

Whole-exome resequencing reveals recessive mutations in *TRAP1* in individuals with CAKUT and VACTERL association

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Congenital abnormalities of the kidney and urinary tract (CAKUT) account for approximately half of children with chronic kidney disease and they are the most frequent cause of end-stage renal disease in children in the US. However, its genetic etiology remains mostly elusive. VACTERL association is a rare disorder that involves congenital abnormalities in multiple organs including the kidney and urinary tract in up to 60% of the cases. By homozygosity mapping and whole-exome resequencing combined with high-throughput mutation analysis by array-based multiplex PCR and

next-generation sequencing, we identified recessive mutations in the gene *TNF receptor-associated protein 1* (*TRAP1*) in two families with isolated CAKUT and three families with VACTERL association. *TRAP1* is a heat-shock protein 90-related mitochondrial chaperone possibly involved in antiapoptotic and endoplasmic reticulum stress signaling. *Trap1* is expressed in renal epithelia of developing mouse kidney E13.5 and in the kidney of adult rats, most prominently in proximal tubules and in thick medullary ascending limbs of Henle's loop. Thus, we identified mutations in *TRAP1* as highly likely causing CAKUT or VACTERL association with CAKUT.

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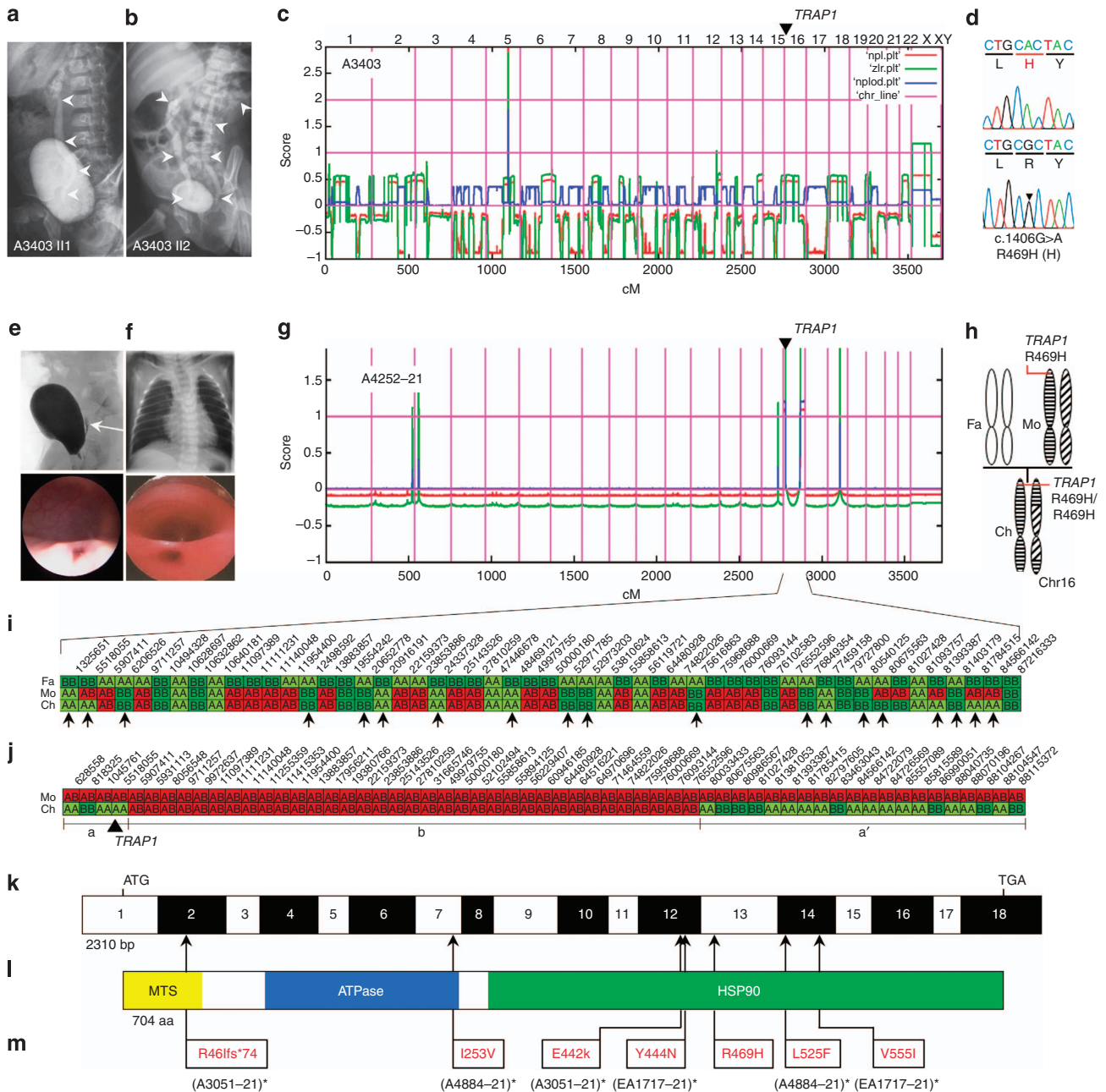
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Congenital abnormalities of the kidney and urinary tract (CAKUT) occur in 3–6 per 1000 live births. CAKUT are the most frequent cause for chronic kidney disease in children

