Review

NATURALLY OCCURRING AQP5 MUTATIONS IN RATS AND HUMANS AND THEIR AFFECTED PHENOTYPES

HOSOI Kazuo[1,2,] *, KARABASIL Ratko Mileva[3], MURDIASTUTI Kwartarini[4]

[1]Department of Molecular Oral Physiology, Division of Oral Science, Graduate School of Biomedical Sciences, Tokushima University, Tokushima-shi, Tokushima 770-8504, Japan;
[2]Kosei Pharmaceutical Co. Ltd., Osaka-shi, Osaka, 540-0039, Japan;
[3]Ritz Dental Clinic, Dubai Festival City, Dubai, United Arab Emirates;
[4]Department of Periodontology, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

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Abstract

Thirteen members of aquaporin (AQP), a water channel, are expressed in mammals. In this review, we briefly overview these mammalian AQPs, then focus on AQP5, an exocrine gland-type AQP. Namely, we discuss: (1) the mechanism for coupling of AQP5 dynamics with the secretion and restoration cycle of amylase after isoproterenol (IPR) in the parotid gland (PG); (2) roles of parasympathetic nerve for maintaining AQP5 level in the submandibular gland (SMG), and; (3) AQP5 down-regulation in an experimental pathological model by LPS administration in the PG. We then move to the effects of single nucleotide mutation (SNP) found in rats and humans and its affected phenotypes. That is, G308A point mutation found in rat *AQP5* cDNA resulted in amino acid substitutions of Gly103 for Asp103, and causes diminished expression of its protein product. In humans, several SNPs in *AQP5* are found in European and Chinese families and cause autosomal-dominant diffuse nonepidermolytic palmoplantar keratoderma.

Key Words: aquaporin 5 (AQP5), salivary glands, single nuclear polymorphism (SNP)

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*Corresponding author – e-mail: khosoi@tokushima-u.ac.jp

Abbreviations: AQP, aquaporin; CHIP, channel forming integral protein; CTD, chorda tympani nerve denervation; IPR, isoproterenol; LPS, lipopolysaccharide; Lamp2, lysosomal associated membrane protein 2; MAPK, mitogen-activated protein kinase; MIP, major intrinsic protein; NF-$\varkappa$B, nuclear factor-kappa B; PG, parotid gland; SNP, single nuclear polymorphism; SDS/PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SMG, submandibular gland; TNF-α, tumor necrosis factor-α; TNFR1, TNF receptor 1.

INTRODUCTION

One of the characteristics of cell membranes is their semi-permeability. This feature allows water to enter and exit cells. However, epithelial cells in tissues such as mammalian renal collecting tubules, amphibian skin and bladder are much more water permeable than other tissues. Therefore, the existence of other mechanisms that enable such high permeability was suspected but has long been unsolved.

Aquaporin (AQP) is a membrane protein with a serpentine-type structure which functions as a water channel. It is widely distributed in the living world, from microorganisms to plants and animals, and is intimately involved in water metabolism, essential in living organisms. The first water channel, "channel forming integral protein 28 (CHIP 28)", was cloned from erythrocytes by Agre and his collaborators in 1992 (Preston et al., 1992). Thereafter, CHIP was renamed as aquaporin (AQP) and the name AQP1 was given to CHIP 28 (Agre and Chrispeels, 1993). In the same year, Sasaki's group cloned the second AQP, AQP2 from the kidney (Fushimi et al., 1993). Thereafter, Fujiyoshi et al. succeeded in the X-ray crystallography of the AQP1 molecule and clarified the whole molecular structure (Murata et al., 2000).

On the other hand, a major intrinsic protein (MIP/MP26/MIP26) of the lens in humans had been reported before the discovery of AQP1 (Pisano and Chepelinsky, 1992), while its function remained unknown. Since MIP26 afforded permeation of water and was highly homologous to members of the AQP family, it was later considered to be an AQP (Mulders et al., 1995). Thus the protein and gene of MIP26 became to be referred as AQP0 (Mitton et al., 1996). At present, there are 13 molecular species (AQP0-12) in the mammalian AQP family (Hosoi, 2016).

The general characteristics of this channel molecule are as follows: (1) It has a tandem repeat structure with six transmembrane domains, and usually presents as a tetramer. (2) There are Asp-Pro-Ala sequences (NPA motifs) conserved among family members; the NPA motif exists at two locations in the molecule, which form hemichannel structures. Hemichannels face each other from inside and outside of the cell, forming a pore by which water can pass through in the molecule. (3) In the AQP molecule, phosphorylation target motifs as well as glycosylation sites are present, which could be involved in functional regulation of water channel. (4) AQPs can be classified into four subfamilies; i.e., water-specific channels (AQP0, 1, 2, 4, 5 and 6), water and glycerin transport channels (AQP3, 7, 9 and 10), super AQP (AQP11 and 12), and a short-chain N-terminal aquaporin (AQP8). The water movement across the biological membrane by AQP is simply dependent on osmotic gradient.

In this review, we briefly overview localization and physiological function of each member of mammalian AQPs, then focus on AQP5 of the salivary gland and other tissues in rodents and humans. We will particularly focus on the effects of the *AQP5* gene alterations such as gene knock-out, single nucleotide polymorphism (SNP) on its physiological function.

