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Individuals heterozygous for *ALG8* protein-truncating variants are at increased risk of a mild cystic kidney disease

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Abstract

ALG8 protein-truncating variants (PTVs) have previously been described in patients with polycystic liver disease and in some cases cystic kidney disease. Given a lack of well-controlled studies, we determined whether individuals heterozygous for *ALG8* PTVs are at increased risk of cystic kidney disease in a large, unselected health system-based observational cohort linked to electronic health records in Pennsylvania (Geisinger-Regeneron DiscovEHR MyCode study). Out of 174,172 patients, 236 were identified with *ALG8* PTVs. Using ICD-based outcomes, patients with these variants were significantly at increased risk of having any kidney/liver cyst diagnosis (Odds Ratio 2.42, 95% confidence interval: 1.53–3.85), cystic kidney disease (3.03, 1.26–7.31), and nephrolithiasis (1.89, 1.96–2.97). To confirm this finding, blinded radiology review of computed tomography and magnetic resonance imaging studies was completed in a matched cohort of 52 thirty-plus year old *ALG8* PTV heterozygotes and related non-heterozygotes. *ALG8* PTV heterozygotes were significantly more likely to have cystic kidney disease, defined as four or more kidney cysts (57.7% vs. 7.7%), or bilateral kidney cysts (69.2% vs. 15.4%), but not one or more liver cyst (11.5% vs. 7.7%). In publicly-available UK Biobank data, *ALG8* PTV heterozygotes were at significantly increased risk of ICD code N28 (other disorders of kidney/ureter) (3.85% vs. 1.33%). *ALG8* PTVs were not associated with chronic kidney disease or kidney

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failure in the MyCode study or the UK Biobank data. Thus, PTVs in *ALG8* result in increased risk of a mild cystic kidney disease phenotype.

Graphical Abstract

Keywords

autosomal dominant polycystic kidney disease (ADPKD); polycystic liver disease; genetics; chronic kidney disease (CKD); renal cyst; genotype-first

Introduction:

While *PKD1* and *PKD2* variants account for the majority of autosomal dominant polycystic kidney disease (ADPKD), several other genes have been implicated, including *GANAB*, *ALG9*, and *DNAJB11.* [1–3] These genes are involved in the endoplasmic-reticulum processing and maturation of the *PKD1* product polycystin-1. Polycystin-1 is expressed on the renal tubular epithelium and hepatic bile duct epithelium and abnormal polycystin-1 expression leads to polycystic kidney and liver disease. [4–6] Regarding ADPKD, pathogenic variants in genes such as *GANAB, ALG9*, and *DNAJB11* present with heterogenous and less severe presentations of cystic kidney disease compared to ADPKD due to *PKD1* or *PKD2*, which typically results in enlarged polycystic kidneys and early-onset end-stage kidney disease (ESKD). [3,7,8] The rapidly growing field of nephrogenetics continues to expand on the clinical presentations and genetic heterogeneity of ADPKD.

Another gene encoding an endoplasmic reticulum resident protein responsible for glycosylation of proteins such as polycystin-1 is *ALG8*[9]. In a cohort of 159 unrelated individuals with PCLD, Besse et al. identified 5 individuals with *ALG8* variants including p.Arg364Ter (n=3), p.Arg179Ter (n=1), and a tenth exon splice variant (n=1). [2] These patients had ≥10 liver cysts, and four of five individuals presented with kidney cysts, ranging from one to nine cysts. In another cohort of 212 patients with ADPKD who underwent a gene panel analysis that included *PKD1, PKD2*, and 14 other cystogenes, there was 1 proband with a heterozygous *ALG8* p.Leu149Arg variant who presented with renal and

hepatic cysts with normal kidney function.[10] The proband's mother also carried the same variant and had multiple renal and hepatic cysts, and computational predictions suggested the variant may be associated with increased risk.

In vitro experiments show that *ALG8* knockout cells express reduced polycystin-1 glycosylation and total polycystin-1 levels, which are returned to normal levels upon re-expressing the *ALG8* gene. [2] Decreased levels of functional polycystin-1 within the kidney tubular and liver bile duct epithelium may explain the potential associations of *ALG8* variants with cystic kidney disease and autosomal dominant polycystic liver disease (PCLD), respectively. A number of clinical case reports describe homozygous and compound heterozygous *ALG8* variant presentations in the congenital disorder of glycosylation, CDG-Ih. [11–13] CDG-Ih is a multi-organ disease that can include edema, protein-losing enteropathy, seizures, ataxia, coagulopathy, elevated transaminases, cataracts, and renal tubular dysgenesis, resulting in neonatal or infantile death. Although a few ClinVar submissions have identified additional patients with *ALG8*-related liver and kidney cysts, little data exists on the phenotypic spectrum of pathogenic *ALG8* variants. Given the heterogeneity in the genetic causes of PKD, next-generation sequencing (NGS) is becoming an increasingly used tool for the diagnosis of genetic kidney disease and specifically PKD; several panels include *ALG8*. [1,8] However, the ClinGen Kidney Cystic and Ciliopathy Disorders Gene Curation Expert Panel (Clinical Genome Resource. https://search.clinicalgenome.org/kb/affiliate/10066?page=1&size=25&search= [12/17/21].) currently has classified the level of evidence for *ALG8* in causing polycystic liver disease with or without kidney cysts as limited. [14]

In this study we examine the phenotypic spectrum of *ALG8* protein truncating variants (PTVs) using data from the Geisinger-Regeneron MyCode DiscovEHR study. We hypothesized that rare *ALG8* PTVs increase the risk of cystic kidney disease. To answer this question, we examined whether *ALG8* PTV heterozygotes were at increased risk of cystic kidney disease ICD diagnoses, and then performed a matched analysis with blinded radiologist review of imaging data to evaluate whether ClinVar pathogenic/likely pathogenic *ALG8* PTV heterozygotes were at increased risk of cystic kidney disease, compared to related non-heterozygotes. Findings were further confirmed using a publicly-available United Kingdom Biobank (UKBB) dataset.