(~50%)^{1,2} in the United States. The acronym ‘CAKUT’ comprises heterogeneous malformations involving the kidney (e.g., renal agenesis, hypodysplasia) and the urinary tract (e.g., vesicoureteral reflux (VUR), ureteropelvic junction obstruction).³ These congenital anomalies are related because a part of their pathogenesis is an impaired co-development of nephrogenic tissues derived from the metanephric mesenchyme and the ureteric bud.⁴ Twenty monogenic causes of isolated CAKUT in humans have been published to date, as reviewed recently by Yosypiv.⁵ However, they only account for ~10–20% of all cases indicating a broad genetic heterogeneity of CAKUT. A recent study on copy number variations in a large cohort of individuals with CAKUT and

two publications identifying novel monogenic causes of CAKUT bring further evidence that CAKUT is a condition of extensive genetic heterogeneity.^{6–8} CAKUT most frequently occur isolated, but they might be associated with extrarenal phenotypes, for instance, with VACTERL association (MIM #192350). The acronym ‘VACTERL’ describes the combination of at least three of the following congenital anomalies: vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal malformations (R), and limb defects (L). VACTERL association is a rare disease that occurs mostly sporadic in 1/10,000–40,000 live births.⁹ Its etiology is enigmatic, although animal models suggest an



involvement of Sonic hedgehog signaling.¹⁰ In humans, *ZIC3* mutations are the cause of a closely related nonclassic VACTERL condition (VACTERL-X, MIM #314390).^{11,12} In addition, there are six case reports published of individuals with VACTERL association in conjunction with mitochondrial dysfunction, as summarized recently by Siebel and Solomon.¹³ To identify new recessive genes that cause isolated CAKUT or CAKUT in VACTERL association, we performed homozygosity mapping and whole-exome resequencing (WER) in 24 affected individuals with CAKUT from 16 families, and in 4 individuals with CAKUT in VACTERL.

RESULTS

WER identifies a homozygous mutation in tumor necrosis factor (TNF) receptor-associated protein 1 (*TRAP1*) in CAKUT and in VACTERL association

By homozygosity mapping in a family of two siblings (A3403) with unilateral and bilateral VUR III^o, respectively (Figure 1a and b and Table 1), we identified a short 5.2-Mb segment of homozygosity on chromosome 5 (Figure 1c), indicating distant consanguinity of the parents. This finding suggested that in this family CAKUT are most likely caused by a homozygous recessive mutation in an unknown CAKUT gene. We performed WER in individual A3403-21, as described previously by the authors.^{14,15} In order not to miss either a homozygous mutation in a short run of homozygosity or a compound heterozygous mutation (which, as in this case, cannot be excluded *a priori* in families with remote consanguinity),¹⁶ we considered variants not only in the homozygosity peak but within regions of genetic linkage for both siblings (coverage ≥ 4 ; minor variant frequency ≥ 0.2). After variant filtering, we retained 38 variants in 13 genes for Sanger confirmation and segregation analysis (Supplementary Table S1 online). Only a single homozygous missense mutation (R469H) in the gene *TRAP1* on chromosome

16p13.3 survived the variant filtering process and segregation analysis (Figure 1d). This homozygous variant in *TRAP1* in A3403-21 and A3403-22 was positioned in an ~ 1.5 -Mb run of apparent homozygosity that was not detected by homozygosity mapping (Figure 1c), because the threshold for detection of 'homozygosity peaks' is 2.1 Mb.¹⁷

