

**PROCEEDING****Aquaporin 1 water channel is overexpressed in the plasma membranes of pancreatic ducts in patients with autoimmune pancreatitis**

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**Abstract :** Chronic pancreatitis with all kinds of etiologies is characterized by pancreatic exocrine dysfunction especially impaired fluid secretion from pancreatic ducts. However, the molecular mechanism of this impaired fluid secretion in chronic pancreatitis is largely unknown. Aquaporin water channels are intrinsic membrane proteins expressed most of the cell types which have high osmotic water permeability. Among them aquaporin 1 (AQP1) is a predominant water channel expressed in the plasma membranes of human pancreatic ducts. Exocrine function test revealed that fluid secretion was severely impaired in AIP. immunohistochemical analysis revealed that AQP1 is localized mainly in the apical and lateral membranes of small pancreatic ducts in control subjects. AQP1 expression was significantly increased in plasma membranes of pancreatic ducts in AIP. Upregulation of AQP1 expression seen in pancreatic ducts of patient with AIP may be caused by the reduced fluid secretion from the pancreas as compensation. Further study would be required to elucidate the precise molecular mechanism for the role of AQP1 in pancreatic fluid secretion from the pancreas in diseases characterized by the impaired ductal fluid secretion such as cystic fibrosis. *J. Med. Invest.* 56 Suppl. : 318-321, December, 2009

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**PANCREATIC EXOCRINE FUNCTION WAS SEVERELY IMPAIRED IN AUTOIMMUNE PANCREATITIS**

Chronic pancreatitis is a disease with progressive

chronic inflammation of the pancreas. Most frequent cause of chronic pancreatitis is an excessive ingestion of alcohol, called alcoholic chronic pancreatitis. At advanced stage, chronic pancreatitis is characterized by endocrine (diabetes mellitus) and exocrine

Abbreviations used are :

AIP, Autoimmune pancreatitis ; AO, amylase output ; AQP1, Aquaporin 1 ; MBC, maximum HCO<sub>3</sub><sup>-</sup> concentration

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dysfunction (maldigestion), especially impaired fluid secretion from pancreatic ducts, as a result of chronic inflammation. Autoimmune pancreatitis is a recently established disease entity in chronic pancreatitis and is characterized by the diffuse swelling of the gland and high serum Immunoglobulin G (1). Since weight loss and maldigestion are often the primary symptoms at the onset of the disease, it is suspected that pancreatic exocrine function in autoimmune pancreatitis is severely impaired (2). However, the molecular mechanism of this impaired fluid secretion from the pancreas of autoimmune pancreatitis is largely unknown.

We studied 5 patients with definite autoimmune pancreatitis, who came to Aichi Cancer Center Hospital or Nagoya University Hospital. All patients met the revised Japanese clinical diagnostic criteria for AIP in 2006. Pancreatic exocrine function was evaluated by secretin test (3) in autoimmune pancreatitis. Two parameters, secreted fluid volume for an hour (Volume) and maximum bicarbonate concentration in the juice (MBC), report ductal function and total amylase output for an hour corresponds to acinar cell function. In autoimmune pancreatitis, fluid volume was decreased to  $146.4 \pm 40.7$  ml/h ( $n=5$ , normal  $\geq 183$  ml/h) and MBC was decreased to  $44.9 \pm 7.3$  mEq/L (normal  $\geq 80.0$  mEq/L). Total amylase output for an hour was also decreased to  $7,219 \pm 4,606$  IU/h, less than 10% of the lower limit of normal (normal  $\geq 99,000$  IU/h). All three parameters by secretin test were significantly decreased and these patients were diagnosed as having truly pancreatic insufficiency by this direct exocrine function test.

### AQP1 IS OVEREXPRESSED IN PANCREATIC DUCT CELLS IN AUTOIMMUNE PANCREATITIS

Aquaporin water channels are the water and small solutes selective pore in the plasma membranes of most of the cell types which have high osmotic

water permeability (4). Among them AQP1 is believed to be a major channel for water in the pancreas since AQP1 is relatively widely expressed in pancreatic ducts (5). The molecular role of AQP1 in pancreatic fluid malsecretion seen in chronic pancreatitis is unknown.

To elucidate the role of AQP1 expression in fluid malsecretion in autoimmune pancreatitis, immunohistochemical analysis was performed using pancreatic sections from patients with written informed consent. Aquaporin 1 (AQP1) immunolocalization was examined using an anti-rat AQP1 IgG (Alpha diagnostics). Figure 1 shows immunoreactivity for AQP1 in the human pancreas. In normal subjects AQP1 expression is mainly confined to apical and lateral plasma membranes in pancreatic duct cells from centroacinar cells, intercalated cells, to medium sized interlobular ducts where most of the fluid secretion occurs in the human pancreas. In AIP, disappearance of acinar cells and massive fibrosis were evident in these tissues. In pancreatic ducts AQP1 expression was remarkably increased in both apical and lateral plasma membranes from centroacinar cells to interlobular ducts. It was also evident that marked deformed cell shapes and nuclei in pancreatic duct cells in this disease. Table 1

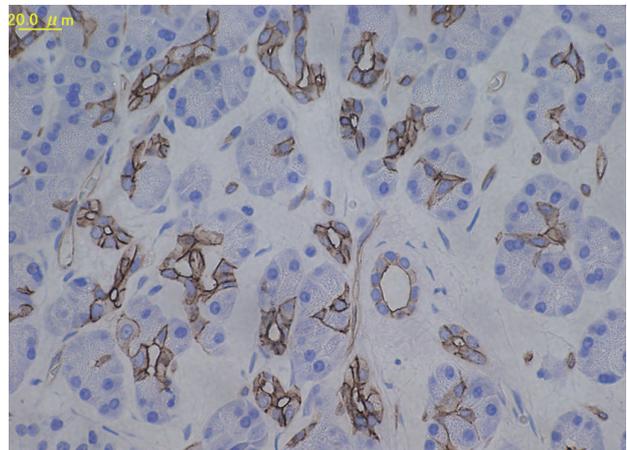


Figure 1 Immunolocalization of AQP1 in human pancreas. AQP1 is expressed in the plasma membranes of centroacinar cells and mainly in the apical and lateral plasma membranes from intercalated to small interlobular ducts.

