Editorial Commentary

Expanding congenital abnormalities of the kidney and urinary tract (CAKUT) genetics: basonuclin 2 (BNC2) and lower urinary tract obstruction

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Chronic kidney disease (CKD): the 21-century epidemic

CKD is characterized by chronic (>3 months), often irreversible, evidence of kidney injury or dysfunction with consequences for health (1). A diagnosis is made in the presence of decreased glomerular filtration rate (GFR) or if there is analytical (most commonly pathological albuminuria), histological or imaging evidence of kidney injury. The criterion "with consequences for health" implies that CKD is associated with an increased risk of progression to end-stage renal disease (ESRD) requiring renal replacement therapy, which is the best-known consequence of CKD. However, CKD is also associated with an increased risk for premature death and in fact, CKD is projected to become 1 of the top 5 causes of death in the world by 2040 and similar trends have been described in individual countries (2,3). While the most frequent causes of CKD are acquired, including kidney disease secondary to diabetes and hypertension, the influence of genetic factors has been increasingly recognized, including genetic defects leading to congenital abnormalities of the kidney and urinary tract (CAKUT). CAKUT may be caused by hereditary genetic defects, as recently exemplified by the description of basonuclin 2 (BNC2) nonsense variants as causing congenital lower urinary-tract obstruction (LUTO) (4).

What is CAKUT?

In PubMed, publications using the term CAKUT date from 1999, 20 years ago (5). CAKUT is considered the leading cause of pediatric ESRD and the most common cause of CKD before 30 years of age (6). The spectrum of anomalies includes kidney abnormalities (agenesis, hypoplasia or dysplasia as well as supernumerary, ectopic or fused kidneys) and urinary tract abnormalities (e.g., ureter duplication, ureteropelvic junction obstruction, primary megaureter or ureterovesical junction obstruction, vesicoureteral reflux, ureterocele, and posterior urethral valves, which are a cause of LUTO) (*Figure 1*). Genetic defects have been increasingly recognized as causing CAKUT. However, monogenic mutations currently explain only 14% of CAKUT cases (7). In addition to classical gene variants in developmental genes (missense or nonsense mutations, deletions, frameshift mutations), the spectrum of genetic defects causing CAKUT keeps expanding. Thus, CAKUT may also result from copy number variations (CNV) and mutations in genes, such as *SON*, regulating the splicing of CAKUT-causing genes (8,9). There is even a genotype-phenotype correlation at this level, with kidney anomaly cases being most enriched for exonic CNVs (8). Interestingly, genomic disorders causing CAKUT may also increase the risk of neurocognitive impairment,

