Large-Scale Whole-Exome Sequencing Association Studies Identify Rare Functional Variants Influencing Serum Urate Levels

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Supplementary Information

Supplementary Methods

Secondary meta-analyses. To assess potential heterogeneity between race groups, we conducted race-specific single variant and SKAT-O meta-analyses using the same variant filtering criteria and methods as the primary meta-analysis (**Supplementary Figure 10**). To evaluate whether a score integrating additional annotation sources might improve association signals, we conducted race-specific and race-combined SKAT-O meta-analyses that included all putative damaging variants regardless of MAF weighted by their Combined Annotation–Dependent Depletion (CADD) scores.[1] For genes that were significantly associated with serum urate in the primary meta-analysis, we conducted a burden test for gout using uniform weight to evaluate the association per copy of variant.

Follow-up analysis of urate-associated rare variants and blood pressure

Given the long standing controversy around the potential influence of serum urate on blood pressure,[2-4] the association between the rare variants with large effect on serum urate and systolic blood pressure (SBP) was evaluated in the ARIC study, overall and stratified by sex. Any significant variants ($p<0.05$) were evaluated for replication in an exome-wide association study of blood pressure,[5] and the CoLaus and SHIP-Trend studies (**Supplementary Data 19**).[5-7] The methods of this SBP association analysis were the same as in Yu et al.[5] Briefly, if a participant was treated for hypertension, 15 mm Hg was added to SBP levels. Participants with SBP <60 mm Hg without hypertension treatment were excluded. SBP levels were further winsorized at the 99.9th percentile. We performed inverse variance weighted fixed effect meta-analysis to combine the results from all replication studies as well as from ARIC and the replication studies. The replication significance was set at p-value <0.05.

Interrogation of primary meta-analysis results for urate-associated coding variants in *SLC2A9* and *SLC22A12*. We conducted a search for reported coding variants associated with serum urate in *SLC2A9* and *SLC22A12* using the keywords: “human”, “mutation”, and “urate”, combined with “*SLC2A9*“ or “*SLC22A12*“. Then we excluded publications that were non-English or reviews, reported only known variants, functional or lab method studies, or AKI only studies. Altogether 21 publications were identified.[8-28] We then interrogated the primary single variant meta-analysis results of serum urate for the coding variants found in the 21 publications.

Interrogation of primary meta-analysis results for candidate variants and genes from GWAS, Mendelian diseases, and mouse models. We investigated whether genome-wide significant loci identified from GWAS might harbor low frequency or rare variants associated with our outcomes. Index variants of known loci for serum urate, eGFR and UACR were identified using the NHGRI-EBI Catalog of Published Genome-wide Association Studies[29] based on the following criteria: i) disease traits that matched the selected kidney function or serum urate related traits in **Supplementary Data 20**; ii) index variants with p-value $<5x10^{-8}$ with replication; iii) in studies of adult populations. The list of GWAS index SNPs identified are reported in **Supplementary Data 21** for eGFR, UACR and serum urate. In addition, genes that have been linked to Mendelian forms of kidney dysfunction or serum urate dysregulation were evaluated for associated low frequency or rare variants in our population-based studies. These genes were identified based on a comprehensive gene panel for diagnosis of genetic nephropathies (**Supplementary Data 22**).[30] Genes linked to Mendelian forms of serum urate dysregulation were identified using the Online Mendelian Inheritance in Man (OMIM)[31] database by searching for phenotypes with known molecular basis linked to hyperuricemia or hypouricemia (**Supplementary Data 22)**. Finally, genes linked to kidney function or serum urate dysregulation in

genetically manipulated mice were retrieved from the Mouse Genome Informatics (MGI) database using previously described methods.[32; 33] Briefly, phenotype ontology terms related to abnormal kidney function or serum urate in genetically manipulated mice were selected (**Supplementary Data 20)**, followed by linking to the human ortholog through the Human-Mouse:Disease Connection (HMDC) database.[34] The resulting genes for abnormal kidney morphology, for abnormal kidney physiology, and for abnormal urate levels are also listed in **Supplementary Data 22**. The significance thresholds for the interrogation of the primary meta-analysis results for variants mapping to these genes were determined by the Bonferroni method as reported in **Supplementary Data 23**.

Selection of variants for experimental studies. To prioritize variants detected through WES in *SLC22A12* for experimental studies of the encoded urate transporter URAT1, we established a protocol to identify the variants that were most likely to have functional or regulatory effects based on the long isoform and the corresponding mRNA amino acid sequence (NP_653186; **Supplementary Figure 2)**. Using an in-depth literature search, we created maps of the predicted transmembrane domains and other domains or elements that may have functional or regulatory consequences. The amino acid sequence was subjected to a bioinformatic analysis of phosphorylation sites using GPS 3.0,[35] a program that evaluates the probability that any given S, T, or Y is phosphorylated based on known kinase binding motifs. Finally, we produced two protein sequence alignments: one with other human protein sequences of transporters of the same family; and a second with the same protein in a representative sampling of other vertebrates. We compared sequence conservation and looked for discordant conservation in the two alignments as a potential indicator of urate specific function or regulation. After our literature based "maps" were constructed, we then located the newly identified variants and looked for

those in areas of predicted functional or regulatory significance. **(Supplementary Data 16).**

Molecular Biology. Primers used for SLC22A12 mutagenesis: K536T FW 5' GAA CCA GGC AGT AAC GAA GGC AAC ACA TG 3', RV 5' CAT GTG TTG CCT TCG TTA CTG CCT GGT TC 3'; R405C FW 5' GAG CCA CCT GGG CTG CCG CCC CAC GCT G 3', RV 5' CAG CGT GGG GCG GCA GCC CAG GTG GCT C 3'; T467M FW 5' GTG CTC AGG ATG ATG GCA GTG GGC TTG 3', RV 5' CAA GCC CAC TGC CAT CAT CCT GAG CAC 3'; R325W FW 5' CTT TCA GCC ATG TGG GAG GAG CTG AG 3', RV 5' CTC AGC TCC TCC CAC ATG GCT GAA AG 3'.

Supplementary Note 1

Variants in known kidney-function associated genes are associated with eGFR and UACR

Single variant analysis of eGFR confirmed known associations of common variants in *GATM,* its neighboring gene *SPATA5L1* as well as in *CPS1* as exome-wide significant (**Supplementary Data 3**).[36] These eGFR-associated variants were not associated with UACR ($p>0.05$). The SKAT-O meta-analysis of eGFR with MAF <5% confirmed the known association of low frequency and rare variants in *SLC47A1* ($p=1.1 \times 10^{-6}$, **Supplementary Data 4**).[37]

Single variant meta-analysis of UACR confirmed the known association of rs334, the sickle cell trait variant at *HBB* among populations of African ancestry, as exome-wide significant (**Supplementary Data 5 and 6**).[38] The A allele, which was associated with higher UACR, was also nominally associated with lower eGFR ($p=6.1 \times 10^{-4}$).

Overall, WES association analyses in 18,800 population-based individuals did not identify any low frequency or rare variants associated with eGFR or UACR that mapped outside of known GWAS loci.

Interrogation of eGFR and UACR primary meta-analysis results for candidate variants or genes implicated in GWAS of eGFR, Mendelian form of kidney disease, and abnormal kidney morphology and physiology based on genetically manipulated mouse models.

We interrogated the primary meta-analysis results of eGFR and UACR for variants in known GWAS loci, genes linked to Mendelian form of kidney disease, and genes linked to abnormal kidney function from genetically manipulated mouse models listed in **Supplementary Data 20** based on significance thresholds reported in **Supplementary Data 23**. Interrogation of the eGFR primary single variant meta-analysis results identified significant eGFR-associated common variants in the following genes: *CPS1*, *GATM*, and *SLC28A2* among the 56 eGFR GWAS loci ($p < 6.4 \times 10^{-6}$**)**; the neighboring *ALMS1* and *NAT8* at $p < 1.2 \times 10^{-5}$ among the 301 genes linked to Mendelian form of kidney disease; and *ALSM1* among the 366 genes linked to abnormal murine kidney physiology (**Supplementary Data 7**). Interrogation of the UACR primary single variant meta-analysis results confirmed the association of *APOL1* risk variant rs73885319 (G1) with increased UACR (**Supplementary Data 8**). Interrogation of primary SKAT-O results of eGFR and UACR did not identify any significant associations.

Follow-up analysis on the association between urate-associated rare variants at *SLC22A12* and blood pressure

Given the large effect of rs150255373 and rs147647315 on serum urate levels and the long-standing debate around the relationship of serum urate levels and blood pressure,

we conducted association analyses of these variants and SBP in the ARIC study. Consistent with the reported relationship of hyperuricemia and hypertension, the urate-lowering T allele of rs150255373, found exclusively in EA, was significantly associated with lower SBP in ARIC EA overall and in males (overall: n=7,391, beta=-8.2 mm Hg, p=0.03; males: n=3,421, beta=-18.1 mm Hg, p=0.004). The A allele of rs147647315, found largely in AA, was not significantly associated with SBP in ARIC AA participants (p>0.7 in AA overall and sex-stratified analysis, **Supplementary Data 11**). In replication studies, the association between rs150255373 and SBP was in the same direction but not significant (overall: n=16,533, beta=-5.0 mm Hg, p=0.16; males: n=6,520, beta=-2.2 mm Hg, p=0.69, **Supplementary Data 11**).

Interrogation of serum urate primary meta-analysis results for candidate variants or genes implicated in GWAS of serum urate, abnormal serum urate levels based on Mendelian inheritance or genetically manipulated mouse models.

In the interrogation of the single variant primary meta-analysis results of serum urate for 31 urate-associated GWAS loci, 34 genes linked to Mendelian forms of abnormal urate levels, and 36 genes linked to abnormal urate levels from genetically manipulated mouse models, we confirmed the association of common variants in 8 genes and no additional low frequency or rare variants were identified (**Supplementary Data 15**). In the interrogation of the SKAT-O primary meta-analysis results of serum urate in the above 3 sets of genes or regions, in addition to confirming the association of *SLC22A12* and *SLC2A9*, *HPRT1* on chromosome X was identified as associated with serum urate levels among 34 genes linked to Mendelian form of abnormal urate levels (SKAT-O $p=1.4x10^{-3}$, **Supplementary Data 14**). This is the first instance that a variant in a gonosomal gene was identified as associated with serum urate in population-based association studies.

Supplementary Note 2 Analysis Plans

Exome Sequencing Analysis Plan for Serum Urate and Gout
February 2015

Please contact Adrienne Tin (atin@jhsph.edu) or Anna Kottgen (anna.koettgen@uniklinik-freiburg.de) if you have any questions regarding this analysis plan.

Purpose of this analysis plan:

- This analysis plan is for generating score statistics into an R object using data from one cohort. The meta-analysis team will use the score statistics in the R object to perform meta-analyses for single SNP test, gene-based burden and SKAT tests. The functional and allele frequency criteria for variants in the gene-based tests will be decided at the meta-analysis stage.

General guideline for all analyses:

- Perform all analyses stratified by self-reported race, European American (EA) vs. African American (AA).
- Restrict analyses to individuals with available information on urate, sex, age, and BMI for the urate analyses, and gout, age, sex for the gout analyses (include study center or cohort status if applicable)
- Please account for individual relatedness in the appropriate way if necessary.
- Individual studies should adjust for measures of population stratification through the use of principal components if available and necessary.
- Please do not exclude individuals with gout (treated or not) from the analyses of serum urate.
- Serum urate measures should be in mg/dL. To convert umol/L to mg/dL, please divide by 59.48. Your mean serum urate should be approximately 5-6.5 mg/dl. Please do not impute missing values of urate.
- Include all variants in your data, even if they are monomorphic.
- Assume additive genetic model.

Phenotype definitions:

Please use the following definitions to define gout in cohorts that have this information available:

a. Self-report of gout at a study exam or a questionnaire. If not available, please use:
b. Gout defined based on ICD-codings (ICD-9 code 274.0, 274.1, 274.8, or 274.9; ICD-10 M10.0, M.10.3, M.10.4, M10.9) from hospital discharge records or death certificates. If not available, please use:
c. Intake of previous or current gout-specific medication: probenecid, benzbromarone, sulfinpyrazone, pegloticase, rasburicase, febuxostat or allopurinol
d. If you are using another definition, please let us know what this definition is in the accompanying excel spreadsheet.

