Molecular Pathogenesis of Nephrogenic Diabetes Insipidus

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Congenital nephrogenic diabetes insipidus (NDI) is a rare inherited disorder characterized by inability to concentrate the urine in spite of the presence of vasopressin. Most of the patients, if early diagnosis and proper management are delayed, suffer from recurrent episodes of hypernatremic dehydration in neonatal or early infant period, which result in growth and developmental retardations. The mode of transmission is X-linked recessive in about 90% of the patients [arginine vasopressin V2 receptor gene (*AVPR2*) mutation] and autosomal recessive or dominant in less than 10% of the patients [aquaporin 2 (*AQP2*) gene mutation]. Part of AVPR2 missense mutations are known to translate mutant receptors which can not be translocated to the cell membrane normally (intracellular mistrafficking) and retained in the endoplasmic reticulum (ER) due to misfolding. These mutant proteins can be functionally rescued by using so-called chemical chaperones. In this study, the genetic analysis of the *AVPR2* and *AQP2* genes were performed in patients with clinical diagnosis of NDI. In addition, the functional rescue of the some mutant AVPR2 molecules using chemical chaperones was tried. Considering the limited effectiveness of the current pharmacological management of NDI and the practical difficulties in applying gene therapies, chemical chaperones can provide a novel and more easily applicable therapeutic strategy.

Key Words : Congenital nephrogenic diabetes insipidus (NDI), Arginine vasopressin V2 receptor, Arginine vasopressin V2 receptor gene (*AVPR2*), Aquaporin 2, Aquaporin 2 gene (*AQP2*), Chemical chaperone, Protein misfolding, Functional rescue of mutant protein

Introduction

Congenital nephrogenic diabetes insipidus (NDI) is a rare inherited disorder characterized by the insensitivity of the distal nephron to the antidiuretic effect of vasopressin[1]. Defect of urine concentration causes polyuria, hyposthenuria, nocturia, enuresis, functional obstruction and compensatory polydipsia[2]. The lack of response to exogenous vasopressin differentiates this disorder from central diabetes insipidus. NDI is frequently complicated by recurrent episodes of hypernatremic dehydration especially in neonates or early infants. So, if early correct diagnosis and proper management are delayed, most of the affected babies will have serious complications of growth and developmental retardations. According to a long-term clinical follow-up study, the intelligence was normal in only about 10% of NDI patients without early treatment[3]. Currently, NDI patients are managed by adequate hydration, low salt diet, and thiazide diuretic alone or in combination with a potassium sparing diuretic or a prostaglandin inhibitor[4]. However, these managements are not curative but only partial and symptomatic. So, many researchers are now studying some forms of novel therapy using genetic approach.

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Genetics studies

After the complicated processes of renal concentrating mechanisms including counter current multipli-

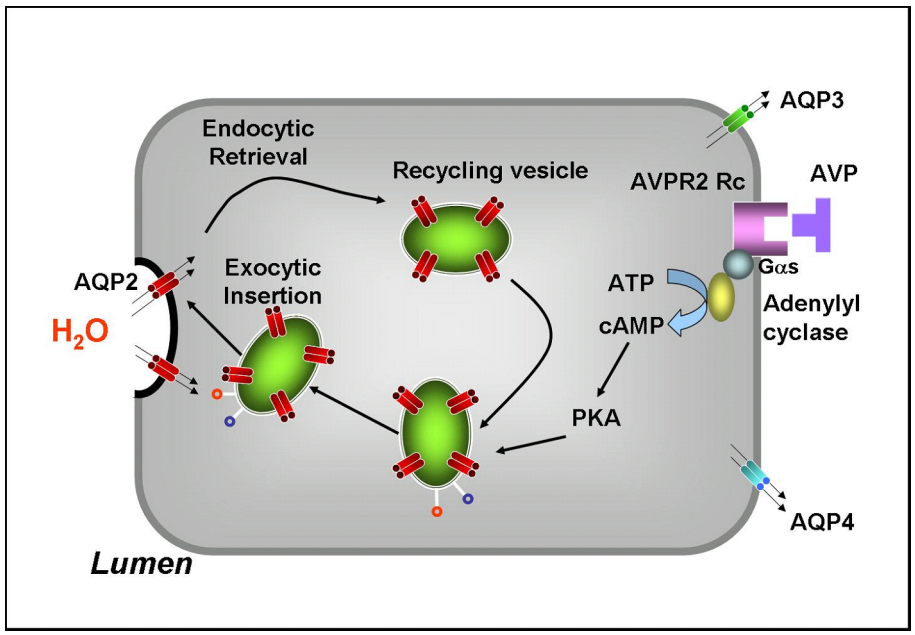

Fig. 1. *Roles of arginine vasopressin (AVP), V2 receptor (AVPR2) and aquaporin 2 (AQP2) in the process of water reabsorption in the renal collecting duct epithelial cell.*

