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Rare Single Nucleotide and Copy Number Variants and the Etiology of Congenital Obstructive Uropathy:  
Implications for Genetic Diagnosis

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**Abstract:**

Background: Congenital obstructive uropathy (COU) is a common cause of developmental defects of the urinary tract, with heterogeneous clinical presentation and outcome. Genetic analysis has the potential to elucidate the underlying diagnosis and help risk stratification.

Methods: We performed a comprehensive genomic screen of 733 independent COU cases, which consisted of individuals with ureteropelvic junction obstruction (UPJO; n=321), ureterovesical junction obstruction/congenital megaureter (UVJO; n=178), and congenital hydronephrosis not otherwise specified (COU-NOS; n=234). We identified pathogenic single nucleotide variants (SNVs) in 53 (7.2%) cases and genomic disorders in 23 (3.1%) cases. No significant differences in the overall diagnostic yield among COU sub-phenotypes nor pathogenic SNVs in several genes were associated with any of the three categories.

Discussion: Although COU may appear phenotypically heterogeneous, COU phenotypes are likely to share common molecular bases. However, mutations in *TNXB* were more often identified in COU-NOS cases, demonstrating the diagnostic challenge in discriminating COU from hydronephrosis secondary to vesicoureteral reflux, particularly when diagnostic imaging is incomplete. Pathogenic SNVs in only six genes were found in more than one individual, supporting high genetic heterogeneity. Finally, convergence between data on SNVs and genomic disorders suggest *MYH11* as a dosage-sensitive gene possibly correlating with severity of COU.

Conclusion: We established a genomic diagnosis in 10.0% of COU individuals. The findings underscore the urgent need to identify novel genetic susceptibility factors to COU to better define the natural history of the remaining 90% of cases without a molecular diagnosis.

**Significance Statement:**

Congenital obstructive uropathy (COU) is a prevalent human developmental defect with highly heterogeneous clinical presentations and outcomes. Genetics may refine diagnosis, prognosis, and treatment, but the genomic architecture of COU is largely unknown. Comprehensive genomic screening study of 733 cases with three distinct COU sub-phenotypes revealed disease etiology in 10.0% of them. We detected no significant differences in the overall diagnostic yield among COU sub-phenotypes, with characteristic variable expressivity of several mutant genes. Our findings therefore may legitimize a “genetic first” diagnostic approach for COU, especially when burdening clinical and imaging characterization is not complete or available.

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**Rare Single Nucleotide and Copy Number Variants and the Etiology of Congenital Obstructive Uropathy: Implications for Genetic Diagnosis**

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**ABSTRACT**

**Background:** Congenital obstructive uropathy (COU) is a common cause of developmental defects of the urinary tract, with heterogeneous clinical presentation and outcome. Genetic analysis has the potential to elucidate the underlying diagnosis and help risk stratification.

**Methods:** We performed a comprehensive genomic screen of 733 independent COU cases, which consisted of individuals with ureteropelvic junction obstruction (UPJO; n=321), ureterovesical junction obstruction/congenital megaureter (UVJO; n=178), and congenital hydronephrosis not otherwise specified (COU-NOS; n=234). We identified pathogenic single nucleotide variants (SNVs) in 53 (7.2%) cases and genomic disorders in 23 (3.1%) cases. No significant differences in the overall diagnostic yield among COU sub-phenotypes nor pathogenic SNVs in several genes were associated with any of the three categories.

**Discussion:** Although COU may appear phenotypically heterogeneous, COU phenotypes are likely to share common molecular bases. However, mutations in *TNXB* were more often identified in COU-NOS cases, demonstrating the diagnostic challenge in discriminating COU from hydronephrosis secondary to vesicoureteral reflux, particularly when diagnostic imaging is incomplete. Pathogenic SNVs in only six genes

were found in more than one individual, supporting high genetic heterogeneity. Finally, convergence between data on SNVs and genomic disorders suggest *MYH11* as a dosage-sensitive gene possibly correlating with severity of COU.

Conclusion: We established a genomic diagnosis in 10.0% of COU individuals. The findings underscore the urgent need to identify novel genetic susceptibility factors to COU to better define the natural history of the remaining 90% of cases without a molecular diagnosis.

ACCEPTED

## INTRODUCTION

Obstructive uropathy is caused by structural or functional defects of the urinary tract that constrain urinary flow from the kidneys to the bladder<sup>1,2</sup>. Congenital hydronephrosis is the main presenting indicator for urinary tract obstruction, which is diagnosed in 2 to 29 cases per 10,000 live births<sup>3</sup>. Because hydronephrosis is clinically silent in most cases and neonates and children do not undergo screening by imaging studies, this incidence is vastly underestimated. In humans, kidney and urinary tract development starts at the fifth gestational week, when protrusion of the ureteric bud into the metanephric mesenchyme enables the formation of a patent ureter and fetal urinary flow from the metanephros to the embryonic bladder<sup>4,5</sup>. At the same time, the bladder and urethra are formed from the urogenital sinus<sup>6,7</sup>. Unilateral or bilateral perturbations in these tightly regulated processes may therefore affect every level of the kidney and urinary tract, resulting in obstructive uropathy categorized into four main phenotypic groups based on their anatomical localization: (1) uretero-pelvic junction obstruction (UPJO), (2) uretero-vesical junction obstruction (UVJO) leading to a (non-refluxing) megaureter, (3) dysfunction of the bladder e.g. caused by either neurogenic (e.g. spina bifida) or non-neurogenic causes, and, (4) lower urinary outflow tract obstruction caused by posterior urethral valves (PUV), urethral atresia or prolapsing ureterocele<sup>8,9</sup>. Based on a clinical perspective, UPJO and UVJO are often considered as congenital obstructive uropathy (COU) phenotypes, whereas bladder dysfunction and PUV are conditions stratified under lower urinary tract obstruction (LUTO). Depending on their severity and/or co-occurrence of other perturbations in development, all of the above mentioned diagnoses can lead to significant morbidity and mortality after birth<sup>10-12</sup>, and the potential requirement for early surgical interventions to prevent progression of kidney failure<sup>13</sup>. Genetics has the potential to aid in the ascertainment of diagnosis, prognosis and treatment of COU patients<sup>2,14</sup>. However, despite the fact that COU is a common human developmental defect, its molecular etiology remains largely elusive<sup>15</sup>. Notwithstanding a clear familial occurrence for COU phenotypes, genetic discoveries of monogenic causes or copy number variants (CNV, i.e. large deletions or duplications within the genome) lag behind when compared to other congenital anomalies of the kidney and

urinary tract (CAKUT) such as kidney hypodysplasia<sup>14,16,17</sup>. One major explanation is that relatively small and heterogeneous cohorts of individuals with COU have been subjected to genetic testing until now<sup>2,14,18</sup>. Nevertheless, our current genetic knowledge for COU shows strong overlap with other CAKUT phenotypes, as Mendelian forms caused by rare pathogenic single nucleotide variants (SNVs) or genomic disorders caused by pathogenic CNVs have both been identified in individuals with COU as well as congenital kidney hypodysplasia<sup>16,17,19,20</sup>. As such, this hallmark of variable expressivity within the complex etiology of CAKUT may hamper our ability to ascertain risk and prognosis, thus resulting in suboptimal clinical management and genetic counseling of patients.

