PROCEEDING

# Effects of natural point mutation of rat aquaporin 5 expressed *in vitro* on its capacity of water permeability and membrane trafficking

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**Abstract : In the colony of Sprague-Dawley (SD) strain, we found that there were rats expressing a mutant AQP5, which has a point mutation at nt 308 (G308A), leading to a replacement of $^{103}$Gly with $^{103}$Asp in the 3rd transmembrane domain. The mutant molecule scarcely expressed in the acinar cells, probably because of ineffective trafficking. The mutant molecule, however, showed normal water permeability when assessed by the oocyte system. J. Med. Invest. 56 Suppl. : 398-400, December, 2009**

***Keywords*** **:** *AQP5 mutant, trafficking, salivary gland*

## INTRODUCTION

AQP is a channel protein expressed in virtually all living cells. There are 13 members in mammals and they are generally responsible for rapid water movement across the plasma membrane in almost all cells (1, 2). AQP5, a member of this family proteins is expressed in the apical membrane of multiple secretory glands, including the lacrimal, salivary, and airway submucosal glands, type 1 alveolar cells (3, 4), sweat glands (5), corneal epithelium (6), and duodenal Brunner's gland (7). We found that the expression level of the AQP5 protein in the submandibular glands (SMG) was divergent among individual SD rats, and identified a point mutation in AQP5 gene of rats expressing AQP5 protein at low level. In the present study, we characterized this mutant AQP5 with respect to its ability to afford water permeability and to undergo membrane trafficking/translocation.

## METHODS

Water permeability of wild type and mutant AQP5 was determined by *Zenopus* oocytes osmotic assay. Western blotting, RT-PCR/real-time PCR were employed for analysis of AQP5 proteins and its mRNA. Trafficking of AQP5 protein was measured under a confocal laser scanning microscope by using the MDCKII cells transiently expressed with GFP-AQP5.

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## RESULTS AND DISCUSSION

A greater than 2-fold diversity in the expression level of aquaporin 5 (AQP5) protein has been observed in the membrane fraction of the SMG in SD rats (8). Breeding between brother and sister rats was repeated within high AQP5-producers and low ones to obtain inbred offspring. By Western blotting, levels of AQP5 protein in the parotid and lacrimal glands, and lungs were all low in low producers, whereas they were all high in high producers, implying genetic variations of the gene for this water channel. Despite this implication, AQP5 mRNA levels were almost the same between the 2 groups by Northern blotting and real-time RT-PCR, suggesting the irrelevance of transcriptional regulation for this diversity. AQP5 cDNAs from the SMGs of the 2 groups were sequenced. The nucleotide sequence of AQP5 cDNA from low producers indicated the existence of a point mutation at nt 308 (G308A), leading to a replacement of $^{103}$Gly with $^{103}$Asp in the 3rd transmembrane domain (Fig. 1) ; but no alteration was detected in the Kozak area (9). The existence of such a mutation was confirmed by the assessment of genomic DNA also. The mutant AQP5 expressed in *Xenopus* oocytes showed water permeability similar to those expressed by the normal molecule. The mutant and wild-type GFP-AQP5's

