HUMAN INTER-ALPHA-TRYPSIN INHIBITOR (ITI) SILENT ALLELE FOUND IN A CASE OF DISPUTED PATERNITY

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INTRODUCTION

Inter-alpha-trypsin inhibitor (ITI) is a 180-kDa glycoprotein having the role of a serine protease inhibitor. It has been revealed that ITI is not a single polypeptide chain structure, but a complex of three kinds of subunits, two heavy (H1 and H2) chains and a light (L) chain (Bourguignon et al. 1983) with chondroitin sulfate cross-links (Jessen et al. 1988). In situ hybridization showed that the H1, H2 and L chains were encoded by separate genes located on chromosomes 3, 10 and 9, respectively (Diarra-Mehrpour et al. 1989). Genetic variation of ITI on an isoelectric point was reported by Vogt and Cleve (1990) being controlled by mainly three alleles (ITI*1, ITI*2 and ITI*3) with autosomal codominant inheritance. The utility of ITI system has been supported from the results of several population studies (Luckenbach et al. 1991; Vogt et al. 1991a, 1991b; Yuasa et al. 1991; Vogt et al. 1992). However, Vogt et al. (1991b) indicated the cautious application of ITI for paternity testing due to an incomplete expression of ITI phenotypes among infants.

We present here a case of disputed paternity with ITI silent allele (ITI*Q0) in a mother-child pair, supposing from the fact that no inconsistent result of maternity in 26 other genetic marker systems containing the rare variant allele in alpha-2-HS-glycoprotein system (AHSG*15) was observed.

MATERIALS AND METHODS

Serum samples obtained from individuals in paternity testing were treated with neuraminidase and subjected to isoelectric focusing (IEF) on polyacrylamide gel (PAG) containing 2.5 M urea and 2.5% carrier ampholyte of either pH 3.5-9.5 or pH 5.5-7.7 (pH 6-8 : pH 7-9 = 3 : 1). After IEF, ITI bands were visualized by immunoblotting using anti-ITI antiserum (Dako-patts) mostly according to the previous report (Nakayashiki and Katsura 1989). In this case, following genetic markers were phenotyped; 7 red cell antigens (ABO, MNSs, Rh, Duffy, Kidd, Lewis, P), 10 serum proteins (HP, GC, TF, PI, AHSG, GM, KM, HF, ZAG, ITI), 5 red cell enzymes (ACP1, PGM1, PGD, GPT, ESD) and 3 HLA antigens (HLA-A, -B, -C). Additionally, 2 DNA polymorphisms (VNTRs: ApoB and YNZ22) by PCR technique were tested.

RESULTS AND DISCUSSION

Fig. 1 shows ITI phenotypes by means of PAGIEF and immunoblotting. In comparison with common types, the mother and her child were phenotyped as ITI 2 and ITI 1, respectively (Fig. 1, lane 3 and 4), thus resulting in the denial of maternity. However, no additional incompatibility between mother and child was observed in other genetic marker systems including AHSG demonstrating the transmission of a rare allele (AHSG*15) from mother to child (Fig. 2, lane 2 and 3). Although we could not observe apparent and considerable decrease of ITIs in both sera judging from the intensity of ITI bands (Fig. 1) and a quantitative rocket immunoelectrophoresis (data not shown), we considered that the inconsistency might be elucidated by the presence of a silent allele (ITI*Q0).

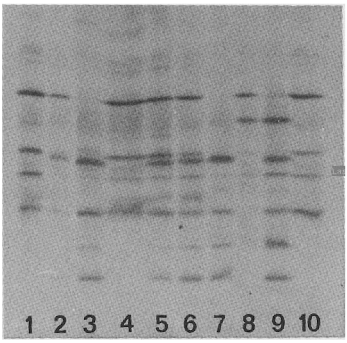

Fig. 1. Band patterns of ITI phenotypes of desialized sera analyzed by IEF in 2.5 M urea and the range of pH 5.5-7.7 followed by immunoblotting. Anode is at the top.

Lane 1 and 10	:	ITI 1
2 and 6	:	2-1
3(mother)	:	2
4(child)	:	1
5(father)	:	2-1
7	:	2
8	:	3-1
9	:	3-2

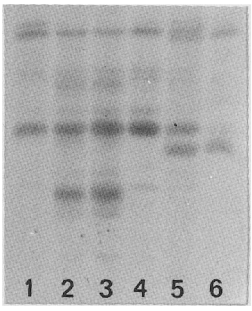

Fig. 2. AHSG phenotypes of desialized sera analyzed by IEF in 2.5 M urea and the range of pH 5-6 followed by immunoblotting. Anode is at the top.

Lane 1	:	AHSG 1
2(mother)	:	15-1
3(child)	:	15-1
4(father)	:	1
5	:	2-1
6	:	2

Vogt et al. (1991b) reported one paternity case excluded fatherhood in ITI system, and suggested that the ITI phenotype in younger children below 18 months having genotype ITI*1-2 might be typed as homozygous phenotype of ITI 1 or ITI 2 by reason of incomplete expression of ITI. This assumption still remains in the present case because the child was 9 months old when she was tested.

In the present method, the polymorphism on ITI has been detected with the use of a commercially available anti-ITI antiserum that reacts with all of the ITI subunits. In subsequent experiments, we found that there was an ability to detect ITI variation in anti-H chains, but not in anti-L chain fractionated from the antiserum by affinity chromatography (data not shown). Recently, the cDNA sequences of H1, H2 (Salier et al. 1987) and that of L coding genes (Bourguignon et al. 1985) were determined and moreover DNA polymorphisms in the ITI H2 gene were demonstrated (Léveillard et al. 1990a, 1990b). Relationship of ITI polymorphisms between protein and cDNA is still unknown, however, it might be solved in further studies.

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