In this study, we aimed to elucidate the genetic etiology of upper urinary tract obstruction by performing a large clinical genomic screen of individuals with UPJO, UVJO and congenital hydronephrosis not otherwise specified (COU-NOS), by using a combined approach of exome sequencing for single nucleotide variants (SNVs) and copy number variation (CNV) analysis. Given the common embryonic background of a disturbed outgrowth of the ureteric bud, we hypothesize that the genetic backgrounds of COU subcategories display strong molecular overlap.

## **METHODS**

### **Study participants**

The study involving human subjects was conducted in accordance with the Declaration of Helsinki. All participants and/or guardians provided written informed consent, and the study was approved by the Institutional Review Board (IRB) of Columbia University Irving Medical Center (CUIMC) and the local ethics committees of participating recruitment sites.

The COU study participants consisted of 733 unrelated and affected individuals recruited from 24 participating sites in 7 countries, Italy (n=296), Poland (n=204), Macedonia (n=118), United States (n=61), Croatia (n=47), The Netherlands (n=4), and Turkey (n=3). Diagnosis was based on the ICD10 –code provided by the recruiting physician. The inclusion criteria included individuals who have been clinically ascertained for UPJO, UVJO or COU-NOS. Individuals with a primary hierarchical

diagnosis of other CAKUT phenotypes including kidney anomaly (KA), duplicated collecting system, vesicoureteral reflux (VUR), horseshoe kidney/ectopic kidney (HK-EK) and LUTO/PUV, in isolation or in addition to COU were excluded, as well as individuals with neurogenic obstructive uropathy (e.g. spina bifida) or non-neurogenic neurogenic bladder. 21,498 population controls with available DNA microarray data describe in our prior publication were used for comparisons of CNV frequencies<sup>16,17,19-22</sup>. An in-house 11,818 multiethnic population controls dataset from the Institute for Genomic Medicine (IGM) was used for allelic frequency estimation of our prioritized SNVs<sup>23</sup>.

### **Exome sequencing, variant and base calling**

Genomic DNA was isolated from whole blood according to standard protocols. Proband-only exome sequencing was performed at three sequencing facilities using either the Illumina HiSeq2500 sequencing platform (Yale Mendelian Genomics Center, YMGC; Columbia Institute for Genomic Medicine, IGM) or the Illumina HiSeqX10 sequencing platform (New York Genome Center), on the following capture kits: IDT Exome Enrichment Panel, Agilent V4, and Roche NimbleGen SeqCap Exome EX v3.0. The DRAGEN v3 platform was utilized to map sequenced reads to the reference genome (hs37d5.fa, Ensembl -GRCh37.73), GATK 3.6 was subsequently used for base quality recalibration, indel realignment and variant calling. ClinEff was used for variant annotation with Ensembl (version GRCh38), EVS-v.0.0.30, ExAC 0.3<sup>24</sup>, gnomAD Exome and gnomAD Genome version 2.1<sup>25</sup>, dbNSFP 4.1a, HGMD 2021.4, Clinvar 2022-01-10<sup>26</sup>, ACMG v3, and REVEL 2016-06-03. Resulting variant calls, sample-level site coverages data and annotations were stored in the ATAV centralized database and queried<sup>23</sup>.

### **Variant-level quality control and prioritization**

We first used a manually curated list of 625 nephropathy-associated genes<sup>27</sup>, from which we further prioritized 382 genes that, when mutated, are known to cause Mendelian forms of isolated or syndromic CAKUT (**Supplementary Table S1**).

Variants-level data from the 733 COU individuals within the 382 prioritized genes were queried using the variant annotation function implemented in the Analysis Tool for Annotated Variants (ATAV)<sup>23</sup>, the analysis variant engine that powers our exome-genome sequencing warehouse (**Supplementary Figure S1**). Variant filtering was performed to require a quality score > 50, quality by depth score >= 2, genotyping quality score >=20, mapping quality score >=40, coverage >= 10, alternate read percentages was within the range of 0.3 and 0.7 for heterozygous genotypes. To further ensure the removal of sequencing artifacts, variants that occurred >=20 within the COU cohort and variants that appeared >=500 within the internal ATAV controls cohort were removed. For variant prioritization, we used the Diagnosticator (<https://diagnosticator.com>) and Varsome (<https://varsome.com/>) web-based platforms that implement the American College of Medical Genetics (ACMG) guidelines<sup>28</sup> as a first-pass screen to predict an ACMG verdict for each uploaded variant for further clinical variant interpretation and genotype-phenotype correlation for all 382 genes. We used the following criteria to define a positive genetic finding for our clinical research variant adjudication. First-tier positive findings were considered if the genotype was already reported as pathogenic or likely pathogenic in ClinVar<sup>26</sup> or classified as pathogenic or likely pathogenic by strict ACMG criteria via individual variant curation in Varsome. Since missense variants and variants never observed in public databases such as gnomAD rarely meet ACMG P/LP criteria and are often classified as US (unknown significance), in order to define our second-tier positive genetic finding we used the following criteria: absent or exceedingly rare in public databases as well as in our in-house 11,818 multiethnic population controls from the Institute for Genomic Medicine (IGM)<sup>23</sup>; a Revel score >=0.5<sup>29</sup>; plausibility of the genetic mutation to be associated to the observed COU phenotype. Additionally, variants with the PVS1 classifier (i.e. null variant in a gene where loss-of-function is a known mechanism of disease) were classified as positive findings even if other criteria were not fulfilled. We next confirmed prioritized variants through Sanger sequencing in the patient DNA and, when available, in family members for segregation analysis in order to add support to our pathogenicity adjudication.



## Exome CNV calling and prioritization

For robust analysis of CNV using exome sequencing data, we first divided our COU exome-sequencing (ES) cohort into four batches grouped by exome-capture kits. GATK DepthOfCoverage (v3.6) and exome Hidden Markov Model, XHMM (v1.0) were used for exome CNV discovery<sup>30</sup>. CNVs were called based on hg19 coordinates. For each batch, we computed coverage statistics from the base-recalibrated and indel realigned, “analysis-ready” bam files restricting coverage computations to the exome-captured intervals in each kit. Raw coverages were merged, outlier targets and samples were removed, and mean centered-data were normalized with PCA to construct a normalized read depth for CNV calling. A subset of 434 COU cases have also been analyzed using chromosomal microarray data. The identification of pathogenic genomic disorders in 162 out of these 434 individuals has been previously published (**Supplementary Figure S1**)<sup>16</sup>. The DNA array CNV calls were detected using PennCNV as described<sup>16,17,19-21</sup>, and the results used for comparison, calibration, and validation of the exome CNV calls. After DNA array and exome-based CNV analysis we used the bedtools intersect function to compare the putative start and end breakpoints of the XHMM-derived CNV against the putative start and end breakpoints of the PennCNV-derived CNV as an orthogonal method to test for congruency. CNVs were annotated with overlapping RefGenes, Known CNVs with reported association with a genomic disorder and curated gene sets. Using the same criteria for CNV prioritization as previously described, CNVs were classified as “pathogenic” (GD-CNV) or “likely pathogenic”<sup>16,17,19-22</sup>. Burden of rare CNVs and pairwise comparisons were conducted using Fisher’s exact or Chi Square, as appropriate.

