Genetic loci influencing kidney function and chronic kidney disease in man

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Abstract

Chronic kidney disease (CKD), the result of permanent loss of kidney function, is a major global problem. We identify common genetic variants at chr2p12-p13, chr6q26, chr17q23 and chr19q13 associated with serum creatinine, a marker of kidney function ($P=10^{-10}$ to 10^{-15}). SNPs rs10206899 (near *NAT8*, chr2p12-p13) and rs4805834 (near *SLC7A9*, chr19q13) were also associated with CKD. Our findings provide new insight into metabolic, solute and drug-transport pathways underlying susceptibility to CKD.

In North America and Europe, chronic kidney disease (CKD) affects ~11% of the adult population. CKD is associated with high morbidity, and in the advanced stage requires life-supporting treatment by renal dialysis or transplantation.[1] CKD is also a major risk factor for myocardial infarction and stroke.

CKD is a multifactorial disorder with an important genetic component.[2] A number of monogenic disorders underlying CKD have been identified, although these account for only a small proportion of the total burden of kidney disease. Recent studies have identified common genetic variants at the *UMOD, SHROOM3, GATM* and *MYH9* loci associated with kidney function in European and African American populations.[2, 3] We carried out a genome-wide association and replication study, to identify genetic loci associated with serum creatinine levels. Though creatinine levels may be partially influenced by non-renal factors including diet and generation from muscle metabolism, serum creatinine is a validated measure of glomerular filtration rate.[4]

Genome-wide association was done in 23,812 European white participants from nine studies; characteristics of participants and genotyping arrays used are summarised (Supplementary Methods and Supplementary Tables 1). Creatinine levels were $\log_{10}$ transformed to achieve approximate normality, and SNP associations tested by linear regression using an additive genetic model, and adjustment for age and sex. Principal component scores were included as ancestry covariates in regression analyses, and test statistics corrected for the genomic control inflation factor to adjust for population substructure (Supplementary Methods).[5] Analyses were performed separately in each cohort, followed by meta-analysis using z-scores weighted by square root of sample size. QQ plots showed good adherence to null expectations (Lambda=1.024, Supplementary Figure 1). The genome-wide association study had 80% power to detect SNPs associated with 0.14% of population variation in creatinine levels at $P<5\times10^{-7}$.

There were 109 SNPs associated with creatinine at $P<5\times10^{-7}$, distributed between 5 genetic loci (chr2p12-p13, chr4q21, chr6q26, chr17q23 and chr19q13, Figure 1 and Supplementary Figure 2). At four of these loci (chr2p12-p13, chr6q26, chr17q23, chr19q13) common variants have not been reported to be associated with kidney function or CKD; at each locus we selected the most strongly associated SNP for replication testing against creatinine in a further sample of 16,626 Europeans (Supplementary Methods and Supplementary Table 2). All four SNPs showed strong replication with creatinine ($P=2.4\times10^{-3}$ to 7.0×10^{-9}, Table 1 and Supplementary Table 3). At chr4q21, the most closely associated SNP was rs9992101 ($P=5.9\times10^{-9}$), which is located in *SHROOM3*, and which is in high LD with rs17319721 ($r^2=0.78$, HapMap CEU population), a SNP previously reported to be associated with glomerular filtration rate.[2]

Next we tested the four top-ranking SNPs for association with estimated glomerular filtration rate (eGFR) and cystatin-c (both additional measures of kidney function[4]), and also with CKD, amongst the participants from the replication sample (Supplementary Methods). SNPs rs10206899 (chr2p12-p13) and rs4805834 (chr19q13) were associated with eGFR,

cystatin-c and CKD (Table 1 and Supplementary Table 4). In contrast, rs3127573 (chr6q26) and rs8068318 (chr17q23) were associated with eGFR, but not with cystatin-c or CKD. None of the four SNPs were associated with weight, hypertension, diabetes or other clinical parameters known to influence creatinine levels (Supplementary Table 5), and the relationships of these SNPs with creatinine were similar amongst people with and without diabetes or hypertension (Supplementary Table 6).

