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Genetic Variants in Nicotinic Acetylcholine Receptor Genes Jointly Contribute to Kidney Function in American Indians: The Strong Heart Family Study

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Abstract

Background—Cigarette smoking negatively affects kidney function. Genetic variants in the nicotinic acetylcholine receptors genes (nAChRs) have been associated with nicotine dependence, and are likely to influence renal function and related traits. While each single variant may only exert a small effect, the joint contribution of multiple variants to the risk of disease could be substantial.

Methods—Using a gene-family approach, we investigated the joint association of sixty-one tagging SNPs in seven genes encoding the nicotinic acetylcholine receptors with kidney function in 3,620 American Indians participating in the Strong Heart Family Study, independent of known risk factors. Kidney function was evaluated by estimated glomerular filtration rate (eGFR), urinary albumin/creatinine ratio (UACR), albuminuria and chronic kidney disease (CKD). The joint impact of smoking-related variants was assessed using the weighted truncated product method.

Results—Multiple SNPs showed marginal individual effect on renal function variability, only a few survive multiple comparison correction. In contrast, a gene-family analysis considering the joint impact of all 61 SNPs reveals significant associations of the nAChR gene family with kidney function variables including eGFR, UACR, and albuminuria (all P's 0.0001) after adjusting for established risk factors including cigarette smoking.

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Conclusions—Genetic variants in nAChRs genes jointly contribute to renal function or kidney damage in American Indians. The effects of these genetic variants on kidney function or damage are independent of traditional risk factors including cigarette smoking *per se*.

Keywords

nicotinic acetylcholine receptors genes; cigarette smoking; joint associations; gene-family analysis; kidney function; American Indians

Introduction

Chronic kidney disease (CKD) is a complex trait characterized by the progressive loss of renal function, manifested by a decline in the glomerular filtration rate (GFR) and the onset of abnormal albuminuria. The pathogenesis of CKD involves genes and environment as well as gene-environment interactions.¹ Genome-wide association studies (GWAS) have identified over 30 genetic variants influencing interindividual variation in renal function,²⁻⁵ but these polymorphisms collectively only explain a small proportion of the estimated heritability for renal function or CKD. It is well accepted that complex traits, such as CKD and renal function, are regulated by many genes, each of which only has a small to moderate individual effect, but their joint impact on disease susceptibility could be substantial.⁶ Thus, testing each SNP one at a time often results in low statistical power in genetic analysis of complex traits. In contrast, statistical methods taking into account the joint contribution of multiple variants in one or more biological pathways may capture a large proportion of the associated genetic variants, and thus should have a higher power than single-gene analysis in dissecting the complex genetic architecture of renal function and its related disorders.

Cigarette smoking is an independent risk factor for the development of proteinuria,⁷ CKD and its progression,⁸ and end-stage renal disease (ESRD).⁹ American Indians have the highest prevalence of cigarette smoking among all US groups,¹⁰ and also suffer from high rates of CKD.¹¹ Epidemiologic studies have demonstrated that, cigarette smoking significantly increases the risk of CKD¹² and there was a dose-response relationship between number of cigarettes smoked and the levels of proteinuria¹³ and glomerular filtration rate (eGFR).¹⁴ Cigarette smoking may also accelerate the progression of CKD of diverse etiologies including diabetes,^{15, 16} hypertension¹⁷ and post kidney transplantation.¹⁸ However, the biological mechanisms linking cigarette smoking to reduced renal function or CKD remain to be determined. Identifying independent preventable risk factors will provide not only novel insights into the pathophysiological mechanisms but also optimal strategies to mitigate the loss of renal function, thereby preventing or slowing down CKD or eventual kidney failure.

Nicotine, the major bioactive component of cigarette smoke, may cause renal damage by increasing blood pressure¹⁹ or renal oxidative stress.²⁰ At the molecular level, nicotine acts by binding to nicotinic acetylcholine receptors (nAChRs), a superfamily of ligand-gated ion channels that is widely present within neuronal and non-neuronal cell types.²¹ Genetic polymorphisms in the nAChRs genes have been shown to affect renal function in a mouse model.²² However, limited research has investigated the potential impact of nAChRs

variants on renal function variability in human. Moreover, existing studies focused on single gene analysis, which is less powerful in detecting small genetic effect and cannot capture the joint contribution of multiple genes. The goal of this study is therefore to conduct a gene-family analysis to examine the joint impact of 61 tagging SNPs in seven nAChRs genes on kidney function in a large, well-characterized population of American Indians participating in the Strong Heart Family Study (SHFS).

