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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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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°, 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 $\geq$4; minor variant frequency $\geq$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 $\geq$0.55; coverage $\geq$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 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