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The First Successful Prenatal Diagnosis on a Korean Family with Citrullinemia

Kyeong-Man Hong[1], Moon-Kee Paik[1], Ook-Joon Yoo[2], and Si-Houn Hahn*

Department of Pediatrics, Ajou University School of Medicine, Suwon 442-749, Korea;

[1] Department of Biochemistry, Wonkwang University College of Medicine, Iksan 570-749, Korea;

[2] Department of Biological Science, Biomedical Research Center, KAIST, Taejon 305-701, Korea.

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DNA prenatal diagnosis was successfully performed on a family with citrullinemia. The father carried the G324S mutation and the mother carried the IVS6-2A > G mutation in the argininosuccinate synthase gene. They had a previous child with citrullinemia who died in the week after birth owing to complicated hyperammonemia. The lost child turned out to be a compound heterozygote. DNA was extracted from the cultured amniotic cells after amniocentesis done at 18-week gestation. For the detection of the G324S mutation, the PCR and restriction fragment length polymorphism method was used, and for the IVS6-2A > G mutation, allele-specific PCR was performed. The fetus was found to carry G324S but not IVS6-2A > G, suggesting a heterozygote carrier. Pregnancy was continued and a healthy boy was born. Plasma amino acid analysis performed on the third day after birth was normal and the serial ammonia level was in the normal range. A molecular study on his genomic DNA after birth also agreed with the previous fetal DNA analysis. He is now 2-months old with normal growth and development.

Introduction

Citrullinemia is an autosomal recessive disease showing citrullinemia, hyperammonemia, mental retardation, and early death when untreated. This is caused by a deficiency of argininosuccinate synthetase (ASS; EC6.3.4.5), which catalyzes the conversion of citrulline, aspartate, and ATP into argininosuccinate, AMP, and pyrophosphate. The enzyme activity is found in the liver, where the enzyme functions in the urea cycle to eliminate ammonia although ASS is also present in other tissues and cultured cells. The human ASS mRNA of 1.67 kb encodes a 412 amino acid sequence of a homotetramer with a subunit molecular mass of 46 kDa (Bock *et al*., 1983; Kobayashi *et al*., 1990). The expressed gene consists of 16 exons and is located in chromosome 9q34, spanning 63 kb, and multiple pseudogenes were also reported (Beaudet *et al*., 1986).

The mutations of the ASS gene were reported in Caucasian, Japanese, and Korean patients with citrullinemia (Kobayashi *et al*., 1990, 1994, 1995; Hong *et al*., 2000a). IVS6-2A > G and G324S are shown to be the most frequent mutations in Korean patients (Hong *et al*., 2000b), so these frequent mutations could be useful for molecular diagnosis and prenatal diagnosis of citrullinemia. Kakinoki *et al*. (1997) reported a DNA diagnosis system to detect 14 out of 23 reported mutations. Hong *et al*. (2000b) also reported a simple DNA diagnostic method to detect the IVS6-2A > G mutation. In this report, we present a prenatal diagnosis case of citrullinemia with DNA diagnostic tests.

Materials and Methods

Subject and DNA isolation A mother with a previous child with citrullinemia who died in the week after birth owing to complicated hyperammonemia had another baby. The lost child had the IVS6-2A > G and the G324S mutations (Hong *et al*., 2000b). Amniocentesis was performed for the amniotic cell culture at the 16th week of gestation, and the amniotic cells were cultured in a minimal essential medium (Gibco BRL) supplemented with 10% fetal bovine serum. Genomic DNA samples were purified from the cultured cells of the fetus and the whole blood of the parents using a DNA purification kit (Qiagen).

Detection of the IVS6-2A > G and the G324S mutations in genomic DNA The genomic DNA samples from the parents' blood and the fetal cells were used for the detection of the

* To whom correspondence should be addressed.
Tel: 82-31-219-5166; Fax: 82-31-219-5169
E-mail: omik@madang.ajou.ac.kr

IVS6-2A > G and the G324S mutations. The sequence including partial intron 6, exon 7, and partial intron 7 was amplified by the method reported by Hong *et al.* (2000b).