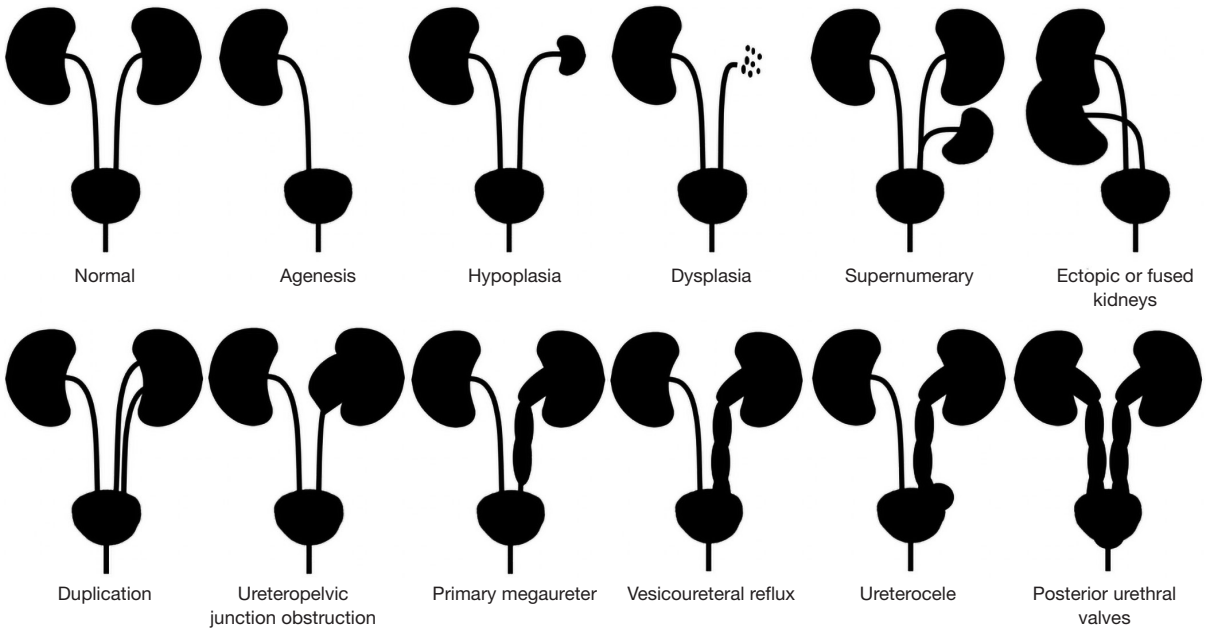

Figure 1 Key manifestations of CAKUT. Examples of key CAKUT manifestations. All manifestations shown to affect one kidney may also be bilateral. CAKUT, congenital abnormalities of the kidney and urinary tract.

whose early recognition can impact clinical care (10). CAKUT may also form part of a spectrum of extrarenal and kidney abnormalities with very variable expression in terms of frequency, severity and type of CAKUT (11). Hypospadias and LUTO are also part of the CAKUT, spectrum. Hypospadias is a common congenital anomaly of the external male genitalia, in which the urethral meatus is abnormally placed in a ventral position. The pathogenesis is considered multifactorial: it may be influenced by environmental factors that negatively affect androgenic stimulation, but it may be related to single gene mutations (12). Hypospadias may be part of syndromes associated with other CAKUT, tumors and other systemic manifestations; and may present as non-syndromic hypospadias (12). Hypospadias is not usually associated with progressive CKD (13). However, LUTO may be associated with progressive CKD. LUTO is a rare condition characterized by obstruction of the bladder outflow, leading to secondary retrograde dilatation of the urinary tract. The diagnosis may be made in utero, especially if severe, in childhood or, for milder forms, in adulthood, when it presents as repeated urinary tract infections. The most common anatomical cause of LUTO is the presence of posterior urethral valves that occurs only in males, at the level of the prostatic urethra. Another less frequent cause of LUTO is urethral atresia, which can occur in both sexes (4).

What are zinc finger proteins?

Zinc finger proteins are transcription factors that regulate gene expression by binding to DNA and regulate numerous physiological processes like cell proliferation, differentiation and apoptosis (14). Zinc finger proteins are characterized by the presence of zinc fingers. A zinc finger is a small protein structural motif that contains a zinc ion and binds specific DNA sequences known as GC boxes (14,15). The zinc ion is ligated to a combination of cysteines and histidines, thus stabilizing the folds of the fingers (16). Different types of fingers are recognized based on the number and order of these amino acids. Cys2His2 is the classic zinc finger, characterized by two cysteines in one chain and two histidines in other (14). Zinc finger proteins may also be classified according to their overall shape into Cys2His2-like, treble clef, and zinc ribbon (17). The crystallographic structure of the classical zinc finger has two β-sheets and one α-helix (14). A few amino acids in the α-helix that juxtaposes three base pairs on DNA confer the DNA binding specificity (14).

What is BNC2?

BNC2 is an extremely conserved Cys2His2 zinc finger protein orthologous to BNC1 (18). Genes encoding