Please provide information on proportion with gout and gout case counts in the excel spreadsheet even if the number of gout cases is too small for analysis (<50 cases).

- Use the following definitions for diabetes and hypertension for **Table 1** information:

- **Diabetes**:
  - **Preferred definition:** fasting plasma glucose ≥ 126 mg/dl (7.0 mmol/L) OR treatment for diabetes
  - **If fasting glucose is not available:** casual glucose (non-fasting) ≥ 200 mg/dl (11.0 mmol/L) OR treatment for diabetes
  - **If glucose is not available:** self-reported diabetes status
  - **If an alternative definition was used:** please specify this in your methods write-up
- **Hypertension**:
  - **Preferred definition:** systolic blood pressure ≥ 140 mm Hg OR diastolic blood pressure ≥ 90 mm Hg OR treatment for hypertension
  - **If measured blood pressure is not available:** self-reported hypertension.
  - **If an alternative definition was used:** please specify in your methods write-up

Analysis steps

Step 1: Download seqMeta package and snp information file.

A) The **seqMeta** package (version 1.6.5) can be downloaded from CRAN.
B) Version 13 of the SNP information file can be downloaded from the 'Annotation' folder in Google Drive: (https://drive.google.com/drive/folders/0BzYDtCo_doHJT0JVeXYyRXh2NmM)

Step 2: Perform inverse normal transformation of the adjusted residuals of serum urate as outcome. The covariates should include age, sex, BMI, appropriate PCs, and study centers if applicable.

```
     # Example R code for inverse normal transformation of the
adjusted residuals of serum urate
        library("GenABEL")
        urate_resid = resid(lm(urate~age+sex+BMI+pc1+pc2))
        urate_resid_invnorm = rntransform(urate_resid)
```

Step 3: Generate score statistics using the prepScores function (for chromosome X, use prepScoresX and code the genotypes in male as 0/2) in the seqMeta R Package with the inverse normal transformed residuals of serum urate as a continuous outcome. Some example R code are provided at the end of the analysis plan.

a. Please conduct all analyses by chromosome because different chromosomes may have genes with the same name that should be aggregate separately.

b. **prepScores** will generate the score statistics for all SNPs. Please upload the results as Rdata file.
   I. The outcome cannot contain missing value.
   II. Individual data for genotype and outcome need to be in the same order.
   III. Use the SNP information file downloaded in **Step 1** to define the aggregation of the SNPs.
   IV. The coding allele of the genotype needs to be the same as the alternate (ALT) allele in the snpinfo file.
   V. In the regression model, include adjustment for relatedness if necessary. An example of the regression model using the inverse normal transformed

residuals of urate as outcome in R syntax is provided in the example code below (see command 2).

c. Save the Rdata file using the following naming convention (one output for each chromosome in each ethnic group):

[YOURSTUDYNAME]_ES_URATE_INVNORM_[RACE]_chr[N]_[MMDDYYYY].Rdata where

RACE is either EA or AA;
N=1 to 22 and X for the chromosomes.
MMDDYYYY is the time stamp.

For example, the file for chromosome 1 in European Americans in the ARIC study is:
ARIC_ES_URATE_INVNORM_EA_chr1_02132015.Rdata

Step 4: Generate score statistics using the prepScores function (for chromosome X, use prepScoresX and code the genotypes in male as 0/2) in the seqMeta R Package with gout as a binary outcome. Some R code examples are provided at the end of the analysis plan.

a. **prepScores** will generate the score statistics for all SNPs. Please upload the results as Rdata file.
   I. The outcome cannot contain missing value.
   II. Individual data for genotype and outcome need to be in the same order.
   III. Use the SNP information file downloaded in **Step 1** to define the aggregation of the SNPs.
   IV. In the regression model, include adjustment for relatedness if necessary. An example of the regression model in R syntax is provided in the example code below (see command 2).

b. Save the Rdata file using the following naming convention (one output for each chromosome in each ethnic group):

[YOURSTUDYNAME]_ES_GOUT_[RACE]_chr[N]_[MMDDYYYY].Rdata where

RACE is either EA or AA;
N=1 to 22 and X for the chromosomes.
MMDDYYYY is the time stamp.

For example, the file for chromosome 1 in European Americans in the ARIC study is:
ARIC_ES_GOUT_EA_chr1_02132015.Rdata

Step 5. Upload cohort results. Each outcome, e.g. residuals of serum urate, will have 23 files for each ethnic group (one for each chromosome). For file upload, please zip up the output for one outcome into 1 or 2 files.

Example R code for the cohort level analysis:

Command 1: Load CHARGE SNP information file

```
snpinfo = get(load("snpinfo_WES_v13_Analytic_ChrN_10202015.Rdata"))
```

Command 2A: Create a seqMeta object containing one cohort's contributions for unrelated individuals

```
# for residuals of serum urate
cohort_score_urate = prepScores(Z = genos_chrN, formula =
"urate_resid_invnorm ~ 1", data = pheno, SNPInfo = snpinfo,
snpNames="SNP", aggregateBy = "SKATgene")

# for gout
cohort_score_gout = prepScores(Z = genos_chrN, formula = "gout ~ age +
sex + pc + center", family=binomial(), data = pheno, SNPInfo = snpinfo,
snpNames="SNP", aggregateBy = "SKATgene")
```

Command 2B: Create a seqMeta object containing one cohort's contributions for related individuals

```
# for residuals of serum urate
cohort_score_urate = prepScores(Z = genos_chrN, formula =
"urate_resid_invnorm  ~ 1", fullkins = makekinship(fullped$fam,
fullped$id, fullped$fa, fullped$mo), data = pheno, SNPInfo = snpinfo,
snpNames="SNP", aggregateBy = "SKATgene")

# for gout
cohort_score_gout = prepScores(Z = genos_chrN, formula = "gout ~ age +
sex + pc + center", family=binomial(),fullkins =
makekinship(fullped$fam, fullped$id, fullped$fa, fullped$mo), data =
pheno, SNPInfo = snpinfo, snpNames="SNP", aggregateBy = "SKATgene")
```

Command 3: Save the result as Rdata file

```
save(cohort_score_urate,file =
"COHORTA_ES_URATE_INVNORM_EA_chrN_02132015.Rdata")

save(cohort_score_gout,file = "COHORTA_ES_GOUT_EA_chrN_02132015.Rdata")
```

After you have performed these analyses. Please fill in the attached tables and upload your results files within 4 weeks to the following Google Drive:

https://drive.google.com/drive/folders/0BzYDtCo_doHJM0dwZ3dqSEZxZkU
CHARGE – Urate exome sequencing

To access this Google Drive:

1) Sign up for a Google Account ID (username@gmail.com) and password at: http://accounts.google.com/SignUp

2) E-mailed your Google Account ID, your study name and the requested Google Drive (CHARGE – Urate exome sequencing) to chargeco@u.washington.edu and you will be added to the access list (please cc Anna Kottgen (anna.koettgen@uniklinik-freiburg.de). Once your ID has been added, you will receive an email from chargeco.

Renal Trait Analysis Plan using Genotypes from Exome Sequencing
February 2016

This analysis plan for exome sequence genotype is based on our prior trait creation so that we can remain consistent with our prior work. The traits of interest are eGFRcrea, eGFRcys, UACR, and CKD. (Details for CKD will be specified in a later time because the methods for controlling type 1 errors for gene-based test of rare variants for binary traits are still in development.)

Please contact Anna Kottgen (anna.koettgen@uniklinik-freiburg.de) and Adrienne Tin (atin1@jhu.edu) if you have any questions regarding trait creation or variable definitions.

General guideline for all analyses:

- Perform all analyses stratified by self-reported race (European American [EA] vs. African American [AA]).
- If different phenotypes were collected at different times in your study, please use the **covariates** from the respective time point of the main phenotype collection in the analyses. For example, if eGFRcrea was collected at visit 4 of the study, please use age at visit 4 for that analysis.
- If you have more than one visit with measurement of serum creatinine, use the visit with the largest sample size. This applies to all other traits where you may have more than one measure; we would like to maximize sample size.
- All genotypes are called from the + strand using hg19 as the reference and the reference and alternate alleles need to match the alleles in the CHARGE SNP information file
- Please include information on all SNPs in your sample, even if they are monomorphic
- The numbers in Table 1 should refer to the time point of serum creatinine measurement used to calculate eGFRcrea overall.
- Fill in Table 1 as you go along; please use the following definitions to define diabetes and hypertension for Table 1 information:
    - **Diabetes** should be defined as:
        - **Preferred definition:** fasting plasma glucose ≥ 126 mg/dl (7.0 mmol/L) OR treatment for diabetes
        - **If fasting glucose is not available:** casual glucose (non-fasting) ≥ 200 mg/dl (11.0 mmol/L) OR treatment for diabetes
        - **If glucose is not available:** self-reported diabetes status
        - **If an alternative definition was used:** please specify this in your methods write-up
    - **Hypertension** should be defined as:
        - **Preferred definition:** systolic blood pressure ≥ 140 mm Hg OR diastolic blood pressure ≥ 90 mm Hg OR treatment for hypertension
        - **If measured blood pressure is not available:** self-reported hypertension.
        - **If an alternative definition was used:** please specify in your methods write-up
    - **Define CKD as eGFRcrea<60**

Step by Step Analysis Plan:

TRAIT CREATION (STEPS 1 TO 5). These trait definitions are the same as those in the exome chip analysis plan.

Step 1: Calibrate the serum creatinine. The goal is to have a similar mean serum creatinine for each age group to the one in NHANES, a nationally representative US survey. Here are the SAS commands for the calibration. **START BY MAKING SURE YOUR CREATININE IS IN MG/DL units.** If your creatinine is in μmol/l, please divide by 88.4 to obtain mg/dl. Your mean creatinine should be approximately 0.7-1.1 mg/dl.

For European ancestry participants, use Step 1a.
For African ancestry participants, use Step 1b

Step 1a: SAS code for calibrating the serum creatinine in European ancestry participants

```
/* Notation:
age1= age, creatin1=creatinine,
sex = 1 for men and sex = 2 for women */

data analyze;
  set YOURDATA;
  if sex ne . and age1 >=20;  /* ne is SAS code for: not equal */
  if creatin1 >=0;
run;

data analyze;
  set analyze;
  if  20 <= age1 < 40  then agegrp=1;
  else if  40 <= age1 < 60  then agegrp=2;
  else if  60 <= age1 < 70  then agegrp=3;
  else if age1 >=70  then agegrp=4;
run;

proc sort; by sex agegrp; run;

proc means noprint;
  var creatin1; by sex agegrp;
  output out=meanc mean=meancrt;
run;

data analyze;
  set analyze;
  merge analyze meanc;
  by sex agegrp;
  /* generate calibrated means based on the NHANES agegroup-and sex-
specific means */
  if sex=1 and agegrp=1 then nhcreat1= creatin1 - meancrt + 0.9101763;
  else if sex=1 and agegrp=2 then nhcreat1= creatin1 - meancrt + 0.9347561;
  else if sex=1 and agegrp=3 then nhcreat1= creatin1 - meancrt + 0.9957202;
  else if sex=1 and agegrp=4 then nhcreat1= creatin1 - meancrt + 1.127975;
  else if sex=2 and agegrp=1 then nhcreat1= creatin1 - meancrt + 0.7016764;
  else if sex=2 and agegrp=2 then nhcreat1= creatin1 - meancrt + 0.7304324;
  else if sex=2 and agegrp=3 then nhcreat1= creatin1 - meancrt + 0.8134599;
  else if sex=2 and agegrp=4 then nhcreat1= creatin1 - meancrt + 0.8771303;
run;
```

```
data analyze;   /* Remove variables meancrt and agegrp, because they
are not needed in the following steps */
  set analyze;
  drop meancrt agegrp;
run;
```

Step 1b: SAS code for calibrating the serum creatinine in African ancestry participants

```
/* Notation:
age1= age, creatin1=creatinine,
sex = 1 for men and sex = 2 for women */

data outdat;
        set indat;
if age1 < 20  then  delete;
if creatin1 <0   then delete;
/* calculate gfr */;

if      20 <=  age1 < 40   then  agegrp=1;
else if   40 <=  age1 < 60   then  agegrp=2;
else if   60 <=  age1 < 70   then  agegrp=3;
else if        age1>= 70  then  agegrp=4;
run;

proc  sort; by sex agegrp;
proc  means noprint;
var  CREATIN1 ; by sex  agegrp;
output  out=meanc  mean=meancrt;
run;
proc print  data=meanc;

data  all; merge outdat meanc; by sex agegrp;
run;
data all;
        set all;

    if  sex=1  and agegrp=1  then  nhcreat1= CREATIN1  -meancrt + 1.00501;
else if  sex=1  and agegrp=2  then  nhcreat1= CREATIN1  -meancrt + 1.026986;
else if  sex=1  and agegrp=3  then  nhcreat1= CREATIN1  -meancrt + 1.132971;
else if  sex=1  and agegrp=4  then  nhcreat1= CREATIN1  -meancrt + 1.200909;
else if  sex=2  and agegrp=1  then  nhcreat1= CREATIN1  -meancrt + 0.7514;
else if  sex=2  and agegrp=2  then  nhcreat1= CREATIN1  -meancrt + 0.774523;
else if  sex=2  and agegrp=3  then  nhcreat1= CREATIN1  -meancrt + 0.905134;
else if  sex=2  and agegrp=4  then  nhcreat1= CREATIN1  -meancrt + 0.968041;
run;
```

Step 2a: Calculating eGFRcrea from the MDRD equation in European Ancestry participants. Be sure to use the calibrated serum creatinine measurements from Step **1a**.

eGFRcrea = $186.3*(\text{nhcreat1})^{-1.154} * \text{age}^{-0.203}$ * (0.742 if female).