Methods

Study population

The Strong Heart Family Study (SHFS) is a multicenter, family-based prospective study designed to identify genetic factors for cardiovascular disease (CVD), diabetes and their risk factors in American Indians. A total of 3,665 tribal members (aged 18 years and older) 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 phenotype collection have been described previously.^{23, 24} 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. Fasting blood sample was obtained to measure lipid levels, fasting glucose, fasting insulin and inflammatory biomarkers. A spot urine sample was collected to measure albumin and creatinine. Laboratory methods were reported previously.^{23, 25} All participants have given informed consent for genetic study of cardiovascular disease, diabetes and associated risk factors. The SHFS protocol was approved by the Institutional Review Boards from the Indian Health Service and the participating centers.

Assessments of renal function or kidney damage

Methods for the measurement of urinary albumin and creatinine have been described previously.²⁶ In brief, serum and urine creatinine were measured by the picric acid method.²⁷ Urine albumin content was measured by a sensitive nephelometric technique.²⁸ Serum and urine creatinine were assayed by a kinetic alkaline picrate method and urine albumin by a sensitive nephelometric method, both on the Hitachi 717 platform (Roche Diagnostics, Indianapolis, IN). Urine albumin excretion was estimated as urinary albumin/creatinine ratio (UACR, mg/g) and estimated glomerular filtration rate (eGFR) was calculated using the Modified Diet and Renal Disease (MDRD) equation.²⁹ Renal function was assessed using eGFR, whereas UACR was used as a measure of kidney damage. Based on UACR values, participants were classified as either normal (UACR <30 mg/g), or microalbuminuria (30 ≤ UACR < 300 mg/g) or macroalbuminuria (UACR ≥ 300 mg/g). CKD was defined as eGFR < 60 mL/min/1.73m² or UACR ≥ 30mg/g.³⁰

Measurement of risk factors

Body weight (kg) and height (cm) were measured when participants wore light clothes and no shoes by trained research staff. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Waist circumference was measured at

the level midway between the lowest rib and the uppermost iliac crest with the subjects standing. Hip circumference was measured at the level of widest circumference over greater trochanters with the legs close together. Waist/hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Cigarette smoking was assessed via questionnaire and classified as current smokers, former smokers and never smokers. 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. Never smokers are those who smoked fewer than 100 cigarettes or never smoked in their life time. Based on the history of alcohol consumption, subjects were categorized into current drinkers, former drinkers and never drinkers. 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. Hypertension was defined as blood pressure levels of 140/90 mm Hg or higher or use of antihypertensive medications. According to the 1997 American Diabetes Association (ADA) criteria,³¹ 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 6.1-7.0 mmol/L. Fasting glucose <6.1 mmol/L was defined as normal.

TagSNPs selection and genotyping

A total of 3,665 SHFS participants were genotyped for sixty-one tagSNPs in seven candidate genes from the nAChRs gene family (*CHRNA3-A6*, *CHRNA2-B4*). These genes were frequently reported to be associated with cigarette smoking in previous studies. For tagSNP selection within each candidate gene, we used the computer program Haploview 4.2³² with an r^2 threshold of 0.80 for linkage disequilibrium (LD). The following criteria were also considered: minor allele frequency (MAF $>5\%$), SNP location (i.e., coding region) and Illumina design scores (quantifying how likely a SNP can be genotyped). SNPs that could not be tagged (i.e., singletons) were included as long as their design score was greater than 0.15. 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%. Details of the 61 tagSNPs were shown in Table S1.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) of each SNP was tested by PLINK using genotype data of founders. Descriptive analysis of continuous variables was performed using generalized estimating equation (GEE), which accounts for correlations among family members. Chi-square test was used for categorical variables. Prior to statistical analyses, continuous variables were log-transformed to improve normality. Participants with missing information on smoking status (N=15) or renal function (N=30) were excluded from further analyses. All analyses were done using R 3.0.1 (R Development Core Team) and SAS 9.3 (SAS Institute Inc., Cary, NC).

