Alpha₁-Antitrypsin Phenotype of Children with Liver Diseases in Thailand

Voranush Chongsrisawat[1], Podchanad Jantaradsamee[1], Boosba Vivatvakin[1], Praneet Pongpaew[2] and Yong Poovorawan[1]

Alpha₁-antitrypsin (AAT) is a glycoprotein synthesized by the liver, macrophages and the epithelial cells lining the intestinal wall.[1] AAT represents a major inhibitor of human neutrophil elastases, mainly those of the serine protease type.[2] AAT is composed of a chain of 394 amino acids, with three carbohydrate chains linked to three different amino acids (Asn^{46}, Asn^{89}, Asn^{247}). The salt bridges (Glu^{342}-Lys^{290} and Glu^{264}-Lys^{387}) constitute important parts of the protein structure because if not formed, as in the Z pathological variant, the protein cannot be secreted properly and accumulates in the cisterna of the rough endoplasmic reticulum.[3,4] The normal serum level of AAT ranges from 150 to 350 mg/dl with a half-life of 3-5 days. The AAT gene is located on chromosome 14 at q 31-32.3.[5] The gene is composed of seven exons and six introns, with only the last four exons containing the structural information for the protein. Hepatocytes synthesize AAT at a rate approximately 200-times higher than monocytes and alveolar macrophages.

AAT is an electrophoretically heterogeneous protein producing eight different bands in isoelectric focusing. This electrophoretic heterogeneity is due to differences in its amino acid sequences as well as the respective carbohydrate chain location. This electrophoretic pattern is genetically determined by two co-dominant alleles expressed independently. PiM (Pi = protease inhibitor) represents the most common normal allele. PiS homozygotes constitute approximately 60% and PiZ homozygotes approximately 15% of the serum AAT level in PiM homozygotes.[6]

SUMMARY Alpha₁-antitrypsin deficiency (PiZZ) constitutes not only the most common hereditary cause of liver diseases, but also of the most prevalent metabolic diseases in need of liver transplantation. It is a codominantly inherited disorder which predisposes to chronic liver disease, usually beginning in early infancy. The purpose of the present study has been to investigate α₁–antitrypsin phenotype in pediatric patients with various liver diseases. Phenotypic identification of α₁–antitrypsin variants has been carried out in 69 children with various liver diseases and 100 healthy controls using isoelectric focusing on polyacrylamide gel slabs. PiMM represents the most common phenotype detected in both groups (92% in the group with liver diseases and 88% in normal controls). We could detect PiZZ in only one healthy child but in none of those with liver diseases. Consequently α₁-antitrypsin deficiency does not appear to be a common cause for liver disease among children in Thailand. Further studies are necessary to elucidate the frequency of various α₁-antitrypsin variants and the clinical relevance with respect to liver diseases in Thailand.

From the [1]Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and Hospital, Bangkok 10330, Thailand and [2]Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand. Correspondence: Yong Poovorawan

Liver disease has been associated with AAT deficiency since 1969 after AAT globules had been detected in hepatocytes of PiZ patients with infantile cirrhosis.[7] PiMZ has been found associated with adult cirrhosis in Thailand.[8] As yet, serum Pi types have not been reported in connection with Thai children with liver diseases. Therefore, we performed this study to reveal the association between Pi types and various liver diseases.

MATERIAL AND METHODS

Population study

Children with liver diseases

The Pi phenotype was determined from sera of 169 children of whom 24 were suffering from idiopathic infantile cholestasis, 30 from extrahepatic biliary atresia, 5 from chronic hepatitis, 2 from cirrhosis, 8 from miscellaneous liver diseases including hepatoblastoma, extrahepatic portal hypertension, Caroli disease, congenital hepatitic fibrosis, Alagille syndrome, and also in 100 normal children. Chil-

dren in the idiopathic infantile cholestasis group seropositive for TORCH, hepatitis A or B, syphilis, exhibiting abnormal results regarding urinary $FeCl_3$ or reducing substance, along with children showing abnormal biliary sonography were not included in this study.

