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A genome-wide association for kidney function and endocrine-related traits in the NHLBI's Framingham Heart Study

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Abstract

Background: Glomerular filtration rate (GFR) and urinary albumin excretion (UAE) are markers of kidney function that are known to be heritable. Many endocrine conditions have strong familial components. We tested for association between the Affymetrix GeneChip Human Mapping 100K single nucleotide polymorphism (SNP) set and measures of kidney function and endocrine traits.

Methods: Genotype information on the Affymetrix GeneChip Human Mapping 100K SNP set was available on 1345 participants. Serum creatinine and cystatin-C (cysC; n = 981) were measured at the seventh examination cycle (1998–2001); GFR (n = 1010) was estimated via the Modification of Diet in Renal Disease (MDRD) equation; UAE was measured on spot urine samples during the sixth examination cycle (1995–1998) and was indexed to urinary creatinine (n = 822). Thyroid stimulating hormone (TSH) was measured at the third and fourth examination cycles (1981–1984; 1984–1987) and mean value of the measurements were used (n = 810). Age-sex-adjusted and multivariable-adjusted residuals for these measurements were used in association with genotype data using generalized estimating equations (GEE) and family-based association tests (FBAT) models. We presented the results for association tests using additive allele model. We evaluated associations with 70,987 SNPs on autosomes with minor allele frequencies of at least 0.10, Hardy-Weinberg Equilibrium p-value ≥ 0.001, and call rates of at least 80%.

Results: The top SNPs associated with these traits using the GEE method were rs2839235 with GFR (p-value 1.6*10⁻⁵), rs1158167 with cysC (p-value 8.5*10⁻⁹), rs1712790 with UAE (p-value 1.9*10⁻⁵), and rs6977660 with TSH (p-value 3.7*10⁻⁶), respectively. The top SNPs associated with these traits using the FBAT method were rs6434804 with GFR (p-value 2.4*10⁻⁵), rs563754 with cysC (p-value 4.7*10⁻⁵), rs1243400 with UAE (p-value 4.8*10⁻⁵), and rs4128956 with TSH (p-value 3.6*10⁻⁵), respectively. Detailed association test results can be found at http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?id=phs000007. Four SNPs in or near the CST3 gene were highly associated with cysC levels (p-value 8.5*10⁻⁹ to 0.007).
Background

Kidney disease affects 19 million adults in the United States [1]. Chronic kidney disease (CKD) is associated with cardiovascular disease [2-4], stroke [5], peripheral arterial disease [5,6], and all-cause mortality [7,8]. CVR risk factors are associated with the development of kidney disease [9], and the prevalence of traditional and novel CVR risk factors is elevated among those with kidney disease [7,10]. Urinary albumin excretion (UAE) is an early marker of kidney function that predicts CKD progression [11-14]. While glomerular filtration rate (GFR) and UAE are both measurements for kidney function, they represent different phenotypes and identify different subsets of at-risk individuals [15].

Genetic factors play a role in the progression of renal disease. Familial aggregation of end-stage renal disease has been identified [16]. Linkage analyses of kidney function have been conducted [17-21], and novel loci have been mapped to chromosomes 1 [18], 2 [21,22], 3 [17], 7 [22], 10 [19,20,22], and 18 [22]. In the Framingham Heart Study, we have shown that kidney function is heritable [23], suggesting a role for genetic mechanisms in its etiology. Results of the linkage study from the Framingham Heart Study suggested linkage between kidney disease and a locus on chromosome 4 with a LOD score of 2.2 [23]. Familial clustering of UAE has been observed in siblings of subjects with diabetes [24], and UAE has been shown to be heritable among the offspring of diabetic subjects [25]. Genome-wide linkage analyses have mapped novel loci to chromosomes 12 [26] and 19 [26] among families enriched for hypertension. Among families with more severe forms of nephropathy, suggestive evidence for linkage has been found on chromosome 10p [27] and 9q31-32 [28]. In the Framingham Heart Study, we observed a LOD score of 2.2 for UAE on chromosome 8 [29].

