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Multiple gene mutations in patients with type 2 autoimmune pancreatitis and its clinical features

FENG DONG[1], QING-QUAN CHEN[2*], ZE-HAO ZHUANG[3], QING-LIANG HE[4], FENG-QING WANG[5], QI-CAI LIU[5], HE-KUN LIU[6], YU WANG[2]*

*These authors contributed equally to this manuscript.

[1]Department of Radiation Oncology, Fujian Medical University, Fuzhou, PR China
[2]Department of Laboratory Medicine, Medical Technology and Engineering College, Fujian Medical University, Fuzhou, PR China
[3]Department of Gastroenteropathy, Fujian Medical University, Fuzhou, PR China
[4]Department of Surgery, Fujian Medical University, Fuzhou, PR China
[5]Department of Laboratory Medicine, the First Affiliated Hospital, Fujian Medical University, Fuzhou, PR China
[6]Department of Cell Biology and Genetics, Fujian Medical University, Fuzhou, PR China

Abstract

Background: *It is now clear that there are two histological types (type 1 and type 2) of autoimmune pancreatitis (AIP). The histological substance of type 1 AIP is known as lymphoplasmacytic sclerosing pancreatitis (LPSP) or traditional AIP, and type 2 AIP is characterized by distinct histology called idiopathic duct centric pancreatitis (IDCP). Serum IgG4 increase is considered as a marker for type 1 AIP. Far less is known about type 2 and it lacks predicting markers, so it easily leads to missed diagnosis and misdiagnosis.*

The aim of this study *was to describe multi-gene mutations in patients with type 2 AIP and its clinical features.*

Material and methods: *Three unrelated patients with type 2 AIP, 10 cases with type 1 AIP, 15 cases with other chronic pancreatitis and 120 healthy individuals were studied. The mutations and polymorphisms of 6 genes involved in chronic pancreatitis or pancreatic cancer — PRSS1, SPINK1, CFTR, MEN1, PKHD1, and mitochondrial DNA – were sequenced. Information of clinical data was collected by personal interview using a structured questionnaire.*

Results: *Novel mutations were found in the genes encoding for MEN1 (p.546 Ala > The) and PKHD1 (c. 233586 A>G and c. 316713 C>T) from patients with type 2 AIP. What is more, the serum TCR (T cell receptor) level is relatively higher in patients with type 2 AIP than in patients with type 1 AIP and other chronic pancreatitis or normal controls. Weight loss was the major manifestation and no patients had extrapancreatic involvement in type 2 AIP.*

Conclusions: *Type 2 AIP may occur with multi-gene mutations. For screening purposes, it is more reasonable to evaluate TCR levels in serum.*

Key words: *autoimmune pancreatitis, type 2, gene mutations, serum TCR.*

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Introduction

Most of the early literature pertaining to autoimmune pancreatitis (AIP) came from Japan [1-3]. According to these criteria, AIP is classified into 2 types [4-9]. The histological substance of type 1 AIP is known as lymphoplasmacytic sclerosing pancreatitis (LPSP), and type 2 AIP is characterized by distinct histology called idiopathic duct centric pancreatitis (IDCP). IgG4 positive plasma cells are considered a marker for type 1 AIP, it can be detected in the pancreas and a variety of other tissues and increased serum IgG4 were non-invasive biomarkers [1, 4]. Type 2 AIP is generally seronegative and lacks other organ involvement in contrast to type 1 AIP. Type 2 AIP more easily leads to missed diagnosis and misdiagnosis. Histological differentiation is becoming more important for diagnosing type 2 AIP. However, a surgically resected specimen can only be obtained from a patient who was

Correspondence: Dr. Qi-cai Liu, Department of Laboratory Medicine, First Affiliated Hospital, Fujian Medical University, 20 Cha Zhong Road, Taijiang, Fuzhou 350005, China, tel. 86-591-87981972, e-mail: lqc673673673@163.com
Liu HK, PhD, Department of Cell Biology and Genetics, Fujian Medical University, Fuzhou 350005, China, tel. 86-591-87982733, e-mail: lqclqc2013@163.com

misdiagnosed with pancreatic cancer and had a surgical operation [10, 11]. Further studies are needed to clarify if cases with normal serum IgG4 are a precursor of type 1 or type 2 AIP or other diseases, and its molecular mechanism is still unknown.