Classifications and functions of mammalian AQPs

Water-selective aquaporins (AQP0, 1, 2, 4, 5, 6)

AQP0 is expressed in the lens tissue of the eye. AQP0 controls osmotic substances and water content of the lens tissue. Gene abnormality results in cataract development (Berry et al., 2000; Francis et al., 2000), which frequency increases with age (Gu et al., 2007; Geyer et al., 2006).

AQP1 was initially found in the erythrocyte, a highly water-permeable cell (Preston et al., 1992). AQP1 is also present in the kidney proximal convoluted tubule and descending limb of Henle's loop and is involved in counter current concentration of urine. Its deletion causes defective urinary concentration (Papadopoulos and Verkman, 2005). AQP1 is also localized in the lung (vascular endothelium in pulmonary alveolus). Lung AQP1 is important for aspiration of the amniotic fluid at birth. Defect in AQP1 shows a protective action against lung edema (King et al., 1996).

AQP2 was cloned from the kidney (Fushimi et al., 1993) and is shown to be localized in the luminal membrane of kidney collecting duct cells. Long-term action of vasopressin regulates gene expression of *AQP2 via* cAMP. Short-term action of vasopressin induces trafficking of AQP2-bearing vesicles from the cytosol to the apical membrane (DiGiovanni et al., 1994). Mutation of *AQP2* gene in humans causes nephrogenic diabetes insipidus (Guyon et al., 2009). A possibility is suggested that abnormal up-regulation of vasopressin or its receptor affects movement of inner ear AQP2, causing endolymphatic hydrops (Meniere's disease; Maekawa et al., 2010).

AQP4 was cloned from the lung and brain independently (Hasegawa et al., 1994; Jung et al., 1994); brain cDNA library was reported to express a specific *AQP4* cDNA in rather high amounts (Jung et al., 1994). The major AQP4-expressing tissues in the brain are capillary, synaptic space, and endfeet of astrocytes adjacent to the node of Ranvier. Cytotoxic brain edema is relatively mild in AQP4-deficient mice, but it aggravates vasogenic edema by causing leakage of cerebrospinal fluid (Papadopoulos et al., 2005). AQP4 is involved in formation and disappearance of edema in the brain. AQP4 in astrocytes plays important roles in brain edema due to brain ischemia, injury, inflammation or metabolic failure. Recently, evidence has accumulated suggesting that AQP4 is involved in a variety of functions, including control of extracellular volume, potassium buffer, cerebrospinal fluid circulation, interstitial regulation, water absorption, waste removal, neuroinflammation, osmotic stimulation, cell migration, calcium signaling etc. (Nagelhus and Ottersen, 2013).

AQP5 was fist cloned from the salivary gland (Raina et al., 1995). AQP5 is involved in fluid secretion from the salivary glands and lung (Verkman, 2007; Ma et al., 1999). The salivary gland AQP5 level is regulated by the autonomic nervous system under normal conditions (Azlina et al., 2010; Li et al., 2008), while lipopolysaccharide down-regulates AQP5 expression *via* the nuclear factor-kappa B (NF-κB) pathway (Yao et al., 2010; described later in detail).

AQP6 is expressed in the kidney collecting duct cells. AQP6 is localized in vesicles inside the collecting duct cells and has been suggested to play a role in anion-channel function (Yasui et al., 1999). The anion transport activity of AQP6 is high at low pH, whereas its water permeability is low. This water channel is possibly involved in acid-base balance (Promeneur et al., 2000; Yasui et al., 1999). AQP6 is found also in the retina (Iandiev et al., 2011), acinar cells of parotid gland (PG) (Matsuki-Fukushima et al., 2008), and inner ear (Taguchi et al., 2008).

Aquaglyceroporins (AQP3, 7, 9, 10)

AQP3, having been cloned from the kidney, is the first AQP gene shown to participate in transport of glycerol and urea (Ishibashi et al., 1994). AQP3 is also expressed in the cell membrane of keratinocytes in the basal layer of the epidermis, where it has an important role in glycerol transport. In the normal skin, its expression is elevated in response to skin stress in diseases such as atopic eczema. AQP3 helps to supply water to the skin and provides elasticity. Its deficiency results in failure of wound healing and formation of new skin (Hara-Chikuma and Verkman, 2005).

AQP7 and 9 are involved in glycerol transport. AQP 7 is expressed in fat tissues and 9, in the liver at sinusoidal surface. In the fat tissue, *AQP7* mRNA decreases and increases during food intake and fasting, respectively. This change accords with changes in the serum glycerol level, and is opposite to the changes in the serum insulin concentration. AQP9 is supposed to be a glycerol channel in liver cells. Transcriptional activities of *AQP7* and *9* are both negatively regulated by insulin (Kishida, et al., 2000; Kishida et al., 2001; Tsukaguchi et al., 1999).

AQP10 is expressed in the duodenum and jejunum. AQP10 transports water and glycerol in these digestive organs (Ishibashi et al., 2002).

Super-aquaporins (AQP11, 12)

AQP11 is expressed in the testis, liver, and kidney proximal convoluted tubules. Its function is unknown. Animals deleted of AQP11 by gene knockout develop polycystic kidney disease and die within two months (Morishita et al., 2005).

AQP12 is expressed in the pancreatic acinar cells and is localized in the cytosol of these cells. Based on knockout data, this channel protein has been implicated in exocrine water secretion. (Ohta et al., 2009; Itoh et al., 2005).

