR	%BF	SNP	Gene	BMI	WC	WHR	%BF
rs1051730	CHRNA3	0.1877	0.0412	0.0365	0.8535	rs905739	CHRNA5	0.3011	0.1160	0.3512	0.4066
rs11637630	CHRNA3	0.2825	0.2414	0.3157	0.5119	rs951266	CHRNA5	0.3051	0.0853	0.1057	0.5417
rs12910984	CHRNA3	0.2631	0.1942	0.2390	0.5500	rs2304297	CHRNA6	0.6362	0.4043	0.3585	0.3681
rs12914385	CHRNA3	0.0980	0.0218	0.0178	0.1244	rs2072658	CHRNA2	0.1954	0.1876	0.3787	0.6123
rs1317286	CHRNA3	0.0915	0.0167	0.0117	0.5951	rs2072659	CHRNA2	0.3214	0.1891	0.0823	0.2303
rs1878399	CHRNA3	0.4281	0.8369	0.6572	0.3977	rs2072660	CHRNA2	0.8459	0.5275	0.0926	0.2502
rs3743074	CHRNA3	0.3945	0.3921	0.2356	0.1417	rs2072661	CHRNA2	0.7452	0.4177	0.0584	0.2765
rs3743078	CHRNA3	0.3160	0.2671	0.3977	0.6535	rs3811450	CHRNA2	0.1970	0.1414	0.0260	0.1095
rs578776	CHRNA3	0.3284	0.2962	0.5690	0.1495	rs10958726	CHRNA3	0.0594	0.0225	0.1464	0.0168
rs6495308	CHRNA3	0.3171	0.2105	0.1912	0.5500	rs13277254	CHRNA3	0.1179	0.0535	0.2173	0.0491
rs660652	CHRNA3	0.6294	0.6067	0.3738	0.2455	rs13280604	CHRNA3	0.1150	0.0479	0.2607	0.0513
rs7177514	CHRNA3	0.2710	0.1923	0.2423	0.5869	rs4950	CHRNA3	0.1437	0.0542	0.2759	0.0635
rs2236196	CHRNA4	0.2525	0.1869	0.1944	0.6613	rs4952	CHRNA3	0.0470	0.1484	0.5240	0.1034
rs2273504	CHRNA4	0.1865	0.2433	0.5767	0.1302	rs4953	CHRNA3	0.0470	0.1484	0.5240	0.1034
rs3787116	CHRNA4	0.1821	0.2634	0.2975	0.1245	rs4954	CHRNA3	0.6603	0.5589	0.9305	0.3235
rs3787137	CHRNA4	0.4825	0.4961	0.1098	0.1499	rs6474413	CHRNA3	0.0741	0.0284	0.1514	0.0257
rs6122429	CHRNA4	0.1202	0.2037	0.8469	0.2186	rs11633223	CHRNA4	0.1690	0.2089	0.2744	0.5478
rs11633585	CHRNA5	0.3977	0.4575	0.5277	0.9635	rs11636605	CHRNA4	0.1269	0.1615	0.1820	0.2898
rs11637635	CHRNA5	0.6294	0.5317	0.3256	0.2455	rs12440014	CHRNA4	0.9045	0.7610	0.3659	0.6559
rs16969968	CHRNA5	0.1503	0.0381	0.0344	0.7120	rs12914008	CHRNA4	0.7220	0.5278	0.1373	0.6542
rs17483686	CHRNA5	0.0221	0.0257	0.2607	0.2522	rs1316971	CHRNA4	0.1115	0.1543	0.1947	0.2898
rs17486278	CHRNA5	0.1903	0.0605	0.0660	0.7120	rs16970006	CHRNA4	0.3634	0.2398	0.2761	0.2166
rs2036527	CHRNA5	0.1006	0.0228	0.0161	0.6179	rs17487223	CHRNA4	0.3746	0.3187	0.1100	0.0967
rs514743	CHRNA5	0.6566	0.5117	0.3174	0.2507	rs1948	CHRNA4	0.3430	0.7518	0.4674	0.5464
rs569207	CHRNA5	0.3125	0.2745	0.3082	0.7889	rs1996371	CHRNA4	0.0797	0.0216	0.0118	0.3807
rs588765	CHRNA5	0.7221	0.8917	0.5775	0.3476	rs3813567	CHRNA4	0.1407	0.0573	0.0996	0.5025
rs615470	CHRNA5	0.4599	0.6513	0.5450	0.1476	rs3971872	CHRNA4	0.1438	0.3257	0.2571	0.7550
rs637137	CHRNA5	0.3166	0.2928	0.3302	0.8278	rs7178270	CHRNA4	0.4334	0.3123	0.1807	0.5865
rs680244	CHRNA5	0.6953	0.6374	0.4410	0.5823	rs8023462	CHRNA4	0.4391	0.8614	0.2181	0.4958
rs684513	CHRNA5	0.9469	0.7493	0.6940	0.7512	rs950776	CHRNA4	0.6998	0.4449	0.0702	0.8830
rs8034191	CHRNA5	0.1258	0.0199	0.0187	0.3158						