Single SNP analysis—We first tested the association of each SNP with renal function variables, including eGFR (continuous), UACR (continuous), albuminuria (microalbuminuria vs. macroalbuminuria vs. normal), and CKD (yes/no) using multivariate

GEE, adjusting for age, sex, study center, BMI, history of diabetes or hypertension or CVD, smoking status (ever smoker vs. never), alcohol drinking (current vs. former vs. never), physical activity level, and socioeconomic status. To examine whether population stratification will affect our results, we further validated the results by family-based association test (FBAT) using FBAT.³³ Multiple testing was corrected using the Storey's q-value method.³⁴

Gene-based and gene-set analysis—Association of a candidate gene (including all SNPs within the gene) with each measure of kidney function was assessed by combining P-values from single SNP association analysis. This was done using a weighted truncated product method (wTPM)³⁵ with effect size of each single SNP analysis as the weight. A gene-set analysis was then performed by combining P-value 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-set analyses have been described previously.³⁶

Sensitivity analyses—Renal function and CKD are strongly associated with CVD,^{37, 38} diabetes³⁹ or hypertension.⁴⁰ To examine their potential impact on our results, we conducted sensitivity analyses by excluding participants with CVD (N=153), diabetes (N=820) or hypertension (N=1,205). To investigate whether the observed gene-family associations are primarily driven by the most significant SNPs in single gene analysis, we performed secondary analyses by removing SNPs showing the most significant association with renal variables.

Results

Baseline characteristics of the study participants

Table 1 presents baseline characteristics of study participants according to smoking status. Compared to never smokers, ever smokers (current plus former smoker) were older, more likely to be males, more likely to be centrally obese, and had higher levels of total cholesterol and triglyceride. No difference was observed for other risk factors between never and ever smokers.

Association of individual SNP with renal function variables

The pattern of linkage disequilibrium (LD) and allele frequencies of the studied SNPs stratified by study center (AZ, OK and DK) were described previously.³⁶ It appears that subjects from different centers have heterogeneous genetic background. However, this should not be a concern for our analysis because we validated our results using family-based association test (FBAT), which are robust to population substructure.³³

Results of single SNP association analysis are listed in Table 2. After adjustments for covariates and multiple testing, eight SNPs (six in *CHRNA3*, two in *CHRNA5*, all p's 0.0006) were significantly associated with eGFR, and one SNP in *CHRNA4* (rs1996371, p=0.006) was significantly associated with UACR. No SNP was individually associated with CKD or albuminuria. Effect sizes of the SNPs showing significant associations were shown in Table S2.

Gene-based and gene-set association with kidney function

Gene-based analysis indicated that, after adjusting for multiple testing, the *CHRNA3* gene was significantly associated with eGFR ($P = 0.0001$), UACR ($P = 0.0001$) and albuminuria ($P = 0.002$). The *CHRNA5* gene was significantly associated with eGFR and UACR (both P 's 0.0001), and the *CHRNA4* gene was significantly associated with eGFR ($P = 0.0001$) and albuminuria ($P = 0.002$). In addition, the *CHRNA4* gene was associated with albuminuria ($P = 0.004$). Gene-family analysis comprising all seven genes showed significant associations with eGFR, UACR and albuminuria (all p 's 0.0001). No association was observed for CKD at either the gene-level or the pathway-level. Results for gene- and gene-family analyses are shown in Table 3.

Results of sensitivity analysis

Kidney disease is strongly related to diabetes, hypertension and CVD. To examine whether and how these chronic disorders influence our results, we conducted sensitivity analyses by stratifying statistical analyses according to the status of diabetes, hypertension or CVD. The observed gene-family associations with eGFR, UACR, or albuminuria persisted among participants with neither (Table S3) or one of these chronic conditions (Tables S4-6). It shows that excluding participants with hypertension and/or CVD has no or little effect on the observed associations with the four renal phenotypes, but excluding diabetic patients resulted in different results. For instance, the gene-family association with CKD was statistically nonsignificant in analysis of all study participants (though diabetes was adjusted in the statistical model), but after excluding participants with diabetes, the gene-family association became highly significant ($P < 0.0001$). This may suggest a larger impact of diabetes on the relationship between smoking variants and kidney function. In addition, removing the most significant SNP from gene-set analysis did not change our results, indicating that the observed gene-family associations may not be driven by the SNP showing the most significant association with eGFR, UACR, CKD or albuminuria (Table S7). The gene-based and gene-family associations using P values obtained by FBAT are listed in Table S8, which indicates that population structure has no appreciable effect on our results. Furthermore, additional adjustments for smoking status or pack-years did not change the results of gene- or gene-family analyses, suggesting that the observed associations are independent of smoking *per se*.

