Variant functionalization data strengthen the genetic association of SLC6A19 loss-of-function with improved outcomes in chronic kidney disease

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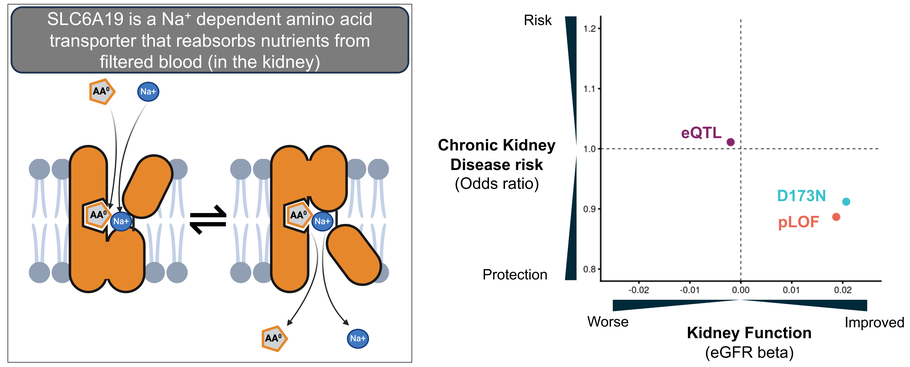
SLC6A19 LOF associated with improved kidney function

Chronic kidney disease (CKD) is characterized by loss of kidney function and may progress to kidney failure. Loss of function (LOF) variants in *SLC6A19* are strongly associated with better kidney function (UK Biobank: estimated glomerular filtration rate (eGFR), beta = 0.15, p = 5.35e-36). SLC6A19 recaptures free neutral amino acids in the proximal tubule of the kidney. One low-frequency 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 previously evaluated D173N and 9 other known LOF missense variants, and all showed reduced abundance of SLC6A19 and loss of amino acid transport activity, demonstrating that low abundance is a strong predictor of functional impact. Here, we expand upon our prior work, compiling *SLC6A19* variants from multiple genotyped cohorts and quantified protein abundance. 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.



Experimental schematic

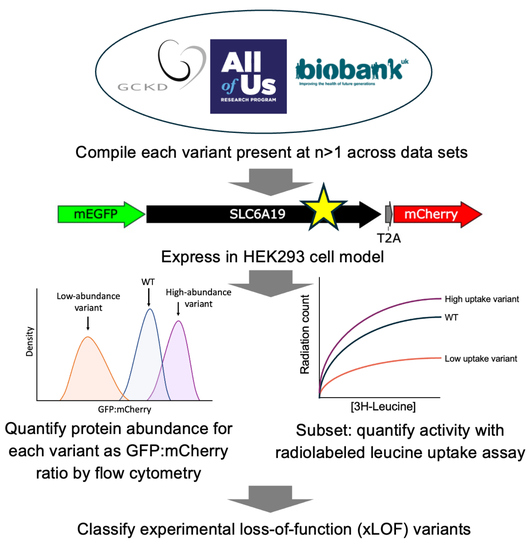


Figure 1. SLC6A19 missense variants functionalized in cellular model. SLC6A19 coding variants including all observed in UK Biobank, All of Us, and German Chronic Kidney Disease cohorts with AC >1 across all three datasets were compiled for screening (n=365). *SLC6A19* cDNA containing each variant was 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. For a subset of variants, activity was quantified by radiolabeled leucine uptake assay. Variants causing total SLC6A19 protein abundance significantly lower than WT (Dunnett's test p<0.05) 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 uptake activity