Genetic factors have been identified in patients with chronic pancreatitis (CP) and these factors are believed to play an important role in the pathogenesis of CP. Mutations in protease serine 1 (*PRSS1*) (OMIM 276000), cystic fibrosis transmembrane conductance regulator (*CFTR*) (OMIM 602421), and pancreatic secretory trypsin inhibitor (*SPINK1*) (OMIM 167790), were causally linked to the pathogenesis of CP. Although there is no direct evidence of vascular involvement in the pancreas of patients with the A3243G mutation, it is known that the organ is susceptible to ischemic injury, and that perturbations of the systemic and pancreatic microvascularization play a significant role in the pathogenesis of pancreatitis [12, 13]. Moreover, polycystic kidney and hepatic disease 1 (*PKHD1*) gene mutation may be a genetic factor for pancreatitis. Williams produced a mouse model of autosomal recessive polycystic kidney disease by replacing exons 1-3 of Pkhd1 with a lacZ reporter gene utilizing homologous recombination. Dilatation of pancreatic exocrine ducts was uniformly seen in Pkhd1 (lacZ/lacZ) mice, with pancreatic cysts arising less frequently. The expression of beta-galactosidase, Pkd1, and Pkd2 was reduced in the kidneys of Pkhd1 (lacZ/lacZ) mice compared with wild-type littermates. These results indicate that deletion of exons 1-3 leads to loss of Pkhd1 expression and results in kidney cysts, pancreatic cysts. Therefore, there are reasons to believe that there is an association between *PKDH1* gene mutation and pancreatitis [14].

Material and methods

Patients and controls

A total of three patients with type 2 AIP, ten cases with type 1 AIP, 15 cases with other chronic pancreatitis and 120 healthy individuals in the past three years were included in the study, there was no history of tobacco smoking or alcohol consumption in these patients. All patients originated from the Han ethnicity in the mainland of China. AIP diagnostic criteria: I. Pancreatic imaging studies show diffuse narrowing of the main pancreatic duct with an irregular wall (more than 1/3 of length of the entire pancreas). II. Laboratory data demonstrate abnormally elevated levels of serum gamma globulin and/or IgG, or the presence of autoantibodies. III. Histopathologic examination of the pancreas shows fibrotic changes with lymphocyte and plasma cell infiltrate. For diagnosis, criterion I (pancreatic imaging) must be present with criterion II (laboratory data) and/or III (histopathologic findings).

DNA extraction and molecular genetic analysis

Genomic DNA was extracted from peripheral blood and other tissue specimens using a QIAamp DNA mini kit (Qiagen, Germany). Six genes involved in pancreatitis/pancreatic cancer – *PRSS1*, *CFTR*, *SPINK1* and multiple endocrine neoplasia 1 (*MEN1*), polycystic kidney and hepatic disease 1 (*PKHD1*) and mtDNA – were sequenced according to references [15].

