

Copy Number Variant Analysis and Genome-wide Association Study Identify Loci with Large Effect for Vesicoureteral Reflux

Miguel Verbitsky,¹ Priya Krithivasan,¹ Ekaterina Batourina,² Atlas Khan,¹ Sarah E. Graham,³ Maddalena Marasà ,¹ Hyunwoo Kim,² Tze Y. Lim,¹ Patricia L. Weng,⁴ Elena Sánchez-Rodríguez,¹ Adele Mitrotti,^{1,5} Dina F. Ahram,¹ Francesca Zanoni,¹ David A. Fasel ,¹ Rik Westland,^{1,6} Matthew G. Sampson,⁷ Jun Y. Zhang,¹ Monica Bodria,⁸ Byum Hee Kil,¹ Shirlee Shril,⁹ Loreto Gesualdo,¹⁰ Fabio Torri,¹¹ Francesco Scolari,¹² Claudia Izzi,¹³ Joanna A.E. van Wijk,⁶ Marijan Saraga,^{14,15} Domenico Santoro,¹⁶ Giovanni Conti,¹⁷ David E. Barton,^{18,19} Mark G. Dobson,^{19,20} Prem Puri,^{20,21} Susan L. Furth,²² Bradley A. Warady ,²³ Isabella Pisani,²⁴ Enrico Fiaccadori,²⁴ Landino Allegri,²⁴ Maria Ludovica Degl'Innocenti,⁸ Giorgio Piaggio,⁸ Shumyle Alam,²⁵ Maddalena Gigante,¹⁰ Gianluigi Zaza,²⁶ Pasquale Esposito,²⁷ Fangming Lin,²⁸ Ana Cristina Simões-e-Silva ,²⁹ Andrzej Brodkiewicz,³⁰ Dorota Drozd,³¹ Katarzyna Zachwieja ,³¹ Monika Miklaszewska ,³¹ Maria Szczepanska,³² Piotr Adamczyk,³² Marcin Tkaczyk ,³³ Daria Tomczyk,³³ Przemysław Sikora ,³⁴ Malgorzata Mizerska-Wasiak,³⁵ Grazyna Krzemien,³⁵ Agnieszka Szmigielska,³⁵ Marcin Zaniew,³⁶ Vladimir J. Lozanovski,^{37,38} Zoran Gucev,³⁸ Iuliana Ionita-Laza,³⁹ Ian B. Stanaway,⁴⁰ David R. Crosslin,⁴⁰ Craig S. Wong,⁴¹ Friedhelm Hildebrandt,⁹ Jonathan Barasch,^{1,2} Eimear E. Kenny,^{42,43,44} Ruth J.F. Loos,⁴⁵ Brynn Levy,⁴⁶ Gian Marco Ghiggi ,⁸ Hakon Hakonarson,⁴⁷ Anna Latos-Bieleńska,⁴⁸ Anna Materna-Kiryluk,⁴⁸ John M. Darlow,^{19,20} Velibor Tasic,³⁸ Cristen Willer,^{3,49,50} Krzysztof Kiryluk,¹ Simone Sanna-Cherchi,¹ Cathy L. Mendelsohn,² and Ali G. Gharavi¹

Due to the number of contributing authors, the affiliations are listed at the end of this article.

ABSTRACT

Background Vesicoureteral reflux (VUR) is a common, familial genitourinary disorder, and a major cause of pediatric urinary tract infection (UTI) and kidney failure. The genetic basis of VUR is not well understood.

Methods A diagnostic analysis sought rare, pathogenic copy number variant (CNV) disorders among 1737 patients with VUR. A GWAS was performed in 1395 patients and 5366 controls, of European ancestry.

Results Altogether, 3% of VUR patients harbored an undiagnosed rare CNV disorder, such as the 1q21.1, 16p11.2, 22q11.21, and triple X syndromes (OR, 3.12; 95% CI, 2.10 to 4.54; $P=6.35 \times 10^{-8}$). The GWAS identified three study-wide significant and five suggestive loci with large effects (ORs, 1.41–6.9), containing canonical developmental genes expressed in the developing urinary tract (*WDPCP*, *OTX1*, *BMP5*, *VANGL1*, and *WNT5A*). In particular, 3.3% of VUR patients were homozygous for an intronic variant in *WDPCP* (rs13013890; OR, 3.65; 95% CI, 2.39 to 5.56; $P=1.86 \times 10^{-9}$). This locus was associated with multiple genitourinary phenotypes in the UK Biobank and eMERGE studies. Analysis of *Wnt5a* mutant mice confirmed the role of *Wnt5a* signaling in bladder and ureteric morphogenesis.

Conclusions These data demonstrate the genetic heterogeneity of VUR. Altogether, 6% of patients with VUR harbored a rare CNV or a common variant genotype conferring an OR >3. Identification of these genetic risk factors has multiple implications for clinical care and for analysis of outcomes in VUR.

JASN 32: 805–820, 2021. doi: <https://doi.org/10.1681/ASN.2020050681>

Vesicoureteral reflux (VUR), the retrograde flow of urine from the bladder toward the kidneys, caused by malfunction at the vesicoureteral junction, is a common cause of febrile urinary tract infection (UTI) and pediatric kidney failure.^{1,2} VUR occurs most often in isolation, but can arise in conjunction with more complex defects, such as hydronephrosis and renal dysplasia.^{3–5} VUR has a prevalence of 1%–2% in European populations^{6–9} and is much more prevalent in females (>70%), although males predominate in patients diagnosed between 0 and 2 years of age.^{10,11} VUR is highly familial, with a reported occurrence rate of 27%–51% among siblings, and 66% among offspring of affected individuals.⁹ Segregation analyses and linkage studies suggested the contribution of rare variants with large effects and multiple modes of inheritance.^{12–19}

Urinary tract formation in the mouse begins at E9, when nephric ducts form and extend, joining the cloaca. Cloacal septation separates the hindgut from the urogenital sinus (UGS) between E11 and E14. Subsequently, the UGS differentiates into the bladder and urethra. The ureteric bud (UB) sprouts from nephric duct on E11, and its distal portion will form the ureter; however, it is initially joined to the nephric duct and not directly to the bladder. Mature ureter-bladder connections form between E10 and E13. Abnormalities in timing or morphogenesis at any stage can alter the site of ureter insertion, resulting in ectopic ureters or an abnormal connection at the vesicoureteral junction, causing VUR and obstruction.^{20–23}

Relatively little is known about the genetics of VUR in humans.²⁴ VUR is often present in syndromic disorders featuring congenital anomalies of the kidney and urinary tract (CAKUT).²⁵ Mutations in *ROBO2*,²⁶ *SOX17*,²⁷ and *TXNB28* have been implicated in rare families with isolated VUR, but most nonsyndromic patients are genetically unexplained. Genome-wide association studies (GWAS) of VUR have not been adequately powered.^{15,16,29} Recently, we reported that rare copy number variant (CNV) disorders also contribute to many urinary tract malformations, including VUR.³⁰ Although CNV disorders were detected in 7.7% of patients with kidney malformations, they accounted for only 1%–2% of VUR patients.³⁰ These findings motivated additional investigation of the genetics of VUR in larger cohorts, to confirm the contribution of CNV disorders, and explore alternative genetic models. Here, we combined genome-wide association and rare CNV analyses in the largest VUR cohort studied to date.

Significance Statement

Vesicoureteral reflux (VUR) is associated with progressive kidney disease. Familial aggregation supports a hereditary basis; however, its genetic architecture remains to be elucidated. The largest VUR copy number variant analysis and genome-wide association study to date accounts for multiple modes of inheritance and sex-specific effects in VUR, identifying three study-wide significant and five suggestive loci with large effects, containing canonical developmental genes including *WDPCP* and *WNT5A*. Results of experiments in mice support novel roles of *Wnt5a* in urogenital development. Altogether, 6% of patients carried high-risk genotypes. These findings have important implications for VUR screening.

We complemented these studies by analysis of expression pattern and developmental defects in *Wnt5a* mutant mice.

METHODS

Subjects and Genotyping

All aspects of the study involving human research participants adhered to the principles of the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Boards of Columbia University Medical Center and each participating recruitment site. Signed written informed consent by the participant and/or their parents or guardians was obtained according to the protocol of local Institutional Review Boards.

VUR patients and controls were obtained from the following study cohorts: VUR patients and healthy controls from Columbia University and US and international collaborating sites, patients from the Randomized Intervention for Children with VUR (RIVUR) study cohort,³¹ reflux nephropathy patients from the CKD in Children (CKiD) study,^{32,33} the Population Architecture using Genomics and Epidemiology consortium³⁴ convenience controls and an Irish cohort comprising VUR-affected children, and unrelated controls.¹⁵ As we reported previously,³⁰ 21,498 convenience controls were obtained from collaborators and from dbGaP (see Data Availability and Accession Numbers), which comprised participants of genome-wide genotyping studies of complex traits that are not associated with VUR or other developmental defects. VUR patients from the RIVUR and Irish cohorts were diagnosed by voiding cystourethrogram (VCUG); CKiD VUR patients had a primary diagnosis of reflux nephropathy; patients from the Columbia cohort, including US and international collaborators, were ascertained by VCUG or other diagnostic imaging such as renal scintigraphy. All of the samples from the Columbia University and RIVUR cohorts were genotyped for this study on the Illumina MEGA array. For the Population Architecture using Genomics and Epidemiology cohort, genotyped on MEGA arrays, and the Irish cohort, genotyped on Affymetrix SNP 6.0 chips, we obtained access to intensity level raw genotyping data. Of these patients, 660 were included in our prior study.³⁰

Received May 18, 2020. Accepted December 4, 2020.

M.V. and P.K. contributed equally to this work.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Ali G. Gharavi or Cathy L. Mendelshon, Columbia University, Medicine, New York, NY. Email: ag2239@cumc.columbia.edu or clm20@cumc.columbia.edu

Copyright © 2021 by the American Society of Nephrology

Initial Data Processing and Quality Control

Raw intensity probe level data were processed for each cohort using Illumina GenomeStudio v2011 (Illumina) or Affymetrix Power Tools software (Affymetrix) to generate genotyping calls. Using PLINK software,³⁵ markers with call rate (CR) $\geq 95\%$ and minor allele frequency (MAF) $> 1\%$ were used to select subjects based on sample CR ($\geq 95\%$ or 99% , for association, or copy number analyses, respectively). We used KING software³⁶ to estimate relatedness between samples within and across cohorts, and retained only unrelated samples (kinship coefficient < 0.0884). After examining the possibility of sex chromosomal abnormalities that could be reportable in our CNV analysis (e.g., Klinefelter syndrome), we excluded samples with discrepancies between genotyped sex and self-reported sex. Principal component analyses (PCA) were carried out using Smartpca.³⁷

CNV Analysis

PennCNV software³⁸ was used to determine CNV calls and quality; CNVs with confidence scores < 30 were excluded.^{30,38,39} CNV analyses were performed on hg18 coordinates, and subsequently mapped to hg19 using University of California Santa Cruz LiftOver software (<https://genome.ucsc.edu/cgi-bin/hgLiftOver>). Illumina Genome Viewer 1.9.0 or Affymetrix ChAS were used to visualize CNVs and evaluate possible artifacts. CNVs were classified as pathogenic (known genomic disorders [GDs]) or likely pathogenic CNVs, based on previously described criteria³⁰ adapted from recommendations from the American College of Medical Genetics guidelines^{40,41} and making use of annotations with RefGene, the Database of Genomic Variation and Phenotype in Humans using Ensembl Resources and International Standards for Cytogenomic Arrays (ISCA) databases, and curated lists of genes from the Online Mendelian Inheritance in Man and Mouse Genome Informatics databases and the literature. As previously described,^{30,42,43} a CNV was classified as pathogenic (known GD) when its genomic coordinates overlapped 70% or more of those of a well-characterized syndromic CNV, as defined in Database of Genomic Variation and Phenotype in Humans using Ensembl Resources⁴⁴ and the literature^{39,45–47}; whereas large, exon-intersecting CNVs with frequency in controls $\leq 0.02\%$, which did not overlap a benign or likely benign CNV in the ISCA database, were classified as likely pathogenic when they met one or more of the following conditions: overlapped 70% or more of the span of a pathogenic or likely pathogenic CNV in the ISCA database; intersected a gene associated with kidney disease or genitourinary tract defects or other developmental disorders in humans or mice; or overlapped 70% or more with the coordinates of a well-characterized syndromic CNV, with opposite copy number sign (loss or gain).