Table 1. Mutations of *AVPR2* and *AQP2* Genes Detected in This Study

AVPR2 gene	Missense point mutations (8)	A98P, R113W, S167L, R202C T205N, L219P, L274P, P322L
	Deletion (3)	409delT, 93delG, 913del5
	Insertion (1)	315ins9 (duplication of 3 amino acids)
	Compound gene rearrangement (1)	
AQP2 gene	Missense point mutations (2)	A70D, R187H

cation and exchange, the final concentration of urine is determined in the collecting duct[1]. The epithelial cells of the collecting duct have arginine vasopressin V2 receptors at the basolateral membrane. If vasopressin binds with this receptor, the intracellular signaling pathways are activated and finally the aquaporin 2, the water channel, moves from the recycling endosome to the luminal side of plasma membrane which facilitates water reabsorption and increase the concentration of urine (Fig. 1)[5]. Genetically, about 90 % of the patients have the X-linked recessive mode of transmission associated with the abnormal arginine vasopressin V2 receptor gene (*AVPR2*) in chromosome X, and the rest are the autosomal recessive and dominant forms associated with abnormal the aquaporin 2 (*AQP2*) gene in chromosome 12[6]. Both genes are composed of only 3 and 4 exons, respectively. So, genetic tests, which are essential for early diagnosis during intrauterine period or right after birth, are rather simple and easy to apply clinically[7].

By the mutational analysis of *AVPR2* and *AQP2* genes in the patients with clinical diagnosis of NDI, we have found 13 hemizygous mutations in *AVPR2* and 2 heterozygous mutations in *AQP2* (Table 1). Among the 13 *AVPR2* mutations, 8 were missense point mutations, 3 were short deletions, 1 was duplicated insertion, and 1 was a compound rearrangement. Seven out of the 8 missense mutations were located in the transmembrane domains (TMDs) of the AVPR2 protein (Fig. 2), and 4 of those (A98P, L219P, L274P, and P322L) were novel mutations.

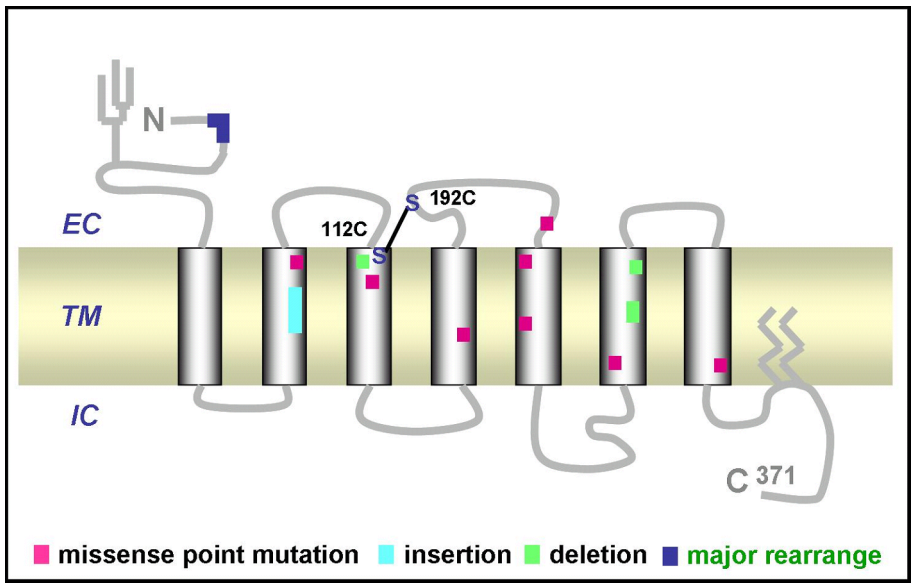

Fig. 2. *The location of 13 mutations of AVPR2 gene in the AVPR2 protein molecule (EC, extracellular; TM, transmembrane; IC, intracytoplasmic).*

