

## **Mechanisms of Lower Bile Duct Stricture in Autoimmune Pancreatitis**

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**Running title:** Lower bile duct stricture in AIP

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## **Abstract**

### **Objectives**

We attempted to clarify the mechanism underlying lower bile duct stricture in autoimmune pancreatitis.

### **Methods**

Imaging and histology of the bile duct were assessed for 73 patients with autoimmune pancreatitis to clarify whether IgG4-related biliary inflammation or pancreatic head swelling is associated with lower bile duct stricture.

### **Results**

Lower bile duct stricture was found in 59 patients (81%). Pancreatic head swelling was significantly more frequent among patients with lower bile duct stricture than those without (53 (90%) versus 4 (29%);  $p < 0.01$ ). Intraductal ultrasonography revealed lower bile duct wall thickening in 21 of 22 patients with lower bile duct stricture (95%), and lower bile duct wall of patients with pancreatic head swelling was significantly thicker than those without ( $p = 0.028$ ). Among 38 patients with lower bile duct biopsies, 14 (37%) exhibited abundant IgG4-bearing plasma cell infiltration. Among lower bile duct

stricture patients, an IgG4-related inflammation seemed to exert a dominant effect under limited conditions, including concomitant middle bile duct stricture and neither pancreatic swelling nor pancreatic duct stricture in the head region.

### **Conclusions**

Both pancreatic head swelling and IgG4-related biliary inflammation influence the occurrence of lower bile duct stricture, which may be included in IgG4-related sclerosing cholangitis. Pancreatic head swelling affects IgG4-related biliary wall thickening.

### **Key words**

IgG4, IgG4-related disease, IgG4-related sclerosing cholangitis, autoimmune pancreatitis

## INTRODUCTION

IgG4-related disease is a recently proposed disease concept characterized by high serum IgG4 concentrations and abundant IgG4-bearing plasma cell infiltration in the affected organs.<sup>1,2</sup> IgG4-related disease consists of systemic diseases, including autoimmune pancreatitis (AIP),<sup>3</sup> Mikulicz's disease,<sup>4</sup> retroperitoneal fibrosis,<sup>5</sup> and sclerosing cholangitis.<sup>6</sup> Sclerosing cholangitis is a major member of the disease group and is now recognized as IgG4-related sclerosing cholangitis (IgG4-SC) due to the infiltration of IgG4-positive plasma cells observed histologically within and around bile ducts, as well as in the pancreas.<sup>7</sup> IgG4-SC has been classified based on cholangiographic findings of duct stricture.<sup>7,8</sup> According to Nakazawa's classification, stricture in the lower part of the common bile duct is only classified as type 1, stricture diffusely distributed in the intrahepatic and extrahepatic bile duct as type 2, stricture distributed in both the hilar hepatic region and lower part of the common bile duct as type 3, and stricture in only the hilar hepatic region of the bile duct as type 4. According to this classification, lower bile duct stricture mimics the stricture caused by pancreatic cancer, whereas intrahepatic or hilar bile duct stricture mimic those caused by primary

sclerosing cholangitis (PSC) or cholangiocarcinoma.<sup>7,9</sup> Intrahepatic or hilar bile duct strictures are generally accepted to correspond to genuine IgG4-SC and are considered to be typical lesions of IgG4-SC in international consensus diagnostic criteria (ICDC) for autoimmune pancreatitis.<sup>10</sup> However, controversy exists whether lower or intra-pancreatic bile duct stricture, which is frequently complicated with AIP, should be included in IgG4-SC. Some reports have insisted that lower bile duct stricture is secondary to compression due to pancreatic head swelling caused by AIP based on a close association between the two conditions.<sup>11</sup> In contrast, other reports support classifying these lesions as IgG4-SC based on wall thickening revealed by intraductal ultrasonography (IDUS) or IgG4-bearing plasma cell infiltration in the affected tissues.<sup>12</sup> In recently proposed Japanese diagnostic criteria for IgG4-SC, lower bile duct stricture was classified as IgG4-SC.<sup>9</sup>

Some lower bile duct stricture lesions found in AIP are resistant to corticosteroid therapy, and residual lesions require more intensive therapy.<sup>13-15</sup> In these cases, an IgG4-related inflammatory process in the bile duct wall may exacerbate lower bile duct stricture, and a comprehensive therapeutic strategy is needed for effective management.

