DIAGNOSIS OF X-LINKED ICHTHYOSIS USING PCR AMPLIFICATION

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X-Linked ichthyosis (XLI) is an inherited skin disorder due to the deficiency of steroid sulfatase (STS) activity. So far, XLI has been diagnosed before birth by assaying STS activity in cultured aminiotic cells. Recently, STS gene was cloned and it was found that most of XLI patients in Europe and USA had a large deletion across the STS genome. DNA amplification by polymerase chain reaction (PCR) has been widely used in analysis of genetic diseases. This method circumvents the tedious and timeconsuming cell culture and the use of a radioisotope reagent for the enzyme assay. In this study, we applied this method for genetic diagnosis of XLI.

STS gene is about 140 kbp in size with 10 exons. We designed two sets of oligonucleotide primers for PCR analysis. One set was in exon 1 and the other set was in exon 10. DNA amplification was performed according to the standard PCR amplification protocol. Primers were annealed at 59°C and thirty cycles of amplification were performed. As far as we examined the amplified DNA products of 214 bp (exon 1) and 414 bp (exon 10) were obtained from normal human leukocytes, however, DNAs from XLI patients were unable to produce both fragments of DNA. These results indicated deletion of STS gene was also the possibile etiology of XLI in Japan.

PRENATAL DIAGNOSIS OF ORGANIC ACIDEMIA - PROPIONIC ACIDEMIA

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Propionic acidemia(PA) and methylmalonic acidemia(MMA) are a group of inborn error of amino acid metabolism. The disorders may present in the first week of life with feeding difficulties, lethargy, vomiting and life threatening acidosis. The metabolites of propionyl-CoA most characteristic of propionic acidemia are 3-hydroxypropionic , methylcitric and propionic acid., and the compound most characteristic of methylmalonic acidemia is methylmalonic acid. The GCMS can separate and identify those metabolites has made it the method most widely used to diagnose the disorders of organic acidemia.

During the past years, we have investigated four families with history of organic acidemia(2PA & 2MMA)and proceeded six prenatal diagnoses with stable isotope dilution method by GCMS. Mid-trimester amniocenteses were performed between 14-17 weeks of gestation age. The concentration assay of methylcitrate and methylmalonate in amniotic fluid were used to prenatal diagnosis of propionic acidemia and methylmalonic acidemia respectively. Two cases were diagnosed to have propionic acidemia and confirmed by PA incorporation and propionyl-CoA carboxylase activity determination from skin fibroblasts taken after termination of pregnancy.

We conclude that measurement of methylcitrate and methylmalonate concentration in amniotic fluid with stable isotope dilution method by GCMS is very useful in prenatal diagnosis of propionic acidemia and methylmalonic acidemia.

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