Next, set all eGFRcrea values>200 to 200 and those <15 to 15.

```
/*SAS-Code:*/
data analyze;
  set analyze;
  if sex=1 then eGFRcrea1=186.3*(nhcreat1** (-1.154))*(age1** (-
0.203));
  else if sex=2 then eGFRcrea1=186.3*(nhcreat1**(-1.154))*(age1**(-
0.203))*0.742;
  if eGFRcrea1>200 then eGFRcrea1=200;
  if eGFRcrea1<15 then eGFRcrea1=15;
run;
```

Step 2b: Calculating eGFRcrea from the MDRD equation in African ancestry participants. Be sure to use the calibrated serum creatinine measurements from Step **1b**.

eGFRcrea = $186.3*(\text{nhcreat1})^{-1.154} * \text{age}^{-0.203}$ * (0.742 if female)*(1.21 if African ancestry).

Next, set all eGFRcrea values>200 to 200 and those <15 to 15.

```
/*SAS-Code:*/
data analyze;
  set analyze;
  if sex=1 then eGFRcrea1=186.3*(nhcreat1** (-1.154))*(age1** (-
0.203))*1.21;
  else if sex=2 then eGFRcrea1=186.3*(nhcreat1**(-1.154))*(age1**(-
0.203))*0.742*1.21;
  if eGFRcrea1>200 then eGFRcrea1=200;
  if eGFRcrea1<15 then eGFRcrea1=15;
run;
```

Step 3: define CKD as eGFRcrea<60. Please fill in eGFRcrea and CKD information into **Table 1**, along with information about age and sex.

Step 4: Calculate eGFRcys using cystatin C

a. Make sure your cystatin C is in mg/L
b. Create eGFRcys using the following equation

egfrcys = $76.7*(\text{serum cystatin C})^{-1.19}$
if egfrcys >200 then egfrcys=200;
if egfrcys <15 then egfrcys=15;

```
/**SAS code**/
egfrcys = 76.7 * serumcys**(-1.19);
if egfrcys > 200 then egfrcys = 200;
else if egfrcys < 15 then egfrcys =15;
```

General guideline for analyses of urinary albumin and urinary creatinine

- Dealing with urine albumin levels below the assay's detection threshold: values that are below the detection limit of your particular urinary albumin assay should not be missing or zero.

- If you have urine albumin levels below the detection threshold, please note how often you observe this in your overall sample (proportion of total population with the lowest detection limit value) and the detection threshold value of the assay in Table 1.
- Please set the values below the detection limit to the lower limit of your urinary albumin assay; this value should not be ZERO. In our experience, the majority of studies use a urinary albumin assay with a lower limit of detection of 3 mg/L. If this applies to your study, then please set all values below this value of 3 mg/L to 3 mg/L. Your study may have used an assay with a different level of detection. If this is the case, then assign those with urinary albumin below the assay's detection threshold [<assay] to this level. Divide this new value by the urinary creatinine value to obtain an ACR value for each of the participants in your dataset.

Step 5: Create UACR

a. Define UACR as [urinary albumin(mg/L) / urinary creatinine(mg/dl)]*100; Important: your urinary albumin and urinary creatinine values may have different units, so adapt the formula as needed to obtain UACR measures in mg/g.
b. If your ACR is in the unit "mg/mmol", convert to mg/g by using the following information: 1g creatinine = 8.84mmol creatinine (or 1mg/dL creatinine = 88.4 µmol/L creatinine).

   For example, ACR of 3.4mg albumin / mmol creatinine is equivalent to 8.84x3.4mg = 30.056 mg albumin / g creatinine.

c. In general population studies, the median of ACR is usually around 1.5 mg/g.
d. Enter the median and 25% and 75% percentile values of your distribution into Table 1.

COHORT ANALYSES (STEPS 6 to 10)

Step 6: Perform two transformations of the traits: log transformation (Step 6a) and inverse normal transformation of the residuals from the log transformation (Step 6b).

Step 6a: Perform log transformation of eGFRcrea, eGFRcys, and UACR that you created in steps 1, 2, 4, and 5.

```
# Example R code for log transformation of eGFRcrea
eGFRcrea_log = log(eGFRcrea)
```

Step 6b: Perform inverse normal transformation of the residuals from the log transformation of the trait after regressing it against the covariates

```
# Example R code for inverse normal transformation of
log(eGFRcrea) residuals
library("GenABEL")
eGFRcrea_log = log(eGFRcrea)
egfr_log_resid = resid(lm(eGFRcrea_log~age+sex+pc1+pc2))
egfr_log_resid_invnorm = rntransform(egfr_log_resid)
```

Step 7: Perform cohort analyses of the log transformation of the traits (eGFRcrea, eGFRcys, and UACR) and inverse normal transformation of the residuals from the log transformation of the traits.

Please use ALL SNPs, including monomorphic ones, in the analysis. The SNP selection criteria for burden test and SKAT test, including MAF threshold and function, will be decided at the meta-analysis stage.

a. Please conduct all analyses by chromosome because different chromosomes may have genes with the same name that should be aggregate separately.

b. For the log transformation of the trait from **step 6a**, please include covariates (age, sex, study center, and PCs).
c. For the inverse normal transformation of the residuals from **step 6b**, please do not include covariates.
d. Perform analyses using the prepScores() function for chromosomes 1 to 22 and the prepScoresX() function for chromosome X in the seqMeta package (version 1.6.5 , cran.r-project.org/web/packages/seqMeta/), aggregate by SKATgene column in the SNP annotation file
e. For either prepScores or prepScoresX, the order of the individuals in the genotype and the phenotype files need to be identical.
f. The coding allele of the genotype needs to be the same as the alternate (ALT) allele in the snpinfo file.
g. Be sure to use the snpinfo files that include all variants discovered in your cohort. Version 13 of the snpinfo files can be downloaded from the 'Annotation' Google Drive folder (https://drive.google.com/drive/folders/0BzYDtCo_doHJT0JVeXYyRXh2NmM)
h. Studies with family data should use the kins argument to provide the kinship matrix for prepScores or prepScoresX
i. prepScoreX requires an additional parameter "male" for calculating allele frequency. The genotype for male at chromosome X should be coded as 0 or 2 to account for X-inactivation.
j. Perform all analyses stratified by ethnicity/race
k. Phenotype data cannot contain null values
l. Phenotype and genotype data need to be in the same order
m. Column names in the genotype data need to be the same as the SNP names in the SNP annotation data
n. Use the alternate allele as the coding allele and assume an additive genetic model
o. Save the Rdata file using the following naming convention:

[YOURSTUDYNAME]_ES_[TRAIT]_[TRANSFORMATION]_[SAMPLE]_[RACE]_chr[N]_[MMDDYYYY].Rdata where

TRAIT is either eGFRcrea, eGFRcys, or UACR;
TRANSFORMATION is either log or invnorm;
SAMPLE is either overall, DM, or nonDM;
RACE is either EA or AA;
N=1 to 22 and X for the chromosomes.

Each outcome, e.g. log(eGFRcrea), will have 23 files for each ethnic group (one for each chromosome). For file upload, please zip up the output for one outcome into two or three files.

Examples of file name for one study are given below. :

I. **European Americans (or Europeans):**

ARIC_ES_eGFRcrea_log_overall_EA_chr1_01202014.Rdata
ARIC_ES_eGFRcrea_invnorm_overall_EA_chr1_01202014.Rdata
ARIC_ES_eGFRcys_log_overall_EA_chr1_01202014.Rdata
ARIC_ES_eGFRcys_invnorm_overall_EA_chr1_01202014.Rdata
ARIC_ES_UACR_log_overall_EA_chr1_01202014.Rdata
ARIC_ES_UACR_invnorm_overall_EA_chr1_01202014.Rdata

II. **African Americans:**

ARIC_ES_eGFRcrea_log_overall_AA_chr1_01202014.Rdata
ARIC_ES_eGFRcrea_invnorm_overall_AA_chr1_01202014.Rdata
ARIC_ES_eGFRcys_log_overall_AA_chr1_01202014.Rdata
ARIC_ES_eGFRcys_invnorm_overall_AA_chr1_01202014.Rdata
ARIC_ES_UACR_log_overall_AA_chr1_01202014.Rdata
ARIC_ES_UACR_invnorm_overall_AA_chr1_01202014.Rdata

<u>Step 8</u>: Repeat the analysis for log eGFRcrea and UACR and inverse normal transformation of their residuals among those with diabetes

Examples of file name for eGFRcrea and UACR with diabetes are given below. :

I. **European Americans (or Europeans):**

ARIC_ES_eGFRcrea_log_DM_EA_chr1_01202014.Rdata
ARIC_ES_eGFRcrea_invnorm_DM_EA_chr1_01202014.Rdata
ARIC_ES_UACR_log_DM_EA_chr1_01202014.Rdata
ARIC_ES_UACR_invnorm_DM_EA_chr1_01202014.Rdata

II. **African Americans:**

ARIC_ES_eGFRcrea_log_DM_AA_chr1_01202014.Rdata
ARIC_ES_eGFRcrea_invnorm_DM_AA_chr1_01202014.Rdata
ARIC_ES_UACR_log_DM_AA_chr1_01202014.Rdata
ARIC_ES_UACR_invnorm_DM_AA_chr1_01202014.Rdata

<u>Step 9</u>: Repeat the analysis for log eGFRcrea and UACR and inverse normal transformation of their residuals among those without diabetes

Examples of file name for eGFRcrea and UACR without diabetes are given below. :

I. **European Americans (or Europeans):**

ARIC_ES_eGFRcrea_log_nonDM_EA_chr1_01202014.Rdata
ARIC_ES_eGFRcrea_invnorm_nonDM_EA_chr1_01202014.Rdata
ARIC_ES_UACR_log_nonDM_EA_chr1_01202014.Rdata
ARIC_ES_UACR_invnorm_nonDM_EA_chr1_01202014.Rdata

II. **African Americans:**

ARIC_ES_eGFRcrea_log_nonDM_AA_chr1_01202014.Rdata
ARIC_ES_eGFRcrea_invnorm_nonDM_AA_chr1_01202014.Rdata
ARIC_ES_UACR_log_nonDM_AA_chr1_01202014.Rdata
ARIC_ES_UACR_invnorm_nonDM_AA_chr1_01202014.Rdata

Example R code for the cohort level analysis in unrelated individuals:

Command 1: Load CHARGE SNP information file

```
snpinfo = get(load("snpinfo_WES_v13_Analytic_ChrN_10202015.Rdata"))
```