Aquaporin with short N-terminal chain (AQP8)

AQP8 is expressed in the large intestine, pancreatic acinar cells, and liver. SNP found in *AQP8* could be associated with polycystic ovary syndrome among Chinese Han women (Li et al., 2013).

Roles of AQP5 in water secretion from the salivary gland

As described earlier, cDNA for *AQP5* was first cloned from the salivary gland; it is known as an exocrine-type water channel with a unique tissue expression (Raina et al., 1995). In experiments using Northern blot analysis and *in situ* hybridization, strong expression of *AQP5* occurs in many exocrine gland tissues, i.e., the salivary gland, eye, lacrimal gland, lung, and trachea (Raina et al., 1995; Lee et al., 1991). Parvin et al. also showed the expression of AQP5 in the duodenum Brunner's gland (Parvin et al., 2005; Parvin et al., 2002). AQP5 is thought to play a fundamental role in the water movement for formation of saliva, tears, and other exocrine secretion (King and Agre, 1996). An *AQP5* knockout experiment conducted in mice resulted in production of saliva that was significantly hypertonic, viscous, and smaller in volume. This fact directly indicates that AQP5 has an essential role in saliva secretion (Ma et al., 1999).

Dynamics of the salivary gland AQP5 under normal physiological conditions

It was revealed that AQP5 is involved in water secretion in the salivary glands. For better understanding of the physiological roles of this water channel, it is important to reveal dynamic changes of the salivary gland AQP5 under different physiological conditions. Some of such studies are described below. The salivary gland is innervated by both sympathetic and parasympathetic nerves. The parasympathetic nerve that innervates the submandibular gland (SMG) is a chorda tympani nerve, the center of which is located in superior salivary nucleus in the medulla oblongata. The experimental results of chorda tympani nerve denervation (CTD, parasympathectomy) suggest the AQP5 protein level in the SMG is regulated by the parasympathetic nerves/M3 muscarinic receptor agonist not at transcriptional level but by a post-transcriptional mechanism (Li et al., 2008). Furthermore, CTD showed a gradual increase in the amount of lysosomal-associated membrane protein 2 (Lamp2), a lysosomal marker in the SMG. Confocal immunohistochemical analysis disclosed an increase in number of lysosome-like structures positive for both AQP5 and Lamp2 in the SMG acinar cells after CTD. This suggests that AQP5 enters autophagosomes and/or lysosomes by CTD for degradation (Azlina et al., 2010).

On the other hand, β-adrenergic agonists and their second messenger, cAMP, mimic amylase secretion from the PG (Nederfors et al., 1994; Baum, 1993). Thus, the effects of isoproterenol (IPR), a β-adrenergic agonist, on AQP5 in the mouse PG were examined (Chen et al., 2014). The results suggest a rapid appearance of AQP5 in the plasma membrane is induced by IPR, and such appearance is thought to be

the result of exocytotic translocation of AQP5 from the granule membrane to the plasma membrane. This result agrees well with the research showing that expressions of *AQP5* mRNA and protein are increased by cAMP and its derivatives in MLE-12, a cultured mouse lung cell line (Yang et al., 2003).

These results suggest the AQP5 levels in the SMG is maintained by the parasympathetic nerve, and that this water channel in the PG is translocated to the apical membrane in conjunction with trafficking/docking of secretory granules containing amylase upon stimulation with β-adrenergic agents (Hosoi et al., 2020).

Down-regulation of salivary gland/lung AQP5 under pathologic condition

Yao et al. found that lipopolysaccharide (LPS) downregulates the expression of AQP5 in the cultured PG *in vitro*, and studied its mechanism (Yao et al., 2010). They showed that repression of transcription of *AQP5* by LPS was completely suppressed by PDTC and MG132, which are an I-𝜘B kinase inhibitor and a proteasome inhibitor, respectively. Therefore, this result reveals that I-𝜘B, accumulated in the presence of each of these inhibitors, produced free NF-𝜘B, which consequently downregulates *AQP5* transcription. However, in addition to this fact, transcriptional repression of *AQP5* by LPS was completely blocked by the mitogen-activated protein kinase (MAPK) inhibitors AG126 and SP600125 (inhibitors of ERK1/2 and JNK, respectively), resulting in inhibition of phosphorylation of c-Jun and c-Fos. Thus, both the NF-𝜘B pathway and the MAPK pathway are thought to be involved in repression of *AQP5* transcription by LPS.

Furthermore, another fact revealed from this study is that suppressing the production of any one of these three transcription factors, pc-Jun, pc-Fos and NF-𝜘B, un-suppresses the LPS-induced down-regulation of AQP5, implying that all three of these transcription factors are indispensable for AQP5 down-regulation by LPS (Yao et al., 2010). Actually, immunoprecipitation experiments using antibody column verified that pc-Jun, pc-Fos and NF-𝜘B form a complex.

In the lung, AQP5 expression is reduced by acute viral infection; tumor necrosis factor-α (TNF-α), which is elevated by viral infection, is considered to be a mediator of this reduction (Towne et al., 2000). By using MLE-12 cultured lung epithelial cells, transcription of *AQP5* mRNA was shown to be suppressed by TNF-α *in vitro*. The NF-𝜘B pathway mediated by TNF receptor 1 (TNFR1) is involved in this down-regulation (Towne et al., 2001). The MAPK system is generally activated in the signal transduction system mediated by TNFR1. However, they argue that the MAPK system is not involved in the suppression of *AQP5* mRNA transcription by TNF-α in MLE-12 cells. Anyway AQP5 in the PG and lung is negatively regulated under pathologic conditions where NF-𝜘B pathway is involved.