## RESULTS

### Study cohort

The total cohort included 733 independent COU cases, of whom 321 (43.8%) individuals had UPJO, 178 (24.2%) had UVJO, and 234 (31.9%) individuals were diagnosed with COU-NOS (**Table 1**). The majority of cases were of European ancestry (599, 81.7%; **Supplementary Figure S2**). There was a strong male predominance in COU cases (male 502 (68.5%) vs female 231 (31.5%)). Additional kidney and urinary

tract defects were present in 123 (16.7%) cases, of which reflux nephropathy was most prevalent (6.4% of cases). Extrarenal phenotypes were identified in 127 (17.3%) COU-cases, with abnormalities in musculoskeletal system (n=18, 2.5%), central nervous system (n=14, 1.9%) and cardiac defects (n=19, 2.6%) as predominant conditions. One in five patients had a family history of kidney disease, which was higher in cases with COU-NOS than in individuals with UPJO or UVJO (**Table 1**; OR 2.74, 95% confidence interval (CI) 1.91 – 3.92; Fisher's exact *P*-value =  $3.1 \times 10^{-8}$  vs combined group of UPJO/UVJO).

### **Exome sequencing identifies rare pathogenic SNVs in 7.2% of COU cases**

We queried ES data for 382 manually-curated genes in 733 COU cases using ATAV (**Supplementary Table S1, Supplementary Figure S1**)<sup>23</sup>. Of these 382 genes known to be associated to CAKUT when mutated, 127 were associated with dominant inheritance (119 autosomal and 8 X-linked), 240 with recessive inheritance (225 autosomal, 1 digenic, and 14 X-linked), and 15 genes associated with both dominant and recessive inheritance (10 autosomal, 5 X-linked). We retrieved 8,525 raw variants from the ATAV database and annotated them using Diagnosticator. After standard quality control, 1,677 variants were removed. Additional 1,181 variants were further removed because affecting non coding regions (ex 5' or 3' UTR) or classified as "Benign", "Likely Benign" or "Benign/Likely Benign" in ClinVar at the time of analysis. We next analyzed the remaining 5,667 variants based on the reported mode of inheritance for all CAKUT disorders associated with the genes. To further prioritize rare variants, we removed variants with more than 0.05% minor allele frequency and more than 1% allele frequency for dominant and recessive genes, respectively, in all populations from the public repositories ExAC<sup>24</sup> and gnomAD v2.1.1 genomes<sup>25</sup>. Finally, we individually curated the remaining variants using Diagnosticator and VarSome as decision support tools and applied the first- and second- tier criteria to adjudicate positive findings as described above. Finally, we identified positive genetic findings in 53 (7.2%) COU cases. Of these, 40 (75.4%) individuals had an autosomal dominant genetic cause of COU, 6 (11.3%) individuals harbored pathogenic SNVs in genes with an autosomal dominant or recessive mode of inheritance, and 7 (13.2%) individuals had

an autosomal recessive form of COU, demonstrating a significantly skewed distribution towards genes with an autosomal dominant mode of inheritance in our study cohort. Comparison of COU subcategories did not reveal statistically significant differences in proportions of identified disease-associated SNVs between the three diagnosis groups (Chi square 2x3,  $P = 0.57$ ; **Figure 1**). An overview of all identified SNVs is summarized in **Table 2**.

When zooming in at the contribution of each single gene to the etiology of COU, we identified a striking genetic heterogeneity (33 distinct genes affected in 53 cases) with only a handful of genes harboring pathogenic variants in more than one individual (**Table 2**). The latter included *TNXB* (n=6), *HNF1B* (n=4), *TBX18* (n=3), *PAX2* (n=2), *ALDH18A1* (n=2) and *TP63* (n=2). Genes with an autosomal dominant mode of inheritance predominantly encoded for transcription factors or proteins with a pivotal role in transcription (13/30, 43%; e.g. *HNF1B*, *PAX2*, *TBX18*, *SALL1*, *EYA1*, *FOXC1*, *BMP4*, *BMP7* and others). Interestingly, variants in these genes were identified across all three COU sub-phenotypes, indicating the highly variable expressivity within the genetic etiology of urinary tract malformations, and suggesting that, at least for a fraction of genes involved in COU etiology, the underlying sub-phenotype has no predictive value for the underlying molecular cause. Other important subgroups of autosomal dominant genes known to be associated with COU encode signaling molecules that play a role in multiple developmental processes and cell fate decisions, such as *BRAF*, *NOTCH2* and *SHH*<sup>31-33</sup>. This is in contrast with the variants found in genes with an autosomal dominant/recessive or autosomal recessive mode of inheritance, where the molecular action of genes was much more heterogeneous, including genes that encode for extracellular matrix proteins (*TNXB*, *FREM1*)<sup>34,35</sup>, muscle proteins (*MYH11*)<sup>36</sup>, nuclear factors (*SDCCAG8*)<sup>37</sup>, and transmembrane proteins (*DYNC2H1*)<sup>38</sup>. In addition to the aforementioned genetic pleiotropy of genes underlying the different COU phenotypes, we observed mutations in *TNXB* mostly in cases with COU-NOS (4 out of 5 individuals). As variants in *TNXB* have been previously predominantly associated to VUR<sup>35,39</sup>, this specific finding most likely reflects the challenging diagnostic interpretation of hydronephrosis and its distinction from VUR when a voiding cystourethrogram (VCUG) has not been performed or available.

Segregation analysis using in a subset of affected cases for whom parental DNA was available for analysis (**Table 2**) identified *de novo* pathogenic SNVs in two cases. In another two individuals with COU, we could establish familial segregation of the variant in affected individuals. Finally, 10 variants were either maternally or paternally inherited from parents with an unknown urinary tract phenotype. Due to the retrospective nature of the study and the different infrastructure at individual recruitment sites, parental DNA was not available for most cases, thus preventing complete assessment of the variants' inheritance patterns in our cohort.

Further annotation of variants identified additional 8 COU cases (UPJO n=3, UVJO n=2, COU-NOS n=3) that carried a heterozygous SNV in 8 distinct genes with an autosomal dominant mode of inheritance that were completely absent in public repositories and were also predicted to be deleterious according to different publicly accessible prediction tools but did not fulfill our first- or second- tier criteria (**Supplementary Table S2**). These 8 SNVs were classified as variants of uncertain significance pending additional genetic or segregation support.