Thyroid disease, including Hashimoto's thyroiditis and Graves' disease, has a known familial component [30], and the same genes may underlie both conditions [31]. Measures of thyroid function have been shown to be heritable [32-34], and linkage has been reported to chromosome 18 for autoimmune thyroid disease in at least 2 studies [35,36].

As part of the Framingham Heart Study 100K Project, we sought to test the relation of multiple kidney and endocrine traits to 70,987 SNPs. In this manuscript, we focus the results of association studies for GFR, UAE, cysC, and thyroid stimulating hormone (TSH), a sensitive measure of thyroid function.

Methods

Overall, 1345 participants were genotyped for the Affymetrix GeneChip Human Mapping 100K SNP set. For this manuscript, we focused on GFR from examination 7, UAE from examination 6, serum cysC from examination 7, and mean TSH from examinations 3 and 4. Phenotypes were available in 1010 participants for GFR at exam cycle 7, 822 participants for UAE at exam cycle 6, 981 participants for cysC at exam cycle 7, and 810 participants for mean TSH at exam cycles 3 and 4. Details about the selection process and genotyping are provided in the Overview [37]. Age-sex- and multivariable-adjusted residuals were generated; we present here only the results for multivariable-adjusted traits (all available results can be found in the website http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?id=phs000007). We evaluated associations with 70,987 SNPs on autosomes with minor allele frequencies of at least 0.10, HWE p-value ≥ 0.001, and genotypic call rates of at least 80%.

Phenotype assessment

Serum creatinine was measured using the modified Jaffe method at exams 2 (1978–1981), 5 (1991–1995), 6 (1995–1998), and 7 (1998–2001), and glomerular filtration rate (GFR) was estimated using the simplified Modification of Diet in Renal Disease Study equation [38,39]. CKD was defined based on the National Kidney Foundation Kidney Disease Outcome Quality Initiative working group, and modified slightly as previously described [9]. Urinary albumin concentration (UAE) was measured by immuno-turbidimetry (Tina-quant Albumin assay; Roche Diagnostics, Indianapolis, IN) during the sixth examination cycle (1995–1998). Urinary albumin was indexed to urinary creatinine (as the urine albumin/creatinine ratio, UACR) in order to account for differences in urine concentration. UACR is a validated and reliable single-sample measure of urinary albumin excretion and is highly correlated with albumin excretion rates assessed by 24-h urine collection [40,41]. Cystatin-C (cysC) was measured using particle enhanced immunonephelometry (Dade Behring BN 100 nephelometer; Dade Behring – Cystatin C reagent) with an inter-assay and intra-assay coefficient of variation of 3.3 and 2.4%, respectively. We have previously published correlates of CKD in the Framingham Heart Study, including hypertension, diabetes, smoking, obesity, and low HDL cholesterol [9,42].

Conclusion: Kidney function traits and TSH are associated with SNPs on the Affymetrix GeneChip Human Mapping 100K SNP set. These data will serve as a valuable resource for replication as more SNPs associated with kidney function and endocrine traits are identified.
TSH was measured using a chemiluminescence assay (London Diagnostics, Eden Prairie, Minn) with a lower limit of detection of 0.01 mU/L. Luteinizing hormone (LH), follicle stimulating hormone (FSH), and dehydroepiandrosterone sulfate (DHEAS) were measured as previously described [43,44]. Briefly, DHEAS concentrations were measured on serum samples via radioimmunoassay (Diagnostic Products Corp, CA). Calcium and phosphorous were measured at the second examination cycle using a standard colorimetric method (Roche Diagnostics, Alameda, CA), and uric acid was measured at the second examination cycle using an autoanalyzer with a phosphotungstic acid reagent.

**Genotyping**

Genotyping was performed using the 100K Affymetrix GeneChip. Please see the Overview [37] for details.