All P-values adjusted for age, sex, study center, smoking status, alcohol intake, levels of physical activity, total daily energy intake, social economics status and diabetes status.
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Table 3. Gene-based and gene-family association of nAChRs variants with obesity (n = 3,640).

	BMI	WC	WHR	%BF
CHRNA3	0.1730	0.0360	0.0320	0.4870
CHRNA4	0.2380	0.2220	0.2150	0.1460
CHRNA5	0.3050	0.0001	0.0160	0.5870
CHRNA6	0.6352	0.4033	0.3575	0.3671
CHRNB2	0.3840	0.3010	0.0190	0.2110
CHRNB3	0.0470	0.0370	0.1980	0.0300
CHRNB4	0.1890	0.0560	0.0950	0.3310
Gene-family association	0.1970	0.0001	0.0001	0.2910

P-values in bold indicates significant association after adjusting for multiple testing by FDR.
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abdominal fat distribution. Moreover, this association was independent of general obesity because additionally adjustment for BMI did not attenuate the gene-family association (**Table S2**). In addition, removing the most significant SNPs from gene-family analysis did not change our results, indicating that the observed gene-family association may not be driven by SNPs showing the most significant association with WC or WHR (**Table S3**).

Discussion

In this study, we demonstrated for the first time that multiple SNPs in the nAChRs gene family, each of which has a small individual effect, exhibited joint association with abdominal obesity among American Indians participating in the SHFS. This association is independent of body mass index and cigarette smoking *per se*. Although the potential role of this pathway in abdominal obesity awaits further research, the identified genetic pathway appears to highlight novel biological mechanisms involved in the regulation of central adiposity in American Indians.

It is generally accepted that BMI and percent body fat are measures of the overall level of adiposity or general obesity, whereas waist circumference and WHR estimate the amount of visceral or abdominal fat, and are indicators of central obesity.[35] Our finding for an association of the nAChRs gene family with WC and WHR, but not BMI and percent body fat, is in agreement with previous studies demonstrating an association of smoking with abdominal or visceral obesity but not overall fatness,[16,36,37] and suggested a differential effect of the nAChRs pathway on body fat distribution among American Indians. Previous studies have reported a differential negative effect of general or central fat accumulation on cardiometabolic risk, with abdominal or visceral fat showing particularly adverse consequences on health.[38] This suggests that different measures of body fat composition may have different pathophysiological roles in leading to cardiometabolic risk. Moreover, genome-wide association studies (GWAS) have identified multiple genetic loci associated with body fat distribution, but there was incomplete overlap with the loci influencing central obesity (e.g., WC, WHR) and general obesity (e.g., BMI, %BF), implying that physiological variation in abdominal fat accumulation and overall level of adiposity may attribute to different susceptibility variants. The observed differential effect of the nAChRs genetic pathway on central obesity but not general obesity in our study lends further support for a potential different genetic impact of the nAChRs variants on body fat distribution in American Indians. In a previous study, we observed that nAChRs genetic pathway is

associated with type 2 diabetes.[30] Moreover, among those diagnosed with type 2 diabetes, more than 93% were also overweight. These findings suggest that obesity and diabetes may share some same genetic background, consistent with previous studies[39,40].

The link between cigarette smoking and body weight is evident, but the mechanisms through which smoking influences body weight or body fat distribution are complex and incompletely understood. Body weight is determined by daily calorie intake and energy expenditure. Nicotine in cigarette smoke may influence body weight by decreasing appetite, and hence calorie intake, or by raising metabolic rate or decreasing metabolic efficiency through its potential effects on central nervous system.[41] For example, a recent study shows that smoking stimulates the activity of pro-opiomelanocortin via activation of hypothalamic $\alpha_3\beta_4$ nAChRs.[42] This process may also involve the stimulation of the sympathetic nervous system,[43] leading to the release of neurotransmitters such as higher cortisol concentrations,[44] thereby influencing visceral or abdominal adiposity.[45] These results demonstrated neural control of cigarette smoking on body fat distribution or related phenotypes. In line with these findings, the association of the nicotine genetic pathway with abdominal adiposity identified in our study is independent of cigarette smoking *per se*, lending further support for a role of the central nerve system in mediating the association between cigarette smoking and body fat distribution.