### **Rare pathogenic CNVs make up the genetic architecture of an additional 3.1% of COU cases**

Under the hypothesis that a fraction of the 680 “unsolved” COU cases might be attributable to CNVs associated to genomic disorders, we conducted an exome-wide CNV analysis using GATK DepthOfCoverage and exome Hidden Markov Model (XHMM) using ES data from the entire cohort (**Supplementary Figure S1**). Out of 733 cases, 468 (63.8%) had also an available Illumina DNA microarray that was used to call CNVs as previously described<sup>16,17,19-22</sup> and results used for cross-validation of the CNV calls from ES. Using this combinatorial approach, we identified 18 distinct genomic disorders in 23 (3.1%) unique COU cases (**Table 3**). When compared to 134 (0.6%) genomic disorders in 21,498 in controls, this represented a highly significant burden excess of GD in COU (OR 5.16, 95% CI 3.14-8.14; Fisher's exact  $P = 2.09 \times 10^{-9}$ ). Similar to what is observed for the SNVs above, the landscape of CNVs showed high genetic heterogeneity with 18 pathogenic CNVs at 15 chromosomal loci in 23 independent COU cases. In fact, we observed only four loci that were copy number

variable in more than one individual: the chr.1q21.1 TAR syndrome region (one deletion and one duplication; both UVJO); the chr.16p13.11 locus (four deletions, one duplication; four UVJO, one COU-NOS); the 17q12 RCAD syndrome region (three deletions; two COU-NOS, one UPJO); and the chr.22q11.2 microdeletion syndrome region (i.e. DiGeorge/Velocardiofacial Syndrome), for which one UVJO case carried a 22q11.2 microdeletion between low-copy-repeats (LCR) B-D, while one individual with UPJO carried a 22q11.2 microdeletion between LCR A-D (**Table 3**). Taken together, these 4 genomic disorders loci explained nearly half (11/23, 47.8%) of the GD carriers in our COU cases. Although cases with UVJO had a higher burden of GD (8/178, 4.5%) as compared to UPJO or COU-NOS cases (9/321, 2.8%; 6/234, 2.6%, respectively), this difference was not statistically significant (Chi Square 2x3,  $P = 0.48$ ; **Figure 1**). Larger sample size cohorts are required to verify if indeed the UVJO subcategory is more frequently caused by GD as compared to the other classes of COU. Interestingly, COU cases were enriched for deletions compared to duplications (16 deletions vs. 7 duplications), implicating reduced gene dosage via haploinsufficiency as the main molecular mechanism that underlies obstructive uropathies.

Additional annotation of CNVs identified 4 microdeletions and 1 microduplication in 5 COU individuals that all were <100 kb in size, intersected with known CAKUT genes in humans or mice, and were completely absent in 21,498 controls (**Supplementary Table S3**). Since these CNVs have not (yet) been linked to a known genomic disorder that includes CAKUT, we defined these additional CNVs as variants of unknown significance.

Importantly, pathogenic CNVs were identified in three individuals with a pathogenic or likely pathogenic SNV or genotype (and vice versa) (**Table 3**), supporting a correct causality attribution in the two independent analyses for the majority of patients. Of these cases carrying a potentially pathogenic SNV as well as a CNV, one subject with UVJO (P74) carried two ultrarare and potentially pathogenic SNVs in *FGFR3* (with a autosomal dominant or recessive mode of inheritance) or *TBX6* (a driver of the CAKUT phenotypes in the chromosome 16p11.2 microdeletion syndrome<sup>16</sup>) as well as a 349 kb deletion at the incompletely penetrant chromosome 1q21.1 susceptibility locus for TAR-syndrome. Another subject (P30) with UPJO carried a very rare ClinVar pathogenic

SNV in *TBX18*, a known gene associated to ureter maldevelopment<sup>40</sup>, as well as a 919 kb deletion at chromosome 1q21.1, a locus shown to display incomplete penetrance and variable expressivity<sup>41</sup>. Finally, in the last subject, affected by COU-NOS (P04), we identified another very rare SNV in *TBX18* and the typical 1.4 Mb RCAD deletion at chromosome 17q21. Interestingly, these 3 COU cases did not present with a more severe urinary or extra-urinary phenotype as compared to the rest of the cohort. Hence, in order to dissect the exact pathomechanisms leading to COU in these individuals, additional genetic and functional studies need to be performed.

**The overall genomic architecture of COU indicates a diagnosis in about one in 10 individuals, identifies both commonalities and differences among COU sub-phenotypes, and supports convergence between SNVs and CNVs on COU genetic drivers**

In our cohort of individuals with COU, the diagnostic yield of candidate pathogenic SNVs and CNVs in cases was 73/733 (10.0%) cases (**Figure 1**). As expected, the overall diagnostic yield of 127 individuals with COU and an extrarenal, syndromic, phenotype (30/127 (23.6%)) was much higher than in 606 individuals with isolated, non-syndromic, COU (43/606 (7.1%)) (OR 4.05, 95% CI 2.42 – 6.77; Fisher's exact  $P=3.16 \times 10^{-7}$ ). In contrast, the presence of a positive family history was not different between cases with a genomic diagnosis (16/73, 21.9%) and cases without a genomic diagnosis (148/660, 22.4%; OR 1.03, 95% CI 0.57-1.85; Fisher's exact  $P=1.00$ ). The genomic landscape across COU sub-phenotypes show remarkable overlap in molecular etiologies, but also differences between categories were observed. First, the distribution of the overall diagnostic yield among phenotypes was n=30 (9.4%), n=23 (12.9%) and n=20 (8.6%) for UPJO, UVJO and COU-NOS, respectively (**Supplementary Figure S3**). Comparison between COU phenotypes did not show differences in the distribution of diagnostic yield (2 x 3 Chi-square  $P=0.3$ ). Another example of the overlap between COU-subcategories is the well-known pleiotropic phenotype that is related to haploinsufficiency of *HNF1B*. In fact, we identified pathogenic SNVs in *HNF1B* across all COU-phenotypes. In addition, we detected chr.17q12 microdeletions (*HNF1B* locus) in 2 cases with COU-NOS and 1 case with UPJO. These findings are in accordance

with the fact that the *HNF1B*-related diseases play a role throughout urinary tract development resulting in anomalies across the entire CAKUT spectrum. Convergence of SNV and CNV data also provides remarkable lead points into the potential pathomechanistic pathways of candidate genes for CAKUT. We identified 5 COU cases (4 deletions and one duplication) harboring microdeletions within the chr.16p13.11 locus, encompassing *MYH11*, as well as one case affected by severe bilateral obstructive megaureters, additional urinary tract anomalies and extrarenal developmental defects, with biallelic mutations in this gene. *MYH11* encodes a heavy chain of myosin expressed in the kidney and the musculature of the urinary tract and bladder. Interestingly, recessive mutations in *MYH11* cause megacystis-microcolon-intestinal hypoperistalsis syndrome 2 (MMIHS2, OMIM 619351), in which affected individuals manifest, among other phenotypes, megabladder, dilated ureters and hydronephrosis<sup>36,42</sup>. Conversely, heterozygous mutations in *MYH11* have been associated to visceral myopathy-2 (VSCM2, OMIM 619350), a less severe form of smooth muscle myopathy with variable phenotypic expression, which also feature urinary tract obstruction<sup>43,44</sup>. Taken together, our findings support the implication that *MYH11* is a key dosage-sensitive gene in the urinary tract with its expression possibly correlating with the severity of COU.

