Supplementary Information - Table of Contents

Supplementary Table 1. Fourteen recessive, 25 dominant and 1 X-lined gene that represent monogenic causes of human CAKUT, if mutated.

(Note that underlined gene indicates that we identified a variant in this gene as the causative mutation in one or more of the 232 families with CAKUT in this study)

Gene	Protein	Reference
Autosomal recessive (AR)		
ACE	Angiotensin I-converting enzyme	Gribouval *Nat Genet* 37:964, 2005
AGT	Angiotensinogen	Gribouval *Nat Genet* 37:964, 2005
AGTR1	Angiotensin II receptor, type 1	Gribouval *Nat Genet* 37:964, 2005
CHRM3	Muscarinic acetylcholine receptor M3	Weber *AJHG* 19:634, 2011
ETV4	ETS translocation variant 4, E1A enhancer binding protein	Chen *IJPCH* 4:61, 2016
FRAS1	Extracellular matrix protein FRAS1	Kohl *JASN* 25:1917, 2014
FREM1	FRAS1 related extracellular matrix protein 1	Kohl *JASN* 25:1917, 2014
FREM2	FRAS1 related extracellular matrix protein 2	Kohl *JASN* 25:1917, 2014
GRIP1	Glutamate receptor interacting protein 1	Kohl *JASN* 25:1917, 2014
HPSE2	Heparanase 2 (Inactive)	Bulum *Nephron* 130:54, 2015
ITGA8	Integrin α8	Humbert *AJHG* 189:1260, 2014
REN	Renin	Gribouval *Nat Genet* 37:964, 2005
TRAP1	Heat-shock protein 75 (also known as TNF receptor-associated protein 1)	Saisawat *KI* 85:880, 2014
FGF20	Fibroblast Growth Factor 20	Barak *Dev Cell* 22:1191, 2012
Autosomal dominant (AD)		
BMP4	Bone morphogenic protein 4	Weber *JASN* 19:891, 2008
CHD1L	Chromodomain helicase DNA binding protein 1-like	Brockschmidt *NDT* 27:2355, 2012
CRKL	CRK Like Proto-Oncogene, adaptor protein	Lopez-Rivera *NEJM* 376:742, 2017
DSTYK	Dual serine/threonine and tyrosine protein kinase	Sanna-Cherchi *NEJM* 369:621, 2013
EYA1	Eyes absent homolog 1	Abdelhak *Nat Genet* 15:157, 1997
GATA3	GATA binding protein 3	Pandolfi *Nat Genet* 11:40, 1995; Van Esch *Nature* 406:419, 2000
GREB1L	Growth Regulation By Estrogen In Breast Cancer 1 Like	Brophy *Genetics* 207:215, 2017
HNF1B	HNF homeobox B	Lindner *Hum Mol Genet* 24:263, 1999
MUC1	Mucin 1	Kirby *Nat Genet* 45:299, 2013
NRIP1	Nuclear Receptor Interacting Protein 1	Vivante *JASN* 28:2364, 2017
PAX2	Paired box 2	Sanyanusin *Hum Mol Genet* 4:2183, 1995
RET	Proto-oncogene tyrosine-protein kinase receptor Ret	Skinner *AJHG* 82:344, 2008
ROBO2	Roundabout, axon guidance receptor, homolog 2 (*Drosophila*)	Hwang *Hum Genet* 134:905, 2015; Lu *AJHG* 80:616, 2007

SALL1	Sal-like protein 1 (also known as spalt-like transcription factor 1)	Kohlhase *Nat Genet* 18:81, 1998
SIX1	SIX homeobox 1	Ruf *Proc Nat Acad Sci* 101: 8090, 2004
SIX2	SIX homeobox 2	Weber *JASN* 19:891, 2008
SIX5	SIX homeobox 5	Hoskins *AJHG* 80:800, 2007
SLIT2	Slit homolog 2	Hwang *Hum Genet* 134:905, 2015
SOX17	Transcription factor SIX-17	Gimelli *Hum Mut* 31:1352, 2010
SRGAP1	SLIT-ROBO Rho GTPase activating protein 1	Hwang *Hum Genet* 134:905, 2015
TBX18	T-Box transcription factor	Vivante *AJHG* 97:291, 2015
TNXB	Tenascin XB	Gbadegesin *JASN* 24:1313, 2013
UMOD	Uromodulin	Hart *JMG* 39:882, 2002
UPK3A	Uroplakin 3A	Jenkins *JASN* 16:2141, 2005
WNT4	Protein Wnt-4	Biason-Lauber *NEJM* 351:792, 2004; Mandel *AJHG* 82:39, 2008; Vivante *JASN* 24:550, 2013
X-linked (XL)		
KAL1	Anosmin 1	Hardelin *PNAS* 89:8190, 1992

Supplementary Table 2. 179 genes (91 recessive, 54 dominant, 11 mixed autosomal dominant & recessive, 6 *de novo*, 14 X-linked genes and 3 unclassified inheritance) that represent monogenic causes of human syndromic CAKUT, if mutated

(Note that underlined gene indicates that we identified a variant in this gene as the causative mutation in one or more of the 232 families with CAKUT)

Gene	Protein	Reference
Autosomal recessive (AR)		
AHI1	Abelson Helper Integration Site 1	Parisi *J Med Gen* 43:334, 2005
ARL6	AT-Rich Interaction Domain 1B	Pretorius *PLoS Genet* 19;6, 2010
B3GALTL	Beta 3-Glucosyltransferase	Lesnik Oberstein *Am J Hum Genet* 79:562, 2006
BBS1	Bardet-Biedl Syndrome 1	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BBS10	Bardet-Biedl Syndrome 10	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BBS12	Bardet-Biedl Syndrome 12	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BBS2	Bardet-Biedl Syndrome 2	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BBS4	Bardet-Biedl Syndrome 4	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BBS5	Bardet-Biedl Syndrome 5	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BBS6	Bardet-Biedl Syndrome 7	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BBS7	Bardet-Biedl Syndrome 7	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BBS8	Bardet-Biedl Syndrome 8	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BBS9	Bardet-Biedl Syndrome 9	Tieder *Int J Pediatr Nephrol* 3:199, 1982
BSCL2	BSCL2, Seipin Lipid Droplet Biogenesis Associated	Haghighi *Clin Genet* 89: 434, 2016
CD151	CD151 Molecule (Raph Blood Group)	Karamatic *Blood* 104:2217, 2004
CD96	CD96 Molecule	Kaname *AJHG* 81:835, 2007
CEP290	Centrosomal Protein 290	Valente *Nat Genet* 68:623, 2006
CHRNG	Cholinergic Receptor Nicotinic Gamma Subunit	Vogt *J Med Genet* 49:21, 2012
CISD2	CDGSH Iron Sulfur Domain 2	Amr *AJHG* 81:673, 2007
CTU2	Cytosolic Thiouridylase, subunit 2	Shaheen *AJMG* 170:3222, 2016
CYP21	Cytochrome P450 Family 21	Martul *Arch Dis Child* 55:324, 1980
DACH1	Dachshund Family Transcription Factor 1	Schild *Nephrol Dial Transplant* 28:227, 2013
DHCR7	7-Dehydrocholesterol Reductase	Löffler *AJHG* 13;95:174, 2000
DYNC2H1	Dynein Cytoplasmic 2 Heavy Chain 1	Baujat *J Med Genet* 50:91, 2013
EMG1	EMG1, N1-Specific Pseudouridine Methyltransferase	Armistead *AJHG* 84:728, 2009
ESCO2	Establishment Of Sister Chromatid Cohesion N-Acetyltransferase 2	Vega *J Med Genet* 47:30, 2010
ETFA	Electron Transfer Flavoprotein Alpha Subunit	Lehnert *Eur J Pediatr* 139:56, 1982
ETFB	Electron Transfer Flavoprotein Beta Subunit	Lehnert *Eur J Pediatr* 139:56, 1982

ETFDH	Electron Transfer Flavoprotein Dehydrogenase	Lehnert *Eur J Pediatr* 139:56, 1982
EVC	EVC Ciliary Complex Subunit 1	Moudgil *Pediatr Nephrol* 12:20, 1998
EVC2	EVC Ciliary Complex Subunit 2	Kurian *Indian J Dent Res* 18:31, 2007
FANCA	Fanconi Anemia Complementation Group A	Joenje & Patel *Nat Rev Genet* 2:466, 2001
FANCB	Fanconi Anemia Complementation Group B	McCauley *Am J Med Genet* A 155A:2370, 2011
FAT4	FAT Atypical Cadherin 4	Alders *Hum Genet* 133:1161, 2014
FOXP1	Forkhead Box P1	Bekheirnia *Genet Med* 19:412, 2017
GLIS2	GLIS Family Zinc Finger 2	Attanasio *Nat Genet* 39:1018, 2007
HES7	Hes Family BHLH Transcription Factor 7	Sparrow *Hum Mol Genet* 17:3761, 2008
HYLS1	HYLS1, Centriolar And Ciliogenesis Associated	Paetau *J Neuropathol Exp Neurol* 67:750, 2008
IFT172	Intraflagellar Transport 172	Friedland-Little *Hum Mol Genet* 20:3725, 2011
IFT27	Intraflagellar Transport 27	Schaefer *J Hum Genet* 61:447, 2016
IFT46	Intraflagellar Transport 46	Lee *Dev Biol* 400:248, 2015
IFT52	Intraflagellar Transport 52	Walczak-Sztulpa *Am J Med Genet* A 173:1364, 2017
IFT57	Intraflagellar Transport 57	Bruel *J Med Genet* 54:371, 2017
IFT74	Intraflagellar Transport 74	Cevik *PLoS Gene* 9:e1003977, 2013
IFT80	Intraflagellar Transport 80	Beales *Nat Genet* 39:727, 2007
IFT81	Intraflagellar Transport 81	Perrault *J Med Genet* 52:657, 2015
INPP5E	Inositol Polyphosphate-5-Phosphatase E	Travaglini *Eur J Hum Genet* 21:1074, 2013
INVS	Inversin	Otto *Nat Gene* 34:413, 2003
ITGA3	Integrin Subunit Alpha 3	Yalcin *Hum Mol Genet* 24:3679, 2015
JAM3	Junctional Adhesion Molecule 3	Mochida *Am J Hum Genet* 10;87:882, 2010
LFNG	LFNG O-Fucosylpeptide 3-Beta-N-Acetylglucosaminyltransferase	Sparrow *Am J Hum Genet* 78:28, 2006
LMNA	Lamin A/C	Klupa *Endocrine* 36:518, 2009
LRP2	LDL Receptor Related Protein 2	Kantarci *Nat Genet* 39:957, 2007
LRP4	LDL Receptor Related Protein 4	Li Am *J Hum Genet* 86:696, 2010
MESP2	Mesoderm Posterior BHLH Transcription Factor 2	George-Abraham *Am J Med Genet* A 158A:1971, 2012
MKKS	McKusick-Kaufman Syndrome	Yamamura *Clin Exp Nephro* 21:136, 2017
MKS1	Meckel Syndrome, Type 1	Kyttälä *Nat Genet* 38:155, 2006
MKS3	Meckel Syndrome Type 3 Protein	Baala *Am J Hum Genet* 80:186, 2007
NEK1	NIMA Related Kinase 1	Thiel *Am J Hum Genet* 88:106, 2011
NPHP1	Nephrocystin 1	Hildebrandt *Nat Genet* 17:149, 1997
NPHP3	Nephrocystin 3	Olbrich *Nat Genet* 34:455, 2003
NPHP4	Nephrocystin 4	Otto *AJHG* 71:1161, 2002

PEX5	Peroxisomal Biogenesis Factor 5	Sundaram *Nat Clin Pract Gastroenterol Hepato*l 5:456, 2008
PKHD1	PKHD1, Fibrocystin/Polyductin	Bergmann *Kl* 67:829, 2005
PMM2	Phosphomannomutase 2	Horslen *Arch Dis Child* 66:1027, 1991
PROK2	Prokineticin 2	Madan *Mol Genet Metab Rep* 12:57, 2017
RECQL4	RecQ Like Helicase 4	Siitonen *Eur J Hum Genet* 17:151, 2009
ROR2	Receptor Tyrosine Kinase Like Orphan Receptor 2	Wiens *Clin Genet* 37:481, 1990
RPGRIP1L	RPGRIP1 Like	Suzuki *Clin Genet* 90:526, 2016
RPS19	Ribosomal Protein S19	Hoefele *Pediatr Nephrol* 25:1255, 2010
SCARF2	Scavenger Receptor Class F Member 2	Anastasio *Am J Hum Genet* 87:553, 2010
SDCCAG8	Serologically Defined Colon Cancer Antigen 8	Airik *J Am Soc Nephrol* 25:2573, 2014
STRA6	Stimulated By Retinoic Acid 6	Golzio *Am J Hum Genet* 80:1179, 2007
TMCO1	Transmembrane And Coiled-Coil Domains 1	Xin *Proc Natl Acad Sci U S A* 107:258, 2010
TMEM231	Transmembrane Protein 231	Shaheen *J Med Genetics* 50:160, 2013
TMEM216	Transmembrane Protein 216	Edvardson *Am J Hum Genet* 86:93, 2010
TMEM67	Transmembrane Protein 67	Kumar *Am J Med Genet* 61:122, 1996
TRIM32	Tripartite Motif Containing 32	Chiang *Proc Natl Acad Sci U S A* 103:6287, 2006
TWIST2	Twist Family BHLH Transcription Factor 2	Stevens *Am J Med Genet* 107:30, 2002
UBR1	Ubiquitin Protein Ligase E3 Component N-Recognin 1	Vanlieferinghen *Genet Couns* 14:105, 2003
PEX1	Peroxisomal Biogenesis Factor 1	Crane *Hum Mutat* 26:167, 2005
PIGL	Phosphatidylinositol Glycan Anchor Biosynthesis Class L	Schnur *Am J Med Genet* 72:24, 1997
PIGO	Phosphatidylinositol Glycan Anchor Biosynthesis Class O	Krawitz *Am J Hum Genet* 91:146, 2012
PIGN	Phosphatidylinositol Glycan Anchor Biosynthesis Class N	Ohba *Neurogenetics* 15:85, 2014
PIGT	Phosphatidylinositol Glycan Anchor Biosynthesis Class T	Nakashima *Neurogenetics* 15:193, 2014
PIGV	Phosphatidylinositol Glycan Anchor Biosynthesis Class V	Horn *Eur J Hum Genet* 22:762, 2014
PIGY	Phosphatidylinositol Glycan Anchor Biosynthesis Class Y	Ilkovski *Hum Mol Genet* 24:6146, 2015
PTF1A	Pancreas Specific Transcription Factor, 1a	Gurung *Mol Med Rep* 12:1579, 2015
WFS1	Wolframin ER Transmembrane Glycoprotein	Salih *Acta Paediatr Scand* 80:567, 1991
WNT3	Wnt Family Member 3	Niemann *Am J Hum Genet* 74:558, 2004
ZMPSTE24	Zinc Metallopeptidase STE24	Chen *Am J Med Genet A* 149A:1550, 2009
Autosomal dominant (AD)		
ACTB	Actin Beta	Rivière *Nat Genet* 44:440, 2012
ACTG1	Actin Gamma 1	Rivière *Nat Genet* 44:440, 2012
BICC1	BicC Family RNA Binding Protein 1	Kraus *Hum Mutat* 33:86, 2012

BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase	Sarkozy *Hum Mutat* 30:695, 2009
CDC5L	Cell Division Cycle 5 Like	Groenen *Genomics* 49:218, 1998
CREBBP	CREB Binding Protein	Kanjilal *J Med Genet* 29:669, 1992
EP300	E1A Binding Protein P300	Roelfsema *Am J Hum Genet* 76:572, 2005
ESRRG	Estrogen Related Receptor Gamma	Harewood *PLoS One* 5:e12375, 2010
FBN1	Fibrillin 1	Tokhmafshan *Pediatr Nephrol* 32:565, 2017
FGF10	Fibroblast Growth Factor 10	Milunsky *Clin Genet* 69:349, 2006; Bamforth *Am J Med Genet* 43:932, 1992
FGF8	Fibroblast Growth Factor 8	Falardeau *J Clin Invest* 118:2822 2008
FGF3	Fibroblast Growth Factor 3	Marquis-Nicholson *Sultan Qaboos Univ Med J.* 13:80, 2013
FMN1	Formin 1	Dimitrov *J Med Genet* 47:569, 2010
FOXC1	Forkhead Box C1	LeHeup *Eur J Pediatr* 154:130, 1995
FOXF1	Forkhead Box F1	Hilger *Hum Mutat* 36:1150, 2015
GDF3	Growth Differentiation Factor 3	Karaca *Am J Med Genet A* 167A:2795, 2015
GFRA1	GDNF Family Receptor Alpha 1	Chatterjee *Hum Genet* 131:1725, 2013
GLI2	GLI Family Zinc Finger 2	Carmichael *J Urol* 190:1884, 2013
HOXA13	Homeobox A13	Halal *Am J Med Genet* 30:793, 1998
HOXD13	Homeobox D13	Garcia-Barceló *Am J Med Genet A*146A:3181, 2008
JAG1	Jagged 1	Kamath *Nat Rev Nephrol* 9:409, 2013
KAT6B	Lysine Acetyltransferase 6B	Campeau *Am J Med Genet* 90:282, 2012
KCTD1	Potassium Channel Tetramerization Domain Containing 1	Marneros *Am J Hum Genet* 92:621, 2013
KCNH2	Potassium Voltage-Gated Channel Subfamily H Member 2	Caselli *Am J Med Genet* 146A:1195, 2008
KRAS	KRAS Proto-Oncogene, GTPase	Schubbert *Nat Gene* 38:331, 2006
LMX1B	LIM Homeobox Transcription Factor 1 Beta	Dreyer *Nat Genet* 19:47, 1998
LPP	LIM Domain Containing Preferred Translocation Partner In Lipoma	Hernández-García *Am J Med Genet A* 158A:1785, 2012
MLL2/ KMT2D	Myeloid/Lymphoid Or Mixed-Lineage Leukemia Protein 2	Banka *Eur J Hum Genet* 20:381, 2012
MYCN	Feingold Syndrome	Marcelis *Hum. Mut*. 29:1125, 2006
NFIX	Nuclear Factor I X	Malan *Am J Hum Genet* 87:189, 2010
NOTCH2	Notch 2	Kamath *Nat Rev Nephrol* 9:409, 2013
PAX8	Paired Box 8	Meeus *J Clin Endocrinol Metab* 89:4285, 2004
PKD1	Polycystin 1, Transient Receptor Potential Channel Interacting	Rossetti *J Am Soc Nephrol* 18:2143, 2007
PKD2	Polycystin 2, Transient Receptor Potential Cation Channel	Rossetti *J Am Soc Nephrol* 18:2143, 2007
PROKR2	Prokineticin Receptor 2	Sarfati *Front Horm Res* 39:121, 2010
PTPN11	Protein Tyrosine Phosphatase, Non-Receptor Type 11	Bertola *Am J Med Genet* 130A:378, 2004

RAF1	Raf-1 Proto-Oncogene, Serine/Threonine Kinase	Razzaque *Nat Genet* 39:1013, 2007
RAI1	Retinoic Acid Induced 1	Vilboux *PLoS One* 6:e22861, 2011
SALL4	Spalt Like Transcription Factor 4	Kohlhase *GeneReviews®Book Section,* 1993
SEMA3A	Semaphorin 3A	Young *Hum Reprod* 27:1460, 2012
SETBP1	SET Binding Protein 1	Schinzel *Am J Med Genet* 1:361, 1978
SHH	Sonic Hedgehog	Lurie Am *J Med Genet* 35:286, 1990
SF3B4	Splicing Factor 3b Subunit 4	Bernier *Am J Hum Genet* 90:925, 2012
SOS1	SOS Ras/Rac Guanine Nucleotide Exchange Factor 1	Ferrero *Eur J Med Genet* 51:566, 2008
SOX9	SRY-Box 9	Airik *Hum Mol Genet* 19:4918, 2010
SRCAP	Snf2 Related CREBBP Activator Protein	Hood *Am J Hum Genet* 90:308, 2012
TBX1	T-Box 1	Kujat *Am J Med Genet A* 140:1601, 2006
TBX3	T-Box 3	Meneghini *Eur J Med Genet* 49:151, 2006
TFAP2A	Transcription Factor AP-2 Alpha	Milunsky *Am J Hum Genet* 82:1171, 2008
TP63	Tumor Protein P63	Celli *Cell* 99:143, 1999
TRPS1	Zinc finger transcription factor; Trichorhinophalangeal syndrome	Tasic *Ren Fail* 36:619, 2014
TSC1	Tuberous Sclerosis 1	Curatolo *Lancet* 372:657, 2008
TSC2	Tuberous Sclerosis 2	Kumar *Hum Mol Genet* 4:1471, 1995
WNT5A	Wnt Family Member 5A	Roifman *Clin Genet* 87:34, 2015; Person *Dev Dyn* 239:327, 2010

Autosomal recessive & dominant (AR & AD)		
ARID1B	AT-Rich Interaction Domain 1B	Levy *J Med Genet* 28, 1991
DIS3L2	DIS3 Like 3'-5' Exoribonuclease 2	Astuti *Nat Genet* 5;44:277, 2012
FGFR2	Fibroblast Growth Factor Receptor 2	LeHeup *Eur J Pediatr* 154:130, 1995
FGFR3	Fibroblast Growth Factor Receptor 3	Prontera *Genet Couns* 17:407, 2006
GDF6	Growth Differentiation Factor 6	Tassabehji *Hum Mutat* 29:1017, 2008
GLI3	GLI Family Zinc Finger 3	Cain *PLoS One* 4:e7313, 2009
PCSK5	Proprotein Convertase Subtilisin & Kexin Type 5	Nakamura *BMC Res Notes* 8:228, 2015
PTEN	Phosphatase And Tensin Homolog	Reardon *J Med Genet* 38:820, 2001
RPS24	Ribosomal Protein S24	Yetgin *Turk J Pediatr* 36:239, 1994
TTC21B	Tetratricopeptide Repeat Domain 21B	Davis *Nat Genet* 43:189, 2011
VANGL1	VANGL Planar Cell Polarity Protein 1	Bartsch *Mol Syndromol* 3:76, 2012

***De novo* inheritance**		
AXIN1	Axin 1	Oates *Am J Hum Genet* 79:155, 2006
AXIN1	Axin 1	Oates *Am J Hum Genet* 79:155, 2006
H19	H19, Imprinted Maternally Expressed Transcript (Non-Protein Coding)	Hur *Proc Natl Acad Sci U S A* 113:10938, 2016
KCNQ1OT1	KCNQ1 Opposite Strand & Antisense Transcript 1 (Non-Protein Coding)	Chiesa *Hum Mol Genet* 21:10, 2012
NIPBL	NIPBL, Cohesin Loading Factor	Rohatgi *Am J Med Genet* 152A:1641, 2010

CDKN1C	Cyclin Dependent Kinase Inhibitor 1C	Mussa *Pediatr Nephrol* 27:397, 2012
CHD7	Chromodomain Helicase DNA Binding Protein 7	Janssen *Hum Mutat* 33:1149 2012
X-linked (XL)		
AMER1	APC Membrane Recruitment Protein 1	Pellegrino *Am J Med Genet* 16:159, 1997
ATP7A	ATPase Copper Transporting Alpha	Vulpe *Nat Genet* 3:7, 1993
BCOR	BCL6 Corepressor	Ng *Nat Genet* 36:411, 2004
FAM58A	Family With Sequence Similarity 58 Member A	Green *J Med Genet* 33:594, 1996; Unger *Nat Genet* 40:287, 2008
FLNA	Filamin A	Robertson *Am J Med Genet* A 140:1726, 2006
GPC3	Glypican 3	Cottereau *Am J Med Genet* C *Semin Med Genet* 163:92, 2013
MID1	Midline 1	Preiksaitiene *Clin Dysmorphol* 24:7, 2015
NSDHL	NAD(P) Dependent Steroid Dehydrogenase-Like	König *J Am Acad Dermatol* 46:594, 2002
OFD1	Oral-Facial-Digital Syndrome 1 Protein	Bisschoff *Hum Mutat* 34:237, 2013
PIGA	Phosphatidylinositol Glycan Anchor Biosynthesis Class A	Johnston *Am J Hum Genet* 90:295, 2012
PORCN	Porcupine O-Acyltransferase	Suskan *Pediatr Dermatol* 7:283, 1990
SMC1A	Structural Maintenance Of Chromosomes 1A	Deardorff *GeneReviews® Book Section Seattle(WA),* 1993
UPF3B	UPF3B, Regulator Of Nonsense Mediated MRNA Decay	Lynch *Eur J Med Genet* 55:476, 2012
ZIC3	Zic Family Member 3	Chung *Am J Med Genet* 155:1123, 2011
Inheritance unknown		
GDF11	Growth Differentiation Factor 11	Tsuda *Eur J Pediatr Surg* 21:238, 2011
TTC30A	Tetratricopeptide Repeat Domain 30A	Hilger *Hum Mutat* 36: 1150, 2015
SH2B1	SH2B Adaptor Protein 1	Sampson *Am J Med Genet* 152:2618, 2010

Supplementary Table 3. 185 monogenic genes that cause murine CAKUT if mutated.

185 genes that if mutated cause murine CAKUT phenotypes were retrieved from the MGI database (http://www.informatics.jax.org/) by searching for the following terms: "abnormal mesonephric mesenchyme", "abnormal mesonephric mesenchyme morphology", "abnormal metanephric mesenchyme", "abnormal metanephric mesenchyme morphology", "abnormal metanephric ureteric bud development", "abnormal ureter development", "abnormal ureter morphology", "abnormal ureteric bud elongation", "abnormal ureteric bud invasion", "abnormal ureterovesical junction", "abnormal urinary system development", "absent kidney", "absent metanephric mesenchyme", "absent metanephros", "absent ureter", "dilated ureter", "double kidney pelvis", "double kidney pelvis", "double ureter", "duplex kidney", "ectopic ureter", "ectopic ureteric bud", "hydroureter", "impaired branching involved in ureteric bud morphogenesis", "pelvic kidney", "renal hypoplasia", "short ureter", "single kidney", "small metanephros", "ureter hypoplasia", "abnormal nephrogenic mesenchyme morphogenesis", "ureteropelvic junction obstruction". Mouse CAKUT genes are listed here in parallel with the corresponding human phenotype that was extracted from the OMIM database (www.omim.org). Human phenotypes containing CAKUT are underlined (source: clinical synopsis table, OMIM, www.omim.org).