Therefore, clarifying the pathogenesis of lower bile duct stricture in AIP or determining whether this lesion is caused by pancreatic head swelling or IgG4-related bile duct inflammation is important. The present study aimed to resolve this issue by analyzing cholangiography, IDUS, and computed tomography (CT), and bile duct histology findings.<sup>16</sup>

## **PATIENTS AND METHODS**

### *Study subjects*

Ninety-three patients were diagnosed with AIP at Shinshu University Hospital between August 1992 and July 2011. We retrospectively enrolled 73 of these patients whose cholangiography was available (54 men and 19 women, mean age $\pm$ SD: 65 $\pm$ 8.5 years). Diagnosis of AIP was based on the Asian diagnostic criteria for AIP.<sup>17</sup>

### *Image analysis*

ERCP findings of lower bile duct stricture and pancreatic duct narrowing in the head region were assessed by three expert endoscopists. Subsequently, IDUS was performed using a 20 MHz probe (2.0 mm diameter, UM-G20-29R, Olympus Optical Co, Tokyo,

Japan). The IDUS findings for the lower and middle common bile duct were assessed.

An inner low echoic layer thickness of more than 0.8 mm was defined as bile duct wall thickening.<sup>12</sup> Though some patients received stent treatment for obstructive jaundice, ERCP and IDUS procedures were done for these patients before stenting. Bile duct biopsy of the lower bile duct lesion was performed at least three times using biopsy forceps (FB-45Q-1; Olympus Optical Co.). Pancreatic swelling was determined using the Haaga criteria or by a marked decrease in size after corticosteroid therapy.<sup>18</sup> ERCP findings of middle bile duct stricture were also evaluated to assess the correlation between lower and middle bile duct stricture.

### *Histopathology*

Specimens were stained with hematoxylin and eosin and immune-stained with anti-IgG4 antibody (AU009, The Binding Site, Birmingham, UK) and HRP-labeled rabbit anti-sheep immunoglobulin (p 0163, DakoCytomation, Glostrup, Denmark).

Infiltration of IgG4-positive plasma cells was defined by the presence of 10 and more IgG4-positive plasma cells in a high power (HP) field ( $\times 400$ ).

### *Statistical analysis*

The Fisher's exact test, Pearson's chi-square test and Mann-Whitney test were adopted to test for differences between subgroups of patients. P values less than 0.05 were considered to be significant.

### *Ethics*

This study was approved by the ethics committee of Shinshu University (approval number 1976).

## **RESULTS**

### *Contribution of pancreatic head swelling*

At diagnosis, lower bile duct stricture was found with cholangiography in 59 (81%) of the 73 patients. Among these 59 patients, swelling of the pancreatic head lesion and irregular narrowing of the main pancreatic duct at the head lesion was found in 53 (90%) and 56 patients (95%), respectively. Among the 14 patients without lower bile duct stricture, swelling of the pancreatic head lesion was found in only 4 patients (29%), and irregular narrowing of the main pancreatic duct at the head lesion was found in only 3 patients (21%). Swelling and irregular narrowing of the main pancreatic duct at the

pancreatic head region occurred significantly more frequently in patients with lower bile duct stricture compared to those without ( $p < 0.01$ , Table 1). These results suggest that pancreatic head swelling could induce pancreatic duct narrowing in a manner significantly related to lower bile duct stricture in AIP patients.

#### *Correlation between pancreatic head swelling and bile duct wall thickening*

The IDUS (Figure 1) data available for 22 of the 59 patients with lower bile duct stricture is given in Table 2, showing lower bile duct wall thickening in 21 of 22 patients (95%) and middle bile duct thickening in 17 (77%). The correlation between pancreatic head swelling and bile duct wall thickening was assessed for 23 patients who underwent IDUS among 73 all patients and included one patient without lower bile duct stricture. The lower bile duct wall, but not the middle bile duct wall, was significantly thicker in the 19 patients with pancreatic head swelling compared to the 4 patients without pancreatic head swelling ( $p = 0.028$ ) (Table 3).

