infiltration of IgG4-positive plasma cells and T lymphocytes, storiform fibrosis and obliterative phlebitis [1–3]. Since AIP is frequently associated with various sclerosing extrapancreatic lesions, such as sclerosing cholangitis, sclerosing sialadenitis, and retroperitoneal fibrosis, showing similar histological findings to the pancreas, AIP can be considered to be a pancreatic lesion of IgG4-related systemic disease, and its extrapancreatic lesions are clinical manifestations of organs involved in this systemic disease [4–6].

Another characteristic feature of AIP is significant elevation of serum IgG4 levels [1–3]. Hamano et al. [7] reported that the serum IgG4 level was >135 mg/dl in 19 of 20 AIP patients and that an IgG4 cutoff value of 135 mg/dl resulted in high accuracy (97%), sensitivity (95%), and specificity (97%) in distinguishing AIP from pancreatic cancer. However, in a recent study, the sensitivity of an elevated serum IgG4 level was 68% [8] to 81% [1].

From retrospective, histological examination of the resected pancreases of patients with mass-forming chronic pancreatitis, American and European pathologists described another unique histological pattern, which they called idiopathic duct-centric pancreatitis (IDCP) [9] or AIP with granulocyte epithelial lesions (GEL) [10]. It is histologically characterized by ductal epithelial granulocytic infiltration leading to ductal damage and obstruction, a feature not seen in LPSP. The lobular infiltrate contains neutrophils. Obliterative phlebitis is uncommon in IDCP, and the tissue does not generally stain for IgG4-positive cells [3, 9–11]. Although the typical clinical features of IDCP have not yet been clarified, typical serological abnormalities seen in AIP are not seen in IDCP [3]. IDCP is sometimes detected in Western countries, but it is uncommon in Japan and Korea [2].

In this study, to clarify the pathophysiology of AIP patients without elevated serum IgG4 levels (SIgG4-negative AIP), clinicopathological differences detected between AIP patients exhibiting elevated serum IgG4 levels (SIgG4-positive AIP) and SIgG4-negative AIP patients were examined.

Patients and methods

Study patients

Serum IgG4 levels were measured by nephelometry using IgG subclass kits (BS-NIA IgG4, Medical & Biological Laboratories, Nagoya, Japan) in 58 AIP patients [43 males and 15 females; average age 63.2 ± 12.9 (mean ± SD) years] before steroid therapy or surgical resection. They were diagnosed as having AIP according to the Asian diagnostic criteria [12] based on radiological, serological and histological findings, and responsiveness to steroid. The serum IgG4 cutoff value was 135 mg/dl, which has been used widely [7]. Serum IgG4 levels were measured at least more than twice in patients without an elevated serum IgG4 level on the first examination.

Pancreatic resection and bypass operation were performed on suspicion of pancreatic cancer in 7 and 4 patients, respectively; steroid therapy was performed finally in 42 patients; and 6 patients have been followed conservatively. Steroid therapy was started at 0.6 mg/kg/day of prednisolone and gradually tapered to a maintenance dose over a period of 3–6 months. To prevent relapse, steroid maintenance therapy (2.5 mg–5 mg/day) was performed for 1–3 years. Relapse of AIP was defined as reappearance of symptoms with the development or reappearance of pancreatic and/or extrapancreatic abnormalities on imaging studies [13, 14].

Clinical, serological, and radiological analysis

The following clinical factors were retrospectively assessed: age at the time of diagnosis; sex ratio; drinking and smoking habits; present or past history of allergic diseases such as acute allergic rhinitis, atopic dermatitis, and bronchial asthma; and treatment. Drinking habit was defined as drinking more than 80 g of alcohol/day for more than 7 years, and smoking habit was defined as smoking more than 20 pack-years (the number of packages of cigarette per day times years of smoking). Acute pancreatitis was diagnosed when both severe upper abdominal pain and elevated serum amylase levels (>3 times normal) were met. Furthermore, SIgG4-negative AIP patients were subdivided into 2 groups according to the degree of serum IgG4 levels: slightly lower SIgG4 patients (IgG4 70–135 mg/dl) and extremely lower SIgG4 patients (IgG4 <70 mg/dl), and clinical features were compared between the 2 groups.

Serologically, serum IgG levels ($n = 58$), autoantibodies ($n = 56$) including antinuclear antigen and rheumatoid factor, serum IgE levels ($n = 30$), peripheral eosinophil count ($n = 49$), and serum amylase levels ($n = 58$) were reviewed.

Radiologically analyzed findings were as follows: enlargement of the pancreas (diffuse/segmental) and extrapancreatic lesions on CT, fluorine-18 fluorodeoxyglucose (FDG) uptake and maximum standardized uptake value (SUV) on FDG positron emission tomography (PET) ($n = 13$) [15, 16], and high signal intensity and apparent diffusion coefficient (ADC) value on diffusion-weighted magnetic resonance imaging (DWI) ($n = 13$) [17]. The presence of 4 extrapancreatic lesions that were detected with a relatively high frequency in AIP patients (sclerosing cholangitis of the hilar or intrahepatic bile duct, sclerosing

cholecystitis, sclerosing sialadenitis, and retroperitoneal fibrosis) was evaluated. Though stenosis of the lower bile duct occurred frequently in AIP patients, sclerosing cholangitis as an extrapancreatic lesion of AIP was defined as presence of stenosis of the hilar or intrahepatic bile duct on cholangiography to rule out stenosis of the lower bile duct induced by compression by the swollen pancreas. Sclerosing cholecystitis was defined as gallbladder wall thickening of more than 4 mm. The presence of salivary gland swelling and retroperitoneal fibrosis was determined based on the CT findings. FDG uptake was first evaluated visually. A region of interest (ROI) was placed over the entire area of any abnormal FDG uptake. SUVmax (maximum ROI activity/injected dose/body weight) was then computed at the early period [15, 16]. DWI was obtained using a single-shot echo-planar imaging sequence, and the high signal intensity area was assessed. All ADC values were calculated on a workstation with standard software (ShadeQuest; Yokogawa Electric, Tokyo, Japan). The ADC values were determined by measurements of the ROI created on each ADC map [17].

To assess glucose tolerance, fasting serum glucose and glycosylated hemoglobin levels were examined in all patients. *N*-Benzoyl-L-tyrosyl-*p*-aminobenzoic acid (BT-PABA) excretion tests (normal $\geq$70%) were performed on 9 patients to assess pancreatic exocrine function.

Salivary and lacrimal gland functions

If AIP and its extrapancreatic lesions are clinical manifestations of organs involved in IgG4-related systemic disease [4–6], salivary and lacrimal gland functions may be impaired in AIP patients. Therefore, we investigated salivary gland function with sialochemistry and lacrimal gland function with Schirmer's test.

Salivary fluid is normally isotonic with plasma, and Na^+ and Cl^- are extensively resorbed via the ductal system to produce a hypotonic secreted fluid. Salivary Na^+ concentrations are increased in patients with Sjogren's syndrome, since resorption is altered in Sjogren's syndrome by the periductal lymphocytic infiltration [18]. The salivary β2-microglobulin level shows high specificity for inflammation of the salivary glands, and the salivary β2-microglobulin level increases in Sjogren's syndrome [19]. Saliva was collected without stimulation in 25 patients. Patients allowed saliva to drain continuously from the lower lip or spit it out for 30 min in the morning. Salivary concentrations of Na^+ and β2-microglobulin were investigated [20]. The sialochemistry data of 30 normal individuals were used as controls.

To examine tear production as a measure of lacrimal gland function, Schirmer's test was performed prospectively in the 14 AIP patients before steroid therapy. Schirmer's test involves measuring the amount of wetting of a special filter paper, which is 5-mm wide and 35-mm long. First, the filter paper is folded 5 mm from one end and inserted between the middle and outer third of the lower lid. The patient is then asked to keep his eyes open and to blink as necessary. After 5 min, the filter strip is removed, and the amount of wetting from the fold is measured. A normal eye will wet between 10 and 25 mm during that period. Measurements between 6 and 10 mm are considered borderline, and values of 5 or <5 mm are indicative of impaired secretion [21]. A participant with one or both eyes yielding abnormal test results was defined as having tear secretion dysfunction. The lower of the right and left lacrimal gland test results was used for the analysis [22].

Histological and immunohistochemical examination

Surgically resected ($n = 7$) and surgically or US-guided biopsied ($n = 7$) pancreatic specimens, and endoscopic ultrasonography-guided fine-needle aspiration EUS-FNA specimens taken with a 19-gauge needle ($n = 2$) and 21-gauge needle ($n = 5$) were examined histologically and immunohistochemically using anti-CD3, anti-CD20, and anti-IgG4 antibodies. Endoscopically biopsied specimens from the stomach ($n = 24$) were stained with anti-IgG4 antibody, and the number of IgG4-positive plasma cells was counted per high power field (hpf).

Statistical analysis

Data were expressed as mean $\pm$ SD. The data were compared between S. IgG4-positive and S. IgG4-negative AIP. For statistical analyses, Student's t test, Fisher's exact probability test, and Mann-Whitney's U test were employed. A value of <0.05 was considered statistically significant. When repeated comparisons were made, a value of <0.01 was considered significant.