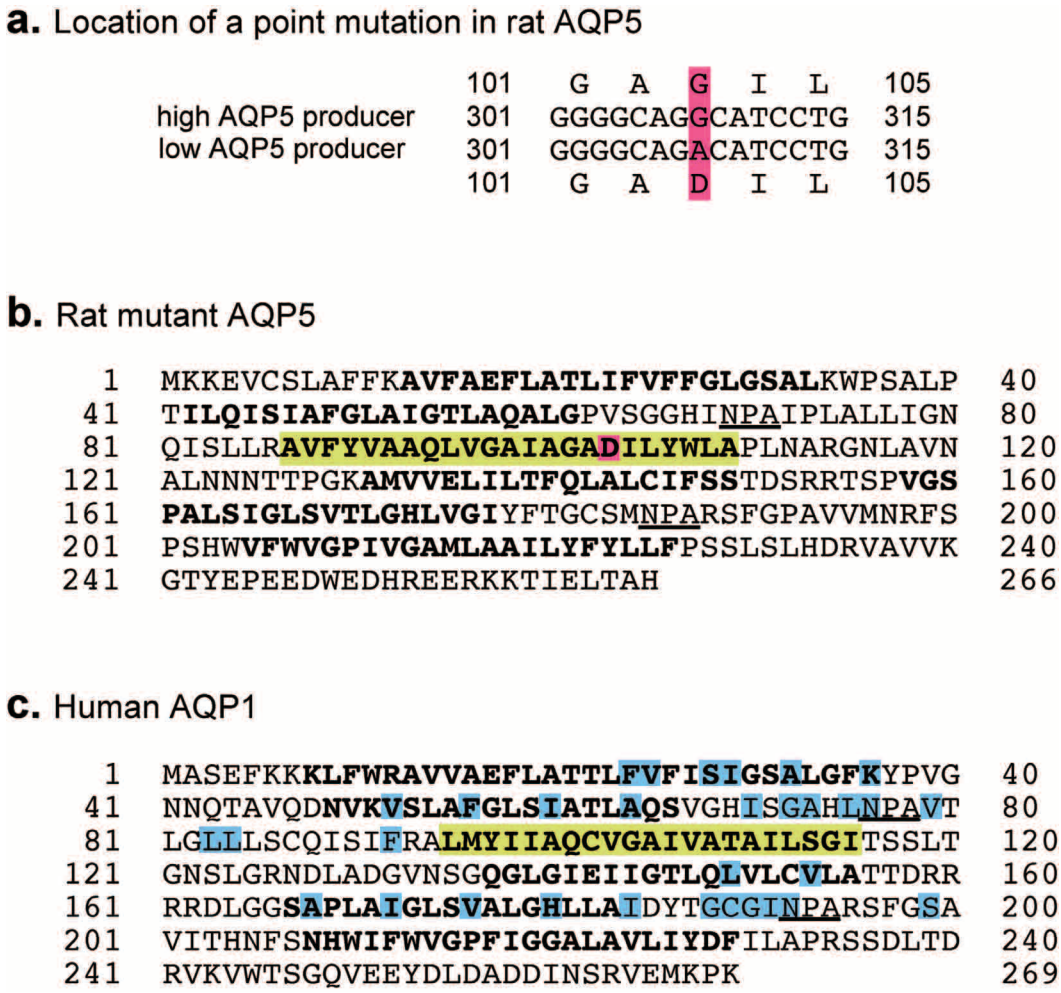


Fig. 1 Location of a point mutation in the AQP5 cDNA and the deduced amino acid sequence as compared with human AQP1. Point mutation is located in the 3rd transmembrane domain, where glycine is replaced with aspartic acid. Point mutation is located at the remote site from the aqueous pore in the membrane, implying that mutation may not affect the AQP5 function. From Murdiastuti, *et al*.(9)

Location of the point mutation in rat AQP5 molecule

Amino acid residue facing to the inside of the aqueous pore in the membrane From Murata, *et al*. (10).

Amino acid residues of the 3rd transmembrane domain, which are located at the remote site from the aqueous pore in the membrane.

Underlines, the NPA motif conserved throughout the family. Bold letter, trans membrane domains.

expressed in MDCK-II cells stayed in the cytoplasmic compartment by 12 h, and were then translocated to the apical plasma membrane at 24 and 48 h. During translocation, involvement of microtubules, but not phosphorylation of AQP5 at Ser/Thr PKA target motif ($^{152}$SRRTS) were suggested. At 24 and 48 h, the apical localization of mutant GFP-AQP5 was less than that of the wild-type molecule. Thapsigargin, an inhibitor of ER $Ca^{2+}$-ATPase, induced the rapid trafficking of AQP5 ; and the mutant molecule showed significantly reduced membrane trafficking comparing to the wild-type molecule (11). In frozen sections of the SMG from mutant rats, but not in those of the wild-type gland, a relatively large number of AQP5-positive structures appeared in the cytoplasm of the acinar cells, which structures were also immuno-positive for LAMP2, a lysosome-associated membrane protein, suggesting that most of the mutant AQP5 molecule entered lysosomes for degradation.

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