Genomic fragments including exon 13 were amplified by PCR using the following primers, which were modified by removing the restriction enzyme sites of the original primers (Kobayashi *et al.*, 1997): int-12F; 5′-CAG TTT GGG TTT CAT GCG-3′ and int-13B; 5′-ACT TTG GGA TCC CTT GTG AG-3′. The reaction mixture was incubated at 94°C for 5 min to denature the DNA and was subjected to 25 cycles of PCR with the following conditions: 95°C for 30 s, 55–60°C for 1 min, and 72°C for 1 min. After the amplification, the amplified product was digested with *Msp*I restriction enzyme (from Promega).

Results and Discussion

The prenatal diagnosis of citrullinemia was successfully performed. The father carried the G324S mutation (Fig. 1) and the mother carried the IVS6-2A > G mutation (Fig. 2). The fetus carried the G324S mutation, but not the IVS6-2A > G mutation, indicating that the fetus is a heterozygote carrier. Pregnancy was continued and a healthy boy was born with a birth weight of 3.59 kg at 39 weeks of gestation. Plasma and urine amino acid analyses performed on the third day after birth were normal. The plasma citrulline level was 24.50 nmol/ml (normal range: 10–45 nmol/ml) and the urine level was 0.084 nmol/g creatinine (normal range: 0.03–0.18 nmol/g creatinine). The serial ammonia levels performed on the same day were also in the normal range: they were 41.412 μM at 09:14, 28.56 μM at 16:10, and 33.558 μM at 23:44. A molecular study on his genomic DNA was also performed and it agreed with the previous fetal DNA analysis. He is now 2-months old and growing normally.

Kakinoki *et al.* (1997) reported two prenatal diagnosis cases with the PCR and restriction fragment length polymorphism method. All the parents were IVS6-2A > G heterozygotes. One fetus was detected to be a heterozygous carrier; the other fetus was shown to be a citrullinemia patient with homozygous IVS6-2A > G, and the baby had elevated serum citrulline after birth.

The diagnostic methods to detect mutations in citrullinemia using genomic DNA were reported by a Japanese group (Kobayashi *et al.*, 1990, 1994, 1995; Kakinoki *et al.*, 1997). By combining PCR and restriction enzyme digestion, 12 mutations, i.e. R157H, R272C, R279Q, G280R, R304W, G324S, G390R, IVS6-2A > G, IVS13 + 5, Δ13bp/Ex15&IVS15, IVS15-1 and Δ11bp/IVS15, can be detected. Two other mutations could be detected by Southern blot analysis. An insertional mutation, c1125–1126ins67-bp, could also be detected by PCR amplification (Hong *et al.*, 2000a). For the remaining missense mutations, DNA diagnostic analysis are not available. Until the establishment of DNA diagnostic methods for all mutations, three DNA polymorphisms (Northrup *et al.*, 1989, 1990) and a microsatellite VNTR (Kwiatkowski *et al.*, 1991) may be useful.

