Translational Nephrology

Mouse models for congenital nephrogenic diabetes insipidus: what can we learn from them?*

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Introduction

To get rid of waste products and to keep blood ion concentrations and body waste products below toxic levels, our blood volume of 5-6 l daily passes the glomerular filter 30–40 times. In order not to dehydrate, this puts our kidneys to the enormous task of reabsorbing nearly 180 l of water from the pro-urine, for which Aquaporin (AQP) water channels are essential [1]. While AQPs in the proximal nephron account for 90% of constitutive water reabsorption, the remaining 15–20 l can be reabsorbed in the connecting tubule (CNT) and collecting duct (CD) and are under the tight control of the pituitary hormone arginine vasopressin (AVP). In these segments, AVP binding to the vasopressin type-2 receptor (V2R) increases AQP2 expression, phosphorylation by protein kinase A (PKA) at S256, and translocation from intracellular vesicles to the apical (pro-urinary side) membrane. As the apical membrane is the rate-limiting barrier, this insertion of AQP2 allows transcellular water reabsorption, which is driven by an osmotic gradient of sodium and urea, and production of concentrated urine.

The essential role of the V2R and AQP2 in the maintenance of body water homeostasis became clear when it was shown that mutations in their genes cause nephrogenic diabetes insipidus (NDI), a disorder in which the kidney is unable to concentrate urine in response to AVP [2–4]. Ninety percent of NDI patients have mutations of the V2R gene, which resides on the X-chromosome and is thus most prominent in males. In the remaining 10% of patients, NDI is caused by AQP2 gene mutations and can be inherited either as an autosomal recessive or as an autosomal dominant trait. AQPs are expressed as homotetramers, while a monomer forms the functional channel, and each monomer consists of cytosolic N- and C-terminal tails, connected by the core region, comprising 6 transmembrane domains and 5 connecting loops (A–E), in which loops B and E form the water pore. Interestingly, nearly all missense mutations found in recessive NDI are located in the core region and in vitro expression studies revealed that these mutations lead to misfolded and unstable AQP2 proteins that are retained in the endoplasmic reticulum (ER). Moreover, their inability to interact with wild-type (wt) AQP2 explains recessive NDI [2,5,6].

In contrast, all mutations found in dominant NDI are located in the AQP2 C-terminal tail. As the encoded mutants interact with wt-AQP2 and the mutations mostly introduce a missorting signal, the formed heterotetramers are misrouted to other organelles in the cell, resulting in insufficient amounts of AQP2 in the apical membrane, thereby explaining the dominant nature of the mutations [5,7–9]. Although these in vitro studies provided explanations, in vivo evidence supporting this was lacking. Recently, however, four congenital NDI mice strains saw the light [10–13], providing novel and important in vivo information on the role of AQP2 in water conservation in health and disease. In this commentary, I attempt to place these findings in perspective with the in vitro studies and NDI in humans.

Phenotype of NDI: similar in mice and man?

Through clever crossing of mice carrying a floxed third exon encoding the AQP2 E-loop with mice driving ubiquitous (EIIa promoter) or collecting duct specific (homeobox b7 promoter) expression of Cre recombinase, Rojek et al. [10] generated mice lacking functional AQP2 completely (AQP2-total-KO) or only in the collecting ducts (AQP2-CD-KO).
heterozygous mice, although this was clearly reduced significantly increase the urine concentration in the heterozygous mice survived. Moreover, dDAVP did compared with recessive NDI, the AQP2-763-772del and that the disorder is sub-clinically 'milder' as age of onset of dominant NDI in humans is often later

Consistent with the complete lack of functional AQP2 in the homozygous knock-in of an AQP2 mutant in recessive NDI, AQP2-T126M [14], AQP2-total-KO mice were identical to their healthy siblings at birth, but subsequently failed to thrive and died within 2 weeks, due to the severe urinary concentrating defect and massive contraction of extracellular fluid volume. Moreover, the kidneys showed papillary atrophy and an increase in pelvic space, which are clear signs of hydronephrosis. Figure 1 shows the location of the mutations in AQP2.

AQP2-CD-KO mice, which showed a nearly 100% absence of AQP2 in their CDs but clear AQP2 in the CNTs, survived to adulthood, but did show growth retardation and progressive hydronephrosis, which was due to a 10-fold increased urine volume compared with controls. Consistently, their urine osmolality was 10-fold decreased. Similar phenotypes were found for the other three congenital NDI mice [11–13], and even led to increased blood urea nitrogen levels, indicative of renal failure, in the AQP2-763ΔΔL NDI mice [13]. While growth retardation is a well-known feature of uncontrolled NDI in humans, hydronephrosis and renal failure have rarely been noted [15,16] and reveal that NDI is more severe in mice.