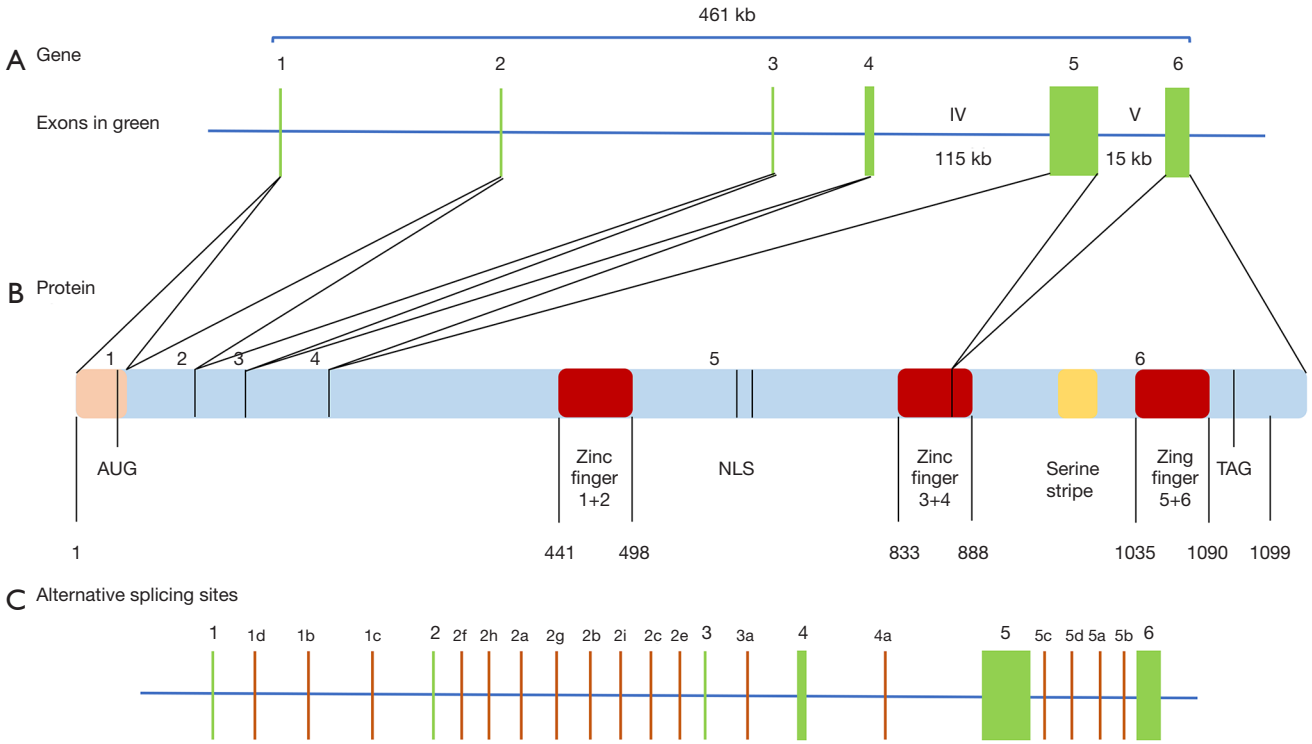

Figure 2 Structure and function of basonuclin 2 (BNC2). Location of mutations associated with different diseases. Murine *BNC2* gene and protein. (A) Gene; (B) protein: the 6 zinc fingers and the serine stripe are represented; (C) potential alternative splice sites that may originate multiple different proteins. Drawn from information found in (21,22). NLS, nuclear location signal.

both proteins differ in size and are located on different chromosomes, but they have a common evolutionary origin and BNC2 is thought to be older and to have remained largely invariant retaining its original function (19,20). The *BNC2* gene is located at 9p22 and contains six exons encoding a 1,099-residue protein with three pairs of zinc fingers, a putative nuclear location signal (NLS) and a serine stripe (19,21) (*Figure 2*). The 15th exon encodes the NLS and the first three zinc fingers and a part of the 4th zinc finger and the 6th exon encodes the remaining part of the 4th finger and the 5th and 6th zinc fingers (22). However, *BNC2* may undergo alternative splicing with 23 alternative exons and has the potential to generate 89,468 mRNA isoforms (22). This huge number of potential isoforms and the presence of multiple zinc finger pairs, each potentially binding to a different target sequence, may explain the pleiotropic effects of BNC2 (18,21). BNC2 localizes in nuclear speckles and has an additional presumed function in nuclear pre-mRNA processing (18,19). The tissue distribution of BNC2 is wide and it is abundant in testis, skin, kidney, uterus and intestine (21). In addition, to the disease associations, discussed below, in male gonocytes, BNC2 represses meiosis and mitosis and also regulates hair follicle cycles (23).

What are the disease associations of BNC2?

