Molecular Diagnostic Analysis Reveals High Diagnostic Rate in Congenital Obstructive Uropathy

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Background

- Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) constitute a very heterogenous group of birth defects encompassing conditions with very different prevalence and severity. As a result, the reported diagnostic yield in CAKUT were extremely variable, likely because of ascertainment bias and the diverse phenotypic composition of the cohorts studied[1].

- Congenital Obstructive Uropathy (COU) is a subset of CAKUT and is the most frequent urinary tract anomaly occurring in up to 2% of pregnancies, constituting a leading cause of pediatric chronic kidney disease. The precise genetic architecture of COU is mostly unknown.

- Here we aimed to define the contribution of point mutations (SNVs) and structural variants (SVs or CNVs) to the diagnosis of COU based on an exome sequencing (ES) study of 822 COU cases, encompassing three main classes of congenital urinary obstructions: a) Ureteropelvic Junction Obstruction (UPJO; N=338), b) Ureterovesical Junction Obstruction/ megaloureter (UVJO; N=217), and c) COU not otherwise specified (COU-NOS; N=267).

Cohort Selection & Methods

- The cohort subjects were described in Table 1.
- ES was conducted on the entire cohort of 822 COU cases; Illumina DNA microarray for CNV analysis was performed for a subset 461 COU individuals.
- Clinical annotation to identify candidate diagnostic/pathogenic Mendelian mutations and structural variants was conducted based on the American College of Medical Genetics and Genomics (ACMG) guidelines for clinical variant interpretation.

Gender	**n**	**%**
Male	557	67.76%
Female	265	32.24%
Total	822	100.00%
Clinical Phenotype	**n**	**%**
Ureteropelvic Junction Obstruction (UPJO)	338	41.12%
Ureterovesical Junction Obstruction (UVJO) or Congenital Megaloureter	217	26.40%
COU not otherwise specified (COU-NOS)	267	32.48%
Total	822	100.00%
Extrarenal phenotype	**n**	**%**
Neural	18	2.19%
Craniofacial	12	1.46%
Cardiac	17	2.07%
Musculoskeletal	21	2.55%
Gastrointestinal	13	1.58%
Genital	15	1.82%
General Developmental Delay	5	0.61%
Total	101	12.29%

Table 1. Baseline clinical characteristics of our COU cohort. 101 of the 822 COU cases had reported extrarenal phenotypes.

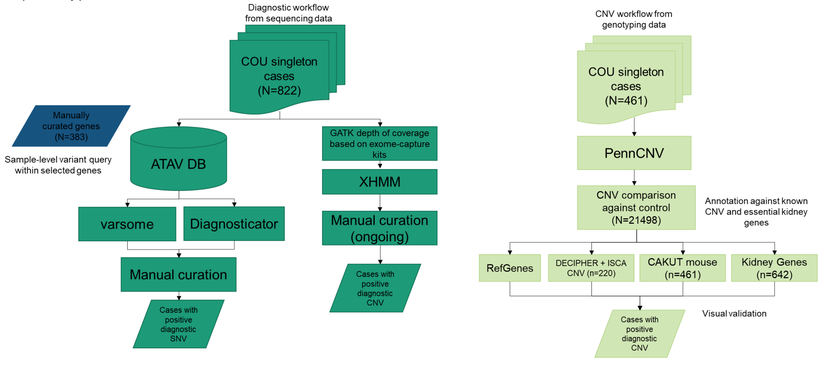

Figure 1. Analysis workflow to annotate single nucleotide variants and structural variants (or CNV). We performed molecular diagnostic annotation using the infrastructure from the Analysis Tool for Annotated Variants (ATAV)[2], along with VarSome[3], and Diagnosticator (https://diagnosticator.com) as reference, with additional manual curation according to the most recent ACMG recommendations[4,5,6].SNVs were annotated against 383 genes which have been characterized with CAKUT involvement[7,8].We simultaneously ran a genome-wide SV analysis on 461 of the 822 exome cases with existing genotyping data from Illumina DNA arrays with PennCNV[9] according to established methods[10].

