Functional classification of population variants demonstrates that SLC6A19 loss-of-function is associated with reduced progression to kidney failure

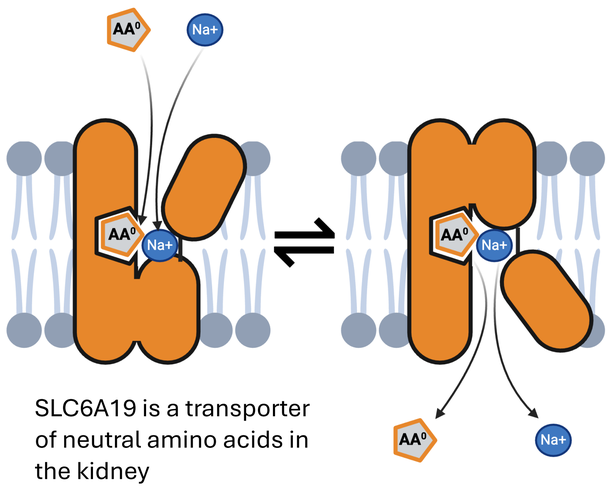
Hannah N. De Jong[1], Sahar Mozaffari[1], Casper Wong[1], Nora I. Scherer[2], Elena Butz[2], Nathan Fastman[1], Julie Ullman[1], Maarten Hoek[1], Robert R. Graham[1], Victoria Assimon[1], Nathan Sallee[1], Anna Köttgen[2], Karol Estrada[1]

[1]Maze Therapeutics, USA [2]University of Freiburg, Germany

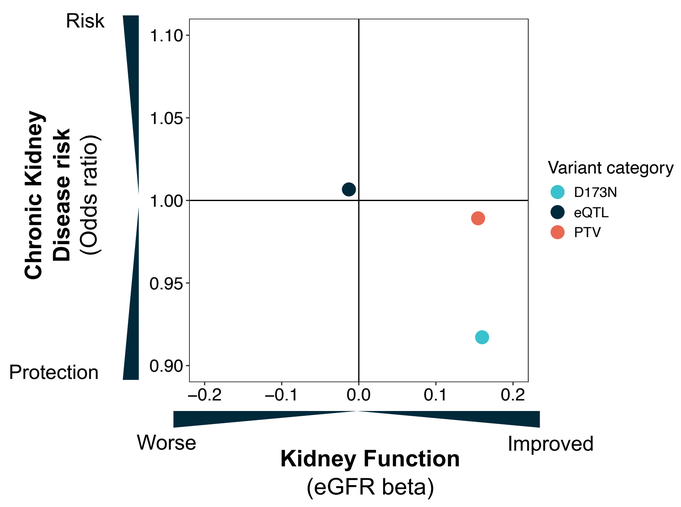
SLC6A19 LOF associated with improved kidney function

SLC6A19 is a transporter expressed in the kidney. It recaptures free amino acids from the diet before excretion in the urine. Protein-truncating variants in *SLC6A19* are strongly associated with improved estimated glomerular filtration rate (eGFR) (UK Biobank: beta = 0.15, p = 5.35e-36), a metric for kidney function. One low-frequency missense variant, D173N (AF = 0.4%), is associated with higher eGFR (beta = 0.14, p = 9.38e-23) and protection from CKD (OR = 0.87, p = 2.64e-3).[1] However, there are hundreds of naturally occurring missense variants that have not yet been characterized.

We therefore compiled 365 *SLC6A19* variants for assay, including all those present at an allele count >1 among UK Biobank (UKBB), All of Us (AOU), and the German Chronic Kidney Disease study (GCKD). Expressing each variant in HEK293 cells, we quantified SLC6A19 protein abundance by flow cytometry survival of treatment with the nephrotoxic compound S-(1,2-dichlorovinyl)-L-cysteine (DCVC) as a proxy for uptake activity. Of these, 83 variants were classified as experimental loss-of-function ("xLOF") by low protein abundance alone, 17 as xLOF by DCVC survival alone, and 125 as xLOF by both assays. This enabled us to identify, at scale, variants expected to cause LOF, and then perform burden analyses to clarify the role of *SLC6A19* LOF in CKD progression.