Table 1 Summary of AQP1 expression levels in control and autoimmune pancreatitis. AQP1 is uniformly overexpressed in all parts of the plasma membrane from centroacinar cells to the middle sized interlobular ducts.

|         | Acinar cells |    | Centroacinar cells | Intercalated cells |     | Intralobular ducts |    | Interlobular ducts |    | Large ducts |    |
|---------|--------------|----|--------------------|--------------------|-----|--------------------|----|--------------------|----|-------------|----|
|         | AP           | BL |                    | AP                 | BL  | AP                 | BL | AP                 | BL | AP          | BL |
| Control | -            | -  | ++                 | ++                 | ++  | +                  | ++ | +                  | +  | -           | -  |
| AIP     | -            | -  | +++                | +++                | +++ | +++                | ++ | ++                 | -  | -           | -  |

AP, apical plasma membrane  
 BL, basolateral plasma membrane  
 AIP, autoimmune pancreatitis

summarizes immunohistochemical analysis of AQP1 expression in autoimmune pancreatitis.

## PANCREATIC EXOCRINE FUNCTION IS CRITICAL FOR PROPER DIGESTION OF FOOD

Pancreatic exocrine function was severely impaired in autoimmune pancreatitis. Among three parameters which report exocrine function, pancreatic fluid volume secreted after an intravenous administration of pancreatic secretagogue, secretin, was evidently decreased compared to normal subjects. In addition Aquaporin 1 water channel was significantly overexpressed in autoimmune pancreatitis.

Pancreas is a gland which acts as both an endocrine and exocrine organ and is critical to maintain optimal blood glucose levels and proper digestion of food. Chronic pancreatitis with any etiology is characterized by exocrine dysfunction because of destruction of the gland due to progressive chronic inflammation of the pancreas. The most frequent cause of the disease is excessive ingestion of alcohol in developed countries and even in developing countries. When patients have pancreatic insufficiency, maldigestion results in malnutrition without a life-long proper digestive enzyme replacement therapy, especially in diseases such as cystic fibrosis (6).

## ROLES OF AQUAPORIN WATER CHANNEL IN PANCREATIC DUCTAL SECRETION

Aquaporin water channel comprises a pore for water and small solutes in most of the cells with high osmotic water permeability. In the pancreas several isoforms of aquaporin are known to be expressed (5). Among them only three isoforms are confirmed to be expressed in the human pancreas. AQP8 is an isoform expressed mainly in the plasma membrane of hepatocytes (7) and is also expressed in the apical membrane of human and rodents pancreatic acinar cells (8), although AQP8 knockout mice did not show any defect in digestive enzyme secretion (9). AQP5, which is a major isoform expressed in salivary acinar cells (10) and lacrimal duct cells (11) and is responsible for the saliva and tear formation, is exclusively expressed in the apical plasma membrane of intercalated ducts and small interlobular ducts (5), although no other study

could confirm the AQP5 expression in the human pancreas. AQP1 is expressed in the plasma membrane from centroacinar cells to middle sized interlobular ducts (5). Indeed, in a previous study with rat pancreas we have shown that isolated interlobular ducts have high mercury-sensitive osmotic water permeability and AQP1 expression was confined by immunohistochemical analysis mainly to the apical and lateral plasma membranes of interlobular ducts where most of the fluid is secreted into the luminal space in this species (12).

The findings that pancreatic fluid secretion was remarkably decreased in autoimmune pancreatitis, but in contrast AQP1 expression was increased in the plasma membrane of pancreatic ducts are puzzling. If AQP expression was upregulated in the plasma membrane, one would expect that the transcellular water movement is increased due to overexpression of pores for water in the membrane. A possible explanation for this discrepancy is that expression of ion channels or transporters localized in the apical plasma membrane of pancreatic ducts such as CFTR chloride channel (13) or SLC26 family members of anion transporters (14) was decreased by contrast to overexpression of AQP1 water channel. Aquaporin water channel is a bidirectional pore for water, small solutes or ions in the plasma membrane. Therefore expression levels of ion channels or transporters, not the expression level of aquaporin, determine the direction of fluid movement (secretion or absorption) in most of the epithelial cells. Another possible explanation is that this increase of AQP1 expression in chronic pancreatitis is just a reflection of a compensatory mechanism for the reduced fluid secretion by pancreatic ducts. This explanation would further be supported by the finding that overexpression of AQP1 is also observed in alcoholic chronic pancreatitis, a most frequent entity in chronic pancreatitis (data not shown). Further study would be required to elucidate the precise molecular mechanism for the role of AQP1 expression in pancreatic ducts and fluid secretion from the pancreas in diseases characterized by the impaired ductal fluid secretion such as chronic pancreatitis and cystic fibrosis.

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