□ Case report □

A case of hereditary pancreatitis with a N29I mutation in the cationic trypsinogen gene

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Hereditary pancreatitis is an autosomal dominant disease characterized by recurrent episodes of pancreatitis, often beginning in childhood, with a positive family history involving at least two other affected family members with no known other precipitating factors. Most forms of hereditary pancreatitis are caused by one of two common mutations, i.e., R122H in exon 3 and N29I in exon 2 of the cationic trypsinogen (CT) (PRSS1) gene, located on chromosome 7. The authors describe the case of a 15-year-old boy who had suffered from recurrent attacks of pancreatitis since age three. His mother and grandmother had chronic pancreatitis and diabetes mellitus. Mutation analysis was performed on the family due to the suspicion of hereditary pancreatitis. The CT gene was analyzed in DNA samples extracted from the peripheral blood of three family members, the mother, the proband, and the proband's sister. Two members of the family, the mother and the proband, were found to have a N29I mutation in the CT gene. The authors document the first family with hereditary pancreatitis associated with the N29I mutation in Korea. **(Korean J Pediatr 2006;49:1111-1115)**

Key Words : Hereditary pancreatitis, Cationic trypsinogen, Korean

Introduction

Hereditary pancreatitis was first described by Comfort and Steinburg in 1952[1], and since this first report more than 200 families have been identified world-wide, the majority of which are of Caucasian ancestry, though a few cases have been reported in Japan[2]. Hereditary pancreatitis accounts for about 1 percent of all chronic pancreatitis cases[3], and is characterized by recurrent episodes of pancreatitis (often beginning in childhood), a positive family history with at least two other affected members, the frequent presence of calcified stones in the pancreatic duct, and the absence of other known precipitating factors[4]. The mode of inheritance is autosomal dominant with an 80% penetrance rate. Most forms of hereditary pancreatitis are caused by one of two common mutations, i.e., R122H in the third exon or N29I in the second exon of the cationic trypsinogen gene (protease 1, PRSS1) located on chromosome 7q35. In 1996 the first disease-specific mutation (R122H) was identified in cationic trypsinogen by Whitcomb et al.[5], and this was followed by a report of a second common disease-specific mutation (N29I)[6]. In Korea, the first family of hereditary pancreatitis with a cationic trypsinogen gene mutation revealed an arginine to histidine amino acid substitution at residue 122[7]. Here, we report the first family with hereditary pancreatitis associated with the N29I mutation in Korea.

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Case reports

This case concerns a 15 year old boy who had suffered from recurrent abdominal pain since he was 30 months old. He was of normal height (176.1 cm, 90-97 percentile) and weight for age (64.1 kg, 50-75 percentile). When he was 3 years old, he was admitted to Seoul National University Hospital for abdominal pain and mild fever and was diagnosed as having hemorrhagic pancreatitis. At the time, serum amylase was 559 IU/L, serum calcium level 10.6 mg/dL, WBC count 9,170/μL, Hb 10.4 g/dL, and platelets

372/μL. He underwent massive irrigation and multiple drain insertions and was then discharged with clinical improvement. However, at age 15 years, he visited the emergency room complaining of epigastric pain and vomiting. On this occasion, his serum amylase was 827 IU/L, lipase 74,767 U/L, serum triglyceride 42 mg/dL, WBC count 14,900/μL, Hb 16.4 g/dL, and platelets 231/μL. Serum total bilirubin, AST and ALT were 1.8 mg/dL, 21 IU/L, and 17 IU/L, respectively. Magnetic resonance cholangiography revealed peripancreatic fluid collection and a pancreatic duct with a slightly 'beaded' appearance, which suggested chronic pancreatitis with acute exacerbation without any evidence of congenital malformation (Fig. 1). His epigastric pain improved after a nine-day fast. Serum amylase and serum lipase normalized within 3 days and 3 weeks, respectively. Five months later, follow-up magnetic resonance cholangiography revealed normally calibered pancreatic duct.

Family history taking showed that 3 members of his family had chronic pancreatitis or pancreatic calculus. His mother had chronic pancreatitis, diabetes mellitus, and pancreatic calculus, and his grandmother also had diabetes mellitus and pancreatic calculus. Neither of his two maternal aunts nor his younger sister had pancreatitis (Fig. 2).