(Note underlined gene indicates that we identified a variant in this gene as the causative mutation in one or more of the 232 families with CAKUT)

Gene	Protein	PubMed or MGI Reference ID	Human Phenotype (contains CAKUT)	Human phenotype OMIM #
Ace	Angiotensin I converting enzyme	8642790	Renal tubular dysgenesis	267430
Acvr2b	Activin A Receptor Type 2B	9242489	Heterotaxy, visceral, 4, autosomal	613751
Adamts1	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 1	10811842	-	-
Agt	Angiotensinogen	8675666	Renal tubular dysgenesis	267430
Agtr1a	Angiotensin II receptor, type 1a	10024874	Renal tubular dysgenesis	267430
Agtr1b	Angiotensin II receptor, type 1b	10024874	Renal tubular dysgenesis	267430
Agtr2	Angiotensin II Receptor Type 2	10024874	-	-
Aldh1a2	Aldehyde Dehydrogenase 1 Family Member A2	20040494	-	-
Amer1	APC Membrane Recruitment Protein 1	21571217	Osteopathia striata with cranial sclerosis	300373
Anp32b	Acidic Nuclear Phosphoprotein 32 Family Member B	21636789	-	-

Aprt	Adenine Phosphoribosyltransferase	8864750	Adenine phosphoribosyl-transferase deficiency	614723
Aqp2	Aquaporin 2	3184310	Diabetes insipidus, nephrogenic	125800
Arhgap1	Rho GTPase Activating Protein 1	17227869	-	-
Arhgap35	Rho GTPase Activating Protein 35	26859289	-	-
Arid5b	AT-Rich Interaction Domain 5B	17143286	-	-
Arl3	ADP Ribosylation Factor Like GTPase 3	16565502	-	-
Atmin	ATM Interactor	24852369	-	-
Atp7a	ATPase Copper Transporting Alpha	11534785	Menkes disease; Occipital horn syndrome; Spinal muscular atrophy, distal, X-linked 3	309400; 304150; 300489
Axin1	Axin 1	17246824 13340237	Hepatocellular carcinoma, somatic	114550
Bag6	BCL2 Associated Athanogene 6	16287848	-	-
Bcl2	BCL2, Apoptosis Regulator	8623928	Leukemia/ lymphoma, B-cell, 2	n/a
Bmp4	Bone Morphogenetic Protein 4	10749566	Microphthalmia, syndromic 6	607932
Bmp5	Bone Morphogenetic Protein 5	5692092	-	-
Bmp7	Bone Morphogenetic Protein 7	7590254	-	-
Bmper	BMP Binding Endothelial Regulator	17035289	Diaphano-spondylo-dysostosis	608022
Cc2d2a	Coiled-Coil And C2 Domain Containing 2A	J:175213	COACH syndrome; Joubert syndrome 9; Meckel syndrome 6	216360; 612285; 612284
Cdc42	Cell Division Cycle 42	23555292	Takenouchi-Kosaki syndrome	616737
Cdh4	Cadherin 4	11839813	-	-
Cdh6	Cadherin 6	10864459	-	-
Chrm3	Cholinergic Receptor Muscarinic 3	10944224	Prune belly syndrome	100100
Cntrl	Centriolin		-	-

Crb3	Crumbs 3, Cell Polarity Complex Component	26631503	-	-
Crim1	Cysteine Rich Transmembrane BMP Regulator 1	22511315	-	-
Ctdnep1	CTD Nuclear Envelope Phosphatase 1	23360989	-	-
Ctnnb1	Catenin Beta 1	20454682	Colorectal cancer, somatic; Hepatocellular carcinoma, somatic; Medullo-blastoma, somatic; Mental retardation, autosomal dominant 19; Ovarian cancer, somatic; Pilomatricoma, somatic	114500; 114550; 155255; 615075; 167000; 132600
Ctnnbip1	Catenin Beta Interacting Protein 1	17803964	-	-
Cxcr4	C-X-C Motif Chemokine Receptor 4	J:175213	WHIM syndrome	193670
Cyp26a1	Cytochrome P450 Family 26 Subfamily A Member 1	11157778	-	-
Dact1	Dishevelled Binding Antagonist Of Beta Catenin 1	20145239	-	-
Dchs1	Dachsous Cadherin-Related 1	21303848	Mitral valve prolapse 2; Van Maldergem syndrome 1	607829; 601390
Dhcr7	7-Dehydrocholesterol Reductase	11230174	Smith-Lemli-Opitz syndrome	270400
Dlg1	Discs Large MAGUK Scaffold Protein 1	17172448	-	-
Dlg5	Discs Large MAGUK Scaffold Protein 5	17765678	{Inflammatory bowel disease 20}	612288
Dnah11	Dynein Axonemal Heavy Chain 11	J:175213	Ciliary dyskinesia, primary, 7, with or without situs inversus	611884
Dnah5	Dynein Axonemal Heavy Chain 5	J:175213	Ciliary dyskinesia, primary, 3, with or without situs	608644

			inversus	
Dym	Dymeclin	18852472	Dyggve-Melchior-Clausen disease; Smith-McCort dysplasia	223800; 607326
Dync2h1	Dynein Cytoplasmic 2 Heavy Chain 1	J:175213	Short-rib thoracic dysplasia 3 with or without polydactyly	613091
Efnb2	Ephrin B2	15223334	-	-
Emx2	Empty Spiracles Homeobox 2	9165114	Schizencephaly	269160
Esrrg	Estrogen Related Receptor Gamma	21138943	-	-
Etl4/Etn2	Early transposon element insertion site 2	23436999	Epilepsy, familial temporal lobe, 4	611631
Etv4	ETS variant 4	19898483	-	-
Etv5	ETV variant 5	19898483	-	-
Exoc5	Exocyst complex component 5	26046524	-	-
Eya1	EYA Transcriptional Coactivator And Phosphatase 1	10471511	Branchiooto-renal syndrome 1, with or without cataracts; Branchiootic syndrome 1; Anterior segment anomalies with or without cataract; Otofaciocervical syndrome	113650; 602588; 602588; 166780
Fat4	FAT Atypical Cadherin 4	21303848	Van Maldergem syndrome 2; Hennekam lymph-angiectasia-lymphedema syndrome 2	615546; 616006
Fgf10	Fibroblast Growth Factor 10	11062007	Aplasia of lacrimal and salivary glands; LADD syndrome	180920; 149730
Fgf7	Fibroblast Growth Factor 7	9876183	-	-
Fgf8	Fibroblast Growth Factor 8	16049111	Hypo-gonadotropic hypogonadism 6 with or without anosmia	612702

Fgfr2	Fibroblast Growth Factor Receptor 2	15843416	Antley-Bixler syndrome; Apert syndrome; Beare-Stevenson cutis gyrata syndrome; Bent bone dysplasia syndrome; Craniofacial-skeletal-dermatologic dysplasia; Crouzon syndrome; Gastric cancer, somatic; Jackson-Weiss syndrome; LADD syndrome; Pfeiffer syndrome; Saethre-Chotzen syndrome; Scaphocephaly, maxillary retrusion, and mental retardation	207410; 101200; 123790; 614592; 101600; 123500; 613659; 123150; 149730; 101600; 101400; 609579
Fgfrl1	Fibroblast Growth Factor Receptor-Like 1	19715689	-	-
Fmn1	Formin 1	7517224	-	-
Foxc1	Forkhead Box C1	5500588	Anterior segment dysgenesis 3, multiple subtypes; Axenfeld-Rieger syndrome, type 3	601631; 602482
Foxd1	Forkhead Box D1	8666231	-	-
Foxd2	Forkhead Box D2	10648626	-	-
Foxg1	Forkhead Box G1	16109771	Rett syndrome, congenital variant	613454
Fras1	Fraser Extracellular Matrix Complex Subunit 1	12766769	Fraser syndrome	219000
Frem1	FRAS1 Related Extracellular Matrix 1	12766769	Bifid nose with or without anorectal and renal anomalies; Manitoba oculotrichoanal syndrome; Trigonocephaly 2	608980; 248450; 614485
Frem2	FRAS1 Related Extracellular Matrix Protein 2	12766769	Fraser syndrome	219000
Fstl1	Follistatin Like 1	22485132	-	-

Fzd4	Frizzled Class Receptor 4	21343368	Exudative vitreoretinopathy 1; Retinopathy of prematurity	133780; 133780
Fzd8	Frizzled Class Receptor 8	21343368	-	-
Gata2	GATA Binding Protein 2	18233958	Emberger syndrome; Immunodeficiency 21	614038; 614172
Gata3	GATA Binding Protein 3	16319112	Hypopara-thyroidism, sensorineural deafness, and renal dysplasia	146255
Gdf11	Growth Differentiation Factor 11	12729564	-	-
Gdnf	Glial Cell Derived Neurotrophic Factor	11422733	Central hypoventilation syndrome	209880
Gfra1	GDNF Family Receptor Alpha 1	23542432	-	-
Glce	Glucuronic Acid Epimerase	12788935	-	-
Gli3	GLI Family Zinc Finger 3	11978771	Greig cephalopoly-syndactyly syndrome; Pallister-Hall syndrome; Polydactyly, postaxial, types A1 and B; Polydactyly, preaxial, type IV	175700; 146510; 174200; 174700
Gpc3	Glypican 3	10402475	Simpson-Golabi-Behmel syndrome, type 1; Wilms tumor, somatic	312870; 194070
Grem1	Gremlin 1, DAN Family BMP Antagonist	15201225	-	-
Grip1	Glutamate Receptor Interacting Protein 1	10974668	Fraser syndrome	219000
Hnf1b	HNF1 Homeobox B	23362348	Diabetes mellitus, noninsulin-dependent; Renal cysts and diabetes syndrome	125853; 137920
Hoxa11	Homeobox A11	7596412	Radioulnar synostosis with amegakaryo-cytic thrombocyto-penia 1	605432

Hoxd11	Homeobox D11	12050119	-	-
Hoxa13	Homeobox A13	12783783	Guttmacher syndrome; Hand-foot-uterus syndrome	176305; 140000
Hoxc10	Homeobox C10	19623272	-	-
Hoxc11	Homeobox C11	12050119	-	-
Hpse2	Heparanase 2	25510506	Urofacial syndrome 1	236730
Hs2st1	Heparan Sulfate 2-O-Sulfotransferase 1	9637690	-	-
Hsd17b2	Hydroxysteroid 17-Beta Dehydrogenase 2	18048640	-	-
Hspa4l	Heat Shock Protein Family A (Hsp70) Member 4 Like	16923965	-	-
Htr3a	5-Hydroxytryptamine Receptor 3A	15201326	-	-
Id2	Inhibitor Of DNA Binding 2, HLH Protein	15569159	-	-
Ilk	Integrin Linked Kinase	19829382	-	-
Itga3	Integrin Subunit Alpha 3	10433923	Interstitial lung disease, nephrotic syndrome, and epidermolysis bullosa, congenital	614748
Itga6	Integrin Subunit Alpha 6	10433923	Epidermolysis bullosa, junctional, with pyloric stenosis	226730
Itga8	Integrin Subunit Alpha 8	9054500 17537792	Renal hypodysplasia/ aplasia 1	191830
Itgb1	Integrin Subunit Beta 1	19439520	-	-
Kif26b	Kinesin Family Member 26B	20439720	-	-
Lama5	Laminin Subunit Alpha 5	10625553	-	-
Lamc1	Laminin Subunit Gamma 1	12015298	-	-
Lgr4	Leucine Rich Repeat Containing G Protein-Coupled Receptor 4	21523854 22738954	{Bone mineral density, low, susceptibility to}	615311

Lhx1	Luteinizing Hormone/Choriogonadotropin Receptor	16216236	-	-
Lin7c	Lin-7 Homolog C, Crumbs Cell Polarity Complex Component	17923534	-	-
Lrp4	LDL Receptor Related Protein 4	20454682	Sclerosteosis 2; Cenani-Lenz syndactyly syndrome; ?Myasthenic syndrome, congenital, 17	614305; 212780; 616304
Lzts2	Leucine Zipper Tumor Suppressor 2	21949185	-	-
Megf8	Multiple EGF Like Domains 8	18043505	Carpenter syndrome 2	614976
Mks1	Meckel Syndrome, Type 1	21045211	Meckel syndrome 1	249000
Mmp14	Matrix Metallopeptidase 14	20727881	?Winchester syndrome	277950
Mmp17	Matrix Metallopeptidase 17	21347258	-	-
Mycn	V-Myc Avian Myelocytomatosis Viral Oncogene Neuroblastoma Derived Homolog	1459449	Feingold syndrome 1	164280
Ndst1	N-Deacetylase And N-Sulfotransferase 1		Mental retardation, autosomal recessive 46	616116
Nf1	Neurofibromin 1	7926784	Neurofibromatosis, type 1	162200
Nfia	Nuclear Factor I A	17530927	Brain malformations with or without urinary tract defects	613735
Nmnat2	Nicotinamide Nucleotide Adenylyltransferase 2	23082226	-	-

Nog	Noggin	18028901	Brachydactyly, type B2; Multiple synostoses syndrome 1; Stapes ankylosis with broad thumb and toes; Symphalangism, proximal, 1A; Tarsal-carpal coalition syndrome	611377; 186500; 184460; 185800; 186570
Notch2	Notch 2	20299358	Alagille syndrome 2; Hajdu-Cheney syndrome	610205; 102500
Npnt	Nephronectin	17537792	-	-
Osr1	Odd-Skipped Related Transciption Factor 1	16790474	-	-
Parva	Parvin Alpha	19829382	-	-
Pax2	Paired Box 2	8575306	Papillorenal syndrome; Glomerulo-sclerosis, focal segmental, 7	120330; 616002
Pax8	Paired Box 8	12435636	Hypothyroidism, congenital, due to thyroid dysgenesis or hypoplasia	218700
Pbx1	PBX Homeobox 1	12591246	Leukemia, acute pre-B-cell	176310
Pcnt	Pericentrin (kendrin)	25220058	Microcephalic osteodysplastic primordial dwarfism, type II	210720
Pcsk5	Proprotein Convertase Subtilisin/Kexin Type 5	18519639	-	-
Pdgfra	Platelet Derived Growth Factor Receptor Alpha	19217431	Gastrointestinal stromal tumor, somatic; Hyper-eosinophilic syndrome, idiopathic, resistant to imatinib	606764; 607685
Pds5a	PDS5 Cohesin Associated Factor A	19412548	-	-
Plxnb1	Plexin B1	18799546	-	-
Plxnb2	Plexin B2	21035938	-	-
Plxnd1	Plexin D1	J:175213	-	-