Results

Clinical, serological, and radiological differences

Serum IgG4 levels were elevated in 45 (78%) of the 58 AIP patients (604.2 $\pm$ 526.0 mg/dl) and ranged from 11 to 123 mg/dl in the other 13 patients. No significant differences in age, presence of drinking and smoking habit, and allergic disease history were found. The female ratio tended to be higher in SIgG4-negative AIP patients, but the difference was not significant. As an initial symptom, obstructive jaundice was significantly more frequent in SIgG4-positive AIP patients (71%, $p = 0.002$), and abdominal pain was

more frequent in SIgG4-negative AIP patients (38%, $p = 0.01$). Steroid therapy was effective in both groups, but relapse was detected only in SIgG4-positive patients. SIgG4-positive AIP patients frequently underwent steroid therapy, and SIgG4-negative AIP patients were sometimes followed conservatively (31%, $p = 0.019$) (Table 1). Six SIgG4-positive AIP patients who were followed conservatively at first were later treated with steroids because of exacerbation of AIP. There were no significant differences in clinical findings between 5 slightly lower SIgG4 patients and 8 extremely lower SIgG4 AIP patients (Table 2).

Serum IgG4 and IgG levels were significantly higher in SIgG4-positive AIP patients ($p < 0.001$). There were no differences in presence of autoantibodies, serum IgE, and amylase levels, and peripheral eosinophil count (Table 3).

Radiologically, pancreatic diffuse swelling was frequently detected in SIgG4-positive AIP patients (51%, $p = 0.027$), and segmental swelling of the pancreatic body and/or tail was more frequent in SIgG4-negative AIP patients (46%, $p = 0.018$). FDG-PET revealed intense FDG uptake in all patients, and there were no differences in maximum SUV. On DWI, high signal intensity was detected in all patients, and there were no differences in ADC values (Table 4).

Sclerosing extrapancreatic lesions, especially sclerosing cholecystitis and sclerosing sialadenitis, were frequently detected in SIgG4-positive AIP patients (51%, $p = 0.008$). Acute pancreatitis was more frequent in SIgG4-negative AIP patients (23%, $p = 0.45$). Ulcerative colitis was associated in one SIgG4-positive and SIgG4-negative AIP patient, respectively. There were no significant differences in frequencies of diabetes mellitus and pancreatic exocrine dysfunction (Table 5).

Salivary gland and lacrimal gland functions

Salivary Na^+ concentration increased significantly in both SIgG4-positive AIP patients and SIgG4-negative AIP patients compared with controls ($p < 0.001$), but it was significantly higher in SIgG4-positive AIP patients than in SIgG4-negative AIP patients ($p = 0.034$). The salivary $\beta 2$ microglobulin concentration increased significantly in both SIgG4-positive AIP patients ($p < 0.001$) and SIgG4-negative AIP patients ($p = 0.003$) compared with controls, but it was significantly higher in SIgG4-positive AIP patients than in SIgG4-negative AIP patients ($p = 0.024$) (Table 6).

On Schirmer's test, tear secretion dysfunction was detected only in 5 SIgG4-positive AIP patients; the average level of the lower of the two eyes' test results was significantly lower in SIgG4-positive AIP than in SIgG4-negative AIP patients ($p = 0.015$) (Table 7).

Histological and immunohistochemical findings

The 5 resected and the 5 biopsied pancreatic specimens of SIgG4-positive AIP patients revealed LPSP with abundant infiltration of CD3-positive T lymphocytes and IgG4-positive plasma cells (Fig. 1a), and abundant infiltration of IgG4-positive plasma cells was detected in the peripancreatic retroperitoneal regions and peripancreatic lymph nodes. EUS-FNA of 3 SIgG4-positive AIP patients could not confirm the diagnosis due to inadequate materials.

Table 1 Clinical differences between SIgG4-positive and SIgG4-negative AIP patients

	SIgG4-positive	SIgG4-negative	*p* value
Number of patients	45	13	
Age at diagnosis (years), mean ± SD (range)	63.7 ± 10.2 (27–83)	61.5 ± 20.7 (29–83)	0.610
Male/female	36/9	7/6	0.077
Alcohol intake +/−	3/32 (9%)	1/12 (8%)	>0.999
Smoking +/−	21/14 (60%)	6/7 (46%)	0.516
Present and/or past history of allergic diseases +/−	11/26 (30%)	2/6 (33%)	>0.999
Initial symptoms			
Obstructive jaundice	35 (78%)	4 (31%)	0.002
Abdominal pain	3 (7%)	5 (38%)	0.010
Asymptomatic	7 (16%)	4 (31%)	0.243
Therapy			
Steroid	36 (80%)	6 (46%)	0.031
Responsiveness	36/36 (100%)	6/6 (100%)	>0.999
Relapse	5/36 (14%)[a]	0/6 (0%)[b]	>0.999
Resection	5 (11%)	2 (15%)	0.647
Bypass operation	3 (7%)	1 (8%)	>0.999
Follow-up	2 (4%)	4 (31%)	0.019

[a] Observation period: 50.2 ± 38.6 (6–173) months

[b] Observation period: 57.3 ± 45.4 (8–140) months

Table 2 Clinical differences between slightly lower SIgG4 and extremely lower SIgG4 AIP patients

	Slightly lower SIgG4	Extremely lower SIgG4	*p* value
Number of patients	5	8	
Age at diagnosis (years), mean ± SD (range)	70.4 ± 10.5 (63–83)	57.3 ± 23.8 (29–83)	0.464
Male/female	2/3	5/3	0.592
Alcohol intake +/−	0/5 (0%)	1/7 (13%)	>0.999
Smoking +/−	2/3 (40%)	4/2 (67%)	>0.999
Present and/or past history of allergic disease +/−	1/3 (25%)	1/3 (25%)	>0.999
Initial symptoms			
Obstructive jaundice	2 (40%)	2 (25%)	>0.999
Abdominal pain	0	5 (63%)	0.075
Asymptomatic	3 (60%)	1 (12%)	0.216
Acute pancreatitis +	0	3 (38%)	0.230
Ulcerative colitis	0	1 (12%)	>0.999
Therapy			
Steroid	0	6 (75%)	0.021
Responsiveness		6 (100%)	
Relapse		0	
Resection	1 (20%)	1 (12%)	>0.999
Bypass operation	1 (20%)	0	0.384
Follow-up	3 (60%)	1 (12%)	0.216

Table 3 Serological differences between SIgG4-positive and SIgG4-negative AIP patients

	SIgG4-positive ($n = 45$)	SIgG4-negative ($n = 13$)	*p* value
Serum IgG4, mg/dl (range)	604.2 ± 526.0 (144–2490)	62.4 ± 40.5 (11–123)	<0.001
Serum IgG, mg/dl[a] (range)	2344.8 ± 966.3 (1220–5580)	1396.2 ± 277.4 (984–1836)	<0.001
Autoantibody +/−	20/24 (45%)	7/5 (58%)	0.552
Serum IgE >580 IU/ml +/−	10/13 (43%)	1/6 (14%)	0.214
Serum IgE (IU/ml)[a]	838.4 ± 1022.8	1429.1 ± 3266.7	0.212
Eosinophils >600/mm^3 +/−	3/33 (8%)	3/10 (23%)	0.321
Eosinophils (cell/mm^3)	283.3 ± 214.6	354.0 ± 247.6	0.377
Elevation of serum amylase +/−	12/33 (27%)	4/9 (31%)	0.739
Serum amylase (IU/l)	199.8 ± 305.3	242.6 ± 313.3	0.399

[a] Mean ± SD

Two resected, 1 biopsied, and 1 EUS-FNA (19G-needle) pancreatic specimen of SIgG4-negative AIP patients revealed LPSP with abundant infiltration of T lymphocytes and IgG4-positive plasma cells. However, abundant infiltration of IgG4-positive cells was not detected in the peripancreatic retroperitoneal region or peripancreatic lymph nodes in 2 resected pancreatic materials. The number of IgG4-positive plasma calls infiltrating the resected or biopsied pancreas was not significantly different in the SIgG4-positive AIP (55.5 ± 23.9/hpf) and SIgG4-negative AIP (31.6 ± 24.6/hpf); the average number of IgG4-positive plasma cells in the pancreas was only 15/hpf in resected AIP, with a serum IgG4 level of 43 mg/dl (Fig. 1b). EUS-FNA of 2 SIgG4-negative AIP patients could not confirm the diagnosis because of inadequate materials. In the pancreas of 1 surgically biopsied and 1 EUS-FNA (19G needle) specimen, marked fibrosis (Fig. 2a) and abundant infiltration of CD20-positive B lymphocytes (Fig. 2b) rather than CD3-positive T lymphocytes (Fig. 2c), and destruction of acinar cells were detected, but few IgG4-positive plasma cells were observed (Fig. 2d), and neutrophilic infiltration was not detected (Table 8).

The number of IgG4-positive plasma cells infiltrating the gastric mucosa was significantly higher in S. IgG4-positive AIP than in SIgG4-negative AIP patients ($p = 0.004$) (Table 9).