Methods:

Study Population

The Geisinger Institutional Review Board approved this study. Participants completed informed consent in the MyCode™ Community Health Initiative starting in 2007 and underwent exome sequencing as part of the Geisinger-Regeneron DiscovEHR collaboration. [15] There are no particular eligibility requirements to participate in MyCode and patients are recruited at clinics in the Geisinger Health System in central and northeast Pennsylvania.

Genotyping

As previously described, exome sequencing was performed in collaboration with Regeneron Genetics Center. [15] Probes from NimbleGen (VCRome) or a modified version of the xGEN probe from Integrated DNA Technologies (IDT) were used for target sequence capture. [16] Sequencing was performed by paired end 75bp reads on either an Illumina HiSeq2500 or NovaSeq. Coverage depth was sufficient to provide more than 20% coverage over 85% of the targeted bases in 96% of the VCR samples and 90% coverage for 99% of IDT samples. Alignments and variant calling were based on GRCh38 human genome reference sequence. We required participants to have alternative allele >3 and allele balance ≥0.333.

We identified patients who were heterozygous for *ALG8* variants that were protein-truncating variants (i.e. start-loss, frameshift, canonical splice or early termination/stop-gain of the encoded protein), determined by Ensembl. [17] Among patients with *ALG8* PTVs, we also searched for moderate-high impact variants (minor allele frequency <0.01) in *PKD1, PKD2, PKHD1, PRKCSH, ALG9, DNAJB11, GANAB, HNF1B, IFT140, OFD1, SEC61B*, and *SEC63 (hereafter referred collectively as "cystic genes"*) to evaluate whether detected cystic kidney disease in these patients could be due to other variants. While data from this cohort indicate that high-quality exome sequencing can be used to reliably detect *PKD1* [18], others have suggested limitations with exome sequencing for *PKD1*[19]; potential applicability requires validation and correlation in other cohorts. Mean depth of coverage for *ALG8* was 55.3 (SD 18.4) (see Supplementary Table S1 for additional details on depth coverage for other cystic genes). Variants were cross-referenced with ClinVar (accessed 11/22/2021) to evaluate whether they had previously been listed as pathogenic or likely pathogenic for any disease. Genetic relatedness was determined using Pedigree Reconstruction and Identification of a Maximum Unrelated Set (PRIMUS). [20]

Phenotyping

We used data from the electronic health record (EHR) to ascertain whether participants had International Classification Diseases (ICD) diagnosis codes for cystic kidney disease-related outcomes (see Supplementary Table S2 for full set of ICD codes).

To rigorously examine the association between *ALG8* PTV heterozygotes and a milder cystic kidney disease phenotype, we conducted focused chart review in patients with previously described ClinVar pathogenic or likely pathogenic *ALG8* variants vs. a matched control group of relatives of *ALG8* PTV carriers by at least 2 reviewers, including a nephrologist. Chart review focused on kidney and liver imaging data, cerebral aneurysms, history of dialysis, transplant, and family history of ADPKD. Imaging phenotyping was performed by an independent, blinded radiologist (GS) with difficult calls reviewed with a senior radiologist (WT), following similar procedures as done in a prior study that confirmed another cystic gene, *ALG9,* as a cause of cystic liver and kidney disease. [7]

Outcomes

For ICD code-based analyses, the primary outcome was a composite outcome that included ADPKD (Q61.2, Q61.3, 753.13, 753.12), cystic kidney disease (Q61.9, 753.10), congenital kidney cyst (Q61.00, Q61.01, A61.02, 753.11, 753.19), acquired kidney cysts (N28.1,

593.2), liver cystic disease (Q44.6, 573.8, 751.62). Additional outcomes included cystic kidney disease-related conditions— nephrolithiasis, ESKD, eGFR <60 ml/min/1.73m^2, kidney failure (dialysis, kidney transplant or last eGFR <15 ml/min/1.73m^2), hypertension (defined by ICD codes, blood pressure values and medications); we also examined kidney cancer (Supplementary Table S2 for full details).

For imaging-based analyses, the primary outcome was ≥ 4 cysts on computed tomography (CT), or magnetic resonance imaging (MRI). We used a ≥ 4 cyst cutoff as a study of health organ donors found that among adults > age 50 years of age 1, 2, or 3 cysts seen in 26%, 9,8%, and 4.3% of healthy organ donors while only 1.2% had ≥ 4 cysts. [21] Secondary imaging outcomes included ≥ 4 cysts or too small to characterize (TSTC) hypodense lesions, bilateral kidney cysts, bilateral kidney cysts or TSTC hypodense lesions, ≥ 1 liver cyst, ≥1 liver cyst or TSTC hypodense lesions, nephrolithiasis (clinical history or on imaging). These outcomes were examined as TSTC hypodense lesions and subclinical nephrolithiasis may be seen on imaging and could reflect early manifestations of genetic cystic kidney disease.

Statistical Analyses

We examined whether *ALG8* PTV heterozygotes were at higher risk for ADPKD-related outcomes compared to controls (without any cystic gene PTVs) using logistic regression, adjusted for age, sex, genetic ancestry, and clustered by family network.