**Statistical methods**

Phenotypes used for the analysis were created by generating normalized residuals. We generated both age-sex adjusted and multivariable adjusted residuals for each trait. Table 1 shows the covariates included in the multivariable adjustment; all data in this manuscript represents the multivariable-adjusted traits. All association analyses were performed using the generalized estimating equations or family based association tests; details are provided in the Overview [37]. Methods to verify family structure, generate identity-by-descent for these 1345 participants with genotype information as well as the markers used for linkage analysis, is detailed in the Overview [37]. To assess the clustering of significance between each SNP and phenotypes that were repeatedly measured in several examination cycles (see the third table in this article), we generated the geometric mean of p-values for SNPs that fit the following criteria: at least 4 out of 6 p-values of <0.01 in GEE or FBAT analyses for 6 GFR traits (change in serum creatinine from exam 2 to 7; GFR at exam 2; GFR at exam 5; GFR at exam 6; GFR at exam 7; mean GFR exams 2, 5, 6, 7); one out of two UACR traits (UACR; UACR in a sample enriched for hypertension); three out of three of TSH traits (TSH at exam 3; TSH at exam 4; mean TSH at exams 3 and 4). Among the GFR traits, Pearson correlation coefficients ranged from 0.18 (p < 0.001) between GFR at exam 2 and exam 7, to 0.77 (p < 0.001) for the mean of GFR at exams 2, 5, 6, and 7 and GFR at exam 7. Linkage analysis was performed using the variance components methods on a subset of 100K markers and Marshfield short-tandem repeats; please see the variance components methods on a subset of 100K and GFR at exam 7. Linkage analysis was performed using a standard colorimetric method (Roche Diagnostics, Alameda, CA), and uric acid was measured at the second examination cycle using an autoanalyzer with a phosphotungstic acid reagent.

**Results**

A description of all traits and phenotypes, including relevant examination cycles and multivariable-adjustments, is presented in Table 1. The median eGFR among individuals with CKD in our sample is 53.7 ml/min/1.73 m². Table 2a presents the top 25 SNPs with the lowest p-values obtained via GEE for GFR, cysC, UAE, and mean TSH; additional results can be found on the National Center for Biotechnology Information website [http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/]

**Additional findings**

We also identified several other plausible candidate genes that appear in our list of top 500 SNPs for each trait (see [http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/]

**Table 3** presents the top SNPs for our multiple phenotype analysis for GFR, UAE, and TSH with a total of 24 SNPs showing consistently significant associations with multiple related phenotypes. Tables 4a and 4b present results looking at replication of genes that have been associated with kidney traits in the published literature. Four SNPs in or near the CST3 gene were highly correlated with cysC levels (p-value 8.5*10-09 to 0.007). All four SNPs have minor allele frequencies greater than 10% and none were in linkage disequilibrium (defined by R² > 0.8) as shown on Table 4. The proportion of the cysC variation that can be explained by these SNPs is shown in Table 4. rs1158167 accounts for 2.5% of the cysC variation. We found nominal significance between a SNP near the APOE gene and CKD (p = 0.04).
For UAE, we observed strong association with between cysC levels and 4 SNPs in or near the GeneChip. We found strong evidence for association related traits and TSH with SNPs on the Affymetrix 100K strong evidence for association between multiple kidney-