SNP rs10206899 (chr2p12-p13), linked with creatinine, eGFR, cystatin-c and CKD, is close to a number of genes including *NAT8*, *NAT8B*, *ALMS1*, *DUSP11* and *TPRKB* (Figure 1). *NAT8* is a biologically compelling candidate for the observed association. NAT8 is a member of the GCN5-related N-acetyltransferase (GNAT) superfamily, a group of enzymes that catalyze transfer of an acetyl group from acetyl-coenzyme A to a wide range of acceptor substrates.[6] *NAT8* is strongly, and almost exclusively, expressed in kidney (Supplementary Figure 3), in particular by tubular cells of the renal cortex (Supplementary Figures 4 and 5). Acetylation is a key metabolic pathway for the detoxification of nephrotoxic substances such as aminoglycosides, inhalational anaesthetics and environmental toxins including industrial solvents such as trichloroethylene.[7, 8] SNP rs10206899 is in high LD (r^2=1.0) with the only common non-synonymous SNP in *NAT8*, rs15358 (A595G). SNP rs15358 gives rise to a non-conservative amino acid change (F143S) within the acetyl-coenzyme A binding site an effect predicted to influence acetylation by NAT8 (Supplementary Figure 6). SNP rs15358 was also closely associated with creatinine levels in the genome-wide study (P=1.8×10^{-8}). Our findings raise the possibility that common genetic variation in *NAT8* may influence acetylation pathways, disturbances of which are known to be associated with drug and toxin induced kidney injury.

NAT8B is highly homologous with NAT8, also contains an acetyltransferase domain, but is only expressed at low levels in kidney (Supplementary Figure 3). Mutations in *ALMS1* are responsible for Alström Syndrome, a rare autosomal recessive multisystem disorder characterised by progressive kidney and hepatic failure, obesity and insulin resistance, blindness and hearing loss.[9] Though *DUSP11* and *TPRKB* are also in proximity to rs10206899, neither has been implicated in kidney function. DUSP11 is a dual specificity protein phosphatase, *TPRKB* encodes p53-related protein kinase-binding protein, and is of unknown function. Neither is strongly or preferentially expressed in kidney.

SNP rs4805834 (chr19q13) is close to *SLC7A9*, a cationic amino acid transporter, highly expressed in kidney tubular cells (Supplementary Figure 3).[10] *SLC7A9* is a strong candidate for the association of rs4805834 with creatinine, eGFR, cystatin-c and CKD; mutations in *SLC7A9* cause cystinuria and nephrolithiasis, and are associated with increased risk of CKD.[10] SNP rs4805834 is also near *CCDC123* and *C19orf40*. The latter (also known as *FAAP24*) has been identified as a component of the Fanconi anemia core complex which plays a crucial role in DNA damage response,[11] but has no reported relationship to kidney function. The function of *CCDC123* is not known.

SNPs rs3127573 (chr6q26) and rs8068318 (chr17q23) were associated with creatinine and eGFR. SNP rs3127573 is near to *SLC22A2*, an organic cation transporter strongly and preferentially expressed in kidney (Supplementary Figure 3), which contributes to excretion of creatinine and other substrates by renal tubular epithelial cells.[12] Common variants at this locus are reported to influence kidney injury by nephrotoxic drugs such as cisplatin.[13] SNP rs8068318 is located in *TBX2*, a member of the highly conserved T-box family of transcription factors.[14] *TBX2-/-* mutants have a range of morphological defects including limb deformities and cardiac anomalies, but a renal phenotype has not been described.[14] *TBX2* is widely expressed in tissues, including developing and adult kidneys[15], though the structural and functional roles of TBX2 in the kidney are not known. SNP rs8068318 is also

near *BCAS3* and hypothetical gene *C17orf82. BCAS3* may be involved in angiogenesis, though is not known to be involved in kidney function.