## DISCUSSION

COU is a subcategory of CAKUT that includes highly heterogeneous phenotypes with a variable clinical presentation and a virtually unpredictable outcome. The major reason for this is related to the fact that the molecular architecture of COU is characterized by high genetic heterogeneity, incomplete penetrance and variable expressivity, which both hamper personalized prognostication and treatment, as well as genetic discovery. Our current study incorporates individuals with developmental ureteral defects who have been subjected to a comprehensive genetic screen that includes whole exome sequencing for SNV as well as CNV analyses. In our hands, this genomic evaluation demonstrated a diagnostic yield of 10.0% of cases. Despite restricting our analysis to only upper urinary tract obstruction phenotypes and conducting subgroup investigation, we found significant overlap between COU's genetic background and other CAKUT

phenotypes such as kidney hypodysplasia and VUR<sup>16,17,19-22,45</sup>. Our findings alone are yet another confirmation that developmental defects of the kidney and urinary tract are part of a spectrum of congenital malformations that, at least in part, arise from similar molecular alteration. This in turn intimates that even with detailed clinical and imaging workup, our ability to predict the underlying genetic defect is marginal. In fact, the strong variable expressivity of these genetic defects underlies the clinical observation that multiple CAKUT phenotypes occur within individual families, and even within the same individual, notwithstanding the fact that all members carry the same genetic mutation<sup>46-48</sup>. The finding that the molecular aetiology of COU subcategories shows strong similarities is important because the exact clinical definition of COU phenotypes, and CAKUT at large, is often challenging. Therefore, if our capability to ascertain diagnosis, prognosis, and treatment at the individual level is currently limited, this observed genetic heterogeneity and variable expressivity of COU legitimize a “genetic first” diagnostic approach to these developmental defects, even when extensive (and potentially burdening) clinical and imaging characterization is not completely available or uniform. The same therefore might be true for genetic discovery studies: while there is an obvious value to obtain detailed clinical phenotyping for studies targeted at specific CAKUT subcategories, the aggregation of large cohort of kidney and urinary tract defects at large is likely to lead to the identification of novel susceptibility genes and variants that predispose to CAKUT in its more broad manifestations. Our study also demonstrates that the genetic architecture of COU is likely less well-defined and more complex as compared to other CAKUT subgroups. In fact, congenital kidney anomalies usually show a higher yield of pathogenic SNVs and CNVs<sup>49-53</sup>, indicating a “more Mendelian” nature of kidney parenchymal defects as compared to ureteric conditions. One explanation for this observation can be traced back to selective pressure: while kidney malformations significantly affect early life morbidity and mortality and hence are likely to be enriched in highly deleterious mutations that are classified as pathogenic in a clinical genetic diagnostic framework, COU, showing more variable and, on average, more benign course, is likely to be characterized by a more complex genetic determination. Another explanation for this difference with other CAKUT subgroups is the fact that not all COU phenotypes originated from aberrant urinary tract



development by definition, as for example UPJO may also be caused by ectopic vasculature compression or dynamic dysfunction of the ureteral smooth muscle cells<sup>54</sup>. In our cohort, we could not make a clear distinction between these non-developmental causes of COU purely based on clinical and imaging grounds. Although the genomic diagnostic yield is strongly dependent on cohort characteristics and enrolment criteria, our findings are in line with the clinical observation that COU-related phenotypes show different incidence and severity as compared to kidney hypodysplasia<sup>55</sup>. In this study we provide evidence that, by simultaneously assessing SNVs and CNVs with large effect size, we can deliver a genetic diagnosis in up to one in 10 COU cases. The diagnostic yield should be interpreted in the context of a predictive algorithm to implicate pathogenicity, which, in order to favour accuracy, maybe penalizing for the interpretation of missense variants of variants that escape the clear-cut definition of Mendelian mutations and may incorporate inconsistencies<sup>56</sup>. Our findings particularly indicate that genetic testing has high-yield when extrarenal manifestations are present in individuals with COU, which is in line with recent clinical practice recommendations for genetic testing in CAKUT<sup>57</sup>. At the same time, our study points out that a molecular etiology cannot be identified in about 90% of patients. This large unsolved fraction of COU might be attributable to yet undiscovered Mendelian genes or structural variants, common variants with small effect size and a complex polygenic background, low-frequency variants with moderate effect size that are more difficult to assess, and/or a combination of all of the above. Epigenetic, environmental and stochastic factors are also likely to play a significant role. As large sequencing and genotyping efforts are being undertaken, all these different modes of genetic determination will be tested.

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## DISCLOSURES

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## **DATA SHARING STATEMENT**

All authors approve adherence to the FAIR data principles. There are some restrictions for this data as follows: Data sharing is possible on the basis of anonymity Exome

sequencing data is available in dbgap submitted as part of the Yale Center for Mendelian Genomics (YCMG)

## SUPPLEMENTARY MATERIAL

Supplementary Figure S1. Analytical workflow for SNV and CNV analyses of the study cohort

Supplementary Figure S2. Genetically-determined ancestry proportions of the study cohort

Supplementary Figure S3. Overall diagnostic yield in each COU subcategory

Supplementary Table S1. List of 382 prioritized genes for developmental defects of the kidney and urinary tract (Excel File)

Supplementary Table S2. Ultrarare SNVs of uncertain significance identified in genes with an autosomal dominant inheritance (Excel File)

Supplementary Table S3. Rare structural variants of uncertain significance. (Excel File)

SDC ----<http://links.lww.com/JSN/E403>

SDC Table ----<http://links.lww.com/JSN/E404>

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## TABLES

**Table 1. Study cohort characteristics**

Characteristics	UPJO (N = 321)	UVJO (N = 178)	COU-NOS (N = 234)	Overall COU cohort (N = 733)
<b>Gender</b>				
Female	117 (36.4)	51 (28.7)	63 (26.9)	231 (31.5)
Male	204 (63.6)	127 (71.3)	171 (73.1)	502 (68.5)
<b>Laterality</b>				
Bilateral	31 (9.7)	35 (19.7)	64 (27.4)	130 (17.7)
Left	164 (51.1)	95 (53.4)	95 (40.6)	354 (48.3)
Right	94 (29.3)	33 (18.5)	62 (26.5)	189 (25.8)
Unknown	32 (10)	15 (8.4)	13 (5.6)	60 (8.2)
<b>Genetically-determined ancestry</b>				
European participants	264 (82.2)	148 (83.1)	187 (79.9)	599 (81.7)
Admixed participants	40 (12.5)	22 (12.4)	27 (11.5)	89 (12.1)
Hispanic participants	6 (1.9)	5 (2.8)	12 (5.1)	23 (3.1)
South Asian participants	5 (1.6)	2 (1.1)	2 (0.9)	9 (1.2)
African participants	3 (0.9)	1 (0.6)	4 (1.7)	8 (1.1)
East Asian participants	3 (0.9)	0	2 (0.9)	5 (0.7)
<b>Additional renal phenotype</b>				
Bladder defect	2 (0.6)	3 (1.7)	5 (2.1)	10 (1.4)
DCS	8 (2.5)	5 (2.8)	2 (0.9)	15 (2)
Ectopia	5 (1.6)	4 (2.2)	4 (1.7)	13 (1.8)
Glomerular	3 (0.9)	0	2 (0.9)	5 (0.7)
KHD	19 (5.9)	5 (2.8)	7 (3)	31 (4.2)
Nephronopthisis	1 (0.3)	0	0	1 (0.1)
Reflux nephropathy	14 (4.4)	23 (12.9)	10 (4.3)	47 (6.4)
Tubular defect	0	0	1 (0.4)	1 (0.1)
<b>Non-urinary defect</b>				
Neural	8 (2.5)	5 (2.8)	1 (0.4)	14 (1.9)
Craniofacial	8 (2.5)	7 (3.9)	4 (1.7)	19 (2.6)
Cardiac	5 (1.6)	5 (2.8)	9 (3.8)	19 (2.6)
Musculoskeletal	6 (1.9)	8 (4.5)	4 (1.7)	18 (2.5)
Gastrointestinal	6 (1.9)	5 (2.8)	1 (0.4)	12 (1.6)
Genital	12 (3.7)	5 (2.8)	10 (4.3)	27 (3.7)
General Developmental Delay	6 (1.9)	8 (4.5)	4 (1.7)	18 (2.5)
Other syndromes*	0,00	0,00	2 (0.9)	2 (0.3)
<b>Family history of kidney disease</b>	47 (14.6)	35 (19.7)	82 (35)	164 (22.4)

