

Immunohistochemical Study of Aquaporin 1 and 2 in the Hepatobiliary System of Qinghai Lizard (*Phrynocephalus vlangalii*)

Estudio Immunohistoquímico de Aquaporin 1 y 2 en la Sistema Hepatobiliar del Lagarto de Qinghai (*Phrynocephalus vlangalii*)

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SUMMARY: A serous membrane covering the liver and the hepatic parenchyma, consists of hepatocytes, arteries, veins, hepatic sinusoids and biliary ductuli. There are erythrocytes, thrombocytes, melanin particles and Kupffer cell in the hepatic sinusoids and the blood vessels. The gall bladder wall consists of a mucous layer a muscle layer and a serous layer. The bottom of the epithelium abounds with round or oval secretory. In liver, immunohistochemistry results show that AQP1 have intense reaction in hepatic lobule, Kupffer cells (*Macrophagocytus stellatus*), hepatocytes, portal tract, blood islands ,vein and artery, but almost no reaction of AQP2 was detected. In gallbladder, mucous epithelium, endothelial cells from vein and artery all have strong AQP1 expression, AQP2 showed minor diffused positive reaction in gallbladder, which suggesting that AQP1 may have the main role in the absorption and transportation of fluid in hepatobiliary system of Qinghai Lizard.

KEY WORDS: Aquaporin1; Aquaporin2; Immunohistochemistry; Qinghai Lizard.

INTRODUCTION

It had been well accepted that the transport of water was due to simple diffusion through the lipid bilayer until the aquaporins (AQPs) were found (Gomes *et al.*, 2009). AQPs allow the transcellular water transport driven by osmotic forces. So far, thirteen AQPs have been cloned in mammals. They are classified into essentially permeable to water, permeable to water but also to glycerol, urea, and small solutes and unorthodox AQPs with highly variable NPA motifs (Ishibashi *et al.*, 2014). Different AQPs have specific tissue distribution and execute particular physiological function.

Although the exact function of AQPs and the related mechanism still remain unknown, studies have focused on the role of AQPs in the liver and in bile formation (Portincasa *et al.*, 2003) and it has been demonstrated that there are differences between hepatocytes and cholangiocytes regarding their AQPs equipment (Marinelli *et al.*, 2000). In hepatobiliary system, significant water movements take place throughout the duct, they are fundamentally important to the fluid transport in the bile. AQPs have been shown to be expressed in the liver and

biliary epithelium (Masyuk & LaRusso, 2006). Cells from the hepatobiliary tract including hepatocytes, cholangiocytes, gallbladder epithelial cells, and endothelial cells from blood vessels have indeed been shown to express various AQPs that could account for transcellular water transport (Portincasa & Calamita, 2012). Besides, the existence of additional pathways of water movement other than AQPs was suggested following biophysical studies using rat liver mitochondria (Calamita *et al.*, 2006). AQP-1 protein expression was detected by various methods, including immunocytochemistry, in the bile duct cells of rats and humans (Nielsen *et al.*, 1993; Nihei *et al.*, 2001). AQP-1 was also expressed in human liver capillary endothelium and the endothelial cells of other organs (Mobasher & Marples, 2004).

Qinghai Lizard belong to *Reptilia*, *Squamata*, *Lacertilia*, *Agamidae* of *Phrynocephalus*. They are special species in China (Jin & Liu, 2007), occupying a special evolutionary status and playing vital roles in ecosystem balance. Qinghai Lizard usually survives in severe drought areas, so fluid secretion or absorption are prerequisite for

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them when confronting water shortage. Bile mainly secreted from the hepatobiliary system consists of more than 98 % water and water transportation in hepatobiliary system are essential for appropriate hepatobiliary tract function, but the genetic strategies confronting water shortage for them is unknown. Therefore, the present study was carried out to investigate the distribution of AQP-1/AQP-2 in hepatobiliary tract of Qinghai Lizard, which may provide not only morphological structure but also histochemical data for water transportation in Qinghai Lizard.

MATERIAL AND METHOD

Animals and tissues preparation. The present study was carried out on Qinghai Lizard, body length above 50 mm and randomly selected during July to September from GeErMu, QingHai Province, China. Liver and gall bladder were fixed with 4 % paraformaldehyde for no less than 24 h, then the tissues were thoroughly washed in PBS, dehydrated in graded ethanol and embedded in paraffin. Five micrometer-thick sections were cut from each tissue.