Ppp3r1	Protein Phosphatase 3 Regulatory Subunit B, Alpha	15057312	-	-
Prickle1	Prickle Planar Cell Polarity Protein 1	25190059	Epilepsy, progressive myoclonic 1B	612437
Ptch1	Patched 1	22792366	Basal cell carcinoma, somatic; Basal cell nevus syndrome; Holopros-encephaly 7	605462; 109400; 610828
Pten	Phosphatase And Tensin Homolog	17540362	Bannayan-Riley-Ruvalcaba syndrome; Cowden syndrome 1; Endometrial carcinoma, somatic; Lhermitte-Duclos syndrome; Macrocephaly/autism syndrome; Malignant melanoma, somatic; Squamous cell carcinoma, head and neck, somatic; VATER association with macrocephaly and ventriculo-megaly	153480; 158350; 608089; 158350; 605309; 155600; 275355; 276950
Ptprf	Protein Tyrosine Phosphatase, Receptor Type F	19273906	?Breasts and/or nipples, aplasia or hypoplasia of, 2	616001
Pygo1	Pygopus Family PHD Finger 1	17425782	-	-
Pygo2	Pygopus Family PHD Finger 2	17425782	-	-
Rara	Retinoic Acid Receptor Alpha	9376317	Leukemia, acute promyelocytic	612376
Rdh10	Retinol Dehydrogenase 10 (All-Trans)	21930923 17473173	-	-
Rere	Arginine-Glutamic Acid Dipeptide Repeats	23451234	Neurodevelopmental disorder with or without anomalies of the brain, eye, or heart	616975

Ret	Ret Proto-Oncogene	16452504	Central hypoventilation syndrome, congenital; Medullary thyroid carcinoma; Multiple endocrine neoplasia IIA; Multiple endocrine neoplasia IIB; Pheochromo-cytoma	209880; 155240; 171400; 162300; 171300
Robo1	Roundabout Guidance Receptor 1	J:175213	-	-
Robo2	Roundabout Guidance Receptor 2	17357069	Vesicoureteral reflux 2	610878
Rspo2	R-Spondin 2	17904116 12782276	-	-
Sall1	Spalt Like Transcription Factor 1	11688560	Townes-Brocks syndrome; Townes-Brocks branchio-otorenal-like syndrome	107480
Sall4	Spalt Like Transcription Factor 4	17216607	Duane-radial ray syndrome; IVIC syndrome	607323; 147750
Sc5d	Sterol-C5-Desaturase	J:175213	Lathosterolosis	607330
Scarb2	Scavenger Receptor Class B Member 2	12620969	Epilepsy, progressive myoclonic 4, with or without renal failure	254900
Sema3a	Semaphorin 3A	18249526	{Hypogonado-tropic hypogonadism 16 with or without anosmia}	614897
Sestd1	SEC14 And Spectrin Domain Containing 1	23696638	-	-
Shh	Sonic Hedgehog	12399320	Holopros-encephaly 3; Microphthalmia with coloboma 5; Schizencephaly; Single median maxillary central incisor	142945; 611638; 269160; 147250
Six1	SIX Homeobox 1	14695375	Branchiootic syndrome 3; Deafness, autosomal dominant 23	608389; 605192
Six2	SIX Homeobox 2	17036046	-	-

Slit2	Slit Guidance Ligand 2	15130495	-	-
Slit3	Slit Guidance Ligand 3	14550534	-	-
Sox4	SRY-Box 4	16109771	-	-
Sox9	SRY-Box 9	20881014	Acampomelic campomelic dysplasia; Campomelic dysplasia; Campomelic dysplasia with autosomal sex reversal	114290
Spry1	Sprouty RTK Signaling Antagonist 1	15691764	-	-
Sulf1	Sulfatase 1/ Sulfatase 2	17593974	-	-
Sulf2	Sulfatase 1/ Sulfatase 2	17593974	-	-
Tbc1d32	TBC1 Domain Family Member 32	J:175213	-	-
Tbx18	T-Box 18	24016759	Congenital anomalies of kidney and urinary tract 2	143400
Tbx6	T-Box 6	4073528	Spondylocostal dysostosis 5	122600
Tcf21	Transcription Factor 21	10572052	-	-
Tfcp2l1	Transcription Factor CP2-Like 1	17079272	-	-
Tgfb2	Transforming Growth Factor Beta 2	9217007	Loeys-Dietz syndrome 4	614816
Tns1	Tensin 1	23095816	-	-

Trp53	Transformation related protein 53	11780111	Adrenal cortical carcinoma; Breast cancer; Choroid plexus papilloma; Colorectal cancer; Hepatocellular carcinoma; Li-Fraumeni syndrome; Nasopharyngeal carcinoma; Osteosarcoma; Pancreatic cancer	202300; 114480; 260500; 114500; 114550; 151623; 607107; 259500; 260350
Trps1	Transcriptional Repressor GATA Binding 1	19820125	Trichorhino-phalangeal syndrome, type I; Trichorhino-phalangeal syndrome, type III	190350; 190351
Tshz3	Teashirt Zinc Finger Homeobox 3	18776146	-	-
Tyr	Tyrosinase	J:179802	-	-
Umod	Uromodulin	15611339	Medullary cystic kidney disease 2; Hyperuricemic nephropathy, familial juvenile 1; Glomerulocystic kidney disease with hyperuricemia and isosthenuria	603860; 162000; 609886
Upk3a	Uroplakin 3A	11085999	-	-
Wasl	WAS/WASL Interacting Protein Family Member 1	23555292	-	-
Wdpcp	WD Repeat Containing Planar Cell Polarity Effector	24302887	?Congenital heart defects, hamartomas of tongue, and polysyndactyly	217085
Wnt11	Wnt Family Member 11	12783789	-	-
Wnt4	Wnt Family Member 4	7990960	Mullerian aplasia and hyperandro-genism; ?SERKAL syndrome	158330; 611812
Wnt5a	Wnt Family Member 5A	J:175213	Robinow syndrome, autosomal dominant 1	180700

Wnt7b	Wnt Family Member 7B	19060336	-	-
Wnt9b	Wnt Family Member 9B	16054034	-	-
Wt1	Wilms Tumor 1	18040647	Denys-Drash syndrome; Frasier syndrome; Meacham syndrome; Mesothelioma, somatic; Nephrotic syndrome, type 4; Wilms tumor, type 1	194080; 136680; 608978; 156240; 256370; 194070
Xpl	X-linked polydactyly	7391545	Orofaciodigital syndrome I	311200
Yap1	Yes Associated Protein 1	23555292	Coloboma, ocular; Coloboma, ocular, with or without hearing impairment, cleft lip/palate, and/or mental retardation	120433
Zbtb14	Zinc Finger And BTB Domain Containing 14	J:175213	-	-

Supplementary Table 4. Information on identified novel variants per family.
Genes are sorted by corresponding gene category: Murine CAKUT gene (pink); novel CAKUT candidate gene (red); multiple candidate genes per family (green).

Family ID	Gene	Nucleotide change	Amino acid change	State	Evolutionary conservation[A]	PP2 SIFT MT	EVS[B]	gnomAD[B]	Biobase
A2220	***TNS1***	c.5102C>T	p.Thr1701Met	het	*D. melanogaster*	0.997 Del. /	0/30/4270	1/728/ 276128	Gene
	TNS1	c.4169G>A	p.Arg1390Gln	het	*D. rerio*	0.470 Del. /	/	0/5/ 246220	Gene
B1305	***MEGF8***	c.3952G>A	p.Val1318Met	het	*X. tropicalis*	0.002 Tol. D.C.	/	0/3/ 192132	Gene
	MEGF8	c.331A>G	p.Ile111Val	het	*C. intestinalis*[1]	0.265 Tol. D.C.	/	0/3/ 245650	Gene
B1397	***LAMA5***	c.6422G>A	p.Arg2141His	het	*D. melanogaster*[2]	0.707 Del. D.C.	/	0/22/ 263718	Gene
	LAMA5	c.1520G>A	p.Arg507Gln	het	*D. rerio*	0.371 Tol. D.C.	0/1/4291	1/24/ 270776	Gene
A3859	***FOXC1***	c.203_204 insGCCGCA	p.Gln72_Pro73dup	het	-	/	/	0/3/ 245502	Gene
B1402	***FOXC1***	c.926_940 del	p.Ser309_Ile313del	het	-	/	/	0/1/ 89244	Gene
A976	***CNTN6***	c.575G>A	p.Gly192Asp	het	*D. melanogaster*[3]	1.00 Del. D.C.	/	0/1/ 245228	Gene
	CNTN6	c.908G>A	p.Arg303Gln	het	*G. gallus*	0.985 Del. D.C.	1/82/4215	9/1583/ 274042	Gene

A899	***TOP1MT***	c.955G>C	p.Asp319His	hom	*C. intestinalis*	0.998 Del. D.C.	0/51/2152	0/361/ 277036	No
B22	***SCN8A***	c.5674C>T	p.Arg1892Cys	hom	*D. melanogaster*	0.999 Del. D.C.	/	0/1/ 246136	Gene
A1090	***CCDC170***	c.1033G>A	p.Glu345Lys	hom	*D. melanogaster*	0.453 Del. D.C.	0/63/4055	1/1474/ 276910	Gene
B259	***CAST***	c.1186C>A	p.Pro396Thr	hom	*X. tropicalis*	1.00 Del. D.C.	/	0/16/ 244640	Gene
B911	***ATAD3C***	c.830C>T	p.Ala277Val	hom	*D. melanogaster*	0.997 Del. D.C.	/	0/0/ 239626	No
F0088	***DLG1***	c.476C>A	p.Pro159Gln	het	*C. elegans*[4]	0.101 Del. D.C.	/	/	Gene
B1004	***ZNF12***	c.136T>A	p.Ser46Thr	hom	*X. tropicalis*	0.596 Del. P.M.	/	0/1/ 246182	Gene
A538	***ZNF423***	c.2278C>T	p.Arg760Cys	het	*D. melanogaster*	0.995 Del. D.C.	/	0/11/ 276836	Gene
A3948	***CLCNKA***	c.1939C>T	p.Leu647Phe	hom	*D. melanogaster*[5]	0.990 Del. D.C.	/	3/280/ 277204	Gene
A5069	***CLIP1***	c.2451+1 G>T	100% ESS	hom	-	/	0/1/4299	1/90/ 246224	Gene
B266	***FANK1***	c.71G>T	p.Ser24Ile	hom	*C. intestinalis*	0.998 Del. D.C.	/	0/1/ 246174	No
B374	***STEAP2***	c.969A>C	p.Leu323Phe	hom	*D. rerio*	0.980 Del. D.C.	/	/	No

B1282	***NAT10***	c.3057_3058 insA	p.Lys1021Thrfs*16 (Stop gain)	hom	-	/	/	/	Gene
B1288	***ALKBH4***	c.497T>G	p.Ile166Ser	hom	*D. melanogaster*	1.00 Del. D.C.	0/10/4260	0/297/ 267008	No
B1293	***MYH7B***	c.3189_3191 delGGA	p.Glu1063del	het	-	/	/	/	No
	MYH7B	c.5901_5903 delCAA	p.Asn1967del	het	-	/	/	1/131/ 276856	No
B1397	***DCAF4L2***	c.1139G>A	p.Gly380Glu	hom	*D. rerio*	1.00 Del. /	/	0/12/ 244148	No
B1399	***DNASE1***	c.619C>T	p.Arg207Cys	hom	*D. rerio*	1.00 Del. D.C.	0/7/4293	1/334/ 277042	Yes
B1407	***MAPK15***	c.406C>T	p.Arg136Trp	het	*S.cerevisiae*	1.00 Del. D.C.	/	0/13/ 233920	No
	MAPK15	c.637C>T	p.Arg213Trp	het	*M. musculus*	0.983 Del. D.C.	/	0/20/ 242992	No
B20	***ASB9***	c.374G>A	p.Gly125Glu	hom	*C. intestinalis*	0.985 Del. D.C.	/	1/27/39/ 177450	No
B1420	***SLC26A4***	c.1195T>C	p.Ser399Pro	het	*C. elegans*[6]	0.968 Tol. D.C.	/	0/33/ 245782	Yes
	SLC26A4	c.1198delT	p.Cys400Valfs*32	het	-	/	/	0/6/ 245792	Gene
A361	***PRELID2***	c.184G>A	p.Val62Met	hom	*X. tropicalis*	0.998 Del. D.C.	0/10/4290	0/250/ 274480	No
	FBN2	c.1592G>C	p.Gly531Ala	hom	*G. gallus*	0.352 Del. D.C.	0/21/4279	1/389/ 276800	Gene

