Genome-wide association study for serum urate concentrations and gout among African Americans identifies genomic risk loci and a novel URAT1 loss-of-function allele

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Serum urate concentrations are highly heritable and elevated serum urate is a key risk factor for gout. Genome-wide association studies (GWAS) of serum urate in African American (AA) populations are lacking. We conducted a meta-analysis of GWAS of serum urate levels and gout among 5820 AA and a large candidate gene study among 6890 AA and 21 708 participants of European ancestry (EA) within the Candidate Gene Association Resource Consortium. Findings were tested for replication among 1996 independent AA individuals, and evaluated for their association among 28 283 EA participants of the CHARGE Consortium. Functional studies were conducted using ^{14}C-urate transport assays in mammalian Chinese hamster ovary cells. In the discovery GWAS of serum urate, three loci achieved genome-wide significance ($P < 5.0 \times 10^{-8}$): a novel locus near *SGK1/SLC2A12* on chromosome 6 (rs9321453, $P = 1.0 \times 10^{-9}$), and two loci previously identified in EA participants, *SLC2A9* ($P = 3.8 \times 10^{-32}$) and *SLC22A12* ($P = 2.1 \times 10^{-10}$). A novel rare non-synonymous variant of large effect size in *SLC22A12*, rs12800450 (minor allele frequency 0.01, G65W), was identified and replicated (beta -1.19 mg/dl, $P = 2.7 \times 10^{-16}$). ^{14}C-urate transport assays showed reduced urate transport for the G65W URAT1 mutant. Finally, in analyses of 11 loci previously associated with serum urate in EA individuals, 10 of 11 lead single-nucleotide polymorphisms showed direction-consistent association with urate among AA. In summary, we identified and replicated one novel locus in association with serum urate levels and experimentally characterize the novel G65W variant in URAT1 as a functional allele. Our data support the importance of multi-ethnic GWAS in the identification of novel risk loci as well as functional variants.

INTRODUCTION

Uric acid is the breakdown product of purines in humans, who lack uricase. An elevated level of serum urate, hyperuricemia, is a key risk factor for gout (1). Gout is the most common inflammatory arthritis in men, with a prevalence of 1–2% (2). Serum urate levels are highly heritable (3). Several Mendelian syndromes featuring hypo- or hyperuricemia and gout are known [OMIM ID #220150 (*SLC22A12*), #300322 (*HPRT*), #300661 (*PRPS1*), #612076 (*SLC2A9*)]. It is therefore of interest to identify both genetic variants that increase and those that decrease serum urate levels to obtain a complete understanding of an individual's predisposition to hyperuricemia and gout. Genome-wide association studies (GWAS) have identified common variants that are associated with serum urate levels and gout among individuals of European ancestry (EA) (4–10). Follow-up projects discovered that the proteins encoded by two of the identified genes, *SLC2A9* (7,11,12) and *ABCG2* (13–14), function as urate transporters, furthering our knowledge of urate metabolism in humans.

Despite this progress, GWAS for common genetic risk variants for hyperuricemia and gout have only been reported from EA study participants as well as a Japanese study population (15). Additionally, the reported higher cumulative incidence of gout among African American (AA) men compared with EA men (10.9 versus 5.8%) (16) as well as the reported higher prevalence of gout among middle-aged AA compared with EA study participants (8.8 versus 5.4%) (8) motivates the search for genetic risk variants in AA populations.

Thus, the objectives of this work were to identify novel variants influencing serum urate levels and gout risk among AA participants of the Candidate Gene Association Resource (CARe) Consortium (17), a consortium of nine studies with ~8000 AA participants with information on ~2.5 million genotyped and imputed single-nucleotide polymorphisms (SNPs) as well as ~40 000 EA and AA participants with information on 50 000 genotyped SNPs with dense coverage in cardiovascular disease and inflammation pathway candidate genes

across ~2000 loci (IBC array). Because of the strong observational link between serum urate concentrations and cardiovascular disease and its risk factors (18) and the link between gout and inflammation (19), genotyping of the IBC chip may provide information beyond that obtained from a genome-wide SNP platform. Urate transport assays were conducted to assess functionality of novel rare non-synonymous variants in urate transporters.

RESULTS

Study sample characteristics by genotyping array are shown in Table 1. For the GWAS of serum urate, the overall sample size was 5820 AA participants [mean serum urate levels 5.8 mg/dl (SD 1.7)]. The GWAS of gout were conducted among 2831 individuals (177 gout cases). For the analysis of SNPs on the IBC chip, 6890 AA and 21 708 EA participants contributed information; sample size information for gout and mean urate levels are reported in Table 1. Within all AA study samples, median European ancestry ranged from 15.2 to 20.5% (Table 1).

GWAS for serum urate concentrations and gout among AA participants

Figure 1 presents a plot of $-\log_{10}(P\text{-values})$ by genomic position for the GWAS of serum urate among AA participants. The quantile–quantile plots are shown in Supplementary Material, Figure S1 for serum urate with and without genomic regions known to contain urate-associated genes among EA populations (A, B) and for gout (C). Significant associations ($P < 5.0 \times 10^{-8}$) were observed for SNPs in one novel region on chromosome 6 (closest genes *SGK1* and *SLC2A12*) as well as two genomic regions previously reported among EA participants (*SLC2A9* and *SLC22A12*; P-value range 2.1×10^{-10} to 3.8×10^{-32}; Table 2). Regional association plots of the novel region providing information on both