By homologous recombination of part of the last exon of the human AQP2 mutant (763–772del), Sohara et al. [12] generated the first mouse model of dominant NDI. In line with the observations that the age of onset of dominant NDI in humans is often later and that the disorder is sub-clinically ‘milder’ as compared with recessive NDI, the AQP2-763–772del heterozygous mice survived. Moreover, dDAVP did significantly increase the urine concentration in the heterozygous mice, although this was clearly reduced compared with wt mice. While an increased urine osmolality with dDAVP was also observed in their human counterparts and some other families with dominant NDI, this is not common with dominant NDI. Most interestingly, however, treatment of these mice with the phosphodiesterase(PDE)-4 inhibitor rolipram, but not PDE-3 or PDE-5 inhibitors, resulted in an increased urine concentration and probably (although this is not shown) decreased urine volume. If reproducible in man, PDE-4 inhibitors may be useful in treating dominant NDI.

Mechanism of NDI: similarities and surprises

The ‘ER’ glycosylation of AQP2-T126M in the inbred mice [14] and the ever-beautiful immunohistochemical figures of the Nielsen team, revealing a ‘cytosolic’ and weak expression of the AQP2 mutant in the AQP2-total-KO mice [10], show that misfolding, ER-retention and degradation also underlie recessive NDI in vivo (Figure 2). Moreover, the wt-mutant interaction and their missorting to the basolateral membrane in the AQP2-763–772del heterozygous mice illustrated that the mechanism underlying dominant NDI in vivo is also similar to the in vitro situation [12].

The other two mice, however, came with clear surprises. McDill et al. [13] found that NDI in their mouse strain was caused by an S256L mutation in AQP2, which, as they nicely showed, was due to the lack of S256 phosphorylation by PKA. Interestingly, in humans, the R254L mutation also causes NDI because it precludes AQP2 phosphorylation at S256 [17]. Surprisingly, however, the S256L mutation causes recessive NDI in mice, whereas the R254L mutation causes dominant NDI in humans. As anticipated from the location of the mutations in the C-terminal tail, both AQP2 mutants interacted with wt-AQP2. However, in contrast to our cell culture experiments, a considerable portion of wt-AQP2 (and possibly AQP2-S256L) was found in the apical membrane of the AQP2-S256L heterozygote. This clearly indicates that with AQP2 mutants interacting with wt-AQP2, recessive or dominant NDI depends on the relative strengths of the apical sorting signal in wt-AQP2 vs the missorting signal in the AQP2 mutant, and that this strength depends on the mutation and its location within the AQP2 C-tail. These data are consistent with our findings that the AQP2-P262L mutant causes recessive, instead of dominant, NDI in humans, because it is sorted to the apical membrane upon co-expression and interaction with wt-AQP2, while it is intracellularly retained when expressed alone [18].

An even greater surprise was found by Lloyd et al. [11], who discovered that recessive NDI in their mice was due to an F204V mutation. In line with its location in the AQP2 core region, this mutation mainly resulted in an ER-retained protein. However, this mutant was ‘reducedly’ misfolded, as some mature AQP2 mutant made it to the apical membrane, which explains why this mutation was not lethal to the mice. This ‘reduced severity of NDI’ in these mice seems similar to our
NDI patients encoding AQP2-V168M, as this mutant was also less ER-retained than other AQP2 mutants in recessive NDI, and several humans homozygous for AQP2-V168M had no or less severe NDI than most patients with recessive NDI [19].

Additionally surprising, but convincingly shown in vitro and in vivo by Lloyd et al. [11], AQP2-F204V also interacted efficiently with wt-AQP2 in the heterozygote, resulting in the targeting of the wt-mutant complex to the apical membrane. This reveals for the first time that an AQP2 mutant with a mutation in the core region can interact with and can be rescued in its cell surface expression by wt-AQP2. It remains to be determined whether and to what extent AQP2 mutants in recessive NDI in humans are able to interact with wt-AQP2.

The practicing nephrologists: how will this affect my clinical work?

Although congenital NDI is rare (1:100,000), it is important to identify it very early in life to prevent dehydration and its consequences (see earlier). Subsequent conventional treatment by giving adequate supply of fluid, combined with a decreased solute diet and administration of diuretics, such as hydrochlorothiazide and amiloride, to decrease the water excretion, are important and usually sufficient to prevent development of hydronephrosis (http://www.ndif.org [6]). Although mice develop a more extreme renal phenotype of NDI than usually encountered in humans, the above studies indicate that the underlying mechanisms and responses are quite similar. Based on these in vitro and in vivo data, it is therefore likely that there will be humans with ‘congenital NDI’ who have a mildly reduced urine-concentrating ability and intermediate urine volume production that remain sub-clinical under normal conditions, but may become apparent when patients are not able to consume sufficient amounts of water (e.g. anesthetized patients). Moreover, as NDI in mice is more severe than in humans, a congenital sub-clinically impaired urine-concentrating ability may then also be caused by mutations in genes causing non-lethal NDI in mice, as reviewed recently [6]. In addition, the data from Sohara et al. [12] indicate that dominant NDI might be relieved by treatment with PDE-4 inhibitors. While it remains to be established whether these inhibitors also relieve dominant NDI in humans, it is unlikely that they are of use in patients with recessive NDI.

As illustrated by Sohara et al. [12], the generated mice are ideal models to develop for therapies for congenital NDI. Because the anticipated therapies are probably specific for the different forms of NDI, genetic diagnosis of congenital NDI in patients becomes even more important.

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References


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