Our primary tests were to compare the burden of (1) the set of all large, rare CNVs and (2) a predefined set of 148 known GDs, between VUR patients and controls. The corrected *P* value threshold for testing whether VUR patients and

controls significantly differ in the proportion of carriers of these two sets of variants is $P = 0.05/2 = 0.025$. We next performed *post-hoc* tests for 18 individual GDs observed in VUR, with a corrected threshold of $P = 0.05/18 = 2.7 \times 10^{-3}$. Rare CNV burden analyses were performed on autosomal, gene-intersecting CNVs of size ≥ 100 kb in patients and controls, with a frequency $\leq 0.1\%$ in population subgroups based on PCA (Supplemental Figure 1), to avoid including CNVs that might be relatively common within ancestry groups represented in controls. Six controls, outliers in PCA without matching patients, were removed before burden analyses. For CNV burden analyses, the R v3.5.1 implementation of the two-sided Fisher's exact test was used to compare proportions, and Kaplan–Meier survival curves representing the largest CNV per genome were compared between patients and controls using the nonparametric log-rank test implemented in the Python package lifelines (<https://github.com/CamDavidsonPilon/lifelines/>).

GWAS

For the purpose of GWAS, samples were first grouped by genotyping platform into four case-control datasets (Affymetrix SNP6.0; Illumina MEGA; Illumina Omni 1, 2.5, or OmniExpress; Illumina 660, 610, or 550 arrays).

After conducting PCA on each platform-specific dataset, only subjects of European ancestry were selected and the MEGA array dataset was further subdivided based on ancestry (Northwestern European, Polish, Italian, and Southeastern European), making a total of seven genetically matched case-control GWAS cohorts, referred to in the Results section. PCA was then performed separately on each of these seven GWAS cohorts. Tracy-Widom statistics⁴⁸ from each of these PCAs were used to identify statistically significant PCs to be used as covariates in the association tests.

Imputation was performed separately for each cohort. After filtering out genotyped single nucleotide polymorphisms (SNPs) with a CR $< 95\%$, MAF $< 1\%$, or Hardy-Weinberg equilibrium exact test *P* value < 0.0001 in controls and updating or removing SNPs with discrepancies in strand, alleles, position, and frequencies (1000 G-check-bim.pl perl script from the McCarthy Group; <https://www.well.ox.ac.uk/~wrayner/tools/>), the Michigan Imputation Server (<https://imputationserver.sph.umich.edu/>) was used to carry out phasing (Eagle v2.3), and imputation (Minimac3) with the Haplotype Reference Consortium (r1.1 2016) reference panel. Imputation of chromosome X markers was run separately for males and females. DosageConvertor (v1.0.3 for autosomes, v1.0.4 for chromosome X) was used to convert dosage files from Minimac3 format to PLINK dose format. Only SNPs with high imputation quality ($r^2 \geq 0.8$) were retained.

Association analyses under additive, recessive, and dominant logistic regression models were then performed with Plink using logistic regression separately for each cohort with cohort-specific significant PCs included as covariates. For the additive model, dosage values for each SNP were

used. For the recessive and dominant models, dosage values were first converted to “best guess” genotypes (*i.e.*, genotypes with highest probability). All analyses were also repeated for males and females separately.

Association summary statistics from these GWAS, for 6,131,010 variants common to all seven cohorts, were meta-analyzed using the fixed-effects inverse variance weighted method implemented in METAL.⁴⁹

We used a *P* value threshold of 7.58×10^{-9} , ($5 \times 10^{-8} \div 2.2 \div 3$) as a conservative threshold for genome-wide statistical significance of associations and 1.52×10^{-7} ($20 \times 5 \times 10^{-8} \div 2.2 \div 3$) for suggestive associations, to account for testing of multiple variants: the value of 5×10^{-8} is accepted as the standard threshold for genome-wide significant associations⁵⁰; dividing by 2.2 accounts for the three genetic models tested, as shown by González *et al.*⁵¹; and dividing by three accounts for testing in males and females, both separately and combined.

Genomic inflation factor lambda was calculated using the *estlambda* function from the R package GenABEL. Quantile-quantile and Manhattan plots were generated in R using the qqman package. Regional plots for top were created with LocusZoom⁵² (<http://csg.sph.umich.edu/locuszoom/>), using LD information (r^2) from the 1000 Genomes European population (November 2014).

Heritability and Polygenic Risk Scores

We used LDSC software⁵³ (<https://github.com/bulik/ldsc>) to estimate VUR heritability and LDPred⁵⁴ (<https://github.com/bvilhjal/ldpred>), to derive a polygenic risk score (PRS) for VUR, based on the summary statistics from our VUR GWAS under an additive model. Next, we used LDPred to generate the VUR-PRS for individuals of European ancestry in the CKiD study cohort ($n=432$). As opposed to genotypic risk scores, which are selectively based on top signals, or PRS estimated using linkage disequilibrium (LD)-based marker pruning and a *P* value threshold, PRS estimation from GWAS summary statistics with LDPred, uses a Bayesian approach with *a priori* on effect sizes and LD information from an external reference panel (1000 Genomes panel) to derive the posterior mean effect size of each marker.⁵⁴ All common markers in our VUR GWAS summary statistics intersecting the reference panel, with a defined effect estimate value in the meta-analysis were used ($n=5,061,103$ markers).

Single Variant PheWAS in the Electronic Medical Records and Genomics and UK Biobank Datasets

Single variant PheWAS analysis under an additive model was performed for our GWAS eight top variants, using UK Biobank (UKBB; <http://pheweb.sph.umich.edu/SAIGE-UKB/>) and Electronic Medical Records and Genomics (eMERGE; <https://emerge-network.org/>) datasets. In the latter, we conducted the analyses in the whole cohorts and separately in the subgroup of participants under 21 years of age. International Classification of Disease (ICD) codes were converted

ICD-9 codes (<https://www.cms.gov/Medicare/Coding/ICD9ProviderDiagnosticCodes/codes>) for PheWAS analysis,⁵⁵ which mapped to totals of 1817 distinct phecodes. PheWAS was performed using the PheWAS R package.⁵⁶ The package uses predefined “control” groups for each phecode “case” grouping. The case definition requires a minimum of two ICD-9 codes from the “case” grouping of each phecode. All phecodes were tested with logistic regression with each phecode case-control status as an outcome and genotype coded according to the GWAS model (additive, recessive, or dominant), adjusted for age, sex, site, and three PCs of ancestry as a predictor. We set the Bonferroni corrected statistical significance threshold to account for the number of phecodes used ($0.05/1817=2.75 \times 10^{-5}$). Additionally, we meta-analyzed PheWAS from the eMERGE (all age) and UKBB analyses, as implemented in the PheWAS R package.

Mice

All animal experiments followed protocols approved by the Institutional Animal Care and Use Committee at Columbia University. Hoxb7-Gfp: 129S.Cg-Tg (Hoxb7-EGFP)33Cos/J, Frank Costantini, Columbia University.⁵⁷ Wnt5a Mutants: 004758 - B6; 129S7-Wnt5a^{tm1Amc}/J; Andrew P McMahon, University of Southern California.⁵⁸ All work with mice was approved by and performed under the regulations of the Columbia University Institutional Animal Care and Use Committee. Animals were housed in the animal facility of Irving Cancer Research Center, Columbia University.

Mouse Genotyping

For Wnt5a ko genotyping was performed with the following primers: WT forward 5'-GAG GAG AAG CGC AGT CAA TC-3'; common 5'-CAT CTC AAC AAG GGC CTC AT-3' and Mut forward 5'-GCC AGA GGC CAC TTG TGT AG-3'; wild type shows 484bp, mutant 400bp.

Histology

Histology was carried out using hematoxylin and eosin staining with the standard protocol. Paraffin sections were cleared in three washes with xylene for 2 minutes each. Samples were rehydrated by incubating successively in 100% ethanol for 5 minutes per change, two changes of 95% ethanol for 5 minutes per change, and two changes of 70% ethanol for 5 minutes per change. Slides were rinsed in tap water and samples were stained in hematoxylin solution for 3 minutes, rinsed, then incubated in 95% ethanol for 4 minutes. Slides were then stained with eosin Y solution for 2–3 minutes, then dehydrated with successive changes of ethanol and cleared with the slides xylene and mounted in Permount.

Immunostaining

Embryos were embedded in paraffin and serial sections were generated. For immunohistochemistry, paraffin sections were deparaffinized using HistoClear and rehydrated through a series of ethanol and 1XPBS washes. Antigen retrieval was performed by boiling slides for 15 minutes in pH 9 buffer or 30 minutes in pH 6 buffer. Primary antibodies in 1% horse serum were incubated

overnight at 4°C. The next day, slides were washed with PBST three times for 10 minutes each and secondary antibodies were applied for 2 hours at room temperature (RT). 4',6-diamidino-2-phenylindole (DAPI) was either applied as part of the secondary antibody cocktail or for 10 minutes, for nuclear staining, and then the slides were sealed with coverslips. E-cadherin (Ecad) staining: goat anti-mouse Ecad antibody (500 ng/ml, Paraffin or Cryo, #AF748; R&D Systems).

Whole-mount *In Situ* Hybridization

Embryos were fixed overnight at 4°C on a rocking table with 4% paraformaldehyde (PFA) in PBS. After fixation, embryos were washed three times for 10 minutes each in PBS with 0.1% Tween (PTW) at RT, incubated in 6% H₂O₂ for 15 minutes at RT, and then washed three times in PTW for 10 minutes each time. After the last wash, the PTW was replaced with 1 µg/ml of proteinase K for 15 minutes at RT. Proteinase K digestion was terminated by washing twice for 5 minutes each wash at RT with PTW, then tissue was postfixed for 10 minutes in 4% PFA/PTW/0.2% glutaraldehyde, washed three times for 5 minutes each in PTW, then placed in hybridization buffer (50% Formamide [Fluca,#47671], 1.3x SSC, pH 5.3; 5 mM EDTA pH 8; Yeast RNA [50 µg/ml, #R6750; Sigma], 0.1% Tween 20; 0.4% Chaps; Heparin [100 µg/ml]) first, for 5 minutes at RT, and then for 1 hour at 68°C. Hybridization buffer was replaced with 0.4 ml hybridization buffer containing probe, and samples were incubated overnight at 68°C. Hybridization buffer was replaced with warmed hybridization buffer without probe at 68°C, washed three times for 30 minutes each, then washed in warmed hybridization buffer with Tris-buffered saline with 0.1% Tween 20 (TBST) three times for 30 minutes each at 68°C, and in TBST twice for 5 minutes each at RT. Embryos are preblocked in 0.5 ml 1× TBST + 10% heat treated goat serum then placed in blocking solution with fresh 1× TBST + 10% serum containing a 1:5000 dilution of alkaline phosphatase-conjugated anti-digoxigenin Fab fragments for o/n at 4°C. After the incubation with antibody samples were washed two times at RT with TBST, then placed in fresh TBST overnight at 4°C, and washed twice in alkaline phosphatase buffer (NTMT [100 mM Tris, pH 9.5, 50 mM MgCl₂, 100 mM NaCl, 0.1% Tween 20 1%]). Finally, samples were incubated in substrate solution (NTMT, nitro blue tetrazolium chloride, 5-bromo-4-chloro-3-indolyl-phosphate; Roche) containing 0.5 mg/ml levamisole for 15 minutes to 2 hours at RT in the dark. Reactions were stopped by washing twice for 5 minutes each in TBST. Embryos were then postfixed in 4%PFA/PTW for o/n at 4°C.