Immunohistochemistry. Tissue sections were deparaffinized in xylene, washed in alcohol and rehydrated in PBS. Antigen retrieval was performed in a microwave oven in 0.01M PBS (pH 7.4) for 15 min, then the sections were cooled at room temperature and washed again in PBS. Endogenous peroxidase was blocked by using 3 % hydrogen peroxide for 30 min. After washing in PBS for three times, goat serum (10 %) was used for 20 min to avoid any non-specific reactions. Then, primary antibody,

polyclonal rabbit anti-AQP1, AQP2 were applied (sigma, dilution 1:500) and incubated in a moist chamber at 4 °C overnight. Sections were incubated with biotin-labeled secondary antibodies and avidin-HRP third antibodies, positive staining was detected using DAB. The sections were counterstained with hematoxylin. Negative control sections had the same procedure except omitting primary antibody.

Analysis. A light microscopy was utilized for the histology studies of the sections (Zeiss, Germany), and photomicrographs were recorded with a digital camera (Germany).

RESULTS

Histological Structure of liver and Gallbladder.

Histologically, the liver was composed of hepatocytes extensively interspersed with most areas. There are several hepatocytes around the central venule forming a circle which is considerably similar to the acinus (Fig. 1.A, B). The tissue structure was unorganized with no lobular structure or bile duct network evident, but they have many hepatic sinusoids (Fig. 1.C). The hepatocytes are round or ellipsoid or multilateral, different in size, with one nucleus in most cells (Fig. 1.D). There are erythrocytes, monocyte, granulocyte, lymphocyte, thrombocyte and melanin particles in hepatic sinusoid (Fig. 1.C, D).

The gallbladder has three layers: mucosa, muscular layer and serosa (Fig. 1.E-H). The mucosa is high columnar

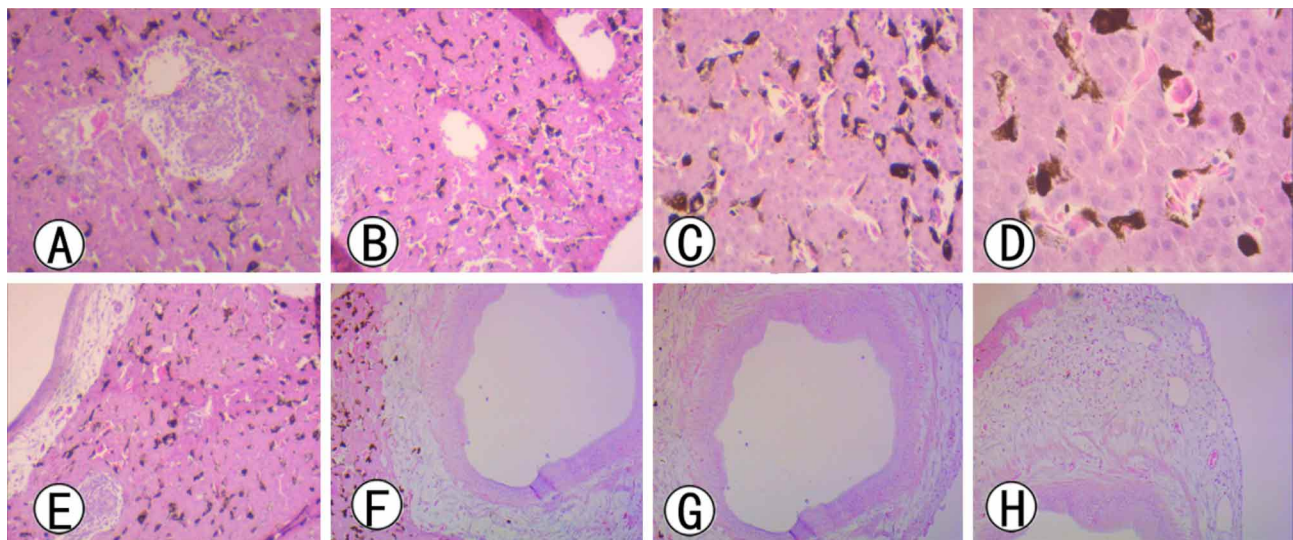


Fig.1. Microstructure of the liver and gallbladder from Qinghai Lizard. (A-H) view of the liver from *Phrynocephalus vlangalii* shows: central vein (Cv); hepatic sinusoids(Hs); biliary ductule (Bd); portal venule (P); Kupffer cell (Kc); endothelial cell (Ec); interlobular vein(Iv); interlobular artery (Ia); interlobular bile duct (Ibd); erythrocytes (E).