In family A4252 with CAKUT in VACTERL, we performed WER in an affected individual (A4252-21). This girl was born with a right double kidney and duplex ureter, left VUR, esophageal atresia type IIIb, and anal atresia with a vestibular fistula (Figure 1e and f and Table 1). Although there was no consanguinity reported in this family, homozygosity mapping showed unusually broad homozygosity peaks on chromosome 16 on the p-terminus and q-terminus (5.5 and 9.6 Mb, respectively; Figure 1g). In this case, we hypothesized that CAKUT in VACTERL is caused by a homozygous mutation within these homozygous regions. When evaluating WER data in this individual, the 512,733 variants initially detected (minor variant frequency ≥ 0.55 ; coverage ≥ 2) were reduced to only 11 variants within the 'homozygosity peaks' on chromosome 16 and 18 (Supplementary Table S2 online). The only variant that was confirmed by Sanger sequencing and that altered a conserved amino-acid residue was *TRAP1* R469H, the same allele as in family A3403. By comparison of SNPs in the affected girl and her parents, we demonstrated that partial maternal isodisomy of chromosome 16 with two recombinants (one located on the p-arm and one located on the q-arm) was the underlying cause of homozygosity for *TRAP1* R469H (Figure 1g-j).

The *TRAP1* allele c.1406G>A, p.R469H alters an evolutionary highly conserved amino-acid residue, and it is predicted to be deleterious for protein function by publically available software programs (Table 1 and Supplementary Figure S1 online). In the Exome Variant Server database, R469H has a minor allele frequency (MAF) of 0.9% in

Figure 1 | Homozygosity mapping and whole-exome resequencing identifies mutations in tumor necrosis factor (TNF) receptor-associated protein 1 (*TRAP1*) as causing congenital abnormalities of the kidney and urinary tract (CAKUT) or VACTERL association. (a, b) Voiding cysturethrograms (VCUG) of CAKUT siblings A3403-21 and A3403-22 showing unilateral vesicoureteral reflux (VUR) grade III and bilateral VUR, respectively (white arrow heads). **(c)** Nonparametric LOD (NPL) scores across the human genome in two affected siblings. X axis represents Affymetrix 250k Styl array single-nucleotide polymorphism (SNP) positions across human chromosomes concatenated from the p-terminus (left) to the q-terminus (right). Genetic distance is given in cM. A single peak indicates distantly related parents. **(d)** Chromatogram of newly identified homozygous missense mutation (arrow head) in the gene encoding TNF receptor-associated protein 1 (*TRAP1*) over wild-type control. **(e)** VCUG (upper panel) and cystoscopy (lower panel) demonstrating VUR and a dilated ureteral orifice, respectively. **(f)** Chest X-ray (top panel) and esophagoscopy (bottom panel) showing esophageal atresia and esophagotracheal fistula in individual A4252-21 with CAKUT in VACTERL association. **(g)** NPL score in an individual A4252-21 with VACTERL association. Two maximum peaks indicate homozygosity at the p-terminus and q-terminus of chromosome 16. **(h)** Panel on the right illustrates maternal heterodisomy of chromosome 16 and partial uniparental isodisomy (p-ter and q-ter) of the child (Fa, father; Mo, mother; Ch, child). **(i)** Partial haplotypes of selected markers and their physical positions across chromosome 16 in the Fa, the Mo, and the affected Ch of CAKUT family A4252. Selected markers (biallelic SNPs; minor allele frequency (MAF) = 0.496–0.5) homozygous in the father are shown in green (alleles AA) and light green (alleles BB). The fact that for 19 of 52 alleles there is paternal noncontribution in the child strongly suggests maternal isodisomy of chromosome 16. No paternal noncontribution was observed in the child on any other chromosome (data not shown). **(j)** Selected markers (biallelic SNPs; MAF = 0.497–0.5) heterozygous in the Mo of family A4252 are shown for alleles coded in red (AB; phase unknown). Note that in the central segment **(b)**, separated by vertical lines, the Ch haplotype is identical to the mother's haplotype. In the p-ter (a) and q-ter (a') segments (a, a'), the child is homozygous, indicating maternal isodisomy in these segments. **(k)** Exon structure of human *TRAP1* complementary DNA. Positions of start codon (ATG) and of stop codon (TGA) are indicated. **(l)** Domain structure of the *TRAP1* protein. HSP, heat-shock protein; MTS, mitochondrial targeting sequence. **(m)** Translational changes of detected mutations are shown relative to their positions in *TRAP1* complementary DNA (see **l**) and *TRAP1* protein (see **m**) for affected individuals with CAKUT or CAKUT in VACTERL association with recessive *TRAP1* mutations. Family numbers are shown in parentheses. *Denotes an individual with compound heterozygous mutations in *TRAP1*.

