Aquaporin 2 and Nephrogenic Diabetes Insipidus

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Renal Diabetes Insipidus

Nephrogenic Diabetes Insipidus (NDI) is a condition characterized by production of excessive volumes of diluted urine, up to 20L per day. Common complications include dehydration, abnormal thirst, large dilatation of urinary tract and bladder that eventually can lead to chronic renal failure.

The condition has various causes such as deficient secretion of arginine vasopressin hormone, renal insensitivity to its effects, increased metabolism of vasopressin during pregnancy, or hereditary mutations of aquaporin 2 (AQP2) protein, a membrane protein that serves as a water channel. In the last case, symptoms can be observed within the first few weeks of infant's life. If left untreated, mental retardation occurs due to repeated episodes of brain dehydration and edema.

Diabetes insipidus is not related to diabetes mellitus. Both result in excretion of abnormally large volumes of urine which gave the origin to the Latin name.

Aquaporin 2

Aquaporin 2 (AQP2) is a protein found in apical cell membranes of the kidney's collecting duct principal cells. *AQP2* is encoded in chromosome region 12q13 and consists of four exons and three introns. The protein consists of 271 residues and forms a tetramer that contains four independent water channels. It has six transmembrane, three extracellular, and four cytoplasmic domains.

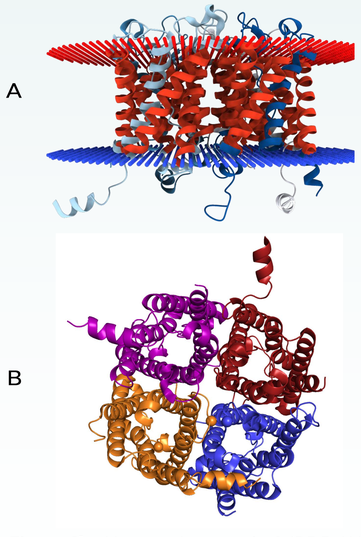

Figure 1[1] : Human Aquaporin 2 (PDB 4NEF)
(A) Transmembrane View
(B) Water Channels

AQP2 Function and Regulation

AQP2 is a water channel regulated by arginine vasopressin hormone that is secreted by posterior pituitary gland in response to dehydration. Vasopressin binds to its receptor (AVP2 also known as V2R) in the renal collecting ducts which then stimulates the production of cyclic adenosine monophosphate (cAMP). This causes the activation of protein kinase A (PKA) that phosphorylates AQP2. Phosphorylation is thought to stimulate vesicles containing the AQP2 protein to translocate. Once they reach the plasma membrane, AQP2 fuses with it increasing the number of the water channels and therefore resulting in a more effective urine concentration. Once AVP concentration drops, the AQP2 water channels are removed from the apical membrane and return to the cytoplasm via endocytosis.

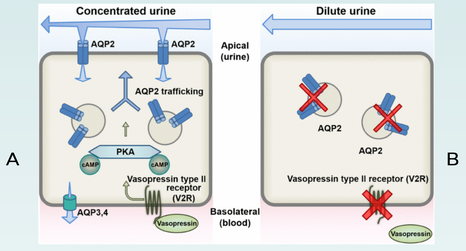

Figure 2[2] : Regulation of Permeability of the Collecting Duct
(A) Regulation pathways function properly
(B) Loss-of-function mutation in AVP2 or AQP2

Mutations

Mutations in genes encoding either arginine vasopressin (*AVP*), arginine vasopressin receptor 2 (*AVPR2*), or aquaporin 2 (*AQP2*) result in very similar phenotypes as they all inactivate the same regulatory pathway.

Mutated Gene	Type of Diabetes Insipidus
AVP	Neurohypophyseal
AVPR2	X-linked Nephrogenic
AQP2	Non-X-linked Nephrogenic

About 50 mutations inactivating AQP2 have been identified in 40 families. 65% of those mutations have been classified as missense mutations, 23% as frameshift mutations caused by nucleotide deletions or insertions, 8% as nonsense mutations, and 4 % are splice site mutations. They all result in loss of water only, as opposed to loss of water and ions which is the case in some other forms of diabetes insipidus.

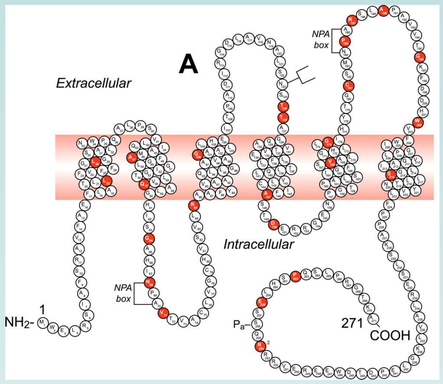

Figure 3[3]: Mutations Sites in AQP2
NPA box : Conserved asparagine-proline-alanine boxes
Pa : site of PKA phosphorylation
Orange shading of amino acid : location of the missense or nonsense mutations.