In our analysis of kidney-related traits, we have found

Discussion

Table 1: Traits names, Framingham Heart Study examination cycle, and multivariable adjustments

| Trait                                | Sample size | Exam cycle/s | Adjustment† |
|--------------------------------------|-------------|--------------|-------------|
| Serum Creatinine                     | 840–1010    | 2, 5, 6, 7   | 0 Age and sex; multivariable* |
| Change in serum creatinine           | 854         | 2, 7         | 0 Age and sex; multivariable* |
| Glomerular Filtration Rate (GFR)     | 840–1010    | 2, 5, 6, 7   | 0 Age and sex; multivariable* |
| Chronic Kidney Disease               | 1010        | 7            | 0 Age and sex; multivariable* |
| Cystatin C                           | 981         | 7            | 0 Age and sex; multivariable* |
| Uric acid                            | 912–1031    | 1, 2         | 0 Age and sex; multivariable* |
| Urinary Albumin Excretion ≥ 30 mg/g  | 822         | 6            | 0 Age and sex; multivariable* |
| Mean of TSH exam 3 & 4               | 810         | 3, 4         | 0 Age and sex; age, sex, body mass index, smoking, menopausal status, thyroid hormone use |
| Luteinizing hormone (LH)             | 508         | 3            | 0 Age and sex; multivariable*** |
| Follicle stimulating hormone (FSH)   | 509         | 3            | 0 Age and sex; multivariable*** |
| Dehydroepiandrosterone sulfate (DHEAS)| 850         | 3            | 0 Age and sex; multivariable*** |

*Multivariable adjustment include age, sex, systolic blood pressure, hypertension treatment, HDL-cholesterol, smoking, diabetes, body mass index
**Men and post-menopausal women only with natural menopause not using hormone replacement treatment or oral contraceptive pills
*** Age, diabetes mellitus, impaired fasting glucose, smoking, systolic blood pressure, diastolic blood pressure, body-mass index, hypertension treatment, prevalent cardiovascular disease, total cholesterol/HDL ratio and alcohol intake

study.cgi?id=phs0000007). For GFR, we identified LRP1B (GEE p-value = 0.0006, rs1049688), ADRBK2 (GEE p-value = 0.002, rs1048312), APOB (GEE p-value = 0.003, rs1048312), several genes in the chromosome 17 cytokine gene cluster including CCL3, CCL4, and CCL18 (GEE p-value 0.004, rs1818816), SCARB1 (GEE p-value = 0.004, rs1902569), NFKB1 (FBAT p-value = 0.001, rs230489), TGFB1 (FBAT p-value = 0.003, rs2072239), and PPARG (FBAT p-value = 0.005, rs709157). For UAE, we also identified a SNP in the LRP1B gene (GEE p-value = 0.004, rs1049687). For cysC, we identified SNPs in the LRP1B gene (GEE p-value = 0.001, rs1463615), ANGPT1 (GEE p-value = 0.005, rs4354281), NFKB1 (FBAT p-value = 0.004, rs2991716), and PPARG (FBAT p-value = 0.004, rs1051041). For mean TSH, we identified SNPs in ADRBK2 (GEE p-value = 0.002, rs3888397), TRDH (GEE p-value = 0.002, rs2044305) and DIO2 (GEE p-value = 0.003, rs54566), the SCD4 gene (rs10516679 GEE p-value = 0.0007 FBAT p-value = 0.02), VLDLR (FBAT p-value = 0.002, rs4084415) and APOBEC2P (FBAT p-value = 0.005, rs722442).

Discussion

In our analysis of kidney-related traits, we have found strong evidence for association between multiple kidney-related traits and TSH with SNPs on the Affymetrix 100K GeneChip. We found strong evidence for association between cysC levels and 4 SNPs in or near the CST3 gene. For UAE, we observed strong association with ADAM23, a gene involved in the metalloproteinase family, which may be involved in the pathophysiology of glomerulosclerosis [46], and PCDH9, a gene that is a member of the cadherin superfamily. For TSH, we observed significant association with the HSPA4L gene with a mean p-value for all three TSH measurements, a gene that is part of the heat shock protein family, which may be involved in the pathophysiology of thyroid disease [47]. We also observed association with the SCD4 gene, a gene involved in the conversion of saturated to monounsaturated fatty acids; TSH is an important correlate of lipid levels [48].