As we expected ICD code diagnoses would not capture milder cystic kidney disease, our primary analyses focused on a matched subcohort of *ALG8* P/LP PTV heterozygotes and related non-heterozygotes above the age of 30 who had complete imaging of both kidneys by either CT or MRI. *ALG8* P/LP PTV heterozygotes were matched 1:1 on imaging modality (CT with IV contrast, CT without IV contrast, or MRI) and age quartile (at time of imaging) with participants in the non-heterozygote relative cohort. Logistic regression was used to calculate odds ratios (OR) for imaging-based categorical outcomes. In addition, we conducted a sensitivity analysis that included *ALG8* P/LP PTV of any age with complete imaging of both kidneys by either CT or MRI, matched 1:1 with non-heterozygotes by imaging modality and age quartile (at time of imaging). P values <0.05 were considered statistically significant, and all analyses were conducted using Stata/MP 15.1 (College Station, TX).

UK Biobank replication cohort

To confirm associations between *ALG8* P/LP PTV and cystic kidney disease-related traits in an independent cohort, we examined publicly-available association analyses on the Genebass web browser (app.genebass.org) of 4,529 phenotypes on 394,841 individuals in the UK BioBank. [22] Quality control included minimum coverage of 20x, minimum of 2 variants per group test. Gene-based burden association tests were done using scalable generalized mixed-model region-based association tests (SAIGE-GENE). [23] Cystic kidney disease-related phenotypes included self-reported (patient interview) polycystic kidney disease and hypertension, ICD code diagnoses Q61 (includes ADPKD, cystic kidney disease, and congenital kidney cyst ICD codes), N28 (other disorders of kidney and ureter, not elsewhere classified; includes acquired kidney cyst ICD code), N20 (calculus of kidney

and ureter), and N18 (chronic kidney disease [CKD]). These combinations of ICD codes differed and were less specific than definitions used in the MyCode cohort (see Table 1 for details), and thus we considered all outcomes equally and used a Bonferroni-adjusted p value threshold of <0.0071 for statistical significance.

Results:

MyCode cohort of *ALG8* PTV heterozygotes and controls without cystic gene PTVs

Out of 174,172 participants, there were 236 participants with an *ALG8* PTV (Figure 1). Demographics were similar for *ALG8* heterozygotes and 171578 controls without any cystic gene PTVs (mean age 55.9 vs. 57.5 y; female sex 60.2% vs. 60.6%; European American 92.3% vs. 94.3%; follow-up time 13.2 vs. 12.9 years; Supplementary Table S3). *ALG8* PTV heterozygotes were at increased risk of the primary composite outcome—any kidney/liver cyst ICD code (8.47% vs. 4.08%; OR 2.42, 95% CI: 1.53–3.85; p= 1.8e-04), cystic kidney disease (2.14% vs. 0.78%; OR 3.03, 95% CI: 1.26–7.31; p=0.01), congenital kidney cyst (3.42% vs. 1.07%; OR 3.53, 95% CI: 1.73–7.21; p=0.001), as well as nephrolithiasis (9.83% vs. 5.80%; OR 1.89, 95% CI: 1.96–2.97; p=0.006) (Table 1). *ALG8* PTVs were not associated with ICD diagnoses of ADPKD, liver cystic disease, hypertension, kidney failure, kidney cancer, or eGFR <60 ml/min/1.73m^2.

Matched cohort of ClinVar Pathogenic/likely pathogenic *ALG8* PTV Heterozygotes and related non-heterozygotes

Focused chart and imaging review was performed on 103 participants from 85 families who had a PTV that was listed as pathogenic (P) or likely pathogenic (LP) in Clinvar and 55 non-heterozygous relatives. P/LP variants included Arg364Ter (n=85), Arg179Ter (n=9), Arg41Ter (n=7), and a splice-donor variant c.368+2T>G (n=1). Demographic characteristics and availability of CT imaging (44.5% and 56.4%) and MRI imaging (3.9% and 10.9%) were similar in *ALG8* heterozygotes and non-heterozygotes (Supplementary Table S3). The most common indications for CT imaging studies were abdominal pain (38%) flank pain/nephrolithiasis (16%), trauma (11%), and then evaluating renal or liver lesion (8%). Among heterozygotes and non-heterozygotes with US imaging, kidney sizes were not significantly different (right kidney 11.0 vs. 11.0 cm; left kidney (11.3 vs. 11.5 cm). Images are shown for Arg364Ter heterozygotes, Arg179Ter heterozygotes, Arg41Ter heterozygotes, and the splice-donor variant carrier (Figures 2,3). Full individual-level details are shown in Supplementary Tables S6–8.

Blinded imaging-based analyses

Out of 42 *ALG8* PTV heterozygotes and 28 related non-heterozygotes who were at least 30 years of age and had CT or MRI imaging, a total of 26 heterozygotes were matched 1:1 to 26 non-heterozygotes on imaging modality, age quartile (at time of imaging), and sex. Mean age at time of imaging was 58.0 and 58.6 years for matched heterozygotes and non-heterozygotes (Supplementary Table S5). The most common imaging modality used for evaluation was CT with IV contrast (85%), followed by CT without IV contrast (12%), and then MRI without contrast (4%). *ALG8* PTV heterozygotes had increased prevalence of ≥4 kidney cysts (57.7% vs. 7.7%; OR 16.36, 95% CI: 3.13–85.63; p=9.3e-04), ≥4 kidney cysts

or TSTC hypodense lesions (73.1% vs. 19.2%; OR 11.40, 95% CI: 3.05–42.56; p=2.9e-04), bilateral renal cysts (69.2% vs. 15.4%; OR 12.38, 95% CI: 3.16–48.48; p=3.0e-04), and bilateral renal cysts or TSTC hypodense lesions (84.6% vs. 23.1%; OR 18.33, 95% CI: 4.45–75.57; p=5.7e-05) (Table 2). *ALG8* PTV heterozygotes did not have significantly increased prevalence of liver cysts (11.5% vs. 7.7%; OR 1.57, 95% CI: 0.23–10.43; p=0.64) or liver cysts/TSTC hypodense lesions (23.1% vs. 15.4%; OR 1.65, 95% CI: 0.40–6.80; p=0.49) in this matched imaging-based analysis.