A4671	***USP35***	c.103C>T	p.Arg35Cys	het	*X. tropicalis*	0.446 Del. D.C.	/	/	No
	USP35	c.1711G>T	p.Gly571*	het	-	/	/	0/10/ 246106	No
	TBX3	c.381G>C	p.Lys127Asn	het	*D. melanogaster*	0.989 Del. D.C.	/	/	Gene
A1808	***SEC14L3***	c.1025A>G	p.Tyr342Cys	hom	*D. melanogaster*	0.258 Del. D.C.	0/21/2182	0/145/ 276228	No
	CHTF18	c.2564G>A	p.Arg855Gln	hom	*D. melanogaster*[7]	0.909 Tol. D.C.	0/7/4112	0/57/ 268878	No
	OSM	c.479C>T	p.Ser160Leu	hom	*M. musculus*	0.954 Del. /	/	0/3/ 276654	No
B23	***ZNF343***	c.1666C>T	p.Arg556*	hom	-	/	0/0/4300	0/19/ 276792	No
	PPP2R1B	c.1510C>T	p.Arg504Cys	hom	*S.cerevisiae*	0.009 Tol. D.C.	/	0/35/ 277012	Gene
A391	***BIN1***	c.622G>A	p.Glu208Lys	hom	*D. melanogaster*	0.900 Del. D.C.	/	/	Gene
	UGGT1	c.2752G>A	p.Glu918Lys	hom	*S.cerevisiae*[8]	0.638 Tol. D.C.	0/9/4291	0/131/ 277122	No
	PHKA2	c.1832C>T	p.Ser611Leu	hom	*D. melanogaster*[9]	0.975 Del. D.C.	/	/	Gene

A1323	***CAP1***	c.1369T>G	p.Phe457Val	het	*C. elegans*	0.808 Del. D.C.	/	/	No
	RAPGEF4	c.2053C>T	p.Arg685Trp	het	*D. melanogaster*	0.985 Del. D.C.	0/1/6235	0/20/ 277134	Gene
	CALR	c.904G>A	p.Asp302Asn	het	*D. melanogaster*	0.742 Del. D.C.	0/1/4299	0/10/ 246270	Gene
A1886	***TUT1***	c.11del	Pro4Leufs*79	hom	-	/	28.02.61	2/583/ 208896	Gene
	MAST2	c.1285C>T	Arg429Cys	hom	*S.cerevisiae*	0.998 Del. /	/	0/1/ 246156	Gene
A3954	***ADORA3***	c.422G>A	p.Trp141*	hom	-	/	/	/	Gene
	DUSP23	c.53G>A	p.Gly18Glu	hom	*D. rerio*	0.992 Del. D.C.	/	0/43/ 166174	Gene
A4666	***RPGR***	c.1099C>G	p.Pro367Ala	hemi	*D. rerio*	0.994 Del. D.C.	/	0/6/15/ 177312	Gene
	STARD8	c.918A>C	p.Lys306Asn	hemi	*C. intestinalis*	0.347 Del. D.C.	0/5/2/4055	0/71/210/ 196549	No
A4962	***RGS3***	c.2854C>T	p.Arg952*	het	-	/	/	0/3/ 241844	No
	VAMP8	c.38_39 dupTG	p.Arg14Cysfs*15	het	-	/	/	/	No
A5071	***AGAP3***	c.1396C>T	p.Arg466Cys	het	*D. rerio*	0.993 Del. D.C.	/	0/10/ 269988	No
	CASKIN2	c.79A>G	p.Lys27Glu	het	*D. rerio*[10]	0.994 Del. D.C.	/	0/1/ 245878	No

B255	***DVL3***	c.758A>G	p.Asn253Ser	het	*D. melanogaster*	0.928 Del. D.C.	/	0/24/ 277158	Gene
	CASKIN1	c.745G>A	p.Val249Met	het	*D. rerio*[11]	0.947 Del. D.C.	/	0/9/ 246038	No
B1160	***TM4SF19***	c.619_630 del	p.Ala206_Asp209del	hom	-	/	/	/	Gene
	ZNF607	c.1741C>T	Arg581*	hom	-	/	/	0/9/ 246072	Gene
	ADH1B	c.937C>T	Arg313Cys	hom	*D. rerio*	1.00 Del. D.C.	/	0/24/ 277046	Gene
	N4BP2	c.1324C>A	Leu442Ile	hom	*D. rerio*	1.00 Del. /	/	/	Gene
	IFRD1	c.1211C>T	Pro404Leu	hom	*D. rerio*	0.631 Del. D.C.	/	0/24/ 277122	Gene
B1292	***HIPK3***	c.852C>G	p.Asp284Glu	hom	*C. elegans*	0.799 Del. D.C.	/	0/8/ 276890	No
	CXorf36	c.196A>C	p.Thr66Pro	hemi	*C. intestinalis*	0.998 Del. D.C.	/	0/2/2/ 178627	No
B1413	***HID1***	c.1576C>T	p.His526Tyr	het	*D. melanogaster*[12]	0.734 Del. D.C.	/	0/2/ 246176	Gene
	HID1	c.346G>A	p.Gly116Ser	het	*C. intestinalis*	0.199 Del. D.C.	/	0/1/ 244236	Gene
	RBMXL3	c.2288G>A	p.Gly763Asp	hemi	*M. musculus*	0.992 Del. P.M.	0/6/3/794	0/59/154/ 131258	No

B1461	***ENOX2***	c.1108C>T	p.Arg370Cys	hemi	*D. melanogaster*	0.980 Del. D.C.	/	0/1/1/ 162899	No
	RRH	c.730delA	p.Ile244Serfs*2	hom	-	/	/	1/66/ 277200	Gene
B1494	***MED26***	c.1184_1186 delAGA	p.Lys395del	hom	-	/	/	0/71/ 276922	No
	LAMC1	c.3051A>C	p.Glu1017Asp	het	*D. melanogaster*	0.998 Del. D.C.	/	/	Gene
B1140	***DNAH6***	c.6643C>T	p.Pro2215Ser	het	*D. melanogaster*	0.996 Tol. D.C.	/	0/7/ 182512	Gene
	DNAH6	c.11669G>A	p.Arg3890His	het	*D. rerio*	0.978 Tol. D.C.	/	0/61/ 181994	Gene
B1524	***SHROOM2***	c.3521C>T	p.Pro1174Leu	hom	*D. melanogaster*	0.514 Del. /	/	/	No
B1649	***AADACL3***	c.583G>A	p.Gly195Arg	hom	*C. elegans*	1.00 Del. P.M.	0/1/4146	0/15/ 277012	No
	PLCE1	c.4000G>A	p.Val1334Met	hom	*D. rerio*	0.962 Tol. D.C.	/	0/10/ 277006	Gene
B1651	***ARHGEF10***	c.1439C>T	p.Ser480Leu	hom	*C. intestinalis*	0.972 Tol. /	/	0/11/ 276612	Gene
	RDH13	c.302T>G	p.Leu101Arg	hom	*S.cerevisiae*[13]	0.787 Del. P.M.	0/4/4172	0/88/ 277184	No

D.C., Disease Causing; **Del.**, Deleterious; **del**; deletion, **delins**, deletion insertion; **dup**., duplication; **ESS**, essential splice site; **EVS**, Exome Variant Server; **fs**, frameshift; **gnomAD**, Genome Aggregation Database; **hemi**, hemizygous; **het**, heterozygous; **hom**, homozygous; **ins**,insertion; **MT**, Mutation Taster; **PP2**, PolyPhen 2; **P.M.**, Polymorphism; **SIFT**, Sorting intolerant from tolerant, **Tol.**, Tolerated.
* frameshift, stop loss, stop gain or nonsense; double underline denotes finding in more than 2 categories; /, denotes data not available.
[A]Evolutionary conservation was assessed across phylogeny over 8 species: *M. muscularis, Mus musculus; G. gallus, Gallus gallus; X. tropicalis, Xenopus tropicalis; D. rerio, Danio rerio; C. elegans, Caenorhabditis elegans; C. intestinalis, Ciona intestinalis; D. melanogaster, Drosphilia melanogaster; S. cerevisiae, Saccharomyces cerevisiae.* If conservation is interrupted in one species but otherwise preserved across phylogeny a numerical reference is provided as outlined below. [1]Leucine present *D. rerio*, [2]Glutamine present *G. gallus, D. rerio* and *C. intestinalis*, [3]Lysine present *C. elegans*, [4]Asparagine present *C. intestinalis*, [5]Isoeucine present *D. rerio*, [6]Glutamine present *C. intestinalis*, [7]Leucine present *C. elegans*, [8]Alanine present *D. melanogaster*, [9]Threonine present *C. elegans*, [10]Arginine present *X. tropicalis*, [11]Isoleucine present *X. tropicalis*, [12]Proline present *C. elegans*, [13]Phenylalanine present *C. elegans*.
[B]Variant frequencies listed for homozygous/ hemizygous (if applicable)/ heterozygous/ total alleles detected in the population.
[C]Biobase HGMD Human Genome Mutation Database, (https://portal.biobaseinternational.com/hgmd). If the gene but not the exact variant has been reported for the corresponding phenotype "**Gene**" is indicated in this column.

Supplementary Table 5A. Sidedness of total CAKUT pathologies in the 273 of the 319 affected individuals with either unilateral (130/319) or bilateral (143/319) CAKUT that were submitted for WES analysis.

Specific CAKUT phenotypic pathologies (Cranial to caudal)	Distribution of pathologies left versus right in the total cohort* n (%)			Distribution of pathologies left versus right in the solved cohort* n (%)		
	Total	**Left**	**Right**	**Total**	**Left**	**Right**
Renal Agenesis	**39 (9%)**	25 (6%)	14 (3%)	**5 (8%)**	4 (6%)	1 (1.5%)
RHD	**96 (23%)**	48 (12%)	48 (11%)	**18 (28%)**	10 (15%)	8 (12%)
MCDK	**30 (7%)**	14 (3%)	16 (4%)	**10 (15%)**	3 (5%)	7 (11%)
Hydronephrosis	**27 (7%)**	15 (4%)	12 (3%)	**4 (6%)**	3 (5%)	1 (1.5%)
UPJO	**27 (7%)**	18 (4%)	9 (2%)	**3 (5%)**	2 (3%)	1 (1.5%)
Hydroureter	**3 (<1%)**	1 (<1%)	2 (<1%)	**0**	0	0
VUR	**125 (30%)**	68 (16%)	57 (14%)	**21 (32%)**	9 (14%)	12 (18%)
Ectopic/ Horseshoe	**23 (6%)**	15 (4%)	8 (2%)	**1 (1.5%)**	1 (1.5%)	0
Duplex system	**27 (7%)**	15 (4%)	12 (3%)	**2 (3%)**	1 (1.5%)	1 (1.5%)
UVJO	**10 (2%)**	4 (1%)	6 (1%)	**1 (1.5%)**	0	1 (1.5%)
Cryptorchidism	**9 (2%)**	4 (1%)	5 (1%)	**0**	0	0
Total	**416 (100%)**	227 (55%)	189 (45%)	**65 (100%)**	33 (51%)	32(49%)

A total of 416 pathologies were present in 273 individuals with unilateral and bilateral CAKUT. Total number of pathologies were calculated independent of laterality and, if present, in combination with other pathologies in the same individual. Solved cohort indicated individuals in whom we detected a mutation in a known or syndromic CAKUT gene or a mutation in a gene known to phenocopy CAKUT (**Figure 1A-B**).

Supplementary Table 5B. Sidedness and distribution of CAKUT pathologies in affected individuals with either unilateral (130/319) or bilateral (143/319) CAKUT that were submitted for WES analysis

Specific CAKUT phenotypic pathologies (Cranial to caudal)	Distribution of pathologies in patients with unilateral renal pathology n (%)			Distribution of pathologies in patients with bilateral renal pathologies** n (%)		
	Total	**Left**	**Right**	**Total**	**Left**	**Right**
Renal Agenesis	**28 (22%)**	19 (15%)	9 (7%)	**11 (4%)**	6 (2%)	5 (2%)
RHD	**23 (18%)**	12 (9%)	11 (8%)	**73 (25%)**	36 (13%)	37 (13%)
MCDK	**6 (5%)**	3 (2%)	3 (2%)	**24 (8%)**	11 (4%)	13 (4%)
Hydronephrosis	**6 (5%)**	6 (5%)	0	**21 (7%)**	9 (3%)	12 (4%)
UPJO	**21 (16%)**	15 (12%)	6 (5%)	**6 (2%)**	3 (1%)	3 (1%)
Hydroureter	**0**	0	0	**3 (1%)**	1 (<1%)	2 (1%)
VUR	**21 (16%)**	14 (11%)	7 (5%)	**104 (37%)**	54 (19%)	50 (18%)
Ectopic/ Horseshoe	**7 (5%)**	5 (4%)	2 (2%)	**16 (6%)**	10 (3%)	6 (2%)
Duplex system	**13 (10%)**	7 (5%)	6 (5%)	**14 (5%)**	8 (3%)	6 (2%)
UVJO	**3 (2%)**	1 (<1%)	2 (1%)	**7 (2%)**	3 (1%)	4 (1%)
Cryptorchidism	**2 (1%)**	2 (1%)	0	**7 (2%)**	2(1%)	5 (2%)
Total	**130 (100%)**	84 (65%)	46 (35%)	**286 (100%)**	143 (50%)	143 (50%)

130 pathologies were present in 130 individuals with unilateral CAKUT. In the 143 individuals with bilateral CAKUT, a total of 286 pathologies were present. The bilateral renal pathologies consist of individuals with both bilateral concordant (111/319) and bilateral discordant (32/319) CAKUT. The total number of pathologies were calculated independent of both laterality and whether the bilateral pathologies were concordant or discordant in the same individual.