Command 2: Create a seqMeta object containing one cohort's contributions

```
# for log(eGFRcrea)
cohort_score = prepScores(Z = genos_chrN, formula = "eGFRcrea_log ~ age
+ factor(gender) + factor(center) + PC1", data = pheno, SNPInfo =
snpinfo, snpNames="SNP", aggregateBy = "SKATgene")

# for inverse normal transformed residuals of log(eGFRcrea)
cohort_score_2 = prepScores(Z = genos_chrN, formula =
"egfr_log_resid_inv ~1", data = pheno, SNPInfo = snpinfo,
snpNames="SNP", aggregateBy = "SKATgene")
```

Command 3: Save the result as Rdata file

```
save(cohort_score,file =
"COHORTA_ES_eGFRcrea_log_overall_EA_chrN_04042014.Rdata")
```

Example R code for cohort level analysis in related individuals:

Command 1: Load in the central SNP information fil

```
snpinfo = get(load("snpinfo_WES_v13_Analytic_ChrN_10202015.Rdata"))
```

Command 2: Create a seqMeta object containing one cohort's contributions

```
# for log(eGFRcrea)
cohort_score = prepScores(Z = genos_chrN,formula = "eGFRcrea_log ~ age
+ factor(gender) + factor(center) + PC1", data = pheno, SNPInfo =
snpinfo, fullkins = makekinship(fullped$fam, fullped$id, fullped$fa,
fullped$mo), snpNames="SNP", aggregateBy = "SKATgene")

# for inverse normal transformed residuals of log(eGFRcrea)
cohort_score = prepScores(Z = genos_chrN,formula = "egfr_log_resid_inv
~1", data = pheno, SNPInfo = snpinfo, fullkins =
makekinship(fullped$fam, fullped$id, fullped$fa, fullped$mo),
snpNames="SNP", aggregateBy = "SKATgene")
```

Command 3: Save the result as Rdata file

```
save(cohort_score, file =
'COHORTB_ES_eGFRcrea_log_overall_EA_chrN_04042014.Rdata')
```

After you have performed these analyses. Please fill in the attached tables and upload your results files <u>within 4 weeks</u> to the following Google drive:

"CHARGE Renal exome sequencing upload site" at
https://drive.google.com/drive/folders/0BzYDtCo_doHJRjlwZTFLajl4YXM

To access this Google drive:

1) Sign up for a Google Account ID (username@gmail.com) and password at: http://accounts.google.com/SignUp

2) Once they receive their ID, the ID and your study name should be emailed to Mira (chargeco@uw.edu) and you will be added to the access list (please cc Anna Kottgen (anna.koettgen@uniklinik-freiburg.de) and Adrienne Tin (atin1@jhu.edu)
Please also include the name of the Google drive folder you are requesting access to (CHARGE - Renal exome sequencing upload site). Once your ID has been added, you will receive an email from chargeco@uw.edu.

3) Please remember to email us the excel worksheet with your study sample information.

Any questions? Please contact Anna Kottgen (anna.koettgen@uniklinik-freiburg.de) and Adrienne Tin (atin1@jhu.edu)

Renal and Urate Traits Meta-analysis Plan using Genotypes from Whole Exome Sequencing
June 2016

1) Traits covered in this plan: both renal and urate traits:

Trait	Stratum	Transformation	Ancestry	Participating studies	Approx. sample size
eGFRcrea	overall	inverse normal, log	EA	ARIC, CHS, FHS, RS, ESP, ERF, CoLaus, Cilento	14363
eGFRcrea	DM	inverse normal, log	EA	ARIC, CHS, FHS, RS, ESP, ERF, CoLaus, Cilento	1355
eGFRcrea	nonDM	inverse normal, log	EA	ARIC, CHS, FHS, RS, ESP, ERF, CoLaus, Cilento	11530
eGFRcys	overall	inverse normal, log	EA	ARIC, CHS, FHS, RS, ESP	10859
UACR	overall	inverse normal, log	EA	ARIC, CHS, FHS, RS, ESP, CoLaus	11004
UACR	DM	inverse normal, log	EA	ARIC, CHS, FHS, RS, ESP, CoLaus	1003
UACR	nonDM	inverse normal, log	EA	ARIC, CHS, FHS, RS, ESP, CoLaus	8856
Gout	overall	N/A	EA	ARIC, CHS, FHS, RS, ESP	7416
Urate	overall	inverse normal	EA	ARIC, CHS, FHS, RS, ESP, CoLaus	11896
eGFRcrea	overall	inverse normal, log	AA	ARIC, ESP	4080
eGFRcrea	DM	inverse normal, log	AA	ARIC	479
eGFRcrea	nonDM	inverse normal, log	AA	ARIC	2339
eGFRcys	overall	inverse normal, log	AA	ARIC	1872
UACR	overall	inverse normal, log	AA	ARIC, ESP	1901
UACR	DM	inverse normal, log	AA	ARIC	270
UACR	nonDM	inverse normal, log	AA	ARIC	1627
Gout	overall	N/A	AA	ARIC	1979
Urate	overall	inverse normal	AA	ARIC	3626

2) Primary analyses
   A) Regarding transformations, the analyses using inverse normal transformation are our primary analyses for determining statistical significance. The analyses using the log transformed outcomes are mainly for obtaining beta estimates for comparison with published results.
   B) Overall vs stratified analysis. We will use the overall analyses as our primary analyses. The number of variants and genes in the overall analysis will be used for setting the significance threshold.
   C) Gene-based tests. Section 4B lists 4 gene-based tests. The primary analyses are the test including MAF < 1% and MAF < 5%.

3) Filters that are applicable to all tests:
   A) Single variant test filters:

i) Variant annotation filter: none
ii) Minor allele count filter: 10 will likely yield a qq-plot without showing p-value inflation and a genomic control that is close to 1 using the inverse normal transformed traits. We may adapt this threshold based on the qq-plot of the meta-analysis.

B) Gene-based test filters:
i) Variant annotation filters
We will include the union of the following 3 sets of variants: a) nonsynonymous and splice sites as defined by sc_nonsynSplice; b) predicted splice sites defined by splicing_consensus_ada_score ≥ 0.6, splicing_consensus_rf_score ≥ 0.6, or absolute value of SPIDEX_dpsi_max_tissue > 5; c) coding indels defined by sc_indel_coding.
ii) Gene filter: contain 2 or more variants in meta-analysis
iii) Cumulative MAF filters: requiring at least 10 copies in a gene. We may adapt this threshold based on the GC parameter and qq-plot of the meta-analysis.

C) Gene-based test weight
i) beta distribution of MAF with parameters alpha=1, beta=25 unless specified otherwise below.

4) Meta-analysis within EA, AA, and combined EA and AA populations

A) Single variant test
B) Gene-based tests
i) Two primary SKAT-O tests: 1) MAF < 1%, 2) MAF < 5%
ii) Two secondary SKAT-O tests: 1) no limit on MAF weighted by beta distribution, 2) no limit on MAF weighted by CADD phred score (aka CADD scaled score). Variants without CADD phred scores will be assigned a score that is the median of variants in sc_nonsynSplice by MAF bin (<0.1%, ≥ 0.1% to <1%, ≥1% to <5%, and ≥5% based on MAF in CHARGE Freeze 5).

5) Statistical significance threshold

A) Single variant test:
i) Kidney function traits: 0.05/(number of variants after MAC filter in the EA and AA combined overall analysis of eGFRcrea)
ii) Serum urate: 0.05/(number of variants after MAC filter in the EA and AA combined overall analysis of serum urate)

B) Gene-based tests:
i) Kidney function traits: 0.05/(number of genes * number of primary tests in the EA and AA combined analysis of eGFRcrea)
ii) Serum urate: 0.05/(number of genes * number of primary tests in the EA and AA combined analysis of serum urate)

Renal and Urate Exome Sequencing Project
Interrogation of Susceptibility Loci Analysis Plan (October 2016)

Overview of Interrogation Analysis

The purpose of these analyses is to evaluate whether known kidney or uric acid susceptibility variants or genes are associated with the corresponding traits in the exome sequencing meta-analysis results.

Sources of known kidney or uric acid susceptibility variants or genes will be retrieved from the following data sources with details described in the sections below on the specific traits:

1) GWAS catalog
2) Mendelian genes: published lists of genes causing Mendelian forms of the disease in human
3) Mouse model genes: genes associated with corresponding traits in mouse models in the Mouse Genome Informatics (MGI) databases.

Targets of queries will be the results of the primary analyses in EA+AA of the corresponding traits, which will be used for determining statistical significance. The results from EA and AA will also be retrieved to inform heterogeneity across ethnic groups. The primary analyses in EA+AA are:

1) Single variant meta-analysis filtered by ≥ 10 copies of the minor allele
2) SKATO meta-analysis of putative functional variants with MAF < 1%
3) SKATO meta-analysis of putative functional variants with MAF < 5%

The tables generated from this analysis plan should contain all information necessary for publication plus other useful columns that may be trimmed later (see spreadsheet).

Definition of query regions and significance threshold

1) **GWAS catalog**. The query region will be defined as 500kb on both sides of the index SNP in the GWAS catalog. Recombination hot spots are not considered here because significant index SNPs in GWAS tend to lie in regulatory regions. The causal regulating SNP and the target gene may be separate by recombination hot spot. If two index SNPs are less than 500kb apart, then the query region will be defined as 500kb around the middle of the two index SNPs.
    A) **Single variant query significant threshold**. Since exonic variants tend to have low LD, the p-value significant threshold will be defined as 0.05 divided by the number of variants with ≥ 10 copies of the minor allele in the query region in the EA+AA meta-analysis.
    B) **Gene-based test query significant threshold**. 0.05 divided by the number of genes with ≥ 2 variants in the query region * 2 for two SKATO tests in EA+AA meta-analysis.

2) **Mendelian genes**. The query region and significant threshold are defined similar to the procedure in Jing et al. KI, 2016 with adaptation for exonic variants. The query region will be defined as 10kb upstream or downstream of the gene.
   A) **Single variant query significant threshold**: 0.05 divided by the **total** number of **index variants** across **all** genes in the corresponding trait. An **index variant** of a gene is defined as the variant with the lowest p-value in the gene region and a minor allele count ≥ 10 copies.
   B) **Gene-based test query significant threshold**: 0.05 divided by the total number of genes with ≥ 2 variants in our meta-analysis of the corresponding trait * 2 for two SKATO tests in EA+AA.
3) **Mouse model genes**. The query region and the significant threshold will be defined as the same as in the query using the Mendelian genes.