Results

- Overall, 6.2% of 822 COU individuals carried a pathogenic genotype at a CAKUT Mendelian gene by ES analysis
- 4.1% of 461 COU individuals with DNA microarray available carried a P/LP CNV, for a combined yield of 8.5% (Fig. 2).
- Incomplete CNV analysis (361 individual under analysis of CNVs from ES) suggest a yet underestimation of the total diagnostic rate for COU.
- Similarities and differences among the 3 COU subcategories are shown in Fig. 2,4 and Tab. 2,3.

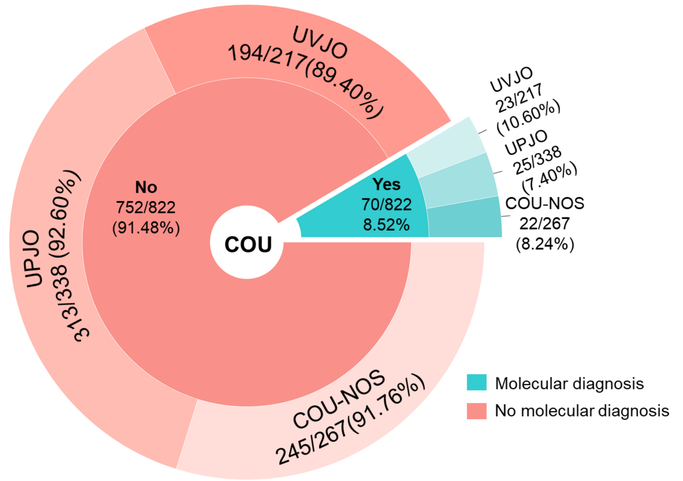

Figure 2. The overall in-silico diagnostic yield of the candidate pathogenic SNVs and SVs in the COU cohort is 8.52% (70 of 822 patients), with rare and potentially pathogenic SNVs detected in 51 (6.20% of 822) COU cases and rare and potentially pathogenic SVs detected in 19 (2.31% of 822) COU cases.

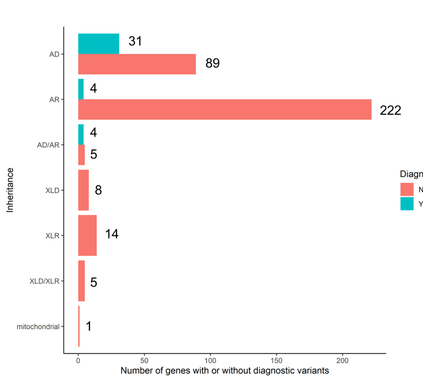

Figure 3. Distribution of genes carrying candidate pathogenic SNVs based on mode of inheritance. The list encompassed a total of 383 genes, of which 128 genes were associated with dominant-inheritance disorder (120 autosomal, AD and 8 X-linked, XLD), 240 genes with recessive disorder (226 autosomal, AR and 14 X-linked, XLR), and 14 genes with both dominant and recessive inheritance (AD/AR and XLD/XLR).

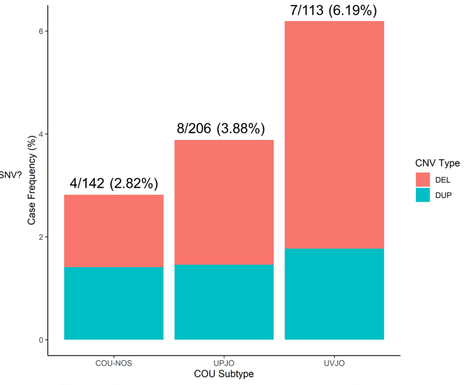

Figure 4. Large and rare pathogenic and likely pathogenic SVs were detected in 19/461 cases (4.1%) with 12 copy number losses (DEL) and 7 copy number gains (DUP). Interestingly, there were ~1.6x more potentially pathogenic CNVs in the UVJO cases compared to the UPJO cases, and these predominantly comprised the 16p13.11 recurrent microdeletion.