Table 1. Study sample characteristics in the individual studies, CARe

Study	Sample size (uric acid/gout)	Median European Ancestry, % (25, 75 percentile)[a]	Age in years (SD)[b]	Women % (n)[a]	UA, mg/dl (SD)	Gout % (n)
Affy 6.0—African Americans						
ARIC	2749/1908	15.3 (10.7, 22.1)	53 (5.8)	62.9 (1728)	6.28 (1.66)	8.33 (159)
CARDIA	937/923	16.8 (12.4, 23.4)	24 (3.8)	60.9 (571)	5.12 (1.34)	1.95 (18)
JHS	2134/NA	15.7 (11.8, 21.1)	50 (12.1)	60.8 (1297)	5.45 (1.69)	NA
Total	5820/2831	15.7 (11.4, 21.9)	47 (13.3)	61.8 (3596)	5.79 (1.69)	6.25 (177)
IBC chip—African Americans						
ARIC	2927/2010	15.2 (10.5, 22.8)	53 (5.8)	62.9 (1840)	6.30 (1.68)	8.66 (174)
CARDIA	1268/1254	16.8 (11.8, 23.7)	24 (3.8)	58.9 (747)	5.12 (1.38)	1.83 (23)
CHS	727/NA	20.5 (12.4, 32.7)	73 (5.7)	62.9 (457)	5.79 (1.63)	NA
JHS	2027/NA	15.9 (11.5, 21.9)	50 (12.0)	60.6 (1229)	5.43 (1.68)	NA
Total	6890/3264	16.2 (11.2, 23.3)	49 (15.5)	61.5 (4273)	5.78 (1.69)	6.04 (197)
IBC chip—European Americans						
ARIC	9581/7719	NA	54 (5.7)	53.5 (5128)	5.93 (1.50)	5.22 (403)
CARDIA	1430/1411	NA	26 (3.4)	53.3 (762)	5.34 (1.37)	1.28 (18)
CHS	3938/NA	NA	73 (5.6)	56.1 (2208)	5.67 (1.52)	NA
FHS	6759/6757	NA	38 (9.6)	53.4 (3607)	5.42 (1.47)	2.19 (148)
Total	21708/15887	NA	51 (15.4)	53.9 (11705)	5.69 (1.50)	3.58 (569)
Replication—African Americans						
HANDLS	989/NA	16.1 (11.2, 22.0)	48 (9.0)	55.7 (551)	5.55 (1.65)	NA
HUFS	1007/NA	19.7 (14.3, 26.9)	48 (13.2)	58.9 (593)	5.53 (1.64)	NA
Total	1996/NA					

[a]Summary statistics based on uric acid samples and similar to those from the gout samples.
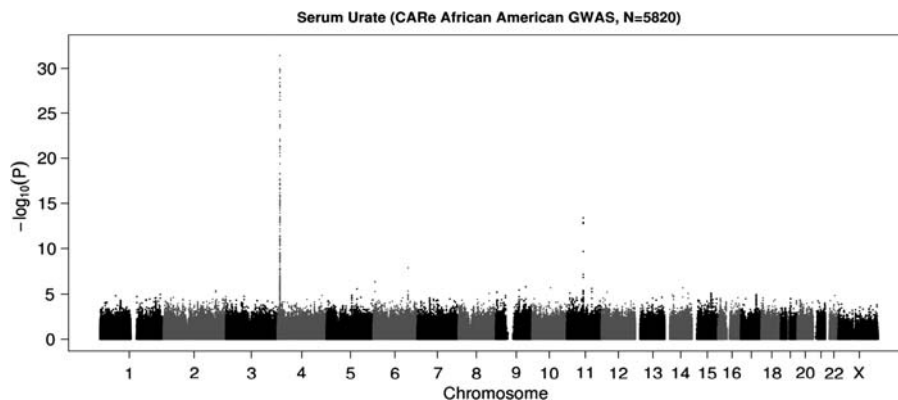[b]Age of serum urate data collection.

Figure 1. $-\log_{10}(P\text{-value})$ by genomic position plot for the GWAS of serum urate among 5820 AA participants.

r^2 and D' as measures of linkage disequilibrium (LD) are presented in Figure 2. A search of publicly available expression data sets (http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/) did not provide evidence that the novel SNP rs9321453 was associated with the transcription of either *SGK1* or *SLC2A12* in any of the 13 data sets queried.

At the known loci *SLC2A9* and *SLC22A12*, multiple signals in low LD were observed (rs13129697 and rs7663032 with $r^2 = 0.31$, *SLC2A9*; rs606458 and rs493573 with $r^2 = 0.02$, *SLC22A12*). Based on multivariable regression analyses in the ARIC AA sample ($n = 2749$) that included the SNPs simultaneously, the two common genome-wide significant SNPs in *SLC2A9* accounted for 3.2% of the serum urate variance (separately 2.6 and 2.3%). The three genome-wide significant SNPs in the *SLC22A12* region were moderately correlated

with r^2 ranging from 0.02 to 0.45. Together, the two rare and one common SNP accounted for 1.2% of the serum urate variance (separately 0.5 and 0.7% for the two rare variants, rs12800450 and rs493573, respectively, and 0.6% for rs606458).

Two significant SNPs in the *SLC22A12* region were coding variants of low minor allele frequency: non-synonymous rs12800450 [(G65W), minor allele frequency (MAF) 0.01, $P = 4.0 \times 10^{-14}$] in *SLC22A12*, which encodes the known urate transporter URAT1, and synonymous rs493573 in nearby *MAP4K2* [(A806A), MAF 0.03, $P = 1.3 \times 10^{-13}$]. The two variants were correlated ($r^2 = 0.45$, $D' = 1$). The effect of the variants on serum urate levels was ~6- to 10-fold larger than the one observed for more common variants in *SLC22A12* (Table 2). No genome-wide significant associations were observed in GWAS of gout.