We extracted genomic DNA form whole blood of three family members - the mother, the proband, and the proband's sister - using a Wizard genomic DNA purification kit according to the manufacturer's instruction (Promega, Madison, WI, U.S.A.). Exon 2 and exon 3 of the CT gene were amplified by polymerase chain reaction. The primer sequencess are shown in Table 1 and annealing temperature was 56℃. And then DNA products were sequenced using an ABI Prism Automated DNA Sequencer (model 310, PE AppliedBiosystems, USA). The sequence was compared with the reference sequence NM_014251 in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov). This showed the presence of a heterozygous A to T transition in exon 2 of the cationic trypsinogen gene (N29I) (Fig. 3). The proband's mother also had the N29I mutation but his sister did not.

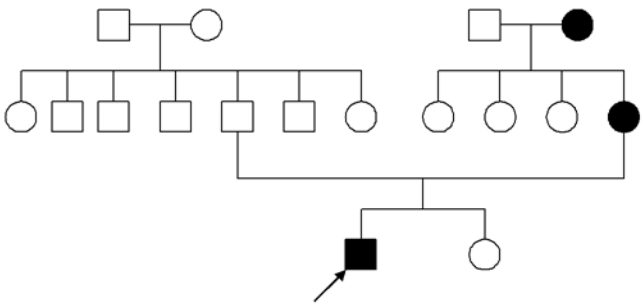

Fig. 2. Pedigrees of patients with hereditary pancreatitis. In this family, the N29I mutation in exon 2 was identified in two affected members [proband (arrowed) and his mother].

Table 1. PCR Primers used for the Detection of Cationic Trypsinogen Mutations

Gene			Primer sequences (5′-3′)
Exon	N29I	forward	CCA TCT TAC CCA ACC TCA GAT G
		reverse	TGA TGA CAG ATC GTT GGG GGG TAG A
Exon3	R122H	forward	GGT CCT TCT CAT ACC TT
		reverse	GGG TAG GAG GCGT TCA CAC TT

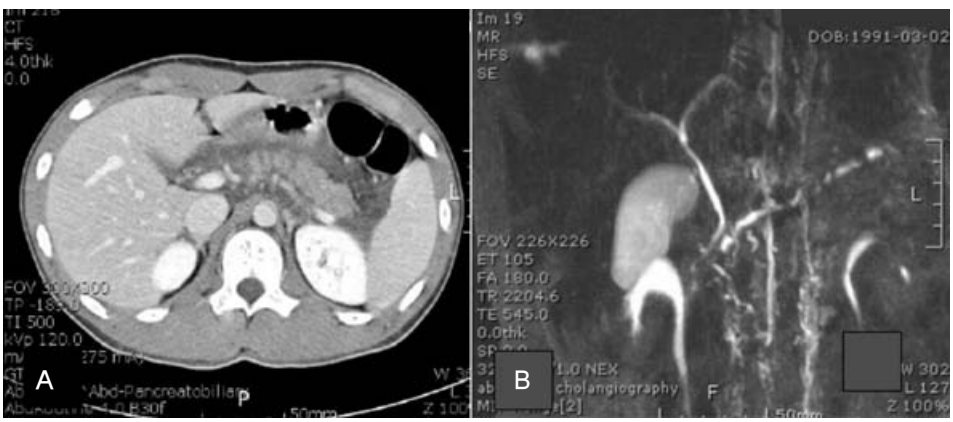

Fig. 1. (A) Abdominal computed tomography revealed mild swelling of pancreas with peripancreatic fluid collection. (B) Magnetic resonance cholangiography shows a pancreatic duct with a slightly 'beaded' appearance.