Table 1 | Mutations of TRAP1 in five families with isolated CAKUT or CAKUT in VACTERL association

Family -Individ. (sex)	Ethnic origin	Nucleotide alteration ^a	Deduced protein change	Continuous amino-acid sequence conservation	MutT ^b	Poly-Phen2 ^c	SIFT ^d	MAF in EVS ^e	Exon (state; segregation)	Urinary tract phenotypes	Other phenotypes
A3403 -21 (F)	Serbian	c.1406G>A	p.R469H	<i>E. coli</i> (<i>C. elegans</i> has L)	0.99	0.997	0.00	0.77%	13 (Hom; Fa, Mo)	-21: VUR-III° R -22: VUR-III° R and L	None
A4252 -21 (F)	Central European	c.1406G>A	p.R469H	<i>E. coli</i> (<i>C. elegans</i> has L)	0.99	0.997	0.00	0.77%	13 (Hom; Mo; partial maternal isodisomy)	Double kidney R VUR L MCDK L	VACTERL association including esophageal atresia IIIb, anal atresia, vestibular fistula
A3051 -21 (M)	Macedonian	c.127_137dup c.1324G>A	p.R46Sfs*75 p.E442K	NA <i>D. rerio</i>	NA 0.99	NA 0.003	NA 0.3	Absent 0.08%	2 (het; Mo) 12 (het; Fa)		None
A4884 -21 (F)	Dutch	c.757A>G c.1573C>T	p.I253V p.L525F	<i>E. coli</i> (<i>X. tropicalis</i> has L) <i>S. cerevisiae</i> has L) <i>E. coli</i>	0.99 0.99	0.433 0.942	0.00 0.00	0.91% Absent	7 (het; Mo) 14 (het; Fa)	Renal agenesis R	VACTERL association including cervical/thoracic hemivertebrae, 5 dysplastic short ribs R, anal atresia with rectoperineal fistula, ASD type II, esophageal atresia, abnormal position of thumbs
EA1717 -21 (F)	Dutch	c.1330T>A c.1663G>A	p.Y444N p.V555I	<i>C. elegans</i> <i>C. intestinalis</i>	0.99 0.99	0.985 0.115	0.03 0.39	0.91% ^f Absent	12 (het; Fa) 14 (het; Mo)	Pyelectasis and VUR L	VACTERL association including anal atresia, esophageal atresia, ASD, VSD, hypoplastic/absent humerus, persistent L vena cava superior, cloaca

Abbreviations: ASD, atrial septum defect; CAKUT, congenital abnormalities of the kidney and urinary tract; cDNA, complementary DNA; *E. coli*, *Escherichia coli*; EVS, Exome Variant Server; F, female; Fa, mutation segregating from the father; L, left; NA, not applicable; M, male; MAF, minor allele frequency; MCDK, multicystic dysplastic kidney; Mo, mutation segregating from the mother; MutT, MutationTaster; R, right; SIFT, sorting intolerant from tolerant; TRAP1, tumor necrosis factor (TNF) receptor-associated protein 1; VSD, ventricular septum defect; VUR-III°, vesicoureteral reflux third degree.

^aTRAP1 cDNA mutations are numbered according to human cDNA reference sequence NM_016292.2, where +1 corresponds to the A of ATG start translation codon.

^bMutationTaster score. Range: 0–1.0, 1.0 being most deleterious.

^cPolyPhen2 (HumVar) score. Range: 0–1.0, 1.0 being most deleterious.

^dSIFT score. Range: 0–1.0, 0 being most deleterious.

^eMinor allele frequency in 8600 alleles of Americans of European descent.

^fOne individual is homozygous for this allele.

Americans of European descent. In our cohort of 675 individuals with CAKUT, most of them European, the MAF is 1.9%. The three affected individuals from two unrelated families with homozygous TRAP1 R469H, as well as six additional heterozygous carriers, share haplotypes at the TRAP1 locus (Supplementary Figure S2 online), which speaks for TRAP1 R469H being a European founder mutation.

Mutation analysis reveals three additional families with TRAP1 mutations

We subsequently analyzed the coding sequence of TRAP1 in a cohort of 675 individuals with isolated CAKUT (Supplementary Table S3 online) and 300 individuals with classic VACTERL association (i.e., VACTERL-X and other related disorders have been excluded) using a bar-coded multiplex PCR approach and consecutive next-generation sequencing, as described previously by the authors.¹⁸ As a control group, we included 800 individuals with the distinct renal phenotype of nephronophthisis.

We detected six additional recessive mutations in TRAP1 in a compound heterozygous state in three additional unrelated families with CAKUT or CAKUT in VACTERL (Table 1, Figure 1k, l and m, Supplementary Figure S1 and S3 online). In individual A3051-21 with a left-sided multicystic dysplastic kidney, we found a maternally inherited protein-truncating frameshift mutation (c.127_137dup, p.R46Sfs*75). This mutation abrogates the N-terminal mitochondrial targeting sequence of TRAP1, which makes this a null allele. The second allele was a missense mutation (c.1324G>A, p.E442K) that segregated from the father.