Single nucleotide polymorphism (SNP) of rat *AQP5* gene

It is well known that abnormalities found in some members of the *AQP* genes cause various diseases and pathological conditions. Some of these examples have been mentioned in the previous section. Also, *AQP5* gene knockout is shown to cause exocrine gland dysfunction. We found naturally occurring SNP in the *AQP5* gene in rats. We considered that rat SNP would be a useful model for analysis of human mutant *AQP5* gene when similarly abnormal *AQP5* gene is discovered in humans in the future. We thus analyzed the naturally occurring *AQP5* mutant gene/protein product and its phenotype in rats.

Discovery of AQP5 mutant in rats

First, Murdiastuti et al. performed Western blotting of AQP5 in the SMG of male rats and found the expression level of this protein (AQP5) differed among individuals (Murdiastuti et al., 2002). This polymorphism did not change with age in the same animals and was also found in females. However, the levels of AQP1, another AQP, expressed in the SMG of the same animal did not differ among individuals. AQP5 high-producing rats and low-producing rats were separately mated and AQP5 expression levels in the third generation off-spring were examined. AQP5 production in the PG, lung, lacrimal gland and the SMG was high in AQP5 high-producing rats and low in low-producing rats. These results suggest differences in AQP5 production among individuals are genetically determined. Actually, after repeated mating of high- and low-producing rats, big differences in AQP5 levels in off-springs are found between them even in the 20th and 19th generations (Fig. 1.; Karabasil et al., 2011); thus they are referred to as wild-type and mutant rats, respectively.
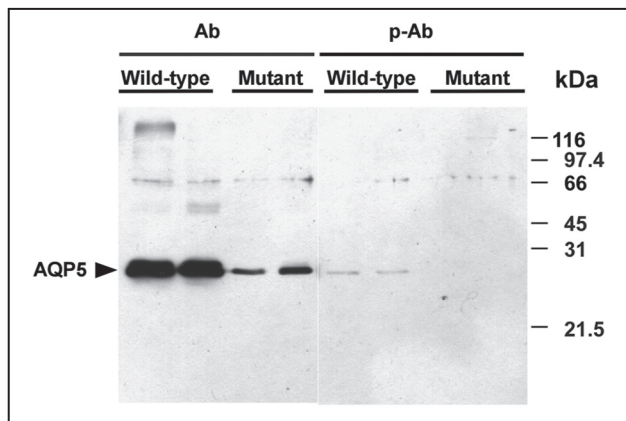

Figure 1. Analysis of AQP5 protein in the SMG of wild-type and mutant rats by Western blotting. The SMG AQP5 levels of 20th- and 19th-generation progenies of wild-type and mutant SD rats were analyzed. Total membrane fraction (5 μg protein) prepared from the SMG was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE), followed by Western blotting. Ab, antiserum; p-Ab, peptide-preabsorbed antiserum (Data from Karabasil et al., 2011).

Effects of AQP5 mutation on salivary secretion

Rates of saliva secretion under un-stimulated condition and upon pilocarpine stimulation were significantly lower in AQP5-mutant rats. Furthermore, water intake was significantly higher in AQP5-mutant rats than in wild-type rats (Murdiastuti et al., 2006). These results are in good agreement with the results of the *AQP5* knockout experiment (Ma et al., 1999), indicating that AQP5 is closely associated with salivary secretion (Murdiastuti et al., 2006; Ma et al., 1999).

Localization of AQP5 mutant protein and the level of its mRNA in the salivary gland

Localization of AQP5 mutant protein in the salivary glands and the level of mutant *AQP5* mRNA were analyzed. As shown in Fig. 2A, AQP5 was localized throughout
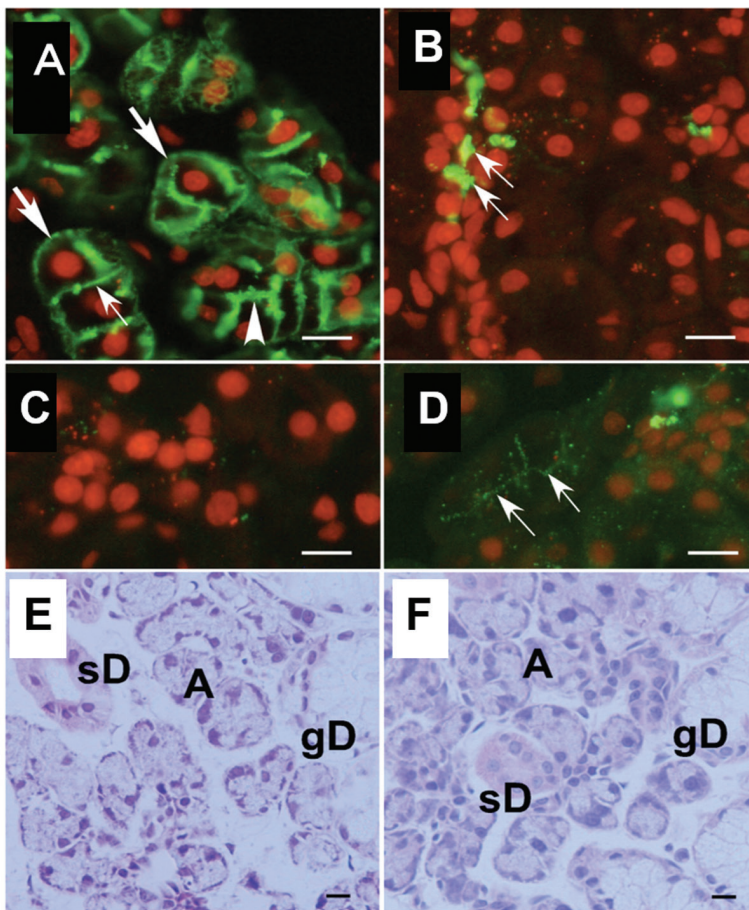

