A SPINK1 GENE MUTATION IN A THAI PATIENT WITH FIBROCALCULOUS PANCREATIC DIABETES

Thiti Snabboon, Wanee Plengpanich, Vitaya Sridama, Sarat Sunthornyothin, Sompongse Suwanwalaikorn and Weerapan Khovidhunkit

ABBREVIATIONS
FCPD - Fibrocalculous Pancreatic Diabetes
TCP - Tropical Chronic Pancreatitis
ICP - Idiopathic Chronic Pancreatitis
MRDM - Malnutrition-related Diabetes Mellitus
TCP - Tropical Chronic Pancreatitis
FCPD - Fibrocalculous Pancreatic Diabetes
Kazal-type 1 (SPINK1, OMIM 167790), also known as pancreatic secretory trypsin inhibitor (PSTI) or the tumor-associated trypsin inhibitor (TATI), is a potent serine protease inhibitor. It has been demonstrated to inhibit up to 20% of potential trypsin activity, thereby postulated to prevent inappropriate activation of the pancreatic digestive enzyme cascade (Threadgold et al, 2002). Recently, mutations in the SPINK1 gene have been reported in association with an increased risk of pancreatitis, especially TCP and FCPD. The SPINK1 gene, located on chromosome 5q32, consists of 4 exons, spanning over 7.5 kb. This gene encodes a protein of 79 amino acids including a signal peptide of 23 amino acids.

INTRODUCTION

Fibrocalculous pancreatic diabetes (FCPD) is a late stage of tropical chronic pancreatitis (TCP), a unique form of idiopathic chronic pancreatitis (ICP). TCP is endemic in tropical developing countries and is more aggressive in character. Patients with TCP tend to have larger calcification or calcific stones in the pancreas and are at higher risk of pancreatic malignancy (Tandon and Garg, 2004). The etiology of TCP is still obscure, but the autodigestion secondary to aberrant intraductal activation of zymogens by trypsin is thought to be a primary common event. FCPD was first classified as a subtype of malnutrition-related diabetes mellitus (MRDM) by the WHO Study Group Report on Diabetes (Alberti and Zimmet, 1998). In the recent Expert Committee on Classification of Diabetes by the American Diabetes Association, however, FCPD is now classified as a secondary cause of diabetes mellitus due to an exocrine pancreatic disease.

Kazal-type 1 (SPINK1, OMIM 167790), also known as pancreatic secretory trypsin inhibitor (PSTI) or the tumor-associated trypsin inhibitor (TATI), is a potent serine protease inhibitor. It has been demonstrated to inhibit up to 20% of potential trypsin activity, thereby postulated to prevent inappropriate activation of the pancreatic digestive enzyme cascade (Threadgold et al, 2002). Recently, mutations in the SPINK1 gene have been reported in association with an increased risk of pancreatitis, especially TCP and FCPD. The SPINK1 gene, located on chromosome 5q32, consists of 4 exons, spanning over 7.5 kb. This gene encodes a protein of 79 amino acids including a signal peptide of 23 amino acids.

We report a case of a young Thai woman with a typical presentation of FCPD, who was found to have a mutation in the SPINK1 gene. To our knowledge, this is the first report of this mutation in a patient from Southeast Asia.

MATERIALS AND METHODS

Subject

A 16-year-old Thai woman was referred for treatment of her poorly controlled diabetes mellitus. She was the first daughter of unrelated parents (Fig 1). She was healthy until 2 years...
prior to this admission when she developed chronic upper abdominal pain, bowel habit change, polyuria, nocturia and a 10 kg weight loss. She has no history of alcohol consumption or other substance use. There was no family history of diabetes mellitus. Physical examination showed a lean woman with a body weight of 35 kg and a height of 152 cm. She had no symptoms or signs of lipodystrophy or thyrotoxicosis. Laboratory findings showed a fasting plasma glucose level of 692 mg/dl, negative serum ketone, and no acidosis. Her liver and renal function tests were normal. A plain abdominal X ray showed calcification along the pancreatic shadow (Fig 2).