epithelium arranged tightly with cilia on top and at basilar part of epithelium is a very thin basement membrane (Fig. 1.E). The muscular layer include smooth muscles, collagen fibers and elastic fibers, and they arrange tightly (Fig. 1.F, G). The thickness of serosa is uneven. There are fibers and blood vessels in serosa (Fig. 1.H).

Immunohistochemistry of AQP1 in liver. AQP1 was expressed in hepatic sinusoids, Kupffer cells (*Macrophagocytus stellatus*) and hepatocytes (Fig. 2.A). AQP-1 immunoreactivity is detected on sinusoidal endothelial cells and microvascular endothelial cells in the portal tract (Fig. 2.B). AQP-1 reacted strongly with the cells in the interlobular vein of liver, and positive reactivity with the ductular structures was also observed (Fig. 2.C). AQP1 was expressed in endothelial cells from venules and sinoids (Fig. 2.D). A negative control, performed in the absence of primary antibody, revealed no unspecific labeling (Fig. 2.E).

Immunohistochemistry of AQP1 in gallbladder. In gallbladder, cells in submucosa have continuous AQP-1 expression (Fig. 3.A, B). AQP1 was expressed in endothelial cells from vein and mucosal sinus, bile duct (Fig. 3.C). AQP1 expression in the serosa, showing in vein, artery and capillary (Fig. 3.D). Reaction products showing AQP1 are localized mainly on endothelial cells of large vessels (Fig. 3.E-G). Negative control was performed in the absence of primary antibody (Fig. 3.H).

Immunohistochemistry of AQP2 in liver and gall bladder. In liver, almost no reaction of AQP2 was detected in the capillary, venules (Fig. 4.A,B), hepatocyte (Fig. 4.C) and endothelial cell (Fig. 4.D). AQP2 showed minor diffused positive reaction in gallbladder (Fig. 4.E-H). A minor reaction was localized in the interlobular vein, interlobular artery (Fig. 4.E), portal venule (Fig. 4.F), interlobular bile duct (Fig. 4.G) and endothelial cell (Fig. 4.H).

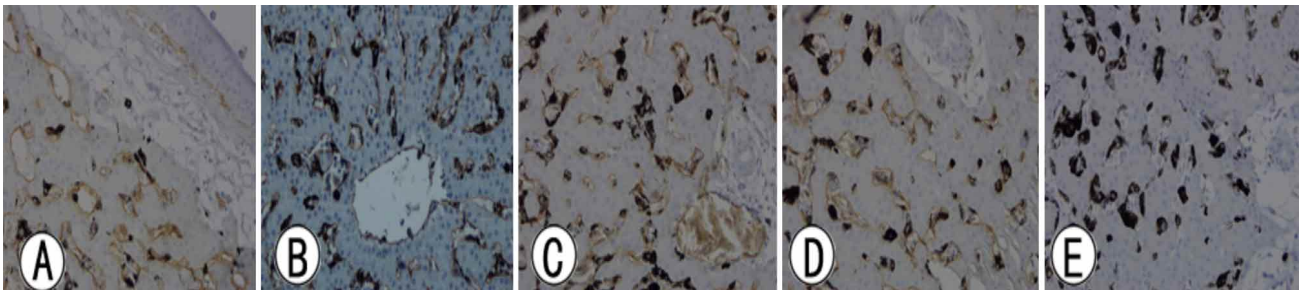


Fig. 2. Immunohistochemistry of AQP1 in liver of Qinghai Lizard. (A) mucous epithelium, hepatic sinusoids, Kupffer cells and hepatocytes express AQP-1 (B)AQP-1 expression in endothelial cells in the portal tract. (C)AQP-1 reacted strongly with the cells in the interlobular vein and interlobular bile duct of liver (D)AQP1 was expressed in endothelial cells from venules and interlobular artery. (E) Negative control was performed in the absence of primary antibody.