#### *Pathological analysis of the bile duct wall*

Forty patients underwent trans-papillary lower bile duct biopsy, and IgG4 immunostaining data was available for 38 patients. Among these 38 patients, 14 (37%)

exhibited abundant IgG4-bearing plasma cell infiltration, reflecting the inflammatory changes of IgG4-related sclerosing cholangitis (Figure 2). Though detailed pathological evaluation for IgG4-related inflammatory findings such as striform fibrosis or obstructive phlebitis was not done because of small sample's volume, these results suggest that bile duct wall thickening might induce the lower bile duct stricture seen in some AIP patients.

#### *Co-occurrence of middle bile duct stricture*

As the finding of wall thickening suggested that the IgG4-related inflammatory process extends from the lower to middle bile duct, an IgG4-related inflammatory lower bile duct stricture would result in simultaneous middle bile duct stricture if IgG4-related inflammation is the dominant factor for lower bile duct stricture. Therefore, we evaluated the presence of middle bile duct stricture in cases with lower bile duct stricture. Cholangiography found that middle bile duct stricture occurred with lower bile duct stricture in only 4 of the 59 patients (Figure 3). IgG4-related inflammatory changes occurring with bile duct stricture appear to extend from the lower to middle bile duct in these 4 cases and exert a dominant effect on lower bile duct stricture.

### *Lower bile duct stricture without pancreatic head swelling*

Among the 59 patients with lower bile duct stricture, 6 patients had no pancreatic head swelling on CT. Four of these 6 patients had co-stricture of the pancreatic duct, in which the inflammatory process affecting the pancreatic head also appears to induce lower bile duct stricture. However, the two patients with a normal pancreatic duct may reflect IgG4-related bile duct inflammation acting as a dominant factor in lower bile duct stricture.

## **DISCUSSION**

The present study found pancreatic head swelling among 90% of AIP cases with lower bile duct stricture, and pancreatic duct narrowing in 95%, indicating that lower bile duct stricture in AIP is closely associated with the compression mechanism due to pancreatic head swelling. Hirano et al. argued that, if the stricture is caused by external compression because of pancreatic head swelling, the frequency should be higher in pancreas head lesion-positive patients; the frequency of lower bile duct stricture was higher among patients with a pancreatic head lesion compared to those without,

suggesting that the lower bile duct stricture in AIP is influenced by the compression from pancreatic head swelling.<sup>11</sup> Similarly, the present study showed that lower bile duct stricture is seen more frequently in cases with pancreatic head swelling compared to those without. In addition, pancreatic head swelling was significantly associated with pancreatic duct narrowing in the pancreatic head region. Coexistence of pancreatic head swelling and pancreatic duct narrowing further supported that pancreatic head swelling induces compression stricture of duct systems, including the lower bile duct. Björnsson et al. argued that lower bile duct stricture should be a part of AIP and not included with IgG4-SC.<sup>19</sup>

The present study, however, showed that inflammatory wall thickening of the lower bile duct possibly influences lower bile duct stricture based on IDUS findings and abundant IgG4-bearing plasma cell infiltration in trans-papillary lower bile duct biopsies. Nakazawa et al. considered biliary stricture limited to the intra-pancreatic area as IgG4-SC.<sup>7,8</sup> Naitoh et al. reported that lower bile duct strictures appeared as marked wall thickening in 93% of patients, and as extrinsic compression in 7% of patients, revealing lower bile duct stricture caused mostly by bile duct wall thickening.<sup>12</sup> Other

studies also support that lower bile duct stricture in AIP is due to inflammatory processes in the bile duct.<sup>20,21</sup> Pathological studies of resected tissue specimens from the pancreatic head revealed abundant lymphoplasmacytic and IgG4-bearing plasma cell infiltration not only in pancreatic tissue, but also in the bile duct wall, indicating that the lower bile duct stricture lesion was due to IgG4-related inflammatory processes in the lower bile duct.<sup>22</sup> Our pathological study of biopsy specimens confirmed IgG4-bearing plasma cell infiltration in 37% of cases.