Table 2. Loci in AA from GWAS: SNP association with uric acid levels

SNP ID	Chr	Position (b36)	Genes within 60 kb	SNP function	Coded allele frequency	Coded allele	Beta (mg/dl)	SE	Discovery P-value	Replication beta	Replication P-value	Meta-analysis beta	Meta-analysis P-value
Novel Loci													
rs9321453	6	134815247	NA	Intergenic	0.42	T	0.15	0.03	1.4×10^{-08}	0.11	2.4×10^{-02}	0.14	1.0×10^{-09}
Known Loci													
rs13129697	4	9536065	SLC2A9	Intronic	0.42	T	0.32	0.03	3.8×10^{-32}	[a]	[a]	[a]	[a]
rs7663032	4	9602936	SLC2A9	Intronic	0.67	T	0.32	0.03	1.2×10^{-30}	[a]	[a]	[a]	[a]
rs12800450	11	64115797	SLC22A12	Non-synonymous coding	0.01	T	−1.21	0.16	4.0×10^{-14}	−1.08	5.0×10^{-03}	−1.19	2.7×10^{-16}
rs606458	11	64302967	MEN1;SF1;MAP4K2; PYGM;RASGRP2; CDC42BPG;NRXN2	Intergenic	0.35	T	−0.21	0.03	2.1×10^{-10}	−0.11	3.9×10^{-02}	−0.18	5.5×10^{-11}
rs493573	11	64313630	MAP4K2	Synonymous coding	0.03	T	−0.81	0.11	1.3×10^{-13}	−0.74	6.9×10^{-05}	−0.80	1.9×10^{-17}

[a]Not sent for replication due to LD ($r^2 > 0.2$) with reported index SNP according to the HapMap CEU and YRI data.

A total of 1996 AA participants from two independent studies, 989 participants from the HANDLS study and 1007 participants from HUFS study, were available for replication of novel findings. The SNP with the lowest P-value at the novel locus on chromosome 6, rs9321453, was of comparable effect size in the replication samples and showed evidence for nominal independent replication in the AA replication samples (P-replication = 0.024) as well as overall replication (P-combined = 1.0×10^{-9}, Table 2). Study-specific results for the replication studies are presented in Supplementary Material, Table S1. The two low-frequency coding variants identified in the GWAS of serum urate also showed evidence for independent replication among the AA replication samples (rs12800450: P-replication = 0.005; rs493573, P-replication = 6.9×10^{-5}) as well as overall replication.

We further evaluated SNPs identified in our AA samples for their effect among 28 283 EA participants of the large international CHARGE Consortium (20). The novel SNP rs9321453 on chromosome 6 showed no association among EA individuals ($P = 0.82$), and the region among EA individuals is depicted in Supplementary Material, Figure S2. The common SNPs at the *SLC2A9* and *SLC22A12* loci listed in Table 2 showed highly significant association with serum urate for the same modeled allele in the CHARGE participants (P-value range 8.0×10^{-6} to 1.5×10^{-242}, Supplementary Material, Table S2). The low-frequency variants in the *SLC22A12* gene region discovered in our analyses were not evaluated among the CHARGE samples because the SNPs were monomorphic in all (rs493573) or some (rs12800450) of the individual studies with frequencies as low as 0.01%, making the estimates not reliable.

IBC chip analyses for serum urate and gout

Supplementary Material, Table S3 shows SNPs with significant association ($P < 2.0 \times 10^{-6}$) with serum urate and gout among the 21 708 EA participants with genotype data from the IBC cardiovascular disease candidate gene chip. There were five regions with evidence of association with serum urate (P-value range 8.3×10^{-8} to 1.9×10^{-44}), all of which had been reported in previous GWAS of serum urate among EA participants (4,10). For gout, the known functional variant rs2231142 in *ABCG2* showed evidence of significant association [odds ratio (OR) = 1.72 per T allele, $P = 3.1 \times 10^{-10}$]. Among 6890 AA participants, no significant associations were observed for either phenotype. Replication of findings from the IBC analyses was not sought, as there were no significant findings for the AA participants, and all the regions identified among EA participants had been reported previously.