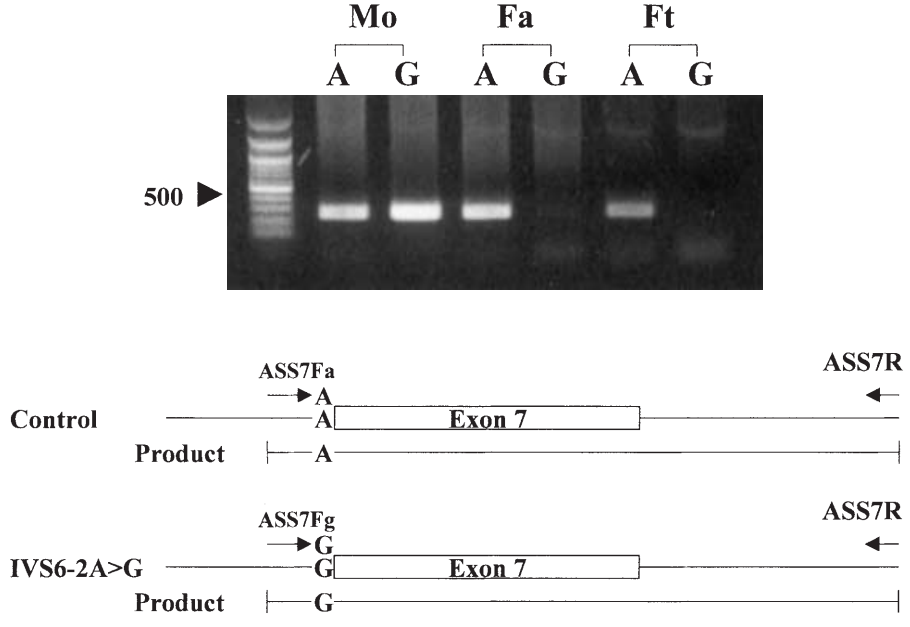

Fig. 1. Detection of IVS6-2A > G in genomic DNA. The upper panel shows the electrophoretic analysis of the amplified DNA from the fetus (Ft), the father (Fa), and the mother (Mo) by using the common reverse primer (ASS7R) and either A-specific primer (ASS7Fa, A) or G-specific forward primer (ASS7Fg, G). A schematic representation of the position of the ASS gene amplified by PCR using these primers is shown in the bottom panel.

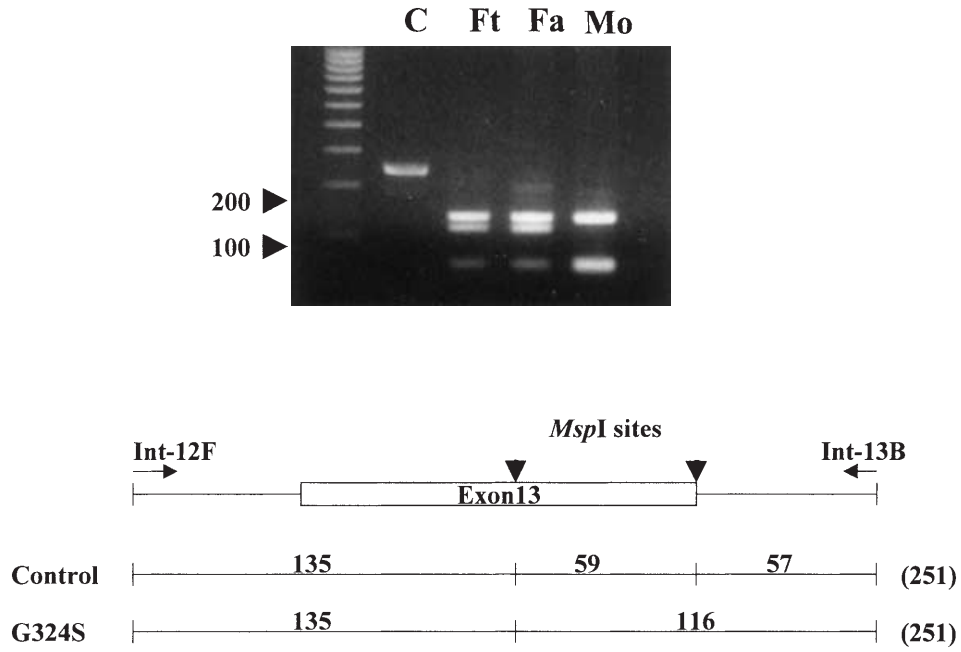

Fig. 2. Detection of G324S in genomic DNA using PCR/restriction enzyme digestion. The exon 13 region of the ASS gene was amplified from the genomic DNA of the fetus (Ft), the father (Fa), and the mother (Mo) as described in **Materials and Methods**. For the detection of the G324S mutation, the amplified products were digested with *Msp*I and the resulting fragments were electrophoresed along with the undigested control (C). The diagram indicates both the position of the *Msp*I sites and the size of the restriction fragments obtained after *Msp*I digestion.

In this study, a prenatal diagnosis on a Korean family with citrullinemia was successfully performed. The family had mutations that could be detected by available DNA diagnostic methods. The allele-specific PCR method for the detection of the IVS6-2A > G mutation used in this study was quite applicable to prenatal diagnosis of citrullinemia. The fetus carried the G324S mutation but not the IVS6-2A > G mutation, suggesting a carrier, and it was healthy after birth.

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