The PCR methods and primers for *PRSS1*, *CFTR*, *SPINK1* and *MEN1* genes were the following reference [15]. And the primers of *PKDH1* gene are as follows: 1F: tgt gaa tca gaa tgg gca gtg, 1R: aac aag ccc tga gga aaa agc, 2F: tgg gga tga tttt atg caa gg, 2R: ggt gtt aag gta ttt gct ttt ggg, 3F: tcc tga tga gtg cag ggt ttt, 3R: gca aag cac agc ataccatga, 4F: tac ccc cag gat ctt agc aca, 4R: ttg ctg tga ttc aat tgc cag, 5F: tga aag gtg act gct ggg aat, 5R: aaa ggc aaa ttg taa atg agc ca, 6F: aca ggc ttg tca agg ttt gga, 6R: tgc ttt ggt ttt acc tct ggg, 7F: ggt ctc cac aga gcc aag aga, 7R: tta tgg tcc ctc atg agc tgg, 8F: gag cca tga gtg catc cct ac, 8R: gat gcc aaa acc ttc ctt gaa, 9F: cct cct ttg agg cca tta caa, 9R: gca aga ttg gtt ctc atg agg a, 10F: ttg gag tct ttg ggc tta tga a, 10R: aat gga agg ggt cca cat ttt, 51F: ttc cca ctg ggt tgt ttt cac, 51R: aat ggg ttg aaa gag gag cag a, 57F: att gca aat ggt ttg gag tca, 57R: ctt ctg ctt gag att ttg tta caa t. The experimental conditions used to generate the fragment was as follows: 50 µl of reaction mixture contained 200 ng of genomic DNA, 10 mmol/l Tris HCl (pH 9.0), 50 mmol/l KCl, 0.1% Triton, 2 mmol/l $MgCl_2$, 0.25 mmol/l dNTPs, 100 ng of upstream primer, 100 ng of downstream primer and 3.0 U Taq-DNA polymerase. Cycling conditions included an initial step at 95°C for 5 minutes, then 30 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 1 minute with a final elongation step at 72°C for 10 minutes. The PCR products were purified for sequencing after electrophoresis on agarose gel. For sequencing, a Perkin Elmer Big Dye Sequencing kit (Perkin-Elmer, Shelton, CT, USA) and an ABI PRISM7700 sequencer (Perkin-Elmer ABI, Foster City, CA, USA) were used.

Pancreatic tissue pathology and electron microscopy

Pancreatic tissue were stained with hematoxylin-eosin (HE), Modified Gomori trichrome (MGT), NADH-tetrazolium reductase (NADH-TR), Periodic Acid-Schiff stain (PSA) and IgG4 special dye.

Detection of serum TCR

Detection of serum TCR was done with ELISA kits (R&D Systems, Minneapolis, MN, USA).

Results

Clinical data of the patients with type 2 AIP

The patients were women with an average age of 43.7 years (38, 49, and 44). Common characteristics: significant

Table 1. Clinical data of patients with type 2 AIP, type 1 AIP, chronic pancreatitis, and normal controls

Item	Type 2 AIP			Type 1 AIP ($\bar{x} \pm s$)	Chronic pancreatitis	Normal controls
	No. 1	No. 2	No. 3			
age of onset	38	49	44	62.6 ±12.5	40.5 ±13.6	–
sex, % men	female	female	female	90% men	60% men	–
abdominal pain	occasionally	–	–	80% constantly	constantly	–
weight loss (kg/12 months)	5	6.2	9	8.6 ±5.2	–	–
anti-nuclear antibody (< 1.0)	0.26	0.33	0.85	1.86 ±0.59	0.68 ±0.32	0.36 ±0.19
immunoglobulin G (IgG) (7-17 g/l)	12.8	14.9	6.8	27.9 ±13.6	15.7 ±4.8	12.3 ±4.3
IgG4 (0.08-1.40 g/l)	1.05	0.12	0.89	12.45 ±8.10	0.78 ±0.30	0.56 ±0.28
Trypsin (2-8) nmol/l	19.85	8.55	7.74	4.1 ±2.3	24.9 ±10.7	6.65 ±3.4
Dispose and lapse	postoperative prednisolone 35 mg/d, improvement	prednisolone 40 mg/d, improvement	prednisolone 40 mg/d, improvement	–	–	–

weight loss (5-9 kg/12 months) and without serum IgG4 increase (Table 1).

Serum TCR of type 2 AIP was 896.3 pg/ml and it did not significantly change during, prior to and post glucocorticoid treatment, but it was significantly higher than in type 1 AIP (521.6 pg/ml), chronic pancreatitis group (603.8 pg/ml) and normal controls (Fig. 1).

It is showed that serum TCR in type 2 AIP is significant higher than in type 1 AIP and chronic pancreatitis group and normal controls.