These previous reports and our findings suggest that both pancreatic head swelling and biliary wall thickening influence lower bile duct stricture in AIP.<sup>11,23</sup> In this respect, lower bile duct stricture found in AIP may be included in IgG4-related SC. However, determining the dominant factor in lower bile duct stricture is difficult because pancreatic head lesions and biliary wall thickening equally coexisted in cases with stricture in which IDUS was performed.<sup>11</sup> Hirano et al. favored pancreatic head swelling as a dominant factor based on the following findings: lower bile duct stricture was rarely seen in cases without pancreatic head swelling, and some patients with alcoholic chronic pancreatitis (ACP), as well as those with AIP, exhibited

intra-pancreatic biliary wall thickening, suggesting that some of the intra-pancreatic biliary wall thickening was reflective of reactive bile duct wall changes secondarily caused by the pancreatic inflammation rather than the inflammatory process of IgG4-SC.<sup>11</sup> We conclude that pancreatic head swelling may affect lower bile duct stricture by exacerbating lower bile duct thickening, because the lower bile duct walls of patients with pancreatic head swelling were significantly thicker than those of patients without pancreatic head swelling.

In this study, we found that, in some cases, IgG4-related SC may be a dominant factor for lower bile duct stricture. Because IDUS showed continuous wall thickening from the lower to middle bile ducts, IgG4-related inflammatory changes in the bile duct seem to extend from the lower to middle bile duct in most cases even though cholangiography showed normal findings.<sup>12</sup> Middle bile duct stricture co-occurred with lower bile duct stricture in cholangiography for 4 cases. Thus, stenotic change in 4 middle bile duct cases appeared to share a common mechanism of IgG4-related biliary wall inflammation with lower bile duct stricture, suggesting that simultaneous lower bile duct stricture and middle bile duct stricture represents dominant IgG4-related bile

duct inflammation. In addition, 2 patients with neither pancreatic swelling nor pancreatic duct stricture in the head region may exhibit IgG4-related bile duct inflammation as a dominant factor in lower bile duct stricture (Figure 4).

## **Conclusion**

Both pancreatic head swelling and biliary wall thickening influence lower bile duct stricture in most AIP cases, which may be included in IgG4-related sclerosing cholangitis. Pancreatic head swelling affects IgG4-related biliary wall thickening.

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## **Conflict of interest**

None of the authors have any conflicts of interest associated with this study.

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## Figure legends

FIGURE 1. IDUS findings for IgG4-SC. A) Cholangiography indicating IDUS position. 1 indicates a non-stricture site, and 2 indicates a stricture site. B) IDUS of non-stricture site 1 in (A), indicating thickening of the inner layer. C) IDUS of stricture site 2 in (A), indicating marked thickening of the inner layer.

FIGURE 2. Histology of lower bile duct tissue specimens. A) HE staining showing abundant lymphoplasmacyte infiltration. B) IgG4 immunostaining showing abundant IgG4-bearing plasma cell infiltration.

FIGURE 3. Lower bile duct stricture pattern based on the co-occurrence of severe middle bile duct stricture (A) or no middle bile duct stricture (B).

FIGURE 4. Mechanisms of Lower Bile Duct Stricture in Autoimmune Pancreatitis.

TABLE 1 Number of Patients Exhibiting Pancreatic Head Swelling or Pancreatic Duct Narrowing at the Head Region Based on the Presence of Lower Bile Duct Stricture

	Lower bile duct stricture (+) n=59	Lower bile duct stricture (-) n=14	P-value
<b>Pancreatic head swelling (+)</b>	53 (90%)	4 (29%)	< 0.01
<b>Pancreatic head swelling (-)</b>	6 (10%)	10 (71%)	
<b>Pancreatic duct narrowing at head region (+)</b>	56 (95%)	3 (21%)	< 0.01
<b>Pancreatic duct narrowing at head region (-)</b>	3 (5%)	11(79%)	

TABLE 2 IDUS Findings for the Lower and Middle Bile Duct in Patients with Lower Bile Duct Stricture (n=22)

	Lower bile duct	Middle bile duct
<b>Wall thickening, n (%)</b>	21 (95%)	17 (77%)
<b>Thickness (mean <math>\pm</math>SD)</b>	1.8 $\pm$ 0.9 mm	1.1 $\pm$ 0.6 mm

TABLE 3 Correlation Between Bile Duct Wall Thickening and Pancreatic Head

Swelling

Wall thickening	Pancreatic head		P-value
	swelling (+)	swelling (-)	
	n=19	n=4	
Lower bile duct	1.7 mm (0.6-4.5)	1.0 mm (0.5-1.6)	0.028
Middle bile duct	1.0 mm (0.2-1.7)	0.9 mm (0.7-1.0)	0.510

Data are given as median (25-75%).