In addition to *SHROOM3*, we also replicate previously reported association of rs12917707 in *UMOD* (P=1.7×10⁻⁵) and rs2467853 in *GATM* (P=6.0×10⁻⁶) with creatinine in the genome-wide association study.[2] Though our study had 80% power to detect a 1% change in creatinine at P<0.05 associated with variants of 4% prevalence, we did not find a relationship of the *MYH9* locus with creatinine.[3] *MYH9* variants may have a less important role in kidney function amongst Europeans than the other variants identified.

Our findings of common genetic variants associated with creatinine, cystatin-c and CKD provide insight into the metabolic, solute and drug-transport mechanisms underlying kidney function and CKD. Further evaluation of these pathways may enable biomarker discovery, and new strategies to protect kidney function and prevent CKD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Levey AS, et al. Chronic kidney disease as a global public health problem: approaches and initiatives - a position statement from Kidney Disease Improving Global Outcomes. Kidney Int. 2007; 72:247–259. [PubMed: 17568785]

2. Kottgen A, et al. Multiple loci associated with indices of renal function and chronic kidney disease. Nat Genet. 2009

3. Kopp JB, et al. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. Nat Genet. 2008; 40:1175–1184. [PubMed: 18794856]

4. Stevens LA, Coresh J, Greene T, Levey AS. Assessing kidney function--measured and estimated glomerular filtration rate. N Engl J Med. 2006; 354:2473–2483. [PubMed: 16760447]

5. Price AL, et al. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38:904–909. [PubMed: 16862161]

6. Dyda F, Klein DC, Hickman AB. GCN5-related N-acetyltransferases: a structural overview. Annu Rev Biophys Biomol Struct. 2000; 29:81–103. [PubMed: 10940244]

7. Kharasch ED. Adverse drug reactions with halogenated anesthetics. Clin Pharmacol Ther. 2008; 84:158–162. [PubMed: 18509326]

8. Lash LH, Fisher JW, Lipscomb JC, Parker JC. Metabolism of trichloroethylene. Environ Health Perspect. 2000; 108(2):177–200. [PubMed: 10807551]

9. Marshall JD, Beck S, Maffei P, Naggert JK. Alstrom syndrome. Eur J Hum Genet. 2007; 15:1193–1202. [PubMed: 17940554]

10. Mattoo A, Goldfarb DS. Cystinuria. Semin Nephrol. 2008; 28:181–191. [PubMed: 18359399]

11. Ciccia A, et al. Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM. Mol Cell. 2007; 25:331–343. [PubMed: 17289582]

12. Fujita T, Urban TJ, Leabman MK, Fujita K, Giacomini KM. Transport of drugs in the kidney by the human organic cation transporter, OCT2 and its genetic variants. J Pharm Sci. 2006; 95:25–36. [PubMed: 16307453]

13. Filipski KK, Mathijssen RH, Mikkelsen TS, Schinkel AH, Sparreboom A. Contribution of Organic Cation Transporter 2 (OCT2) to Cisplatin-Induced Nephrotoxicity. Clin Pharmacol Ther. 2009

14. Naiche LA, Harrelson Z, Kelly RG, Papaioannou VE. T-box genes in vertebrate development. Annu Rev Genet. 2005; 39:219–239. [PubMed: 16285859]