In individual A4884-21 with CAKUT in VACTERL, including right renal agenesis, vertebral malformations, anal atresia with a rectoperineal fistula, atrial septum defect type II, esophageal atresia, and abnormal position of the thumbs (Table 1 and Supplementary Figure S4 online), we detected compound heterozygous missense mutations in TRAP1 located in the ATPase domain (c.757A>G, p.I253V) and in the HSP90 domain (c.1573C>T, p.L525F; Figure 1l).

In individual EA1717 with CAKUT in VACTERL, including pyelectasis, left VUR, a complex anorectal malformation including anal atresia and persistent cloaca, esophageal atresia, cardiac defects, limb defects, and persistent left vena cava superior (Table 1), we detected compound heterozygous missense mutations that are both located in the HSP90 domain of TRAP1 (c.1330T>A, p.Y444N and c.1663G>A, p.V555I).

To exclude the presence of recessive mutations in controls, we sequenced the TRAP1 coding sequence in 800 individuals with the distinct renal phenotype of nephronophthisis. We detected the TRAP1 allele I253V seven times (MAF 0.87%), T444N twice (MAF 0.25%), and R469H twice (MAF 0.025%), all of them as single heterozygous alleles. TRAP1 R46Sfs*75, E442K, L525F, and V555I were absent from our control cohort. Furthermore, no other possibly deleterious variants were present in a homozygous or compound heterozygous state in 800 individuals with nephronophthisis.

Trap1 is expressed in developing and adult kidney

To determine whether TRAP1 has a function during kidney development, we analyzed Trap1 expression in developing

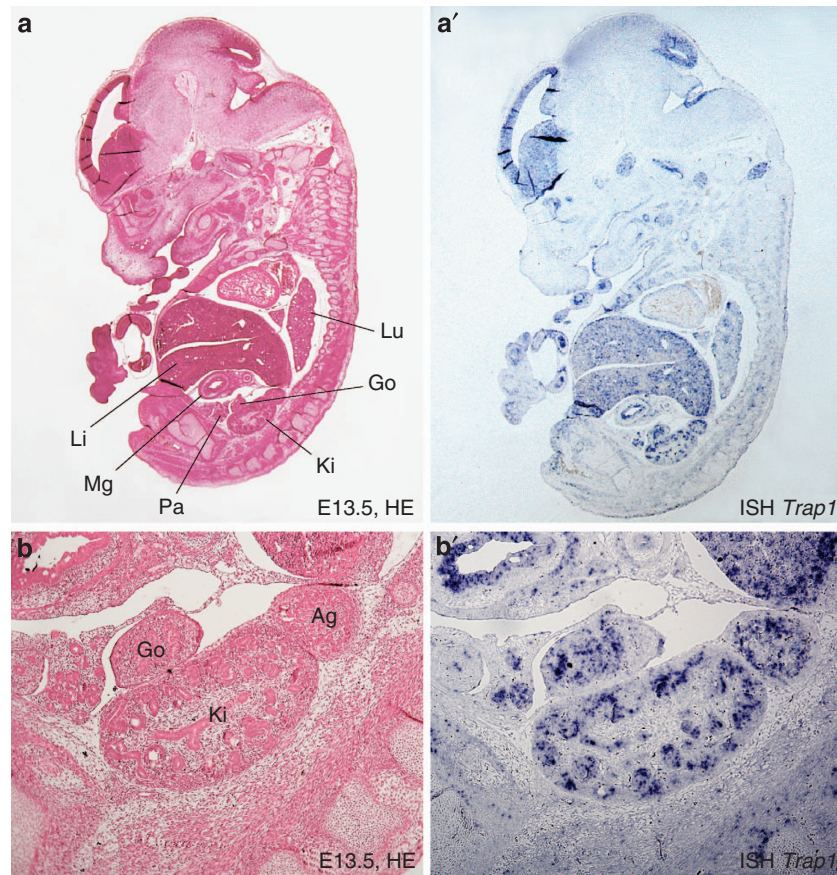


Figure 2 | *Trap1* is highly expressed in the renal epithelia of E13.5 mouse embryos. The upper panel shows an HE-stained sagittal section (a) and a *Trap1*-ISH (a') in consecutive sections of a mouse embryo E13.5. Note the prominent *Trap1* expression in the developing kidney (marked 'Ki' in the left panel). The lower panel shows higher magnifications of E13.5 mouse kidney. (b) HE staining, (b') *Trap1*-ISH. The *Trap1*-ISH staining pattern is consistent with *Trap1* being expressed specifically in renal epithelia (b'). Ag, adrenal gland; Go, gonad; HE, hematoxylin-eosin; ISH, *in situ* hybridization; Ki, kidney (i.e., metanephros); Li, liver; Lu, lung; Mg, midgut; Pa, pancreatic primordium; TRAP1, tumor necrosis factor (TNF) receptor-associated protein 1.