Of the 26 patients with *ALG8* P/LP PTVs who had imaging reviewed and had cystic kidney disease detected, only 2 had a moderate-high impact variant in another cystic gene. This included a patient (STUDY_ID87989 *ALG8*-p.Arg364Ter and a truncating *PKHD1*-p.Trp1248Ter), who had ≥4 bilateral kidney cysts and normal-sized kidneys on a CT scan at age 63; and 1 patient (STUDY_ID124992 *ALG8*-p.Arg364Ter and a variant of unknown significance *HNF1B*-p.Phe62Val) who had bilateral cysts and TSTCs (Supplementary Tables S6–8). We were unable to determine whether these additional variants contributed to these 2 patients' cystic kidney disease, or whether the variants in either patient occurred in cis or trans due to lack of parental data.

Findings were similar in a sensitivity analysis that also included individuals in the cohort under the age of 30 with CT or MRI imaging (Supplementary Table S9). Findings were generally similar across PTVs although sample sizes were limited for formal comparison. Prevalence of kidney and liver phenotypes for each of the *ALG8* variants are shown in Table 3 and Supplementary Tables S10 and S11.

Variability of presentations of *ALG8* Arg364Ter heterozygotes within families

There were 15 families where an *ALG8* Arg364Ter heterozygote had available imaging and at least 1 family member also in our research cohort. The full details of each family are shown in Supplementary Tables S6–8. Among these 15 families, there was variable penetrance within families although data are limited given heterogeneity of availability, type, and age at time of imaging. Nine families had at least 1 *ALG8* Arg364Ter heterozygote with ≥4 cysts on imaging. Of 5 families that had 2 *ALG8* Arg364Ter heterozygotes with sufficient imaging data, ≥4 cysts were seen in 100% of heterozygotes in 3 families, and ≥4 cysts was seen in 50% of heterozygotes in 2 families.

UK Biobank Genebass browser data

There were 468 individuals (cumulative allele frequency 5.82E-4) with 45 *ALG8* PTVs, including 71 with the Arg364Ter variant, 38 with the Arg179Ter variant, 24 with the Arg41Ter variant, and 9 with the splice donor (c.368+2T>G) variant. After correction for multiple comparisons, *ALG8* PTV carriers were at increased risk of ICD code N28 (other disorders of kidney and ureter, not elsewhere specified) (p=6.68E-05). *ALG8* PTV heterozygotes had numerically higher prevalence of Q61 ICD codes (0.43% vs. 0.19%; p=0.279), self-reported polycystic kidney disease (0.21% vs. 0.07%; p=0.293), N20 (calculus of kidney and ureter; 2.35% vs. 1.49%; p=0.162) though these comparisons were not statistically significant (Table 1).

Discussion:

In this study we demonstrate that *ALG8* PTVs increase the risk of cystic kidney disease though the phenotype appears to be weaker than traditional ADPKD and more akin to *ALG9* and *DNAJB11*[1,7]. Despite the increased risk of cystic kidney disease, *ALG8* PTV heterozygotes were not at increased risk of CKD or kidney failure in our cohort or the UK Biobank. Classic ADPKD caused by *PKD1* and *PKD2* typically develop a large quantity of bilateral renal cysts with enlarged kidneys and multiple family members progressing to ESKD. While the cystic kidney disease phenotype observed in our study was mild, *ALG8* PTV heterozygotes were also at increased risk of nephrolithiasis. These data provide support for classifying *ALG8* PTVs as a cause of mild cystic kidney disease. To aid with *ALG8* variant classification efforts, we also provide variant-level data (Supplementary Tables S10–11).

While we found no significant association between *ALG8* PTV heterozygotes and polycystic liver phenotypes, liver cysts were much rarer than kidney cysts, limiting our ability to detect a difference. The presence of *ALG8* variants in polycystic liver disease was previously described in a cohort of patients with unresolved polycystic liver disease. [2] By examining a largely unselected health system-based cohort using a genotype-first approach, we are able to provide deeper insights on penetrance, phenotypic severity, and the full spectrum of clinical presentations associated with *ALG8* gene variants. Additional research with larger and more diverse cohorts of *ALG8* PTV heterozygotes would be helpful to definitively determine risks of liver cysts, nephrolithiasis and kidney failure and aid with variant classification efforts.

Our study adds evidence that alterations in enzymes involved in endoplasmic reticulum processing of polycystin-1 are linked to polycystic kidney disease phenotypes, albeit less severe than PTVs in *PKD1*. [1–3] Given the heterogeneous severity of polycystic kidney disease in our cohort of *ALG8* PTV heterozygotes, the phenotypic severity may depend on a number of additional genetic and environmental factors. Even within families, the cystic kidney disease phenotypes varied widely among relatives carrying *ALG8* PTVs and was often unrecognized in terms of the infrequency of ICD diagnosis codes for cystic kidney disease and reported family history. Kidney length, a reasonable surrogate for total kidney volume (TKV) and kidney failure,[24] was no different compared to non-heterozygotes.

As abdominal imaging has become quite routine in the care of patients presenting with abdominal complaints and for many other reasons, there are many incidental findings of kidney cysts, which could prompt additional anxiety for ADPKD and ESKD. Our study provides important, somewhat reassuring data that individuals found to have *ALG8* PTV are at increased risk of mild cystic kidney disease with far fewer consequences compared to patients with ADPKD due to *PKD1* or *PKD2*. The increased use of large gene panels or exome sequencing in the workup of cystic kidney disease also enables the detection of multiple cystic gene variants simultaneously.[8,10,25] More research is needed to understand whether individuals with multiple variants in different cystic genes are at increased risk for cystic kidney disease and ESKD.