SLC6A19 is a transporter of neutral amino acids in the kidney



Experimental schematic

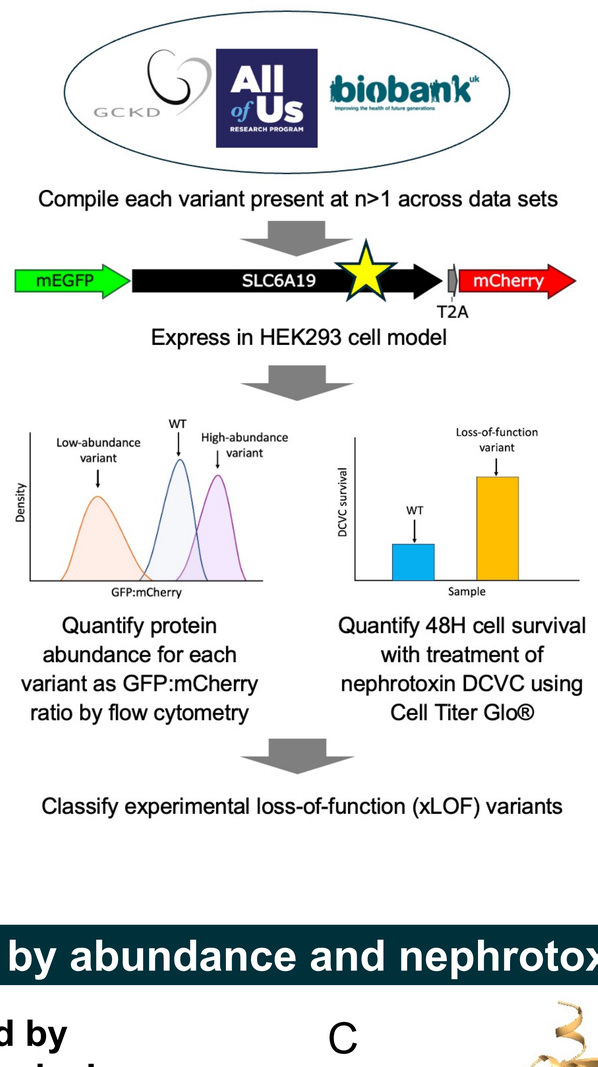


Figure 1. SLC6A19 missense variants functionalized in cellular model. SLC6A19 coding variants including all observed in UK Biobank, All of Us, and German Chronic Kidney Disease cohorts with AC >1 across all three datasets were compiled for screening (n=365). *SLC6A19* cDNA containing each variant was integrated into HEK293 cells expressing the renal chaperone CLTRN. This delivery was done in an arrayed fashion using a Bxb1 landing pad system, resulting in the expression of one copy per cell. Total exogenous SLC6A19 protein abundance was quantified by flow cytometry for each cell using the ratio of an N-terminal GFP tag and a co-expressed mCherry tag. Uptake activity was indirectly quantified by Cell Titer Glo survival readout after 48H treatment with DCVC. Variants causing total SLC6A19 protein abundance significantly lower than WT (Dunnett's test p<0.05) or 50% higher survival than WT in DCVC assay were classified as experimental loss-of-function (xLOF) for the purpose of performing an expanded *SLC6A19* genetic burden analysis.

Experimental loss-of-function (xLOF) variants classified by abundance and nephrotoxin survival