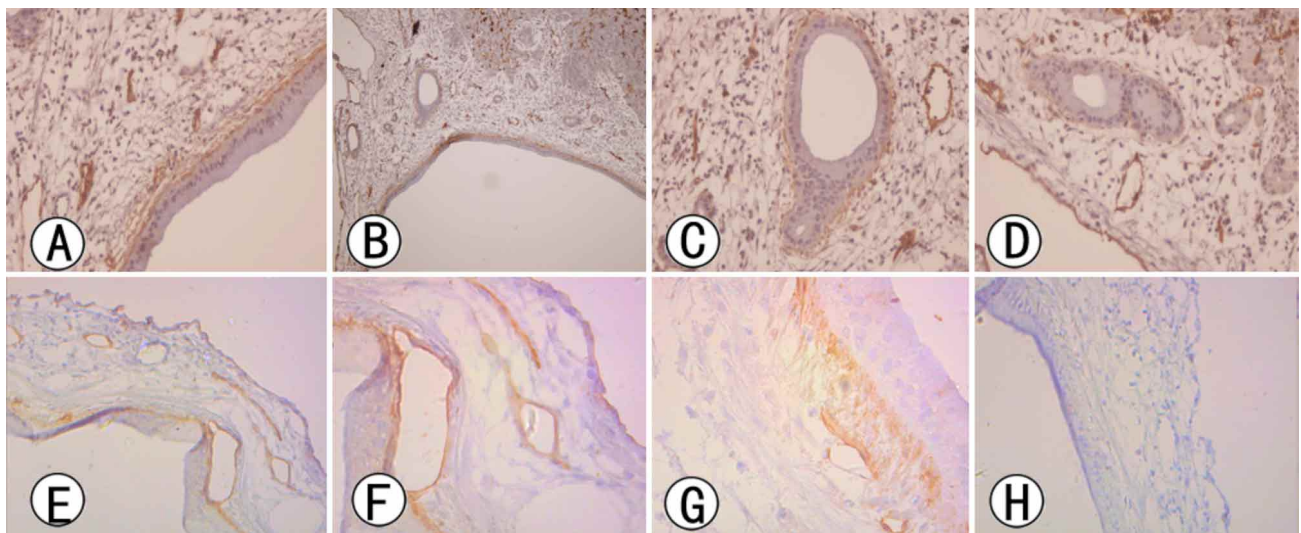


Fig. 3. Immunohistochemistry of AQP1 in gallbladder of Qinghai Lizard. (A)-(B) immunostaining for AQP-1 on the epithelial cells.(C-D) AQP1 expression in the capillary and venules. (E)-(G) Localization of AQP1 in the vein and capillary. (H) the negative control of gallbladder, in which primary antibodies were omitted.