There were several strengths and some limitations in our study. First, we used an unselected patient population to gain a better sense of penetrance, phenotypic severity, and phenotypic spectrum of cystic disease in *ALG8* PTV heterozygotes. Second, we used related family members of *ALG8* PTV heterozygotes as a comparison group, reducing residual confounding since family members will share much of the same environmental and background genetic risk factors. Third, blinded imaging review was performed, which revealed subtle cystic kidney disease in patients without ICD code diagnoses. Lastly, we found consistent findings in UK Biobank data for all codes though only one of the renal phenotypes (other disorders of kidney and ureter) was significant. The relative insensitivity of ICD codes and lack of radiology imaging data in the UK Biobank data included in our study is a limitation given the mild cystic phenotype observed in our carefully reviewed matched imaging analysis. While reliance on routinely collected EHR data is a limitation in MyCode, the proportion of participants with imaging was similar in both groups, and our analysis strategy is likely conservative since non-heterozygote participants with kidney imaging are expected to be more likely to have a kidney-related problem compared to those who have no imaging. Our study was limited to a mostly European ancestry population, reflecting characteristics of the overall Geisinger population; the UK Biobank is 94.6% white and healthier than the general population.[26] Regardless, our study provides supportive data that will be need to evaluated by the ClinGen Kidney Cystic and Ciliopathy Variation Curation Expert Panel to determine whether the strength of gene-disease relationships for *ALG8* with “polycystic liver disease 3 with or without kidney cysts” should be revised.

In conclusion, our study demonstrates that *ALG8* PTVs are associated with a mild cystic kidney disease phenotype and increased risk of nephrolithiasis though not CKD or kidney failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

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Data availability statement:

The data supporting the findings of this study are available within the article and its Supplementary Data files. Additional information for reproducing the results described in the article is available upon reasonable request and subject to a data use agreement.

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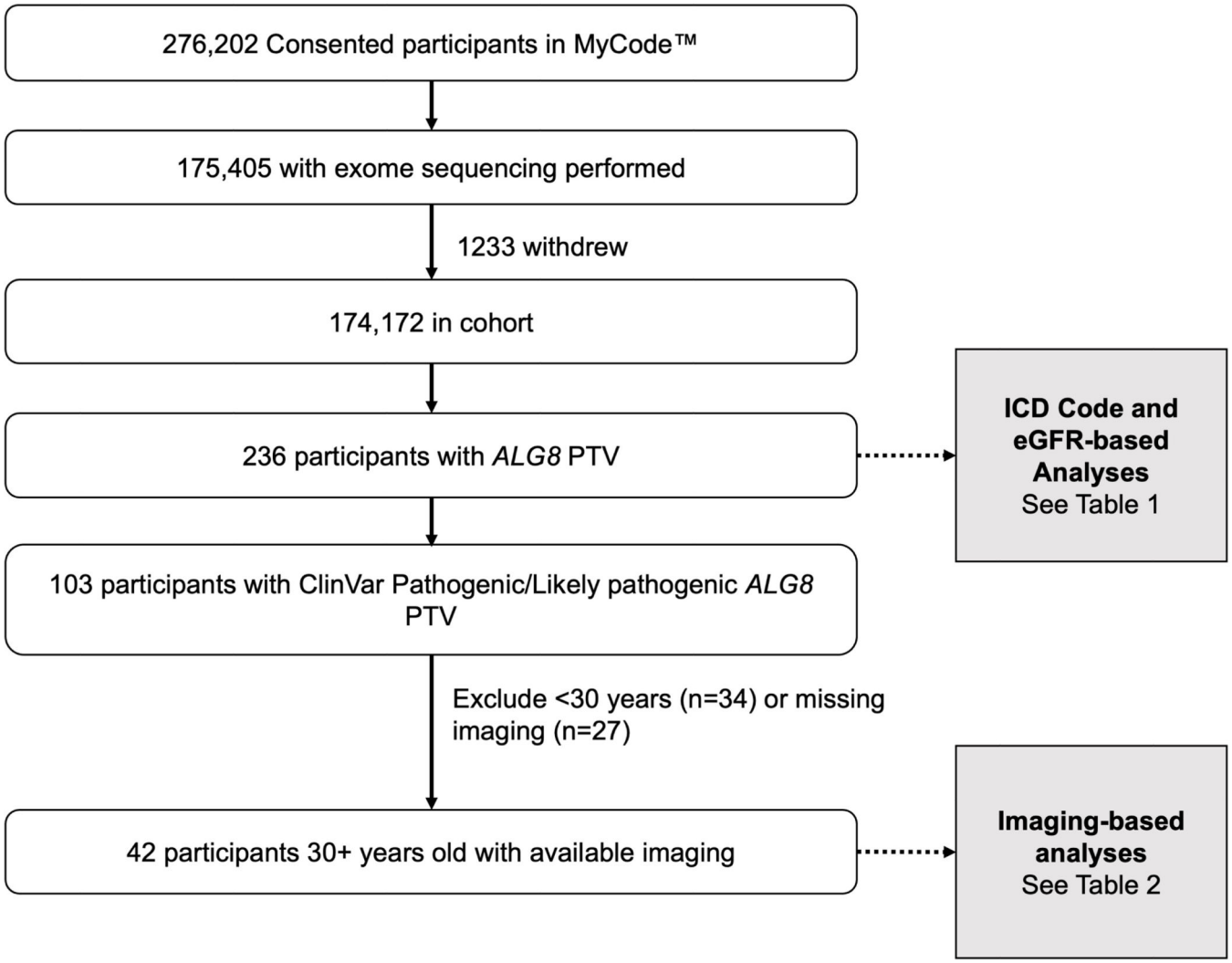