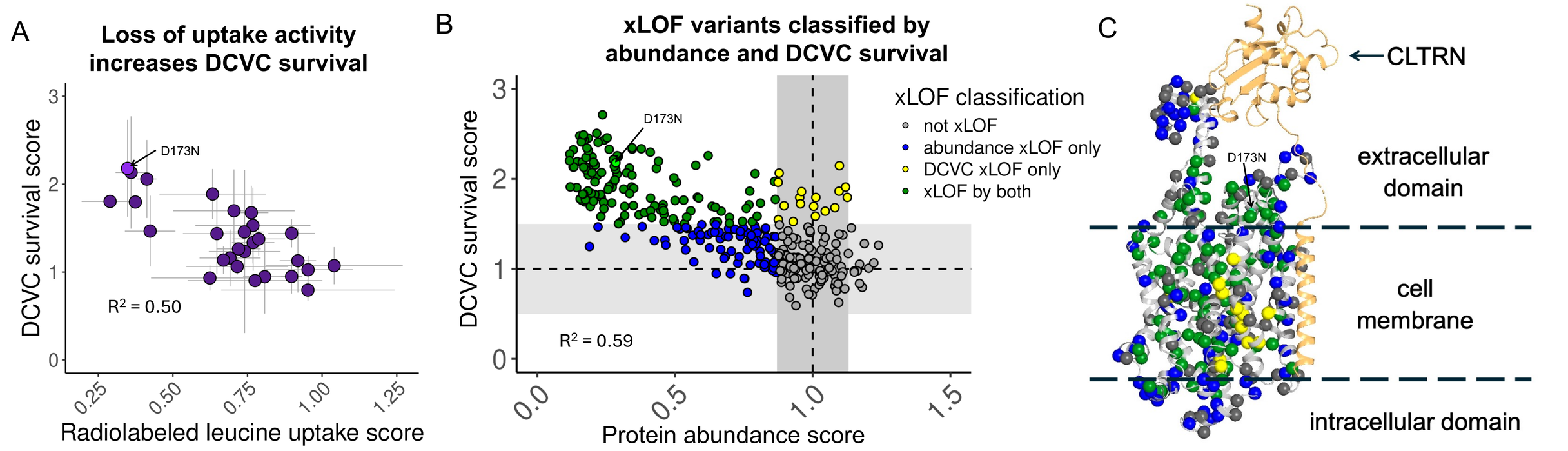
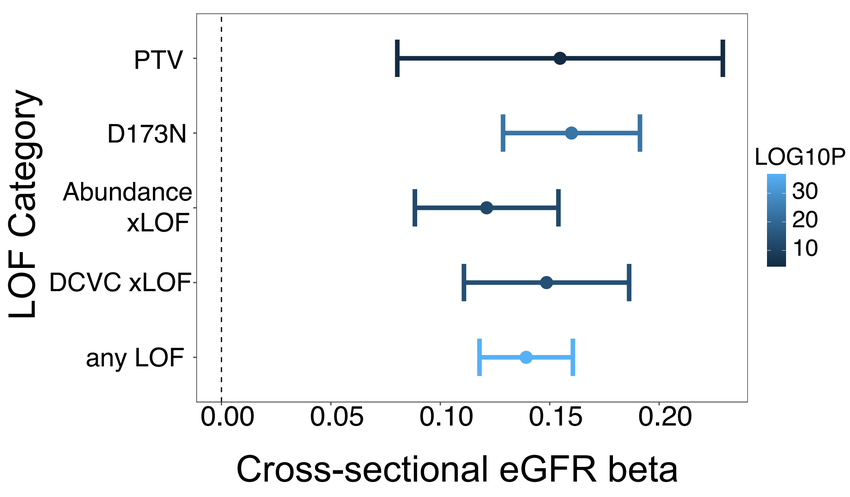


Figure 2. Over half of variants are loss-of-function (xLOF) by abundance and/or DCVC survival assays. A. Survival of nephrotoxin DCVC can be used as a proxy for loss of uptake activity. DCVC survival scores (ratio of Cell Titer Glo luminescence in DCVC-treated vs. untreated cells, normalized within-plate to WT, n=3, error bars show mean+/-SD) are correlated with uptake activity (quantified by radiolabeled leucine uptake assay, normalized to WT, n=2, error bars show mean+/-SD). **B.** DCVC survival scores are shown for each variant tested, plotted against protein abundance scores (mean GFP:mCherry ratio, normalized to within-plate WT SLC6A19 controls, n=3). Dashed lines indicates WT reference ratios, and variants outside of gray bars were classified as xLOF by respective assays (Dunnett's test, p<0.05 for abundance, score > 1.5 for DCVC survival). 83 variants were classified as xLOF by abundance alone (blue), 17 variants by DCVC survival alone (yellow), and 125 variants as xLOF by both assays (green). **C**. Variant residues color-coded by xLOF classification (as in fig. 2B) are mapped onto the protein structure of SLC6A19. White ribbon represents SLC6A19, light orange ribbon represents the SLC6A19 chaperone CLTRN, and spheres represent tested variants.

xLOF variants strengthen association of SLC6A19 LOF with improved kidney function