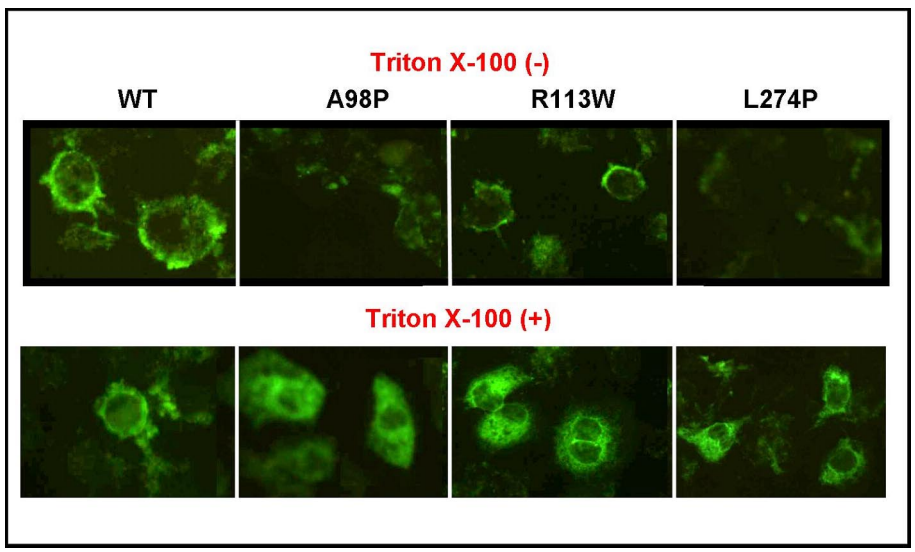

Fig. 3. *The cell surface immunofluorescent microscopic examinations of COS-7 cells transfected with the myc.AVPR2 fused recombinant vector. Upper panel; while the anti-myc antibody reacts with wild-type receptors normally expressed at cell surface, it can not react with intracellularly located A98P and L274P mutant receptors with intact cell membranes. Part of R113W mutant expresses at cell surface. Lower panel; if the cell membranes are permeabilized by triton X-100 treatment, the antibody can enter into cytoplasm and react with intracellular (inside the ER) misfolded/misrouted receptors.*

Intracellular synthesis, processing and trafficking of the AVPR2 molecule

In the epithelial cells of renal collecting duct, newly synthesized AVPR2, as same as other membrane proteins, is translocated into the lumen of the endoplasmic reticulum (ER) where the folding process of the receptor molecule occurs by the interaction with endogenous molecular chaperones. The properly folded receptor, then, can be transported from the ER through the Golgi complex to the cell surface[6]. If the receptor structure is altered by a mutation, the fold-