<u>Specifics for kidney traits (eGFR and UACR)</u>

Criteria for selecting susceptibility variants or genes for kidney traits (eGFR and UACR)

	Criteria
GWAS catalog	1) Index SNPs of the following DISEASE.TRAIT: • Chronic kidney disease • Chronic kidney disease and serum creatinine levels • Glomerular filtration rate • Glomerular filtration rate in chronic kidney disease • Glomerulosclerosis • IgA nephropathy • Kidney function decline traits • Nephrotic syndrome (acquired) • Proteinuria and chronic kidney disease • Proteinuria in chronic kidney disease • Renal function and chronic kidney disease • Renal function-related traits (BUN) • Renal function-related traits (eGRFcrea) • Renal function-related traits (sCR) • Renal function-related traits (urea) 2) GWAS catalog p-value < 5e-8 3) Loci discovered in adult populations
Mendelian genes	The panel in Larsen et al. AJKD, 2016 contains 301 genes. Our queries will include the 265 clinical genes that have known association with nephropathies, but not the 36 “experimental” genes (see spreadsheet)
Mouse model genes	The selection of renal susceptibility genes from the MGI database will follow the same procedure as described in Jing et al. KI, 2016. Briefly, the following

	phenotype from the **Mammalian Phenotype Ontology** will be used to identify susceptibility genes for renal traits in mouse: • MP: 0002847, abnormal GFR • MP: 0002136, abnormal kidney physiology • MP: 0002135, abnormal kidney morphology • MP: 0006315, abnormal urine protein level The corresponding gene in human will be identified using the **Human-Mouse:Disease Connection** database

Targets of queries

All queries for the renal traits will include eGFRcrea overall and UACR overall meta-analysis results in EA+AA, EA, and AA in one table because in the above data sources some phenotypes are associated with both GFR and proteinuria.
The **single variant query** will include the results from the eGFRcrea inverse normal overall meta-analysis in EA+AA, EA, and AA followed by the results from the UACR inverse normal overall meta-analysis in EA+AA, EA, and AA.
The **SKATO query** will include the results from the eGFRcrea inverse normal overall meta-analysis for MAF < 1% in EA+AA, EA, and AA followed by the results from MAF < 5%, then the results from the UACR inverse normal overall meta-analysis for MAF < 1% in EA+AA, EA, and AA followed by the results from MAF < 5%,

<u>Specifics for urate traits (serum urate and gout)</u>

Criteria for selecting susceptibility variants or genes for urate traits (urate and gout)

	Criteria
GWAS catalog	1) Index SNPs with the following values in the DISEASE.TRAIT variable: • Gout • Serum uric acid levels • Urate levels • Urate levels (BMI interaction) • Urate levels in lean individuals • Urate levels in obese individuals • Urate levels in overweight individuals • Uric acid levels 2) GWAS catalog p-value < 5e-8
Mendelian genes	OMIM Advanced Search: select **MIM number Prefix**: "**#** phenotype description, molecular basis known" with the keyword **hyperuricemia** or **hypouricemia**. After the list of phenotypes are displayed, retrieve corresponding **Gene Map**

Mouse model genes	The selection of urate susceptibility genes from the MGI database will follow the procedure as described in Jing et al. KI, 2016 and adapted for urate. Briefly, the following phenotype term in the **Mammalian Phenotype Ontology** will be used to identify susceptibility genes for urate traits in mouse: • MP: 0008820, abnormal blood uric acid level MP: 0008820 includes: • MP: 0008822, decreased blood uric acid level • MP: 0008821, increased blood uric acid level The corresponding genes in human will be identified using the **Human-Mouse:Disease Connection** database

Targets of queries

All queries for the urate trait will include serum urate and gout meta-analysis results in EA+AA, EA, and AA in one table (see table shell example)
The **single variant query** will include the results from serum urate inverse normal meta-analysis in EA+AA, EA, and AA followed by the results from gout meta-analysis in EA+AA, EA, and AA.
The **SKATO query** will include the results from serum urate inverse normal overall meta-analysis for MAF < 1% in EA+AA, EA, and AA followed by the results from MAF < 5%, then the results from gout meta-analysis for MAF < 1% in EA+AA, EA, and AA followed by the results from MAF < 5%.

References

A Custom Targeted Next-Generation Sequencing Gene Panel for the Diagnosis of Genetic Nephropathies. Larsen CP, Durfee T, Wilson JD, Beggs ML. Am J Kidney Dis. 2016 Jun;67(6):992-3.

Combination of mouse models and genomewide association studies highlights novel genes associated with human kidney function. Jing J, Pattero C, Hoppmann A, et al. Kidney International (2016) 90, 764–773;

Replication of Association between rs150255373 at SLC22A12 (Chr 11, 64366298 GRCh37 position, C ->T) and Systolic Blood Pressure (SBP): Analysis Plan

Background

This low frequency variant, T allele, was identified as associated with lower serum urate levels in a multi-cohort exome-wide association study. To follow-up on the long-standing controversy on the relation between serum urate and blood pressure, we found that this variant was also associated with lower blood pressure and wish to replicate this association in additional cohorts.

Outcome Definition and Covariates

The following definition and covariates are the same as in Yu et al. Circ Cardiovasc Genet, 2016

Systolic blood pressure (SBP) definition

1) For individuals under hypertension medication treatment, add 15 mmHg
2) Exclude individuals with untreated SBP < 60 mmHg
3) Winsorize the trait at 99.9th percentile

Covariates: age, age-squared, sex, BMI, genetic principal components, study site if applicable.

Analysis

Please use linear regression and the HRC imputed dosage as predictor of interest. Please carry out the analyses overall, as well as separately in men and women (not including sex as a covariate). If the number of carriers of the T allele is < 5, please provide the unadjusted SBP and hypertension medication status of each carrier of the T allele in the **Readme** spreadsheet.

For our internal information, additional information on associations with blood urate concentrations have been looked up from the urate summary statistics submitted to the CKDGen R4 analyses. The results of your study from this data submission have been prefilled in the **Reg_results** spreadsheet. To gain more insight into this variant, we appreciate if you could provide the additional information in columns M to U in the **Reg_results** sheet for the urate results. In case an interesting observation with SBP is observed, we may be in touch with an official lookup request for this variant on the urate association.

Results

Please provide the results in the attached spreadsheet.

The following table provides a brief description of the columns in the spreadsheet

Columns in the regression result spreadsheet (**Reg_results**):

Variable	Description
Beta	Beta coefficient for each copy of the T allele
SE	Standard error
p-value	p-value of rs150255373
MAF	Minor allele frequency
MAC	Minor allele count

ref	Non-coding allele
alt	Coding allele
N_total	Analyzed sample size
Imp_qual	Mean imputation quality
Num_CT	If the data is imputed, please use 0.5 to 1.5 to convert imputed dosage to imputed genotype. If your study already has a preferred method for conversion to most likely genotype, please use your study's method and note the method.
Num_TT	If the data is imputed and C is the reference, please use dosage > 1.5 to convert imputed dosage to imputed genotype. If your study already has a preferred method for conversion to most likely genotype, please use your study's method and note the method.
Num_urate_med	Number of participants on urate lowering medications if available
Urate_med	If urate lowering medication is available, please note the kind of urate lowering medication included, e.g. allopurino
SBP_mean	Mean SBP of the analyzed sample
SBP_SD	Standard deviation of the SBP in the analyzed sample
Male_pct	Percentage of male participants overall
HT_med_pct	Percentage of participants who were on hypertension medication overall
Carrier_male	Number of carriers of the T allele who are male based on imputed genotype
Carrier_HT_med	Number of carriers of the T allele who were on hypertensive medications based on imputed genotype

Please provide the following information in the **Readme** spreadsheet

Information	Description
Genotype_method	Method for converting imputed dosage to imputed genotype
Genetic_PC_criteria	Criteria for selecting genetic PCs, e.g. first 10 or those significantly associated with SBP among the first 10.

Please address inquiries on this analysis to Adrienne Tin (atin1@jhu.edu) and Anna Kottgen (anna.koettgen@uniklinik-freiburg.de)

Supplementary Note 3 Acknowledgements

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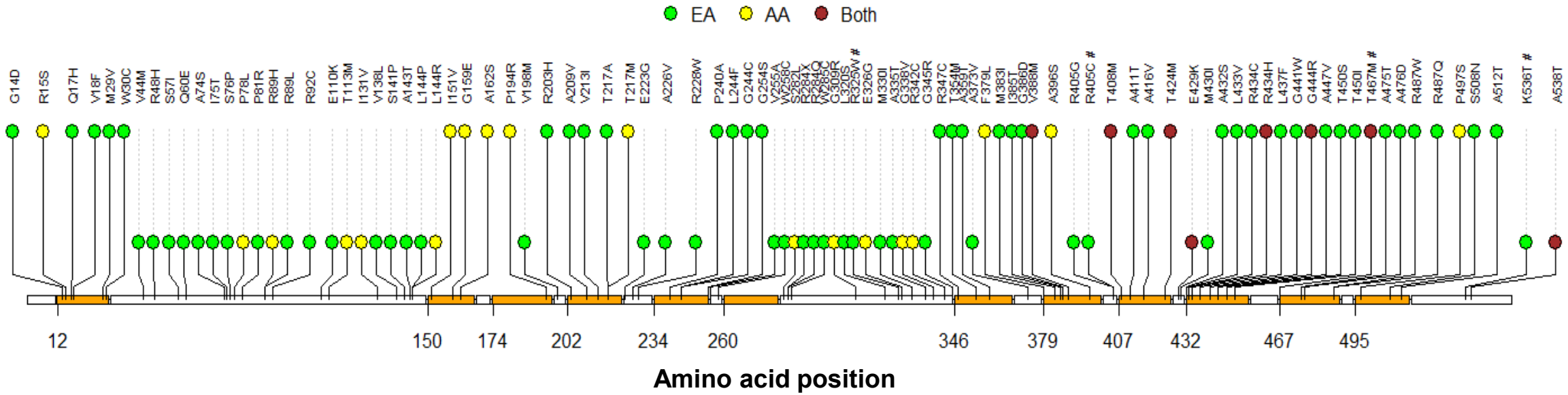
ESP Groups:
[1]Anthropometry Project Team, [2]Blood Count/Hematology Project Team, [3]Blood Pressure Project Team, [4]Data Flow Working Group, [5]Early MI Project Team, [6]ELSI Working Group, [7]Executive Committee, [8]Family Study Project Team, [9]Lipids Project Team, [10]Lung Project Team, [11]Personal Genomics Project Team, [12]Phenotype and Harmonization Working Group, [13]Population Genetics and Statistical Analysis Working Group, [14]Publications and

Presentations Working Group, [15]Quantitative Analysis Ad Hoc Task Group, [16]Sequencing and Genotyping Working Group, [17]Steering Committee, [18]Stroke Project Team, [19]Structural Variation Working Group, [20]Subclinical/Quantitative Project Team

ESP Cohorts:

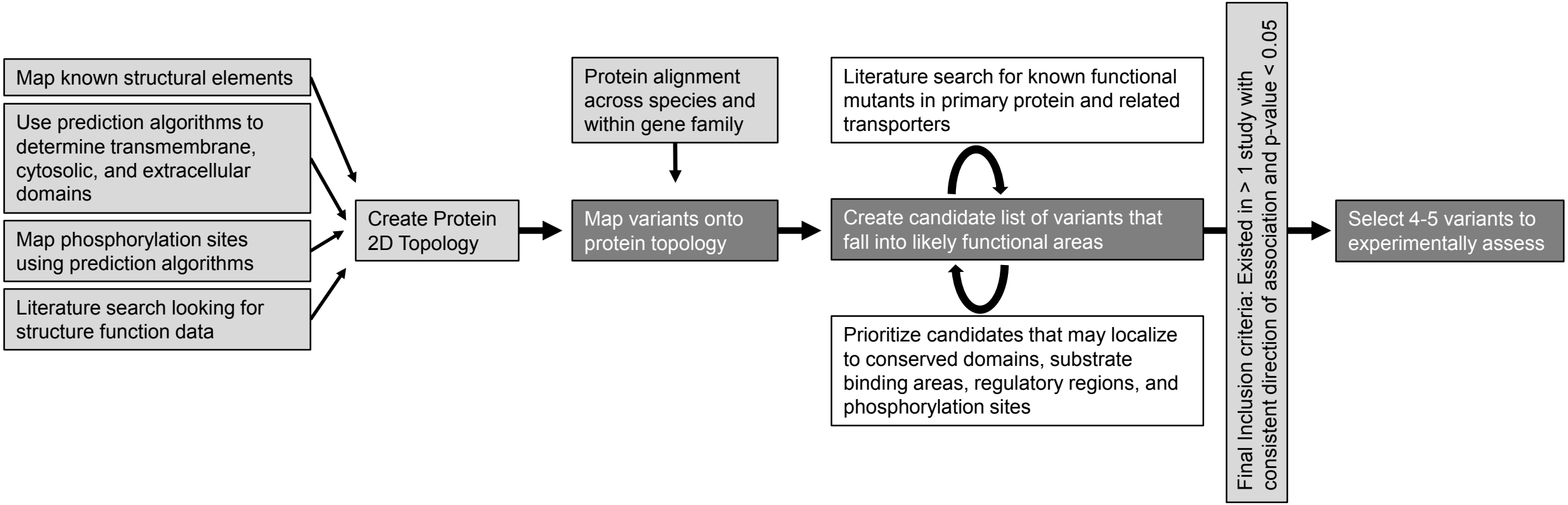
[21]Acute Lung Injury (ALI), [22]Atherosclerosis Risk in Communities (ARIC), [23]Cardiovascular Health Study (CHS), [24]Chronic Obstructive Pulmonary Disease (COPDGene), [25]Coronary Artery Risk Development in Young Adults (CARDIA), [26]Cystic Fibrosis (CF), [27]Early Pseudomonas Infection Control (EPIC), [28]Framingham Heart Study (FHS), [29]Jackson Heart Study (JHS), [30]Lung Health Study (LHS), [31]Multi-Ethnic Study of Atherosclerosis (MESA), [32]Pulmonary Arterial Hypertension (PAH), [33]Severe Asthma Research Program (SARP), [34]Women's Health Initiative (WHI).