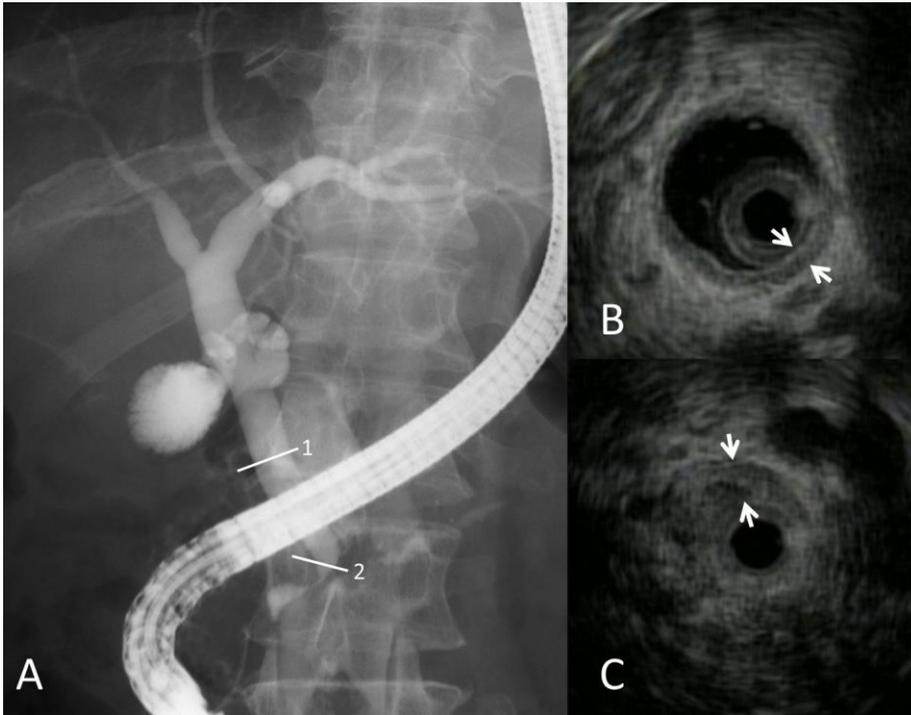


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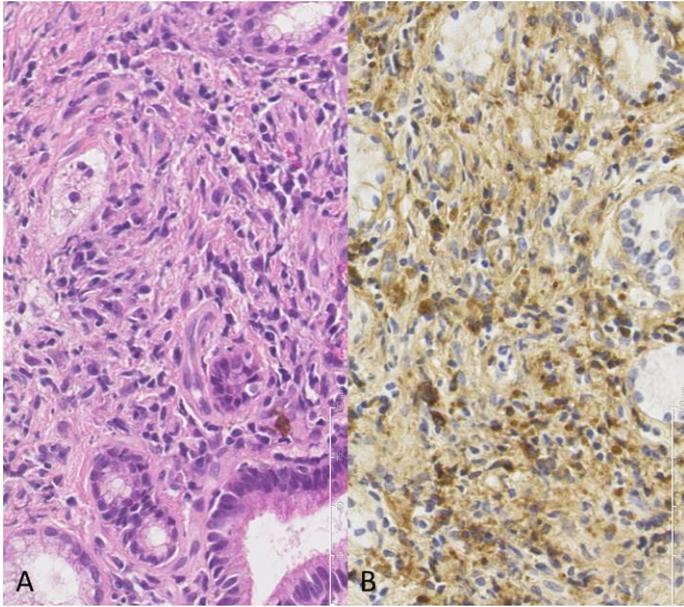


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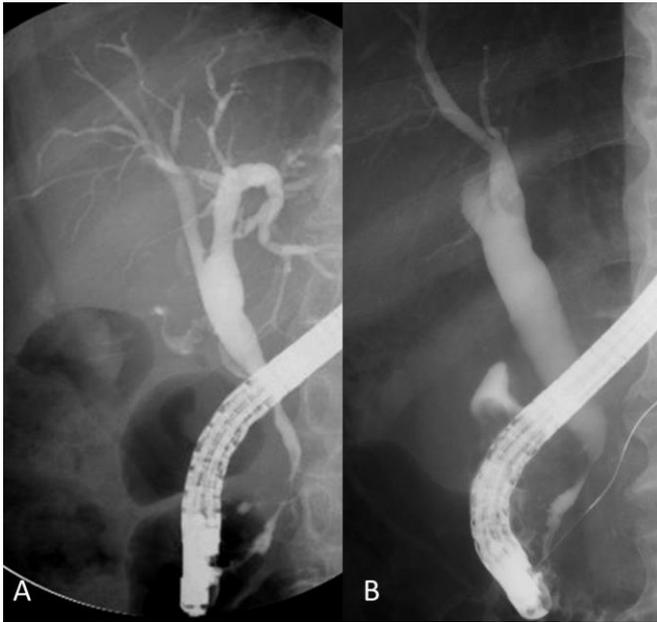