RNAscope

Mouse bladders were fixed in 4% PFA for 24 hours at 4°C, then immersed in 30% sucrose in 1× PBS. Tissue was embedded in optimal cutting temperature compound at −80°C, then 14 µm sections were placed on Superfrost Plus Slides and air-dried for 1–2 hours at −20°C. The slides were then washed with 200 ml 1× PBS for 5 minutes to remove the optimal cutting temperature compound, then baked for 30 minutes

at 60°C. Tissue was postfixed in 10% neutral-buffered formalin for 90 minutes, then dehydrated through a series of ethanol washes followed by another baking step in 60°C for 10 minutes. Three to five drops of RNAscope Hydrogen Peroxide (ACD, 322335) were applied to cover the sections, incubated for 10 minutes then washed in distilled water twice. Target retrieval was performed by boiling slides in RNAscope 1× Target Retrieval Reagent (ACD, 322000) that was heated to at least 99°C for 5 minutes. After rinsing in distilled water for 5 minutes, the slides were transferred to 100% ethanol for 5 minutes. Slides were placed on a humidified chamber and three to five drops of RNAscope Protease III (ACD, 322337) were applied to cover each section for 30 minutes at 40°C. Excess liquid was removed from the slides with distilled water washes, then three to five drops of the Wdpcp-C1 probe (ACD, 502151) was added to cover the sections for 2 hours at 40°C in the humidified chamber. Slides were kept overnight in 5× SSC and washed twice in 1× wash buffer (ACD, 310091) before continuing with the assay. Three to five drops of RNAscope Multiplex FL v2 Amp 1 (ACD, 323101) were applied to the sections then kept in humidified chamber for 30 minutes at 40°C. After two 2 minute washes in 1× wash buffer, three to five drops of RNAscope Multiplex FL horseradish peroxidase-C1 (ACD, 323104) were added onto the slides in the humidified chambers for 15 minutes at 40°C. After two 2-minute washes in 1× wash buffer, 150–200 µl of diluted tyramide signal amplification (ACD 322809) plus Cy3 (NEL744001KT, 1:1500; PerkinElmer) were added to the slides in the humidified chambers for 30 minutes at 40°C. After two 2-minute washes in 1× wash buffer, three to five drops of RNAscope Multiplex FL horseradish peroxidase Blocker (ACD, 323107) were added to the slides in the humidified chambers for 15 minutes at 40°C. DAPI was applied for 30 seconds at RT to each slide and incubated for 30 seconds at RT. DAPI was removed and one to two drops of Prolong Gold Anti-fade Mountant were applied on the slides to seal with coverslips.

Data Availability and Accession Numbers

Raw data that support the findings of this study are available from the corresponding authors on reasonable request. The data are also available on dbGaP (<https://www.ncbi.nlm.nih.gov/gap>) under the following accession numbers: phs001749.v1.p1, phs000650.v3.p1, phs000304.v1.p1, phs000169.v1.p1, phs000092.v1.p1, phs000199.v1.p1, phs000431.v1.p1, phs000356.v2.p1, phs001584.v1.p1 (and other, pending). Some restrictions may apply according to participants' consent and privacy protection. All images generated from mouse experiments reported in this study will also be available from the corresponding authors.

RESULTS

Rare CNV Analysis

We conducted a rare CNV analysis in a multiethnic cohort of 1737 VUR patients and 24,765 population controls. We found

a significant excess burden of large, rare, gene-intersecting CNVs in VUR patients compared with controls (defined as size ≥ 100 kb, frequency in PCA-defined populations $\leq 0.1\%$; Figure 1, Supplemental Figures 2 and 3; $P=3.54 \times 10^{-7}$). This difference was principally attributable to enrichment for larger-sized CNVs (CNVs ≥ 500 kb; Supplemental Tables 1 and 2) in VUR. We estimated that 2.23% of VUR patients can be attributed to carrying a rare CNV of at least 500 kb (odds ratio [OR], 1.40; 95% confidence interval [95% CI], 1.16 to 1.67; $P=3.96 \times 10^{-4}$; Figure 1A, Supplemental Table 1). We next annotated CNVs for pathogenicity. Based on a survey of 148 known GDs, we found that VUR patients were significantly enriched in GDs, with 18 distinct, known GDs in 35 out of 1737 of patients and 162 out of 24,765 of controls (2.01% versus 0.65%, OR, 3.12; 95% CI, 2.10 to 4.54; $P=6.35 \times 10^{-8}$). The GD burden remained highly significant if we only considered the 1077 VUR patients newly reported in

this study (OR, 4.05; 95% CI, 2.60 to 6.11, $P=6.46 \times 10^{-9}$). Recurrent GDs that achieved significance in *post-hoc* tests included 16p11.2 deletion, 22q11.21 duplication, and triple X syndromes (Table 1). GDs were detected among all subcohorts, including the CKiD and RiVUR cohorts, which excluded known chromosomal disorders in their entry criteria.

An additional 16 (0.92%) patients carried a likely pathogenic CNV (Supplemental Table 3). Among them are heterozygous deletions in chr 2q31.1, encompassing *BBS5* and *ITGA6*; 10q24.2, including *HPSE2*; 10q26.11–10q26.3, which includes *FGFR2*; 12p13.33, including *CACNA1C*; 12q21.31–12q21.33, encompassing *NPHP6*; and two instances of heterozygous deletions of *COL4A5* in chr X, in females and duplications in 6q14.3, including *TBX18*; 16q24.1, encompassing *FOXC2* and *FOXF1*; and 17q25.1–17q25.3, including *ITGB4*. Altogether, this analysis of a significantly larger cohort confirms the contribution of rare CNV GDs to the development of VUR and highlights its genetic heterogeneity.

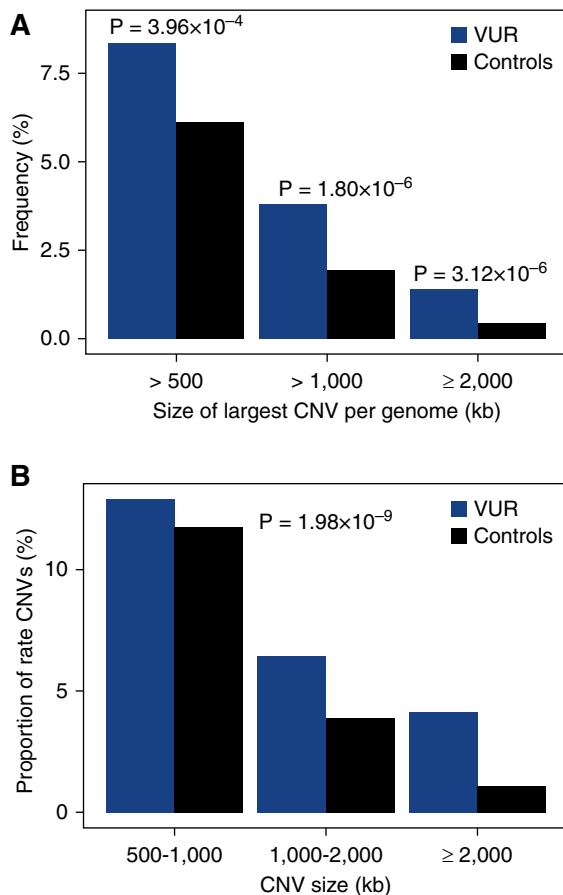


Figure 1. Excess burden of large, rare CNVs in VUR. VUR patients are enriched in large (size ≥ 100 kb), rare (frequency in PCA-defined populations $\leq 0.1\%$), gene-intersecting CNVs. (A) Frequency of subjects with their largest CNV size at or above the indicated size thresholds (kb). (B) Proportion of CNV (size ≥ 100 kb) within the indicated size intervals (kb). The 100–500 kb interval is not shown. Blue bars represent VUR patients and black bars, controls.

Genome-wide Association Study

We investigated the contribution of common variants to VUR by performing a GWAS in seven cohorts of European ancestry (see Methods and Supplemental Table 4). After genetic matching and imputation, we performed meta-analysis in 1395 unrelated VUR patients and 5366 matched population controls, using 6,131,010 imputed variants common to all cohorts. Given that VUR is significantly more prevalent in females and its segregation is consistent with multiple modes of inheritance,^{18,19,59–61} we performed global and sex-specific association analyses under additive, recessive, and dominant models⁶² (Supplemental Tables 5–13, Supplemental Figure 3). Genome-wide significance thresholds were adjusted based on the number of models tested.

Significant and Suggestive GWAS Loci

Altogether we identified three significant and five suggestive loci (Figure 2, Table 2 and Supplemental Figure 4). Remarkably, the three significant loci conferred large effects (OR, 1.41–3.65). The most significant association was in Chr 2p15, under a recessive model (top SNP: rs13013890; OR, 3.65; 95% CI, 2.39 to 5.56; $P=1.86 \times 10^{-9}$), and mapped to an intron of *WDPCP*. Altogether, 3.3% of patients were homozygous for the risk allele, compared with 1.2% of controls. Coding mutations in *WDPCP* cause Bardet-Biedl syndrome (BBS15; MIM 615992), which features VUR as a known manifestation. Studies of *Wdpcp* mutant mice also revealed CAKUT-like phenotypes, including failure of the ureters to join the UGS, the primordium of the bladder and urethra.⁶³ This locus also includes *OTX1*, and heterozygous microdeletions encompassing *WDPCP* and *OTX1* have been reported in patients with genitourinary development defects.⁶⁴ We did not detect such deletions in our CNV analysis of patients with VUR, indicating that this recessive signal is not attributable to hemizyosity at the *WDPCP* locus. Analysis of expression quantitative trait loci (eQTL) studies (Supplemental Tables 14–15) demonstrated

Table 1. VUR patients are enriched in known genomic disorders, compared with controls

Genomic Disorder	Start (Mb)	End (Mb)	Size (Mb)	VUR			Controls <i>n</i> =24,765	OR (95% CI)	<i>P</i>
				Verbitsky et al. 2019 <i>n</i> =660	This Report <i>n</i> =1077	Combined <i>n</i> =1737			
All GDs				7	28	35	162	3.12 (2.10 to 4.54)	6.35×10^{-8}
Individual GDs									
1q21.1 deletion	145.37	147.83	2.21	0	5	5	14	5.10 (1.44 to 15.01)	6.44×10^{-3}
1q21.1 duplication	146.47	147.83	1.36	1	0	1	15	0.95 (0.02 to 6.18)	1
2q11.2 deletion	96.74	98.02	1.28	0	1 ^a	1 ^a	0	∞ (0.37 to ∞)	0.067
2q13 homozygous deletion	110.86	111.98	0.12	0	1	1	0	∞ (0.37 to ∞)	0.067
3pter-p25 deletion	0.45	2.02	1.57	1	0	1	1	14.27 (0.18 to 1110)	0.127
7q11.23 del (Williams-Beuren)	72.72	74.34	1.62	0	1	1	0	∞ (0.37 to ∞)	0.067
7q36 deletion ^b /critical deletion	129.43 [§]	159.12	29.60	1	1	2	0	∞ (2.68 to ∞)	4.29×10^{-3}
15q11.2 deletion (Prader-Willi/Angelman)	23.65	28.54	5.01	1	1	2	0	∞ (2.68 to ∞)	4.29×10^{-3}
16p11.2 deletion	29.59	30.20	0.61	1	3	4	5	11.43 (2.27 to 53.21)	1.77×10^{-3}
16p13.3 deletion (Rubinstein-Taybi)	3.65	5.04	1.39	0	1	1	0	∞ (0.37 to ∞)	0.067
16p13.11 deletion	15.12	16.36	1.24	0	1	1	5	2.85 (0.06 to 25.50)	0.33
16p13.11 duplication	15.49	18.17	2.67	0	1	1	36	0.40 (0.01 to 2.35)	0.51
17p12 deletion (HNPP)	14.10	15.47	1.37	0	2	2	15	1.90 (0.21 to 8.19)	0.31
17q12 duplication	34.82	36.21	1.39	0	1	1	6	2.38 (0.05 to 19.61)	0.38
22q11.21 deletion	20.74	21.46	0.72	1	2 ^a	3 ^a	4	10.71 (1.57 to 63.37)	8.05×10^{-3}
22q11.21 duplication	18.88	21.46	2.53	0	5	5	7	10.21 (2.55 to 37.39)	6.45×10^{-4}
Xp21 hemizygous deletion	6.46	8.14	1.67	0	1	1	3	4.75 (0.09 to 59.28)	0.24
Trisomy X	0.06	155.26	154.91	1	2	3	1	42.81 (3.44 to 2.21×10^3)	1.07×10^{-3}

A screen for 148 known pathogenic deletions and duplications showed a significant excess of genomic disorders in VUR patients ($P=6.35 \times 10^{-8}$). Start, end positions, and size (Mb) are based on genome build hg19. Counts of carriers in VUR patients and population controls, ORs with respect to controls, their 95% CIs, and Fisher's exact test *P* values are shown. VUR counts include patients previously reported, newly reported patients in this study, and the combined counts, shown in three separate columns. Fisher's exact tests are based on the combined VUR counts, compared with controls. In controls, counts of all genomic disorder carriers are larger than the sum of those for individual ones shown in the table, which are only the ones present in VUR patients. HNPP, hereditary neuropathy with liability to pressure palsies.

