Mutations in 12 known dominant disease-causing genes clarify many congenital anomalies of the kidney and urinary tract

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Congenital anomalies of the kidney and urinary tract (CAKUT) account for approximately half of children with chronic kidney disease. CAKUT can be caused by monogenic mutations; however, data are lacking on their frequency. Genetic diagnosis has been hampered by genetic heterogeneity and lack of genotype–phenotype correlation. To determine the percentage of cases with CAKUT that can be explained by mutations in known CAKUT genes, we analyzed the coding exons of the 17 known dominant CAKUT-causing genes in a cohort of 749 individuals from 650 families with CAKUT. The most common phenotypes in this CAKUT cohort were vesicoureteral reflux in 288 patients, renal hypodysplasia in 120 patients, and unilateral renal agenesis in 90 patients. We identified 37 different heterozygous mutations (33 novel) in 12 of the 17 known genes in 47 patients from 41 of the 650 families (6.3%). These mutations include (number of families): *BMP7* (1), *CDC5L* (1), *CHD1L* (5), *EYA1* (3), *GATA3* (2), *HNF1B* (6), *PAX2* (5), *RET* (3), *ROBO2* (4), *SALL1* (9), *SIX2* (1), and *SIX5* (1). Furthermore, several mutations previously reported to be disease-causing are most likely benign variants. Thus, in a large cohort over 6% of families with isolated CAKUT are caused by a mutation in 12 of 17 dominant CAKUT genes. Our report represents one of the most in-depth diagnostic studies of monogenic causes of isolated CAKUT in children.

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Congenital anomalies of the kidney and urinary tract (CAKUT) are observed in three to six per 1000 live births and account for 40–50% of the etiology of chronic kidney disease (CKD) in children worldwide.[1,2] CAKUT cover a wide range of structural malformations that result from a defect in the morphogenesis of the kidney and/or the urinary tract.[3–5] The condition may appear as an isolated feature or as part of a syndrome in association with extra-renal manifestations.[6,7] In addition, CAKUT may either be diagnosed sporadically or be described with familial aggregation in up to 15% of cases.[8,9] In familial cases, the mode of inheritance in most pedigrees is autosomal dominant with variable expressivity and reduced penetrance.[10] The pathogenesis of CAKUT is based on the disturbance of normal nephrogenesis and can be due to genetic abnormalities in the renal developmental genes that direct the process.[1,3–5,11–13] To date, 23 monogenic CAKUT-causing genes have been identified that result in isolated CAKUT or syndromic CAKUT with mild extra-renal manifestations.[14–35] Only a few studies have screened large cohorts of CAKUT patients for disease-causing mutations.[36–41] These studies screened for one to five disease-causing genes and some were preselected for chronic renal insufficiency or severe disease phenotypes.[36–38] Hence, data are lacking on the frequency of monogenic forms of CAKUT in large cohorts.

To address these issues we investigated the frequency of mutations in 17 known dominant CAKUT-causing genes in a

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phenotypically nonselective international cohort of 749 CAKUT individuals out of 650 different families. We show that mutations in known CAKUT-causing genes are present in more than 6% of these families, and we outline possible pitfalls in analyzing autosomal dominant single-gene disorders.

RESULTS

Our cohort of 749 individuals from 650 different families with CAKUT originated from Eastern Europe (63.6%), Western Europe (12.7%), Arab countries (10%), India (7.9%), Romany populations (1.5%), and Asia (0.7%) (Supplementary Table S1 online). There were 414 male (55%) and 331 female (44.2%) individuals. The most common CAKUT phenotype was vesicoureteral reflux ($n = 288$), followed by renal hypodysplasia ($n = 120$) and unilateral renal agenesis ($n = 90$). A total of 161 individuals from 100 families were considered as having familial CAKUT according to clinical questionnaires in our cohort. These families had two to six affected individuals. The most common familial CAKUT phenotypes include vesicoureteral reflux ($n = 68$) and duplex system ($n = 29$), followed by renal hypodysplasia ($n = 19$) and others. For detailed cohort characteristics see Supplementary Table S1 online.