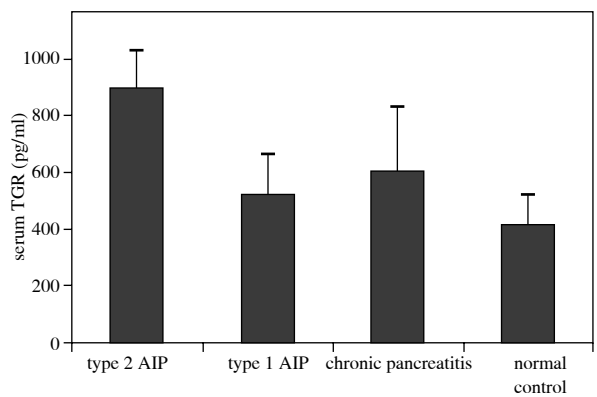

Fig. 1. Different serum TCR in type 2 AIP, type 1 AIP, chronic pancreatitis, and normal controls

Molecular genetic analysis

Heteroplasmy for the A3243G mutation in mtDNA was found in one of the affected patients (Fig. 2A). And novel mutations of *MEN1* (p.546 Ala > The) (Fig. 2B) and *PKHD1* (c. 233586 A>G and c. 316713 C>T) (Figs. 2C, D) gene were found in the pancreatic tissue and blood samples. In addition, in the affected type 2 AIP patients, no mutations were found in the genes coding for *PRSS1*, *SPINK1* and *CFTR*. These mutations were not found in the normal controls and other patients.

Pathological analysis

Histopathologic examination of the pancreas reveals a large number of inflammatory cell infiltrations (mainly neutrophils) (Figs. 3A) and IgG4 negative plasma cells (Figs. 3B), and exhibits interstitial fibrosis and acinar cell atrophy in later stages. However, localization and the degree of duct wall infiltration are variable.

Discussion

Recently, two types of AIP have been distinguished [4-9]. They share the symptomatology and some histopathological features such as periductal lymphoplasmacytic infiltrate and storiform fibrosis, but differ in a particular duct change, called granulocytic epithelial lesion, which characterizes type 2 AIP. In addition, type 2 AIP usually has no or very few IgG4-positive plasma cells. Type 2 AIP patients frequently show an association with inflammatory bowel disease and usually lack serological elevation of IgG4 [16, 17]. The main differential diagnosis of AIP is pancreatic ductal adenocarcinoma. In North America, about 2.5% of patients with a preoperative diagnosis of pancreatic cancer are diagnosed with AIP postoperatively. In many instances, the diagnosis of AIP can be made by imaging together with serological markers. In difficult cases, particularly in type 2 AIP, the diagnosis has to be established by core needle biopsy [11, 18]. Type-2 AIP tends to have focal features and it is more commonly surgically

