Protocol

Official Title: The Impact of Genetic and Environmental Factors on the Progression of Chronic Pancreatitis：An Observational Study

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The Impacts of Genetic and Environmental Factors on the Progression of Chronic Pancreatitis

1 Background

Chronic pancreatitis (CP) is a chronic progressive disease and characterized by fibrosis and inflammation of the pancreas. The prevalence rates varied from 36 to 125 per 100 000 population in different countries.[1] Morphological changes, including atrophy, fibrosis, duct distortion and strictures, and calcification, etc., lead to the irreversible damage of pancreatic endocrine and exocrine functions. The loss of pancreatic functions indicates the progression of CP and leads to the poor quality of life and the increased medical resources utilization.[1]

It has been well confirmed that environmental factors, including alcohol and smoking, were independent risk factors for the occurrence of CP.[2, 3] These factors also accelerate the development from acute pancreatitis and recurrent acute pancreatitis to CP.[4] Moreover, several studies explored the effect of alcohol and smoking on the progression of CP. Hao et al[5] found that compared with idiopathic chronic pancreatitis (ICP), alcoholic chronic pancreatitis (ACP) usually had a more severe course, which confirmed the impact of alcohol on the progression of CP. Maisonneuve et al[6] conducted a study with a cohort of 934 patients with ACP and found that tobacco smoking accelerated the appearance of calcification and diabetes independent of alcohol consumption. The impact of smoking was also found in patients with ICP.[7] However, none of these studies considered the impact of genetic background on the progression of CP, which might be a potential mixed factor.

Over the past two decades, it has been increasingly appreciated that genetic factors also played an important role in the development of CP. The rare pathogenic variants in susceptibility genes of CP, such as *SPINK1* (encoding pancreatic secretory trypsin inhibitor), *PRSS1* (encoding cationic trypsinogen), *CTRC* (encoding chymotrypsin C) and *CFTR* (encoding cystic fibrosis transmembrane conductance regulator) genes, are overrepresented in both ICP and ACP, and related to earlier onset of CP.[8-12] However, few studies focused on the effect of genetic factors on the progression of CP. A prospective study conducted by Muller et al found that patients carrying *SPINK1* mutations had longer delay from onset of disease to pancreatic endocrine and exocrine insufficiency development.[13] Another study by Frulloni et al also concluded exocrine and endocrine insufficiency occurred less frequently and later in CP patients with genetic mutations (*CFTR* and *SPINK1*) than those without.[14] Conversely, Sun et al concluded that

CP patients carrying *SPINK1* c.194+2T>C mutation developed diabetes mellitus (DM) significantly since onset of CP.[15] These discrepancies may be attributable to the small numbers of enrolled patients, the different racial backgrounds, and the analysis of limited genes and pathogenic variants. As far as we knew, there are no published studies investigating the influences of both environmental and genetic factors on the progression of CP.

2 Objectives

The study aims to investigate the comprehensive impact of both environmental and genetic factors on the progression of CP, defined as the development of either endocrine or exocrine insufficiency following the onset of CP.

3 Study setting

The study is designed as a prospective observational study and conducted in Changhai Hospital, which is a tertiary referral center and the largest CP medical center in China.

4 Eligibility criteria

Inclusion criteria

Consecutive patients with a diagnosis of CP and agreed to provide a blood sample for genetic tests are enrolled between January 2010 and December 2014.

Exclusion criteria

1) autoimmune pancreatitis;

2) pancreatic cancer diagnosed within two years following the onset of CP;

3) patient had less than two years' follow-up;

4) patients presenting with diabetes and/or steatorrhea at onset of CP.

5 Study design

If inclusion/exclusion criteria are met and written informed consent has been obtained, the patient will be enrolled in the present study and the following data are documented in detail: demographic data (date of birth, gender), alcohol consumption and smoking history, family history, age at disease onset, etiologies of CP, CP-related complications, laboratory and imaging results, and treatment.

All enrolled patients agreed to provide a blood sample for genetic tests. All known rare pathogenic variants of the *PRSS1*, *CFTR*, *SPINK1*, and *CTRC* genes are included in the final analysis. The detailed DNA preparation methods, gene sequencing, and pathogenic variants inclusion criteria have been described previously.[9] Patients with at least one rare pathogenic variant are considered genetic factor/s

positive (denoted as G+).

Patients are considered smokers if they had smoked ≥100 cigarettes before the onset of CP. Additionally, based on their alcohol consumption patterns before the onset of CP, the patients are categorized into four groups, abstainers, light drinkers (≤20 g/d), moderate drinkers (20-80 g/d), and heavy drinkers (>80 g/d). Patients with a history of either smoking or alcohol consumption are considered environmental factor/s positive (denoted as E+).

During follow-up, aside from visits owing to complaints of discomfort caused by CP, all patients will be periodically (annually at least) contacted for clinical and imaging (computed tomography or magnetic resonance imaging) check-ups. The examination results, any changes in alcohol consumption or smoking status, and CP complications are also recorded. Data for the present study will be obtained in 2020. The follow-up time is defined as the duration from the onset of CP (first clinical symptoms attributable to CP or first imaging procedure showing CP lesions) to the end of follow-up, last personal contact, diagnosis of pancreatic cancer, regional or total pancreatectomy, or death, whichever came first. All patients are prospectively observed from enrolment until the cessation of follow-up.

6 Outcomes

The primary endpoint is the development of pancreatic insufficiency (diabetes and/or steatorrhea). The components of the primary endpoint (diabetes and steatorrhea) are set as the secondary endpoints.

Patients with diabetes and/or steatorrhea during the course of CP are considered to have pancreatic insufficiency. Diabetes is diagnosed according to the criteria of the American Diabetes Association, as follows: (1) hemoglobin A1C ≥6.5% (48 mmol/mol); or (2) fasting plasma glucose ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8h; or (3) 2-h plasma glucose ≥200 mg/dL (11.1 mmol/L) during oral glucose tolerance test (OGTT), which is performed using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water; or (4) in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L). Steatorrhea is diagnosed according to one of the following conditions: (1) chronic diarrhea with foul-smelling, oily bowel movements or (2) a positive result in a standard quantification test (fecal fat excretion of greater than 14 g/d).[16]

7 Data management

Data collection

Clinical data regarding demographic data (date of birth, gender), alcohol consumption and smoking

history, family history, age at disease onset, etiologies of CP, CP-related complications, laboratory and imaging results, and treatment are collected during the first hospital admission

Statistical analysis

For continuous variables, tests of data normality are carried out using the Shapiro-Wilk test. Normally-distributed variables are presented as mean ± standard deviation (SD) and compared using Student's t-test. And non-normally distributed data are presented as median (interquartile range, IQR) and compared using Mann-Whitney U test. Categorical variables results are presented as frequency and percentage. Chi-squared analysis or Fisher's exact test is used for comparison.

Cumulative incidence of pancreatic insufficiency after the onset of CP are presented by Kaplan-Meier survival curves. Log-rank test is further used to analyze the difference of cumulative rates of pancreatic insufficiency between two groups. *P* values for pairwise comparisons were adjusted using the Bonferroni correction. Cox proportional hazards regression model is used to identify the potential risk factors. Hazard ratios and 95% confidence intervals are calculated. All statistical tests are 2-sided. Data are analyzed using SPSS 23.0 (SPSS Inc, Chicago, IL) and SAS 9.4 (SAS Institute, Cary, NC).

8 Ethics approval

Ethical approval is obtained from Changhai Institutional Review Board with the ID of CHEC2020-142.

9 Funding

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10 Declaration of interests

The authors declare that they have no competing interests.

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Statistical Analysis Plan

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