Figure 2. Immunohistochemical localization of AQP5 in the SMG of wild-type and mutant rats. Cryostat sections of the SMG were incubated with anti-rat AQP5 antiserum. In the wild-type (**A**), the fluorescence image of FITC indicating the presence of AQP5 is seen on the entire acinar cell membrane,

which includes apical, lateral (small arrow), and basal (large arrows) aspects. The arrowhead in (**A**) indicates the irregularly shaped intercellular secretory canaliculi. In the mutant (**B**), the strong fluorescence signal is seen at only limited areas of the apical membrane in very few cells (small arrows). The rest of apical and lateral membranes expressed AQP5 very weakly (small arrows) as revealed by the same section exposed for longer time (**D**). The section from a wild-type reacted with the anti-AQP5 antibody preabsorbed with its antigen peptide did not show positive reactions (**C**). Hematoxylin-eosin staining of the section showed very similar morphology between wild-type and mutant (**E** and **F**). A, acini; sD, striated ducts; gD, granular duct. Bars, 20 μm. (Data modified from Murdiastuti et al., 2006)

the entire cell membrane of acinar cells in wild-type rats. However, in the mutant rat, only a very small amount of AQP5 was expressed in a limited area of the acinar cell membrane (Fig. 2B), while the rest of the apical and lateral membranes expressed AQP5 very weakly (small arrows) as revealed by the same section exposed for longer time (Fig. 2D; Murdiastuti et al., 2006; Karabasil et al., 2011). These results and the above-mentioned Western blotting data well support the supposition that the production of AQP5 protein is extremely reduced in the mutant SMG.

The *AQP5* mRNA levels of the wild-type and the mutant were, therefore, analyzed by Northern blotting and real-time reverse transcriptase-polymerase chain reaction (RT-PCR); there was no significant difference in the expression level of *AQP5* mRNA between wild-type and mutant rats. These results revealed that decrease in the amount of AQP5 protein produced in the mutant is not due to decrease in transcription activity.

A Kozak sequence (typically, GCCRCCaugG) exists immediately before the translation initiation signal, and is believed to be important for recognition of the translation initiation site. In rat *AQP5*, there was a GGCACCaugA sequence in both wild-type and mutant rats, and no sequence change was observed in mutants. It is considered that translation activity is probably not affected in the mutant rat. The marked decrease in AQP5 protein level and abnormal localization in acinar cells suggest the mutant protein molecule could be rapidly metabolized and degraded by the cell quality control system (described later).

Gene analysis of mutant AQP5

Analysis of the nucleotide sequence of the *AQP5* mutant cDNA reveals that the mutant gene has a G308A point mutation (Fig. 3A; Murdiastuti et al., 2006). The same is true for the genomic DNA. As a result, amino acid substitution of the hydrophobic amino acid with the hydrophilic amino acid (Gly103 > Asp103) takes place in the mutant (Fig.3A). As shown in Fig. 3B, this site exists in the third transmembrane domain, and it is considered that replacement of the hydrophobic amino acid with the hydrophilic amino acid could have a great influence on the higher-order structure and may have changed the protein conformation.

On the other hand, by comparison with human AQP1, this domain, containing mutant amino acid, is not thought to locate on the inner surface of the water pore in AQP5. (Fig. 3C; Murdiastuti et al., 2006). This suggests this mutation may not affect

water permeability. In fact, the osmotic water permeability assay using *Xenopus* oocyte showed no significant difference in the water permeability of the mutant molecule compared with that of the wild-type (see next section).
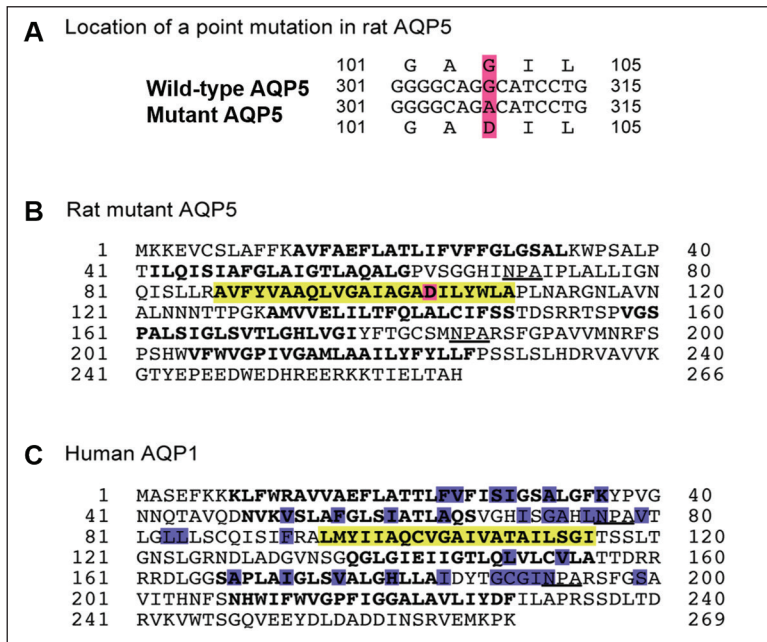