Healthy controls

Blood was taken from healthy infants and children who participated in the long-term follow up of the vaccine trial at the Well Baby Clinic, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Laboratory methods

The sera were stored at $-20°C$ before electrophoresis. AAT phenotypes were determined by thin layer isoelectric focusing (TLIEF) on polyacrylamide gel as described elsewhere.[9, 10]
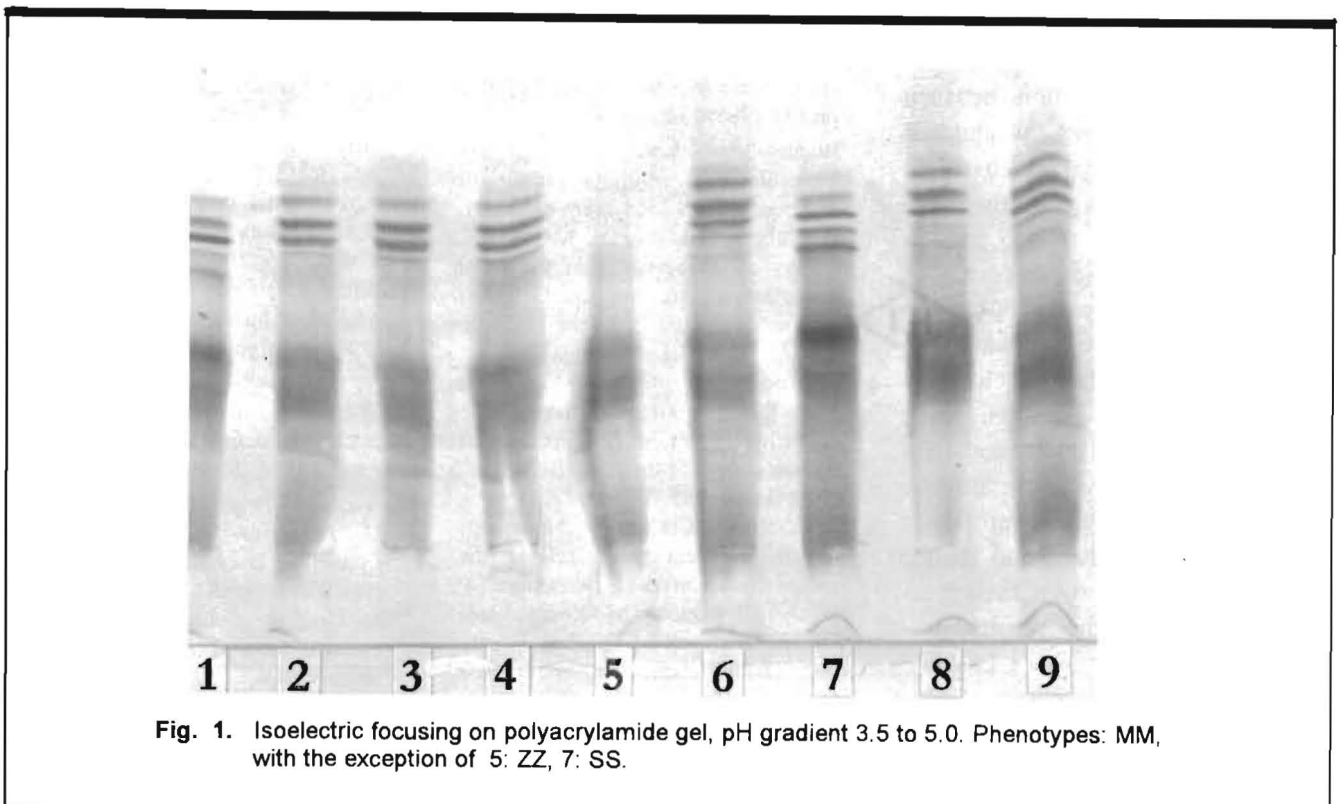
RESULTS

The distribution of AAT

phenotypes in children with liver diseases and controls is shown in Table 1. The age of patients with idiopathic infantile cholestasis, extrahepatic biliary atresia, chronic hepatitis, cirrhosis, miscellaneous liver diseases and healthy controls ranged from 15 days to 2.42 years (mean 0.55 years), 1 month to 10.5 years (mean 2.18 years), 4.92 to 14.75 years (mean 11.1 years), 1.5 to 12.5 years (mean 7 years), 1.25 to 8.17 years (mean 4.28 years), and 1 month to 14 years (mean 6.85 years), respectively. PiMM has been the most common phenotype detected in both groups (92% in the group with liver diseases and 88% in healthy controls). We could demonstrate PiZZ in only one child and PiSS in 3 children in the control group, but none in the group with liver diseases. The electrophoretic phenotypes discernible by isoelectric focusing on polyacrylamide gel slabs are shown in Fig. 1.

