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# Variants in TSHZ3 Encoding a Regulator of Smooth Muscle Differentiation in Human Congenital Anomalies of the Kidney And Urinary Tract

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**Objectives :** Around 180 genes have been associated with congenital anomalies of the kidney and urinary tract (CAKUT) in mice, and represent promising novel candidate genes for human CAKUT. In whole-exome sequencing data of two siblings with genetically unresolved multicystic dysplastic kidneys (MCDK), prioritizing variants in murine CAKUT-associated genes yielded a rare variant in the teashirt zinc finger homeobox 3 (TSHZ3) gene. Therefore, the role of TSHZ3 in human CAKUT was assessed.
**Methods :** Whole-exome or targeted TSHZ3 sequencing was done in 301 CAKUT families. Variant carriers were characterized by reverse phenotyping and immunohistochemical analysis of a nephrectomy specimen. TSHZ3 expression was determined in human tissues and during murine development by qPCR and RNA in situ hybridization. Binding of mutant TSHZ3 was analyzed by co-immunoprecipitation.
**Results :** Taken together, 12 CAKUT patients from 9 of 301 (3%) families carried five different rare heterozygous TSHZ3 missense variants predicted to be deleterious. CAKUT patients with versus without TSHZ3 variants were (significantly) more likely to present with hydronephrosis ($p=0.039$) and hydroureter ($p=0.067$), previously observed in Tshz3-null mutant mice, MCDK ($p=0.055$), genital anomalies ($p=0.029$), and developmental delay ($p=0.037$), similar to patients with heterozygous deletions at 19q12-q13.11 encompassing the TSHZ3 locus. Comparable with Tshz3-null mutant mice, the smooth muscle layer was disrupted in the renal pelvis and proximal ureter of a nephrectomy specimen of a TSHZ3 variant carrier compared to an age-matched control. TSHZ3 was expressed in human fetal kidney, and strongly at embryonic day 11.5-14.5 in mesenchymal compartments of the murine ureter, kidney, and bladder. TSHZ3 variants in a 5' hotspot region were significantly more frequent in CAKUT patients than in gnomAD controls ($p<0.001$). Mutant TSHZ3 harboring N-terminal variants showed significantly altered SOX9 and/or myocardin binding.
**Conclusions :** Our results provide evidence that heterozygous TSHZ3 variants are associated with human CAKUT, particularly hydronephrosis, hydroureter, and MCDK, and, inconsistently, with specific extrarenal features.