Figure 1. Flowchart

Abbreviations: PTV (protein truncating variant), eGFR (estimated glomerular filtration rate), ICD (International Classification of Diseases)

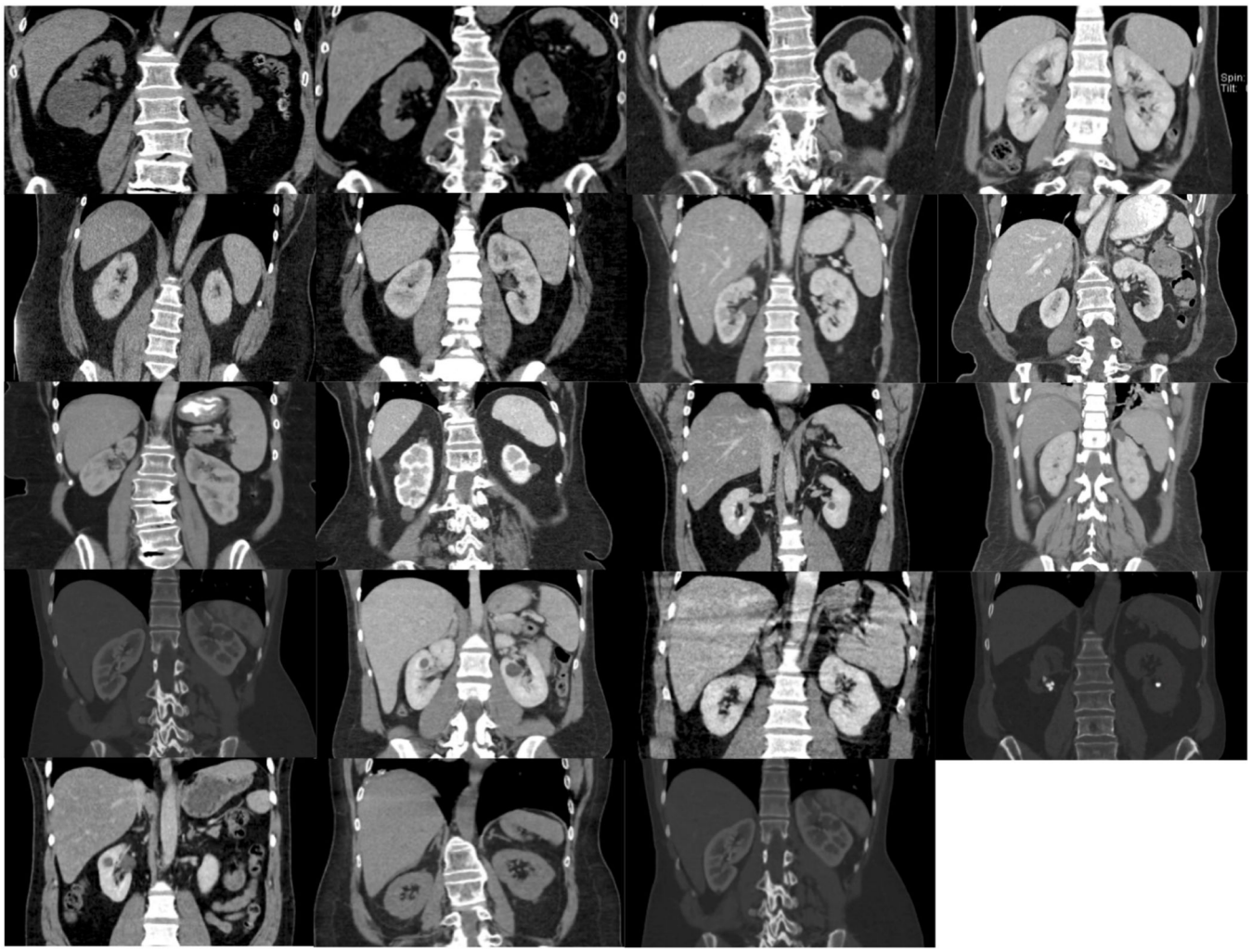

Figure 2. Radiologic Imaging of Families with *ALG8* p.Arg364Ter Heterozygotes

Representative imaging collected from 19 *ALG8* p. Arg364Ter heterozygotes from 9 families.

