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Examination of the Predicted Prevalence of Gitelman Syndrome by Ethnicity Based on Genome Databases

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Background: Gitelman syndrome is an autosomal recessive inherited salt-losing tubulopathy. It has a prevalence of around 1 in 40,000 people, and heterozygous carriers are estimated at approximately 1%, although the exact prevalence is unknown because most cases are thought to be asymptomatic or have nonspecific clinical findings. On the other hand, it has been reported that the non-specific symptoms can reduce the quality of life of patients, and in practice, we have often experienced cases where patients have suffered from these symptoms since childhood, but were not diagnosed and therefore not treated, and were diagnosed in adulthood. It could suggest that there are far more patients and carriers than expected.

Methods: We estimated the predicted prevalence of Gitelman syndrome based on multiple genome databases, HGVD and Jmorp for the Japanese population and gnomAD for other ethnicities, and included all 274 pathogenic missense or nonsense mutations registered in HGMD Professional. The frequencies of all these alleles were summed to calculate the total variant allele frequency in JSC1243 which is the responsible gene for Gitelman syndrome. The carrier frequency and the disease prevalence were assumed to be twice and the square of the total allele frequency, respectively, according to the Hardy-Weinberg principle.

Results: In the Japanese population, the total carrier frequencies were 0.0048 (9.5%) and 0.0068 (8.7%) and the calculated prevalence was 0.00225 (2.3% in 1000 people) and 0.00188 (1.9 in 1000 people) in HGVD and Jmorp, respectively. Other ethnicities showed a prevalence varying from 0.000012 to 0.00083.

Conclusions: These findings indicate that the prevalence of Gitelman syndrome in the Japanese population is higher than expected and that some other ethnicities also have a higher prevalence than previously been considered.

PO1325

An Off-the-Shelf CRISPR Gene Therapy Approach in Human Kidney Organoids

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Background: Gene therapy offers many opportunities to treat kidney diseases. Targeted, off-the-shelf therapeutics are needed for both loss-of-function (e.g. nephropathic cystinosis) and gain-of-function (e.g. ApoL1) disease states. Kidney organoids are complex structures that resemble nephrons and can be used to develop gene therapy approaches. Commonly used gene transfer techniques, such as lentivirus and adenovirus, are size limited, transient, or introduce DNA non-specifically into the genome. While targeted CRISPR gene editing is routinely used in 2D cell cultures, it has been challenging to use this powerful technique in intact organoids.

Methods: To achieve off-the-shelf gene transfer, organoids were transfected with Cas9 and gRNA ribonucleoprotein (RNP) complexes targeting the AATS5 safe harbor locus supplemented with knock-in cassettes encoding green fluorescent protein (GFP) or FLAG-tagged cystinosin (deficient in nephropathic cystinosis). Alternatively, to monitor gene knock-out, organoids expressing GFP from AATS5 were transfected with RNP and either one or two gRNAs to introduce indels in the coding sequence. Genome editing was detected three ways: by confocal microscopy, PCR, and next generation sequencing.

Results: GFP and cystinosin knock-in events in organoids were detected using microscopy and PCR. Immunofluorescence analysis revealed knock-in in proximal tubule epithelial cells (LTL+). In knock-out experiments, live confocal microscopy indicated areas of GFP loss within kidney organoids treated with gRNA targeting GFP, but not with a scrambled guide. Mosaic patches of GFP knockout cells expanded over several days. Staining with nephron markers such as LTL and podocalyxin revealed knockout in proximal tubule cells and podocytes. By next generation sequencing, the two-guide system produced larger deletions and was more efficient (20% knockout), compared to single guide.

Conclusions: The strategy developed here is efficient for knocking in and knocking out genes in kidney epithelium. It uses commercially available reagents to perform CRISPR gene editing. sgRNA sequences or AATS5 knock-in templates can be customized to target or introduce any gene of interest at specific loci. This provides a platform for the development of off-the-shelf gene therapies for diverse kidney disease states.

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The Kidney Genome Atlas: A Resource to Understand APOL1 and Other Genetic Drivers of Adult Proteinuric Kidney Diseases

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Background: Chronic kidney disease (CKD) affects more than 30 million people in the US with African Americans being particularly at risk. There is an unmet need for pharmaceutical therapies that extend or, ideally, restore kidney function.