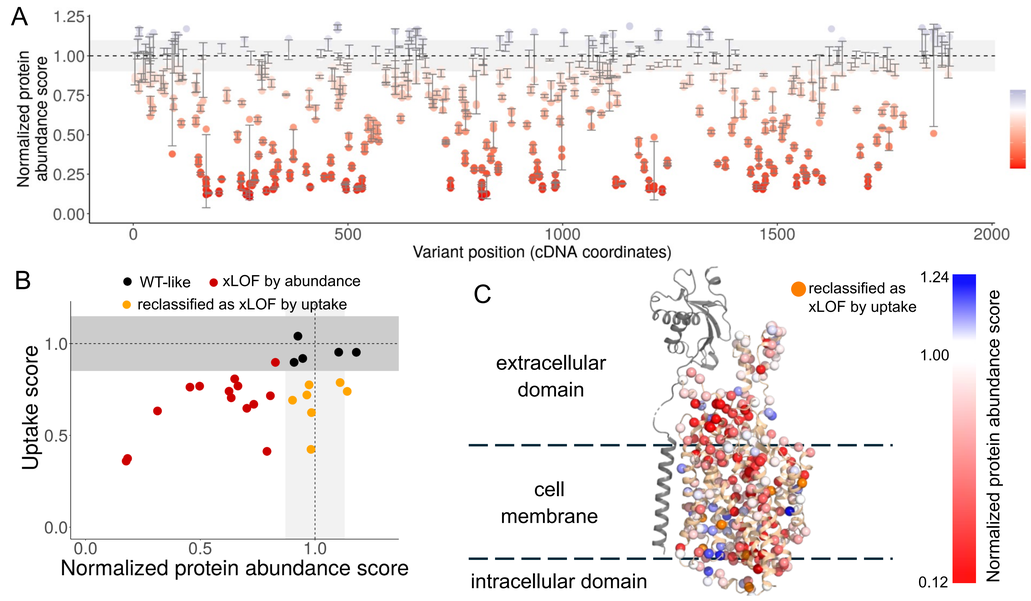


Figure 2. Over half of variants are loss-of-function (xLOF) by abundance and/or uptake activity.

A. Normalized mean GFP:mCherry ratios are shown as abundance scores for each sample (n=3), normalized to same-plate WT SLC6A19 controls. Error bars = mean+/-SD. Dashed line indicates WT reference ratio. 222 variants have significantly lower abundance than WT (Dunnett's test, p<0.05, outside of gray band). Two independent experiments were highly concordant, R^2 = 0.94. **B**. A subset of variants enriched for high allele count and WT-like abundance was tested in radiolabeled leucine uptake assays. Uptake score was plotted against normalized protein abundance score. Dashed lines indicate WT means for each assay; variants outside of gray bands are significantly different than WT (p<0.05, Dunnett's test). Black denotes WT-like variants, red denotes variants classified as xLOF by abundance, and orange denotes variants reclassified to xLOF by uptake assay. **C**. Normalized protein abundance scores for each variant are mapped onto the protein structure of SLC6A19. Beige ribbon represents SLC6A19, gray ribbon represents the SLC6A19 chaperone CLTRN, and spheres represent tested variants. Variants decreasing total SLC6A19 protein abundance are shown in shades of red and are enriched in the core of the protein, and variants increasing abundance are shown in shades of blue and tend to be located on the exterior of the protein. Variants reclassified as xLOF by loss of uptake activity are shown in orange.

xLOF variants strengthen association of SLC6A19 LOF with improved kidney function

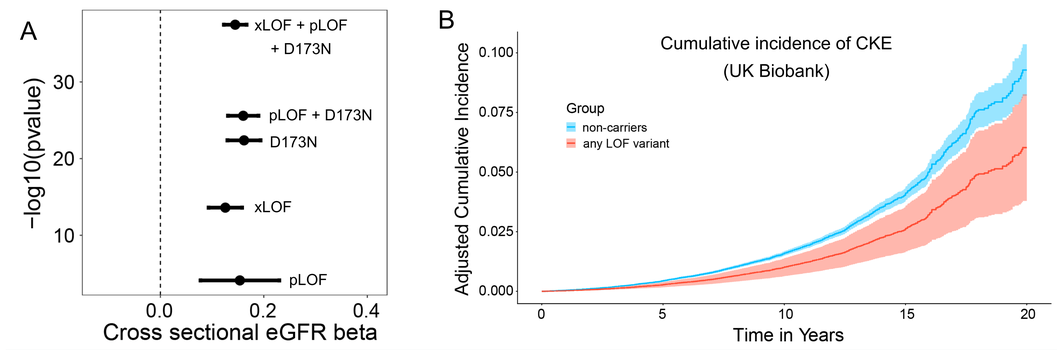


Figure 3. xLOF variants strengthen the association of SLC6A19 with improved renal function.

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 (n=3490, p=2.48e-14). and strengthen the association of all LOF variants with eGFR (n=7615, p=3.45e-38). **B**. xLOF variants in combination with D173N and existing predicted LOF (pLOF) variants 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 = 0.632, p=1.59e-2) [shown in plot, truncated to 20 years]. The same variants are also significantly associated with decreased risk of CKE in CKD cohorts (UK Biobank eGFR<90 and German CKD, HR = 0.51, p = 0.001) [not shown].

Conclusions

- For the first time, 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 229 as experimental loss-of-function (xLOF) by abundance or uptake 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).

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