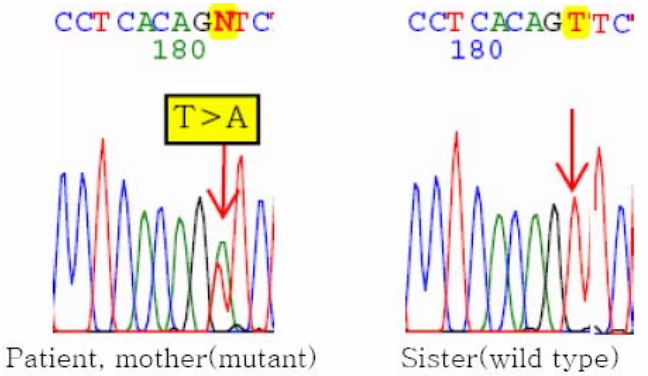

Fig. 3. DNA sequencing electropherograms of human cationic trypsinogen exon 2 revealing a heterozygous missense mutation, 92A>T. Because the sequencing was performed with an anti-sense primer, T was replaced by A. This point mutation is predicted to result in an Asn AAC) to Ile ATC) substitution.

Discussion

Hereditary pancreatitis is an autosomal dominant disease with 80% penetrance, and is indistinguishable from other forms of chronic pancreatitis in terms of its clinical, laboratory, radiological, and histopathological features. Suspicion is raised when a subject presents with recurrent episodes of pancreatitis, beginning in childhood or adolescence in the absence of etiological factors, such as, alcohol abuse, hypertriglyceridemia, and hyperparathyroidism[8].

In hereditary pancreatitis, attacks of acute pancreatitis usually begin in childhood, but age of onset can range from infancy to the fifth or sixth decades of life. Acute attacks may vary from mild abdominal discomfort to severe life-threatening episodes with pancreatic necrosis, splenic vein thrombosis[9], pseudocysts[10] or death[11, 12]. Moreover, a bimodal modus of onset at 1-6 years and 18-24 years of age was revealed[11, 13].

Chronic pancreatitis follows recurrent attacks of acute pancreatitis with all of its common complications, i.e., unrelenting pain, parenchymal and ductal calcifications, duct distortion, fibrosis, maldigestion and diabetes mellitus[11, 12, 14]. These features make hereditary pancreatitis indistinguishable from other causes of acute and chronic pancreatitis, save its relatively early age at onset, the autosomal dominant inheritance pattern, and lack of other identifiable etiologies.

Trypsinogen is a serine protease proenzyme (zymogen) and is synthesized by pancreatic acinar cells. It is converted to trypsin in the duodenum by enterokinase, which hydrolyzes the peptide bond between the eight-amino-acid trypsinogen activation peptide and the mature protein. Trypsin activates a cascade of digestive enzyme precursors, and in turn activates itself, and intestinal digestive enzymes by hydrolyzing the respective activation peptides. Because trypsin is such a potent protease, defense mechanisms exist that can prevent the prolonged action of trypsin if prematurely activated within the pancreas[15]. In fact, it has been hypothesized that the R122H mutation alters a trypsin recognition site, and that this prevents its deactivation within the pancreas, thus prolonging its action[16]. However, the mechanism whereby the N29I mutation causes pancreatitis is unclear, although it has been speculated that this mutation enhances trypsinogen autoactivation, alters the binding of pancreatic secretory trypsin inhibitor (PSTI) to trypsin[6], or impairs trypsin inactivation by altering the accessibility of the initial hydrolysis site to trypsin. Predicted molecular conformational changes in the trypsin structure support this third suggestion[16, 17].

In Korea, the first family with hereditary pancreatitis was described by Kim et al.[7] in 2005, and was proven to be associated with the R122H mutation. In the present case, we discovered that two members of a family, the mother and her son, had a N29I mutation of the CT gene by sequencing exons 2 and 3 of the CT gene, and report this first family with hereditary pancreatitis associated with the N29I mutation in Korea.

Mutations identical with those previously reported in Caucasian and Japanese hereditary pancreatitis kindreds have been previously reported in exons 2 (in our study) and 3[7] of the CT gene in separate Korean hereditary pancreatitis families, thus indicating no racial specificity with respect to the CT gene mutations in patients with hereditary pancreatitis. Though it is known that hereditary pancreatitis patients with the N29I mutation are relatively older at onset than those with the R122H mutation[2, 6], our patient with the N29I mutation had an early age of onset (30 months). A more general genetic survey of hereditary pancreatitis kindreds is required in Korea.