In our linkage results, we observed a region we have previously noted for uric acid [45], albeit with a significantly higher LOD score. We identified a LOD score of 2.78 on chromosome 3, approximately 18 Mb away from a region previously noted in association with kidney function in hypertensive individuals [17], a region that lies within our 1.5 support LOD interval. We also report novel loci for GFR and TSH.

We show significant association between cysC levels and the CST3 gene, an observation that has been previously noted [49]. Our top SNP reaches genome-wide significance, and may represent a true finding. In our candidate gene approach, we found nominal significance for a SNP near the APOE gene, a gene that has been associated with CKD [50]. Unfortunately, poor coverage of the APOE gene by the Affymetrix 100K Genechip precluded a more in-depth test of association with SNPs in the APOE gene and CKD.

Strengths of our study lie in our assessment of multiple measures of kidney function and endocrine traits in a sample unselected for these traits, thus reducing bias. We
### Table 2: Most significant results for GFR (examination 7), UAE (examination 6), cysC (examination 7), and mean TSH (examinations 3 and 4) by GEE (2a), FBAT (2b) and linkage (2c) analyses

#### 2a. Top 25 SNPs for association with GFR (examination 7), UAE (examination 6), cysC (examination 7), and mean TSH (examinations 3 and 4) based on the lowest p value of the GEE test. Corresponding phenotype names on the web are GFRMV7 (GFR), UAELNMV6 (UAE), CYSCMV7 (CysC), and TSHMEAN34MV (TSH)

| TRAIT  | SNP     | Physical Location (Mb) | Chromosome | P value – FBAT | P value – GEE | GENE                |
|--------|---------|-------------------------|------------|----------------|---------------|---------------------|
| CysC   | rs1158167| 23,526,189               | 20         | 0.006          | 8.5*10^-09    | CST9L|CST9|CST3           |
| UAE    | rs1712790| 114,126,679              | 11         | 0.014          | 1.9*10^-06    | FAM55B             |
| TSH    | rs6977660| 19,578,720               | 7          | 0.010          | 3.7*10^-06    |                    |
| TSH    | rs9322817| 105,339,926              | 6          | 0.502          | 6.5*10^-06    | HACE1              |
| UAE    | rs10499559| 21,882,699              | 7          | 0.068          | 8.3*10^-06    | RAPGEFS            |
| UAE    | rs9305354| 28,397,067               | 21         | 0.013          | 8.4*10^-06    |                    |
| CysC   | rs2145231| 23,573,547               | 20         | 0.011          | 1.1*10^-05    | CST9L|CST3|CST4           |
| UAE    | rs723464 | 133,940,196              | 4          | 0.000          | 1.1*10^-05    |                    |
| UAE    | rs2113379| 207,177,180              | 2          | 0.003          | 1.4*10^-05    | ADAM23             |
| GFR    | rs2839235| 46,625,020               | 21         | 0.055          | 1.6*10^-05    | PCNT2              |
| TSH    | rs10493147| 129,095,104            | 4          | 0.040          | 2.1*10^-05    | HSPA4L             |
| TSH    | rs784490 | 39,148,534               | 3          | 0.005          | 2.8*10^-05    | TTC21A             |
| UAE    | rs278021 | 6,698,929                | 1          | 0.958          | 2.9*10^-05    | DNAJC11            |
| UAE    | rs1856190| 33,598,615               | 9          | 0.127          | 3.0*10^-05    |                    |
| UAE    | rs10485409| 91,562,132              | 6          | 0.147          | 3.1*10^-05    |                    |
| UAE    | rs2785980| 216,088,914              | 1          | 4.8*10^-04    | 3.7*10^-05    |                    |
| UAE    | rs837678 | 191,168,583              | 3          | 0.012          | 3.8*10^-05    | LEPREL1            |
| GFR    | rs3095160| 49,844,269               | 13         | 0.002          | 3.8*10^-05    |                    |
| UAE    | rs2761171| 99,278,898               | 13         | 0.078          | 4.1*10^-05    | CLYBL              |
| GFR    | rs10507344| 24,623,069              | 13         | 0.017          | 4.1*10^-05    | PABPC3             |
| GFR    | rs890945 | 157,924,801              | 5           | 0.036          | 4.7*10^-05    |                    |
| UAE    | rs10502192| 114,127,562             | 11         | 0.051          | 4.9*10^-05    | FAM55B             |
| GFR    | rs10489639| 157,492,600             | 11         | 0.194          | 4.9*10^-05    | CD48               |
| TSH    | rs9308765| 118,599,439              | 2           | 0.353          | 5.1*10^-05    |                    |
| TSH    | rs3908399| 12,849,275               | 20         | 8.0*10^-04    | 5.2*10^-05    |                    |