Discussion

AIP is considered to be closely related to IgG4 serologically and histopathologically. In this study, 13 of 58 AIP patients had normal IgG4 levels. The rate of elevated

Table 4 Radiological differences between SIgG4-positive and SIgG4-negative AIP patients

	SIgG4-positive	SIgG4-negative	*p* value
Number of patients	45	13	
Pancreatic swelling			
Diffuse	23 (51%)	2 (15%)	0.027
Segmental			
Head	16 (36%)	5 (38%)	>0.999
Body and/or tail	6 (13%)	6 (46%)	0.018
FDG-PET			
FDG uptake +/−	8/0 (100%)	5/0 (100%)	0.107
Maximum SUV[a] (range)	3.5 ± 1.0 (2.2–5.6)	4.7 ± 1.0 (2.9–5.2)	
Diffusion-weighted MRI			
High signal intensity +/−	8/0 (100%)	5/0 (100%)	
ADC values ($\times 10^{-3}$ mm^2/s)[a] (range)	1.03 ± 0.14 (0.84–1.21)	1.00 ± 0.11 (0.88–1.15)	0.558

FDG fluorine-18 fluorodeoxyglucose, *PET* positron emission tomography, *SUV* standardized uptake value, *ADC values* apparent diffusion coefficient values

[a] Mean ± SD

Table 5 Associated diseases of SIgG4-positive and SIgG4-negative AIP patients

	SIgG4-positive	SIgG4-negative	*p* value
Number of patients	45	13	
Extrapancreatic lesions +	23 (51%)	1 (8%)	0.008
Sclerosing cholangitis +	4 (9%))	1 (8%)	>0.999
Sclerosing cholecystitis +	14 (31%)	0	0.025
Sclerosing sialadenitis +	14 (31%)	0	0.025
Retroperitoneal fibrosis +	3 (7%)	0	>0.999
Acute pancreatitis	2 (4%)	3 (23%)	0.045
Ulcerative colitis +	1 (2%)	1 (8%)	0.401
Diabetes mellitus +	18 (40%)	3 (23%)	0.338
Pancreatic exocrine dysfunction +/−	6/0 (100%)	1/2 (33%)	0.083

Table 6 Differences of sialochemistry between SIgG4-positive and SIgG4-negative AIP patients

	SIgG4-positive ($n = 18$)	SIgG4-negative ($n = 7$)	Controls ($n = 30$)
Na^+ (mEq/l)	32.6 ± 12.2*, **	21.7 ± 3.9*	13.7 ± 8.2
β2-microglobulin (mg/dl)	2.7 ± 1.3*, ***	1.5 ± 0.3****	1.0 ± 0.6

Mean ± SD

* $p < 0.001$ compared with controls

** $p = 0.034$ compared with SIgG4-negative patients

*** $p = 0.024$ compared with SIgG4-negative patients

**** $p = 0.003$ compared with controls

Table 7 Differences of Schirmer's test between SIgG4-positive and SIgG4-negative AIP patients

	SIgG4-positive ($n = 9$)	SIgG4-negative ($n = 5$)	*p* value
Schirmer's test (mm)	5.4 ± 2.4	11.9 ± 0.1	0.015

Mean ± SD

serum IgG4 levels in AIP patients was 78%, which was similar to the 68% [8] to 81% [1] recently reported in the literature. Although serum IgG4 levels sometimes fluctuate, they were measured at least more than twice in AIP patients without an elevated serum IgG4 level on the first examination.

There was no difference in age at diagnosis between SIgG4-positive AIP patients and SIgG4-negative AIP patients, and the female ratio tended to be higher in SIgG4-negative AIP patients. SIgG4-negative AIP patients were less likely to have obstructive jaundice but more frequently with abdominal pain and acute pancreatitis; they also more frequently showed segmental swelling of the pancreatic body and/or tail, but were less likely to have associated sclerosing extrapancreatic lesions. Although the relationship between AIP and allergic aspects is reported [23], there was no difference in allergic manifestations between the 2 groups. No SIgG4-negative AIP patients relapsed after steroid therapy. Finally, SIgG4-negative AIP patients were sometimes followed conservatively. Ghazale et al. [24] compared 34 AIP patients with elevated serum IgG4 levels and 11 AIP patients without elevated serum IgG4 levels, and

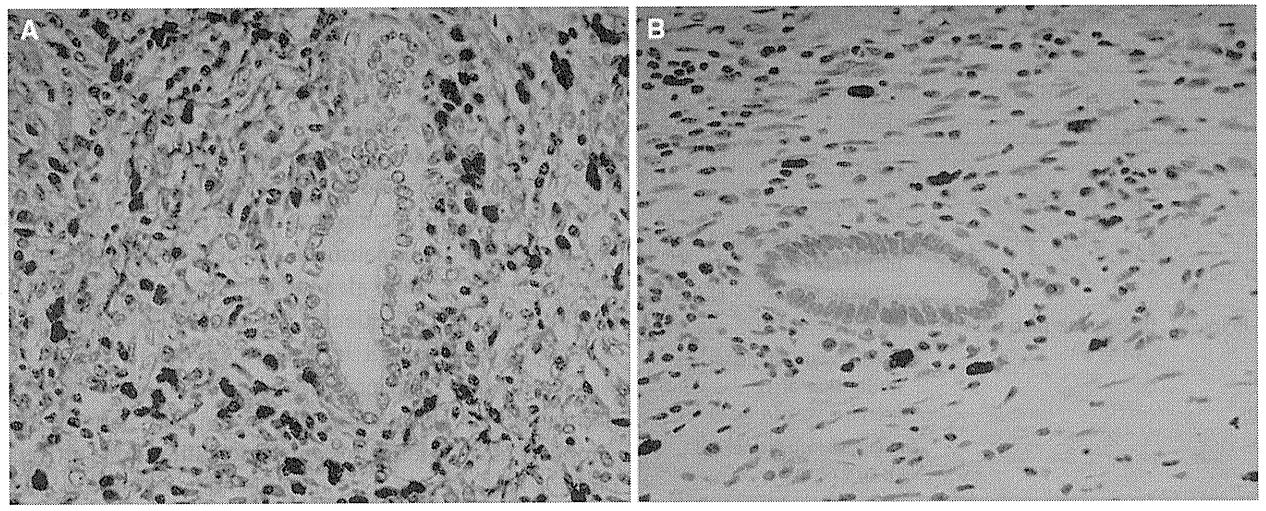

Fig. 1 **a** Abundant infiltration of IgG4-positive plasma cells in the pancreas of SIgG4-positive AIP patient (IgG4 immunostaining). **b** IgG4-positive plasma cells infiltrating the pancreas of SIgG4-negative AIP patient (IgG4 immunostaining). Note the number was less than that of SIgG4-positive AIP patient

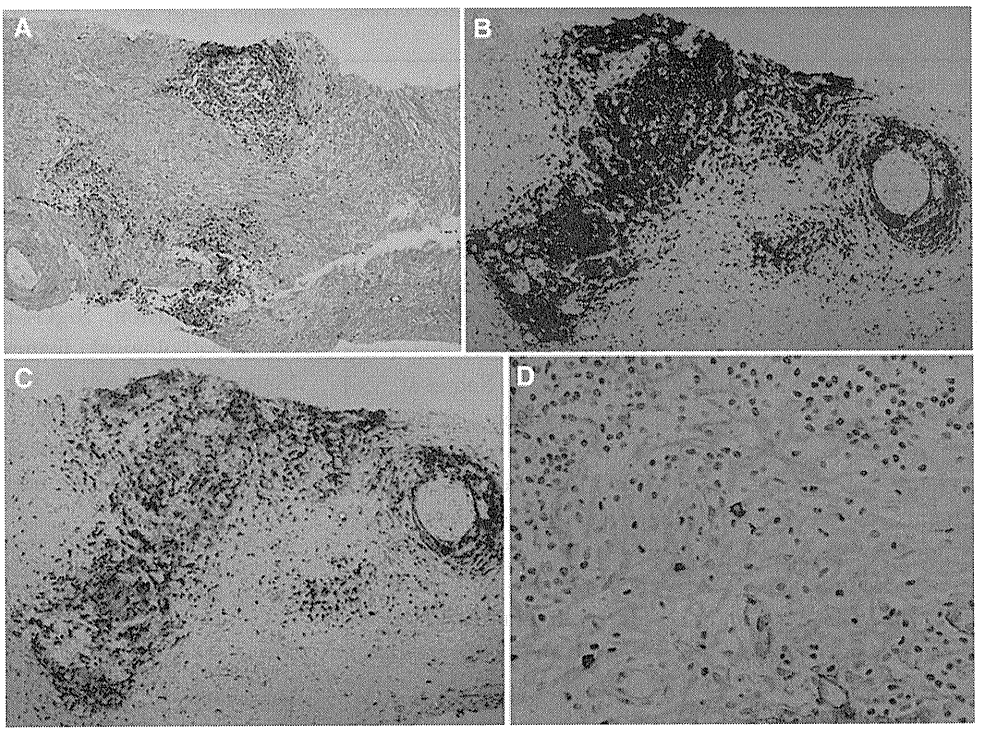

Fig. 2 Surgically biopsied specimen of the pancreas of SIgG4-negative AIP patient. **a** Marked fibrosis with lymphocytic infiltration was detected. Immunohistochemically, **b** infiltration of CD20-positive B lymphocytes was more dominant than **c** that of CD3-positive T lymphocytes. **d** Few IgG4-positive plasma cells were observed (IgG4 immunostaining)

they reported that AIP patients with normal IgG4 levels were more likely to be female (45 vs. 9%, $p = 0.01$) and less likely to present with extrapancreatic lesions (65 vs. 27%, $p = 0.03$).