ID	Gene	Inheritance	Variant	Consequence	ACMG Classification	Sex	Subtype	FHX (Y/N/U)	Additional Genitourinary Phenotype	Extrarenal Phenotype
SNV001	*ALDH18A1*	AD	c.1598T>A	p.Leu533Gln	LP	M	UPJO	N		Prognatism, Hearing loss
SNV002	*ALDH18A1*	AD	c.2061C>G	p.His687Gln	LP	M	UVJO	N	DCS	
SNV003	*ALDH18A1*	AD	c.1930G>C	p.Ala644Pro	LP	M	COU-NOS	U		
SNV004	*ARID1B*	AD	c.189_190insG	p.Gln64fs	LP	M	COU-NOS	N		
SNV005	*BMP4*	AD	c.323dupA	p.Arg109fs	P	F	UVJO	N	RHD	ASD, PDA, RVH, Umbilical hernia
SNV006	*BRAF*	AD	c.716G>A	p.Arg239Gln	LP	M	UVJO	N		
SNV007	*CHD4*	AD	c.5704A>G	p.Thr1902Ala	LP	F	COU-NOS	Y	Bladder Pseudodiverticuli	Spina Bifida
SNV008	*COL5A1*	AD	c.4474G>T	p.Gly1492Cys	LP	M	COU-NOS	N	Cryptorchidism	Bilateral Hip Dysplasia, Bilateral clubfoot
SNV009	*CREBBP*	AD	c.5837delC	p.Pro1946fs	P	M	UVJO	Y		
SNV010	*DSTYK*	AD	c.24G>A	p.Trp8*	P	M	UPJO	N		Epilepsy, Haemangioma
SNV011	*EYA1*	AD	c.1239T>G	p.Cys413Trp	LP	M	COU-NOS	N		
SNV014	*FOXC1*	AD	c.409G>A	p.Val137Ile	LP	M	UPJO	Y	Double Urethra	Psychomotor Disturbance
SNV015	*GREB1L*	AD	c.949+1G>A		P	M	COU-NOS	N		
SNV016	*HNF1B*	AD	c.1484T>A	p.Met495Lys	LP	F	UPJO	N		
SNV017	*HOXD13*	AD	c.923G>A	p.Arg308His	LP	M	UVJO	N		Perianal Protrusion
SNV018	*KANSL1*	AD	c.2902delC	p.Gln968fs	P	M	COU-NOS	N		
SNV019	*KAT6B*	AD	c.3769_3772delTCTA	p.Lys1258fs	P	M	UPJO	N	Micropenis, Cryptorchidism, Piriformis bladder	Genitopatellar Syndrome, Facial Dysmorphism, Agenesis of the Corpus Callosum
SNV020	*KRAS*	AD	c.347A>G	p.Asn116Ser	LP	M	UPJO	N		
SNV021	*LRP5*	AD	c.3814G>A	p.Asp1272Asn	LP	M	COU-NOS	Y		
SNV022	*LRP5*	AD	c.346G>A	p.Asp116Asn	LP	M	UVJO	N		
SNV023	*LRP5*	AD	c.2588G>A	p.Trp863*	P	M	COU-NOS	N		
SNV024	*LRP5*	AD	c.3580C>T	p.Arg1194Cys	LP	F	COU-NOS	N	UTI	
SNV025	*LRP5*	AD	c.2626G>A	p.Gly876Ser	LP	M	UVJO	N	VUR, RHD	
SNV026	*LRP5*	AD	c.340G>A	p.Ala114Thr	LP	F	UVJO	N	VUR	
SNV027	*NFIA*	AD	c.277C>T	p.Leu93Phe	LP	F	UVJO	Y		
SNV028	*NRIP1*	AD	c.3360_3364delTTTAA	p.Asn1120fs	P	M	COU-NOS	Y	Post Infectious Glomerulonephritis	