FIGURE 3. Lower bile duct stricture pattern based on the co-occurrence of severe middle bile duct stricture (A) or no middle bile duct stricture (B).

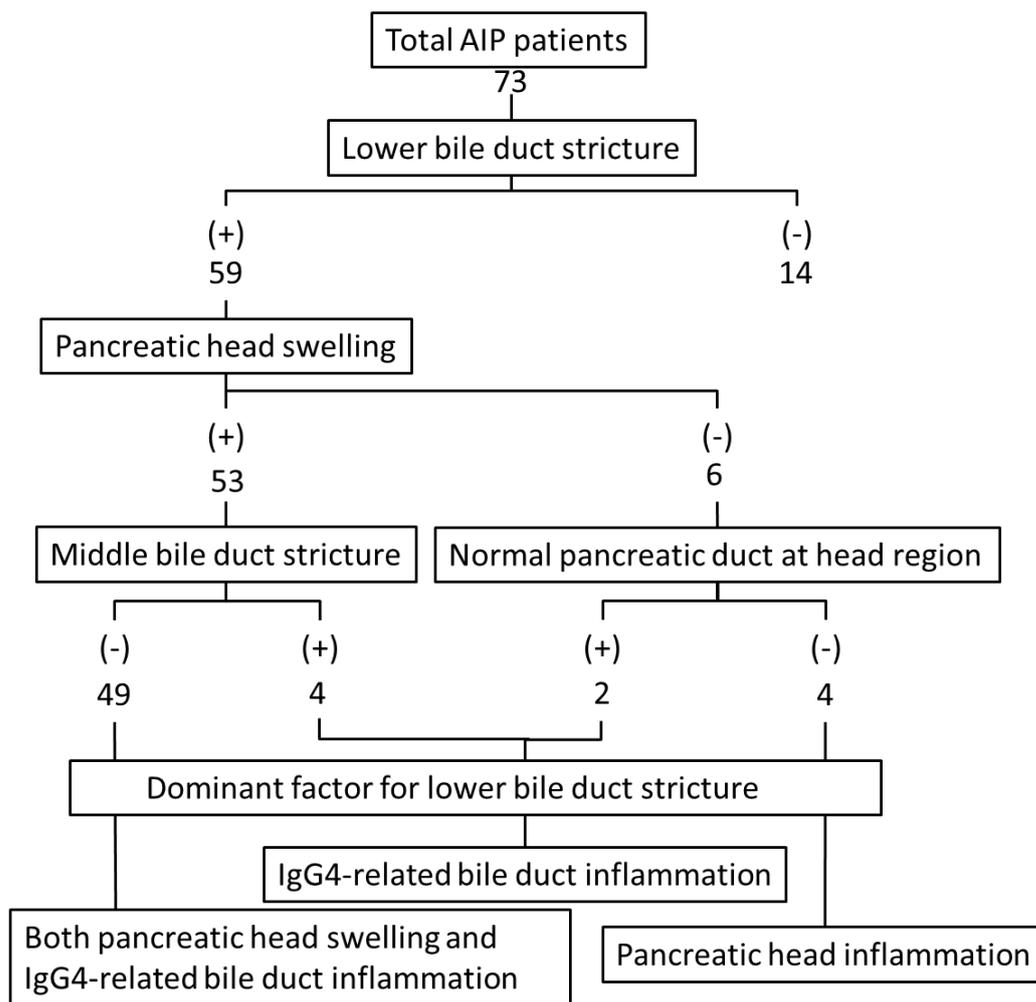


FIGURE 4. Mechanisms of Lower Bile Duct Stricture in Autoimmune Pancreatitis.