Genetic testing is an extremely powerful tool and has both positive and negative implications. The negative aspects tend to be recognized early because of concerns of disease risk for family members, impact on family relationships, career choices, employment, insurability, and other

forms of discrimination. On the other hand, its positive implications impact long term health through therapeutic or lifestyle modifications, although such benefits may take some time to demonstrate in chronic progressive diseases with significant heterogeneity[18].

The role of genetic testing in hereditary pancreatitis has been addressed in a consensus conference report[19], and recommendations are listed on the National Guideline Clearing House website (www.guideline.gov) under the title, "Genetic testing for hereditary pancreatitis : guidelines for indications, counseling, consent and privacy issues".

PRSS1 genetic testing is recommended in symptomatic patients with any of the following: (1) recurrent (two or more separate documented episodes of typical pain with hyper-amylasaemia) attacks of acute pancreatitis for which there is no explanation (for example, anatomic anomalies, ampullary or main pancreatic strictures, trauma, viral infection, gall stones, alcohol, drugs, hyperlipidaemia, etc.), (2) unexplained (idiopathic) chronic pancreatitis, (3) a family history of pancreatitis in a first degree (parent, sibling, child) or second degree (aunt, uncle, grandparent) relative, (4) an unexplained episode of documented pancreatitis occurring in a child that required hospitalization, and where there is significant concern that hereditary pancreatitis should be excluded, or (5) as part of an approved research protocol[19].

In addition, the consensus committed suggested that genetic testing of a child (under the age of 16 years) for PRSS1 mutations should be considered; (1) after an episode of documented pancreatitis of unknown aetiology severe enough to require hospitalization, (2) after two or more documented episodes of pancreatitis of unknown aetiology, (3) after an episode of documented pancreatitis occurring in a child where a relative is known to carry an hereditary pancreatitis mutation, (4) in a child with recurrent abdominal pain of unknown aetiology where a diagnosis of hereditary pancreatitis is a distinct clinical possibility, or (5) in cases of chronic pancreatitis of an unknown aetiology where a diagnosis of hereditary pancreatitis is a distinct clinical possibility[19].

If a major PRSS1 mutation is identified, for example, R122H, or N29I, which are associated with a significant lifetime risk of pancreatic cancer in patients with long-standing chronic pancreatitis (which is lower for non-smokers). There should be a plan for providing counseling and possibly genetic testing for family members if warranted[18].

Though it is well-known that patients with hereditary pancreatitis have a more than 50-fold elevated risk of pancreatic ductal cancer as compared with members of the general population, no screening protocol of proven efficiency exists for the early detection of pancreatic cancer in patients with hereditary pancreatitis. Thus, patients with hereditary pancreatitis need to avoid smoking, drinking, eating fatty foods, and taking any drugs that might induce pancreatitis in order to reduce the risk of pancreatic cancer[3]. Moreover, patients of over 40 years of age should undergo minimally invasive annual screening for pancreatic cancer, as was recommended by an international consensus conference[20].

한 글 요 약

Cationic Trypsinogen N29I 유전자 변이에 의한 유전 췌장염 1례

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신지연 · 오대성 · 류정민 · 심정옥
박지숙 · 고재성 · 서정기

유전성 췌장염은 비교적 젊은 연령에서 다른 이유 없이 반복적으로 급성 췌장염으로 나타나는데 나이가 들면서 만성 췌장염으로 이행된다. 동일 가계 내에 2세대 이상에 걸쳐서 3명 이상의 췌장염 환자가 있을 때 진단이 가능하며 이와 관련된 유전자로 trypsinogen을 만드는 PRSS1 유전자 변이(R122H, N29I)가 가장 대표적으로 알려져 있다. 저자들은 3세부터 반복적인 췌장염으로 입원 치료를 했던 15세 환아와 반복적 췌장염을 앓은 환아모, 환아 동생을 대상으로 CT 유전자의 exon 2, 3의 염기 서열을 분석하여 환아와 환아모에서 국내 처음으로 N29I 변이를 경험하였기에 보고하는 바이다.

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