Supplementary Figure 1. Putative damaging variants with MAF < 5% in *SLC22A12*

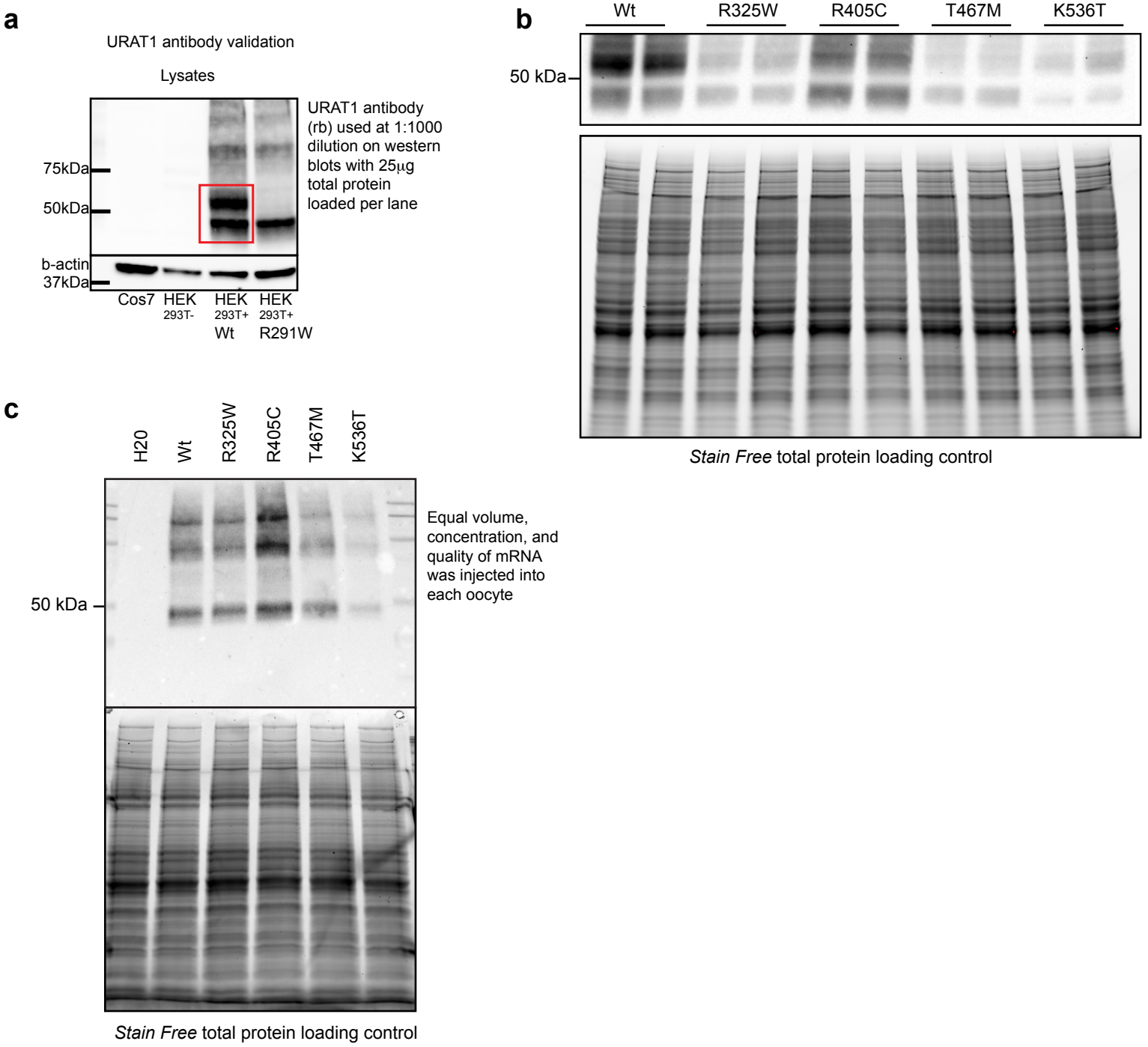


This plot includes 96 of the 97 variants in the primary gene-based test with MAF < 5%.
All 97 variants had MAF < 1%. In *SLC22A12*, no putative damaging variants had MAF between 1 to 5%.
The variant 11:64361276:G:A (MAC=1) is a splice variant and thus not included in this plot.
Orange: predicted transmembrane domains
# denotes the variants selected for functional experiments
Amino acid position based on NP_653186 (ENSP00000366797)

Supplementary Figure 2. Workflow for selecting rare variants in *SLC22A12* for functional study

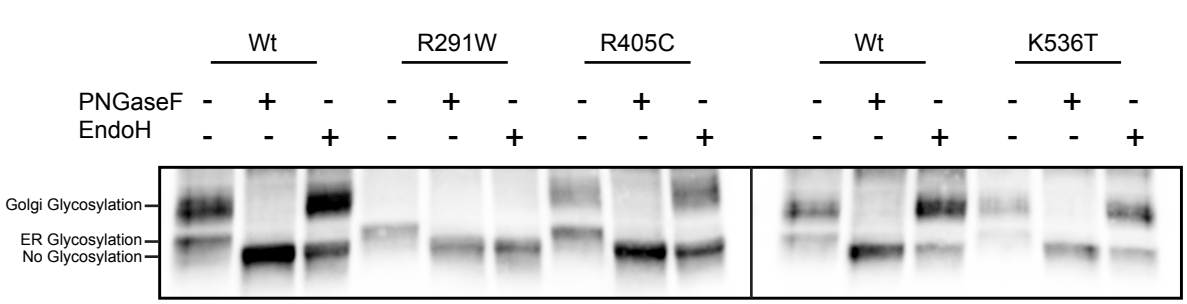


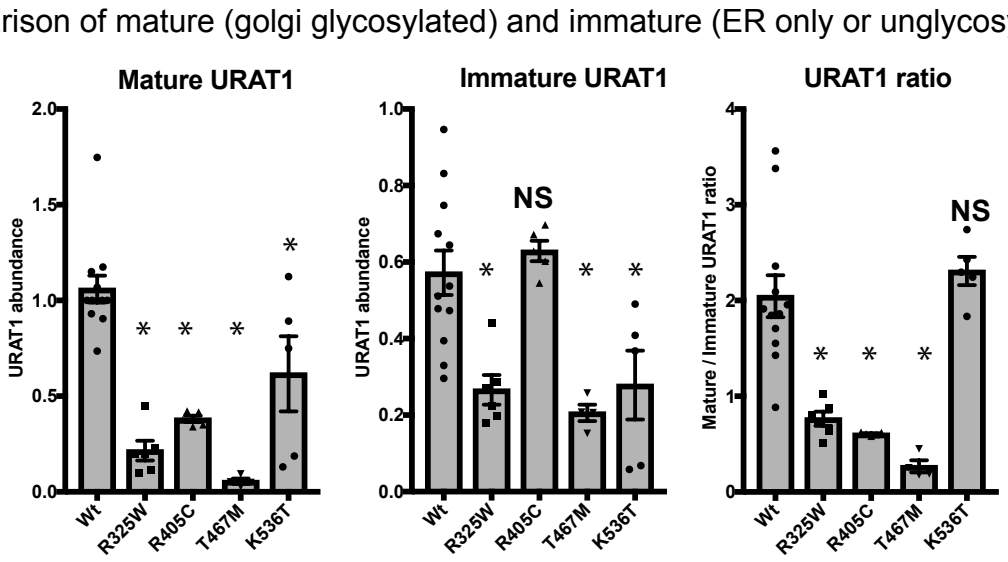
Supplementary Figure 3

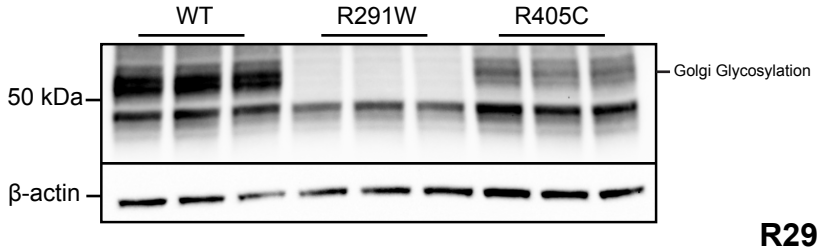


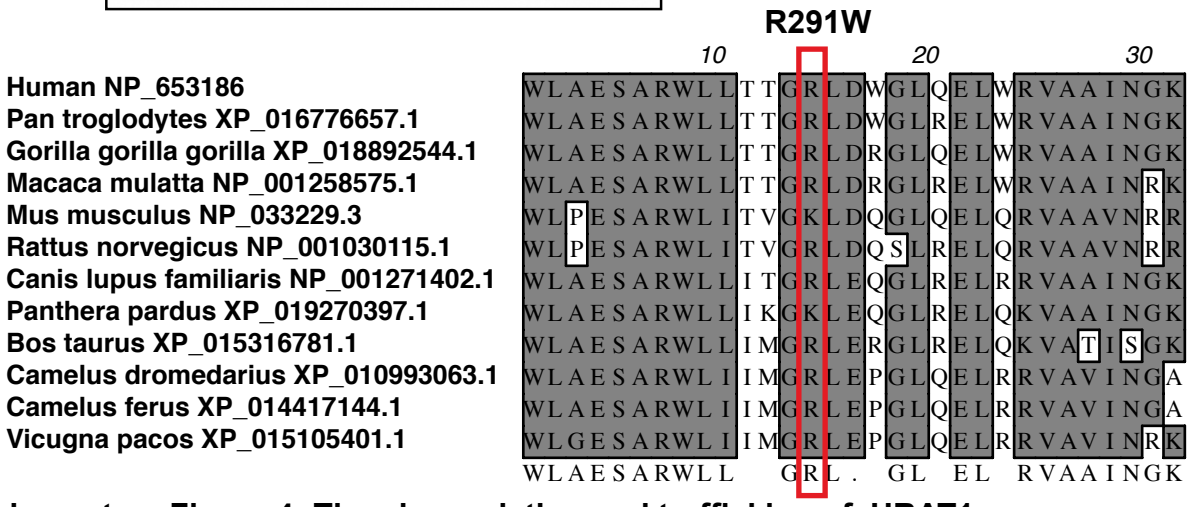
Supplementary Figure 3: Supplemental URAT1 antibody and western blot data
a: URAT1 antibody validation on transiently expressed URAT1 constructs in HEK293T cells and untransfected lysate from COS7 cells. Typical monomer quantification area shown as red box.
b: Total lane protein for Western blot shown in Figure 2B, used as the loading control for quantification (see methods).
c: Full blot and total lane protein for Western blot of URAT1 variants expressed in *Xenopus* oocytes shown in Figure 2C, used as the loading control for quantification (see methods).