DISCUSSION

Alpha$_1$-antitrypsin deficiency (PiZZ) constitutes by far the most common hereditary cause of

Table 1. Pi phenotypes in children with various liver diseases and controls

Group	Pi phenotype (N)							Total (N)
	MM	MZ	IM	MS	FZ	SS	ZZ	
Idiopathic Infantile cholestasis	20	2	-	1	1	-	-	24
Extrahepatic biliary atresia	29	-	1	-	-	-	-	30
Chronic hepatitis	5	-	-	-	-	-	-	5
Cirrhosis	2	-	-	-	-	-	-	2
Miscellaneous	8	-	-	-	-	-	-	8
Controls	88	7	1	-	-	3	1	100

Fig. 1. Isoelectric focusing on polyacrylamide gel, pH gradient 3.5 to 5.0. Phenotypes: MM, with the exception of 5: ZZ, 7: SS.

liver disease.[11,12] In a large epidemiological cohort study of 200,000 Swedish newborns, 127 PiZZ infants were identified of whom 11% had icteric hepatitis (equivalent to 1 in 15,384 live births) and a further 2-3% progressed to cirrhosis. In this survey, up to 70% of PiZZ infants exhibited biochemical evidence of hepatitis at six months of age.[13] Recent reports revealed a favorable prognosis for PiZZ and PiSZ subjects prospectively followed up to the age of 18.[14] None of them presented with any signs of liver disease even though 12% of PiZZ and 15% of PiSZ subjects showed abnormalities of serum alanine aminotransferase or gamma-glutamyl transferase at the age of 18. Alpha₁-antitrypsin deficiency constitutes an increased risk of cirrhosis and hepatoma in adult males above the age of 50.[15] The mechanism of accumulation of AAT glob-

ules in the rough endoplasmic reticulum comprises polymerization favored by a point mutation of the molecule expressed as a substitution of a negatively charged glutamic acid for a positively charged lysine at position 342.[3,16] Intrahepatocyte globules of AAT are nontoxic *per se* because they are present in the PiZZ population as a whole, including those devoid of liver disease.[10,17] The trigger leading to liver disease in AAT deficient patients may be hepatitis C virus or other unidentified viruses.[18] PiZZ individuals with liver disease showed deranged immunomodulation[19,20] and abnormal activation of the complement cascade.[21] Massi[22] has postulated that additional genetic or environmental conditions will probably be identified as cofactors responsible for ensuing liver disease. However, the exact pathogenesis of liver disease

in connection with AAT deficiency remains a challenge. MZ and SZ subjects are not associated with liver injury during infancy but they are more prone to develop liver disease than healthy subjects. Low levels of AAT might contribute to rendering hepatocytes vulnerable to uncontrolled proteolytic damage leading to hepatic function impairment.[23] Proteases are capable of producing oxidants which can damage various tissues by lipid peroxidation of cell membrane components, sulfhydryl enzyme inactivation, and by interaction with DNA.[24,25] In an epidemiological study in Thailand, 4 out of 852 subjects tested turned out to be PiZZ individuals, indicating a rather uncommon prevalence.[10] This suggests a higher prevalence of PiZZ among Asian populations than previously expected (in comparison with the Scandinavian population

with a ratio of 1:1,500). In the present study, we could not determine the connection between various liver diseases in children and AAT deficiency, which might be due, on the one hand, to the small number of patients tested, and on the other, to the fact that various liver diseases such as extra-hepatic biliary atresia were included, instead of concentrating exclusively on cases of icteric hepatitis. Since alpha-1 antitrypsin deficiency triggered by virus may cause inflammation, including other liver diseases in the study would reduce the overall probability of finding an association. Yet, the results presented here can serve as a baseline for further studies necessary to elucidate the genetic frequency and the clinical relevance of ATT deficiency in connection with liver diseases in Thailand.

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