Presented as n (%). \* Other syndromes include Currarino syndrome and Beckwith-Wiedemann. COU-NOS, congenital obstructive uropathy – not otherwise specified; DCS, duplex collecting system; KHD, kidney hypodysplasia, UPJO, ureteropelvic junction obstruction; UVJO, ureterovesical junction obstruction.

ACCEPTED

**Table 2. Identified rare and potentially pathogenic single nucleotide variants**

Autosomal dominant mode of inheritance																
ID	COU Category	Gene	Variant ID	Variant	Consequence	gnomAD Exome global AF	Internal controls AF (N=11,818)	ACMG classification	Clin Var Significance	REVEL Score	Segregation	Gender	FHX (Y/N/U)	Consanguinity (Y/N/U)	Laterality (B/L/R/U)	Additional Genitourinary Phenotype
P75	UVJO	ACTG2	2-74141956-C-T	c.763C>T	p.Arg255Cys	4.04E-06	-	LP	NA	0.937	U	M	N	N	L	
P18	UPJO	ALDH18A1	10-97376235-A-T (rs200452017)	c.1598T>A	p.Leu533Gln	1.37E-04	2.57E-04	US	US	0.915	U	M	N	U	B	
P77	COU-NOS	ALDH18A1	10-97371187-C-G	c.1930G>C	p.Ala644Pro	-	-	US	US	0.652	U	M	U	N	B	
P58	COU-NOS	ARID1B	6-157099426-A-AG	c.189_190insG	p.Gln122AlafsTer110	-	5.68E-04	LB	US	NA	U	M	N	U	L	
P83	UVJO	BMP4	14-54418617-C-CT	c.323dupA	p.Arg109AlafsTer26	-	-	LP	NA	NA	U	F	N	N	U	KHD
P09	UVJO	BMP7	20-55777630-C-	c.661G>A	p.Glu221Lys	-	-	US	NA	0.771	Segregating	M	U	U	R	

			T													
P78	UVJO	<i>BRAF</i>	7-14050 1356-C-T	c.716G>A	p.Arg239Gln	-	-	US	NA	0.863	Inherited maternal	M	N	U	U	
P36	COU-NO S	<i>COL5A1</i>	9-13771 1989-G-T	c.4474G>T	p.Gly1492Cys	-	-	P	P	0.994	U	M	N	U	B	Cryptocidism
P43	UPJO	<i>DSTYK</i>	1-20518 0640-C-T	c.24G>A	p.Trp8Ter	-	-	P	P	NA	Segregating	M	N	U	L	N
P34	COU-NO S	<i>EYA1</i>	8-72129 030-A-C	c.1239T>G	p.Cys413Trp	-	-	US	NA	0.823	U	M	N	U	R	
P63	UPJO	<i>FOXC1</i>	6-16110 89-G-A	c.409G>A	p.Val137Ile	-	-	US	NA	0.621	Paternal	M	Y	N	R	Double urethra
P56	COU-NO S	<i>GREB1L</i>	18-19019 599-G-A	c.949+1G>A	NA	-	-	LP	NA	NA	U	M	N	U	L	
P05	COU-NO S	<i>HNF1B</i>	17-36091 625-G-C (rs138986885)	c.1006C>G	p.His336Asp	1.84E-04	3.83E-04	US	Conflicting	0.702	Inherited maternal	M	Y	N	R	
P51	UPJO	<i>HNF1B</i>	17-36091 625-G-C	c.1006C>G	p.His336Asp	1.84E-04	3.83E-04	US	Conflicting	0.702	U	F	N	U	L	

			(rs138986885)													
P79	UPJO	HNF1B	17-36061038-A-T	c.1484T>A	p.Met495Lys	-	-	LP	Conflicting	0.905	U	F	N	U	R	
P66	UVJO	HNF1B	17-36104610-G-A	c.266C>T	p.Pro89Leu	1.61E-05	-	US	NA	0.961	U	M	N	N	L	
P25	UVJO	HNF1B	17-36091633-C-A	c.998G>T	p.Gly333Val	3.99E-06	-	US	NA	0.572	U	M	N	U	B	
P17	UVJO	HOXD13	2-176959349-G-A	c.923G>A	p.Arg308His	3.18E-05	4.23E-05	US	US	0.791	U	M	N	U	L	
P68	UPJO	KAT6B	10-76788346-GTCTA-G (rs199470470)	c.3769_3772delTCTA	p.Lys1258GlyfsTer13	-	-	P	P	NA	U	M	N	N	B	Micropenis, Cryptorchidism, Piriformis bladder
P72	UPJO	KRAS	12-25378651-T-C (rs202247812)	c.347A>G	p.Asn116Ser	-	-	LP	NA	0.878	U	M	N	N	L	
P3	UVJO	MAP2K1	15-66782	c.1163C>G	p.Thr388Ser	-	-	US	NA	0.32	U	F	U	U	B	



1			934-C-G													
P41	UVJO	NFIA	1-61554070-C-T	c.277C>T	p.Leu93Phe	-	4.24E-05	US	NA	0.651	Inherited maternal	F	Y	U	R	
P85	UPJO	NOTCH2	1-120464967-C-T	c.5105G>A	p.Arg1702Gln	7.95E-06	-	US	Conflicting	0.677	U	M	U	N	L	
P24	UPJO	NOTCH2	1-120462920-G-A (rs372061331)	c.5411C>T	p.Ser1804Leu	1.19E-05	4.24E-05	US	NA	0.611	Inherited paternal	F	U	U	B	
P22	COU-NO S	NRI P1	21-16337149-CTTAA A-C	c.3360_3364delTTTAA	p.Asn1120LysfsTer6	-	-	NA	NA	NA	Inherited maternal	M	Y	U	U	Post Infectious Glomerulonephritis
P73	COU-NO S	NSD1	5-176631267-CAGAA A-C	c.409_412delGAAA	p.Glu406LysfsTer12	-	-	NA	P	NA	U	F	N	N	B	VUR
P38	UVJO	PAX2	10-102510558-C-T	c.320C>T	p.Pro107Leu	7.95E-06	-	LP	US	0.922	U	M	N	U	B	
P12	UPJO	PAX2	10-102510600-AG-A	c.365delG	p.Gly122AlafsTer37	-	-	LP	NA	NA	De novo	M	N	U	L	VUR