Functional studies

Urate transport assays were performed to assess whether the G65W variant of URAT1, encoded by rs12800450 in *SLC22A12*, is a functional allele. This was the only variant selected for functional follow-up studies because of the encoded non-synonymous amino acid substitution, the large effect on serum urate per copy of the minor allele (−1.2 mg/dl), the biological plausibility of the encoded renal urate transporter URAT1 and the predicted damaging
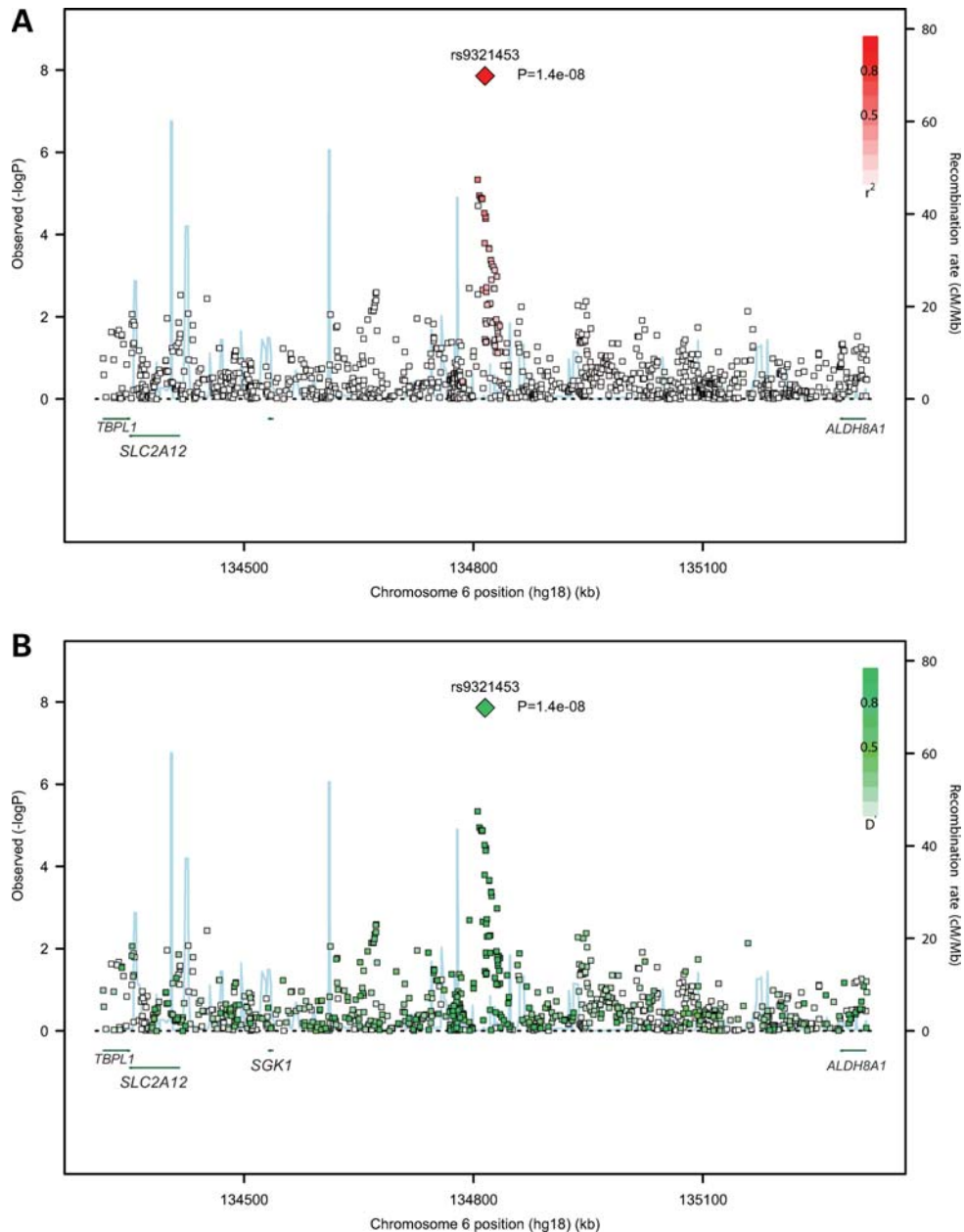
Figure 2. Regional association plots of SNPs at the novel chromosome 6 locus near *SGK1*/*SLC2A12* and their association with serum urate. The upper panel shows r² as a measure of LD (red), and the lower one shows *D*′ (green).

function of the amino acid substitution (PolyPhen2: score 0.99; sensitivity: 0.64, specificity: 0.95) (21). Mammalian Chinese hamster ovary (CHO) cells transiently transfected with human G65W URAT1 exhibited significantly reduced ^{14}C-urate transport in comparison to cells expressing wild-type (WT) human URAT1 ($P < 0.001$, Fig. 3).

Association with gout for SNPs discovered for serum urate

Table 3 presents the association with gout for SNPs that show significant association with serum urate levels among CARe AA participants (GWAS) and CARe EA participants (IBC). Of the six SNPs in AA, the associations between gout and

the two SNPs in *SLC2A9* were significant after correction for multiple testing [P-value $< 8.3 \times 10^{-3}$ ($=0.05/6$)], with OR of 1.4 and 1.46. In EA participants, the functional variant rs2231142 in ABCG2 is associated with gout at a genome-wide significance level as reported previously (4). For all 12 SNPs, directionally consistent effects on serum urate and gout were observed (P-binomial < 0.0001).

Interrogation of known loci associated with serum urate among AA participants

We interrogated genomic regions known to contain SNPs associated with serum urate in individuals of EA among the
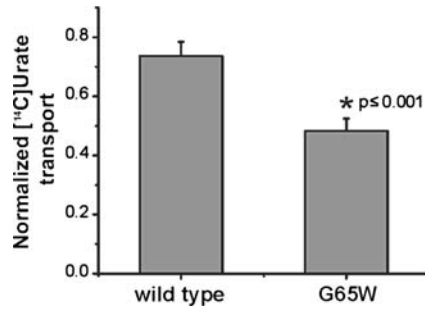
Figure 3. Urate transport in mammalian CHO cells: normalized ^{14}C urate transport rates in CHO cells transiently transfected with either the WT URAT1 or the G65W mutant ($n = 9$ for each; ± SEM).

5820 AA CARe participants with GWAS data. Table 4 lists the 11 genomic regions that were investigated. For the index SNP known from studies in EA participants, 10/11 SNPs showed directionally consistent effects on serum urate for the same modeled allele among AA CARe participants (P-binomial = 0.0005). For six of the index SNPs at five distinct genomic regions, nominally significant P-values were observed among AA participants (SNPs in *PDZK1*, *SLC2A9*, *RREB1*, *SLC22A12* and *R3HDM2*; Table 4). Effect sizes of three SNPs (in *PDZK1*, *SLC22A12*, *R3HDM2*) were over 50% stronger in AA compared with EA individuals. The effect sizes of the other three SNPs (two in *SLC2A9*, one in *RREB1*) were of similar magnitude in EA and AA individuals. No significant signal was observed for the index SNP rs780094 in *GCKR*. Among EA participants, this SNP is in high LD with non-synonymous coding rs1260326 (r^2 of 0.93 in HapMap2 CEU; r^2 of 0.43 in HapMap2 YRI), which has been reported as a functional variant (22). In our AA data, there was no significant association between the rs1260326 T allele and serum urate (beta 0.0135, P-value 0.72, MAF 0.16).

As a second step, we interrogated the region that contained the index SNP known from EA individuals for association with serum urate among CARe AA participants (Supplementary Material, Table S4). Eight of the 11 known EA regions contained SNPs with significant associations (P-value range 1.1×10^{-3} to 3.8×10^{-32}). In the *GCKR* region, independent signals were observed in nearby *RBKS*, which did, however, not replicate (Supplementary Material, Table S4). Supplementary Material, Figure S3A−K shows, for each interrogated region, the AA association results; the index SNP among EA individuals is highlighted. Supplementary Material, Figure S4 shows the LD as measured by r^2 of the CEU and YRI populations for the *GCKR* region, for which LD structure and associations with serum urate differed between EA and AA individuals. Details about genotyping and quality control procedures are reported in Supplementary Material, Table S5, and imputation quality of significant SNPs in Supplementary Material, Table S6. All SNPs that attained genome-wide and IBC-wide significance are listed in Supplementary Material, Table S7.