*Excluding individuals with PUV and epi/ hypospadias as a CAKUT pathology (25/319) and individuals in whom the CAKUT phenotype was undefined (21/319). RHD, renal hypo/dysplasia; MCDK, Multicystic dysplastic kidney; UPJO, ureteropelvic junction obstruction; VUR, vesicoureteric reflux; Ectopic/ Horseshoe kidney; UVJO, ureteropelvic junction obstruction.

Supplementary Table 6. Description of the 15 mutations in genes known to cause *syndromic* CAKUT phenotype identified in 15 families with *isolated* CAKUT.

The exact mutations identified in our patient cohort are listed along with phenotype described in our cohort (column 2 & 3). OMIM number and reported syndromic phenotype for each gene is listed (column 4 & 5). The number and type of previously reported disease causing variant reported in Biobase (i.e. missense versus null variant) for each syndromic gene is described (column 6-12). Data obtained from HGMD® Professional 2017.2 (https://portal.biobase-international.com).

Family ID	Hypomorphic mutation in *syndromic* CAKUT genes identified in a family with *isolated* CAKUT (amino acid change and position)	Phenotype described in our patient cohort	Extra-renal manifestations present in our patient cohort	Syndrome OMIM #	Total # of syndromic variants classified as disease causing in biobase	% of total variants reported that are null variants	# hypomorphic variants reported	# null variants reported			
							Missense	Non-sense	Deletions insertions	Complex rearrang ement	Splice site
B1307	***AMER1* p.Gly62Val**	Right MCDK	No	Osteopathia striata with cranial sclerosis # 300373	30	97%	1	9	20	0	0
A5063	***EP300* p.Thr594Met**	Bilateral MCDK	No	Rubinstein-Taybi syndrome # 180849	74	92%	6	17	45	0	6
B17	***KMT2D* p.Gly4397Val**	Left renal agenesis	No	Kabuki syndrome # 147920	549	86%	78	171	248	1	51
A870	***NOTCH2* p.Tyr1186Asn**	Bilateral VUR reflux	Yes (Nevus pigmentosus & right supranummery nipple)	Alagille syndrome# 118450 Hajdu-Cheney syndrome # 102500 Serpentine fibula-polycystic kidney	48	81%	9	17	20	1	1
A3401	***NOTCH2* p.Arg2256His**	Right RHD & left UVJO	No								
A3957	***NOTCH2* p.Arg2298Trp**	Bilateral hydronephrosis	Yes (Anorectal malformation)								

B1652	***FLNA* p.His2116Gln**	Prune Belly Syndrome Neurogenic bladder BL VUR	No	Periventricular nodular heterotopia 1 #300049	219	65%	77	28	81	4	29
A3095	***NSDHL* p.Arg281His**	Left renal agenesis	Yes (Prune Belly Syndrome)	CHILD syndrome # 308050	28	64%	10	7	9	0	2
A387	***KAT6B* p.Arg718Trp**	Left renal agenesis & right hydro-nephrosis	No	Genitopatellar syndrome # 606170	48	60%	1	9	20	0	0
B1440	***HOXA13* p.Pro9Ser**	PUV with bilateral hydro-nephrosis	Yes (Facial Dysmorphism)	Hand-foot-genital syndrome # 140000	36	50%	18	4	8	6	0
B120	***OFD1* c.517+1G>A 100% ESS**	Right MCDK, left complete duplex and left grade 3 VUR with ureterocoele	No	Oral-facial-digital syndrome 1 # 311200	151	49%	18	20	37	0	17
B1398	***OFD1* c.936-2A>G 100% ESS**	Bilateral VUR with right hydronephrosis									
A3960	***FGFR1* p.Val476Trp**	Left renal agenesis, right VUR, hypospadias with bladder extrophy	No	Kallmann syndrome # 308700	193	35%	125	19	34	1	14
B258	***TP63* p.Val267Ile**	Left UPJO	No	EEC syndrome % 129900	110	22%	86	4	16	0	4
A2904	***FGFR3* p.Val555Met**	Right renal hypo dysplasia	No	Thanatophoric dysplasia # 187600	66	9%	59	3	0	0	3

MCDK; Multicystic dysplasic kidney, **VUR**; vesicoureteric reflux, **PUV**; Posterior urethral value, **UPJO**; ureteropelvic junction obstruction, **UVJO**; ureterovesical junction obstruction, **#**; number; OMIM, Online Mendelian Inheritance in Man, https://www.omim.org.

Supplementary Table 7 Information and potential implications on identified variants in families in whom a variant in a non-CAKUT gene was identified (brown).

Family ID	Gene	Clinical Disease	Potential clinical implications	Mode of transmission	Nucleotide change	Amino acid change	State	Evolutionary conservation[A]	PP2 SIFT MT	CADD SCORE	EVS[B]	gnomAD[B]	ACMG[C]	HGMD[D]	Phenotype	Segregation
A4451	*GPR98*	Usher syndrome Deafness & blindness	Screening for hearing loss and visual impairment	compound heterozygous	c.3509A>G	p.Tyr1170 Cys	het	*X. tropicalis*	0.887 Del. D.C.	18	0/13/4129	0/195/276984	Likely pathogenic	Gene	BL VUR	Yes (unaffected parents heterozgous carriers of each variant, recessive state confirmed in affected individual)
	GPR98				c.17657C>A	p.Ala5886 Asp	het	*D. rerio*	0.974 Del. D.C.	30	0/2/4148	0/115/274630	Likely pathogenic	Gene		
A4672	*SLC3A1*	Cystinuria	Clinical diagnosis confirmed post molecular diagnosis	compound heterozygous	c.647C>A	p.Thr216 Lys	het	*C. intestinalis*	0.947 Tol. D.C.	23	/	/	Pathogenic	DM	R RHD Cystinuria	Yes (unaffected parents heterozgous carriers of each variant, recessive state confirmed in affected individual)
	SLC3A1				c.1400T>C	p.Met467 Thr	het	*D. melanogaster*[1]	0.289 Tol. D.C.	20	0/23/4277	4/669/276866	Pathogenic	DM		
A1070	*ATP7A*	Occipital Horn Syndrome	Screening for occipital horn syndrome	recessive	c.1906C>T	p.Arg636 Trp	hom	*D. rerio*	0.960 Del. D.C.	21	/	/	Likely pathogenic	Gene	R pelvic kidney Renal calculi	Homozygous variant confirmed affected individual, parental DNA NA
A2532	*TSC2*	Tuberous sclerosis	Screening for tuberous sclerosis	dominant	c.2948C>G	p.Ser983 Cys	het	*D. rerio*	0.996 Del. D.C.	25	/	0/7/245804	Likely pathogenic	Gene	BL VUR	Yes (segregates in 2 affected siblings)
B379	*NF1*	Neurofibromatosis 1	Confirm clinical diagnosis of neurofibromatosis	dominant	c.1A>G	p.Met1?	het	-	/	20	/	/	Pathogenic	DM	R RA Café-au lait spots	Yes (inherited from affected father)
A3826	*PYGL*	Glycogen storage disease 6	Screening for hypoglycaemia and institute nutritional support	recessive	c.1762T>G	p.Tyr588 Asp	hom	*S.cerevisiae*	0.998 Del. D.C.	27	/	/	Likely pathogenic	Gene	R VUR, BL hydronephrosis	Homozygous variant confirmed in affected individual, parental DNA NA
A5069	*GARS*	Peripheral neuropathy Charcot-Marie-Tooth disease 2d	Screening for signs of peripheral neuropahty	recessive	c.1754T>C	p.Met585 Thr	hom	*D. melanogaster*[2]	0.385 Del. D.C.	23	0/1/4110	0/6/246240	Likely pathogenic	Gene	Bilateral obstructive megaureter Microcephaly Intellectual disability	Yes (unaffected mother heterozgous carriers of the variant, recessive state confirmed in affected individual, paternal DNA NA)
B255	*RYR1*	Rhabdomyolysis Malignant hyperthermia Central core disease of muscle	Screening to enable monitoring and prophylaxis in the pre-operative setting	dominant	c.10493G>A	p.Arg3498 His	het	*D. rerio*	0.791 Del. D.C.	12	/	0/10/276944	Likely pathogenic	Gene	Left UPJO	Variant inherited from father (affection status unknown)
	RYR1			dominant	c.11407A>T	p.Ile3803 Phe	het	*D. rerio*	0.723 Del. D.C.	13	/	/	Likely pathogenic	Gene		Variant inherited from father (affection status unknown)
B1160	*NUP155*	Atrial fibrillation	Screening and prophylaxis	recessive	c.1052C>G	p.Ser351 Cys	hom	*C. elegans*	0.771 Del. D.C.	29	/	/	Uncertain significance	Gene	R VUR Suprasellar epidermoid cyst	Homozygous variant confirmed in affected individual, parental DNA NA

B1253	***HMGCS2***	Mitochondrial HMG-CoA synthase deficiency	Risk hypoglycaemia upon fasting or infection	recessive	c.634G>A	p.Gly212 Arg	hom	*S.cerevisiae*	1.000 Del. D.C.	38	0/3/4297	1/69/276862	Pathogenic	Gene	BL RHD, hydronephrosis, VUR Absent cranial bone segement Facial malformation	Yes (unaffected parents heterozgous carriers of each variant, recessive state confirmed in affected individual)
	PVRL1	Cleft lip / palate	Genetic counselling on future risk for offspring	recessive	c.677G>T	p.Cys226 Phe	hom	*C. intestinalis*	1.000 Del. D.C.	24	/	/	Likely pathogenic	Gene		
B1398	***BLM***	Breast cancer Bloom syndrome	Screening due to cancer risk	recessive	c. 3416G>C	p.Arg1139 Pro	hom	*C. intestinalis*[3]	0.627 Del.	22	/	0/8/246156	Likely pathogenic	?DM DM	R hydronephrosis BL VUR	Yes (unaffected parents heterozgous carriers of each variant, recessive state confirmed in affected individual)
B1400	***DCHS1***	Mitral valve prolapse	Screening for MVP	dominant	c.7538G>A	p.Arg2513 His	het	*C. intestinalis*[4]	0.973 Del. D.C.	22	/	0/108/270958	Likely pathogenic	DM	BL hydronephrosis, UVJO, R UPJO	Variant inherited from father (affection status unknown)
	SLC5A2	Renal glucosuria	Screening for asymptomatic glucouria	dominant	c.1405G>A	p.Ala469 Thr	het	*C. intestinalis*	0.774 Del. D.C.	22	0/1/4298	0/14/266980	Likely pathogenic	DM		Variant inherited from father (affection status unknown)
B1401	***KCNH2***	Long QT syndrome	Screening due to risk of sudden cardiac death	dominant	c.526C>T	p.Arg176 Trp	het	*M. musculus*	0.973 Del. P.M.	11	/	0/44/107102	Pathogenic	DM	L UVJO	Variant inherited from mother (affection status unknown)
B1441	***PYCR1***	PYCR1 deficiency	/	recessive	c.572G>A	p.Gly191 Glu	hom	*D. melanogaster*	1.00 Del. D.C.	24	/	/	Likely pathogenic	DM	PUV BL VUR & hydronephrosis	Yes (unaffected parents heterozgous carriers the the variant, recessive state confirmed in affected individual)
B1494	***CFHR1***	Susceptibility to atypical haemolytic uraemic syndrome	Monitor for risk of kidney injury due to aHUS	recessive	c.988_991delAGAT	p.Arg330*	hom	-	/	/	/	5/22/259752	Likely pathogenic	Gene	R RA	Yes (unaffected parents heterozgous carriers the the variant, recessive state confirmed in affected individual)
B1524	***OTOF***	Deafness	Screen for deafness to allow for early intervention	recessive	c.5729A>G	p.Glu1910 Gly	het	*D. rerio*	0.998 Tol. D.C.	21	/	/	Likely pathogenic	Gene	PUV	Yes (unaffected parents heterozgous carriers of the variant, recessive state confirmed in affected individual)
	OTOF				c.5711C>T	p.Thr1904 Met	het	*D. rerio*	0.999 Del. D.C.	28	/	0/29/246182	Likely pathogenic	Gene		
	GAA	Glycogen storage disease 2/ Pompe disease	Facilitate early diagnosis and treatment	recessive	c.858+2T>A	100% ESS	het	-	/	NA	/	/	Pathogenic	DM		Yes (unaffected parents heterozgous carriers of the variant, recessive state confirmed in affected individual)
	GAA				c.1828G>A	p.Ala610 Thr	het	*S.cerevisiae*[5]	0.532 Del. P.M.	18	0/1/4275	0/7/240556	Pathogenic	DM		

A3572	***SLC2A9***	Renal hypouricaemia, type 2	Faciliate clinical diagnosis following screening for low uric acid followed by confirmation with Feuric>10%	dominant	c.1343C>T	p.Pro448 Leu	het	*D. melanogaster*[6]	0.997 Tol. D.C.	17	0/0/4300	0/17/277006	Pathogenic	DM	PUV	Variant inherited from mother (affection status unknown)
B1652	***ETFB***	Glutaricacidaemia 2b	Screening for inborn error of metabolism-defects	recessive	c.979G>A	p.Gly327 Ser	hom	*S.cerevisiae*	0.999 Del. D.C.	17	0/0/4300	0/13/246228	Likely pathogenic	Gene	Prune Belly Syndrome Neurogenic bladder with VUR	Yes (unaffected parents heterozgous carriers of the variant, recessive state confirmed in affected individual)

BL, bilateral; **CADD**, Combined Annotation Dependent Depletion; **D.C.**, Disease Causing; **Del.**, Deleterious; **del**; deletion; **EVS**, Exome Variant Server; **Feuric**, fractional excretion of uric acid; **gnomAD**, Genome Aggregation Database; **hemi**, hemizygous; **het**, heterozygous; **hom**, homozygous; **L**, left; MT, Mutation Taster; **NA**, not available; **PP2**, PolyPhen 2; **P.M.**, Polymorphism; **PUV**, posterior urethral valve; **R**, right; **RA,** renal agenesis; **RHD**, renal/hypodysplasia; **SIFT**, Sorting intolerant from tolerant, **Tol.**, Tolerated; **UPJO**, uretero-pelvic junction obstruction; **UVJO**, uretero-vesical junction obstruction; **VUR**, vesicoureteric reflux.