^aOne VUR case carries deletions at both 2q11.2 and 22q11.2; this case is counted only once in the sum of all GD carriers.

^bCoordinates for the 7q36 deletion.

that rs13013890 encodes a human blood eQTL for *OTX1*. We examined expression of *Wdpcp* and *Otx1* in developing mouse embryos, focusing on the UB, which gives rise to the ureter, the bladder, and UGS. Analysis of *Wdpcp* at E11.5 revealed robust expression localized to the UB trunk, which will differentiate into the ureter (Supplemental Figure 5A shows expression at E11). Expression was also observed in the epithelium throughout the

developing bladder and urethra at later stages (not shown). Analysis of *Otx1* expression revealed highest levels in the UGS, detectable from E12 through late stages of development (Supplemental Figure 5B shows expression at E12).

Among females (937 patients with VUR and 3129 controls), we found another genome-wide significant association on Chr 6p12.1 under an additive model (rs1154855; OR, 1.41;

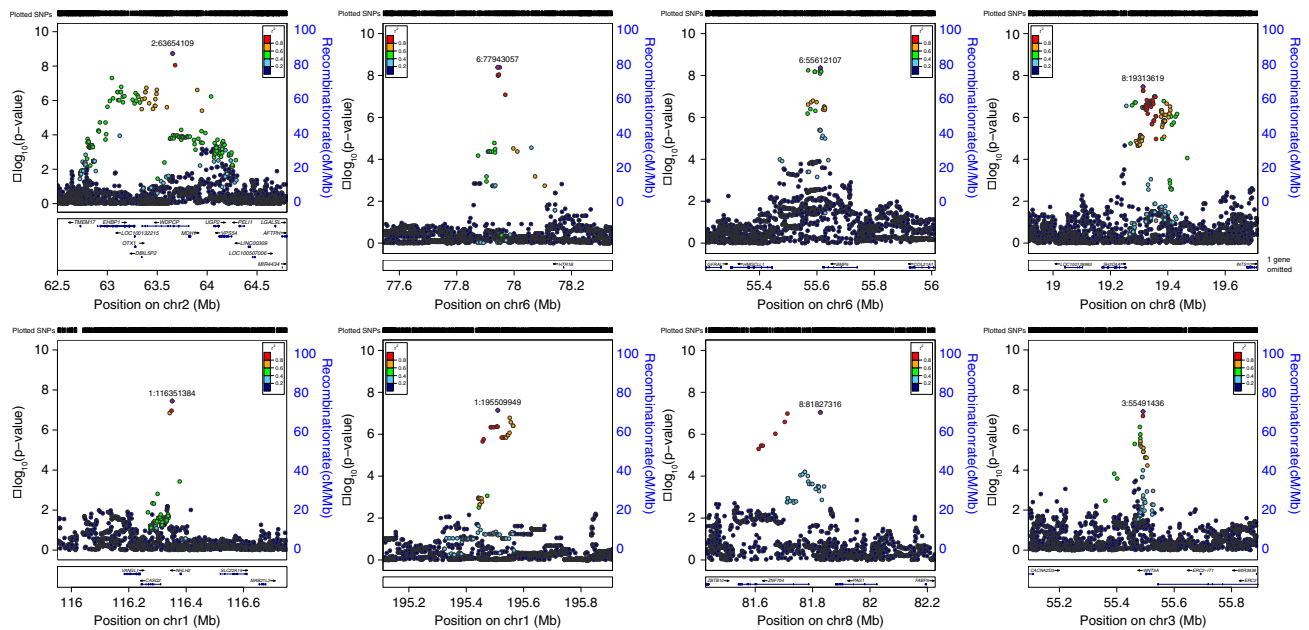


Figure 2. Regional association plots for loci associated with VUR. The calculated $-\log_{10} P$ values are shown according to genomic position (hg19). The most significant SNP is colored purple. Other colors indicate LD (r^2 ; see inset) to top SNP, based on the 1000 Genomes European sample set.

95% CI, 1.26 to 1.59; $P=4.33 \times 10^{-9}$). The top SNP is located within the 3' untranslated region of *BMP5*. We did not detect any eQTL associations for this SNP. The BMP family plays important roles in early embryonic development and urinary tract formation.⁶⁵ *Bmp5* is expressed in stroma abutting the urothelium of the bladder, and in the ureteral mesenchyme. Expression, which was strong at E11, continued through later stages of development (Supplemental Figure 5C shows expression at E14).

Among males (458 VUR patients and 2235 controls), we found a third significant association on Chr 6q14.1 under a recessive model (OR, 2.75; 95% CI, 1.96 to 3.84; $P=4.10 \times 10^{-9}$). Altogether, 15.7% of male patients were homozygous for the risk allele, compared with 9.2% of controls. The top SNP, rs10806089, is located within a low-abundance noncoding RNA (*LOC101928570*) and 203 kb of *HTR1B*. We did not detect any eQTL associations for this SNP. However, serotonergic pathways have been implicated in neural control of the lower urinary tract.⁶⁶ Consistent with these data, *Htr1b* is expressed in cells scattered throughout the bladder mesenchyme and in the urethra, in a thin layer adjacent to the peripheral muscle (white arrowhead Supplemental Figure 5D).

In addition to these significant loci, for which we had adequate power (Supplemental Table 16), we found five suggestive associations (P value range 3.5×10^{-8} – 1.2×10^{-7} , Table 2). Two of these loci, on Chr. 1p31.1 and Chr. 3p14.13, include genes that have been previously implicated in genitourinary developmental defects^{67,68}: the Chr 1p31.1 locus includes *VANGLI1*, a planar cell polarity protein implicated in neural tube defects⁶⁷ and caudal dysgenesis syndrome, which can

include urinary tract defects (rs12759898; OR, 1.61; 95% CI, 1.36 to 1.9; $P=3.56 \times 10^{-8}$; dominant model). The top Chr 3p14.13 signal is downstream of *WNT5A*, which has been implicated in kidney and ureteral development in mice (rs503022; OR, 1.81; 95% CI, 1.45 to 2.25; $P=1.19 \times 10^{-7}$; in males, additive model). *WNT5A* is highly intolerant to mutations and (probability of being loss of function intolerant, pLI, 0.99; missense Z-score, 1.98), and *WNT5A* mutations can cause Robinow syndrome (Online Mendelian Inheritance in Man # 180700). We previously reported a *WNT5A* loss of function variant in a patient with renal agenesis, nominating *WNT5A* as a candidate gene for isolated kidney malformations in humans.⁶⁹

Wnt5a Signaling during Cloacal Morphogenesis is Critical for Nephric Duct Insertion and Ureter Maturation

The association with the *WNT5A* locus prompted us to further investigate the role of *Wnt5a* in development of the cloaca, which gives rise to the urethra and bladder.^{58,68,70,71} *Wnt5a* is expressed in the tail and cloacal mesenchyme at E9 (Figure 3, A and B; www.gudmap.org),⁷² and expression was localized in the mesenchyme surrounding primitive bladder and hindgut at E11 (Figure 3C). At later stages, *Wnt5a* was present in mesenchyme from the bladder neck to the distal urethra (Figure 3, D and E). Whole-mount analysis of *Wnt5a* knockouts at E15 revealed abnormal patterning along the anterior-posterior axis and kidney malformations as previously reported (fused kidneys and renal hypoplasia or renal agenesis, Figure 3, F and N and not shown). The cloaca normally septates between E11

Table 2. VUR associations

Top SNP	Chr.	Position	Nearest Genes	A1/A2	Effect Allele (A1) freq.	OR (95% CI)	P	Model
rs13013890	2	63,654,109	WDPCP, MDH1, OTX1, EHP1, UGP2, VPS54	A/G	0.12	3.65 (2.39 to 5.56)	1.86×10^{-9}	Recessive
rs10806089	6	77,943,057	HTR1B	C/T	0.34	2.75 (1.96 to 3.84)	4.10×10^{-9}	Recessive (males)
rs1154855	6	55,612,107	BMP5	G/T	0.48	1.41 (1.26 to 1.59)	4.33×10^{-9}	Additive (females)
rs76292820	8	19,313,619	CSGALNACT1	T/C	0.16	4.71 (2.72 to 8.17)	3.46×10^{-8}	Recessive (males)
rs12759898	1	116,351,384	NHLH2, VANGL1, CASQ2	A/T	0.08	1.61 (1.36 to 1.9)	3.56×10^{-8}	Dominant
rs16838525	1	195,509,949	AL357932.1	C/T	0.15	6.94 (3.43 to 14.06)	7.21×10^{-8}	Recessive (males)
rs79060316	8	81,827,316	ZNF704, PAG1	T/C	0.02	2.50 (1.79 to 3.50)	9.07×10^{-8}	Dominant
rs503022	3	55,491,436	WNT5A	A/C	0.13	1.81 (1.45 to 2.25)	1.19×10^{-7}	Additive (males)

Top SNP each locus is shown (dbSNP 151). Positions are in University of California Santa Cruz hg19 coordinates. *P* values, ORs, and their 95% CIs are derived from meta-analysis of seven European ancestry cohorts (1395 VUR patients and 5366 controls; detailed counts per cohort are shown in Supplemental Table 4), under the indicated models. Chr., chromosome; freq., frequency; WDPCP, WD Repeat Containing Planar Cell Polarity Effector; MDH1, Malate Dehydrogenase 1; OTX1, Orthodenticle Homeobox 1; EHP1, EH Domain Binding Protein 1; UGP2, UDP-Glucose Pyrophosphorylase 2; VPS54, Vacuolar Protein Sorting-Associated Protein 54; HTR1B, 5-Hydroxytryptamine Receptor 1B; BMP5, Bone Morphogenetic Protein 5; CSGALNACT1, Chondroitin Sulfate N-Acetylgalactosaminyltransferase 1; NHLH2, Nescient Helix-Loop-Helix 2; VANGL1, Vang Gogh-like Planar Cell Polarity Protein 1; CASQ2, Calsequestrin 2; ZNF704, Zinc Finger Protein 704; PAG1, Phosphoprotein Membrane Anchor With Glycosphingolipid Microdomains 1; WNT5A, Wnt Family Member 5A.

and E13, separating the hindgut from the UGS by E15 (Figure 3G) and failure in this process is associated with anorectal malformations. In *Wnt5a* mutants at this stage, the cloaca persisted and remained connected to the hindgut (Figure 3O). UGS defects included rudimentary bladder and urethra (13 out of 18 embryos), absence of the bladder (5 out of 18 embryos) or defective urethral differentiation (Figure 3, N and O).