DISCUSSION

Principal findings

In our GWAS of serum urate levels among AA individuals, we identified and replicated one novel locus, near *SGK1/*

Table 3. Association of urate loci with gout

SNP	Chr	Position (b36)	Genes within 60 kb	Coded allele	Gout beta	Gout SE	Gout OR	95% CI	P-value
African American Affy Loci									
rs9321453	6	134815247	NA	T	0.21	0.12	1.23	0.98, 1.55	7.16×10^{-02}
rs13129697	4	9536065	SLC2A9	T	0.33	0.12	1.40	1.11, 1.76	4.66×10^{-03}
rs7663032	4	9602936	SLC2A9	T	0.38	0.13	1.46	1.13, 1.88	3.97×10^{-03}
rs12800450	11	64115797	SLC22A12	T	−1.36	1.06	0.26	0.03, 2.04	1.98×10^{-01}
rs606458	11	64302967	MEN1;SF1;MAP4K2; PYGM;RASGRP2; CDC42BPG;NRXN2	T	−0.14	0.15	0.87	0.65, 1.16	3.44×10^{-01}
rs493573	11	64313630	MAP4K2	T	−1.51	0.75	0.22	0.05, 0.97	4.51×10^{-02}
European American IBC Loci									
rs1260326	2	27584444	GCKR	T	0.07	0.06	1.08	0.95, 1.22	2.32×10^{-01}
rs2241480	4	9684583	WDR1, SLC2A9	T	0.21	0.09	1.24	1.03, 1.49	2.45×10^{-02}
rs2231142	4	89271347	ABCG2	T	0.54	0.09	1.72	1.45, 2.03	3.13×10^{-10}
rs2051541	6	25934098	SLC17A1 / SLC17A3	A	−0.13	0.08	0.88	0.74, 1.03	1.19×10^{-01}
rs10792443	11	64147733	NRXN2, SLC22A11	C	−0.14	0.10	0.87	0.71, 1.06	1.68×10^{-01}
rs11172147	12	55982944	R3HDM2	A	−0.20	0.09	0.82	0.69, 0.97	1.90×10^{-02}

Table 4. Interrogation of imputed GWAS data in AA for SNPs previously identified in GWAS of urate levels in European ancestry (EA) individuals

Paper	CHR	SNP (proxy)	Position	Gene	Coded allele	Coded allele frequency in EA	Beta in EA	*P*-value in EA	Coded allele frequency in AA	Beta in AA	*P*-value in AA
Kolz (10)	1	rs12129861	144437046	*PDZK1*	A	0.46	−0.06	2.68×10^{-09}	0.39	−0.11	8.73×10^{-05}
Kolz	2	rs780094	27594741	*GCKR*	T	0.42	0.05	1.40×10^{-09}	0.18	−0.03	4.40×10^{-01}
Kolz	4	rs734553	9532102	*SLC2A9*	T	0.77	0.32	5.22×10^{-201}	0.47	0.28	2.68×10^{-24}
Delghan (8)	4	rs6449213	9603313	*SLC2A9*	C	0.18	−0.35	2.20×10^{-104}	0.14	−0.29	2.84×10^{-14}
Kolz	4	rs2231142 (rs4148155)[a]	89273941	*ABCG2*	T	0.11	0.17	3.10×10^{-26}	0.03	0.16	5.50×10^{-02}
Yang (4)	6	rs675209	7047083	*RREB1*	T	0.26	0.07	1.00×10^{-09}	0.48	0.06	4.86×10^{-02}
Kolz	6	rs742132	25715550	*LRRC16*	A	0.70	0.05	8.50×10^{-09}	0.70	0.01	6.35×10^{-01}
Kolz	6	rs1183201	25931423	*SLC17A1, SLC17A3*	A	0.48	−0.06	3.04×10^{-14}	0.13	−0.07	8.20×10^{-02}
Kolz	10	rs12356193	61083359	*SLC16A9*	A	0.83	0.08	1.07×10^{-08}	0.92	0.06	2.86×10^{-01}
Kolz	11	rs17300741	64088038	*SLC22A11*	A	0.51	0.06	6.68×10^{-14}	0.58	0.04	1.53×10^{-01}
Kolz	11	rs505802	64113648	*SLC22A12*	T	0.70	−0.06	2.04×10^{-09}	0.32	−0.13	5.24×10^{-06}
Yang	12	rs1106766	56095723	*R3HDM2*	T	0.23	−0.09	1.90×10^{-11}	0.09	−0.16	6.46×10^{-04}

SNPs were counted as 11 independent loci, as the r^2 between the two SNPs at the *SLC2A9* locus was 0.69 in CEU (HapMap rel 22) and the two SNPs were therefore counted as one locus.
[a]rs4148155, which has an r^2 of 1 with rs2231142, was used because rs2231142 was not available on the GWAS platform.

SLC2A12, and confirmed associations at two loci, *SLC2A9* and *SLC22A12*, previously detected among EA individuals. Using urate transport assays, we showed that the novel rare G65W variant in URAT1, encoded by rs12800450 in *SLC22A12*, is a functional allele that displays reduced urate transport capacity in mammalian cells. In 11 regions previously known to associate with serum urate among EA individuals, we observed similar associations for 10 of the lead EA SNPs in our AA cohorts, of which 6 SNPs at 5 distinct loci were significantly associated with serum urate. Differences in the allelic spectrum of genetic variants between AA and EA individuals and the greater linkage equilibrium in AA may explain the lack of significant association at the other loci.