P80	UPJO	PIK3R2	19-18273294-G-A	c.1087G>A	p.Gly363Ser	-	-	US	NA	0.845	U	M	N	U	L	VUR
P03	UPJO	PKD2 <sup>#</sup>	4-88928887-T-A	c.2T>A	p.Met1Lys	3.62E-05	-	LP	NA	0.318	NA	M	U	U	L	
P55	UPJO	RPS24	10-79796953-A-C	c.281A>C	p.His94Pro	-	-	US	NA	0.676	U	F	N	U	L	
P06	COUNOS	SAL1	16-51175430-C-T	c.703G>A	p.Ala235Thr	7.96E-06	1.27E-04	US	Conflicting	0.713	Inherited maternal	M	Y	N	R	
P64	UVJO	SHH	7-155599442-AT-A	c.1delA	p.Met1CysfsTer19	-	4.82E-05	LP	NA	NA	U	M	N	U	L	
P61	COUNOS	SIX5	19-46272101-A-G	c.2T>C	p.Met1?	7.97E-06	-	LP	NA	0.539	U	M	N	U	L	
P21	UVJO	SPECC1L	22-24718863-C-T	c.1915C>T	p.Arg639Ter	-	-	LP	US	NA	De novo	M	N	U	L	VUR
P20	UPJO	TBX18	6-85453979-C-T	c.1004G>A	p.Arg335Lys	-	-	LP	NA	0.565	Inherited paternal	M	N	U	R	
P30*	UPJO	TBX18	6-85446657-G-A	c.1570C>T	p.His524Tyr	8.06E-06	-	US	P	0.757	U	F	U	U	L	

P04*	CO-NO S	TBX18	6-85466546-G-C	c.641C>G	p.Ala214Gly	7.96E-06	-	US	NA	0.53	U	M	N	N	B	VUR, KHD
P42	UPJO	TP63	3-189608591-T-A	c.1666T>A	p.Leu556Met	-	-	US	NA	0.544	U	F	Y	U	B	Solitary kidney
P40	UPJO	TP63	3-189584503-G-A	c.799G>A	p.Val267Ile	1.19E-05	4.24E-05	US	US	0.599	U	M	N	U	L	
<b>Autosomal Dominant or Recessive mode of inheritance</b>																
ID	COU Category	Gene	Variant ID	Variant	Consequence	gnomAD Exome global AF	Internal controls AF (N=11,818)	ACMG classification	Clin Var Significance	REVEL Score	Segregation	Gender	FHX (Y/N/U)	Consanguinity (Y/N/U)	Laterality (B/L/R/U)	Additional Genitourinary Phenotype
P74*	UVJO	FGFR3	4-1808377-G-A (rs104886024)	c.2135G>A	p.Arg712His	-	-	US	NA	0.896	U	M	N	N	U	
P74*	UVJO	TBX6	16-30100037-C-A	c.745G>T	p.Val249Leu	-	-	US	NA	0.943	U	M	N	N	U	
P33	UVJO	TNXB	6-32010285-C-A	c.12157G>T	p.Glu4053Ter	4.01E-06	-	LP	NA	NA	U	M	N	U	R	
P6	UPJO	TNXB	6-32010	c.12134C>T	p.Thr4045Ile	-	-	US	NA	0.564	U	M	Y	U	L	

5			308-G-A													
P82	CO-UNOS	TNXB	6-32018039-TTG (rs34629684)	c.9174dupC	p.Ile3059HisfsTer30	-	-	LP	NA	NA	U	M	Y	N	R	
P57	CO-UNOS	TNXB	6-32021490-TG	c.8468-2A>C	NA	1.23E-05	-	LP	NA	NA	U	F	Y	U	L	
P59	CO-UNOS	TNXB	6-32035600-GA	c.6382C>T	p.Gln2128Ter	-	-	NA	NA	NA	U	M	N	U	B	
P59	CO-UNOS	TNXB	6-32057150-CCT-C	c.2363_2364delAG	p.Glu788GlyfsTer18	-	-	NA	NA	NA	U	M	N	U	B	
<b>Autosomal Recessive mode of inheritance</b>																
ID	COU Category	Gene	Variant ID	Variant	Consequence	gnomAD Exome global AF	Internal controls AF (N=11,818)	ACMG Classification	Clin Var Significance	REVEL Score	Segregation	Gender	FHX (Y/N/U)	Consanguinity (Y/N/U)	Laterality (B/L/R/U)	Additional Genitourinary Phenotype
P32	UPJO	C5orf42	5-37157912-A-T	c.4511T>A	p.Leu1504Ter	2,39E-05	8,52E-05	NA	P	NA	U	M	N	U	L	
P32	UPJO	C5orf42	5-37226877-TA-T	c.1819delT	p.Tyr607ThfsTer6	1.61E-04	1.27E-04	NA	P/LP	NA	U	M	N	U	L	
P	CO	DYN	11-	c.2delT	p.Met1Arg	4.03	-	NA	US	NA	Inheri	M	Y	U	L	

07	UNOS	C2H1	102980304-AT-A		fsTer23	E-06					ted maternal					
P07	COUNOS	DYNC2H1	11-103175354-G-A	c.11287G>A	p.Ala3763Thr	5.63E-05	1.27E-04	NA	US	0.521	Inherited paternal	M	Y	U	L	
P28	UPJO	FRE M1	9-14842659-C-T	c.1394-1G>A	NA	-	-	NA	NA	NA	U	M	U	U	L	
P28	UPJO	FRE M1	9-14859241-C-T	c.571G>A	p.Gly191Arg	6.09E-04	1.70E-04	NA	Conflicting	0.048	U	M	U	U	L	
P29	UVJO	HPS E2	10-100904148-G-A (rs267606865)	c.457C>T	p.Arg153Ter	1.20E-05	4.24E-05	P	P	NA	U	F	N	U	L	
P50	UPJO	KIAA1109	4-123161006-ATAGTG-A	c.4170_4174delTAGTG	p.Asp1390GlufsTer8	-	-	LP	NA	NA	U	M	N	U	L	
P50	UPJO	KIAA1109	4-123161012-A-C	c.4175A>C	p.Glu1392Ala	-	-	US	NA	0.363	U	M	N	U	L	
P08	UVJO	MYH11	16-15814859-T-G	c.4628A>C	p.Glu1543Ala	3.98E-06	-	US	US	0.918	U	M	N	Y	B	VUR, bladder pseudodiverticuli, hypospa

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P08	UVJO	MYH11	16-15853489-G-A	c.1345C>T	p.Arg449Trp	3.98E-06	-	US	NA	0.742	U	M	N	Y	B		VUR, bladder pseudodiverticuli, hypospadias
P63	UPJO	PKHD1	6-51613307-A-T	c.9107T>A	p.Val3036Glu	-	-	US	Conflicting	0.618	U	M	Y	N	R		
P63	UPJO	PKHD1	6-51924836-G-A (rs376040501)	c.1123C>T	p.Arg375Trp	2.39E-05	4.25E-05	P	P/LP	0.659	U	M	Y	N	R		
P27	UPJO	SDC CAG8	1-243471334-G-T	c.349G>T	p.Glu117Ter	1.19E-05	-	P	P	NA	U	M	N	U	L		
P27	UPJO	SDC CAG8	1-243581270-A-G	c.1310A>G	p.Glu437Gly	-	-	LB	NA	0.114	U	M	N	U	L		

\*Individual also carries a genomic disorder. #This individual with a PKD variant was screened and presented one simple cortical cyst at age 32 detected by abdominal CT scan. AF, allele frequency, B, bilateral, COU-NOS, congenital obstructive uropathy – not otherwise specified; F, female; FHX, family history; KHD, kidney hypodysplasia; M, male; (L)P, (likely) pathogenic; N, no; NA, not available; U, unknown; UPJO, ureteropelvic junction obstruction; US, uncertain significance, UVJO, ureterovesical junction obstruction; VUR, vesicoureteral reflux; Y, yes.