SNV029	*NSD1*	AD	c.409_412delGAAA	p.Glu137fs	P	F	COU-NOS	N	VUR	Generalized Developmental Delay
SNV030	*PAX2*	AD	c.365delG	p.Gly122fs	P	M	UPJO	N	Bilateral VUR	
SNV031	*PAX2*	AD	c.320C>T	p.Pro107Leu	LP	M	UVJO	N		
SNV035	*PIEZO2*	AD	c.4899_4900delGA	p.Lys1634fs	P	F	UVJO	N		Neurofibromatosis Type 1
SNV036	*PIK3R2*	AD	c.1087G>A	p.Gly363Ser	LP	M	UPJO	N		
SNV037	*PKD1*	AD	c.7112T>C	p.Val2371Ala	LP	F	UPJO	N	Bilateral VUR	
SNV038	*RPS24*	AD	c.281A>C	p.His94Pro	LP	F	UPJO	N		
SNV039	*SAMD9*	AD	c.2429delT	p.Leu810fs	P	F	UVJO	Y	DCS	
SNV040	*SHH*	AD	c.1delA	p.Met1fs	LP	M	UVJO	N		
SNV041	*SIX5*	AD	c.2T>C	p.Met1?	P	M	COU-NOS	N		
SNV042	*SPECC1L*	AD	c.1915C>T	p.Arg639*	P	M	COU-NOS	N	Left VUR	
SNV043	*TBX18*	AD	c.1004G>A	p.Arg335Lys	LP	M	UPJO	N		Syringocele
SNV048	*TP63*	AD	c.799G>A	p.Val267Ile	LP	M	UPJO	N		
SNV049	*TP63*	AD	c.1666T>A	p.Leu556Met	LP	F	UPJO	Y	Solitary Kidney	
SNV012	*FGFR2*	AD/AR	c.488C>T	p.Ser163Leu	LP	F	COU-NOS	N	Ectopic Ureter, VUR	
SNV013	*FGFR3*	AD/AR	c.2135G>A	p.Arg712His	LP	M	UVJO	N		
SNV032	*PIEZO1*	AD/AR	c.799C>T	p.Gln267*	P	M	UVJO	N		Preauricular Fistula
SNV033	*PIEZO1*	AD/AR	c.5806C>T	p.Gln1936*	P	M	UPJO	N		
SNV034	*PIEZO1*	AD/AR	c.975dupC	p.Thr326fs	P	M	UPJO	N		
SNV044	*TNXB*	AD/AR	c.12157G>T	p.Glu4053*	P	M	UVJO	N		Facial Dysmorphism
SNV045	*TNXB*	AD/AR	c.8468-2A>C		LP	F	COU-NOS	Y		
SNV046	*TNXB*	AD/AR	c.8468-2A>C		LP	M	COU-NOS	Y		
SNV023	*TNXB*	AD/AR	c.6382C>T c.2363_2364delAG	p.Gln2128* p.Glu788fs	P	M	COU-NOS	N		
SNV047	*TNXB*	AD/AR	c.9174dupC	p.Ile3059fs	P	M	COU-NOS	Y	Phimosis	
SNV051	*C5orf42*	AR	c.4511T>A c.1819delT	p.Leu1504* p.Tyr607fs	P P	M	UPJO	N		
SNV052	*HPSE2*	AR	c.457C>T	p.Arg153*	P	F	UVJO	N	Dysplastic Left Kidney	High Arched Palate, Pes Varus
SNV014	*PKHD1*	AR	c.9107T>A c.1123C>T	p.Val3036Glu p.Arg375Trp	US(P) P	M	UPJO	Y	DCS	Psychomotor Disturbance
SNV050	*SDCCAG8*	AR	c.349G>T c.1310A>G	p.Glu117* p.Glu437Gly	P P	M	UPJO	N		