In the context of the current literature

Our study extends findings from previous GWAS among individuals of EA by identifying one novel locus on chromosome 6 near the *SGK1* and *SLC2A12* genes. We further extend previous knowledge by identifying independent urate-associated signals in the *SLC2A9* and *SLC22A12* regions as well as rare variants in the *SLC22A12* region that was not identified in earlier studies of EA individuals or in the isolated population of the Island of Kosrae (23). More importantly, we evaluate and confirm functionality of an identified low-frequency non-synonymous coding variant in *SLC22A12*, allowing for the conclusion that the minor allele at this variant causes lower serum urate levels and consequently reduced risk of gout. This is in agreement with the knowledge of rare-inactivating mutations in the encoded protein URAT1, which can cause renal hypouricemia (OMIM #220150) (24); affected individuals display serum urate levels as low as 1 mg/dl for individuals homozygous for a loss-of-function variant (25). Recent data from the 1000 Genomes Project suggest that each individual differs from the reference human genome sequence at ∼10 000 non-synonymous sites, but that only ∼400 of these variants are causing premature stops, altered splicing or frame shifts (26). With the accelerated identification of rare non-synonymous

coding variants, it will be increasingly important to assess experimentally whether an identified variant that causes an amino acid substitution indeed is functional.

In this GWAS of serum urate and gout among AA study participants, 10 of the 11 loci known to influence serum urate in EA individuals showed directionally consistent association for the same modeled allele in our AA samples. Six of the EA index SNPs at five distinct loci were nominally significantly associated, and although not significant among our smaller AA samples, the effects of three of the remaining index SNPs were of comparable size in the AA population. This suggests that variants at most genomic regions that influence serum urate levels in individuals of EA also contain associated variants in AA. These results are consistent with observations made for other traits, for example blood lipid-associated variants (17,27). In the *GCKR* gene region, our results do not support rs1260326 as the causal variant responsible for the association with serum urate, as no association was observed among AA participants.

Biological mechanisms

The SNP with the lowest *P*-value at the novel urate-associated locus on chromosome 6, rs9321453, is located ∼134 kb upstream of the next RefSeq gene, *SGK1*. The *SGK1* gene encodes the serine/threonine protein kinase serum/glucocorticoid-regulated kinase 1 (28), which activates certain potassium, sodium and chloride channels. Another gene in the region is the neighboring *SLC2A12*, which encodes GLUT12 (29). GLUT12 is a facilitative glucose transporter that belongs to the same overall family as the known urate transporter GLUT9, encoded by *SLC2A9* (30). It is therefore an interesting candidate emerging from the extended region on chromosome 6, especially since many of the genes identified in our study encode known urate transport proteins (*ABCG2*, *SLC2A9*, *SLC22A11*, *SLC22A12*, *SLC17A1*) or regulators thereof (*PDZK1*). Although recombination hot spots separate rs9321453 from the nearby genes *SGK1* and *SLC2A12*, this SNP displays high LD measured by *D'* with low-frequency SNPs in the

nearby genes based on Hapmap YRI release 22 data (Fig. 2). It is therefore conceivable that variation in these genes could give risk to the observed signal, but additional studies are needed to pinpoint the causal variant as well as to evaluate whether this locus harbors population-specific variants in AA influencing serum urate levels.

The only rare genome-wide significant non-synonymous coding variant we identified, rs12800450 in *SLC22A12*, exhibited up to 10-fold larger effects on mean urate levels compared with more common alleles in *SLC22A12*. *SLC22A12* encodes for the urate/anion exchanger URAT1, which is one of the two main urate transport proteins responsible for the reabsorption of urate from urine into blood and is localized in the apical membrane of renal proximal tubular cells (31). Although the G65W URAT1 variant displayed residual urate transport activity, the fact that each copy of the minor allele is associated with ~1.0 mg/dl lower mean serum urate levels underscores the importance of this transporter in regulating serum urate levels in humans. The reduced urate transport rates and consequently lower mean serum urate concentrations we observe for the G65W variant, together with the localization of URAT1 in the kidney, are consistent with G65W being a partial loss-of-function allele. Genetic variation contributing to each individual's serum urate levels and gout risk is a combination of urate-increasing and urate-lowering alleles. To obtain a more complete picture of the genetic risk of hyperuricemia and gout, it is therefore necessary to identify and characterize both urate-increasing and urate-lowering functional alleles, as done here.

Strengths and limitations

Strengths of our study include the large sample size of ~7000 AA individuals for the discovery analyses of serum urate, as well as the availability of independent replication cohorts of both AA and EA individuals. The genetic variants examined were genotyped on the same genome-wide array in all studies participating in the CARe Consortium, and were complemented by an additional 50 000 SNPs genotyped on a large candidate gene chip. Importantly, we were able to show through functional studies that the G65W allele of human URAT1 leads to reduced urate transport in mammalian cells and therefore is causally related to lower serum urate levels and reduced gout risk.

Some limitations to our study warrant mention. The AA replication samples were of relatively small size, which—together with lower coverage even after imputation—limited statistical power for both the identification of novel loci as well as the replication of previously detected loci. Nevertheless, the magnitude of associations observed for serum urate was similar among AA for loci previously detected in EA populations. We relied on a single measurement of serum urate as well as on self-reported gout, which may have impacted our results to some degree. The fact that we were able to detect highly significant associations between SNPs at known urate- and gout-associated loci underscores the validity of the phenotype definitions.

In summary, our study supports the use of GWAS in populations of non-European ancestry for the discovery of novel trait-associated loci as well as additional risk variants at known loci. It further supports the use of experimental studies to confirm functionality of rare non-synonymous coding variants.

MATERIALS AND METHODS

Serum urate measurement, gout and covariates

Serum urate was measured as described in the Study-specific methods. Gout was defined using cohort-specific definitions based on self-report and medication intake as detailed in the Study-specific methods. Covariates used in analyses were age (years), sex as well as study center if applicable.