Recently, another type of AIP was reported under the name of IDCP [9] or AIP with GEL [10]. It is histologically characterized by ductal epithelial granulocytic infiltration and less presence of IgG4-positive cells, a feature not seen in LPSP. IDCP is sometimes detected in Western countries, but it appears uncommon in Japan and Korea [2]. Although the clinical features of IDCP have not been fully clarified, IDCP appears to affect younger patients and may not have a male preponderance, with no involvement of other organs other than inflammatory bowel disease [3, 9, 10]. Serum IgG4 levels are rarely elevated in IDCP patients [3]. Italian AIP patients had a lower average age (43.4 years), a relatively high proportion of women (38%), a high rate of segmental swelling of the pancreas (63%), a low

Table 8 Histological findings of the pancreas of SIgG4-positive and SIgG4-negative AIP patients

	SIgG4-positive ($n = 13$)	SIgG4-negative ($n = 8$)
Resection	LPSP ($n = 5$)	LPSP ($n = 2$)
Biopsy	LPSP ($n = 5$)	LPSP ($n = 1$)
		Fibrosis with abundant infiltration of B lymphocytes ($n = 1$)
EUS-FNA	Inadequate materials ($n = 3$)	LPSP ($n = 1$)
		Fibrosis with abundant infiltration of B lymphocytes ($n = 1$)
		Inadequate materials ($n = 2$)

Table 9 Differences in the number of IgG4-positive plasma cells infiltrating the gastric mucosa of SIgG4-positive and SIgG4-negative AIP patients

	SIgG4-positive ($n = 17$)	SIgG4-negative ($n = 7$)	p value
Number of IgG4-positive plasma cells (/hpf) mean ± SD (range)	7.0 ± 5.5 (0–20)	1.4 ± 0.9 (0–3)	0.004

prevalence of serum IgG4 elevation (50%), frequent associations with acute pancreatitis (32%) and ulcerative colitis (30%), and rare involvement of other organs [25]. This clinical profile of Italian AIP patients suggests that a fair proportion of Italian AIP patients had IDCP. Sah et al. [26] reported that 3 of 4 IDCP patients presented with acute pancreatitis. Clinical profiles of SIgG4-negative AIP patients are similar to those of IDCP or Italian AIP patients, except for young age and frequent association with ulcerative colitis.

Both inflammatory and neoplastic lesions accumulate FDG on FDG-PET, and showed high signal intensity on DWI. Findings on FDG-PET and DWI were quite similar in SIgG4-positive and SIgG4-negative AIP. In the present study, histological examination of the pancreas was performed in 8 SIgG4-negative AIP patients. LPSP was diagnosed in 4 patients. EUS-FNA of 2 SIgG4-negative AIP patients could not confirm the diagnosis due to inadequate materials, including a 33-year-old male patient with ulcerative colitis. Asian diagnostic criteria can diagnose IDCP from pancreatic imaging and steroid responsiveness. Since histological examination of an adequate pancreatic specimen is necessary to diagnose IDCP, it is possible that IDCP is included in some of the SIgG4-negative AIP patients. On the other hand, in the pancreas of 2 SIgG4-negative AIP patients, marked fibrosis and abundant infiltration of B lymphocytes rather than T lymphocytes, and destruction of acinar cells were detected, but few IgG4-positive plasma cells were observed, and neutrophilic infiltration was not detected. This histology may be another type of SIgG4-negative AIP other than LPSP and IDCP.

Salivary gland function was impaired in all AIP patients, but the degree of impairment was less in SIgG4-negative AIP patients. Lacrimal gland function was impaired only in SIgG4-positive AIP patients. In 2 resected SIgG4-negative AIP patients, abundant infiltration of IgG4-positive cells was confined to the pancreas, and it was not detected in the peripancreatic retroperitoneal region or peripancreatic lymph nodes. Although abundant infiltration of IgG4-positive plasma cells is sometimes detected in the gastric mucosa of AIP patients [5, 27, 28], IgG4-positive plasma cells rarely infiltrated the gastric mucosa of SIgG4-negative AIP patients. It has been reported that serum IgG4 levels reflect disease activity of AIP, and extrapancreatic lesions tend to be more common in patients with higher serum IgG4 levels [7, 29]. Given these findings, though AIP is a systemic disease exhibiting IgG4-related phenomena throughout various organs, these phenomena tended to be rather confined to the pancreas in SIgG4-negative AIP patients. The greatest weakness of this study is that the number of histologically confirmed cases and SIgG4-negative AIP patients is too small to be conclusive. Although AIP is a rare disease, further prospective studies are necessary to clarify this issue.

In conclusion, SIgG4-negative AIP showed different clinicopathological features from SIgG4-positive AIP. Some SIgG4-negative AIP cases are LPSP that is rather confined to the pancreas. SIgG4-negative AIP may include IDCP or sclerosing pancreatitis other than LPSP or IDCP, but further studies are needed to clarify this issue.

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Polymorphism in the *KCNA3* gene is associated with susceptibility to autoimmune pancreatitis in the Japanese population

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Abstract. Autoimmune pancreatitis (AIP), characterized by irregular narrowing of the main pancreatic duct, swelling of the pancreas, and histological evidence of lymphoplasmacytic inflammation by high serum immunoglobulin G4, is distinct from ordinary pancreatitis. However, genetic factors involved in the etiology and pathophysiology of AIP remain unclear. Sixty-four patients with autoimmune pancreatitis (53 men, 11 women; mean age, 62.4 years) and 104 healthy Japanese controls were enrolled in this study. We performed an association analysis using 400 microsatellite markers with an average spacing of 10.8 cM in the genome. We also evaluated the association of AIP with seven single nucleotide polymorphisms (SNPs) within the 20-kb region around the potassium voltage-gated channel, shaker-related subfamily, member 3 gene (*KCNA3*). We identified six statistically significant markers (D1S2726, D5S410, D6S460, D10S548, D15S128, and D20S186; $P < 0.05$) related to susceptibility. The surrounding region showing the strong association ($P = 7.4 \times 10^{-7}$ Pc = 0.0015) contained the *KCNA3* gene. Further analysis by SNP genotyping in *KCNA3* gene revealed that four SNPs (rs2840381, rs1058184, rs2640480, rs1319782) were significantly associated with the AIP susceptibility ($P < 0.007$). *KCNA3* is known to be involved in immunomodulation of autoreactive effector and memory T cell–mediated autoimmune diseases. Our findings provide the first evidence that *KCNA3* is associated with AIP and suggest that *KCNA3* may influence the risk for AIP.

Keywords: AIP autoimmune pancreatitis, SNPs, KCNA3, disease susceptibility

Abbreviations

AIP,	autoimmune pancreatitis;
HWE,	Hardy-Weinberg equilibrium;
HWP,	Hardy-Weinberg proportion;
KCNA3,	potassium voltage-gated channel, shaker-related subfamily, member 3;
LD,	linkage disequilibrium;
SNP,	single nucleotide polymorphism;
Pc-value,	corrected *P*-value.

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1. Introduction

Autoimmune pancreatitis (AIP) is a unique form of chronic pancreatitis characterized by minimal abdominal pain, irregular narrowing of the pancreatic duct, swelling of the pancreatic parenchyma, and predominance in elderly males. It has been referred to by various designations [1–7] and is now generally termed AIP based on clinical features, various serum autoantibodies, hypergammaglobulinemia, histological evidence of lymphoplasmacytic inflammation and fibrosis, and a favorable response to glucocorticoid treatment [3].

Awareness of AIP is a matter of clinical importance because this disease has been frequently misdiagnosed as pancreatic cancer [8]. Furthermore, this disease is

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associated with a variety of extra-pancreatic complications such as sclerosing cholangitis [2,5] sialoadenitis [9,10], hypothyroidism [11], hilar lymphadenopathy [10], retroperitoneal fibrosis [7], interstitial pneumonia [12], and tubulointerstitial nephritis [13], and frequently has been designated as primary sclerosing cholangitis, Sjogren's syndrome, Hashimoto's thyroiditis, sarcoidosis, or primary retroperitoneal fibrosis. Because patients with AIP respond to corticosteroid treatment, the correct diagnosis should be made in order that timely and effective treatment can be implemented.

The etiology and pathogenesis of AIP remain unclear. Previous studies have shown that T-lymphocytes infiltrate the pancreatic tissues and that carbonic anhydrase II and lactoferrin are candidate target antigens [14]. However, there have been conflicting reports regarding the role of cellular immunity and effector cells, and anti-carbonic anhydrase II and anti-lactoferrin autoantibodies have not been proven specific to this condition [15]. The most characteristic feature of AIP is a specific augmentation of serum IgG4 concentration, which was found in over 90% of patients and is indicative of disease activity [6]. Histological findings of abundant IgG4-positive plasma cells are a hallmark of this disease [7,9,16]. These results suggest that IgG4 plays a major role in the pathogenesis of AIP

The development of AIP is likely influenced by multiple interactions between genetic and environmental factors. Our previous report suggested that genetic factors for susceptibility to the disease were premier immune loci, such as the HLA *DRB1*0405-DQB1*0401* haplotype [17], the Fc receptor-like gene 3 (*FCRL3*) [18], and cytotoxic T-lymphocyte antigen 4 gene (*CTLA4*) [19]. However, the genetic factors underlying AIP have not been elucidated conclusively. Moreover, there are numerous candidate genes for AIP susceptibility. Recent progress in molecular genetics has enabled direct approaches for identifying genetic determinants. Here, we aimed to identify potential AIP susceptibility gene regions by performing a genome-wide scan and single nucleotide polymorphism (SNP) genotyping around candidate susceptibility genes.