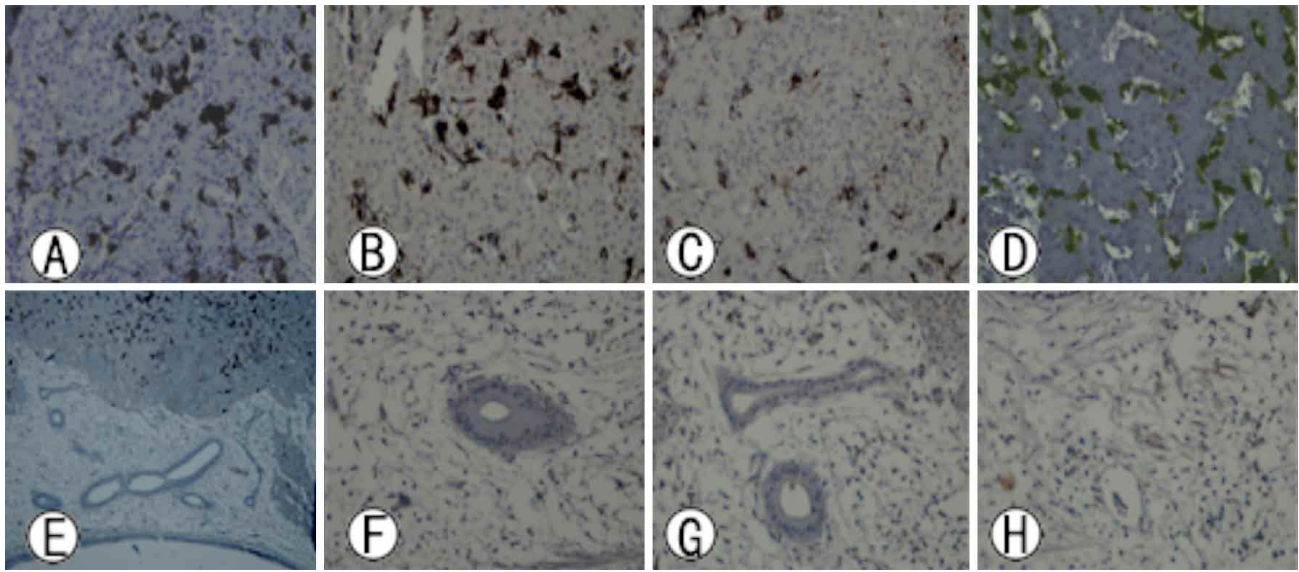


Fig. 4. Immunohistochemistry of AQP2 in liver and gallbladder of Qinghai Lizard. (A-D) Almost no immunostaining for AQP2 in liver. A faint cytoplasmic AQP1 immunoreactivity in gallbladder (E-H). A minor reaction was localized in the interlobular vein, interlobular

DISCUSSION

Qinghai Lizard is a viviparous, agamid sand lizard endemic to the north Tibetan (Qinghai) plateau with a broad altitudinal range from 2000 to 4600 m (Zhao & Adler, 1993). They show extensive variation of morphology along altitudinal gradients (Jin & Liu; Jin *et al.*, 2007). In this study, the outer layer of liver is serous membrane composed of simple squamous epithelium. Together with blood vessels, connective tissue stretch deeply into liver parenchyma and divide into many inconspicuous hepatic lobules surrounding central vein. The central veins are different in size, and the bigger ones pooled by numerous microvessels have obvious lumens and thinner wall with obvious vascular endothelial cells. There are several hepatocytes around venule forming a circle which is similar to the aelnus. All kinds of blood cells and Kupffer cells can be seen in blood vessels and hepatic sinusoids. The gall bladder of Qinghai Lizard has mucosa, muscular layer and serosa. The serosa is the thinnest layer and with high columnar epithelium arranged tightly with cilia on top and at basilar part of epithelium is very thin basement membrane. The muscular layer includes smooth muscles, collagen fibers and elastic fibers, and they arrange tightly. The thickness of serosa is uneven. There are different blood vessels in serosa. There are a lot of melanin particles in the liver, this may be the adaptive mechanism for body when confronting low oxygen.

The hepatic microvascular system comprises all the intrahepatic vessels involved in the delivery and removal of

fluids to and from the hepatic parenchyma; namely, the portal venules, hepatic arterioles, sinusoids, hepatic and lymphatic venules. Arterial blood enters some of the sinusoids principally through branches of the hepatic arterioles, which terminate in sinusoids near their origins from terminal portal venule (McCuskey & Reilly, 1993; Oda *et al.*, 2000). In the present study, AQP-1 immunoreactivity in liver is detected on sinusoidal endothelial cells and microvascular endothelial cells in the portal tract, and is mainly localized in the portal terminal venules and bile ducts, while AQP-1 is hardly detectable in the sinusoids, which is according to the study in human liver (Iguchi *et al.*, 2013). AQP2 showed no reaction in epithelium. AQPs are not only water selective, but also permeable to water and other uncharged molecules (Herrera & Garvin, 2011). Vascular endothelial cells belong to simple squamous epithelium, acting as a barrier for blood and tissue exchanging. Endothelial cells under normal physiological condition can selectively allow small molecule through the vessel wall. AQP1 expression in the capillary and venules of hepatobiliary system may responsible for the rapid absorption of large amount of fluid, thus inevitably influence nutrition supply and hormone transport for Qinghai Lizard. Because of the existence in liver, AQP1 can be considered as integral membrane protein acting to maintain normal fluid in liver. AQP1 is an integral plasma membrane protein and its distribution in the capillary endothelium is compatible with the characteristics of the pore. Expression studies in

oocytes of *Xenopus laevis* have demonstrated that AQP1 is indeed a water-specific channel that can be inhibited by mercurial (Preston *et al.*, 1992).