#### 2b. Top 25 SNPs for association with GFR (examination 7), UAE (examination 6), cysC (examination 7), and mean TSH (examinations 3 and 4) based on the lowest p-value of the FBAT test. Corresponding phenotype names on the web are GFRMV7 (GFR), UAELNMV6 (UAE), CYSCMV7 (CysC), and TSHMEAN34MV (TSH)

| TRAIT  | SNP     | Physical Location (Mb) | Chromosome | P value – FBAT | P value – GEE | GENE                |
|--------|---------|-------------------------|------------|----------------|---------------|---------------------|
| UAE    | rs1243400| 9,016,664               | 10         | 4.8*10^-04    | 0.036         |                    |
| UAE    | rs827640 | 9,028,017               | 10         | 1.5*10^-05    | 0.047         |                    |
Table 2: Most significant results for GFR (examination 7), UAE (examination 6), cysC (examination 7), and mean TSH (examinations 3 and 4) by GEE (2a), FBAT (2b) and linkage (2c) analyses (Continued)

| Trait                        | SNP       | Chromosome | Physical location (Mb) | LOD 1.5 (Lower; Mb) | LOD 1.5 (Upper; Mb) | LOD 1.5  |
|------------------------------|-----------|------------|------------------------|---------------------|---------------------|---------|
| Serum Phosphorous            | rs754958  | 8          | 134307953              | 130413367           | 139409406           | 4.33    |
| Uric acid                    | rs10495487| 2          | 2310945               | 107241              | 3787803             | 4.28    |
| Serum creatinine (exam 6)    | rs10489578| 1          | 229251990             | 224108350           | 230301885           | 3.35    |
| Luteinizing Hormone          | rs10515134| 17         | 52218447              | 45043525            | 59136652            | 3.17    |
| GFR (exam 6)                 | rs1049578 | 1          | 229251990             | 220881707           | 230301885           | 3.08    |
| Serum Calcium                | rs10484370| 6          | 18686366              | 10181754            | 21792915            | 3.03    |
| GFR (exam 7)                 | rs10511176| 3          | 100809191             | 73551217            | 103238533           | 2.79    |
| Serum creatinine (exam 5)    | rs10502302| 18         | 2542886               | 156277              | 3857290             | 2.51    |

2c. Magnitude and Location of Peak LOD scores for regions in which LOD exceeds 2.5. Corresponding phenotype names on the web are: PHOSPHORUSMV2 (serum phosphorous), URICACIDMV2 (uric acid), SCRLNMVNL6 (serum creatinine exam 6), LHMY3 (luteinizing hormone), GFRMVNL6 (GFR exam 6), CALCIUMMV2 (serum calcium), GFRMV7 (GFR exam 7), and SCRLNMVNL5 (serum creatinine exam 5)

*All traits are multivariable-adjusted; see Table 1 for specific covariate adjustments.
Table 3: SNPs showing the top 8 significant association with multiple measurements of GFR, UACR, or TSH phenotypes.