2. Materials and methods

2.1 Patients

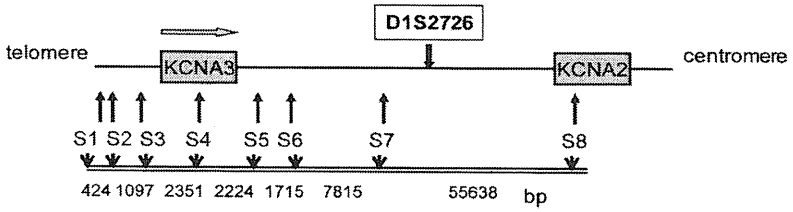
Between September 1994 and October 2004, we treated and followed 64 patients with AIP The 53 men and 11 women were 38-79 years of age (median age, 62.4 years). Diagnostic criteria for AIP included (1) enlarged pancreas on ultrasonography, computed tomography, or magnetic resonance imaging, and irregular narrowing of the main pancreatic duct on endoscopic retrograde cholangio-pancreatography; (2) increased serum gammaglobulin levels, high serum IgG or IgG4 concentrations, or the presence of autoantibodies; and (3) characteristic histological findings of lymphoplasmacytic infiltration and fibrosis [20]. Thirtynine (61%) had concurrent autoimmune diseases, including hypothyroidism (12 patients; 22%) and sclerosing cholangitis (34 patients; 74%), whose diagnosis was described in our prior study [21]. Eight patients had a pathological diagnosis. In western countries, AIP is classified in type 1 and type 2, in which type 1 corresponds to LPSP (lymphoplasmacytic sclerosing pancreatitis; IgG4-related disease) and type 2 to IDCP or AIP with GEL(granulocyte epithelial lesion). The clinical features of type 2 AIP include the following; on average, patients are a decade or more younger in age than patients with type 1 AIP, there is no gender bias, no association with systemic involvements, no elevation of serum IgG4, no or minimal tissue infiltration of IgG4 bearing plasma cells, and there is an association with inflammatory bowel disease in almost 30% of patients. Clinical findings of Japanese AIP definitely different from type 2. All of our patients showed clinical findings of type 1 AIP or LPSP

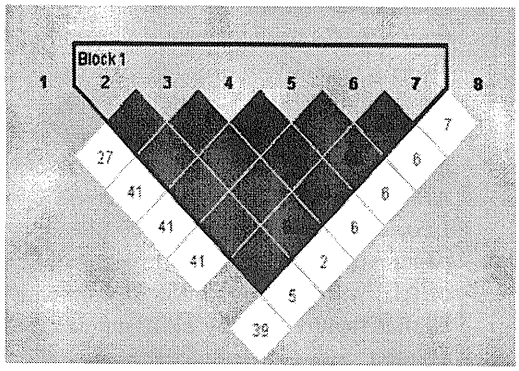
One hundred and four individuals (56 men and 48 women, median age, 54.5 years) for controls were enrolled in this study. The control subjects are all healthy volunteers having regular medical check-ups and they reside in Nagano prefecture.

The institutional ethics committee granted permission for this study; all patients and control subjects provided written performed consent to participate in this study.

2.2. Microsatellite genotyping

The genome scan was carried out using 400 microsatellite markers (ABI Linkage Mapping Set v.2.5 – MD10; Applied Biosystems, Foster City, CA) with an average heterozygosity of 79% and an intermarker distance of 9.4 ± 2.9 cM (mean ± SD). The entire marker set consisted of 28 panels, each containing markers pooled together according to size and fluorescent tag (6-FAM, VIC, NED). The markers were amplified by polymerase chain reaction (PCR) in 10-μl reactions each containing 40 ng of genomic DNA, according to the manufacturer's protocol. After PCR, the pooled





Estimated haplotype frequencies in control

Haplotype	S2	S3	S4	S5	S6	S7	Frequency (n=104)
1	T	A	C	A	T	A	0.428
2	C	G	A	C	C	G	0.389
3	C	A	C	A	T	G	0.130
4	C	G	C	A	T	G	0.029
6	C	G	A	C	C	A	0.019

Estimated haplotype frequencies in patients

Haplotype	S2	S3	S4	S5	S6	S7	Frequency (n=64)
1	C	G	A	C	C	G	0.554
2	T	A	C	A	T	A	0.352
3	C	A	C	A	T	G	0.039
4	C	G	C	A	T	G	0.016
5	C	G	C	A	T	A	0.016
6	C	A	C	A	T	A	0.015

Fig. 1. Structures of linkage disequilibrium (LD) and the haplotype block from rs3762379 to rs3887820 on chromosome 1p13.3. The pairwise LD (D') diagram was delineated using the solid spine method with D' > 0.8 for the 104 Japanese control samples. Haplotype frequencies were estimated by the maximum-likelihood method, with an expectation-maximization algorithm. S1. rs3762379, S2: rs2821557, S3: rs2840381, S4: rs1058184, S5: rs2640480, S6: rs1319782, S7:rs2821548, S8: rs3887820. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-2011-0820)

panels were electrophoresed on an ABI 3130 DNA Analyzer. Semi-automated genotyping was performed using GeneMapper v 3.5 (Applied Biosystems). DNA samples from all patients were collected before steroid therapy.

2.3. Singlel nucleotide polymorphism genotyping

We identified one locus located on chromosome 1p13.3 (microsatellite marker D1S2726) as potentially associated with AIP The gene encoding potassium voltage-gated channel, shaker-related subfamily, member 3 (*KCNA3*) lies 31 kb telomeric of the D1S2726 microsatellite marker and was therefore targeted as a candidate susceptibility gene.

Eight SNPs distributed around *KCNA3* were selected from the National Center for Biotechnology Information (NCBI) dbSNP database (build 36), the JSNP database, and the SNP database of Applied Biosystems (Fig. 1) based on the following criteria: (a) location within the 20-kb region around the candidate microsatellite marker; (b) greater than 10% minor allele frequency in the Japanese population; (c) 0.3 average heterozygosity; (d) marker density of at least one SNP per 8 kb; and (e) availability for validation assays. SNP genotyping was performed using TaqMan® SNP Genotyping Assays, according to manufacturer's instructions.

2.4. Statistical analysis

Frequencies of alleles in each microsatellite marker were estimated by direct counting. To estimate the statistical significance of comparisons between patients with AIP and healthy control subjects, we used the χ^2-test and Fisher's exact probability test for 2×2 contingency tables. We defined a P-value of less than 0.05 as statistically significant. The P-values were corrected by multiplying by the number of different alleles observed in each locus (Pc), and also by multiplying the 400 markers typed in the study. The pairwise relationships between microsatellites, SNPs, and haplotypes were estimated by calculating odds ratios (ORs) and 95% confidence intervals (CIs). The Hardy-Weinberg proportion (HWP) for multiple alleles was calculated by the Markov chain method within the GENEPOP software package (http://wbiomed.curtin.edu.au/genepop/index.html). The linkage disequilibri-

Table 1
Statistically significant STR markers by association for AIP

Chromosome	locus	allele	patient (%) $n = 64$	control (%) $n = 104$	OR	95%CI	c2	P	Pc	Pc*
1	D1S2726	280	27 (42.2)	9 (8.7)	7.70	3.31–17.92	24.507	0.00000074	0.0000037	0.00030
		292	6 (9.4)	27 (26.0)	0.30	0.11–0.76	5.895	0.015	0.076	
	D1S0655i	266	47 (73.4)	47 (45.2)	3.35	1.71–6.59	12.825	0.00034	0.0014	
5	D5S410	331	38 (59.4)	32 (30.8)	3.29	1.72–6.30	13.340	0.00026	0.0067	
6	D6S460	289	23 (35.9)	17 (16.3)	2.87	1.39–5.95	8.383	0.0038	0.0010	
10	D10S548	188	60 (93.8)	69 (66.3)	7.61	2.56–22.65	16.691	0.000097	0.00039	0.039
15	D15S128	203	37 (57.8)	33 (31.7)	2.95	1.55–5.62	11.089	0.00087	0.0070	
20	D20S186	127	35 (54.7)	32 (30.8)	2.72	1.43–5.17	9.450	0.0021	0.021	

Pc was calculated by multiplying the numbers of alleles in the locus.
Pc* was calculated by multiplying the 400 markers typed in the study.

um (LD) patterns, haplotype block structure, and haplotype frequency analysis for SNPs were identified using the block definition of Gabriel et al. [22] and were based on the 95% CI of pairwise LD (D'), as determined with Haploview software [23].

3. Results

Genome-wide linkage association analysis using 400 microsatellite markers identified seven markers as new candidate loci for AIP (Table 1). Strong evidence of positive association was detected for the marker D1S2726 (42.2% vs. 8.7%, $Pc = 0.0000037$) on chromosome (chr) 1p13.1, D5S410 (59.4% vs. 30.8%, $Pc = 0.0067$) on chr 5q31-33, D6S460 (35.9% vs. 16.3%, $Pc = 0.0010$) on chr 6q14, D10S548 (93.8% vs. 66.3%, $Pc = 0.00039$) on chr 10p12, D15S128 (57.8% vs. 31.7%, $Pc = 0.007$) on chr 15q15, and D20S186 (54.7% vs. 30.8%, $Pc = 0.021$) on chr 20p12.2. The observed and expected frequencies of each genotype for the seven markers in both case and control subjects were within Hardy-Weinberg equilibrium (HWE; data not shown).