15. Chapman DL, et al. Expression of the T-box family genes, Tbx1-Tbx5, during early mouse development. Dev Dyn. 1996; 206:379–390. [PubMed: 8853987]
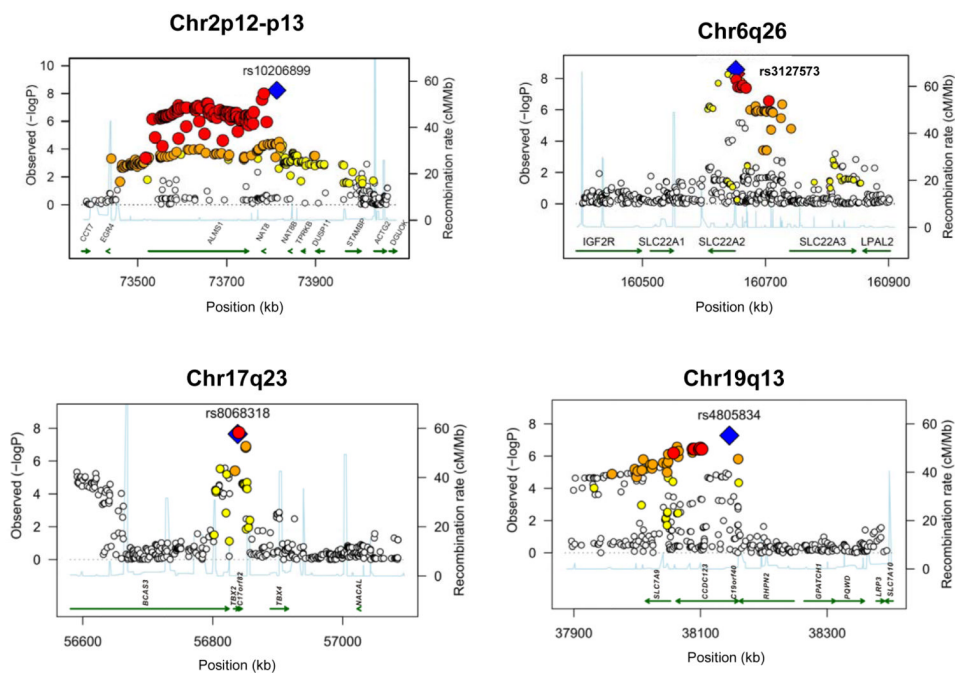
Figure 1.
Architecture of the loci associated with creatinine in the genome-wide association study. The most significant SNP in each region is plotted in blue. LD is based on the HapMap CEU sample and is colour-coded: red (r^2 to top SNP 0.8–1.0), orange (0.5–0.8), yellow (0.2–0.5) and white (<0.2).

Table 1

Genomic context, minor allele frequency (MAF) and association test results for top-ranking SNPs in the genome-wide association (GWA) and replication study. Effect size is % change in serum creatinine (95%CI) or odds ratio for CKD (95% CI) per copy of minor allele, under an additive genetic model, adjusted for age and gender, and for principal component scores in the GWA study. Effect sizes were estimated by meta-analysis of cohort-specific beta-estimates using the inverse variance method and a fixed effects model.

| | | | | Creatinine | | | | | | | | | Chronic Kidney Disease | |
| | | | | GWA sample | | | Replication sample | | | Combined | | | | |
SNP	Locus	Alleles	MAF	N	Effect (95% CI)	P	N	Effect (95% CI)	P	P		P	Odds ratio (95% CI)	P
rs10206899	chr2p12-p13	A / G	0.22	23812	−0.9 (−1.2 to −0.6)	$5.9×10^{-9}$	16167	−1.0 (−1.4 to −0.7)	$7.0×10^{-9}$	−1.0 (−1.2 to −0.7)		$1.2×10^{-15}$	0.85 (0.79 to 0.92)	$5.0×10^{-5}$
rs3127573	chr6q26	A / G	0.13	21857	1.4 (1.0 to 1.8)	$5.0×10^{-9}$	16427	0.7 (0.2 to 1.1)	$2.4×10^{-3}$	1.1 (0.8 to 1.4)		$6.5×10^{-10}$	1.07 (0.97 to 1.17)	0.17
rs8068318	chr17q23	A / G	0.27	23812	0.9 (0.6 to 1.2)	$2.2×10^{-8}$	16350	0.6 (0.2 to 0.9)	$6.1×10^{-4}$	0.8 (0.6 to 1.0)		$3.4×10^{-10}$	1.05 (0.98 to 1.13)	0.16
rs4805834	chr19q13	G / A	0.13	23812	−1.1 (−1.5 to −0.7)	$5.3×10^{-8}$	16241	−0.9 (−1.3 to −0.5)	$4.7×10^{-5}$	−1.0 (−1.3 to −0.7)		$4.5×10^{-11}$	0.84 (0.76 to 0.92)	$3.6×10^{-4}$