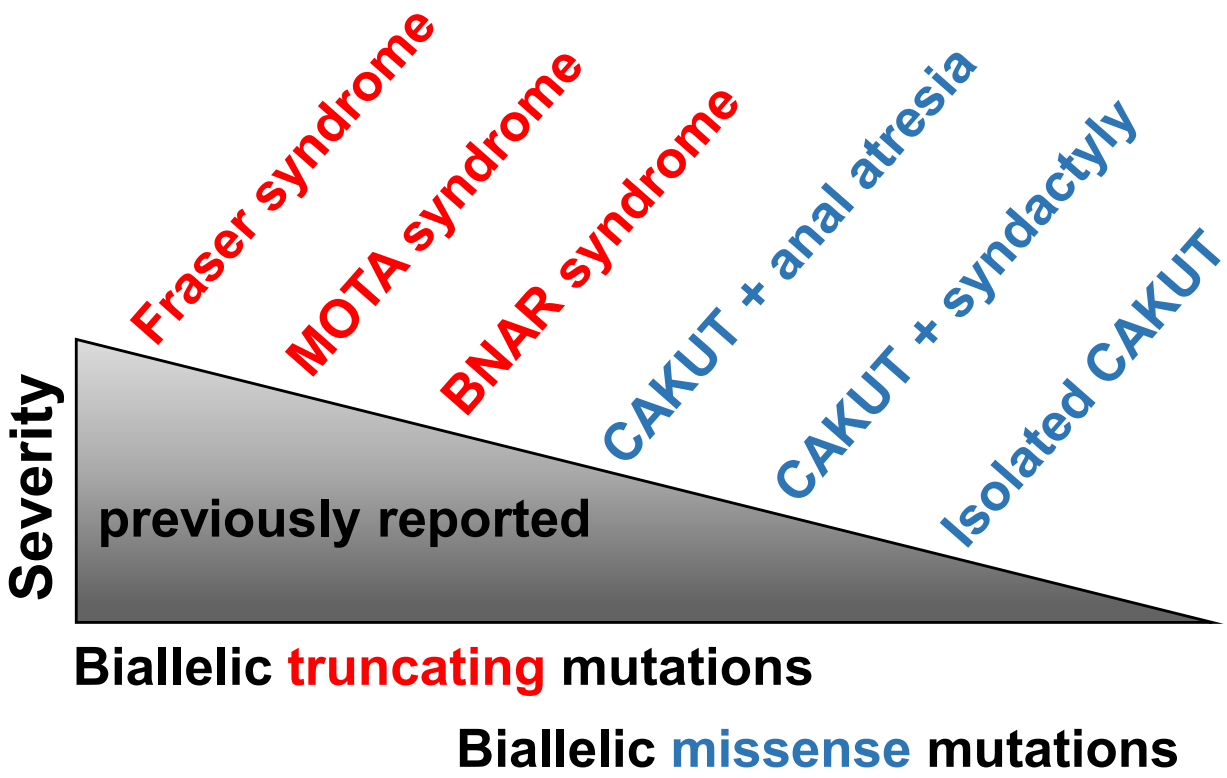
* frameshift, stop loss, stop gain or nonsense; double underline denotes finding in more than 2 categories.

[A] Evolutionary conservation was assessed across phylogeny over 8 species: *M. muscularis, Mus musculus; G. gallus , Gallus gallus; X. tropicalis, Xenopus tropicalis; D. rerio, Danio rerio; C. elegans, Caenorhabditis elegans; C. intestinalis, Ciona intestinalis; D. melanogaster, Drosphilia melanogaster; S. cerevisiae, Saccharomyces cerevisiae.* If conservation is interrupted in one species but otherwise preserved across phylogeny a numerical reference is provided as outlined below. [1]Phenylalanine present *C. intestinalis* & *C. elegans*, [2]Leucine present *C. intestinalis*, [3]Lysine present *D. rerio*, [4]Glutamine present *X. tropicalis*, [5]Serine present *D. rerio* & glycine present *C. intestinalis*, [6]Glycine present *C. intestinalis.*

[B] Variant frequencies listed for homozygous/ hemizygous (if applicable)/ heterozygous/ total alleles detected in the population.

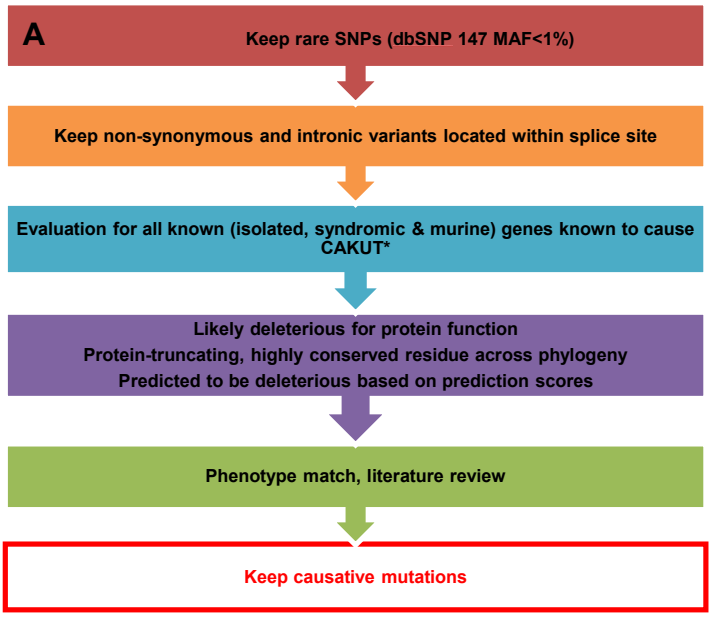
[C] ACMG, American College of Human Genetics Standards and Guidelines Classification as pathogenic, likely pathogenic or uncertain significance (Richards *Genet Med* 17(5):405, 2015)

[D] HGMD Human Gene Mutation Database, https://portal.biobaseinternational.com/hgmd. If the exact variant has previously been reported and classified as a pathogenic mutation to be disease causing, variant denoted as "**DM**". Variant denoted as "**?DM**" if the variant is a likely pathogenic mutation to be disease causing but where the author indicated some degree of doubt or subsequent evidence calls the deleterious nature of the variant into question. If the gene but not the exact variant has been reported for the corresponding phenotype "**Gene**" is indicated in this column.

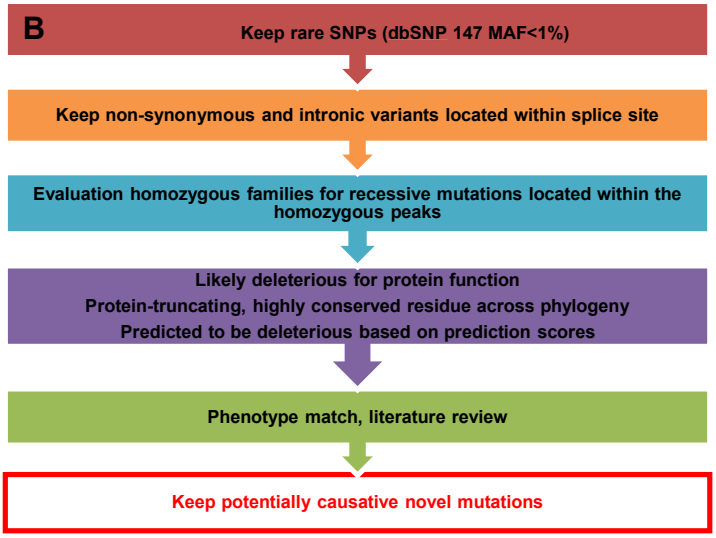


Supplementary Figure 1. Allelism determining syndromic versus isolated CAKUT in the example of genes encoding the Fraser-complex.
Biallelic recessive truncating mutations in genes encoding members of the Fraser-Complex (***FRAS1, FREM1, FREM2)*** and the associated protein ***GRIP1*** are known monogenic causes of the severe, syndromic CAKUT phenotypes Fraser syndrome (***FRAS1, FREM2, GRIP1***) and MOTA/BNAR syndrome (Manitoba-oculo-tricho-anal/ bifid nose with or without anorectal and renal anomalies; ***FREM1***) (Kohl, *JASN*, 25:1917, 2014).

Studies by Kohl et al. revealed, that missense (rather than truncating) mutations in the same Fraser-complex encoding genes may cause isolated CAKUT in a significant proportion (13/590; ~2.2%) of individuals with isolated CAKUT. This indicates that in the case of the ***FRAS1***, ***FREM2***, ***GRIP1*** and ***FREM1*** genes, a mechanism of “allelism” determines the occurrence of syndromic vs. isolated CAKUT, i.e. truncating vs. missense mutations determine whether a syndromic or isolated CAKUT phenotype results.

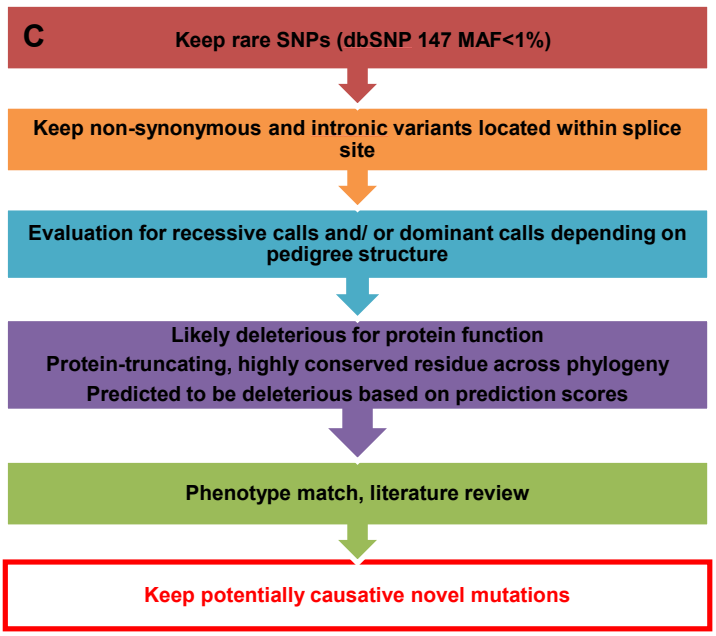


Supplementary Figure 2A. Variant filtering process for the identification of causative mutation <u>in genes known to cause either isolated or syndromic or murine CAKUT</u>.
Schematic overview of the workflow used for filtering of WES data: **i)** Keep rare variants present with a minor allele frequency (MAF) <1% in healthy control cohorts (dbSNP147), **ii)** Keep non-synonymous variants and intronic variants that are located within splice sites, **iii)** Applying known gene approach by selecting all variants detected in known CAKUT genes**, iv)** Ranking of remaining variants based on their predicted likelihood to be deleterious for the function of the encoded protein, **v)** Reviewing literature and delineating whether the detected mutation matches the phenotype. *Depending on the pedigree structure, a recessive or dominant hypothesis was determined for the whole exome sequence evaluation. However, due to incomplete penetrance in families with CAKUT, individuals may have to analyzed under both hypotheses.