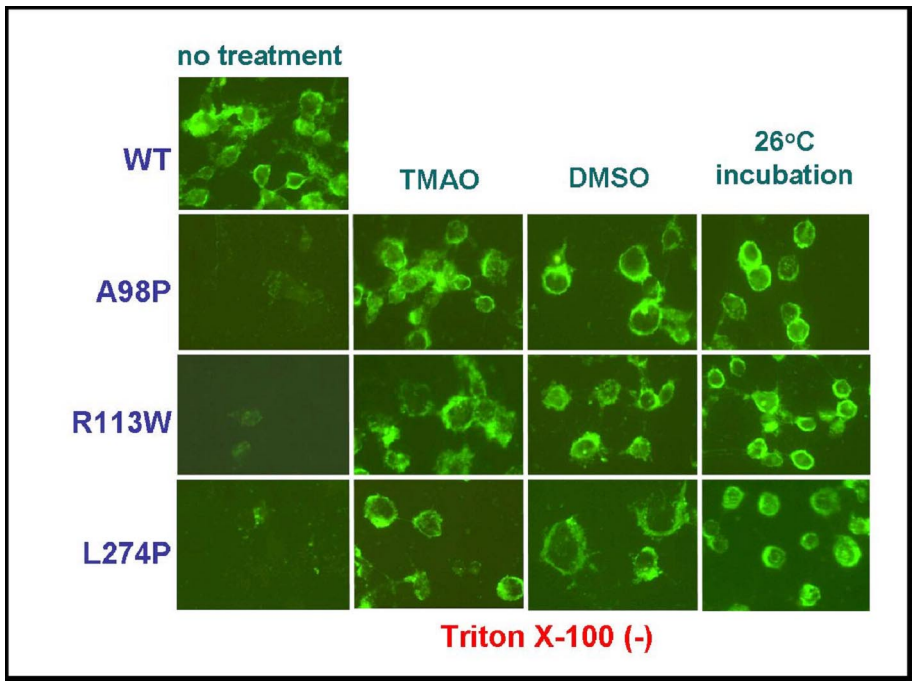

Fig. 4. *Rescue of mutant receptors using chemical chaperones. If the cells are treated with 100 mM TMAO or 2% DMSO for 4 hours or are incubated at 26℃ for 6 hours, most of the misfolded/misrouted mutant proteins can be successfully rescued from the ER to the cell surface. So, they can react with anti-myc antibody without cell membrane permeabilization.*

ing process is not completed successfully. And the misfolded receptor is retained in the ER but not routed to the cell surface[8]. Part of AVPR2 missense mutations, especially those located in the TMDs, are known to translate mutant receptors which are potentially functional but retained in the ER due to misfolding[1, 9].

Functional rescue of misfolded/misrouted proteins using chemical chaperones

Some of misfolded/misrouted proteins can be rescued from the ER to the cell membrane by a pharmacologic strategy using, so called, "chemical chaperones"[10, 11]. Chemical chaperones are a group of low molecular weight compounds which, by unknown mechanisms, facilitates proper folding and reverse the mislocalization of the mutant proteins. Polyols (glycerol), trimethylamines (trimethylamine N-oxide, TMAO), dimethyl sulfoxide (DMSO), 4-phenylbutyric acid, and some membrane-permeable enzyme antagonists, ligands or even substrates can be used as chemical chaperones.

We designed a study for the functional rescue of mutant AVPR2s detected by us.

Total RNAs were extracted from a piece of normal kidney tissue which was surgically removed during the operation of Wilms tumor in a 9-month-old male baby. Then, the whole length of the wild-type human AVPR2 cDNA was amplified by RT-PCR, and cloned into a plasmid vector. By site-directed mutagenesis, we obtained 3 clones of mutant AVPR2 cDNA, A98P, R113W and L274P. The sequences of the wild-type and mutant *AVPR2* cDNAs in the recombinant vectors were confirmed by direct sequencing.

Those wild-type and mutant *AVPR2* cDNAs were excised from the vector and were cloned into another 2 kinds of mammalian expression vectors together with the green-fluorescent protein (GFP) gene and a fragment of *myc* gene encoding 10 amino acids at the N-terminal side, respectively. Then, COS-7 cell, a mammalian cell line, was transfected with these recombinant vectors. The expressions and intracellular

localizations of GFP.AVPR2 or myc.AVPR2 fusion proteins in the transfected cells were checked using self-fluorescence of GFP and an anti-myc antibody, respectively. And, normal cell surface expression of wild-type receptors, absent or very weak surface expression of A98P and L274P mutant receptors, and partial membrane expression of R113W mutant receptors were confirmed (Fig. 3), i.e., most of A98P and L274P and part of R113W mutant proteins were misfolded and failed to be transported to the cell surface. If the cells were treated with several kinds of chemical chaperones (100 mM TMAO or 2% DMSO) for 4 hours or were incubated at 26℃ for 6 hours, most of the misfolded/misrouted mutant proteins were successfully rescued from the ER to the cell surface (Fig. 4). However, treatment of the cells with 1 M glycerol for 4 hours showed no effect (data not shown).

A next study to test whether the rescued mutant receptors will function normally at the cell surface will be performed soon.

Conclusions

Functional rescue of misfolded proteins using chemical chaperones have been tried in a wide range of disorder of protein misfolding and mistrafficking[12] including several other renal diseases such as autosomal recessive NDI associated with *AQP2* gene mutations[13, 14], X-linked hypophosphatemia (also known as vitamin D resistant rickets) associated with *PHEX* gene mutations[15], autosomal recessive steroid-resistant nephritic syndrome associated with *NPHS2* gene (encoding podocin) mutations[16], Finnish type of congenital NS associated with *NPHS1* (encoding nephrin) mutations[17], and Gitelman syndrome associated with *SLC12A3* gene (encoding thiazide-sensitive Na-Cl cotransporter) mutations[18]. And the list is rapidly increasing now.

Considering the current practical difficulties in applying gene therapies clinically, chemical chaperones can provide a novel and more easily applicable therapeutic strategy for many genetic disorders.

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