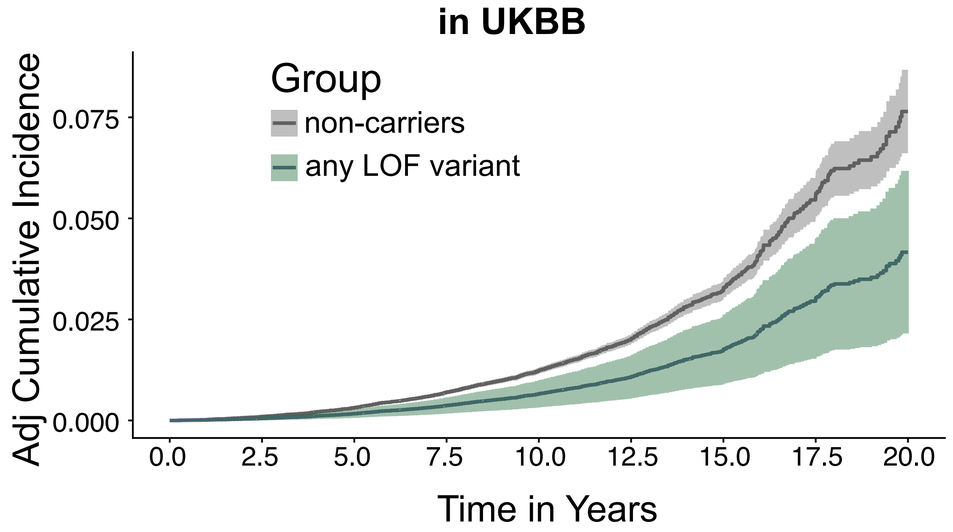


Figure 3. xLOF variants strengthen the association of SLC6A19 with improved renal function.

A. Plot shows eGFR gene burden and p-values for variants with allele frequency < 0.01 in UK Biobank. xLOF variants observed in UK Biobank are significantly associated with higher eGFR (DCVC: n=2423, p=1.21e-14; abundance: n=3216, p=4.40e-13, overlapping classifications) and strengthen the association of all LOF variants with eGFR (n=7670, p=1.76e-37). **B**. xLOF variants in combination with D173N and protein truncating variants (PTVs) are significantly associated with decreased risk of progression to a composite kidney endpoint, or CKE (40% eGFR decline, eGFR <15 mL/min/1.73m^2, dialysis, or transplant) in a general population (UK Biobank, HR = 0.65, p=0.02) [not shown]. The same variants are significantly associated with decreased risk of CKE in CKD cohorts (UK Biobank eGFR<90 [shown in plot, truncated to 20 years, HR = 0.53, p = 0.009] and German CKD [not shown, HR = 0.51, p = 0.059]).

Conclusions

- We have demonstrated that experimentally-classified loss-of-function variants in *SLC6A19* are significantly associated with decreased incidence of CKE.
- Out of 365 tested SLC6A19 variants, we classified 225 as experimental loss-of-function (xLOF) by abundance or nephrotoxin survival assay in a cellular model. These xLOF variants are associated with higher eGFR, and lower risk of CKE in both a general population and a CKD cohort.
- This data provides a strong rationale for investigation of SLC6A19 inhibition as a potential therapeutic approach for CKD. An investigational small molecule inhibitor of SLC6A19, MZE782, is currently being evaluated as a potential therapy for CKD and phenylketonuria (PKU).

Acknowledgements and references: This research has been conducted using data from UK Biobank, a major biomedical database. We acknowledge *All of Us* participants for their contributions, and the National Institutes of Health's All of Us Research Program for making available the participant data examined in this study. The GCKD study was and is supported by the BMBF (FKZ 01ER 0804, 01ER 0818, 01ER 0819, 01ER 0820 and 01ER 0821) and the KfH Foundation for Preventive Medicine. Unregistered grants to support the GCKD study were provided by corporate sponsors (listed at https://gckd.org). The work of AK was funded by DFG Project ID 431984000. We acknowledge Isabel Kerrenbijn of the University of Toronto for her contributions to data analysis. 1. Sveinbjornsson G, Mikaelsdottir E, Palsson R, et al. *Hum Mol Genet*. 2014