We next investigated the fate of nephric ducts and distal ureters. In mutants, the distal ureters, which normally join the bladder neck at E12 and E13, extended partway toward the cloacal region and ended blindly (Figure 3, I and Q). In embryos with rudimentary bladders, ureters generally terminated in the bladder wall outside the lumen, whereas in mutants with no sign of bladder development, ureters generally terminated at an abnormally anterior position and failed to reach the cloacal region (Figure 3, J and R). To investigate the origin of these abnormalities, we analyzed embryos at E9, when nephric ducts normally make primary connections with the cloaca. Ecad staining of control embryos revealed nephric ducts that extended caudally, turned toward the midline, and joined the cloaca at the midpoint between the future hindgut and UGS (Figure 3, K–M). In mutants, cloacae were misplaced to an anterior position and the dorsal cloaca, which differentiates into the UGS, was truncated (Figure 3, S–U; *cl** denotes the truncated portion of the cloaca). In addition to cloacal defects, nephric ducts in *Wnt5a* mutants either joined one another or ended blindly, and did not insert into the cloaca (Figure 3, S–U). The nephric duct termini in mutants were generally larger than normal, but we did not observe any sign of bifurcation as reported in other studies.^{68,70}

PRS, Heritability, and Clinical Associations

Using LD score regression,⁵³ we estimated the SNP-based heritability (h^2) to be 15.24% under the additive model. We computed a VUR-PRS using LDPred⁵⁴ under an additive model. To determine whether the VUR-PRS captures different CA-KUT or kidney disease subtypes, we applied the VUR-PRS to participants of European ancestry in the CKiD study, a cohort composed of pediatric patients with different causes of kidney failure. However, the VUR-PRS was not different between non-VUR nephropathy causes of kidney disease (Supplemental Figure 6). We also did not detect any significant associations of the VUR-PRS with clinical parameters such as the severity of reflux or recurrence of UTI among patients with VUR.

We next performed a phenome-wide association study (PheWAS) of the top GWAS signals using two large cohorts: UKBB and eMERGE, followed by meta-analysis. We also conducted PheWAS on eMERGE participants under 21 years of age (pediatric eMERGE). Homozygosity for the *WDPCP* risk allele (rs13013890-A) was nominally associated with cystitis (Supplemental Tables 17–20; OR, 2.11; 95% CI, 1.22 to 3.65; $P=7.78 \times 10^{-3}$) in males, and with an increased risk of bladder neck obstruction (OR, 5.10; 95% CI, 2.23 to 11.69; $P=1.17 \times 10^{-4}$) and dystrophy of the genital tract (OR, 2.38; 95% CI, 1.39 to 4.07; $P=1.48 \times 10^{-3}$) in females. This locus was associated with 22 genitourinary traits at $P<0.05$. We also observed 46 nominal associations of other VUR risk loci with genitourinary traits (Supplemental Tables 17–20).

DISCUSSION

In the largest genetic study of VUR conducted to date, we detected rare and common variants with large effects on this

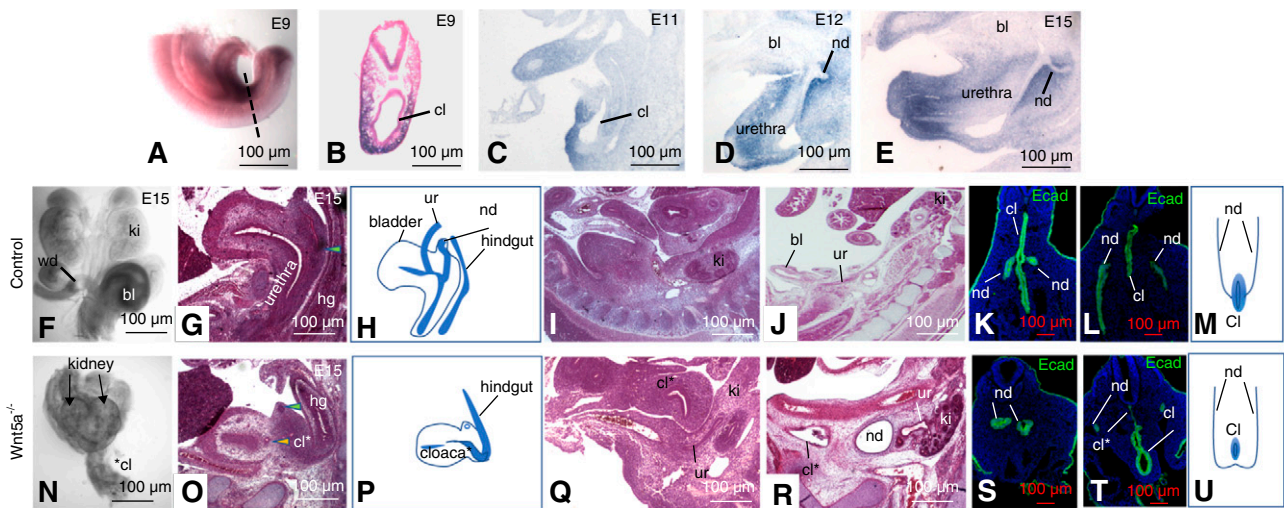


Figure 3. Wnt5a expression and its role in lower urinary tract morphogenesis. (A–E) *In situ* hybridization analysis. (A) Wnt5a expression at E9 in a whole mount embryo. (B) Section through a whole mount embryo showing Wnt5a expression surrounding the cloaca. (C–E) Wnt5a expression in sagittal sections from embryos at E11 (C), E12 (D), and E15 (E). (F and N) Whole-mount urogenital tracts from a control (F) and Wnt5a mutant (N). (G and O) Hematoxylin and eosin-stained sections through the bladder/urethra and hindgut of controls (G) and Wnt5a mutants (O). Green arrowheads point to the nephric ducts. Yellow arrowhead in (O) points to the distal ureter. (H and P) Schematic representation of histology shown in (G and O). (I and Q) Sagittal sections through Wnt5a mutants at E12. (J and R) Sagittal sections through a control (J) and Wnt5a mutant embryo (R). (K, L, S, and T) Ecad staining (green) reveals nephric duct and cloacal epithelia at E9, in controls (K and L) and Wnt5a mutants (S and T). (M and U) Schematic showing nephric duct insertion into the cloaca in a wild-type embryo (M) and in a Wnt5a mutant embryo (U). bl, bladder; cl, cloaca; cl* truncated cloaca in Wnt5a mutant; ki, kidney; nd, nephric duct; ur, ureter; wd, Wolffian duct.

trait. Most studies of developmental disorders have focused on rare variants, but our study now demonstrates that common risk variants also contribute to congenital and developmental disorders. We also found that some risk loci conferred sex-specific effects. Moreover, we searched for loci under nonadditive models and detected two significant loci under a recessive inheritance. These data suggest that more systematic exploration of nonadditive inheritance and sex-specific analyses may increase the yield for GWAS and clarify the architecture of developmental disorders.

We found that 3% of patients with VUR have an unsuspected genomic disorder that is not clinically recognized, including those identified in major national cohorts such as RIVUR and CKiD, where a clinically detectable chromosomal disorder was an exclusion criterion. These findings extend our previous studies³⁰ and clarify the spectrum of CNV disorders that contribute to VUR. In particular, we detected an enrichment of known GD in three loci (Chr. 16p11.2, 22q11.2, and Chr. X). These GD have been implicated in autism, schizophrenia, developmental delay, congenital cardiac, or vertebral defects,^{45,73–77} with variable penetrance. About one third of patients with the 22q11.2 microdeletion syndrome display an urinary tract defect.^{78,79} We previously showed that disruption of *CRKL* and *TBX6* likely account for the kidney and urinary tract defects in the 22q11.2 and 16p11.2 syndromes, respectively.^{30,80,81} Recent studies have also implicated *MAZ*, another gene within the CNV 16p11.2 interval, in genitourinary defects.⁸² The 3% prevalence of CNV disorders has significant clinical implications, given the relatively high prevalence of

VUR in the pediatric population. The detection of pathogenic CNVs can assist with diagnosis and prediction of complications, particularly for identification of patients at risk for poor neurocognitive outcomes.⁸³ Because our dataset was sourced from multiple studies with different ascertainment criteria and phenotyping depth, we lacked access to complete data for all patients on kidney function and renal failure; presence of other genitourinary tract malformations; VUR staging and laterality and other clinical variables. However, we note that the high CNV burden was detected in cohorts with both mild (RIVUR) and severe (CKiD) outcomes, motivating further studies to clarify variability of renal function outcomes.

The GWAS for VUR identified loci with large effect, consistent with prior studies of developmental traits, such as hypospadias^{84,85} and bicuspid aortic valves.⁸⁶ The top associations encompass genes that participate in embryonic development (*WDPCP*, *BMP5*, *WNT5A*, and *VANGL1*). The strongest association was an intronic variant in *WDPCP*, which encodes a planar polarity protein and has been implicated in Bardet Biedl-15⁸⁷ and Oro-Digital-Facial syndromes,⁸⁸ which can feature urinary tract malformations, including VUR.^{89,90} *Wdpcp* is expressed in the ureter during mouse embryonic development and its inactivation results in developmental abnormalities, including cloacal septation defects.⁶³ We did not detect any coding mutations in LD with the top *WDPCP* signal, suggesting the noncoding variant(s) may influence the timing or location of expression of *WDPCP* or other genes in the interval. The large effect

imparted by this locus warrants additional studies to identify the causal allele(s) and its utility in clinical prediction among families with the risk variants. *WDPCP* is also expressed in the lower urogenital tract, motivating further investigation of the potential association with bladder neck obstruction and female genitourinary disorders, as observed in the UKBB and eMERGE PheWAS. In addition, the GWAS findings should be followed up in independent cohorts for replication.

We performed detailed studies of *Wnt5a* mutant mice because heterozygous *WNT5A* mutations cause dominant Robinow syndrome, which can feature urinary tract defects, including VUR.^{91,92} We previously reported a *WNT5A* loss of function mutation in a patient with nonsyndromic kidney malformation.⁶⁹ *WNT5A* signaling with its presumptive receptor ROR2, controls cell migration, convergent extension, planar cell polarity, and numerous aspects of organ development.⁵⁸ *WNT5A* mediates noncanonical Wnt signaling via the calcium-signaling or planar cell polarity pathways.⁹³ *Wnt5a* mutants display urinary tract phenotypes, including duplicated bifid/ureters and kidneys, renal hypoplasia, defective medullary patterning, defective ureteral insertion, and abnormal extension of the intermediate mesenchyme and defective genital tubercle morphogenesis.^{68,70,71} Here, we describe a novel role for *WNT5A* during early stages of bladder and urethral formation. We find that *Wnt5a* expression in the mesenchyme surrounding the cloaca is essential for cloacal morphogenesis and for insertion of nephric ducts, which are precursors of the UBs and the ureters. Defects in the nephric duct insertion process can impair connection between the upper and lower urinary tract and result in CAKUT-like phenotypes. These observations suggest *WNT5A* or a *WNT5A*-dependent signal from the cloaca may be important for directing nephric duct insertion, cloacal septation, and UGS differentiation. Abnormal position or differentiation of the UGS may be sufficient to impair nephric duct and ureter insertion, and may be the cause of the spectrum of phenotypes observed in *Wnt5a* mutants.

Important questions in the clinical management of VUR include risk prediction for complications such as renal failure and UTI, or screening criteria for asymptomatic siblings. In our study, about 6% of VUR patients carried high-risk genotypes, defined as major CNV disorders (3%) or homozygosity for the *WDPCP* risk allele (3%). Assessment of these loci may be useful for selecting siblings for screening. Recent studies have also shown the value of PRS for prediction of complex phenotypes.^{94,95} We had adequate power to detect common risk variants; doubling sample size could yield additional risk loci for VUR at lower MAF and/or OR (Supplemental Table 16) and more robust PRS. There are no long-term follow-up studies of VUR into adulthood, and the PheWAS approach may reveal long-term outcomes associated with genetic risk factors that cause VUR in childhood.