D1S2726 was identified as a marker of interest for further analysis. To further validate the association of the D1S2726 marker with AIP, we examined the association of AIP with an additional marker located 30.7 kb centromeric of D1S2726, D1S0655i. Allele 266 of D1S0655i was also indicated a positive association with AIP (73.4% vs. 45.2% OR = 3.35, $Pc = 0.0014$, Table 1).

To predict novel susceptibility genes within 100-kb of significant markers, we used the NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/mapview/; Table 2). Two genes, *KCNA3 and KCNA2*, were identified within the candidate region around D1S2726. We characterized *KCNA3* as a candidate gene by performing an association analysis using seven SNPs (Fig. 1). Four SNPs (rs2840381 allele G, rs1058184: allele A, rs2640480: allele C, rs1319782: allele C) showed statistically significant association (Table 2). All SNPs were screened in all AIP cases and control subjects. The frequencies of the SNPs for both patients and controls were within HWE. Pairwise LD mapping confirmed that the haplotype structure including*KCNA3* was divided into three blocks (rs3762379, major block; rs2821557 to rs2821548, and rs3887820, Fig. 1). The CGACCG haplotype of the major block was significantly more prevalent in the patient group than in the control group ($P = 0.04$, OR = 1.93).

4. Discussion

Despite recent progress in its clinical, immunological, radiological, and morphological characterization [24], the etiology of AIP still remains unclear. Genetic factors including the HLA DRB1*0405-DQB1*0401 haplotype [17] and polymorphisms of the *FCRL3* and *CTLA4* genes [18,19] have been implicated in the etiology of AIP However these risk factors are identified during analyses performed in a limited region of the genome. A more comprehensive understanding of how the genetic background influences the outcome of AIP requires genome-wide association analyses [25].

This is the first case-control genome-wide association study aimed at identifying candidate genes for AIP pathogenesis, even though the number of enrolled subjects was too small to overcome type I error. Four hundred microsatellite markers were used for this study, providing 10.8-cM genome-wide resolution. This approach is not as efficient as using tens of thousands of microsatellite markers at 100 kb intervals across the human genome [26]; therefore, there are likely to be many undetected genes involved in susceptibility to

Table 2

Association of 8 SNPs around KCNA3 gene with AIP

SNP rs	Observed Allele (1/2)	Frequency		Genotype distribution						*P* value (1/2)	OR 95% CI (1/2)	*P* value (11/12+22)	*P* value (11+12/22)	HWE	
		Allele 1		Allele 1/1		Allele 1/2		Allele 2/2							
		Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls					Patients	Controls
rs3762379	C/T	0.922	0.856	55	75	8	28	1	1	0.0692	1.99 (0.94–4.22)	0.038	0.727	0.290	0.355
rs2821557	C/T	0.648	0.567	27	39	29	40	8	25	0.1407	1.14 (0.89–2.22)	0.546	0.068	0.961	0.027
rs2840381	G/A	0.594	0.438	22	24	32	43	10	37	0.0054	1.88 (1.20–2.94)	0.111	0.005	0.771	0.103
rs1058184	A/C	0.563	0.409	21	22	30	41	13	41	0.0061	1.86 (1.19–2.90)	0.093	0.010	0.703	0.060
rs2640480	C/A	0.563	0.409	21	22	30	41	13	41	0.0061	1.86 (1.19–2.90)	0.093	0.010	0.703	0.060
rs1319782	C/T	0.563	0.409	21	22	30	41	13	41	0.0061	1.86 (1.19–2.90)	0.093	0.010	0.703	0.060
rs2821548	G/A	0.609	0.553	24	37	30	41	10	26	0.3091	1.26 (0.81–1.97)	0.801	0.150	0.902	0.039
rs3887820	C/A	0.586	0.457	25	22	25	51	14	31	0.0214	1.68 (1.08–2.63)	0.012	0.260	0.119	0.904

SNPrs: public reference SNP number from the dbSNP database.
95% CI: 95% confidence interval; OR: odds ratio.
P value was calculated by c2 test 2 × 2 contingency table.
HWE: Hardy-Weinberg Equilibrium.

Table 3
Estimated haplotype frequencies from 3 SNPs in four different populations

SNP	rs1058184	rs2640480	rs1319782	Frequency
Pop				
CEU	C	A	T	0.70
	A	C	C	0.30
CHB	C	A	T	0.56
	A	C	C	0.44
YRI	C	A	T	0.65
	A	C	C	0.34
	C	A	C	0.01
JPA (Cont)	C	A	T	0.59
	A	C	C	0.41
JPA(AIP)	C	A	T	0.44
	A	C	C	0.56

Haplotye frequencies in CEU, CHB, and YRI populations were calculated by HapMap database data.
CEU: Utah residents with ancestry from northern and western Europe.
CHB: Han Chinese in Beijine, China.
YRI: Yoruba in Ibadan, Nigeria.
JAP (Cont): Control group in this study, Japanese.
JAP (AIP): Patients group in this study, Japanese.

AIP Nonetheless, this approach identified seven statistically significant makers (Table 1). In the regions surrounding these markers, we noted interesting genes that might be linked to AIP susceptibility, including *KCNA3*.

KCNA3 is located 30 kb telomeric of D1S2726 and encodes the voltage-gated potassium channel Kv1.3 [27]. Kv1.3 regulates membrane potential and Ca^{2+}-signaling in human T cells and plays an essential role in T-cell proliferation and activation [28–30]. This molecule is expressed in a variety of tissues and hematopoietic cells, particularly in effector memory T cells (T_{EM}). Terminally differentiated T_{EM} cells enter inflamed tissues rapidly and produce copious amount of IFN-γgnd IL-4 [31]. Therefore, suppressing the function of these cells by selectively blocking the Kv1.3 channel offers a potential therapeutic strategy for T cell-mediated autoimmune diseases [32]. Interestingly, Kv1.3 blockers preferentially suppress the proliferation of late memory B cells (CD27+IgG+IgD-) [33], which play an important role in production of IgG antibodies. Consequently, Kv1.3 serves a critical function in modulating immune responses. Thus, the high level production of IgG4 in patients with AIP could be caused by the proliferation of late memory B cells and the elevated expression of Kv1.3 molecules.

Association analysis using eight SNPs in the region of *KCNA3* identified four SNPs (rs2840381, rs1058184, rs2640480, rs1319782) significantly associated with susceptibility to AIP ($P < 0.007$). SNPs in the promoter region of the Kv1.3 gene were examined whether they were associated with impaired glucose tolerance and reduced insulin sensitivity in patients with diabetes mellitus [34]. One of these, rs2821557 (T-1645C) was shown to exhibit differential transcription activity. However, rs2821557 SNP in our analysis was not associated with disease susceptibility to AIP The haplotype (GACC) frequency of these four SNPs involved in the one haplotype block (rs2821557 to rs2821548: CGACCG) was significantly higher in the patient group than in the control group ($P = 0.014$, OR $= 2.45$). This haplotype locates at 6.29 kb upstream of the promoter/5'-untranslated region (UTR) to downstream of the 3'-UTR in *KCNA3* and includes the full length of the *KCNA3* coding region. This result highlights the need to investigate haplotype frequencies in different populations. When comparing the distribution of haplotype frequencies constructed by three of these SNPs (rs1058184, rs2640480, rs1319782) using HapMap data (http://www/hapmap.org/) in three different populations (northern and western Europe, Han Chinese, Yoruba in Ibadan, Nigeria) and in our control group, the CAT haplotype was the most frequent (Table 3). These results provide further support that *KCNA3* is a general susceptibility gene for AIP

Currently, we are searching for candidate susceptibility genes by performing high-resolution microsatellite analysis around the positive markers identified in this study. Future studies also need to focus in the identification of therapy-effectiveness- or disease-severity-related genes. These types of studies could aid in the identification and development of specific therapies for patients with AIP

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Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Risk factors for pancreatic stone formation in autoimmune pancreatitis over a long-term course

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Abstract
Background Autoimmune pancreatitis (AIP) has the potential to progress to a chronic state that forms pancreatic stones. The aim of this study was to clarify the risk factors underlying pancreatic stone formation in AIP.
Methods Sixty-nine patients with AIP who had been followed for at least 3 years were enrolled for evaluation of clinical and laboratory factors as well as computed tomography and endoscopic retrograde cholangiopancreatography findings.
Results During the course of this study, increased or de novo stone formation was seen in 28 patients, who were defined as the stone-forming group. No stones were observed in 32 patients, who were defined as the non-stone-forming group. Nine patients who had stones at diagnosis but showed no change during the course of this study were excluded from our cohort. Univariate analysis revealed no significant differences in clinical or laboratory factors associated with AIP-specific inflammation between the two groups. However, pancreatic head swelling ($P = 0.006$) and narrowing of both Wirsung's and Santorini's ducts in the pancreatic head region ($P = 0.010$) were significantly more frequent in the stone-forming group. Furthermore, multivariate analysis identified Wirsung and Santorini duct narrowing at diagnosis as a significant independent risk factor for pancreatic stone formation (OR 4.4, $P = 0.019$).
Conclusions A primary risk factor for pancreatic stone formation in AIP was narrowing of both Wirsung's and Santorini's ducts, which most presumably led to pancreatic juice stasis and stone development.