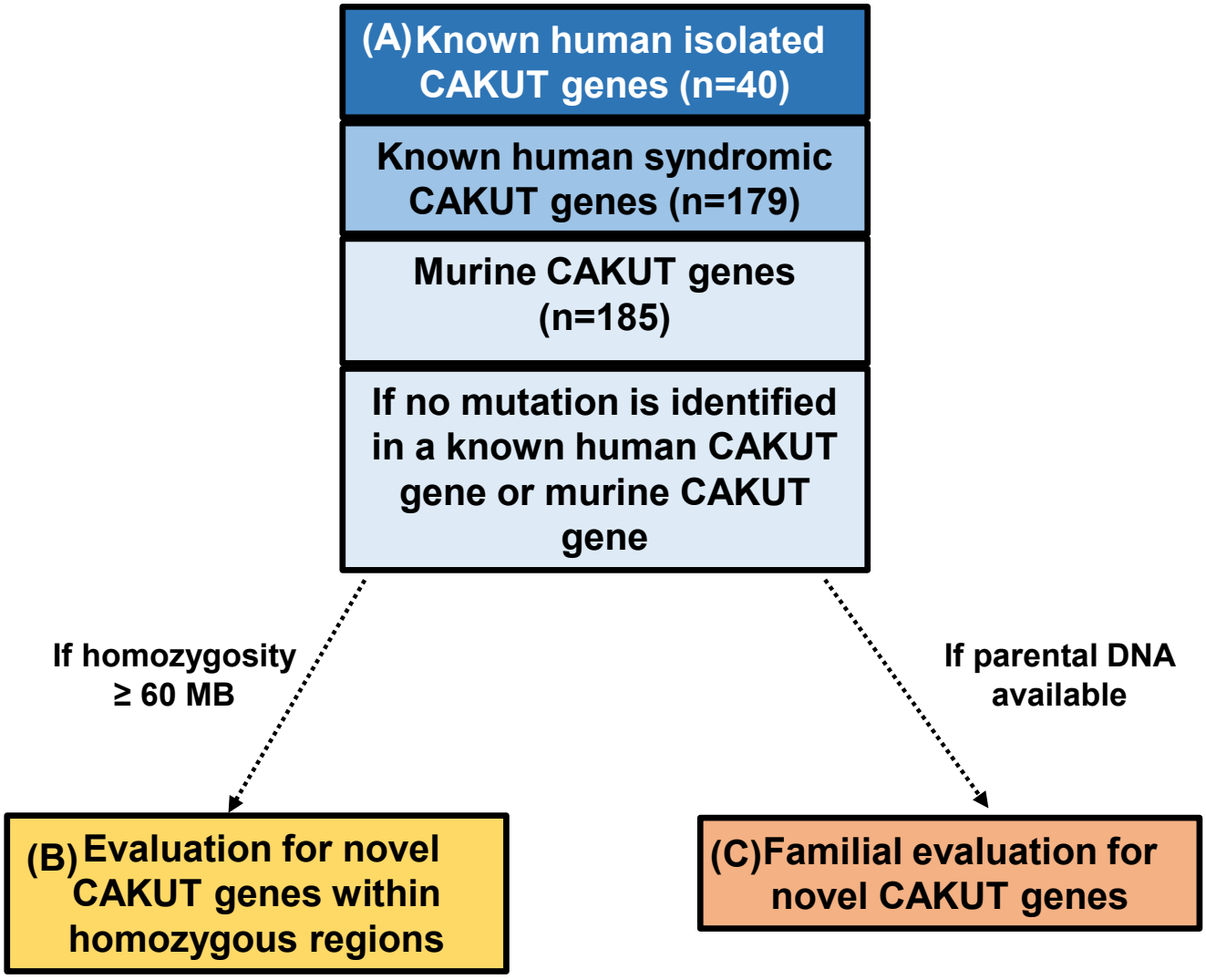
Supplementary Figure 2B. Variant filtering process for the identification of <u>novel mutations in homozygous families with CAKUT.</u>

Schematic overview of the workflow that was used for filtering of WES data: **i)** Keep rare variants present with a minor allele frequency (MAF) <1% in healthy control cohorts (dbSNP147), **ii)** Keep non-synonymous variants and intronic variants that are located within splice sites, **iii)** Applying recessive hypothesis by selecting all variants detected within homozygous peaks**, iv)** Ranking of remaining variants based on their predicted likelihood to be deleterious for the function of the encoded protein, **v)** Reviewing literature and delineating whether the detected mutation matches the phenotype



Supplementary Figure 2C. Variant filtering process for the identification of <u>novel mutations in CAKUT families when parental DNA is available.</u>

Schematic overview of the workflow that was used for filtering of WES data in all families: **i)** Keep rare variants present with a minor allele frequency (MAF) <1% in healthy control cohorts (dbSNP147), **ii)** Keep non-synonymous variants and intronic variants that are located within splice sites, **iii)** Applying recessive hypothesis by selecting homozygous and compound heterozygous variants detected**, iv)** Ranking of remaining variants based on their predicted likelihood to be deleterious for the function of the encoded protein. **v)** Reviewing literature and delineating whether the detected mutation matches the phenotype



Supplementary Figure 3. Evaluation of whole exome sequencing (WES) data in 232 families with CAKUT.

(A) Whole exome sequencing (WES) output of affected individuals was interrogated for causative mutations in 40 known CAKUT genes (**Suppl. Table 1**) in all 232 families with CAKUT. Subsequently, families were evaluated for variants in 179 genes known to cause syndromic CAKUT in humans (**Suppl. Table 2**) and then for 185 murine CAKUT genes (**Suppl. Table 3**) for the presence of potentially disease causing variants.

Evaluation for the presence of mutations in potential novel CAKUT genes was continued depending on additional criteria:

(B) If homozygosity mapping revealed homozygosity ≥ 60 Mbp, an evaluation of all homozygous variants across the exome was performed with special emphasis on homozygous peak regions (n=37);

(C) If parental DNA was available from the proband and either of the parents for WES a familial evaluation was performed (n=65).

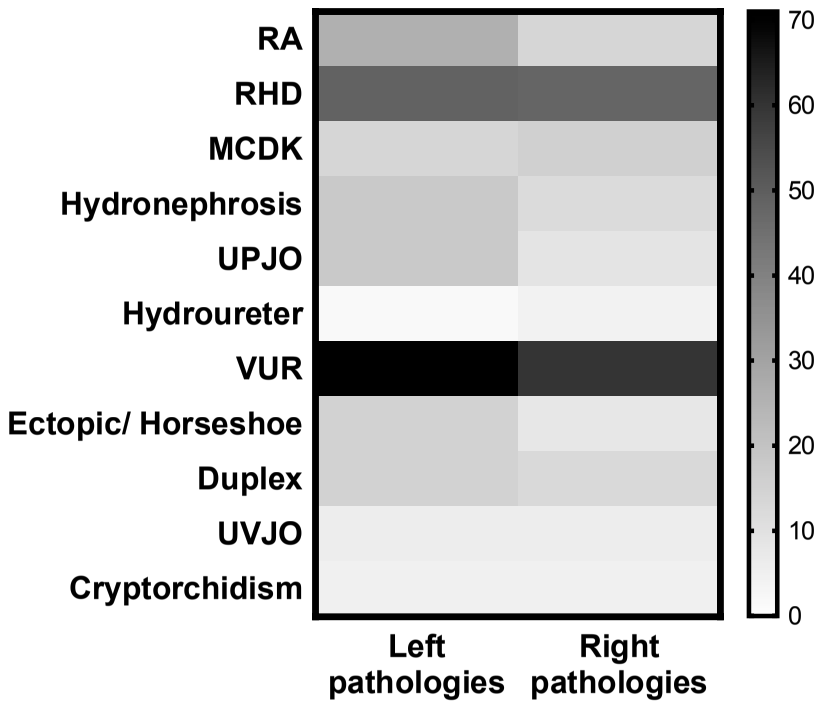
Supplementary Figure 4: Decision making strategy to determine causality of identified variants

Autosomal recessive variants

- **Include homozygous or compound heterozygous alleles as disease causing if:**
  - Truncating mutation (stop, abrogation of start or stop, obligatory splice, frameshift) **or**
  - Missense mutation if at minimum of 3 of the 5 following criteria were met:
    - Continuously conserved at least among vertebrates (or beyond)
    - Previously reported as disease causing or functional evidence implicating causality
    - Loss of function in human allele is supported by functional data
    - Phenotype correlates with the published phenotype for the gene
    - Predicted deleterious for the protein function (at least in two among three prediction programs (Polyphen (>0.5), SIFT (Del.), Mutation taster (D.C.))
- **Exclude allele as disease causing if:**
  - Allele frequency
    - Heterozygous allele frequency >1% (in EVS server, ExAC, gnomAD, 1000 genomes)
    - Compare homozygous allele frequency with control databases (in EVS server, ExAC, gnomAD, 1000 genomes)
  - Non segregation
    - if compound heterozygous variants are in cis or if an affected family member is without the variant or if an unaffected family member is with the allele consider incomplete penetrance and variable expressivity
- **Discussion of genotype-phenotype correlation in a 5-member panel of nephro-geneticists**

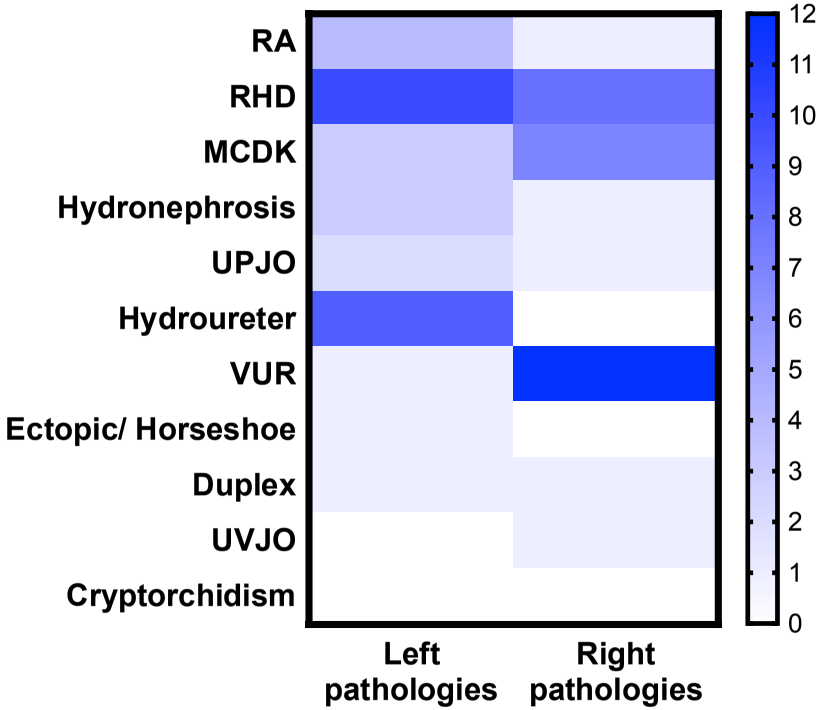
Autosomal dominant variants

- **Include heterozygous alleles as disease causing if:**
  - Truncating mutation (stop, abrogation of start or stop, obligatory splice, frameshift) **or**
  - Missense mutation if at minimum of 3 of the 5 following criteria were met:
    - Continuously conserved at least among vertebrates (or beyond)
    - Previously reported as disease causing or functional evidence implicating causality
    - Phenotype correlates with the published phenotype for the gene
    - Predicted deleterious for the protein function (at least in two among three prediction programs (Polyphen (>0.5), SIFT (Del.), Mutation taster (D.C.))
    - Mode of inheritance resembles known mode of inheritance for the gene (for known genes)
- **Exclude allele as disease causing if:**
  - Allele Frequency
    - Heterozygous allele frequency > 30 individuals for known isolated CAKUT genes or >10 for known syndromic CAKUT genes (ExAC) .
    - If the variant is present homozygously in any individual in ExAC or other databases it can be excluded
  - Non segregation
    - if affected family member is without the variant or if an unaffected family member is with the allele consider incomplete penetrance and variable expressivity
- **Discussion of genotype-phenotype correlation in a 5-member panel of nephro-geneticists**



Supplementary Figure 5A. Heatmap comparing the distribution of the specific phenotypic pathologies in the total cohort of 273 individuals with either unilateral (n=130) or bilateral (n=143) CAKUT that were submitted for WES analysis.

The absolute numbers of pathologies are graded according to color chart displayed on the right. Specific CAKUT phenotypic pathologies are listed from cranial to caudal list on the y-axis. The x-axis is further subdivided into left and right pathologies. Note, 130 pathologies were present in 130 individuals with unilateral CAKUT. In the 143 individuals with bilateral CAKUT, a total of 286 pathologies were present. A total of 416 pathologies were present in 273 individuals with CAKUT.



Supplementary Figure 5B. Heatmap comparing the distribution of the specific phenotypic pathologies in the solved cohort of the 37 individuals with either unilateral (n=14) or bilateral (n=20) CAKUT that were submitted for WES analysis.

The absolute numbers of pathologies are graded according to color chart displayed on the right. Specific CAKUT phenotypic pathologies are listed from cranial to caudal list on the y-axis. The x-axis is further subdivided into left and right pathologies.

Note, 17 pathologies were present in 14 individuals with unilateral CAKUT. In the 20 individuals with bilateral CAKUT, a total of 48 pathologies were present. A total of 65 pathologies were present in 34 individuals with CAKUT. Note, 3 individuals with PUV and 1 individual in whom laterality is unknown are excluded from the above graph.

RA; Renal Agenesis, RHD; Renal Hypoplasia/ Dysplasia, MCDK; Multicystic dysplastic kidney, UPJO; Ureteropelvic junction obstruction , VUR; Vesicourethral reflux, Ectopic/ Horseshoe; Ectopic or horseshoe kidney, Duplex; Duplex collecting system, UVJO; Ureterovesical junction obstruction. The bilateral renal pathologies consist of individuals with both bilateral concordant and bilateral discordant CAKUT. The total number of pathologies were calculated independent of both laterality and whether the bilateral pathologies were concordant or discordant in the same individual. We excluded individuals with a CAKUT pathology of PUV or epi/ hypospadias (n=25) and individuals in whom the CAKUT phenotype is undefined (n=21) in this analysis, due to the lack or inability to determine laterality with these specific pathologies.

Supplementary Figure 6 Web Resources

UCSC Genome Browser, https://genome.ucsc.edu

1000 Genomes Browser, http://www.internationalgenome.org/1000-genom es-browsers

Ensembl Genome Browser, http://www.ensembl.org

Exome Variant Server, http://evs.gs.washington.edu/EVS

Exome Aggregation Consortium, http://exac.broadinstitute.org

Genematcher https://genematcher.org/

gnomAD browser beta, http://gnomad.broadinstitute.org/

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

Polyphen2, http://genetics.bwh.harvard.edu/pph2

Sorting Intolerant From Tolerant (SIFT), http://sift.jcvi.org

MutationTaster http://www.mutationtaster.org

Seqr, http://www.seqr.broadinstitute.org

HGMD, https://portal.biobase-international.com

Conifer software, http://conifer.sourceforge.net

Seqr, https://seqr.broadinstitute.org