Discussion

In this study, we demonstrated that multiple SNPs in the nAChRs gene family, each of which has a small individual effect, jointly contribute to kidney function or renal damage among American Indians participating in the Strong Heart Family Study. This association is independent of known risk factors, including cigarette smoking *per se*. Although the precise mechanisms underlying the observed association await further research, the identified genetic associations or related pathways highlight novel genetic mechanisms involved in the regulation of renal function in this minority population.

Several aspects of our study merit comments. First, consistent with previous research,⁴¹ our results demonstrated that a single SNP may only confer a small or marginal individual effect

on kidney function, but the joint effect of multiple SNPs within a gene or a biological pathway on disease susceptibility could be large. For example, none of the examined SNPs in *CHRNA3* was individually associated with UACR or albuminuria, but gene-based analysis revealed a significant association of this gene with both UACR and albuminuria. Similar phenomenon was also observed for other genes, e.g., *CHRNA4*, *CHRNA5* and *CHRNA4*. Gene-family analysis demonstrates that the 61 examined SNPs as a whole jointly contribute to renal function phenotypes including eGFR, UACR and albuminuria. This reinforces the emerging evidence that it is the cumulative effects of many loci, rather than the individual effect of a single variant, that underlies the susceptibility to disease. Second, multiple variants have been reported to be associated with CKD or related traits,⁴² but none of the SNPs examined in our study was previously associated with kidney function, probably due to the unique genetic background of American Indians, or lack of sufficient genotyping or sequencing coverage of the nAChRs gene regions in previous studies. Third, although eGFR, UACR, albuminuria and CKD are highly correlated clinical phenotypes, their genetic etiologies may not be exactly the same. This is supported by the incomplete overlapping in genetic profiles of different traits at the three genetic levels (i.e., single SNP, gene-based or gene-family). For example, the seven SNPs (Table 2) showing significant associations with eGFR were not associated with UACR, or albuminuria or CKD. A SNP in *CHRNA4* (rs1996371) was associated with UACR, but not other phenotypes. At the gene-level, the *CHRNA3* gene was significantly associated with eGFR, UACR and albuminuria, but not CKD, whereas the *CHRNA5* gene was associated with eGFR and UACR, but not CKD and albuminuria. Moreover, the joint association of all nAChRs variants in gene-family analysis was statistically significant for eGFR, UACR and albuminuria, but not CKD, again highlighting the potential different genetic etiologies underlying these complex traits. In addition, it appears that diabetes has a large impact on the genetic association between nAChRs variants and CKD, because results of gene-family analysis are different before and after excluding participants with diabetes (Tables 3 and S4). Finally, the observed gene-family associations are unlikely to be mediated by cigarettes smoking *per se*, because we adjusted for smoking status in all statistical analyses.

While the link between cigarette smoking and renal function has been well established,¹² biological pathways through which smoking negatively affects renal function remain incompletely understood. It is possible that cigarette smoking influences renal function through its effects on inflammation and/or oxidative stress,⁴³ both of which have been implicated in the pathogenesis of chronic renal failure or CKD.¹⁴ In a recent study, we have shown that genetic variants in the same nAChRs genes included in the current analysis are also associated with insulin resistance,³⁶ a mechanism known to be involved in CKD.⁴⁴ Moreover, it has long been appreciated that renal hemodynamics (i.e., arterial pressure, glomerular filtration rate, and renal blood flow) are regulated by renal sympathetic nerve activity, dysregulation of which may cause renal dysfunction.^{45, 46} Genetic variants in nAChRs genes located on the postganglionic sympathetic nerve terminals could influence renal hemodynamics, thereby contribute to the pathological effects of smoking on the kidney. The association of nAChRs variants with kidney function or damage observed in our study is independent of cigarette smoking *per se*, providing further support that cigarette smoking may affect kidney function through its impact on neuronal nicotinic receptors.