DISCLOSURES

A. Gharavi reports having consultancy agreements with AstraZeneca Center for Genomics Research and Goldfinch Bio; reports receiving research funding

from the Renal Research Institute; reports receiving honoraria from Sanofi; and being a scientific advisor or member of the editorial boards of *JASN*, *Kidney International*, and *Journal of Nephrology*. Because A. Gharavi is an editor of the *JASN*, he was not involved in the peer-review process for this manuscript. A guest editor oversaw the peer review and decision-making process for this manuscript. D. Crosslin reports consultancy agreements with UnitedHealth Group; research funding from UnitedHealth Group; and being a scientific advisor or membership with UnitedHealth Group. L. Gesualdo reports consultancy agreements with Medtronic, Retrophin; research funding from Philanthropic sources, Sanofi Genzyme; honoraria from Sanofi Genzyme, Mundipharma, Estor, AstraZeneca, Retrophin, and GSK; being a scientific advisor or membership with *NDT Journal*, *Journal of Nephrology*, Board of Directors (SIN, RPS, ERA-EDTA); and other interests/relationships: President of the Italian Renal Federation. E. Kenny reports honoraria from Illumina, Inc. and Regeneron Pharmaceuticals; being a scientific advisor or membership as Editorial board member of *Frontiers in Ecology and Evolution* and *American Journal of Human Genetics*; and being an advisory board member of American Society of Human Genetics. B. Levy reports having consultancy agreements with Igenomix, Myriad, Myome, and Natera; reports having an ownership interest in Natera; reports being a scientific advisor or member of Wiley—Prenatal Diagnosis, American College of Medical Genetics Foundation, CAP Cytogenetics committee, Cancer Genomics Consortium, and International Society for Prenatal Diagnosis. M. Miklaszewska reports honoraria from Medycyna Praktyczna – Pediatria. B. Warady reports having consultancy agreements with Akebia, Amgen, Baxter, Bayer, GlaxoSmithKline, Reata, and Relypsa; reports receiving research funding from Baxter Healthcare; reports receiving honoraria from Akebia, GlaxoSmithKline, Reata, Relypsa, and the University of Missouri; reports being a scientific advisor or member of the North American Pediatric Renal Trials and Collaborative Studies, the National Kidney Foundation, and Nephrologists Transforming Dialysis Safety Board of Directors. C. Willer reports their spouse works at Regeneron Pharmaceuticals. C. Wong reports receiving honoraria from UpToDate; being a scientific advisor or member of the Steering Committee for the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) funded CKD in Children study; reports participation in the past year on the *ad hoc* advisory committee to the National Kidney Foundation; reports being on the board of the New Mexico Pediatric Society in Albuquerque, NM; and reports other interests/relationships via a contract with NephCure International as part of the CurGN study (participating site). D. Barton reports being a scientific advisor or member of European Molecular Genetics Quality Network; and other interests/relationships with Rare Disease Ireland. D. Santoro reports receiving honoraria from Alexion, Astellas, AstraZeneca, and Mundipharma. E. Fiaccadori reports being a scientific advisor or member of the Editorial Board of the *Journal of Nephrology*, Editorial Board of *Blood Purification*; and has other interests/relationships as a member of the *Italian Society of Nephrology* and the *European Society of Parenteral and Enteral Nutrition*. F. Hildebrandt reports having consultancy agreements as cofounder of Goldfinch-Bio; reports having an ownership interest in Goldfinch-Bio; reports receiving honoraria from Sanofi; reports being a scientific advisor or member of Goldfinch-Bio; and has other interests/relationships as Cofounder and SAB member of Goldfinch-Bio. F. Lin reports receiving honoraria from lectures and seminars in academic institutions; and being a scientific advisor or member of the *JASN* editorial board. I. Ionita-Laza reports being a scientific advisor or member as the Associate Editor for *Biometrics* and Associate Editor of *Statistics in Biosciences*. I. Stanaway reports sole ownership of an LLC, Byrell Systems, registered in Washington State; and reports other interests/relationships as a member of the community service organization, the Odd Fellows, Ballard Chapter 170. J. Barasch reports having consultancy agreements with Abbvie (one meeting on AKI in 2014); reports receiving research funding from Abbott, and Alere supported one study in the clinical use of Neutrophil gelatinase-associated lipocalin 2009–2010; receiving honoraria from Harvard Medical School (reviewer and lecturer), March of Dimes (as a grant reviewer 2008–2018), and National Institutes of Health (NIH; as a grant reviewer 2020); reports patents and inventions with Abbott and Bioporo, and had licensed patents from Columbia University. K. Kiriyluk reports being a scientific advisor or member of the Scientific Advisory Board of

Gilead Sciences and Goldfinch-Bio; and has other interests/relationships through scientific partnerships with Aeavi Genomics and AstraZeneca. M. Mizerska-Wasiak reports other interests/relationships as a member of the European Society Pediatric Nephrology, and the European Renal Association-European Dialysis Transplantation Association. M. Sampson reports having consultancy agreements with Kohlberg Kravis Roberts & Co. and Janssen Pharmaceutical; and being a scientific advisor or member of Natera. M. Szczepanska reports receiving research funding from Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice; reports receiving honoraria from Baxter, Roche, and Swixx; and reports having other interests/relationships with ESPN, ERA-EDTA, the Polish Society for Pediatrics, and the Polish Society for Pediatric Nephrology. M. Tkaczyk reports having consultancy agreements with Gerson Lehrman Group; and being President of the Polish Society for Pediatric Nephrology. P. Puri reports being Editor-in-Chief of *Pediatric Surgery International*. S. Sanna-Cherchi reports being a scientific advisor or member of Editorial Boards, with no royalties received, of *Acta Biomedica*, *Journal of Nephrology*, *Kidney International*, and *Negative Results*. All remaining authors have nothing to disclose.

FUNDING

The project is supported by NIDDK grants U54 DK104309 06 (to A.G. Gharavi, C.L. Mendelshon, and J. Barasch), R01 DK080099 and U01 HG008680 (to A.G. Gharavi), U01-DK 094530 (to C.L. Mendelshon), R01-DK105124, RC2-DK116690, UH3-DK114926, and U01-HG008680 (to K. Kiryluk), 1R01DK103184, 1R21DK098531, and UL1 TR000040 (to S. Sanna-Cherchi), and R21DK119802-01A (to M. Verbitsky). Genotyping of Irish patients with VUR and support of J.M. Darlow and M.G. Dobson were funded by a grant from The Children's Medical and Research Foundation/National Children's Research Centre, and J.M. Darlow was subsequently supported by the (Irish) Health Research Board grant HRA-POR-2014-693. The eMERGE Phase III Network was funded by the National Human Genome Research Institute through grants U01HG8680 (Columbia University), U01HG8672 (Vanderbilt University Medical Center), U01HG8657 (Kaiser Permanente Washington Health Research Institute/University of Washington), U01HG8685 (Brigham and Women's Hospital), U01HG8666 (Cincinnati Children's Hospital Medical Center), U01HG6379 (Mayo Clinic), U01HG8679 (Geisinger Clinic), U01HG8684 (Children's Hospital of Philadelphia), U01HG8673 (Northwestern University), MD007593 (Meharry Medical College), U01HG8676 (Partners Healthcare/Broad Institute), and U01HG8664 (Baylor College of Medicine).

ACKNOWLEDGMENTS

The authors thank the patients who participated in this study. The POLYGENES network (<http://www.polygenes.org>), a collaborative network of Columbia University in New York and the Polish Registry of Congenital Malformations at Poznań University of Medical Sciences, Poznań, Poland recruited Polish patients with VUR for this study. Genotypes of the Irish controls were kindly provided to David E. Barton by Derek Morris and Prof. Aiden Corvin of Department of Psychiatry, Trinity College Dublin, and the International Schizophrenia Consortium. Parts of this study have also been conducted using the UKBB Resource under UKBB project ID number 41849.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at <http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2020050681/-/DCSupplemental>.

Supplemental Figure 1. PCA for CNV frequency filtering.

Supplemental Figure 2. Excess burden of large, rare CNVs in VUR compared with controls.

Supplemental Figure 3. VUR patients are enriched in larger rare CNVs as compared with controls.

Supplemental Figure 4. GWAS meta-analyses.

Supplemental Figure 5. Expression of *Wdpcp*, *Otx1*, *Bmp5*, and *Htr1b* in the mouse lower urinary tract.

Supplemental Figure 6. Distribution of VUR-PRS values across phenotypes in CKiD participants of European ancestry.

Supplemental Table 1. Excess burden of large, rare CNVs in VUR compared with controls.

Supplemental Table 2. VUR patients are enriched in large, rare CNVs, compared with controls.

Supplemental Table 3. Likely Pathogenic CNV.

Supplemental Table 4. GWAS cohorts.

Supplemental Table 5. GWAS meta-analysis additive model.

Supplemental Table 6. GWAS meta-analysis additive model in males.

Supplemental Table 7. GWAS meta-analysis additive model in females.

Supplemental Table 8. GWAS meta-analysis recessive model.

Supplemental Table 9. GWAS meta-analysis recessive model in males.

Supplemental Table 10. GWAS meta-analysis recessive model in females.

Supplemental Table 11. GWAS meta-analysis dominant model.

Supplemental Table 12. GWAS meta-analysis dominant model in males.

Supplemental Table 13. GWAS meta-analysis dominant model in females.

Supplemental Table 14. Blood eQTL.

Supplemental Table 15. GTEx eQTL.

Supplemental Table 16. Power calculations.

Supplemental Table 17. PheWAS on UKBB and eMERGE.

Supplemental Table 18. PheWAS on eMERGE.

Supplemental Table 19. PheWAS on eMERGE (pediatric only).

Supplemental Table 20. PheWAS on UKBB.

REFERENCES

- Stephens FD, Lenaghan D: The anatomical basis and dynamics of vesicoureteral reflux. *J Urol* 87: 669–680, 1962
- United States Renal Data System: USRDS Annual Data Report: Epidemiology of Kidney Disease in the United States, Bethesda, MD, National Institutes of Health, 2019. Available at <https://www.usrds.org/annual-data-report>. Accessed January 2, 2020
- Farhat W, McLorie G, Geary D, Capolicchio G, Bägli D, Merguerian P, et al.: The natural history of neonatal vesicoureteral reflux associated with antenatal hydronephrosis. *J Urol* 164: 1057–1060, 2000
- Ichikawa I, Kuwayama F, Pope JC 4th, Stephens FD, Miyazaki Y: Paradigm shift from classic anatomic theories to contemporary cell biological views of CAKUT. *Kidney Int* 61: 889–898, 2002
- Ismaili K, Hall M, Piepsz A, Wissing KM, Collier F, Schulman C, et al.: Primary vesicoureteral reflux detected in neonates with a history of fetal renal pelvis dilatation: A prospective clinical and imaging study. *J Pediatr* 148: 222–227, 2006
- Arant BS Jr.: Vesicoureteric reflux and renal injury. *Am J Kidney Dis* 17: 491–511, 1991
- Dillon MJ, Goonasekera CD: Reflux nephropathy. *J Am Soc Nephrol* 9: 2377–2383, 1998
- Peters PC, Johnson DE, Jackson JH Jr.: The incidence of vesicoureteral reflux in the premature child. *J Urol* 97: 259–260, 1967
- Puri P, Gosemann JH, Darlow J, Barton DE: Genetics of vesicoureteral reflux. *Nat Rev Urol* 8: 539–552, 2011
- Steele BT, De Maria J: A new perspective on the natural history of vesicoureteric reflux. *Pediatrics* 90: 30–32, 1992
- Yeung CK, Godley ML, Dhillon HK, Gordon I, Duffy PG, Ransley PG: The characteristics of primary vesico-ureteric reflux in male and female infants with pre-natal hydronephrosis. *Br J Urol* 80: 319–327, 1997