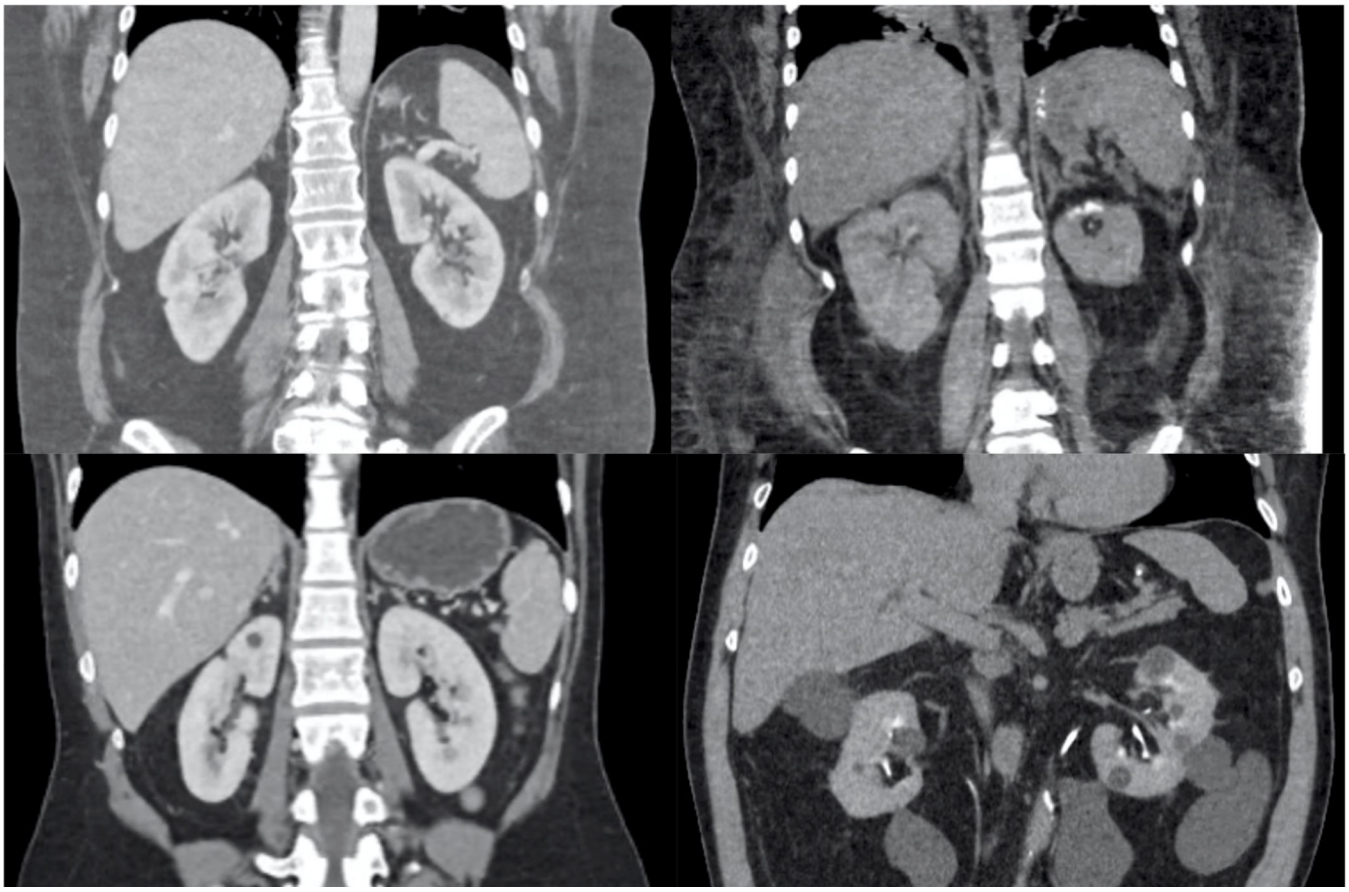

Figure 3. Radiologic Imaging of a Family with *ALG8* p.Arg179Ter Heterozygotes and an individual with ALG8 Arg41Ter

The images on the top and the bottom left show representative imaging from 3 *ALG8* p.Arg179Ter heterozygotes from 2 families. The bottom right image is from an individual heterozygous for *ALG8* Arg41Ter.

Table 1.

ALG8 PTV heterozygotes and risks of PKD-related diagnoses in MyCode and the UK Biobank

MyCode			
Diagnosis	**ICD codes**	**Prevalence in PTV heterozygotes and non-carriers**	**P value***
Any kidney/liver cyst ICD code	Includes ICD codes listed below for ADPKD, cystic kidney disease, congenital kidney cyst, liver cystic disease, acquired kidney cyst	8.47% vs. 4.08%	1.8e-04
ADPKD	ICD-10 **Q61.2, Q61.3**; ICD-9 753.13, 753.12	0.43% vs. 0.11%	0.17
Cystic kidney disease	ICD-10 **Q61.9**; ICD-9 753.10	2.14% vs. 0.78%	0.01
Congenital kidney cyst	ICD-10 **Q61.00, Q61.01, Q61.02**; ICD-9 753.11, 753.19	3.42% vs. 1.07%	0.001
Liver cystic disease	ICD-10 **Q44.6**; ICD-9 573.8, 751.62	1.69% vs. 1.30%	0.50
Acquired kidney cyst	ICD-10 **N28.1**; ICD-9 593.2	2.56% vs. 1.52%	0.11
Nephrolithiasis	ICD-10 **N20**; ICD-9 592.0, 592.1, 592.9, 594.0, 594.1, 594.2, 594.8, 594.9, 274.11	9.83% vs. 5.80%	0.006
Hypertension	See appendix	44.93% vs. 51.74%	0.16
eGFR <60 ml/min/1.73m^2	N/A	18.78% vs. 20.04%	0.73
Kidney failure	See appendix	0.85% vs. 1.67%	0.37
Kidney cancer	See appendix	0% vs. 0.70%	-
UK Biobank			
Diagnosis	**ICD codes**	**Prevalence in PTV heterozygotes and non-carriers**	**P value****
Cystic kidney disease (includes ADPKD and cystic kidney disease)	Q61 (**Q61.00, Q61.01, Q61.02**, Q61.1, **Q61.2, Q61.3**, Q61.4, Q61.5, Q61.8, **Q61.9**)	0.43% vs. 0.19%	0.28
Other disorders of kidney and ureter, not elsewhere specified (includes acquired kidney cyst)	N28 (N28.0, **N28.1**, N28.8, N28.9)	3.85% vs. 1.33%	6.68E-05
Self-reported PKD	N/A	0.21% vs. 0.07%	0.29
Congenital malformations of gallbladder, bile ducts, and liver	Q44 (Q44.0, Q44.1, Q44.2, Q44.3, Q44.4, Q44.5, **Q44.6**, Q44.7)	0.21% vs. 0.05%	0.16
Calculus of kidney and ureter	**N20**	2.35% vs. 1.49%	0.16
Hypertension	Self-reported	22.6% vs. 26.0%	0.05
Chronic kidney disease	N18	4.91% vs. 3.57%	0.11

*In MyCode cohort, p values are from logistic regression analyses adjusted for age, sex, and genetic ancestry, clustered by family.