kidney in mouse embryos 13.5 dpc. *Trap1* seemed to be expressed at this stage in renal vesicles according to *Trap1* transcription assays publically available through the Gudmap project. By *in situ* hybridization (ISH) in E13.5 mouse embryos, we found *Trap1* to be strongly expressed in the kidney, adrenal gland, and gonad. *Trap1* expression specifically localized to renal epithelia (Figure 2a').

To characterize TRAP1 localization in adult kidney, we performed immunofluorescence stainings in rat using a monoclonal TRAP1 antibody in conjunction with established renal markers (Figure 3). TRAP1 is present most prominently in peanut-lectin-marked proximal tubules in the renal cortex (Figure 3a and b). In renal medulla, we detected TRAP1 in peanut-lectin-negative tubular segments and in NKCC-marked ($\text{Na}^+ \text{K}^+ 2\text{Cl}^-$ co-transporter) thick ascending limbs of Henle's loop (Figure 3c and d). TRAP1 colocalizes with mitochondrial marker MTCO1 in renal cortex and medulla.

DISCUSSION

In this study, we identified by WER and high-throughput mutation analysis five unrelated families with CAKUT or

CAKUT in VACTERL association with recessive mutations in *TRAP1*. Two siblings with CAKUT had a homozygous missense mutation (R469H), which segregated from a common ancestor of their parents. A girl with VACTERL association had the identical homozygous mutation due to maternal isodisomy of chromosome 16 p-ter and q-ter. In a cohort of 675 individuals with CAKUT and 300 individuals with classic VACTERL association, we identified 3 additional individuals carrying compound heterozygous mutations in *TRAP1*. Homozygous or compound heterozygous deleterious variants were absent from 800 control individuals. By ISH and immunofluorescence, we showed that *Trap1* is expressed in early mouse renal epithelia, whereas the *Trap1* protein is present only in defined segments of developed nephrons in rat.

In 6500 individuals recorded in the Exome Variant Server server, there are several non-synonymous variants present in *TRAP1*, including heterozygous truncating variants in 11 individuals. However, deleterious alleles in recessive disease genes, unlike in dominant disease genes, do not underlie direct negative selection through evolution. Consequently, the presence of rare deleterious variants in recessive disease genes in a large cohort is an expected finding.