Genetic variants in *BNC2* have been associated with human disease, and in some cases, the relationship has been very well documented in functional animal studies (*Table 1*). *BNC2* single nucleotide polymorphisms (SNPs) have been associated with adolescent idiopathic scoliosis (28). At least one of the SNPs is functional and the susceptibility allele was associated with both higher binding to a transcription factor, YY1 (yin and yang 1), and higher BNC2 enhancer activity than the non-susceptibility allele. Furthermore, *BNC2* overexpression produced body curvature in developing zebrafish, supporting the relevance of the findings (29). Finally, *BNC2* loss-of-function mutations have been identified in diverse cancers (24). Although a tumor suppressor role has been proposed, the molecular mechanisms

Table 1 Disease associations of genetic variants and additional functions of basonuclin 2. General (non-nephrourological) disease associations and nephrourological disease associations are summarized. Additionally, functions not yet related to disease conditions are shown

General disease associations
Cancer (24-27)
Adolescent idiopathic scoliosis (28,29)
Skin color, skin aging and skin cancer (30)
Nephrourological disease associations
Lower urinary-tract obstruction (LUTO)/hypospadias (4,12,31,32)
Blood pressure (33)
Transplant tolerance (34)
Diabetes complications, including kidney disease (35)
Additional functions
Represses of meiosis and mitosis in male gonocytes (23)
Regulation of hair follicle cycles (23)

are unclear. Recently, *BNC2* overexpression was shown to upregulate interferon-stimulated and tumor suppressor genes and to cause growth arrest of cancer cells (25,26) while *BNC2* downregulation increased cancer cell survival (27).

Disruption of the *Bnc2* gene in mice causes neonatal death with cleft palate and craniofacial abnormalities (23). Genetic *BNC2* variants have been also associated with systolic blood pressure, the renal and retinal complications of diabetes (35), skin pigmentation (30) and better tolerance to liver and kidney transplantation (34).

What is the relationship between BNC2 and lower urinary tract obstruction and hypospadias?

However, until now, the most relevant disease association of BNC2 was a form of CAKUT, non-syndromic distal hypospadias (12,31,32). Non-synonymous variations in *BNC2* gene were found in 12.5% of American patients with hypospadias (32). Heterozygous pathogenic mutations in BNC2 where found in Japanese and Vietnamese patients (31). In this regard, $Bnc2^{-/-}$ mice displayed a high frequency of distal urethral defects that were also observed but with reduced penetrance in $Bnc2^{+/-}$ mice (32). In this regard, BNC2 is involved in urethral development. Preclinical data in newborn mice have demonstrated a high BNC2 expression in periurethral tissue (32). In a 7-week embryo, immunohistochemistry localized high BNC2 expression to the urogenital sinus, the precursor of the bladder and urethra, and, using in situ hybridization, high *BNC2* expression was demonstrated during lower urinary tract development (4). A high BNC2 expression is also observed in adult male urethra (4). Only recently, Kolvenbach *et al.* identified a truncating mutation in a family of four affected and a missense variant in a family of two affected members with LUTO with an autosomal dominant inheritance and varying degrees of phenotypic manifestations (4). Upon this finding, they re-sequenced 14 known *BNC2* transcripts in 697 patients with LUTO in the AGORA study of patients and from a multinational collaboration, and found a probably pathogenic *BNC2* variant and two variants of uncertain clinical significance in patients with urethral stenosis or posterior urethral valves (4). The hypothesis that BNC2 disruption indeed causes LUTO was tested in zebrafish, whose embryos expressed bnc2 in the pronephric duct and cloaca, analogs of the mammalian lower urinary tract. Indeed, zebrafish bnc2 knockdown using different methods caused pronephric-outlet obstruction and cloacal dilatation, thus phenocopying human congenital LUTO, and this was rescued by wild-type but not by mutated human *BNC2* mRNAs (4). Thus, Kolvenbach *et al.* have identified and characterized clear pathogenic gene variants causing LUTO with urethral blockade, but were unable to progress in identifying the molecular pathways leading to LUTO or the factors influencing the incomplete penetrance.

What else needs to be known?

Genetic variants in *BNC2* were identified as causing a specific form of CAKUT, LUTO. This will allow to screen for the risk of LUTO in predisposed families. However, the real challenge would be to develop new therapeutic approaches for LUTO or for other BNC2-associated diseases, based on this new knowledge. The answer to some question may allow to progress in this aim: what are the specific BNC2 isoforms implicated? What are the gene targets that are disrupted? And the cell processes involved? What background, genetic or environmental, influences the incomplete penetrance? How can BCN2 dysfunction be targeted during or after development?

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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