Our study has some limitations. Though we have controlled many potential confounders, we cannot rule out the possibility of residual confounding by other unknown or unmeasured factors. The cross-sectional design of our study precludes any causal inference. In addition, our analyses were undertaken among a cohort of American Indians with high prevalence of cigarette smoking and type 2 diabetes. It is unclear whether our results can be generalized to other ethnic groups with different patterns of risk profiles. Moreover, low frequency and/or rare variants may exhibit larger effects on disease phenotypes. In this study, we tagged six low frequency SNPs but none was significantly associated with any of the renal variables. Target resequencing or deep sequencing in future research should provide better chance to capture potentially important low frequency or rare variants with large effects influencing interindividual variability in renal function phenotypes.

In summary, this study provides initial evidence that multiple genetic variants in the nAChRs gene family jointly contribute to kidney function, independent of traditional renal risk factors. The impact of these genetic variants on the susceptibility to renal function may not be mediated by cigarette smoking *per se*. Our results may provide novel insights into disease pathophysiology and also valuable information for personalized prevention or intervention in American Indians who suffer from increasingly high prevalence of diabetes and kidney disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1
Characteristics of the study participants according to smoking status (n=3,620)

	Ever smoker [‡] (n=2,097)	Never smoker (n=1,523)	P*
	(Mean ± SD or %)	(Mean ± SD or %)	
Age (years)	41.2±15.6	37.9±18.5	<0.0001
Male sex (%)	44.5	33.9	<0.0001
Type 2 diabetes (%)	24.2	21.0	0.49
Hypertension (%)	34.2	31.9	0.13
Cardiovascular disease (%)	4.5	3.9	0.29
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	0.2
Waist/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	0.3
Plasma hsCRP (mg/L)	7.0±9.2	6.8±9.9	0.45
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
eGFR (ml/min/1.73m ²)	99.1±27.2	101.8±30.1	0.96
UACR (mg/g) [≠]	24.6 ±58.9	23.7±55.3	0.85
Chronic kidney disease (%)	20.6	20.8	0.08
Microalbuminuria (%)	13.7	13.3	0.69
Macroalbuminuria (%)	3.8	3.7	0.47

* P values were obtained by GEE, adjusting for age and sex when appropriate

[‡] Former plus current smokers

[≠] Excluded outliers with UACR (UACR greater than mean+3SD, total exclusion =8)

Table 2
Association of the 61 SNPs with renal function measures by GEE (n=3,620)