Figure 3. Location of a point mutation in the AQP5 cDNA of mutant rats, its deduced amino acid sequence, and comparison with human AQP1. **A**: partial nucleotide sequences (nt 301–315) of wild-type and mutant *AQP5* (both from 5th generations) and their deduced amino acid sequences. **B**: Complete amino acid sequence of rat mutant AQP5. **C**: Complete amino acid sequence of human AQP1. The location of point mutation in the mutant *AQP5* cDNA, leading to an amino acid replacement of glycine to aspartic acid, is indicated by a red box (**A** and **B**). Bold letters indicate transmembrane domains, whereas underlines indicate the NPA motif conserved within the family (**B** and **C**). Amino acid residues of the 3rd transmembrane domain, where the mutated amino acid is localized, are labeled with yellow boxes (**B**) compared with the identical domain in human AQP1 (**C**). Amino acid residues facing to the inside of the aqueous pore in the membrane are labeled with blue boxes (**C**), implying that the point mutation of rat AQP5 is located at the remote site from the aqueous pore in the membrane. The first nucleotide at the translation initiation site was taken as the first nucleotide for numbering. Data shown in **C** are taken from Murata et al., 2000 (Data modified from Murdiastuti et al., 2006).

Water permeability of AQP5 mutant protein

As mentioned above, it is speculated that the water permeation activity would not be affected by the Gly103>Asp103 point mutation in AQP5. To verify this, an osmotic water permeability assay was performed using *Xenopus* oocytes (Fig. 4A-C) (Karabasil et al., 2011). As shown in Fig 4B, both wild-type and G308A mutant *AQP5s* were expressed in the oocyte cell membrane upon injection of their cRNA. Expression of AQP5 in oocytes was also confirmed by Western blotting (Fig. 4C).

Using *Xenopus* oocytes injected with cRNAs from wild-type and G308A mutant *AQP5*, as well as water, an osmotic water permeability assay was performed according to a standard method. That is, *Xenopus* oocytes were injected with cRNA and incubated for 72 hours; both wild-type and mutant cRNA significantly increased the osmotic water permeability (*Pf* value) compared to the water injection group (Fig. 4A). No significant difference was observed between wild-type and G308A mutant *AQP5* cRNA. This was also the case in the presence of Hg^{2+}, which is an AQP5-specific inhibitor. These studies revealed that the water permeability of AQP5 is not affected by the G308A mutation (Gly103 > Asp103 mutation).
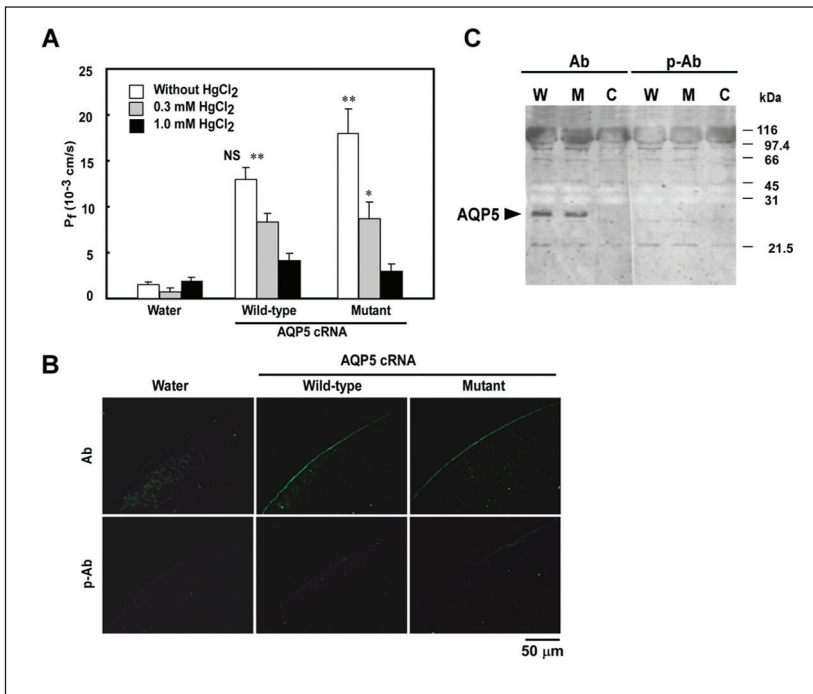