| Trait | chromosome | SNP (rsID) | Physical Location | Genes (in or near) | Mean p-value (GEE) | Mean p-value (FBAT) |
|-------|------------|------------|-------------------|-------------------|--------------------|--------------------|
| GFR   | 21         | rs2839235  | 46625020          | PCNT2             | 6.3*10^-4          | 0.281              |
| GFR   | 17         | rs10512437 | 27046466          |                   | 0.002              | 0.197              |
| GFR   | 13         | rs2480555  | 70785310          | DACH1             | 0.003              | 0.006              |
| GFR   | 7          | rs10486135 | 11301740          |                   | 0.004              | 0.142              |
| GFR   | 7          | rs727087   | 8244570           | ICA1              | 0.004              | 0.223              |
| GFR   | 13         | rs1005066  | 70790573          | DACH1             | 0.004              | 0.022              |
| GFR   | 18         | rs2885618  | 41244839          | SETBP1            | 0.004              | 0.024              |
| GFR   | 2          | rs10496887 | 142198571         | RPR1B             | 0.005              | 0.091              |
| UAE   | 11         | rs1712790  | 114126679         | FAM5SD            | 9.1*10^-7          | 0.009              |
| UAE   | 6          | rs10485409 | 91562132          | EPHA7             | 1.0*10^-5          | 0.067              |
| UAE   | 21         | rs9305354  | 28397067          |                   | 1.9*10^-5          | 0.018              |
| UAE   | 11         | rs10502192 | 114127562         | FAM5SD            | 3.6*10^-5          | 0.041              |
| UAE   | 1          | rs2077678  | 75246848          |                   | 4.4*10^-5          | 0.022              |
| UAE   | 4          | rs723464   | 133940196         |                   | 4.9*10^-5          | 0.000              |
| UAE   | 21         | rs9305355  | 28397088          |                   | 5.0*10^-5          | 0.011              |
| UAE   | 6          | rs10484587 | 143183270         | AIG1              | 5.2*10^-5          | 0.032              |
| TSH   | 7          | rs6977660  | 19578720          |                   | 1.6*10^-5          | 0.022              |
| TSH   | 4          | rs10493147 | 129095104         | HSPA4L            | 2.1*10^-5          | 0.019              |
| TSH   | 7          | rs10495959 | 21882699          | DNAIHI            | 2.8*10^-5          | 0.111              |
| TSH   | 6          | rs9322817  | 105338926         |                   | 7.4*10^-5          | 0.576              |
| TSH   | 2          | rs9308765  | 118759439         | INSIG2            | 7.7*10^-5          | 0.404              |
| TSH   | 6          | rs6942331  | 105298507         |                   | 1.6*10^-4          | 0.541              |
| TSH   | 7          | rs10486365 | 19574604          |                   | 1.9*10^-4          | 0.221              |
| TSH   | 7          | rs10486653 | 34484903          | BMPER             | 2.7*10^-4          | 0.252              |

*see details in methods for criteria for generating mean p-value

Table 4: Results on Association Analysis for Candidate Genes

4a. Results of GEE analysis between SNPs in the CST3 and APOE candidate genes and the kidney function traits with p-value < 0.05. Corresponding phenotype names on the web are CYSMV7 (CysC) and CKDMV7 (CKD).

| Candidate gene | PHENOTYPE | SNP       | CHROMOSOME | Location | Minor allele frequency(%) | P_value (GEE) | Partial R² |
|---------------|-----------|-----------|------------|----------|---------------------------|---------------|------------|
| CST3          | CysC      | rs1158167 | 20         | 23,526,189 | 21                        | 8.0*10^-9      | 2.5        |
| CST3          | CysC      | rs2145231 | 20         | 23,573,547 | 15                        | 1.1*10^-5      | 1.2        |
| CST3          | CysC      | rs726217  | 20         | 23,532,116 | 38                        | 3.1*10^-4      | 0.8        |
| CST3          | CysC      | rs911122  | 20         | 23,573,746 | 37                        | 0.007          | 1.1        |
| APOE          | CKD       | rs3760626 | 19         | 50,148,945 | 45                        |               | -          |