12. Ashraf S, Hoskins BE, Chaib H, Hoefele J, Pasch A, Saisawat P, et al.: Mapping of a new locus for congenital anomalies of the kidney and urinary tract on chromosome 8q24. *Nephrol Dial Transplant* 25: 1496–1501, 2010
13. Burger RH: A theory on the nature of transmission of congenital vesicoureteral reflux. *J Urol* 108: 249–254, 1972
14. Chapman CJ, Bailey RR, Janus ED, Abbott GD, Lynn KL: Vesicoureteric reflux: Segregation analysis. *Am J Med Genet* 20: 577–584, 1985
15. Darlow JM, Darlay R, Dobson MG, Stewart A, Charoen P, Southgate J, et al.: Genome-wide linkage and association study implicates the 10q26 region as a major genetic contributor to primary non-syndromic vesicoureteric reflux [published correction appears in *Sci Rep* 8: 459, 2018 10.1038/s41598-017-17992-w]. *Sci Rep* 7: 14595, 2017
16. Darlow JM, Dobson MG, Darlay R, Molony CM, Hunziker M, Green AJ, et al.: A new genome scan for primary nonsyndromic vesicoureteric reflux emphasizes high genetic heterogeneity and shows linkage and association with various genes already implicated in urinary tract development. *Mol Genet Genomic Med* 2: 7–29, 2014
17. Feather SA, Malcolm S, Woolf AS, Wright V, Blyden D, Reid CJ, et al.: Primary, nonsyndromic vesicoureteric reflux and its nephropathy is genetically heterogeneous, with a locus on chromosome 1. *Am J Hum Genet* 66: 1420–1425, 2000
18. Sanna-Cherchi S, Caridi G, Weng PL, Dagnino M, Seri M, Konka A, et al.: Localization of a gene for nonsyndromic renal hypodysplasia to chromosome 1p32-33. *Am J Hum Genet* 80: 539–549, 2007
19. Weng PL, Sanna-Cherchi S, Hensle T, Shapiro E, Werzberger A, Caridi G, et al.: A recessive gene for primary vesicoureteral reflux maps to chromosome 12p11-q13. *J Am Soc Nephrol* 20: 1633–1640, 2009
20. Georgas KM, Armstrong J, Keast JR, Larkins CE, McHugh KM, Southard-Smith EM, et al.: An illustrated anatomical ontology of the developing mouse lower urogenital tract. *Development* 142: 1893–1908, 2015
21. Liaw A, Cunha GR, Shen J, Cao M, Liu G, Sinclair A, et al.: Development of the human bladder and ureterovesical junction. *Differentiation* 103: 66–73, 2018
22. Rasouly HM, Lu W: Lower urinary tract development and disease. *Wiley Interdiscip Rev Syst Biol Med* 5: 307–342, 2013
23. Williams G, Fletcher JT, Alexander SI, Craig JC: Vesicoureteral reflux. *J Am Soc Nephrol* 19: 847–862, 2008
24. Sanna-Cherchi S, Westland R, Ghiggeri GM, Gharavi AG: Genetic basis of human congenital anomalies of the kidney and urinary tract. *J Clin Invest* 128: 4–15, 2018
25. Sanyanusin P, Schimmenti LA, McNoe LA, Ward TA, Pierpont ME, Sullivan MJ, et al.: Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. *Nat Genet* 9: 358–364, 1995
26. Lu W, van Eerde AM, Fan X, Quintero-Rivera F, Kulkarni S, Ferguson H, et al.: Disruption of ROBO2 is associated with urinary tract anomalies and confers risk of vesicoureteral reflux. *Am J Hum Genet* 80: 616–632, 2007
27. Gimelli S, Caridi G, Beri S, McCracken K, Boccardi R, Zordan P, et al.: Mutations in SOX17 are associated with congenital anomalies of the kidney and the urinary tract. *Hum Mutat* 31: 1352–1359, 2010
28. Gbadegesin RA, Brophy PD, Adeyemo A, Hall G, Gupta IR, Hains D, et al.: TNXB mutations can cause vesicoureteral reflux. *J Am Soc Nephrol* 24: 1313–1322, 2013
29. van Eerde AM, Duran K, van Riel E, de Kovel CG, Koeleman BP, Knoers NV, et al.: Genes in the ureteric budding pathway: Association study on vesico-ureteral reflux patients. *PLoS One* 7: e31327, 2012
30. Verbitsky M, Westland R, Perez A, Kiryluk K, Liu Q, Krithivasan P, et al.: The copy number variation landscape of congenital anomalies of the kidney and urinary tract. *Nat Genet* 51: 117–127, 2019
31. Keren R, Carpenter MA, Hoberman A, Shaikh N, Matoo TK, Chesney RW, et al.: Rationale and design issues of the Randomized Intervention for Children With Vesicoureteral Reflux (RIVUR) study. *Pediatrics* 122 [Suppl 5]: S240–S250, 2008
32. Furth SL, Cole SR, Moxey-Mims M, Kaskel F, Mak R, Schwartz G, et al.: Design and methods of the Chronic Kidney Disease in Children (CKiD) prospective cohort study. *Clin J Am Soc Nephrol* 1: 1006–1015, 2006
33. Wong CJ, Moxey-Mims M, Jerry-Fluker J, Warady BA, Furth SL: CKiD (CKD in children) prospective cohort study: A review of current findings. *Am J Kidney Dis* 60: 1002–1011, 2012
34. Matise TC, Ambite JL, Buyske S, Carlson CS, Cole SA, Crawford DC, et al.: PAGE Study: The Next PAGE in understanding complex traits: Design for the analysis of Population Architecture Using Genetics and Epidemiology (PAGE) Study. *Am J Epidemiol* 174: 849–859, 2011
35. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al.: PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81: 559–575, 2007
36. Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM: Robust relationship inference in genome-wide association studies. *Bioinformatics* 26: 2867–2873, 2010
37. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D: Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 38: 904–909, 2006
38. Wang K, Li M, Hadley D, Liu R, Glessner J, Grant SF, et al.: PennCNV: An integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. *Genome Res* 17: 1665–1674, 2007
39. Sanna-Cherchi S, Kiryluk K, Burgess KE, Bodria M, Sampson MG, Hadley D, et al.: Copy-number disorders are a common cause of congenital kidney malformations. *Am J Hum Genet* 91: 987–997, 2012
40. Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST; Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee: American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genet Med* 13: 680–685, 2011
41. South ST, Lee C, Lamb AN, Higgins AW, Kearney HM; Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee: ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: Revision 2013. *Genet Med* 15: 901–909, 2013
42. Verbitsky M, Sanna-Cherchi S, Fasel DA, Levy B, Kiryluk K, Wuttke M, et al.: Genomic imbalances in pediatric patients with chronic kidney disease. *J Clin Invest* 125: 2171–2178, 2015
43. Westland R, Verbitsky M, Vukojevic K, Perry BJ, Fasel DA, Zwijsenburg PJG, et al.: Copy number variation analysis identifies novel CAKUT candidate genes in children with a solitary functioning kidney. *Kidney Int* 88: 1402–1410, 2015
44. Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, et al.: DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. *Am J Hum Genet* 84: 524–533, 2009
45. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, et al.: A copy number variation morbidity map of developmental delay. *Nat Genet* 43: 838–846, 2011
46. Reddy UM, Page GP, Saade GR, Silver RM, Thorsten VR, Parker CB, et al.; NICHD Stillbirth Collaborative Research Network: Karyotype versus microarray testing for genetic abnormalities after stillbirth. *N Engl J Med* 367: 2185–2193, 2012
47. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al.: Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med* 367: 2175–2184, 2012
48. Patterson N, Price AL, Reich D: Population structure and eigenanalysis. *PLoS Genet* 2: e190, 2006
49. Willer CJ, Li Y, Abecasis GR: METAL: Fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 26: 2190–2191, 2010