Figure 4. Measurement of Pf (osmotic water permeability) by *Xenopus* oocytes injected with wild-type or mutant AQP5 *cRNA*. (**A**) Oocytes injected with water or the indicated cRNA and incubated for 72 h were subjected to the water permeability assay. White, grey and black columns indicate the results obtained in the absence or presence of 0.3 or 1.0 mM $HgCl_2$ respectively. *******P*< 0.05, significantly different from water-injected oocytes in the absence of $HgCl_2$, and *P< 0.05, significantly different from the oocytes injected with mutant *AQP5* cRNA and incubated in the medium without $HgCl_2$ (Mann−Whitney *U*-test). NS, not significantly different from the oocytes injected with the mutant *AQP5* cRNA in the absence of $HgCl_2$. (**B**) Immunohistochemical detection of AQP5 protein expressed in the *Xenopus* oocytes injected with water, wild-type or mutant *AQP5* cRNA. Oocyte sections were probed with 1:500 diluted anti-AQP5 antiserum (Ab; upper panels) or with the same concentration of anti-AQP5 antiserum preabsorbed with 40 μg/ml of antigen peptide (p-Ab; lower panels). Specific staining was observed for oocytes injected with wild-type and mutant *AQP5* cRNA. Scale bar, 50 μm. (**C**) Western blotting of wild-type and mutant AQP5 expressed in *Xenopus* oocyte cell membranes after injection of respective cRNA. W, wild-type *AQP5* cRNA; M, mutant *AQP5* cRNA; C, water. Ab and p-Ab, anti-AQP5 antiserum (1:500) and pre-absorbed antiserum, respectively as described in (**B**) (Data from Karabasil et al., 2011).

Metabolism of mutant AQP5

As mentioned above, the water permeability of the mutant molecule is not significantly different from that of the wild-type. Therefore, decrease in water (saliva) secretions *in vivo* (Murdiastuti et al., 2006) is thought to be caused by reduced expression of the mutant AQP5 in the acinar cells of the SMG. Furthermore, the intracellular localization of AQP5 revealed that the colocalization of AQP5 with the lysosomal marker Lamp2 was low in the wild-type and high in the mutant (Fig. 5A, B). Similarly, it was revealed that the co-localization of cathepsin D, also a lysosomal marker, and AQP5 was also increased in the mutant as compared with the wild-type (Fig. 5C, D); (Karabasil et al., 2011). These results show the mutant molecule is probably metabolically degraded by the lysosomal system under control of the cell quality control system.
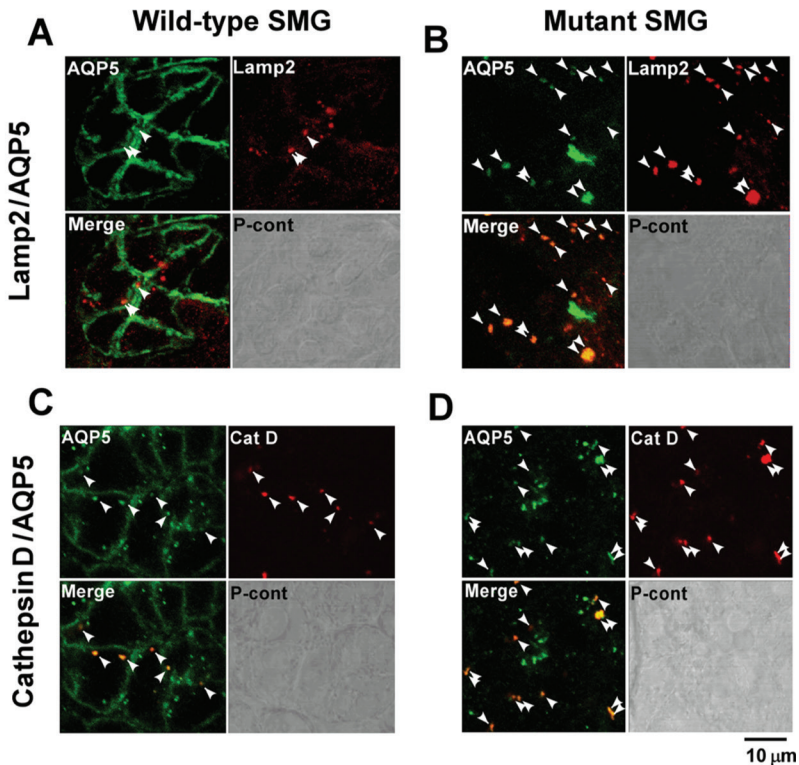

Figure 5. Confocal images of immunohistochemical localization of AQP5 and Lamp2 or cathepsin D in the SMG of wild-type and mutant rats. Twentieth- and 19th-generation progenies of wild-type and mutant SD rats were used in the experiment. (**A**, **B**) Double immunostaining of AQP5 and Lamp2. Green fluorescence of FITC shows the localization of AQP5; and the red fluorescence of Alexa FluorR 594, that of Lamp2. (**C**, **D**) Double immunostaining of AQP5 and cathepsin D. Green fluorescence of FITC shows localization of AQP5; and the red fluorescence of Alexa FluorR 594, that of cathepsin D. (**A**, **C**) Wild-type SMG; (**B**, **D**) Mutant SMG. Arrowheads in the immunofluorescent images indicate the co-localized AQP5 and Lamp2 or cathepsin D. Cat D, cathepsin D; P-cont, phase-contrast image. Scale bar, 20 μm (Data from Karabasil et al., 2011).