SNP	Gene	eGFR	UACR	CKD	albuminuria	SNP	Gene	eGFR	UACR	CKD	albuminuria
rs1051730	CHRNA3	0.0302	0.0029	0.4362	0.0297	rs905739	CHRNA5	0.0028	0.0303	0.7763	0.0208
rs11637630	CHRNA3	0.0004	0.0218	0.9319	0.0133	rs951266	CHRNA5	0.0127	0.0029	0.6346	0.0331
rs12910984	CHRNA3	0.0004	0.0220	0.9206	0.0111	rs2304297	CHRNA6	0.2451	0.6927	0.4334	0.2316
rs12914385	CHRNA3	0.0248	0.0048	0.4886	0.0450	rs2072658	CHRNA2	0.6131	0.2069	0.7974	0.3503
rs1317286	CHRNA3	0.1034	0.0028	0.1572	0.0337	rs2072659	CHRNA2	0.0377	0.2524	0.4495	0.4930
rs1878399	CHRNA3	0.0158	0.4136	0.4555	0.1908	rs2072660	CHRNA2	0.2163	0.8621	0.3385	0.1503
rs3743074	CHRNA3	0.0138	0.3743	0.3741	0.4532	rs2072661	CHRNA2	0.2545	0.9247	0.2728	0.2417
rs3743078	CHRNA3	0.0003	0.0309	0.8764	0.0127	rs3811450	CHRNA2	0.7404	0.1036	0.1743	0.1055
rs578776	CHRNA3	0.0006	0.0179	0.9132	0.0112	rs10958726	CHRNA3	0.2806	0.6389	0.3676	0.2266
rs6495308	CHRNA3	0.0005	0.0212	0.9221	0.0105	rs13277254	CHRNA3	0.3132	0.6483	0.3608	0.1847
rs660652	CHRNA3	0.0163	0.5469	0.3709	0.4167	rs13280604	CHRNA3	0.3690	0.6605	0.3336	0.1336
rs7177514	CHRNA3	0.0004	0.0224	0.8176	0.0121	rs4950	CHRNA3	0.3192	0.6256	0.3290	0.1806
rs2236196	CHRNA4	0.0519	0.7916	0.1314	0.6398	rs4952	CHRNA3	0.5228	0.0472	0.5949	0.3165
rs2273504	CHRNA4	0.9472	0.0809	0.3262	0.0425	rs4953	CHRNA3	0.5230	0.0472	0.5949	0.3164
rs3787116	CHRNA4	0.0706	0.6651	0.2362	0.0358	rs4954	CHRNA3	0.1865	0.9368	0.1439	0.0589
rs3787137	CHRNA4	0.2046	0.1766	0.7300	0.0272	rs6474413	CHRNA3	0.3101	0.6562	0.3607	0.1660
rs6122429	CHRNA4	0.3582	0.1170	0.4992	0.2829	rs11633223	CHRNA4	0.0081	0.5014	0.6752	0.3190
rs11633585	CHRNA5	0.6016	0.4412	0.7536	0.1799	rs11636605	CHRNA4	0.0359	0.0665	0.6838	0.0101
rs11637635	CHRNA5	0.0167	0.5143	0.3830	0.3653	rs12440014	CHRNA4	0.0387	0.0481	0.8297	0.0137
rs16969968	CHRNA5	0.0172	0.0030	0.6397	0.0365	rs12914008	CHRNA4	0.5439	0.6365	0.7895	0.2376
rs17483686	CHRNA5	0.3564	0.1824	0.7189	0.3342	rs1316971	CHRNA4	0.0405	0.0687	0.7230	0.0160
rs17486278	CHRNA5	0.0110	0.0021	0.6710	0.0420	rs16970006	CHRNA4	0.0279	0.2576	0.5716	0.4765
rs2036527	CHRNA5	0.0163	0.0017	0.6981	0.0368	rs17487223	CHRNA4	0.6895	0.0857	0.5508	0.0835
rs514743	CHRNA5	0.0182	0.5228	0.3734	0.4571	rs1948	CHRNA4	0.0535	0.3534	0.4502	0.4930
rs569207	CHRNA5	0.0004	0.0207	0.7626	0.0120	rs1996371	CHRNA4	0.0211	0.0006	0.1459	0.0036
rs588765	CHRNA5	0.0181	0.4002	0.4296	0.1639	rs3813567	CHRNA4	0.6776	0.1304	0.2897	0.3513
rs615470	CHRNA5	0.0175	0.3694	0.3695	0.3445	rs3971872	CHRNA4	0.4949	0.1152	0.2826	0.2506
rs637137	CHRNA5	0.0003	0.0212	0.7725	0.0131	rs7178270	CHRNA4	0.0083	0.5360	0.9714	0.2278

SNP	Gene	eGFR	UACR	CKD	albuminuria	SNP	Gene	eGFR	UACR	CKD	albuminuria
rs680244	<i>CHRNA5</i>	0.0153	0.3609	0.4676	0.1294	rs8023462	<i>CHRNA4</i>	0.0184	0.3012	0.5148	0.3936
rs684513	<i>CHRNA5</i>	0.2843	0.5043	0.3855	0.2053	rs950776	<i>CHRNA4</i>	0.0251	0.5593	0.9804	0.6463
rs8034191	<i>CHRNA5</i>	0.0236	0.0014	0.6348	0.0260						

All P-values adjusted for age, sex, study center, BMI, history of diabetes or hypertension or CVD, smoking status, alcohol drinking, physical activity level, and socioeconomic status. P-values in bold denote statistical significance after correction for multiple testing

Table 3
Gene-based and gene-family associations of seven nAChRs genes with kidney function
using weighted truncated product method

Gene	eGFR	UACR	CKD	albuminuria
<i>CHRNA3</i>	0.0001	0.0001	0.5936	0.0022
<i>CHRNA4</i>	0.0304	0.3436	0.2760	0.0044
<i>CHRNA5</i>	0.0001	0.0001	0.2886	0.0290
<i>CHRNA6</i>	0.2451	0.6927	0.4334	0.2316
<i>CHRNA2</i>	0.1986	0.4552	0.2832	0.2260
<i>CHRNA3</i>	0.2006	0.0872	0.2320	0.1622
<i>CHRNA4</i>	0.0001	0.0180	0.6124	0.0020
The nAChRs gene family	0.0001	0.0001	0.2168	0.0001

P-values in bold indicate statistical significance after correction for multiple testing