DNA sequence analysis

Individual informed consent was obtained from the patient and her family members for research purposes. Genomic DNA was extracted from peripheral blood leukocytes by the phenol-chloroform method. The entire coding sequence of the SPINK1 gene was amplified by PCR using primers and conditions as previously described (Hassan et al, 2002). PCR products were purified by ExoSAP-IT (USB, Ohio, USA) at 37ºC for 15 minutes and 80ºC for 15 minutes and sent for sequencing at Macrogen (Seoul, South Korea).

RESULTS

FCPD was diagnosed and insulin therapy was started. Her daily insulin requirement was approximately 60-80 units to achieve adequate control of her plasma glucose. Oral pancreatic enzyme replacement was also administered to relieve her maldigestion. A subsequent genetic analysis revealed a heterozygous mutation at the splice site of exon 3 (IVS3+2 T>C) of the SPINK1 gene (Fig 3). However, this mutation was not found in her mother or her sister.

DISCUSSION

Pancreatitis is one of the global health care problems, mainly caused by alcohol and gallstones. In a minority of cases, other etiological factors, such as anatomical variations and various metabolic disorders, have been identified.
Approximately 20-30% of the cases are idiopathic (Etemad and Whitcomb, 2001). The pathogenesis of pancreatitis is thought to be due to inappropriate trypsin activation in the pancreatic parenchyma. The discovery of a mutation in the cationic trypsinogen gene (PRSS1) causing hereditary pancreatitis has stimulated the search for other pancreatitis-associated genes (Whitcomb et al, 1996). Up to now, three candidate genes, SPINK1, PRSS1 and CFTR, have been reported in association with ICP (Audrezet et al, 2002).

A number of hypotheses regarding the pathogenesis of TCP and its diabetic stage, FCPD, have been proposed. Environmental factors, such as protein energy malnutrition, cassava consumption and other dietary toxins, were once believed to be the main etiology but this idea has not been substantiated or confirmed. Genetic analyses of the human leukocyte antigen (HLA), the insulin gene, and the islet regenerating (reg) gene have been performed with negative results. In 2000, a significant association between SPINK1 mutations and ICP was first described (Witt et al, 2000). Recent studies in India and Bangladesh have also demonstrated a higher prevalence of SPINK1 mutations in TCP or FCPD patients than patients with ICP (Bhatia et al, 2002; Hassan et al, 2002; Schneider et al, 2002). However, certain mutations of SPINK1 gene were also identified in patients with alcoholic pancreatitis and in the normal population, suggesting its role as a predisposition to pancreatitis with incomplete penetrance or a disease modifier with lowering the threshold for pancreatitis from other genetic or environmental factors (Witt et al, 2001; Threadgold et al, 2002). Up to now, several mutations of the SPINK1 gene have been reported with possible hot spot sites at N34S and P55S. In this study, we reported the single splice site mutation as previously identified (Witt et al, 2000). To our knowledge, this is the first report of this SPINK1 mutation in an FCPD patient in Southeast Asia.

The clinical picture of FCPD consists of a triad of pancreatic calcification, abdominal pain, and diabetes (Mohan et al, 1998). It accounts for 25-90% of TCP cases (Garg and Tandon, 2004). One of the characteristic clinical features of FCPD is that despite requiring insulin for optimal control, patients rarely develop ketosis on insulin withdrawal. This is thought to be attributed by partial preservation of β cells, decreased glucagon reserve, reduced free fatty acid supply, or carnitine deficiency (Mohan et al, 2003). The patients typically present with extreme emaciation, protein energy malnutrition, pain in the abdomen, calcification on the pancreatic shadow, and ketosis resistance. Overt steatorrhea in situation where the pancreatic enzyme production is less than 10%, is observed in less than a third of the patients (Mohan et al, 1998). The pathological findings of the pancreas vary with duration and severity of the disease. The size of the pancreas is usually small, with irregular and nodular surface. Pancreatic calculi in different sizes, shapes, and colors are found throughout the ductal system. They are composed of more than 95% of calcium carbonate and a small amount of calcium phosphate (Govindarajan et al, 2001). A few studies have reported long-term survival in FCPD. The major morbidities are abdominal pain and malnutrition, whereas the majority of death is usually associated with diabetic nephropathy (Mohan et al, 1996).

ACKNOWLEDGEMENTS

This work was supported by the fund of Endocrine and Metabolism Division, Faculty of Internal Medicine, Chulalongkorn University.

REFERENCES