Loss-of-function mutations of *AQP2* described in literature cause NDI via several different mechanisms. Examples include decreased channel function, defective fusion with the plasma membrane, AQP2 misfolding, retention in the endoplasmic reticulum, rapid degradation, or AQP2 misrouting to lysosomes, endosomes, or basolateral plasma membrane where AQP3 and AQP4 should be instead.

C181W and R187C Study

One study[9] looked at point mutations found in patients to better understand the molecular basis for NDI. It was found that both C181W and R187C made the protein nonfunctional as it was unable to leave the endoplasmic reticulum. To investigate if there is any potential for cure, both mutants were incubated with glycerol which is considered to be a "chemical chaperone". While R187C showed almost complete redistribution and some water permeability, it was not the case for R181W which did poorly in both tests. This study highlights the complexity of the molecular basis of NDI and the challenges in developing treatments.

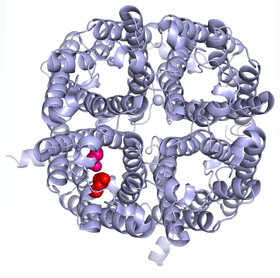

Figure 4 : Mutations in AQP2
Pink : C181W
Red : R187C

Treatment Options

Families with history of NDI are advised to undergo genetic screening. Since the symptoms start to show early in infancy, early diagnosis is very important for proper growth and development of newborns. Usual therapy as of today includes administering thiazide diuretics that inhibit salt reabsorption which stimulates fluid reabsorption in proximal tubules. This way, less fluid is delivered to the distal tubules and collecting ducts. Very low sodium diet is also prescribed but hard to maintain long-term.

When a mutation occurs in *AVP* gene, patients are typically prescribed desmopressin (marketed as DDAVP), which serves as a vasopressin analog. When the problem lies in inactive AVPR2 receptor, some experimental therapies involving *AQP2* upregulation via vasopressin-independent pathways or AVPR2 chaperones are currently under investigation with some success. However, the strategies for patients with mutations in AQP2 are not as straightforward or advanced. Some efforts have been made to look for suitable chaperons but without a major success. It was found that in mice with mutations in C-terminal portion of the protein, that is responsible for AQP2 translocation, a phosphodiesterase 4 (PDE4 or rolipram) reduced the symptoms of NDI. It was shown that this drug increased AQP2 phosphorylation without changing the overall amount of that protein present.

Take Home Messages

- Aquaporin 2 is a membrane protein that serves as a water channel
- It is activated by arginine vasopressin hormone binding to arginine vasopressin receptor 2
- Mutation in any of these three molecules results in similar phenotypes of diabetes insipidus
- Currently, there is no cure for NDI caused by mutations in AQP2

References

1. Frick, A., Eriksson, U.K., Mattia, de, F., Oberg, F., Hedfalk, K., Neutze, R., . . . Tornroth-Horsefield, S. (2014). X-ray structure of human aquaporin 2 and its implications for nephrogenic diabetes insipidus and trafficking. Proceedings of the National Academy of Sciences of the United States of America, 111(17), 6305-6310.
2. Ando, F., & Uchida, S. (2018). Activation of AQP2 water channels without vasopressin: Therapeutic strategies for congenital nephrogenic diabetes insipidus. Clinical and Experimental Nephrology, 22(3), 501-507.
3. Fujiwara, T., & Bichet, D. (2005). Molecular biology of hereditary diabetes insipidus. Journal of the American Society of Nephrology : JASN, 16(10), 2836-46.
4. Wilson, J., Miranda, C., & Knepper, M. (2013). Vasopressin and the regulation of aquaporin-2. Clinical and Experimental Nephrology, 17(6), 751-64.
5. Sohara, Eisei, Rai, Tatemitsu, Yang, Sung-Sen, Uchida, Keiko, Nitta, Kosaku, Horita, Shigeru, . . . Uchida, Shinichi. (2006). Pathogenesis and treatment of autosomal-dominant nephrogenic diabetes insipidus caused by an aquaporin 2 mutation. Proceedings of the National Academy of Sciences of the United States, 103(38), 14217.
6. Serena Milano, Monica Carmosino, Andrea Gerbino, Maria Svelto, & Giuseppe Procino. (2017). Hereditary Nephrogenic Diabetes Insipidus: Pathophysiology and Possible Treatment. An Update. International Journal of Molecular Sciences, 18(11),
7. Moeller, H., Rittig, S., & Fenton, R. (2013). Nephrogenic diabetes insipidus: Essential insights into the molecular background and potential therapies for treatment. Endocrine Reviews, 34(2), 278-301.
8. Tomoki Asai, Michio Kuwahara, Hidetake Kurihara, Tatsuo Sakai, Yoshio Terada, Fumiaki Marumo, & Sei Sasaki. (2003). Pathogenesis of nephrogenic diabetes insipidus by aquaporin-2 C-terminus mutations. Kidney International, 64(1), 2-10.
9. Tamarappoo, B., & Verkman, A. (1998). Defective aquaporin-2 trafficking in nephrogenic diabetes insipidus and correction by chemical chaperones. The Journal of Clinical Investigation, 101(10), 2257-67.