Genotyping platforms for genome-wide genotype and imputation

Genotyping in the CARe Consortium was conducted using two chips: the IBC SNP chip (32) in 7112 AA and 22 539 EA participants, and the Affymetrix 6.0 array in 6089 AA participants. Details about genotyping as well as imputation and data cleaning procedure are provided in Supplementary Material, Table S3. Data cleaning and SNP imputation for the individual cohorts were performed centrally by the CARe analytical group at the Broad institute. For imputation among AA participants, a combined HapMap 2 CEU + YRI reference panel was created, which includes SNPs segregating in both CEU and YRI, as well as SNPs segregating in one panel and monomorphic and non-missing in the other (2.74 million altogether). Imputation results were filtered at an RSQ_HAT threshold of 0.3 and a minor allele frequency threshold of 0.01. The imputation software was MACH 1.0.16 (33). Imputed SNPs were then analyzed as the SNP dosage, a continuous number between 0 and 2 corresponding to the estimated number of copies of the pre-specified allele. As the Affymetrix chip provides better coverage across the entire genome, whereas the IBC chip provides better coverage of selected regions including rare variants and was genotyped in more samples, data from the Affymetrix and IBC chip were analyzed separately.

Statistical methods for discovery stage 1

Phenotypes were ascertained as outlined above. Principal components were generated using EIGENSTRAT (34) with the curated CARe African-American Affymetrix 6.0 genotype data combined with 1178 European Americans (EA) (a multiple sclerosis GWAS graciously offered by Dr Phil de Jager and colleagues) and from 756 Nigerians from the Yoruba region (an hypertension GWAS graciously offered by Dr Richard Cooper and colleagues). The first principal component has a correlation >0.98 with global ancestry, which was generated using ANCESTRYMAP (35) and STRUCTURE (36).

Study-specific association analyses were performed centrally using linear regression for serum urate and logistic regression for gout, assuming an additive genetic model. For serum urate, we analyzed sex-specific residuals controlling for age and study center, when applicable. For gout, covariates were age, sex and study center, when applicable. All analyses were adjusted for 10 principal components; sensitivity analyses

adjusting only for principal components that showed significant association with the outcome yielded similar results. When appropriate, the analyses accounted for relatedness using linear mixed-effect models for serum urate and logistic regression using generalized estimating equations for gout (37).

Meta-analyses of study-specific results were conducted separately for the Affymetrix 6.0 and the IBC chips using inverse-variance weighted fixed-effect models implemented in METAL (38). For the results from the Affymetrix 6.0 platform, genomic control correction was applied to individual-study results with genomic control factor (λ) >1 prior to meta-analysis. After meta-analysis, the lambda statistics were 1.02 (urate) and 0.99 (gout) for the GWAS indicating that there was no appreciable inflation of the test statistics. Genomic control corrections were not applied to the results from the IBC chip, which was not a genomic-wide array.

The statistical significance level was specified *a priori* at the conventional threshold of $\alpha = 5 \times 10^{-8}$ for the GWAS and as $\alpha = 2.0 \times 10^{-6}$ for the IBC chip analyses, which was based on an estimate of 25 000 independent SNPs with variance inflation factor <2 (39). Software used for data management, analysis and graphing included PLINK (40), Eigenstrat (34), METAL (38) and R (v2.9.0).

Stage 2 replication analysis

Six SNPs were selected for replication based on the following criteria: (i) the lead SNP in a locus met genome-wide significance and was not previously reported (rs9321453 in chromosome 6); (ii) the lead SNPs met genome-wide significance in a known locus and were in low LD ($r^2 < 0.2$) with the reported index SNP in EA individuals (rs606458, rs12800450 and rs493573 in chromosome 11); or (iii) SNPs met region-wide significance in the interrogation of known loci in the Affymetrix 6.0 array GWAS platform and were in low LD ($r^2 < 0.2$) with the reported index SNP in EA individuals (rs9679543 on chromosome 2 and rs589691 on chromosome 11).

Replication of these SNPs was attempted using genotyped and imputed *in silico* GWAS data from the replication cohorts. Serum urate was modeled as it was for the discovery cohorts, and an additive genetic model was used. The Study-specific methods provide more detail about the replication cohorts.

After return of the replication results, evidence for independent replication was assessed as one-sided nominal significance from the combined replication samples (1996 individuals, $P < 0.05$). Subsequently, a meta-analysis was conducted for the results from discovery and replication samples. Findings were considered replicated when the effect direction was consistent for the same modeled allele and the combined *P*-value from discovery and replication samples was both genome-wide significant and lower than the *P*-value from the discovery samples alone (41).

Statistical methods for locus interrogation

An interrogation of the 50 kb flanking regions around the known EA loci was conducted in our AA cohorts. In situations where the HapMap CEU haplotype block extended beyond the gene (*SLC22A11/12* and *GCKR* regions), the region of interrogation was extended up to a recombination hotspot or

to 500 kb on both sides of the gene. Significance for this regional interrogation was set at $P < 0.05$ adjusted for the number of independent SNPs in the region as defined by pair-wise variance inflation factor < 2.

In regions in which multiple signals with low LD ($r^2 < 0.5$) were observed in association with serum urate, we conducted regression including multiple index SNPs in the loci as predictors using the ARIC AA sample, the largest sample among our AA cohorts, to assess the independence of the signals and the total contribution of these signals to serum urate variance.

Molecular biology

The use of human URAT1 in the expression vector pcDNA 3.1 was kindly permitted by H. Endou and has been described previously (24). The URAT1 mutation G65W was created using the QuikChange site-directed mutagenesis kit (Stratagene, Santa Clara, CA, USA) and the primers were 5′-CGG CTC AGG CCA GCA TCC TAT GGA GCT TGA GTC CTG AGG CCC-3′ and 5′-GGG CCT CAG GAC TCA AGC TCC ATA GGA TGC TGG CCT GAG CCG-3′. mRNA was prepared from the WT URAT1 and G65W URAT1 expression vectors using the MEGAscript kit (Ambion) according to the manufacturer's protocol.