50. Fadista J, Manning AK, Florez JC, Groop L: The (in)famous GWAS P-value threshold revisited and updated for low-frequency variants. *Eur J Hum Genet* 24: 1202–1205, 2016
51. González JR, Carrasco JL, Dudbridge F, Armengol L, Estivill X, Moreno V: Maximizing association statistics over genetic models. *Genet Epidemiol* 32: 246–254, 2008
52. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Glied TP, et al.: LocusZoom: Regional visualization of genome-wide association scan results. *Bioinformatics* 26: 2336–2337, 2010
53. Finucane HK, Bulik-Sullivan B, Gusev A, Trynka G, Reshef Y, Loh P-R, et al.; ReproGen Consortium; Schizophrenia Working Group of the Psychiatric Genomics Consortium; RACI Consortium: Partitioning heritability by functional annotation using genome-wide association summary statistics. *Nat Genet* 47: 1228–1235, 2015
54. Vilhjálmsson BJ, Yang J, Finucane HK, Gusev A, Lindström S, Ripke S, et al.; Schizophrenia Working Group of the Psychiatric Genomics Consortium, Discovery, Biology, and Risk of Inherited Variants in Breast Cancer (DRIVE) study: Modeling linkage disequilibrium increases accuracy of polygenic risk scores. *Am J Hum Genet* 97: 576–592, 2015
55. ICD-9-CM diagnosis and procedure codes: Abbreviated and full code titles. Baltimore, MD, U.S. Centers of Medicare & Medicaid Services, 2014. Available at www.cms.gov/Medicare/Coding/ICD9ProviderDiagnosticCodes/codes. Accessed May 14, 2017
56. Carroll RJ, Bastarache L, Denny JC: R PheWAS: Data analysis and plotting tools for phenome-wide association studies in the R environment. *Bioinformatics* 30: 2375–2376, 2014
57. Srinivas S, Goldberg MR, Watanabe T, D'Agati V, al-Awqati Q, Costantini F: Expression of green fluorescent protein in the ureteric bud of transgenic mice: A new tool for the analysis of ureteric bud morphogenesis. *Dev Genet* 24: 241–251, 1999
58. Yamaguchi TP, Bradley A, McMahon AP, Jones S: A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* 126: 1211–1223, 1999
59. Kitzler TM, Schneider R, Kohl S, Kolvenbach CM, Connaughton DM, Dai R, et al.: COL4A1 mutations as a potential novel cause of autosomal dominant CAKUT in humans. *Hum Genet* 138: 1105–1115, 2019
60. Pasch A, Hoefele J, Grimminger H, Hacker HW, Hildebrandt F: Multiple urinary tract malformations with likely recessive inheritance in a large Somali kindred. *Nephrol Dial Transplant* 19: 3172–3175, 2004
61. Vivante A, Kleppa MJ, Schulz J, Kohl S, Sharma A, Chen J, et al.: Mutations in TBX18 cause dominant urinary tract malformations via transcriptional dysregulation of ureter development. *Am J Hum Genet* 97: 291–301, 2015
62. Capozza N, Gulia C, Heidari Bateni Z, Zangari A, Gigli S, Briganti V, et al.: Vesicoureteral reflux in infants: What do we know about the gender prevalence by age? *Eur Rev Med Pharmacol Sci* 21: 5321–5329, 2017
63. Cui C, Chatterjee B, Lozito TP, Zhang Z, Francis RJ, Yagi H, et al.: Wdpcp, a PCP protein required for ciliogenesis, regulates directional cell migration and cell polarity by direct modulation of the actin cytoskeleton. *PLoS Biol* 11: e1001720, 2013
64. Jorgez CJ, Rosenfeld JA, Wilken NR, Vangapandu HV, Sahin A, Pham D, et al.: Genitourinary defects associated with genomic deletions in 2p15 encompassing OTX1. *PLoS One* 9: e107028, 2014
65. King JA, Marker PC, Seung KJ, Kingsley DM: BMP5 and the molecular, skeletal, and soft-tissue alterations in short ear mice. *Dev Biol* 166: 112–122, 1994
66. Fan WJ, Chen SC, Hsieh TH, Lai CH, Lin YS, Peng CW, et al.: Influence of serotonergic mechanisms on the urine flow rate in male rats. *Am J Physiol Regul Integr Comp Physiol* 307: R1239–R1250, 2014
67. Iliescu A, Gravel M, Horth C, Gros P: Independent mutations at Arg181 and Arg274 of Vangl proteins that are associated with neural tube defects in humans decrease protein stability and impair membrane targeting. *Biochemistry* 53: 5356–5364, 2014
68. Pietilä I, Prunskaitė-Hyyryläinen R, Kaisto S, Tika E, van Eerde AM, Salo AM, et al.: Wnt5a deficiency leads to anomalies in ureteric tree development, tubular epithelial cell organization and basement membrane integrity pointing to a role in kidney collecting duct patterning. *PLoS One* 11: e0147171, 2016
69. Sanna-Cherchi S, Khan K, Westland R, Krithivasan P, Fievet L, Rasouly HM, et al.: Exome-wide association study identifies GREB1L mutations in congenital kidney malformations [published correction appears in *Am J Hum Genet* 101: 789–802, 2017 10.1016/j.ajhg.2017.09.018]. *Am J Hum Genet* 101: 1034, 2017
70. Yun K, Ajima R, Sharma N, Costantini F, Mackem S, Lewandoski M, et al.: Non-canonical Wnt5a/Ror2 signaling regulates kidney morphogenesis by controlling intermediate mesoderm extension. *Hum Mol Genet* 23: 6807–6814, 2014
71. Yun K, Perantoni AO: Hydronephrosis in the Wnt5a-ablated kidney is caused by an abnormal ureter-bladder connection. *Differentiation* 94: 1–7, 2017
72. Harding SD, Armit C, Armstrong J, Brennan J, Cheng Y, Haggarty B, et al.: The GUDMAP database—an online resource for genitourinary research. *Development* 138: 2845–2853, 2011
73. Al-Kateb H, Khanna G, Filges I, Hauser N, Grange DK, Shen J, et al.: Scoliosis and vertebral anomalies: Additional abnormal phenotypes associated with chromosome 16p11.2 rearrangement. *Am J Med Genet A* 164A: 1118–1126, 2014
74. Glessner JT, Bick AG, Ito K, Homsy J, Rodriguez-Murillo L, Fromer M, et al.: Increased frequency of de novo copy number variants in congenital heart disease by integrative analysis of single nucleotide polymorphism array and exome sequence data. *Circ Res* 115: 884–896, 2014
75. Greenway SC, Pereira AC, Lin JC, DePalma SR, Israel SJ, Mesquita SM, et al.: De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. *Nat Genet* 41: 931–935, 2009
76. Guilmatre A, Dubourg C, Mosca AL, Legallic S, Goldenberg A, Drouin-Garraud V, et al.: Recurrent rearrangements in synaptic and neurodevelopmental genes and shared biologic pathways in schizophrenia, autism, and mental retardation. *Arch Gen Psychiatry* 66: 947–956, 2009
77. Pescosolido MF, Gamsiz ED, Nagpal S, Morrow EM: Distribution of disease-associated copy number variants across distinct disorders of cognitive development. *J Am Acad Child Adolesc Psychiatry* 52: 414–430.e14, 2013
78. Kujat A, Schulz MD, Streng S, Froster UG: Renal malformations in deletion 22q11.2 patients. *Am J Med Genet A* 140: 1601–1602, 2006
79. Wu HY, Rusnack SL, Bellah RD, Plachter N, McDonald-McGinn DM, Zackai EH, et al.: Genitourinary malformations in chromosome 22q11.2 deletion. *J Urol* 168: 2564–2565, 2002
80. Lopez-Rivera E, Liu YP, Verbitsky M, Anderson BR, Capone VP, Otto EA, et al.: Genetic drivers of kidney defects in the DiGeorge syndrome. *N Engl J Med* 376: 742–754, 2017
81. Sanna-Cherchi S, Sampogna RV, Papeta N, Burgess KE, Nees SN, Perry BJ, et al.: Mutations in DSTYK and dominant urinary tract malformations. *N Engl J Med* 369: 621–629, 2013
82. Haller M, Au J, O'Neill M, Lamb DJ: 16p11.2 transcription factor MAZ is a dosage-sensitive regulator of genitourinary development. *Proc Natl Acad Sci U S A* 115: E1849–E1858, 2018
83. Verbitsky M, Kogon AJ, Matheson M, Hooper SR, Wong CS, Warady BA, et al.: Genomic disorders and neurocognitive impairment in pediatric CKD. *J Am Soc Nephrol* 28: 2303–2309, 2017
84. Geller F, Feenstra B, Carstensen L, Pers TH, van Rooij IA, Körberg IB, et al.: Genome-wide association analyses identify variants in developmental genes associated with hypospadias. *Nat Genet* 46: 957–963, 2014
85. van der Zanden LF, van Rooij IA, Feitz WF, Knight J, Donders AR, Renkema KY, et al.: Common variants in DGKK are strongly associated with risk of hypospadias. *Nat Genet* 43: 48–50, 2011

86. Yang B, Zhou W, Jiao J, Nielsen JB, Mathis MR, Heydarpour M, et al.: Protein-altering and regulatory genetic variants near GATA4 implicated in bicuspid aortic valve. *Nat Commun* 8: 15481, 2017
87. Kim SK, Shindo A, Park TJ, Oh EC, Ghosh S, Gray RS, et al.: Planar cell polarity acts through septins to control collective cell movement and ciliogenesis. *Science* 329: 1337–1340, 2010
88. Saari J, Lovell MA, Yu HC, Bellus GA: Compound heterozygosity for a frame shift mutation and a likely pathogenic sequence variant in the planar cell polarity—ciliogenesis gene WPCP in a girl with polysyndactyly, coarctation of the aorta, and tongue hamartomas. *Am J Med Genet A* 167A: 421–427, 2015
89. Atmōş B, Karabay-Bayazıt A, Melek E, Bişgin A, Anarat A: Renal features of Bardet Biedl syndrome: A single center experience. *Turk J Pediatr* 61: 186–192, 2019
90. Sharifian M, Dadkhah-Chimeh M, Einollahi B, Nafar M, Simforoush N, Basiri A, et al.: Renal transplantation in patients with Bardet-Biedl syndrome. *Arch Iran Med* 10: 339–342, 2007
91. Bain MD, Winter RM, Burn J: Robinow syndrome without mesomelic 'brachymelia': A report of five cases. *J Med Genet* 23: 350–354, 1986
92. Roifman M, Marcelis CL, Paton T, Marshall C, Silver R, Lohr JL, et al.; FORGE Canada Consortium: De novo WNT5A-associated autosomal dominant Robinow syndrome suggests specificity of genotype and phenotype. *Clin Genet* 87: 34–41, 2015
93. Kohn AD, Moon RT: Wnt and calcium signaling: Beta-catenin-independent pathways. *Cell Calcium* 38: 439–446, 2005
94. Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, et al.: Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet* 50: 1219–1224, 2018
95. Khera AV, Chaffin M, Wade KH, Zahid S, Brancale J, Xia R, et al.: Polygenic prediction of weight and obesity trajectories from birth to adulthood. *Cell* 177: 587–596.e9, 2019

AFFILIATIONS

¹Division of Nephrology, Department of Medicine, Columbia University, New York, New York

²Department of Urology, Columbia University, New York, New York

³Department of Internal Medicine, Cardiology, University of Michigan, Ann Arbor, Michigan

⁴Department of Pediatric Nephrology, University of California, Los Angeles Medical Center and University of California, Los Angeles Medical Center-Santa Monica, Los Angeles, California

⁵Section of Nephrology, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy

⁶Department of Pediatric Nephrology, Vrije Universiteit University Medical Center, Amsterdam, The Netherlands

⁷Division of Nephrology, Boston Children's Hospital, Boston, Massachusetts

⁸Division of Nephrology, Dialysis, Transplantation, and Laboratory on Pathophysiology of Uremia, Istituto G. Gaslini, Genoa, Italy

⁹Department of Pediatrics, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts

¹⁰Section of Nephrology, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy

¹¹Department of Pediatric Surgery, Spedali Civili Children's Hospital of Brescia, Brescia, Italy

¹²Chair and Division of Nephrology, University and Spedali Civili Hospital, Brescia, Italy

¹³Division of Nephrology and Department of Obstetrics and Gynecology, ASST Spedali Civili of Brescia, Brescia, Italy

¹⁴Department of Pediatrics, University Hospital of Split, Split, Croatia

¹⁵School of Medicine, University of Split, Split, Croatia

¹⁶Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

¹⁷Department of Pediatric Nephrology, Azienda Ospedaliera Universitaria "G. Martino," Messina, Italy

¹⁸University College Dublin School of Medicine, Our Lady's Children's Hospital Crumlin, Dublin, Ireland

¹⁹Department of Clinical Genetics, Our Lady's Children's Hospital Crumlin, Dublin, Ireland

²⁰National Children's Research Centre, Our Lady's Children's Hospital Crumlin, Dublin, Ireland

²¹Department of Pediatric Surgery, Beacon Hospital, University College Dublin, Dublin, Ireland

²²Division of Nephrology, Departments of Pediatrics and Epidemiology, Perelman School of Medicine at the University of Pennsylvania, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

²³Division of Nephrology, Department of Pediatrics, University of Missouri-Kansas City School of Medicine, Children's Mercy Kansas City, Kansas City, Missouri

²⁴Nephrology Unit, Parma University Hospital and Department of Medicine and Surgery, Parma University Medical School, Parma, Italy

²⁵Department of Pediatric Urology, Columbia University College of Physicians and Surgeons, New York, New York

²⁶Renal and Dialysis Unit, Department of Medicine, School of Medicine, University of Verona, Verona, Italy

²⁷Department of Internal Medicine, Nephrology, Dialysis and Transplantation Clinics, Genoa University and IRCCS Policlinico San Martino, Genoa, Italy

²⁸Division of Pediatric Nephrology, Department of Pediatrics, Columbia University, New York, New York

²⁹Department of Pediatrics, Unit of Pediatric Nephrology, Interdisciplinary Laboratory of Medical Investigation, Faculty of Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil

³⁰Department of Pediatrics, Child Nephrology, Dialysis and Management of Acute Poisoning, Pomeranian Medical University, Szczecin, Poland

³¹Department of Pediatric Nephrology and Hypertension, Jagiellonian University Medical College, Krakow, Poland

³²Department of Pediatrics, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Katowice, Poland

³³Department of Pediatrics, Immunology and Nephrology, Polish Mother's Memorial Hospital Research Institute, Lodz, Poland

³⁴Department of Pediatric Nephrology, Medical University of Lublin, Lublin, Poland

³⁵Department of Pediatrics and Nephrology, Medical University of Warsaw, Poland

³⁶Department of Pediatrics, University of Zielona Góra, Zielona Góra, Poland

³⁷University Clinic for General, Visceral and Transplantation Surgery, University of Heidelberg, Heidelberg, Germany

³⁸University Children's Hospital, Medical Faculty of Skopje, Skopje, Macedonia

³⁹Department of Biostatistics, Columbia University, New York, New York

⁴⁰Department of Biomedical Informatics and Medical Education, University of Washington School of Medicine, Seattle, Washington

⁴¹Division of Pediatric Nephrology, University of New Mexico Children's Hospital, Albuquerque, New Mexico

⁴²Institute for Genomic Health, Icahn School of Medicine at Mount Sinai, New York, New York

⁴³Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York

⁴⁴Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York

⁴⁵The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York

⁴⁶Department of Pathology and Cell Biology, Columbia University, New York, New York

⁴⁷Center for Applied Genomics, The Children's Hospital of Philadelphia and Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

⁴⁸Department of Medical Genetics, Poznan University of Medical Sciences, and NZOZ Center for Medical Genetics GENESIS, Poznan, Poland

⁴⁹Department of Human Genetics, University of Michigan, Ann Arbor, Michigan

⁵⁰Department of Computational Medicine and Bioinformatics, Ann Arbor, Michigan