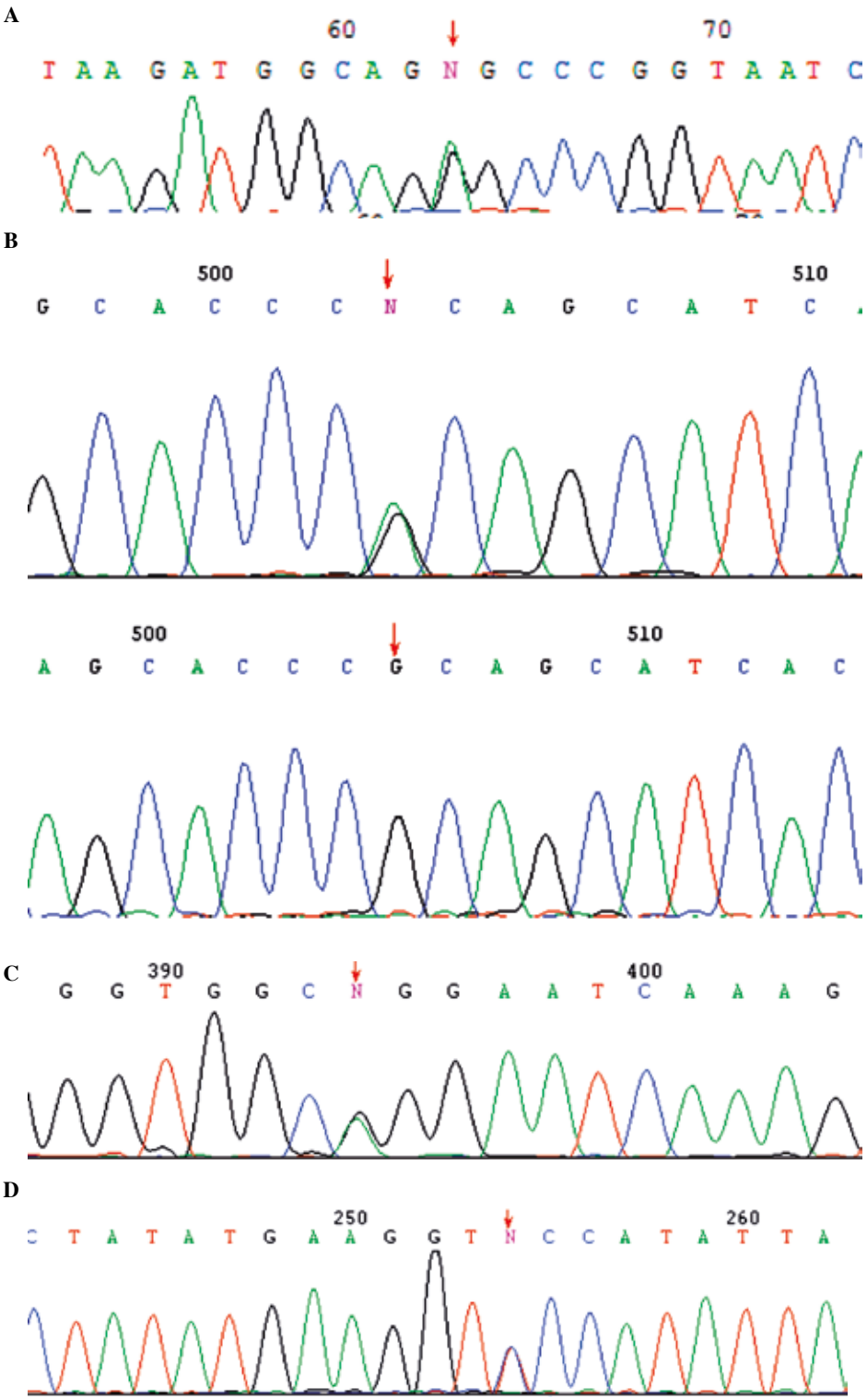

Fig. 2. Sequencing of gene mutations of patients with type 2 AIP. **A**) sequencing of mitochondrial m.A3243G mutation of blood sample of patients with type 2 AIP (No.1); **B**) sequencing of *MEN1* gene mutation (p.546 Ala > The) of blood samples of patients with type 2 AIP(No.1, No.3); **C** and **D**) sequencing of *PKHD1* gene silent mutation c. 233586 A>G (No. 1 and No. 2) and c. 316713 C>T (No. 2 and No. 3) of blood samples of patients with type 2 AIP