**In publicly-available UK Biobank data (app.genebass.org), bolded ICD codes are coded that overlap with ICD codes used in MyCode analyses. P values are from gene burden tests using SAIGE-GENE. As phenotypes in UK Biobank were less specific than ours and could not be customized, we considered each outcome equally and used a Bonferroni-corrected p value <0.0071 for statistical significance.

Abbreviations: ICD (International Classification of Diseases), PTV (protein truncating variant), ADPKD (autosomal dominant polycystic kidney disease),

Table 2.

Kidney and Liver Cystic Phenotypes of matched *ALG8* P/LP PTV heterozygotes and non-heterozygotes 30+ years of age with CT or MRI imaging

	***ALG8* P/LP variant heterozygote s (n=26)**	**Non-heterozygotes (n=26)**	**OR (95% CI)**	**P value**
Primary outcome ≥ 4 kidney cysts	15 (57.7%)	2 (7.7%)	16.36 (3.13–85.63)	9.3e-04
	Secondary outcomes			
≥ 4 kidney cysts or TSTC hypodense lesions	19 (73.1%)	5 (19.2%)	11.40 (3.05–42.56)	2.9e-04
Bilateral kidney cysts	18 (69.2%)	4 (15.4%)	12.38 (3.16–48.48)	3.0e-04
Bilateral kidney cysts or TSTC hypodense lesions	22 (84.6%)	6 (23.1%)	18.33 (4.45–75.57)	5.7e-05
≥1 Liver cyst	3 (11.5%)	2 (7.7%)	1.57 (0.23–10.43)	0.64
≥1 Liver cyst or TSTC hypodense lesions	6 (23.1%)	4 (15.4%)	1.65 (0.40–6.80)	0.49
Nephrolithiasis	14 (53.4%)	8 (30.1%)	2.63 (0.83–8.26)	0.10

Heterozygotes were matched 1:1 to individuals in the non-heterozygote relative cohort by age quartile, sex, and imaging modality (CT with IV contrast (n=22 pairs); CT without IV contrast (n=3 pairs), MRI without contrast (n=1 pair)

Abbreviations: PTV (protein truncating variant), TSTC (too small to characterize), P/LP (pathogenic/likely pathogenic), CT (computed tomography), MRI (magnetic resonance imaging), US (ultrasound)

Table 3.

Kidney and Liver Cystic Phenotypes of *ALG8* P/LP PTV heterozygotes with CT or MRI imaging, by Variant

	NM_004284.5:c.1090C> T (p.Arg364Ter) Age ≥30: n=34 Age <30: n=4	**NM_004284.5:c.5 35C>T (p.Arg179Ter) Age ≥30: n=2 Age <30: n=1**	**NM_004284.5:c.121 C>T (p.Arg41Ter) Age ≥30: n=5 Age <30: n=0**	**NM_004284.5: c.368+2T>G Age ≥30: 1 Age <30: 0**
Consequence	Stop-gained	Stop-gained	Stop-gained	Splice donor
ClinVar classification	Clinvar ID 280116 Pathogenic 8 submission 2 stars	Clinvar ID 492977 Pathogenic 2 submissions 1 star	Clinvar ID 96090 Pathogenic 4 submissions 2 stars	Clinvar ID 863568 Likely pathogenic 1 submission 1 star
Conditions listed in Clinvar	Polycystic liver disease 3 with kidney cysts (AD); Congenital disorder of glycosylation type 1H (AR)	Polycystic liver disease 3 with kidney cysts (AD)	Congenital disorder of glycosylation type 1H (AR)	Congenital disorder of glycosylation type 1H (AR)
≥ 4 kidney cysts	Age ≥30: 18/34 (52.9%) Age <30: 1/4 (25%)	Age ≥30: 0/2 Age <30: 0/1	Age ≥30: 2/5 (60%)	Age ≥30: 1/1 (100%)
≥ 4 kidney cysts or TSTCs	Age ≥30: 25/34 (73.5%) Age <30: 2/4 (50%)	Age ≥30: 2/2 (100%) Age <30: 0/1 (50%)	Age ≥30: 3/5 (60%)	Age ≥30: 1/1 (100%)
Bilateral kidney cysts	Age ≥30: 23/34 (67.7%) Age <30: 2/4 (50%)	Age ≥30: 1/2 (50%) Age <30: 0/1	Age ≥30: 3/5 (60%)	Age ≥30: 1/1 (100%)
Bilateral kidney cysts or TSTCs	Age ≥30: 27/34 (79.4%) Age <30: 2/4 (50%)	Age ≥30: 2/2 (100%) Age <30: 1/1 (100%)	Age ≥30: 4/5 (80%)	Age ≥30: 1/1 (100%)
Liver cysts	Age ≥30: 3/34 (8.8%) Age <30: 0/4	Age ≥30: 0/2 Age <30: 0/1	Age ≥30: 1/5 (20%)	Age ≥30: 1/1 (100%)
Liver cysts or TSTCs	Age ≥30: 10/34 (29.4%) Age <30: 1/4 (25%)	Age ≥30: 0/2 Age <30: 0/1	Age ≥30: 1/5 (20%)	Age ≥30: 1/1 (100%)
Nephrolithiasis	Age ≥30: 18/34 (52.9%) Age <30: 1/4 (25%)	Age ≥30: 0/2 Age <30: 0/1	Age ≥30: 3/5 (60%)	Age ≥30: 1/1 (100%)

Abbreviations: TSTC (too small to characterize)