**Table 3. Identified genomic disorders and likely pathogenic copy number variants**

ID	COU Category	CNV	CNV size (Mb)	Type	GD-CNV	Dosage-sensitive genes	CAKUT (Human)	Gender	FHX	Consanguinity	Additional Genitourinary Phenotype	Extrarenal Phenotype
P74*	UVJO	chr1:145415279-145763815	0,349	DEL	1q21.1 susceptibility locus for Thrombocytopenia-Absent Radius (TAR) syndrome		<i>RBM8A</i>	M	N	N		
P84	UVJO	chr1:145415279-145748587	0,333	DUP	1q21.1 TAR Syndrome region duplication		<i>RBM8A</i>	M	N	N	VUR	
P30*	UPJO	chr1:146496480-147415562	0,919	DEL	1q21.1 recurrent microdeletion			F	N	U		
P15	UPJO	chr7:144702944-148544434	3,841	DEL	7q36.1 deletion	<i>CUL1, EZH2</i>		M	U	U		
P26	COU-NOS	chr15:30918893-32404534	1,486	DEL	15q13.3 microdeletion syndrome			F	N	U		
P60	COU-NOS	chr15:23684690-28557995	4,873	DUP	15q11.2 Prader-Willi/Angelman (Type 1) Reciprocal Duplication			M	N	U		
P11	UPJO	chr16:29675050-30199897	0,525	DEL	16p11.2 deletion	<i>PRRT2</i>	<i>TBX6</i>	M	Y	U	VUR, KHD	Bifid thumbs
P44	UVJO	chr16:15460510-17564653	2,104	DEL	16p13.11 recurrent microdeletion	<i>MYH11</i>		M	N	U		
P45	UVJO	chr16:14960412-16357072	1,397	DUP	16p13.11 duplication	<i>MYH11</i>		M	N	U		Preauricular appendix
P67	UVJO	chr16:14947324-16359036	1,412	DEL	16p13.11 recurrent microdeletion	<i>MYH11</i>		M	N	U	LUTM (Phimosis)	Growth retardation
P76	COU-NOS	chr16:15460510-17353355	1,893	DEL	16p13.11 recurrent microdeletion	<i>MYH11</i>		M	N	N		Perthes disease
P04*	COU-NOS	chr17:34851067-36293050	1,442	DEL	RCAD deletion	<i>ACACA, HNF1B</i>	<i>HNF1B</i>	M	N	N	VUR, KHD	
P10	COU-NOS	chr17:34797485-36340198	1,543	DEL	RCAD deletion	<i>ACACA, HNF1B</i>	<i>HNF1B</i>	M	N	N		Facial dysmorphism, Pervasive developmental disorder
P19	UPJO	chr17:34842442-36104994	1,263	DEL	RCAD deletion	<i>ACACA, HNF1B</i>	<i>HNF1B</i>	M	N	U	KHD	
P37	UPJO	chr17:29051270-30377236	1,326	DEL	NF1-microdeletion syndrome	<i>NF1, SUZ12</i>		F	N	U		Neurofibromatosis type I
P46	UVJO	chr22:20706073-21386101	0,680	DEL	DiGeorge A-D, DiGeorge B-D, DiGeorge C-D, DiGeorge C-E			M	Y	U		Mild glandular hypospadias, high arched palate, slight antimongoloid slant
P70	UPJO	chr22:18893888-20307511 chr22:20706073-21571022	2,279	DEL	DiGeorge A-B, DiGeorge A-D, DiGeorge C-D, DiGeorge A-D, DiGeorge B-D, DiGeorge C-E	<i>DGCR8</i>		M	N	N		Facial dysmorphism, syndactyly, growth retardation

P47	UPJO	chrX:71693492-72092398	0,399	DUP	Triple X		<i>HDAC8</i>	F	N	U		
P69	UVJO	chrX:149638017-155004401	5,366	DEL	Xq28 Rett syndrome	<i>SLC6A8, MECP2, NSDHL, F8, L1CAM, ABCD1, MTM1, RAB39B, FLNA, DKC1, IKBKG, AVPR2</i>	<i>NAA10, NSDHL, CCNQ, DKC1</i>	F	N	N		Interatrial defect
P54	UPJO	chr2:60679700-66798661	6,119	DUP			<i>WDPCP</i>	F	N	U		
P16	UVJO	chr2:141072471-142888527	1,816	DUP		<i>LRP1B</i>		F	N	U		
P23	UPJO	chr9:137320857-137630692	0,310	DUP			<i>COL5A1</i>	F	N	U		
P52	COU-NOS	chr14:31495110-33293979	1,799	DEL		<i>HECTD1, ARHGAP5, AKAP6</i>		F	Y	U		

\*Individual also carries a single nucleotide variant. CAKUT, congenital anomalies of the kidney and urinary tract; COU-NOS, congenital obstructive uropathy – not otherwise specified; CNV, copy number variants, DEL, deletion; DUP, duplication; GD, genomic disorder; F, female; FHX, family history; KHD, kidney hypodysplasia; M, male; N, no; RCAD, renal cysts and diabetes; U, unknown; UPJO, ureteropelvic junction obstruction; UVJO, ureterovesical junction obstruction; VUR, vesicoureteral reflux; Y, yes.



## FIGURE LEGENDS

**FIGURE 1. Diagnostic yield of genomic screen in 733 individuals with congenital obstructive uropathy.** Differences between COU sub-groups for diagnostics SNV and CNV yield were all  $P > 0.05$  by using 2x3 Chi square test. A) The overall *in-silico* diagnostic yield of candidate pathogenic SNV and CNV in the COU cohort is 10.0% (73 of 733 patients). This proportion of genomic contribution to the etiology of COU is in accordance with other congenital kidney and urinary tract phenotypes. B) Distribution of COU cases carrying candidate pathogenic/likely pathogenic SNVs based on mode of inheritance. SNVs in genes with an autosomal dominant mode of inheritance were vastly predominant for all COU subtypes. C) Distribution of genomic disorders, likely pathogenic CNVs, and candidate microdeletions and microduplications covering known genes in COU exome cases. As pathogenic deletions were much more frequently found than duplications in all COU subtypes, our data implicates that haploinsufficiency or dominant negative effects are the main molecular mechanisms leading to congenital obstructive uropathy.

AD, autosomal dominant; AR, autosomal recessive; COU, congenital obstructive uropathy; CNV, copy number variants; Del, deletion; Dup, duplication; NOS, not otherwise specified, SNV, single nucleotide variants, UPJO, ureteropelvic junction obstruction; UVJO, ureterovesical junction obstruction.

