
The Professor Emeritus Visith Sitprija, MD, Ph.D. Endowed Lectureship: From Cloning to Clinic

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Short Biography

Current Selected Positions:

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Committee member, National Subcommittee on Rational Drug Use

Committee member, Patient, Personnel and People Safety National Steering Committee

Consultant, Mahidol scholarship program, Mahidol University

Thailand Quality Award (TQA) Assessor

Awards

Best Internship Award, Bhumibol Adulyadej Hospital (1985)

Outstanding Siriraj Alumni Award for Working alumni with success in every field (2021)

Suankularb Wittayalai Distinguished Alumni Award (OSK 96 - 2023)

Selected Publications

<https://shorturl.at/2qpk5>

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ตำแหน่งปัจจุบัน:

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คำสำคัญ: เลือดเป็นกรด; ยูเรีย; กระบวนการขนส่ง; โปรตีนขนส่ง; โคลนนิ่ง

The involvement of membrane transport proteins in the reabsorption and secretion of solutes, water, and inorganic ions in the kidney has been recognized for a long period of time. From the studies in the whole kidney down to isolated single-tubule perfusion, these investigations enable the detection, classification, and characterization of various transport systems along the distinct segments

of the kidney tubule. Nevertheless, a significant advance in the field of kidney transport was achieved by the introduction of molecular biology techniques to isolate, identify, and characterize membrane transport protein of interest (co-transporter, exchanger, channel, and pump) and to study its regulation/adaptation in physiologic and pathologic states.

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Expression cloning has been one of the most recognized methods in identifying many transport proteins in the past decades. By using the expression vectors to generate the complementary DNA (cDNA) library clones, with each clone containing one gene for one protein, this cDNA library can be screened to find the clones with specific transport properties in the heterologous expression system (e.g., *Xenopus laevis* oocyte). The clone of interest will be recovered for DNA sequencing and hypothetical structure predictions of the protein, for which structure-function analysis can be executed. The knowledge gained from these studies adds to our understanding of the complexity of the kidney tubule processes to maintain homeostasis and to make it accessible for clinical applications.

A few examples in this lecture, which directly or indirectly involved part of my research years in Japan and USA, demonstrate the impact of kidney transport protein cloning on clinical practice.

A Tool for Enhancing Understanding of Kidney Adaptation in Various Clinical Conditions

The first example is urea and the kidney. Historically, urea was the first biomarker used to assess kidney

function, and most nephrologists also use blood urea nitrogen for the assessment of nutritional status (as the end product of protein metabolism) and dialysis adequacy in end-stage kidney disease patients. The functional role of urea in urine concentrating mechanism was not well recognized till the first urea transporter (UT) in the kidney thin descending limb was identified by expression cloning (UT-A2), and subsequently led to the cloning of several related isoforms, including vasopressin regulated apical UT-A1, basolateral UT-A3, and descending vas recta UT-B1 (also in erythrocytes as the Kidd (JK) blood group and other organs). In mammals, all kidney-specific UT-A isoforms are derived by varying splicing from the UT-A gene (SLC14A2) and UT-B1 derived from the UT-B gene (SLC14A1). As a result, the regulation of UT expression in the kidney can be investigated in several conditions that mimic different clinical statuses. These studies highlighted the importance of UT in urine concentration and the relationship between water and nitrogen balance via urea reabsorption and accumulation in the kidney medullary interstitium, as summarized in Table 1 below.

Table 1 Urine urea transporters and their role in water and nitrogen balance

	UT Changes	Results	IM Urea Accumulation
ACUTE AVP EFFECT DEHYDRATION	UT1 Phosphorylation ↑UT2 expression ↓UT1 expression	↑IMCD P _{urea} ↑urea recycling IMCD P _{urea} →	↑ ↑
DIURESIS	↓UT2 expression ↑UT1 expression	↓urea recycling IMCD P _{urea} ↑	↓
LOW PROTEIN DIET	↑UT1 expression	↑urea absorption	BASE > TIP
HYPERCALCEMIA	↑UT1 expression	↑urea absorption	BASE > TIP
GLUCOCORTICOIDS	↓UT1 expression	↓urea absorption	↓

Given the importance of urine concentration, UTs are a potential target for novel drug discovery, particularly diuretic agents. Regarding the site of action in the terminal part of IMCD (UT-A1), it is likely that the compounds

that block urea reabsorption might cause urea induced water diuresis with fewer electrolyte disturbances. Previously known UT inhibitors, such as phloretin or mercurial agents, are relatively non-specific and may be

toxic at specified doses. The identification and validation of urea transporters as potential diuretic targets and the discovery of small molecule UT inhibitors as novel diuretics are under development.

A Candidate for Investigation in Inherited Diseases

The candidate gene approach studies DNA variation in genes known or suspected to play a role in a phenotypic trait of interest, and this is typically followed by functional analysis to confirm that the variant is pathogenic. As for transport protein disorders, the study requires prior information from molecular cloning and heterologous expression systems examining the transport properties. There are many examples, and one relevant to my experience is inherited renal tubular acidosis (RTA). It was conceivable that defects in proteins involved in renal acidification might be associated with RTA, which has been successfully proven by this approach. The culprits include

basolateral sodium-bicarbonate cotransporter NBCe1 for proximal RTA (by Takashi Igarashi, my Japanese colleague) and chloride-bicarbonate exchanger AE1 (or band 3) for dominant distal RTA and also recessive form in Thailand (the first study in collaboration with Thai doctors) and South-East Asia. Studies of AE1 expressed in *Xenopus* oocytes indicated that the corresponding mutations impair bicarbonate transport function. Still, only the G701D mutant causing recessive distal RTA can be rescued for the cell surface expression by erythrocyte Glycophorin A. This explains why the patients with a mutation in the gene that encodes both erythroid and kidney AE1 have normal red cell transport phenotype yet a kidney acidification defect.