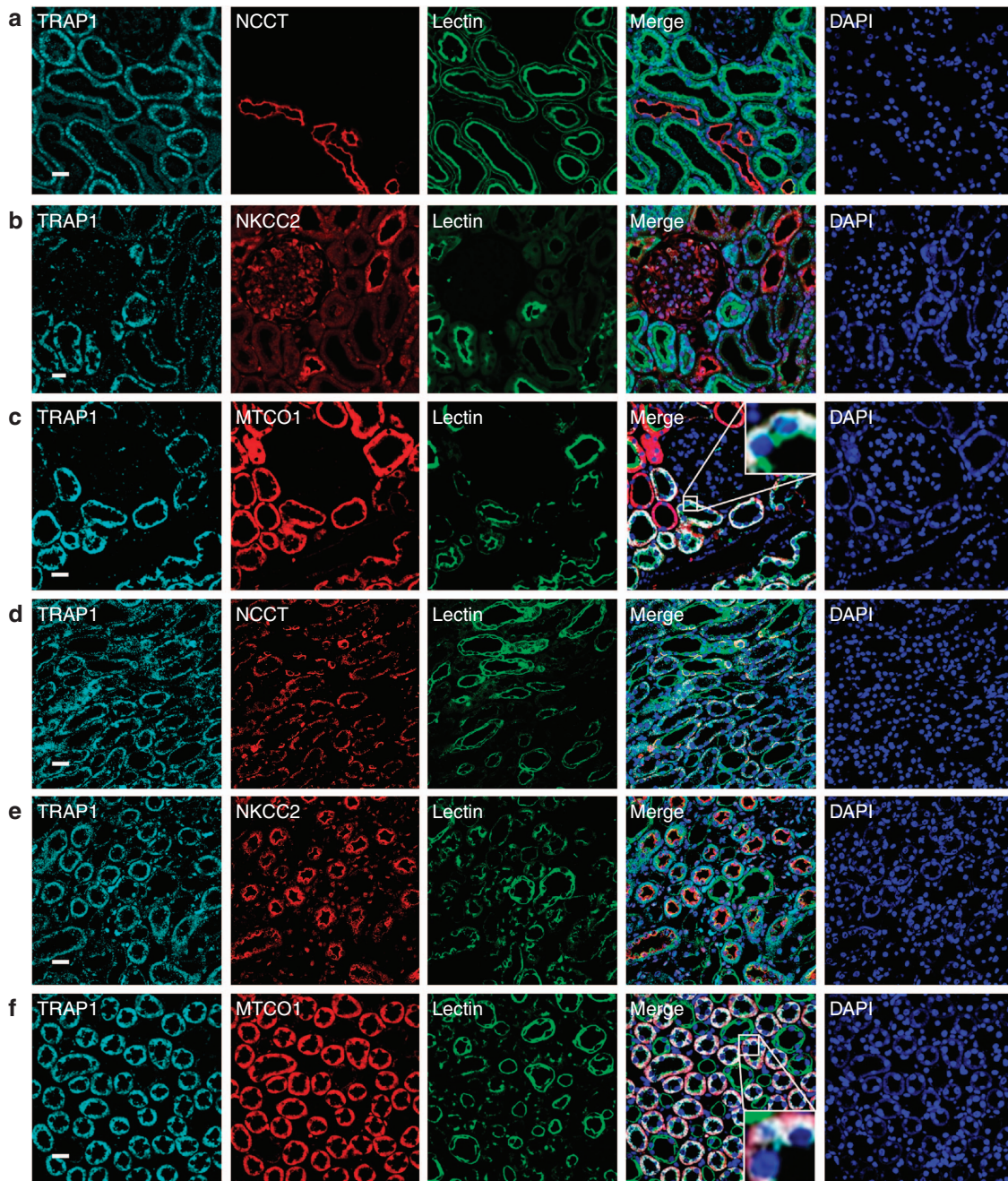


Figure 3 | Renal tubular segmental localization of TRAP1 by immunofluorescence microscopy in adult rat kidney. (a–c) Renal cortex. **(a)** TRAP1 (blue–green) is located in proximal tubules (marked green by peanut lectin) and is absent from distal convoluted tubules (DCT; marked red by NCCT-ab). (False color addition of blue–green and green appears as light green.) **(b)** TRAP1 is mostly absent from cortical thick ascending limb (TAL) of Henle’s loop (marked red by NKCC2-ab). **(c)** TRAP1 colocalizes with mitochondrial marker MTCO1 (marked red by MTCO1-ab) in proximal tubules. (False color addition of blue–green and red appears as white.) **(d–f)** Renal medulla. TRAP1 is absent from peanut-lectin-positive tubular segments. **(d)** TRAP1 expression is present in DCT (marked red by NCCT-ab). **(e)** TRAP1 is expressed in medullary thick ascending limb (mTAL) of Henle’s loop (marked red by NKCC2-ab). **(f)** TRAP1 localizes to mitochondria (marked red by MTCO1-ab) of peanut-lectin-negative tubular segments. Scale bar: 201 m. ab, antibody; DAPI, 4',6-diamidino-2-phenylindole; MTCO1, mitochondrially encoded cytochrome c oxidase 1; NCCT, Na + -Cl- co-transporter; NKCC2, Na + -K + -2Cl- co-transporter; TRAP1, tumor necrosis factor (TNF) receptor-associated protein 1.

The allele *TRAP1* Y444N, detected as a compound heterozygous mutation in an individual with CAKUT in VACTERL, is present homozygously in a single individual of the ESP cohort of 6500 healthy Americans. However, in the context of CAKUT, this does not necessarily mean that the variant is

nonpathogenic. CAKUT frequently remain completely asymptomatic. For instance, a double kidney or unilateral renal agenesis typically are an ‘accidental finding’ in renal ultrasound.

The fact that the homozygous mutation *TRAP1* R469H was found in an individual with CAKUT and an individual

with VACTERL association is surprising. However, in CAKUT and in VACTERL association, intra-familial phenotypic variability is very common.^{19–21} Even in a single individual different CAKUT phenotypes may be present, for instance, left renal agenesis and right VUR.

The frequencies of individuals with recessive *TRAP1* mutations in our cohorts (0.15% in CAKUT, 0.6% in CAKUT with VACTERL) suggest that mutations in *TRAP1* are a rare cause of these conditions. Similarly, mutations in two recently identified CAKUT-causing genes, *WNT4* and *DSTYK*, are rare causes of CAKUT.^{7,8} These findings in humans, along with numerous CAKUT-mouse models, indicate that CAKUT are a common clinical phenotype arising from a multitude of different single-gene causes.