Several aspects of our study merit comments. First, consistent with previous research,[46] our results demonstrated that a single SNP may show no or minimal individual association with a complex trait, but the joint effect of multiple SNPs within a gene or a biological pathway on disease susceptibility could be large. For example, after correction for multiple testing, no variant in *CHRNA5* was individually associated with any of the examined obesity measures, but gene-based analysis showed that this gene was significantly associated with waist circumference. The pathway as a whole was also significantly associated with both WC and WHR. This reinforces the emerging evidence that it is the cumulative effect of many loci that underlies susceptibility to disease. In addition, our results emphasize the important role of statistical approaches that captures the joint contribution of multiple variants simultaneously in better characterizing the complex genetic etiology of common diseases. Second, the gene cluster *CHRNA3/CHRNA5/CHRNB4*, located on chromosome 15q24, has previously been associated with smoking quantity and nicotine dependence.[47,48] In our study, the *CHRNA5* gene was significantly associated with WC, but *CHRNA3* and *CHRNB4*

showed no or weak association with WC or WHR, and none of the three genes was associated with BMI. In a recent meta-analysis, rs1051730 in *CHRNA3* was significantly associated with BMI among ever smokers but not never smokers, indicating a gene \times smoking interaction on BMI.[49] However, we did not observe any effect of this SNP on BMI. These discrepancies are probably attributable to the different genetic background between American Indians and other ethnic populations. Alternatively, these variants may influence obesity susceptibility through pathways beyond cigarette smoking in our sample. Third, the observed pathway association with abdominal fat distribution is unlikely to be mediated by cigarettes smoking *per se* because all our statistical analyses controlled for smoking status. Finally, we observed a joint genetic association with central but not peripheral obesity, and this association is independent of overall adiposity. These findings provide initial evidence for a differential effect of the nAChRs variants on body fat distribution, and may also shed light on the pathophysiological mechanisms underlying the association between cigarette smoking, body fat distribution and cardiometabolic risk.

There are limitations with the study. First, although we have controlled many potential confounders, we cannot rule out the possibility of residual confounding by other unknown or unmeasured factors. Second, based on LD analyses, it appears that subjects from different study center have heterogeneous genetic background, suggesting a possible population admixture among our study participants. However, this should not be an issue for our analysis because we used family-based association analyses, which are robust to population substructure.[31] Third, our study is cross-sectional which precludes any causal inference. Fourth, previous studies indicated that heavy smokers tend to have higher body weight than light or nonsmokers. Our analyses, however, did not differentiate the heaviness of smoking due to lack of detailed clinical information on smoking quantity. In addition, our analysis focused on common genetic variants, but low frequency and/or rare variants may have large effects on disease susceptibility and thus should be investigated in future research. Finally, our analyses were undertaken among a cohort of American Indians with high prevalence of cigarette smoking and diabetes, and with different genetic architecture from other ethnic groups. It is unclear

whether our results can be generalized to other ethnic groups with different patterns of body fat distribution or risk profiles.

In summary, this study provides initial evidence that multiple genetic variants in the nAChRs gene family jointly are jointly associated with central body fat distribution in American Indians. The impact of these genetic variants on the susceptibility to abdominal obesity may not be mediated by cigarette smoking *per se*. Our results may provide novel insights into obesity etiology and also provides valuable information for personalized obesity prevention or intervention in American Indians who suffer from increasingly high prevalence of obesity and diabetes.

Supporting Information

Table S1 Gene-based and gene-family associations of nAChRs variants with obesity among subjects without diabetes by wTPM (n = 2,830).

(DOCX)

Table S2 Gene-based and gene-family associations of the nAChRs variants with central obesity after additional adjustment for BMI (n = 3,640).

(DOCX)

Table S3 Gene-based and gene-family associations of nAChRs variants with obesity measures by wTPM after removing most significant SNPs* (n = 3,640).

(DOCX)

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Author Contributions

Analyzed the data: YZ JY. Wrote the paper: JZ YZ JY. Conceived the study: JZ. Collected genotype data: SAC KH. Reviewed manuscript: SAC KH FY ETL BVH. Contributed to discussion: FY ETL BVH.

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