Keywords Autoimmune pancreatitis · Pancreatic stone · Wirsung duct · Santorini duct

Abbreviations

AIP	Autoimmune pancreatitis
CT	Computed tomography
ERCP	Endoscopic retrograde cholangiopancreatography

Introduction

Autoimmune pancreatitis (AIP) is a specific type of chronic pancreatitis possibly caused by autoimmune mechanisms that is characterized by pancreatic swelling and irregular narrowing of the main pancreatic duct, both of which mimic pancreatic cancer [1]. Other characteristic features of AIP are high serum IgG4 concentration and IgG4-positive plasma cell infiltration in affected pancreatic tissue that also aid in serological and pathological AIP diagnosis [2, 3]. As patients with AIP respond favorably to corticosteroid therapy, the disease was previously believed to be a non-progressive condition which did not progress to an advanced stage of chronic pancreatitis or pancreatic stone formation [4]. However, the short-lived pancreatic swelling and severe lymphoplasmacytic infiltration in acute AIP are now believed to manifest as different clinical features in a chronic state; earlier studies have

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shown that AIP progresses to a chronic stage showing pancreatic stone formation and atrophy resembling ordinary chronic pancreatitis that is closely associated with relapse [5–12]. Moreover, we found that patients with seemingly typical chronic pancreatitis also included several cases with elevated serum IgG4 concentration, which may have been due to chronic stage AIP [6].

Two major mechanisms attempt to explain the formation of pancreatic stones in AIP: severe inflammation specific to AIP and stasis of pancreatic juice due to narrowing of the pancreatic duct [13, 14]. In general, AIP rarely results in severe inflammation or tissue necrosis. Corticosteroid therapy ameliorates irregular narrowing of the pancreatic duct in the majority of patients, although residual stenosis may persist [15]. Additionally, some patients not undergoing corticosteroid therapy show progression of duct changes [16]. On the basis of these findings, we hypothesized that the formation of pancreatic stones in AIP is associated with stasis of pancreatic juice due to stenosis of the pancreatic duct. The aim of the present study was to clarify the risk factors underlying pancreatic stone formation in AIP by comparing the clinical features and frequency of pancreatic stone formation in a long-term follow-up cohort of AIP patients.

Patients and methods

Study subjects

Ninety-three patients with AIP were examined and treated at Shinshu University Hospital between August 1992 and July 2011. Among them, we enrolled 69 patients who had been followed for at least 3 years (median follow-up 91 months, range 36–230 months), which included 54 men and 15 women (median age 64 years, range 38–84 years). Diagnosis of AIP was based on the Asian diagnostic criteria for AIP [17].

Clinical features and laboratory tests

We reviewed the medical charts of our cohort for observation period, age at diagnosis, gender, alcohol consumption, corticosteroid treatment, and relapse. We also compared serum values representative of AIP activity from blood tests at diagnosis, including those for IgG, IgG4, C3, C4, soluble interleukin 2 receptor (sIL2-R), circulating immune complex (CIC), and amylase.

Evaluation of pancreatic stone formation

The presence of pancreatic stones was assessed by using CT images. We evaluated the location of stones with respect to pancreatic region (head, body, or tail), as well as with respect to the pancreatic duct (in the main pancreatic duct or in parenchyma). We also assessed the size and number of stones during the study period. CT scanning was performed using different protocols during the course of this study. At our institute, CT testing was changed to multidetector computed tomography (MDCT) in 2003, which resulted in clearer CT images.

Evaluation of pancreatic swelling

Swelling of the pancreas in CT images was assessed by three pancreatology experts. Pancreatic swelling was determined using the Haaga criteria [18] or a marked decrease in size after corticosteroid therapy and was classified by its location in the pancreas (head, body, or tail). Swelling restricted to either one area or spanning two or three areas was considered to be focal or segmental-diffuse swelling, respectively.

Evaluation of pancreatic duct narrowing

Narrowing of the pancreatic duct seen in endoscopic retrograde pancreatocholangiography (ERCP) was assessed by three expert endoscopists. Pancreatic duct narrowing was classified by its location in the pancreas (head, body, or tail), and narrowing in the head region was further divided into narrowing of Wirsung's duct and narrowing of Santorini's duct. Narrowing restricted to either one area or spanning two or three areas was considered to be focal or segmental-diffuse narrowing, respectively.

Statistical analysis

The Fisher's exact and Pearson's chi-square tests were adopted to test for differences between subgroups of patients. The Mann–Whitney U test was employed to compare continuous data. Multivariate analyses were performed using a logistic regression model. Variables associated with a P value of less than 0.2 in univariate analyses were included in a stepwise logistic regression analysis to identify independent risk factors associated with the formation of pancreatic stones. All tests were performed using the IBM SPSS Statistics Desktop for Japan ver. 19.0 (IBM Japan Inc, Tokyo, Japan). P values of less than 0.05 were considered to be statistically significant.

Ethics

This study was approved by the ethics committee of Shinshu University (approval number 1805).

Results

Pancreatic stone formation

At diagnosis, pancreatic stones were found in 17 of 69 patients and increased in size and number in 8 patients. De novo stone formation was observed in 20 of the remaining 52 patients. In total, increased or de novo stones were seen in 28 patients during the study period, who were collectively defined as the stone-forming group. The 32 patients in whom no stones were found during the course of the study were defined as the non-stone-forming group (Fig. 1). Nine patients who had stones at diagnosis but showed no change during the course of this study were excluded from our cohort.

There were no significant differences in the frequency of pancreatic stone formation among pancreatic areas between the stone increase and de novo stone cases. However, stone formation in the main pancreatic duct was more frequently seen in de novo cases, but not significantly ($P = 0.151$) (Table 1). Thus, there were no fundamental differences in the manner of new stone formation. For de novo stone patients, the median and range of the study period between diagnosis of AIP and stone formation were 57 and 8–138 months, respectively.

Correlation between pancreatic stone formation and clinical and laboratory features associated with AIP-specific inflammation

We next searched for risk factors of pancreatic stone formation by comparing several parameters between the stone-forming and non-stone-forming groups. Univariate analysis revealed no significant differences in observation period, age, gender, alcohol consumption, or corticosteroid treatment between the stone-forming group and the non-stone-forming group. Relapse was more frequently seen in the stone-forming group, but not significantly ($P = 0.093$). We also found no significant differences in serum values of disease activity markers, such as IgG, IgG4, C3, C4, sIL2-R, and CIC, between the two groups (Table 2).

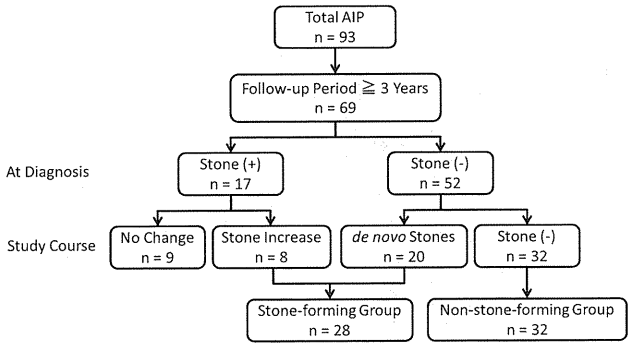

Fig. 1 Study participation flowchart and outcome of 69 patients with AIP who were followed for at least 3 years (mean 91 months, range 36–230 months)

Table 1 Location of pancreatic stone formation

	Stone increase cases ($n = 8$)	De novo stone cases ($n = 20$)	*P* value
Head/body/tail	6/8/5	17/20/15	NS
In MPD/in parenchyma	3/16	18/34	0.151

MPD main pancreatic duct, *NS* not significant

Correlation between pancreatic stone formation and pancreatic swelling

We examined whether pancreatic stone formation was associated with the extent or location of pancreatic swelling. Univariate analysis showed no significant differences in the extent of pancreatic swelling in the focal area versus in the segmental-diffuse area between the stone-forming group and the non-stone-forming group. However, pancreatic head swelling was significantly more frequent in the stone-forming group ($P = 0.006$). No significant differences were seen for pancreatic body or tail swelling (Table 3, Fig. 2).

Table 2 Clinical features and laboratory tests at diagnosis

	Stone-forming group ($n = 28$)[a]	Non-stone-forming group ($n = 32$)[a]	*P* value
Clinical features			
Observation period[b]	100 (36–165)	90 (36–230)	0.524
Age	67 (47–84)	64.5 (38–81)	0.543
Sex (M/F)	24/4	22/10	0.140
Alcohol (+/−)	20/8	19/12	0.582
Prednisolone (+/−)	25/3	28/4	1.000
Relapse (+/−)	11/17	6/26	0.093
Laboratory tests			
Amylase	94 (17–431)	86 (22–478)	0.678
IgG	2,187 (892–7,236)	2,183 (1,194–5,545)	0.686
IgG4	640 (154–2,855)	424 (4–2,970)	0.916
C3	91 (33–157)	87 (29–199)	0.538
C4	20.1 (7.7–39.7)	21.3 (1.1–38.7)	0.627
sIL2-R	738 (132–2,260)	940 (257–4,695)	0.130
CIC	5.1 (1.9–40)	5.5 (1.9–27.5)	0.392

sIL2-R soluble interleukin 2 receptor, *CIC* circulating immune complex

[a] Values are expressed as median (range)

[b] Period from diagnosis of AIP to the most recent observation (months)

Correlation between pancreatic stone formation and pancreatic duct narrowing

We next examined whether pancreatic stone formation was associated with the extent or location of pancreatic duct narrowing. Univariate analysis revealed no significant differences in the extent of pancreatic duct narrowing in the focal area versus in the segmental-diffuse area between the stone-forming group and the non-stone-forming group, nor were there significant differences in the location of pancreatic duct narrowing between the two groups. However, among cases with narrowing of the head region, patients with narrowing of both Wirsung's and Santorini's ducts were significantly more frequent in the stone-forming group ($P = 0.010$) (Table 3, Fig. 3).