Urate transport assays

For the urate transport assays, CHO cells were transiently transfected, using Lipofectamine 2000 (according to the manufacturer's protocol, Invitrogen, USA) with either URAT1, G65W URAT1 or blank vector. Because of the lack of a working commercial antibody, it was not possible to control for equal expression of the two constructs, but all experimental conditions except for the amino acid substitution were kept the same. Radio-labeled ^{14}C uric acid (American Radio-labeled Chemicals) was dissolved in a stock solution of 2 mM NaOH with a final uric acid concentration of 2 mM. After 24 h, the media were replaced with 100 μM ^{14}C urate containing minimal Ham's F12 media with L-glutamine. Cells were incubated for 1 h at 37°C, then washed twice with ice-cold Ham's F-12 media and once with phosphate buffered saline. 1 N NaOH was added to lyse the cells, and the cells and contents scraped, added to a scintillation tube and measured using a Beckman Scintillation counter. Results from each day were normalized to the highest count recorded to allow for cross day comparisons. For each batch of counting, a set of control vials of known volume and concentration of ^{14}C uric acid was included, allowing for a calculation of ^{14}C uric acid concentrations based on the counts measured in any of the experimental samples. URAT1 (or URAT1 G65W) specific transport activity was calculated by subtracting the transport activity measured in the control cells transfected with the blank vector from the one measured in URAT1-expressing cells. Significance was evaluated using a two-tailed Student's *t*-test.

Study-specific methods

ARIC. The ARIC study is a population-based, prospective study in four US communities. From 1987 to 1989, 15 792

mostly white and AA participants aged 45–64 years were recruited by probability sampling and underwent a baseline examination (visit 1), and were examined three more times, roughly every 3 years (42). For this study, participants were excluded for non-consent to genetic research, or if they did not self-identify as black or white. Institutional Review Boards of the participating institutions (Johns Hopkins University, University of Minnesota, Wake Forest University, University of Mississippi, Baylor University, University of Texas and University of North Carolina) approved the study protocols. All participants provided written informed consent.

In the ARIC study, uric acid concentration was measured with the uricase method at visit 1 (43). Gout status was obtained by self-report at visit 4. Age and sex were obtained by self-report at baseline.

CARDIA. Study design details of Coronary Artery Risk Development In Young Adults (CARDIA) have been previously published (44). Briefly, CARDIA recruited a cohort of young black and white adults, age 18–30 at the time of enrollment. From 1985 to 1986, CARDIA recruited participants from four sites: Birmingham, AL, USA; Chicago, IL, USA; Minneapolis, MN, USA; and Oakland, CA, USA. Follow-up examinations occurred at years 2, 5, 7, 10, 15 and 20. Serum urate was from baseline and measured by the uricase method. Gout status was collected at years 7, 10 and 15 by self-report.

CHS. The Cardiovascular Health Study (CHS) is a community-based longitudinal study of risk factors for cardiovascular disease and stroke in adults 65 years of age or older, recruited at four field centers (Forsyth County, NC, USA; Sacramento County, CA, USA; Washington County, MD, USA; and Pittsburgh, PA, USA) (45). A total of 5201 predominantly Caucasian individuals were recruited in 1989–1990 from random samples of Medicare eligibility lists, followed by an additional 687 African-Americans recruited in 1992–1993 (total $n = 5888$). Baseline serum urate was measured on the Kodak Ektachem 700 Analyzer.

FHS. The Framingham Heart Study began in 1948 when the Original Cohort was enrolled (46). Beginning in 1971, the Offspring Cohort was enrolled (5124 participants); the methodology and design has been described (47–48). In 2002, the Third Generation cohort was enrolled ($n = 4095$) (49). Participants for the current study include individuals from the offspring cohort and the Third Generation who attended the first examination. Serum urate was measured at the first examination cycle in every cohort with an autoanalyzer with a phosphotungstic acid reagent (50). Gout was self-reported in the offspring cohort during examination cycles 3 to 7, and in the third-generation group during first examination.

JHS. The Jackson Heart Study is a single-site, longitudinal, population-based study. The sample consists of 5302 African-American women and men selected between 2000 and 2004 (first visit) from the tricounty area encompassing Jackson, MS (51). DNA and consent for data sharing consistent with NIH guidelines were available for 3443 participants for the CARe Consortium. Baseline serum urate was measured using the uricase method.

HANDLS. The Healthy Aging in Neighborhoods of Diversity Across the Life Span study is a prospective longitudinal, multidisciplinary study. Subjects were recruited as a fixed cohort of participants by household screenings from an area probability sample of 12 census segments in the city of Baltimore, MD, USA. These tracts were selected to yield representative distributions of AA and whites, men and women, and individuals with SES $<125\%$ federal poverty level and $\geq 125\%$ federal poverty level. Study design and participant characteristics were previously reported (52). Uric acid was measured in serum from fasting blood samples by spectrophotometry at Quest Diagnostics Laboratories in Chantilly, Virginia.

HUFS. The Howard University Family Study is a population-based study of AA families enrolled from the Washington, D.C. metropolitan area (53). In the first phase of recruitment, a randomly ascertained sample of 350 AA families with members in multiple generations from the Washington, D.C. metropolitan area were enrolled and examined. Families were not ascertained based on any phenotype. In a second phase of recruitment, additional unrelated individuals from the same geographic area were enrolled to facilitate nested case–control study designs. The total number of recruited individuals was 2028, of which 1976 remained after data cleaning. From this sample, 1007 unrelated individuals were included for the serum urate analysis. Serum urate was measured using the COBAS Integra Uric Acid ver.2 assay from Roche Diagnostics (Indianapolis, IN, USA).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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