In conclusion, we propose that recessive mutations in *TRAP1* are a novel rare cause of isolated CAKUT and the first recessive cause of the VACTERL association.

MATERIALS AND METHODS

Human subjects

We obtained blood samples and pedigrees following informed consent from individuals with CAKUT and from individuals with VACTERL association. Approval for human subjects research was obtained from the University of Michigan Institutional Review Board and other institutions involved. The diagnosis of CAKUT and VACTERL association was based on published clinical criteria.⁹

Homozygosity mapping

We performed homozygosity mapping as described previously.¹⁷

Whole-exome resequencing

Exome library preparation and next generation sequencing was conducted using the SeqCap EZ Exome v2 (Nimblegen, Roche NimbleGen, Madison, WI) and Genome Analyzer II (Illumina, San Diego, CA). Subsequent variant detection, filtering, and analysis have been described previously by the authors.^{14,15} All detected variants were confirmed by Sanger sequencing.

Immunofluorescence microscopy

Immunofluorescence was performed as previously described by the authors¹⁴ using a Leica SP5X system (Leica Microsystems GmbH, Wetzlar, Germany) with an upright DM6000 compound microscope (Leica Microsystems GmbH), and images were processed with the Leica AF software suite. The following antibodies were used: TRAP1 (Abcam, Cambridge, MA (TRAP1-6), cat# ab2721), MTCO1 (Abcam cat# ab45918), NKCC2 (LSBio cat# LS-C150446, Seattle, WA), and NCCT (Millipore cat# AB3553, Billerica, MA). Specificity of the anti-TRAP1 antibody for rat TRAP1 was confirmed in immunoblot (Supplementary Figure S5 online).

In situ hybridization

ISH was conducted on sections of wild-type mouse embryos with an Naval Medical Research Institute background at embryonic day 13.5. Mouse embryos were dissected into ice-cold phosphate-buffered saline, fixed overnight in 4% paraformaldehyde/phosphate-buffered saline, and then processed into paraffin wax. ISH was performed on paraffin sections (5 µm) using antisense probes generated by PCR from an E11.0 total embryo complementary DNA library, and specific staining was verified using a sense probe. PCR products

contained 3' T7 and 5' T3 RNA polymerase binding sites for *in vitro* transcription, and probes were purified using G-50 sephadex columns (GE Healthcare, Bio-Sciences, Piscataway, NJ). The 779-bp probe for *Trap1* spans exons 13–17 (accession: NM_026508.2).

ISH was performed according to the protocol from Chotteau-Lelievre *et al.*²² with minor modifications, and detection of alkaline phosphatase activity was visualized using BM Purple (Roche Diagnostics, Mannheim, Germany). After staining, slides were quickly dehydrated in 80% and then 100% ethanol, cleared twice for 1 min in xylene (Roth, Carl Roth GmbH, Karlsruhe, Germany), and coverslips were mounted with Entellan mounting medium (Merck, Merck KGaA, Darmstadt, Germany). Photographs were obtained using the AxioVision software (Zeiss, Carl Zeiss, Oberkochen, Germany) with a Zeiss AxioCam and SterEO Discovery.V12 microscope (Carl Zeiss). Three sections from at least two different embryos were analyzed.

Bioinformatics

Next generation sequencing reads alignment and variant detection was done with Genomics Workbench software (CLC Biotech, Cambridge, MA). Mapping parameters were as follows: global alignment, length fraction = 0.9, and similarity fraction = 0.9. Genetic location is according to the assembly of the Genome Reference Consortium GRCh37.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure S1. Evolutionary conservation of altered TRAP1 amino acid residues.

Figure S2. Haplotype comparison at the TRAP1 locus in individuals carrying the allele TRAP1 R469H.

Figure S3. Clinical findings in individuals A3051-21 with CAKUT and individual A4884-21 with VACTERL association.

Figure S4. Chromatograms of newly identified mutations in TRAP1.

Figure S5. Immunoblot validation of the anti-TRAP1 antibody specificity for rat TRAP1.

Table S1. Whole-exome resequencing (WER) statistics and variant filtering workflow of WER in family A3403 with congenital abnormalities of the kidney and urinary tract (CAKUT).

Table S2. Whole-exome resequencing statistics and variant filtering workflow in individual A4252-21 with VACTERL.

Table S3. Demographics and characterization of 675 individuals with isolated congenital abnormalities of the kidney and urinary tract (CAKUT).

Supplementary material is linked to the online version of the paper at <http://www.nature.com/ki>

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