Interesting inherited kidney diseases associated with defective membrane transport function resulting in kidney tubular secretion and/or reabsorption abnormalities are shown in Figure 1

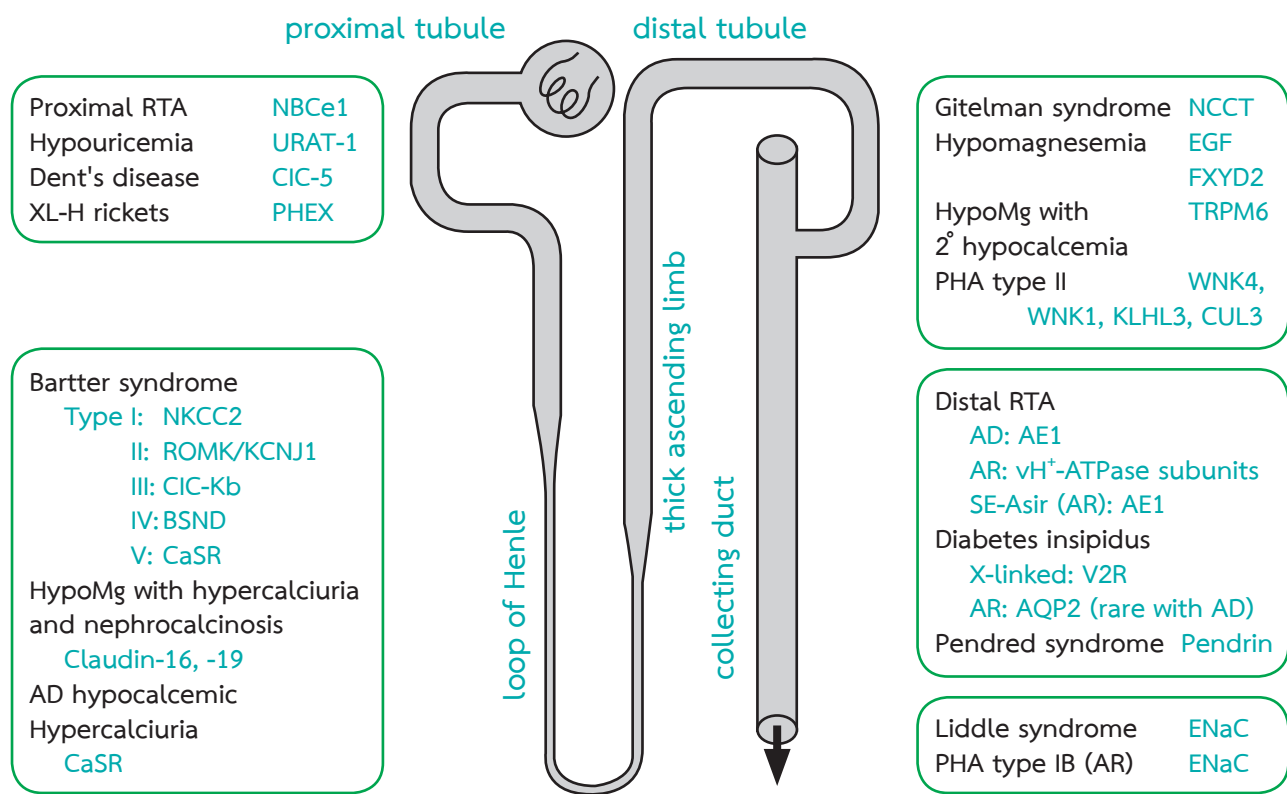


Figure 1 Inherited kidney diseases associated with defective membrane transport function

A target for drug development

Diuretics are among the most commonly used drugs directly targeted at the kidney transporter to produce therapeutic effects. Molecular cloning could accelerate drug discovery and protein by providing information on structure-function relationships and introducing recombinant transport proteins for expression and transport function analysis. Sodium-glucose cotransporter-2 (SGLT2) inhibitor is one of these drugs which has tested to be effective at inhibiting glucose reabsorption mediated by SGLT2 in the heterologous expression system and demonstrated later in the clinical setting with the effects on glucose control in T2DM by renal glycosuria.

SGLT2 (low affinity, high capacity) is the most prevalent and important SGLT subtype and accounts for more than 90% of glucose reabsorption at the early proximal tubule in normoglycemic persons. As a key mechanism for glucose homeostasis in the kidney, SGLT2 inhibitors have been considered promising agents for the treatment of type 2 diabetes after it was cloned and characterized more than 30 years ago (by Yoshikatsu Kanai, my Japanese colleague working in Matthias Hediger's Laboratory). While phlorizin, a naturally occurring competitive SGLT1 and SGLT2 inhibitor, cannot be used because of its short half-life and intestinal side effects, specific inhibitors of SGLT2 were designed and developed by many pharma industries with numbers of throughput and built-in technologies based on the mentioned strategy. Since the synthesis of phlorizin C-glucoside analogs was first studied in 2000 (6 years

after cloning), the first SGLT2 inhibitor was approved and marketed in Europe in 2012, and the United States Food and Drug Administration (FDA) committee also approved this drug for the treatment of T2DM in 2014 (around 20 years after cloning). Currently, the drugs become pillars of therapy for renal, diabetes, and heart failure patients.

Until now, many transport proteins have been characterized and identified, at both the genetic and the cellular level, which have been suggested to play a role in organ function and homeostasis and in the absorption, distribution, or elimination of endogenous, xenobiotic, or drug substrates. For most candidate drugs, an assessment of the role of transporter proteins in their therapeutic effects vs. pharmacokinetic variability and safety from drug-drug interactions needs to be considered.

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