By targeted resequencing of 170 coding exons of 17 genes known to cause autosomal dominant CAKUT we identified 144,382 single-nucleotide variants and 39,081 insertion–deletion variants in the 650 families. After variant filtering, as described in the Materials and Methods, we retained 341 variants as potentially deleterious alleles. Of these, 152 were confirmed by Sanger sequencing, whereas the others represented low-representation artifacts of multiplex polymerase chain reaction (PCR). In order to distinguish benign variants from likely disease-causing mutations we carefully evaluated each variant individually on the basis of the criteria described in the Materials and Methods section. Overall, 105 variants did not meet our criteria for being probably disease-causing. Among these, 43 variants were previously reported as mutations in individuals with CAKUT (Supplementary Table S2 online), and 62 variants were not previously reported (Supplementary Table S3 online) in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php).

In 749 patients with CAKUT from 650 families, disease-causing heterogeneous dominant mutations were identified in 41 unrelated families (6.3%) (Table 1). Mutations were detected in the following genes: *BMP7* (one family), *CDC5L* (one family), *CHD1L* (five families), *EYA1* (three families), *GATA3* (two families), *HNF1B* (five families), *PAX2* (five families), *RET* (three families), *ROBO2* (four families), *SALL1* (nine families), *SIX2* (one family), and *SIX5* (one family) (Table 1). No causative mutations were identified in the genes *SOX17, UMOD, BMP4, SIX1,* and *UPK3A*. In total, 33 of the 37 mutations were most likely novel pathogenic mutations.

DISCUSSION

We examined a large international cohort of 650 unrelated families with CAKUT for the presence of mutations in 17 autosomal dominant known CAKUT-causing genes. We identified 37 different heterozygous mutations in 12 different genes in 41 of the 650 families (6.3%). Thirty-three of the 37 mutations detected were novel.

Our findings also revealed that some variants previously reported as disease-causing cannot be accepted as such on the basis of a finding that shows lack of segregation of these genetic variants in families with multiple affected individuals. For example, the *BMP4* variant p.S91C and the *SIX2* variant p.P241L have been reported to lead to CAKUT among five unrelated patients.[15] We detected these two variants among 13 unrelated families in our cohort and five of them did not segregate with the disease—that is, not all affected family members have the variant. These findings reveal that these two variants cannot be considered as disease-causing variants. These findings encourage us to adhere to our strict definition of disease-causing variants as outlined in 'Materials and Methods' and are consistent with the findings that many alleles published as disease-causing may not reliably have such a role.[42,43] We found that nine variants (43 individuals) in previously CAKUT-related publications and 50 human gene mutation database-unreported variants (62 individuals) did not fulfill our criteria (Supplementary Tables S2 and S3 online, respectively).

This work, to the best of our knowledge, is the most extensive genetic screening of known CAKUT-causing genes. *SALL1, HNF1B,* and *PAX2* were the most prevalent disease-causing genes in our cohort. This is in line with the predominance of *HNF1B* and *PAX2* mutations that has been described in patients with renal hypodysplasia.[36,37,39] *HNF1B* and *PAX2* were previously reported to be disease-causing in 5–20% of CAKUT cases.[36–41] The finding that *PAX2* and *HNF1B* mutations were seen at a higher frequency in previous studies on CAKUT is most likely explained by the fact that these studies were carried out in CAKUT cohorts preselected for CKD and in prenatal findings with severe renal anomalies.[36–38] Our data are consistent with previous publications describing that oligosyndromic CAKUT-causing genes can lead to an isolated CAKUT phenotype.[36]

The fact that we did not identify mutations in *SOX17, UMOD, BMP4, SIX1,* and *UPK3A* suggests that mutations in these genes are rarer. The identification of *SALL1* mutations in $>1\%$ of our cohort suggests that this gene may be a more common cause of CAKUT than previously believed.[36] It should be emphasized that in the current study we did not screen our cohort for copy number variations. It was previously shown that some of the known CAKUT-causing genes may be disrupted by deletions or duplications, such as heterozygous *HNF1B* deletion.[36] Moreover, in a recent study involving 522 patients with CAKUT, 72 distinct known or novel copy number variations in 87 (16.6%) patients were identified, suggesting that kidney malformations can, in part, result from pathogenic genomic imbalances.[44]

Our study supports the observation that CAKUT are a genetically very heterogeneous group of diseases with diverse clinical phenotypes. We provide further evidence that the role of specific oligosyndromic CAKUT genes (i.e., *SALL1*) has a