Supplementary Figure 4









Supplementary Figure 4: The glycosylation and trafficking of URAT1

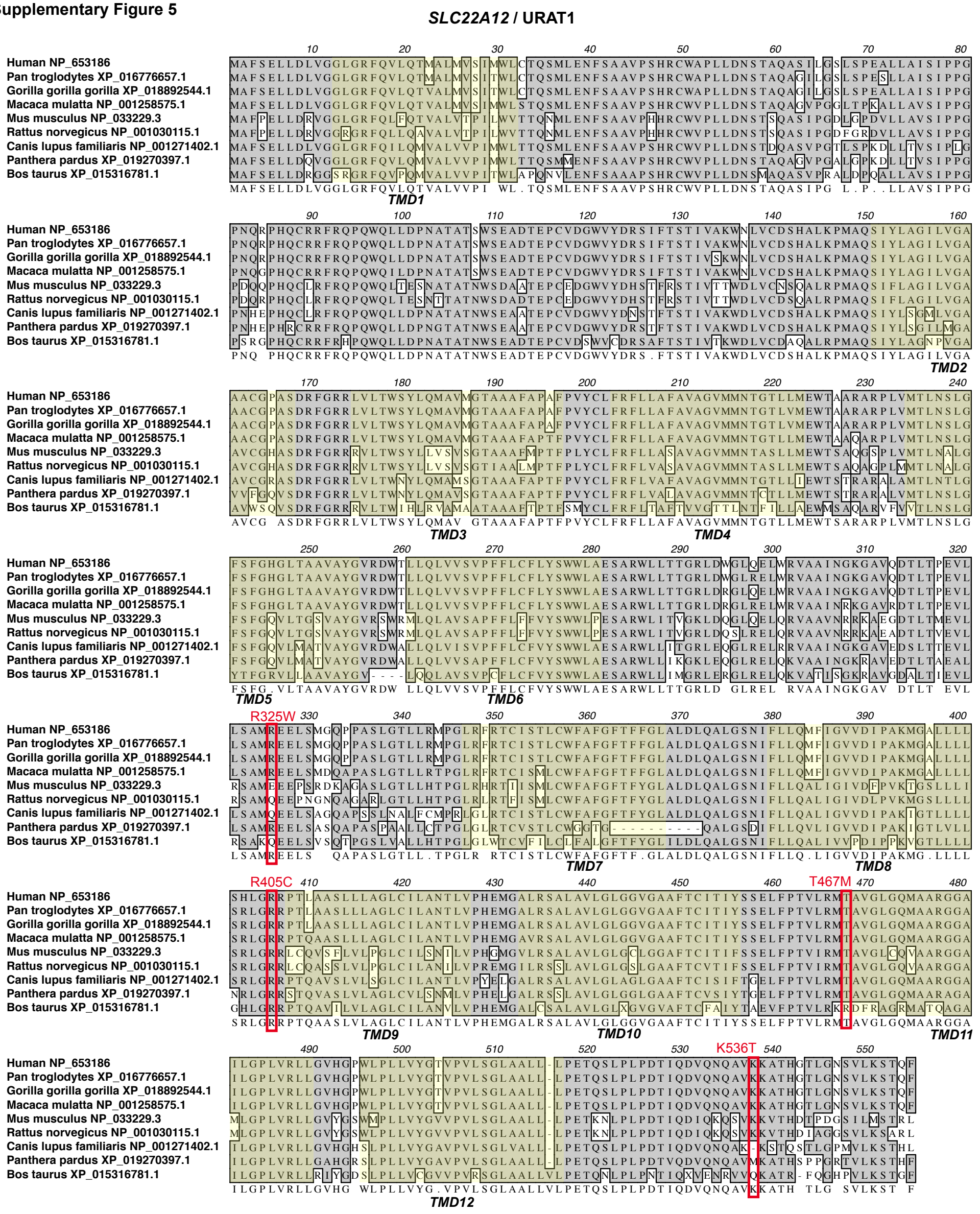
a: Glycosylation of URAT1 and URAT1 mutants revealed by the PNGase and EndoH enzymes on URAT1 protein transiently expressed in HEK293T cell, representative of n=3.

b: Analysis of the mature (golgi glycosylated) URAT1, immature (ER only or unglycosylated) URAT1, and the mature to immature ratio (+/- SEM, n=5-12, P<0.01). Analysis reveals probable processing / trafficking error for the R325W, R405C, T467M mutants. All Western blots processed in parallel.

c: The R291W mutation results in no protein with complex Golgi associated glycosylation when URAT1 is transiently expressed in HEK293T cells; n=3.

d: Alignment of represenative vertebrate URAT1 proteins demonstrating the conserved nature of the R291 residue.

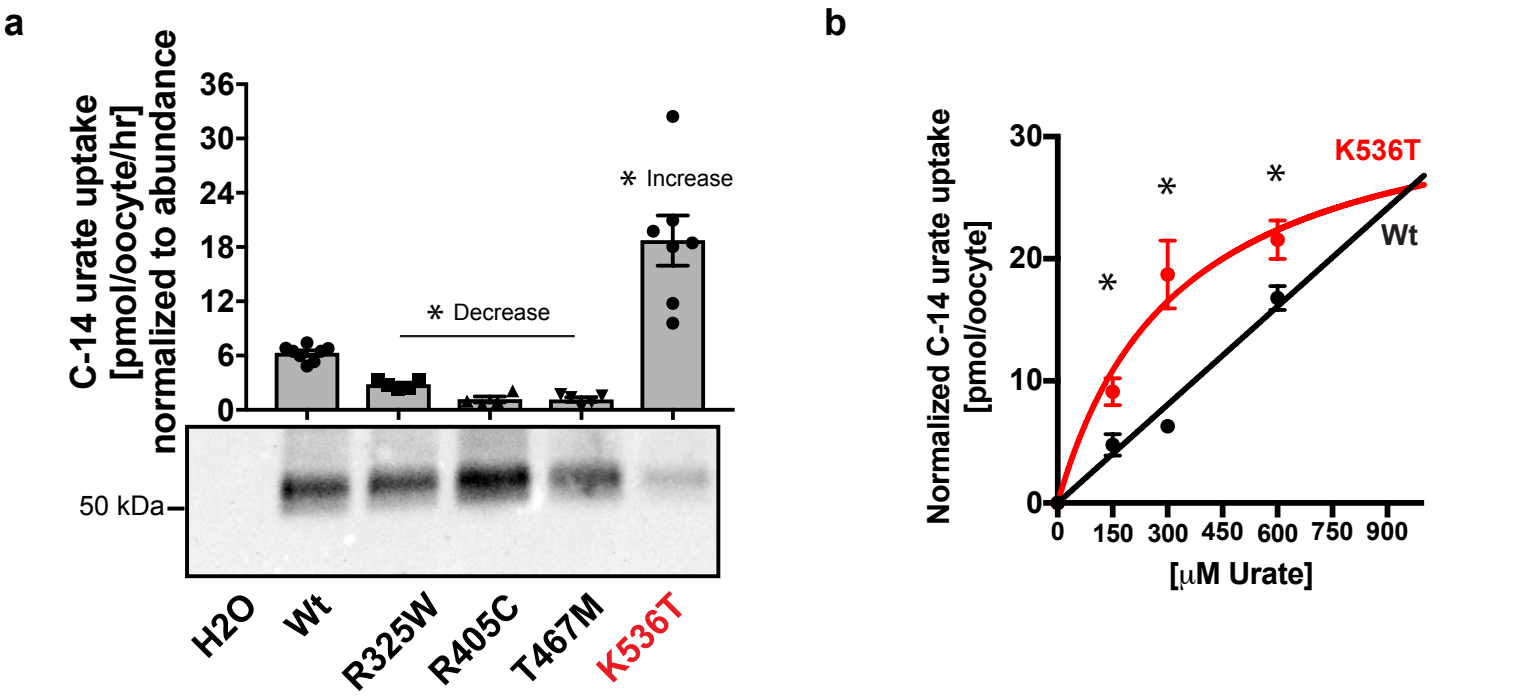
Supplementary Figure 5



Supplementary Figure 5: Alignment of *SLC22A12* / URAT1

Included are human URAT1 and representative URAT1 sequences of other mammals and the amino acid position of significant variants.

Supplementary Figure 6

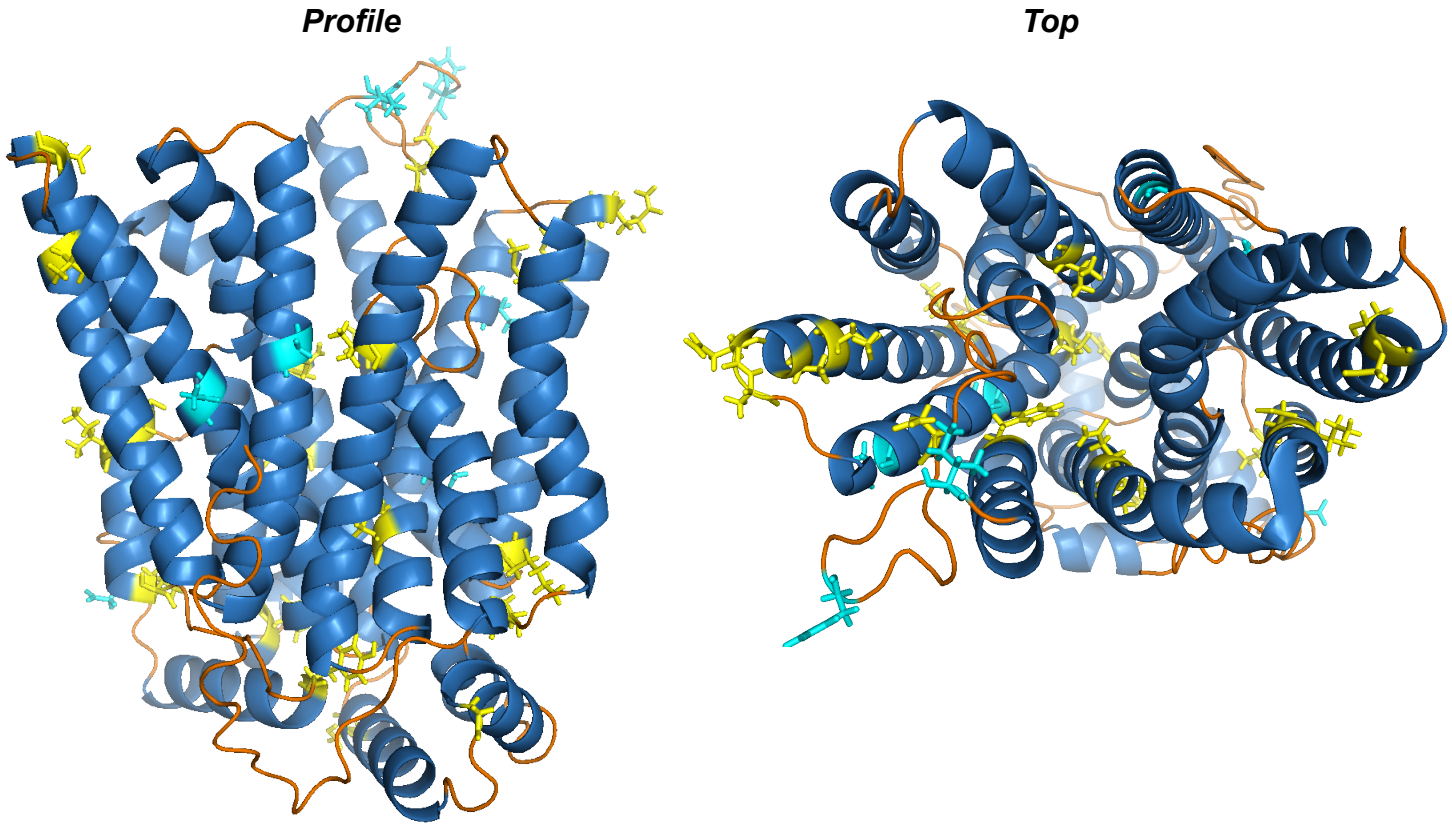


Supplementary Figure 6: Urate transport normalized

a: URAT1 mediated urate transport rates **normalized** to URAT1 protein expression (see Western blot below; lysates from 4-5 pooled oocytes) with the H_2O injected control transport rate subtracted; n=10; +/- SEM; * $p<0.01$.