AQP5 mutation in humans and its affected phenotype

Diffuse nonepidermolytic palmoplantar keratoderma

Recently, missense mutations in the *AQP5* gene were identified in patients suffering from autosomal-dominant diffuse nonepidermolytic palmoplantar keratoderma (referred to as palmoplantar keratoderma Bothnia type), which was mapped to chromosomal region 12q11– 12q13 in Swedish and UK families (Abdul-Wahab et al., 2016; Blaydon et al., 2013). A variant of AQP5 (c.529A>T; p.Ile177Phe) was localized in the plasma membrane in the stratum granulosum in affected palmar epidermis, similarly as in normal subjects, thus indicating that this mutant AQP5 trafficks normally (Blaydon et al., 2013). A year later, another missense mutation of *AQP5* (c.367A>T, p.Asn123Tyr) was identified in a large three-generation family of Chinese Han ethnicity with palmoplantar keratoderma of the Bothnia type (Cao et al., 2014). Study of this mutant showed that the mutant channel is leaky and more sensitive to hypotonic solution than is the wild-type one (Cao et al., 2014). These properties of this mutant AQP5 may account for the intensive cellular swelling that results in the phenotype of diffuse nonepidermolytic palmoplantar keratoderma.

Asthma and other diseases

On the other hand, SNP has been detected in the 3'UTR region of the *AQP5* gene in a certain percentage of asthma patients, resulting in reduced production of the AQP5 protein (Krane et al., 2007). These individuals are hypersensitive toward choline-provoked bronchoconstriction (Krane et al., 2001). Five SNPs in *AQP5* were genotyped in European Americans with chronic obstructive pulmonary disease (n=429), and three of them showed significant association with the rate of decline in lung function (Hansel et al., 2010). Also, a positive association between SNPs in *AQP5* promoter and progesterone receptor was reported (Kasimir-Bauer et al., 2009). Recently, high expression of AQP5 and polymorphism in the *AQP5* promoter were suggested to be associated with peritumoral brain edema in meningioma patients (Lambertz et al., 2013).

CONCLUSION

AQP5 is involved in water secretion from the salivary gland acinar cells *via* a trans-cellular route. Physiologically, the AQP5 level is controlled by parasympathetic nerves in the SMG and sympathetic agonists in the PG, while autophagy and lysosome-mediated degradation are involved in this regulation. Specifically, down-regulation of SMG AQP5 by denervation of the parasympathetic nerve is shown to be due to activation of autophagosomes. However the activation mechanism of AQP5 synthesis after such down-regulation is unknown. Similarly, the mechanism for IPR-induced upregulation of AQP5 transcription in the PG has not yet been determined. These

issues would be important for better understanding of AQP5 function in the salivary glands.

Under pathophysiological conditions such as LPS challenge *in vitro*, NF-𝜅B/MAPK pathways are activated in the PG, leading to the activation of the transcription factors NF-𝜅B and AP-1 (c-Fos/c-Jun). These transcription factors form a complex that binds to the NF-𝜅B-responsive element to potentially down-regulate the transcription of *AQP5* mRNA. The mechanism for LPS-induced down-regulation of AQP5 in the PG is well described. The physiological meaning of the effects of LPS, however, still needs to be elucidated.

SNP has been found in both rats and human. In rats, G308A point mutation is found in the *AQP5* cDNA (and genomic DNA), resulting in amino acid substitutions of Gly103 for Asp103, which exists in the third transmembrane domain of AQP5. This mutant molecule transports water normally. However saliva secretion in mutant rats is significantly reduced, since expression of the mutant molecule is extremely diminished. Rapid degradation of mutant AQP5 by the lysosomal system probably causes low expression of mutant AQP5 in acinar cell membrane.

Mutation of *AQP5* in humans causes autosomal-dominant diffuse nonepidermolytic palmoplantar keratoderma. It remains unclear whether these patients have problems with exocrine function.

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PRIRODNE AQP5 MUTACIJE KOD PACOVA I LJUDI I NJIHOVIH FENOTIPA

HOSOI Kazuo, KARABASIL Ratko Mileva, MURDIASTUTI Kwartarini

Kratak sadržaj

Kod sisara je eksprimirano trinaest akvaporina (AQP), odnosno vodenih kanala. U ovom istraživanju dat je kratak osvrt na te akvaporine, a potom fokus na AQP5 – akvaporine egzokrinih žlezda. Konkretno se diskutuje o (1) mehanizmima kuplovanja dinamike AQP5 sa ciklusom sekrecije i obnavljanja ciklusa amilaze posle pojave izoprenalina u parotidnoj žlezdi, (2) ulogama parasimpatičkog nerva u održavanju nivoa AQP5 u submandibularnoj žlezdi i (3) smanjivanju nivoa AQP5 u eksperimantalnom patološkom modelu administracijom lipopolisaharida (LPS) u parotidnu žlezdu. Zatim se prikazuju efekti jednostrukog nukleotidnog polimorfizma (SNP) koji se sreće kod pacova i ljudi i njihovim fenotipima. Tačkasta mutacija G308A, koja je prisutna u AQP5 u cDNK kod pacova, dovodi do zamene Gly103 za Asp103, što smanjuje ekspresiju proteina. Kod ljudi je prisutno nekoliko SNP-ova u AQP5 kod evropskih i kineskih porodica i oni izazivaju autozomno dominantnu difuznu neepidermolitičku palmoplantarnu keratodermu.

Ključne reči: akvaporin 5 (AQP5), pljuvačne žlezde, jednostruki nukleotidni polimorfizam (SNP)