In the stone-forming group, 4 patients showed duct narrowing in the body and tail regions, but 2 of them showed parenchymal pancreatic stones in the downstream pancreatic region.

Table 3 Pancreatic morphology at diagnosis

	Stone-forming group ($n = 28$)	Non-stone-forming group ($n = 32$)	P value
Swelling (by CT)			
Head (+/−)	26/2	20/12	0.006*
Body (+/−)	20/8	19/13	0.419
Tail (+/−)	17/11	19/13	1.000
Focal/segmental-diffuse	7/21	12/20	0.406
Ductal narrowing (by ERCP)			
Head (+/−)	24/4	22/10	0.140
Wirsung + Santorini (+/−)	21/7	13/19	0.010*
Body (+/−)	15/13	19/13	0.795
Tail (+/−)	22/6	24/8	0.770
Focal/segmental-diffuse	6/22	11/21	0.390

* $P < 0.05$

Multivariate analysis of pancreatic stone formation in AIP at diagnosis

Multivariate analysis was performed for gender, relapse, sIL2-R, pancreatic head swelling, and Wirsung and Santorini duct narrowing, all of which had P values of less than 0.2 in univariate studies. We identified that narrowing of both Wirsung's and Santorini's ducts at diagnosis was a significant determinant of pancreatic stone formation in AIP (odds ratio 4.4, 95% confidence interval 1.3–15.5, $P = 0.019$).

Correlation between pancreatic stone formation and residual pancreatic swelling or residual pancreatic duct narrowing after prednisolone (PSL) therapy

We further assessed whether pancreatic stone formation was associated with the extent or location of residual pancreatic swelling or residual pancreatic duct narrowing 4 weeks after PSL therapy between stone-forming patients and non-stone-forming patients. Univariate analysis showed that residual pancreatic head swelling was more

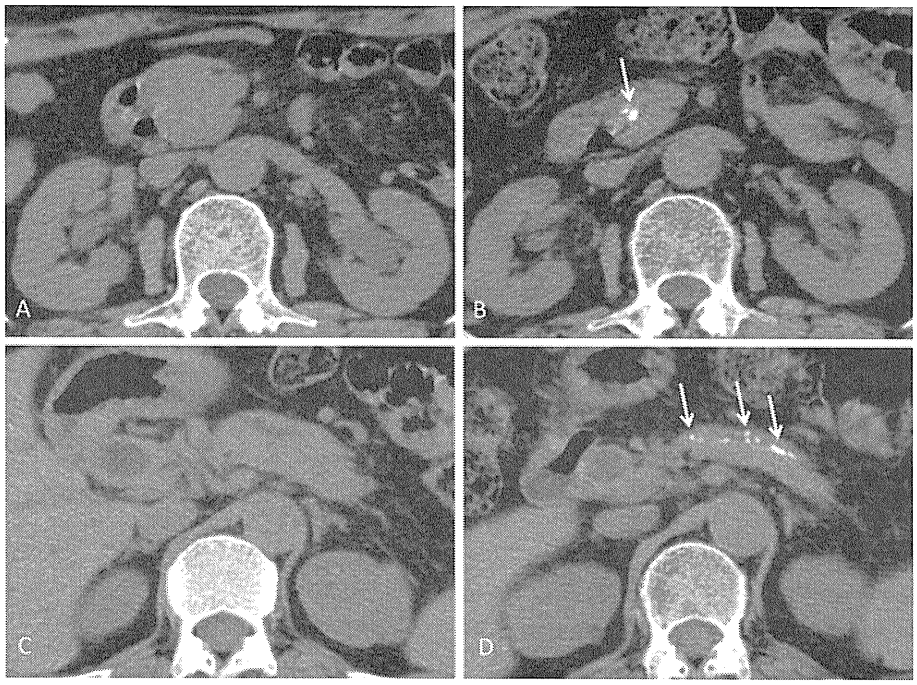

Fig. 2 CT findings in a 67-year-old female with pancreatic head swelling. **a**, **c** CT at diagnosis in May 2005 showing pancreatic head swelling. **b**, **d** CT 27 months later in August 2007 showing pancreatic stone formation (*arrows*) and pancreatic atrophy

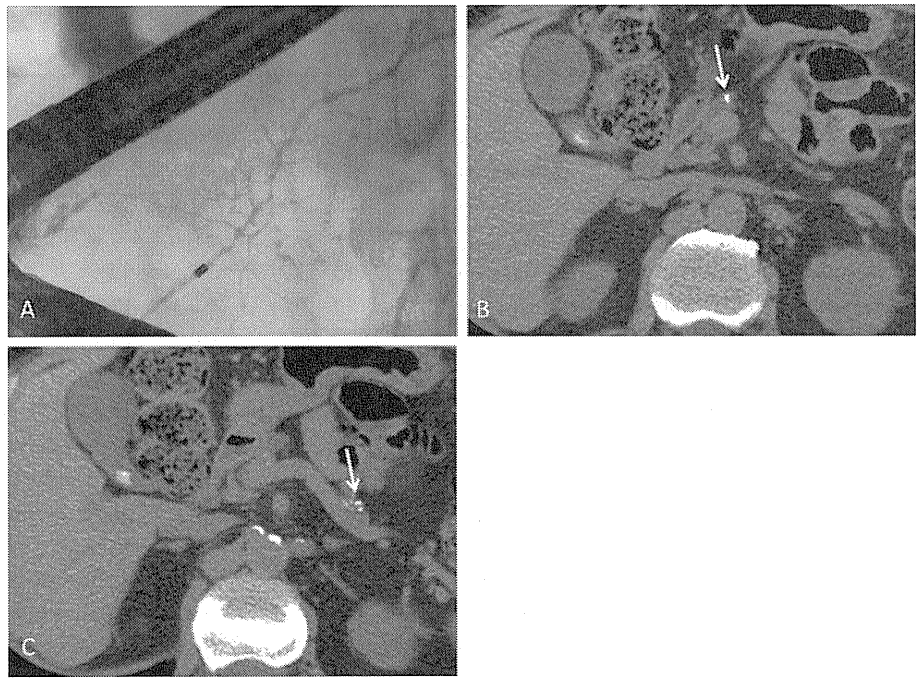

Fig. 3 ERCP and CT findings in a 69-year-old male with narrowing of both Wirsung's and Santorini's ducts. **a** ERCP at diagnosis in April 2001 showing Wirsung's and Santorini's duct narrowing. **b**, **c** CT 105 months later in December 2009 showing pancreatic stone formation (*arrows*) and pancreatic atrophy

Table 4 Pancreatic morphology after corticosteroid therapy

	Stone-forming patients ($n = 24$)	Non-stone-forming patients ($n = 26$)	P value
Swelling (by CT)			
Head (+/−)	7/17	2/24	0.069
Body (+/−)	3/21	3/23	1.000
Tail (+/−)	7/17	6/20	0.866
Focal/segmental-diffuse	7/4	2/4	0.334
	Stone-forming patients ($n = 22$)	Non-stone-forming patients ($n = 20$)	
Ductal narrowing (by ERCP)			
Head (+/−)	17/5	11/9	0.229
Wirsung + Santorini (+/−)	11/11	4/16	0.088
Body (+/−)	4/18	2/18	0.665
Tail (+/−)	7/15	10/10	0.376
Focal/segmental-diffuse	10/8	3/10	0.139

frequently seen in stone-forming patients, but not significantly ($P = 0.069$). In addition, cases with residual narrowing of both Wirsung's and Santorini's ducts in the pancreatic head region tended to be more frequently seen among stone-forming patients ($P = 0.088$) (Table 4).

Correlation between pancreatic stone formation and pancreatic function during the course of the study

We compared serum levels of amylase and HbA1c at diagnosis, at 5 years, and at 8 years among non-stone-forming patients, stone-forming patients, and intraductal stone-forming patients, who seemed to be at a more advanced stage of stone formation. Although we found no significant differences among the groups, both enzyme and HbA1c values tended to be at abnormal levels in intraductal stone-forming patients compared with non-stone-forming patients (Table 5).

Discussion

Autoimmune pancreatitis and pancreatic stone formation

An early study reported that AIP was characterized by the absence of pancreatic stones [5, 6]. Later, hallmark histological findings of marked lymphoplasmacytic infiltration representing acute AIP inflammation were found to give way to other features in the chronic stage; we reported that several patients with AIP formed pancreatic stones during the disease course [5, 6], which has been confirmed by other studies [7]. Since pancreatic stones are a major characteristic of ordinary chronic pancreatitis, such as alcoholic pancreatitis, it appears that chronic stage AIP