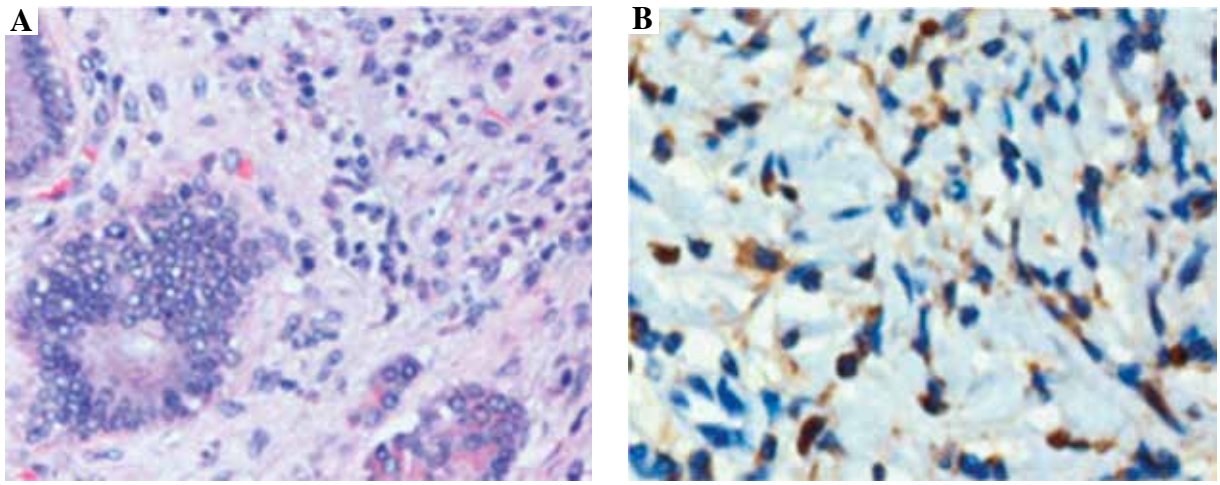

Fig. 3. Histopathologic examination of the pancreas (magnification 400×). **A**) large number of inflammatory cell infiltrations (mainly neutrophils). **B**) IgG4 staining, positive plasma cells < 10%

resected due to the diagnostic difficulty. Although endocrine and exocrine functions of the pancreas are frequently impaired in type 1 AIP, the functions are unknown.

Genetic analyses have identified a specific gene for hereditary pancreatitis and other types of chronic pancreatitis. The first gene is *PRSS1*, some *PRSS1* mutations enhance trypsinogen autoactivation, and other mutations may render some patients more susceptible to pancreatitis in the presence of other insults to the pancreas. Thus, *SPINK1* and *CFTR* genes have been involved in idiopathic recurrent acute pancreatitis and chronic pancreatitis [19-23]. Several authors have paid attention to particular cases of pancreatitis of an autoimmune pattern and some of the cases were associated with DRBI*0405-DQBI*0401. Parkdo reported that substitution of aspartic acid at the 57th position of haploid DQβ1 of the histocompatibility leukocyte antigen is closely related to the recurrence of AIP [15, 24, 25].

In this study, non-hyper-IgG4 was an important clinical feature in patients with type 2 AIP. These findings, however, do raise the possibility that genetic predisposition or histopathology could be useful, particularly when evaluating limited biopsy material. *PKHD1* gene encodes fibrocystin/polyductin (FPC), a type I membrane protein which is expressed in primary cilia [26, 27]. The primary cilium is a solitary, non-motile, tubular organelle extending from the apical plasma membrane of the cell [28, 29]. In recent years, it has been proposed that primary cilia sense and transduction multiple stimuli, such as fluid flow, signals initiated by hormones, morphogenes, growth factors and other physiologically active substances present. FPC is localized in primary cilia and acts as a receptor-like protein. This protein is present in fetal and adult kidney cells, and it is also present at low levels in the liver and pancreas. *PKHD1* gene c.233586 A>G and c.316713 C>T mutations may by changing/may change the expression of β-galactosidase, Pkd1, and Pkd2 compared with wild-type littermates and results in pancreatic cysts. The *MEN1* gene product, menin, functions as an adaptor protein that is involved in interactions with multiple protein partners. Menin is involved in neuroendocrine cell development and function. Later on, it is active in many cellular processes, including gene transcription regulation, DNA replication, DNA repair, and signal transduction. Clinically, mutations in the MEN1 were associated with better prognosis of pancreatic neuroendocrine tumors [30]. In this study, we also found mutations of PKHD1 and MEN1 gene in type 2 AIP patients. Although the pathogenic mechanism needs further studies, this phenomenon may be used as a secondary diagnosis of AIP.

Our findings suggest that type 2 AIP can present with a variety of clinical phenotypes. In addition, molecular genetic studies have elucidated the molecular mechanisms underlying the pathogenesis of type 2 AIP, which is due to the presence of mutations of MEN1 and PKHD1 gene. Additional clinical studies are required to investigate the clinical heterogeneity and molecular pathogenesis of type 2 AIP.

Authors declare no conflict of interest.

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