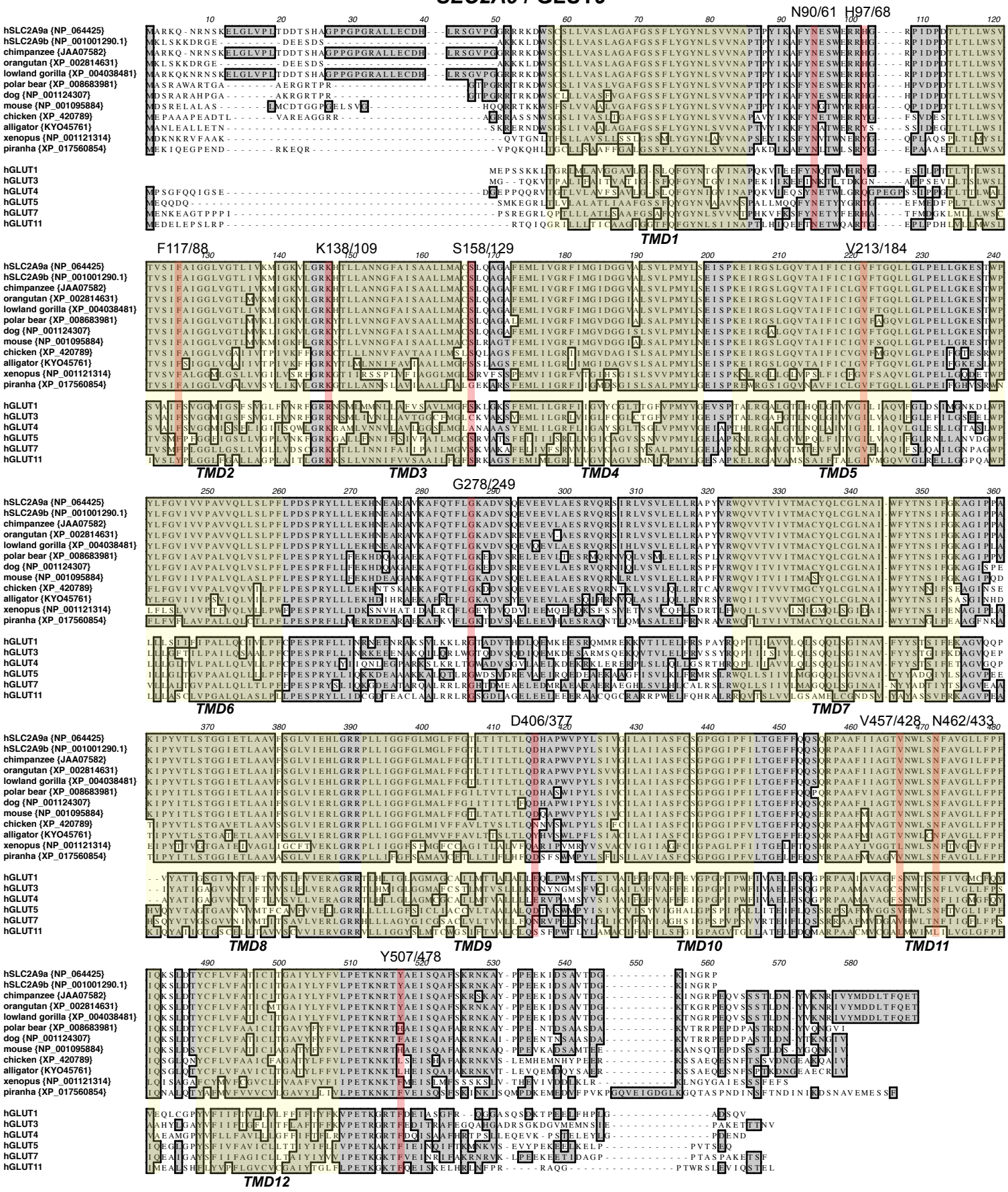
b: The concentration dependence on the rate of transport; n=6 for each concentration; +/- SEM; *$p<0.05$.

Supplementary Figure 7



Supplementary Figure 7: Model of SLC2A9b with newly identified variants
Human SLC2A9b model in the outside open configuration, based on GLUT5 crystal (see methods). All residues depicted are Wt residues. Residues depicted with side chains (sticks) are residues with newly identified SLC2A9 variants with absolute effect >1 standard deviations. Yellow sticks = position of variants associated with lower SUA (Wt residue shown); Cyan = position of variants associated with higher SUA (Wt residue shown).

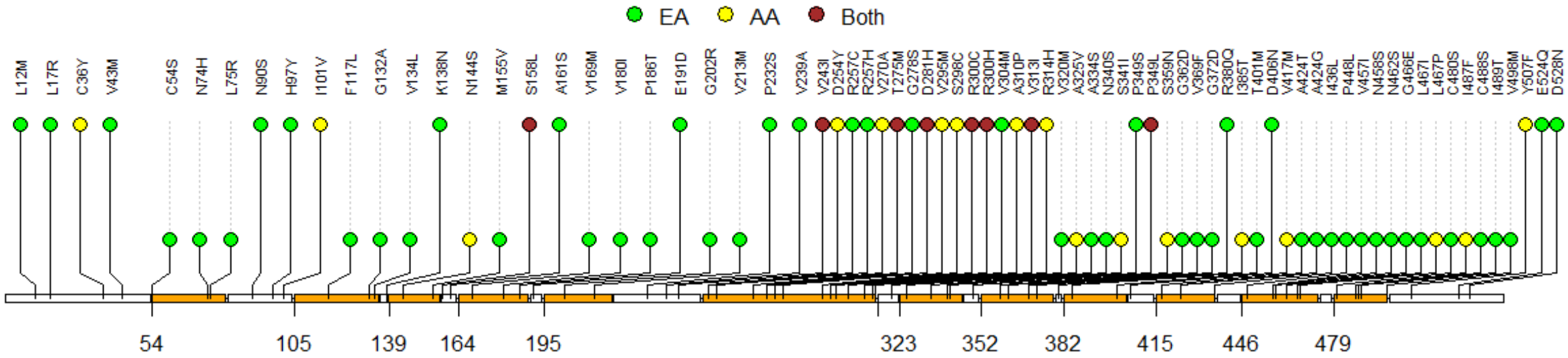
Supplementary Figure 8



Supplementary Figure 8a: Alignment of *SLC2A9* / GLUT9

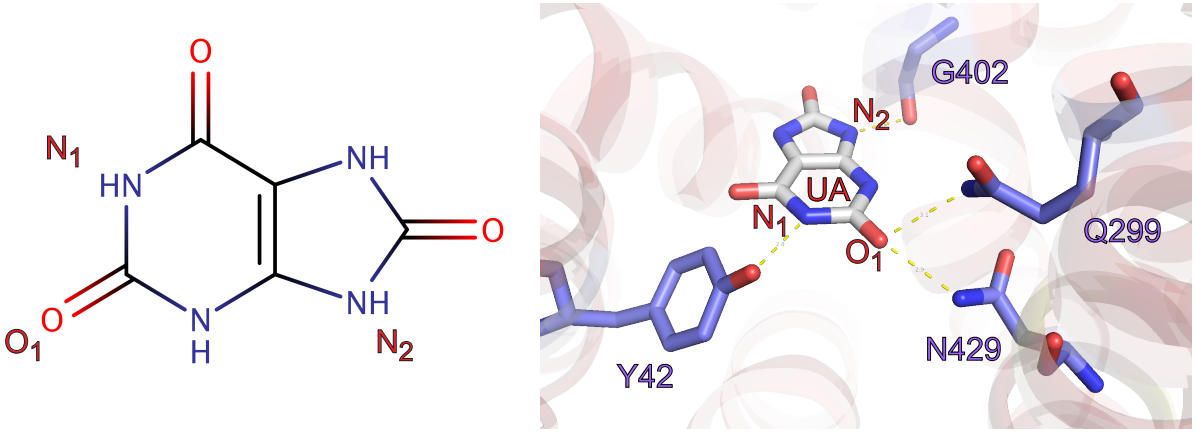
Both human isoforms are included and the amino acid position of significant variants labeled in the numbering scheme of SLC2A9a /SLC2A9b. Included are representative GLUT9 sequences of other mammals and other human GLUT family members [1,3,4,5,7, and 11].

Supplementary Figure 8b. Putative damaging variants with MAF < 5% in *SLC2A9 (*hSLC2A9a*)*



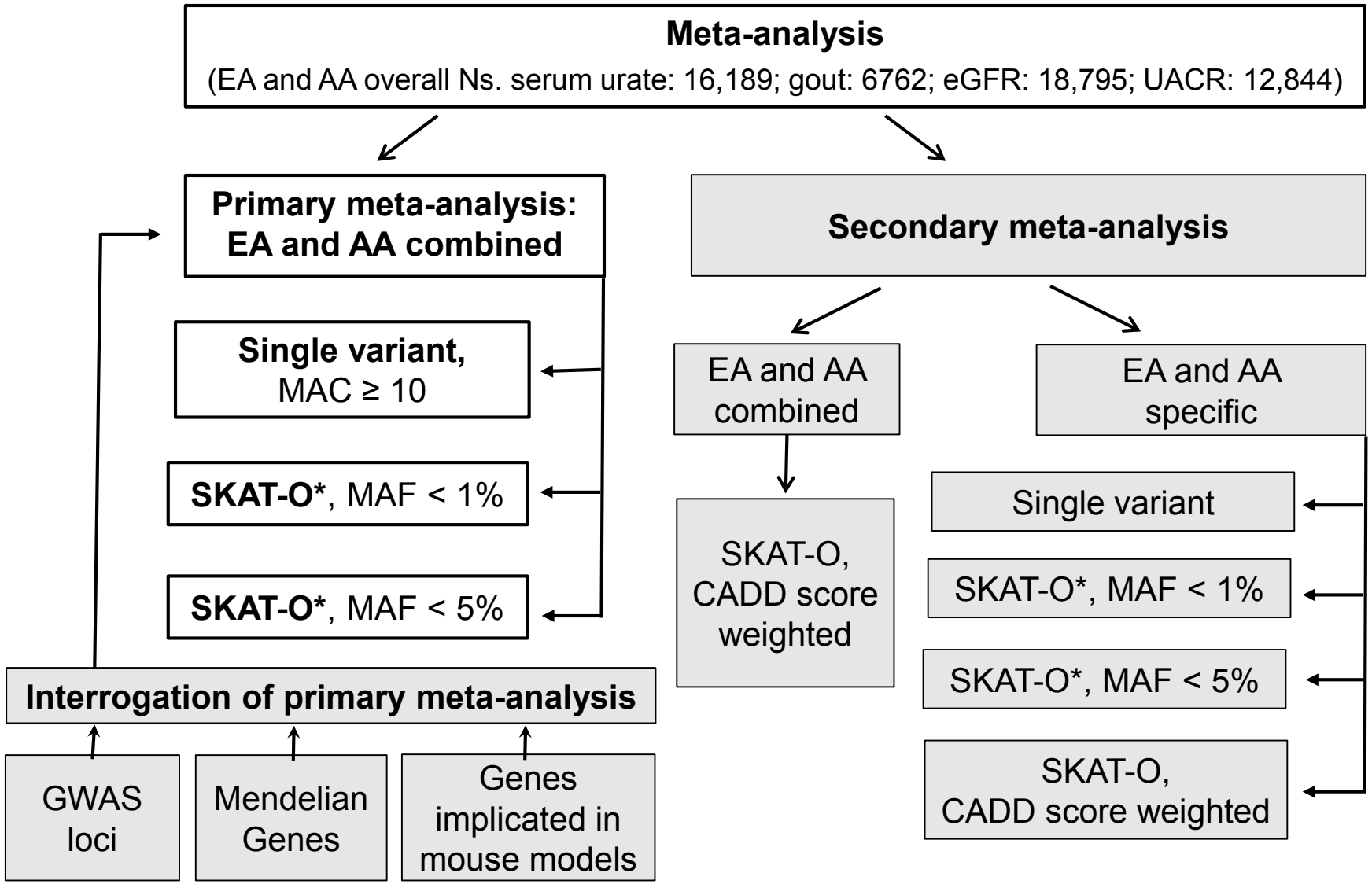
Of the 90 variants included in primary gene-based test, 87 were missense variants, and 3 were splice variants.
Of the missense variants, 76 were found in the hSLC2A9a.
Orange: predicted transmembrane domains
Amino acid position based on NP_064425 (ENSP00000264784)

Supplementary Figure 9



Supplementary Figure 9: Uric acid and the modeled binding pocket of human SLC2A9b
Uric acid molecule schematic and structural model with the key Nitrogens and Oxygen atoms labeled corresponding to nomenclature of the uric acid binding pocket as depicted in Figure 3D.

Supplementary Figure 10. Flow chart of meta-analyses for serum urate, eGFR, and UACR



SKAT-O*: Optimal Sequence Kernel Association Test weight by beta distribution (1, 25). In addition to the MAF filter, only genes with two or more variants were included; CADD: Combined Annotation–Dependent Depletion.
Total number of variants with MAC ≥ 10 in primary single variant meta-analysis: 781,062 in 19,549 genes.

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