Methods: To guide genetically-driven drug development, we have established the Kidney Genome Atlas (KGA), which contains whole-genome sequences (>30X) from adult patients with Focal segmental glomerulosclerosis (FSGS), minimal change disease (MCD) and other, idiopathic, proteinuric disorders as well as public and technically matched controls. By implementing a rigorous quality control procedure, following the gnomAD pipeline, we obtained a high-confidence dataset for downstream analyses. Three genetically inferred ancestries (EUR, AFR, AMR) were included in association testing comparing 1406 cases, including 169 individuals with APOL1 G1/G1, G2/G2 or G1/G2 high-risk haplotypes (APOL1-HRH), with 1468 controls (including 485 APOL1-HRH individuals).

Results: Overall, our common variant cross-ancestry meta-analysis showed minimal impact on potential confounders, such as ancestry or sequencing center differences (lambda=1.03). Using summary statistics from our EUR analysis, we estimated a SNP heritability of 0.15 (SE = 0.028) in proteinuric diseases. Comparison to a recent CKD GWAS (Wuttke et al., 2019) indicated a weak positive genetic correlation (rg) of 0.097 (SE = 0.053). We identified the previously reported significant disease association of APOL1-HRH (p=2x10^-10) in our study. Recent in vitro data suggests amino acid in position 150 (rs2239785) is critical for the pathogenicity of APOL1-HRH (PO1986, ASN 2020) which we confirmed in our cohort of AFR ancestry individuals.

Conclusions: We have built a high-quality, multietnic cohort that enables understanding of genetic drivers of polygenic proteinuric kidney disease. Future analysis including genetic modifiers of APOL1 may provide opportunities for novel therapies and patient stratification.

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Effect of ApoL1 Genotype on Kidney Failure and eGFR Decline in Patients with All-Cause CKD

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Background: ApoL1 risk variants G1 and G2 associate with an increased risk of kidney failure and a higher rate of eGFR loss. We assess the effect of ApoL1 genotype in African American and Latino individuals with chronic kidney disease (CKD) in New York City.

Methods: ApoL1 genotype determined by sequencing. CKD cases with high-risk ApoL1 genotype (n= 242) were compared to CKD cases with a low-risk (ApoL1 genotype (n=885) and African ancestry per Admixture. Kaplan-Meier survival analyses assessed time to kidney failure followed by Adjusted Cox-proportional hazard model and competing risk regression against death both incorporating covariates. Linear mixed-effects modelling evaluated CKD-EPI eGFR, decline rate using the same covariates.

Results: Cases with a high-risk ApoL1 genotype reach kidney failure 10-15 years earlier than low-risk cases. G1/G1 reach kidney failure earliest, followed by G1/G2 and G2/G2 (Fig 1). These data are supported across multiple risk models (Table 1). Cases with a high-risk ApoL1 genotype have a higher eGFR decline rate than low-risk cases with a similar trend per specific genotype (Fig 2). The addition of self-declared or genetically defined ancestry did not confer additional risk.

Conclusions: High-risk ApoL1 genotypes increase the risk of kidney failure at an earlier age, likely due to a higher eGFR decline rate. G1/G1 genotypes appear most affected and G2/G2 least.

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Table 1. Modelling Results by ApoL1 Genotype

| ApoL1 Genotype | Age at Kidney Failure (years) | eGFR Decline Rate (ml/min/1.73m^2/year) | p-value |
|----------------|-----------------------------|----------------------------------------|---------|
| G1/G1          | 53.5 (SD: 12.5)             | 1.62 ± 0.08                            | <0.001  |
| G2/G2          | 56.1 (SD: 12.5)             | 1.60 ± 0.08                            | <0.001  |
| G1/G2          | 54.3 (SD: 12.5)             | 1.59 ± 0.08                            | <0.001  |
| G1/G3          | 55.2 (SD: 12.5)             | 1.57 ± 0.08                            | <0.001  |

* p<0.05 ** p<0.001 *** p<0.0001