A main function of the hepatobiliary system is bile formation. Bile secretion is mainly completed by hepatocytes, then changing its composition by means of absorbing and secretion with epithelial cells and endothelial cells and osmotic pressure gradient was formed. At the same time, water circulation inside the bile duct formed. In this study, AQP-1 have intense expression in gallbladder, namely, mucous epithelium, endothelial cells from vein and artery all have AQP1 reaction products, which showing AQP1 is responsible for the water transportation in bile formation and secretion. Results also show that AQP1 expression in bile duct is higher than in interlobular bile ducts, which may be related to the excretion of bile. In normal mice, AQP1 widely expressed in gallbladder mucosa, with its main function of water secretion to cystic cavity, however this water transportation did not change the composition of bile (Li *et al.*, 2009). Therefore, AQP1 are likely to play a role in water transport that is essential for appropriate hepatobiliary tract function.

Water metabolism in liver is active, so water retention is of a major clinical problem in patients with liver cirrhosis. AQP1 polymorphism could be involved in the genetic susceptibility to develop water retention in patients with liver cirrhosis (Fábrega *et al.*, 2011). Tissue distribution studies of AQP1a mRNA demonstrated that it was expressed in gill, liver, intestine, rectum, kidney, heart, urinary bladder, and whole blood. When sea bream were acclimated to salinity conditions, gill AQP1a transcript was higher (Deane *et al.*, 2011). In cirrhotic liver, of AQP1 at protein and mRNA levels was demonstrated overexpression (Yokomori *et al.*, 2011). Liver endothelial cells surrounding intrahepatic bile ducts and from the gallbladder express AQP1, suggesting a potential functional role of this AQP in facilitating water transport from plasma to bile across cholangiocytes (Yokomori *et al.*). Human and mouse gallbladder epithelial cells express AQPs and AQP1 is localized in intracellular vesicles in mouse gallbladder epithelial cells. While the roles of AQPs in gallbladder physiology remain to be addressed, they are hypothesized to be involved in gallbladder bile formation. Immunohistochemical labeling found that AQP1 was expressed in cholangiocytes and endothelial cells of human liver section and AQP1 was expressed in endothelial cells from venules and Sinoids of primary hepatocytes isolated from female mice (Gregoire *et al.*, 2015). Bt2cAMP or glucagon treated hepatocytes had increased expression of aquaporin in the cellular membrane, increased water transport during cryopreservation, and increased post-thaw viability, suggesting that they play constitutive role in maintaining water balance for animals (Kumar *et al.*, 2015).

Distribution of AQP1 and AQP2 in the hepatobiliary system of Qinghai Lizard was similar to laboratory animals (Pannabecker, 2015), suggesting that they play constitutive role in maintaining water balance, water transmutation and absorption. Living environment for Qinghai Lizard is extremely arid, environment pressure may be promoted the evolution of proteins related to water metabolism, improving animal to adapt to the environment. Although further studies are required, our data may contribute to basic knowledge for Qinghai Lizard.

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AN, F. & ZHANG, Y. Estudio Inmunohistoquímico de acuaporina 1 y 2 en el sistema hepatobiliar del lagarto de Qinghai (*Phrynocephalus vlangalii*). *Int. J. Morphol.*, 37(2):706-711, 2019.

RESUMEN: Una membrana serosa cubre el hígado y el parénquima hepático el cual está formado por hepatocitos, arterias, venas, sinusoides hepáticos y conductos biliares. Se encuentran eritrocitos, trombocitos, partículas de melanina y células de Kupffer en los sinusoides hepáticos y en los vasos sanguíneos. La pared de la vesícula biliar presenta tres capas: mucosa, muscular y serosa. En el hígado, la inmunohistoquímica mostró que AQP1 tiene una reacción intensa en el lóbulo hepático, células de Kupffer, hepatocitos, tracto portal e islotes sanguíneos. En venas y arterias, no se detectó reacción alguna de AQP2. En la vesícula biliar, el epitelio mucoso, las células endoteliales venosas y arteriales tuvieron una importante expresión de AQP1, sin embargo, AQP2 mostró una reacción positiva difusa menor, lo que sugiere que la AQP1 podría tener una función principal en la absorción y transporte de líquido en el sistema hepatobiliar del Lagarto Qinghai.

PALABRAS CLAVE: Aquaporin 1; Aquaporin 2; Inmunohistoquímica; Lagarto de Qinghai.

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