4b. Results of FBAT analysis between SNPs in the CST3 candidate gene and kidney function traits with p-value < 0.05. Corresponding phenotype names on the web are CYSMV7 (CysC) and UAEGE30HTNMV6 (UAE ≥ 30 mg/g)

| Candidate gene | PHENOTYPE | SNP       | CHROMOSOME | Location | P_value |
|---------------|-----------|-----------|------------|----------|---------|
| CST3          | CysC      | rs1158167 | 20         | 23,526,189 | 0.006   |
| CST3          | CysC      | rs2145231 | 20         | 23,573,547 | 0.011   |
| CST3          | UAE ≥ 30* | rs911122  | 20         | 23,573,746 | 0.032   |

*In a sample enriched for hypertensive individuals
also have excellent assessment of potential confounders that we are able to adjust for in our residual creation. Because the Framingham Heart Study has measured multiple traits, we are able to examine phenotype clustering. Limitations exist as well. Kidney function was ascertained by a single serum creatinine measure, which may lead to misclassification. Our sample was not selected for CKD, and as a result, affected individuals had moderate CKD as reflected by the median eGFR of 53.7 ml/min/1.73 m² among participants with CKD. The MDRD equation, which was used to estimate GFR, has been shown to underestimate GFR by 29% in healthy individuals [51]; therefore, we may have introduced additional misclassification into our trait definition. We used a spot urine specimen to assess UAE instead of a 24-hour collection. However, spot UAE approximates 24-hour collections [40], and are not prone to the error inherent in collecting 24-hour urine specimens. We used cysC as a continuous trait and did not use transforming equations to estimate GFR, as most existing equations have been developed in small, selected samples [52,53], or developed using immunoturbimetric method [53,54] instead of nephelometry and therefore we did not feel as though they were appropriate for use in our large population-based cohort. Further, we used cystatin C as a marker of kidney function but can not rule out that it may also reflect cardiovascular disease risk above and beyond its relation to kidney function [55-59]. Our focus on multivariable models may have led us to miss important bivariate associations between SNPs and measures of kidney function. Given that our findings have not yet been replicated, many p-values may represent false positive findings. We used TSH as an indicator of thyroid function, as we do not have measures of free thyroxine or a reliable assessment of thyroid disease in our study sample. Our sample is neither ethnically diverse nor nationally representative, and it is uncertain how our results would apply to other ethnic groups. However, in genetics studies, sample homogeneity is beneficial in order to reduce population stratification. For limitations pertaining to our genotyping or statistical methods, please see the Overview [37].

Conclusion
Kidney function traits and TSH are associated with SNPs on the Affymetrix 100K SNP GeneChip. Replication of association between these traits and SNPs requires follow-up in independent samples. These data will serve as a valuable resource for replication as more SNPs associated with kidney function and endocrine traits are identified.

Abbreviations
CKD = chronic kidney disease; cysC = cystatin-C; DHEAS = dehydroepiandrosterone sulfate; FBAT = family-based association tests; FSH = follicle stimulating hormone; GEE = generalized estimating equations; GFR = glomerular filtration rate; LD = linkage disequilibrium; LH = luteinizing hormone; MDRD = Modification of Diet in Renal Disease; SNP = single nucleotide polymorphism; TSH = thyroid stimulating hormone; UAE = urinary albumin excretion.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
SH generated the phenotype data, participated in the analysis, and drafted the manuscript. CF helped generate the phenotype data, interpret the results, and draft the manuscript. QY generated the phenotype data, interpreted the results, and helped draft the manuscript. JBM helped generate the phenotype data, interpret the results, and revised the manuscript critically for important intellectual content; and has given final approval of the version to be published. EP assisted in the acquisition and cleaning of the TSH data and critically reviewed a draft of the manuscript. All authors gave final approval to the manuscript.

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