Table 2. Rare and potentially pathogenic and likely pathogenic SNVs account for 6.20% (51 of the 822 exome-sequenced COU cases) of the in silico diagnostic yield. Abbrv.:- ASD, Atrial Septal Defect; FHX, family history; N, No; PDA, Patent Ductus Arteriosus; RVH, Right Ventricular Hypertrophy; U, Unknown; Y, Yes.

ID	Subtype	Extrarenal phenotype (Y/N/U)	Chr	Start (Mb)	End (Mb)	Size (Mb)	Type	GD-CNV	CAKUT (Mouse)	CAKUT (Human)
CNV001	UVJO		1	145.37	145.74	0.37	DEL	1q21.1 susceptibility locus for Thrombocytopenia-Absent Radius (TAR) syndrome	-	PEX11B,RBM8A
CNV002	UPJO		1	146.09	147.86	1.52	DEL	1q21.1 recurrent microdeletion	-	CHD1L
CNV003	UPJO		2	57.43	67.56	10.13	DUP	-	Pex13	FANCL,PEX13,WDPCP
CNV004	UVJO		2	141.07	143.70	2.63	DUP	-	-	KYNU
CNV005	UPJO		7	144.60	148.57	3.97	DEL	-	-	-
CNV006	UPJO		9	137.32	137.55	0.24	DUP	-	Rxra	COL5A1
CNV007	COU-NOS		14	31.49	34.11	2.62	DEL	-	-	-
CNV008	COU-NOS		15	23.80	28.21	4.53	DUP	15q11.2 Prader-Willi/Angelman region reciprocal duplication	Oca2	-
CNV009	UVJO	Preauricular appendix	16	14.98	16.30	1.33	DUP	16p13.11 duplication	Myh11	
CNV010	UVJO	Growth retardation	16	15.13	16.29	1.17	DEL	16p13.11 recurrent microdeletion	Myh11	
CNV011	UVJO		16	15.13	16.29	1.17	DEL	16p13.11 recurrent microdeletion	Myh11	
CNV012	COU-NOS		16	15.49	18.17	2.67	DEL	16p13.11 recurrent microdeletion	Myh11	
CNV013	UVJO		16	15.49	18.16	2.67	DEL	16p13.11 recurrent microdeletion	Myh11	
CNV014	UPJO		16	29.60	30.20	0.60	DEL	16p11.2 deletion	Tbx6, Maz	TBX6
CNV015	UPJO	Neurofibromatosis type I	17	29.00	30.38	1.38	DEL	NF1-microdeletion syndrome	Nf1*	NF1*
CNV016	UPJO		17	34.82	36.22	1.41	DEL	RCAD deletion	Lnx1, Hnf1b	HNF1B
CNV017	COU-NOS		21	14.84	48.08	33.14	DUP	Early-onset Alzheimer disease with cerebral amyloid angiopathy	Adamts1, Slc19a1	COL18A1, RIPK4
CNV018	UVJO	Mild grandular hypospadia, high arched palate, slight antimongoliod slant	22	20.73	21.46	0.73	DEL	DiGeorge B-D	-	-
CNV019	UPJO		X	71.81	72.38	0.57	DUP	-	-	-

Table 3. Rare CNVs account for 4.1% of the 461 genotyped COU cases with ~2.3-fold higher yield in UVJO compared to COU-NOS. Abbrv.:- DEL, deletion; DUP, duplication; N, No; Y, Yes; U, Unknown.

Conclusions, Limitations, and Future Directions

This study show a high diagnostic yield for COU, with important ramifications for diagnosis, counselling and risk stratification. While the diagnostic yield appears to be slightly higher for the UVJO group compared to UPJO and COU-NOS, this study had highlighted ample genetic overlap between COU subtypes supporting comprehensive approaches for gene identification in COU. Completion of CNV analysis by ES-CNV and accurate genotype-phenotype correlations are ongoing.

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