Journal of Korean Medical Science
Vol. 3, No. 2, P 73-77, June 1988

Prenatal Diagnosis of A Heterozygote of Salt Wasting Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency by Genetic Linkage Analysis

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For the purpose of prenatal diagnosis of CAH, genetic linkage analysis by HLA genotyping with lymphocytes and cultured amniotic cells were performed in a family at risk in which two consecutive children had been affected with SW CAH. In addition, the response of serum 17-OHP to intravenous ACTH was determinded in obligate carrier parents, and 17-OHP concentration of amniotic fluid was also measured at 16 weeks of gestation. As might be expected, the baseline levels of 17-OHP in obligate parents were significantly higher than that of normal control. Although the post stimulation response of 17-OHP to ACTH in the mother (I-2) was significantly higher than that of normal control, the post stimulation levels of 17-OHP were in normal range in the father (I-1).

The 17-OHP level (5.7ng/ml) in the amniotic fluid showed intermediate value compared to Pang's report (normal <30ng/ml, CAH >12.0ng/ml) suggesting heterozygote of the fetus.

Genetic linkage analysis by HLA genotyping with cultured amniotic cells revealed heterozygote in their fetus (II-3) who has received one chromosome No,6 containing HLA haplotype A24, B40, Cw3 (normal allele for 21-OH) from the father and the other chromosome No,6 containing HLA haplotype A2, Bw62, Cw4 (mutant allele for 21-OH D) from the mother.

In conclusion, attempts to detect heterozygote for 21-OH deficiency by ACTH stimulation test were partially successful and prenatal diagnosis of CAH by the hormone studies in ammiotic fluid requires reliable values in normal, heterozygotes and patients group, respectively.

On the other hand, genetic linkage analysis by HLA genotyping can be utilized for prenatal diagnosis of CAH due to 21-OH deficiency with a high degree of reliability in a family at risk. This is the first report of successful prenatal diagnosis of CAH by genetic linkage analysis in Korea.

Key Words: *Congenital Adrenal Hyperplasia (CAH), HLA haplotype, ACTH stimulation test, genetic linkage analysis.*

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INTRODUCTION

Congenital Adrenal Hyperplasia (CAH) encompass a family fo autosomal recessive inborn errors of adrenal steroidogenesis. Each of these disorders has deficient activity of one of the enzymes necessary for normal cortisol synthesis (Miller & Levine., 1987). Decreased production of adrenal cortisol due to enzyme deficiency will results in increased ACTH secretion, which leads to overproduction of cortisol precursors from which androgen can be synthesized causing characteristic clinical symptoms such as virilization, short stature, dark pigmentation and hirsutism etc (New & Seaman., 1970).

Although CAH was known as a very rare genetic disorder in the past, recent estimates of the incidence of CAH due to 21-hydroxylase (21-O-H) deficiency have ranged from 1:5,000 to 1:15,000. The gene frequency has been estimated to be 1 in 100 and carrier frequency 1 in 50 (New 1986). Thus, CAH is the most common autosomal recessive disorder with approximately 95 percent of CAH is caused by a deficiency of the 21-OH enzyme.

A great advance in investigation of CAH in recent years has occurred as a result of the discovery of genetic linkage between the 21-OH genes and Human Leukocyte Antigen (HLA) genes. Genes are said to be linked to one another if, after meiosis I, they remain together more often than expected by chance and so, they segregate to the same gamete rather than complementary gamete.

Close genetic linkage between HLA complex and 21-OH genes was first described in 1977 (Dupont et al., 1977). In 1979, Pollack et al reported successful prenatal diagnosis of CAH by genetic linkage analysis and this was proved to be a very useful method for the prenatal diagnosis of CAH due to 21-OH deficiency (Pollack et al., 1979). Thus, we performed the genetic linkage analysis by HLA genotyping and hormone studies for prenatal diagnosis of CAH in a family at risk.

MATERIALS AND METHODS

Clinical reports

A para 2, 28-year-old pregnant woman visited our genetic section for genetic counseling and prenatal diagnosis of her fetus. She had previous history of having two consecutive offspring with SW CAH. Her first son (II-1) died of SW CAH at 1 week of age because he had not been treated with hydrocortisone. Her second son (II-2) was also affected with SW CAH but has been treated with hydrocortisone since his birth. Although all her first and second sons showed normal external genitalia and normal blood pressure in newborn periods, they showed dark pigmentation of the skin at that time (Fig. 1). Three year-old second son had been given admission care six times due to occasional convulsive disorder.

Fig. 1. Appearance of the second son affected with SW CAH at 3 months old. Note dark pigmentation of face.

Hormone studies.

Baseline values of 17-hydroxyprogesterone (17-OHP) and stimulation values at 30 minutes, 60 minutes after ACTH injection were measured in the obligate carrier parents and five normal control subjects without CAH. The amniotic fluid was obtained at 16 weeks of gestation by amniocentesis and 17-OHP concentration was also measured. The value of 17-OHP in amniotic fluid was compared to the values of Pang's report (Pang et al., 1980), since the normal have not been established in our laboratory.

Genetic linkage analysis.

The genetic linkage analysis by HLA genotyping

was performed with peripheral blood lymphocytes from the second child (II-2, index case) and parents using the standard microcytotoxicity method. Cultured amniotic cells were harvested from the culture vessels by trypsinization and recultured with Dulbecco's Modified Minimum Essential Medium (DMEM) containing 20 percent Fetal Calf Serum (FCS) with mild stirring for suspension culture. After 1-2 days, the amniotic cells were harvested and HLA genotyping was performed with preabsorbed complement and HLA-ABC typing kit (Terasaki tissue typing tray). The inherited patterns of the 21-OH deficiency mutant genes were then analysed.

Cytogenetic studies.

For the detection of chromosome abnormality, amniotic cells were cultured for 3 weeks and treated with colcemid for metaphase arrest. Chromosome preparations were prepared by the treatment of hypotonic (0.075M KCL) and fixative (3 part methanol+1 part acetic acid) solution. Chromosome analysis was performed on cultured amniotic fluid using QFQ and GTG banding technique.

RESULTS

17-OHP values in obligate parents, normal control and amniotic fluid.

The baseline values of 17-OHP were 3.3ng/ml in the father, 5.1ng/ml in the mother and 1.45±0.6ng/ml in the control group. Thirty and sixty minutes values after ACTH stimulation were 7.0ng/ml and 7.6ng/ml respectively in the mother and 3.41±0.78ng/ml and 3.25±0.48ng/ml respectively in the control. The stimulation values of 17-OHP in the mother were significantly higher than the control values, but the stimulation values of 17-OHP in the father (4.7ng/ml and 4.5ng/ml) were in the normal range. The concentration of 17-OHP in amniotic fluid was 5.7ng/ml, which was higher than the normal upper limit (<3.0ng/ml), but lower than the value of CAH of Pang's report (CAH >12.0ng/ml; Pang et al., 1980). These results suggests that the fetus might be CAH heterozygote (Table 1).

HLA genotyping in family at risk

The HLA antigens of the index case (II-2) were the A2, B-, C-/A2, Bw62, Cw4 and HLA antigens of the father and the mother were A2, B-, C-/A2, B40, Cw3 and A2, Bw62, Cw4/all, Bw35, C- respectively. Thus, we can infer the 21-OH deficiency genes are located on the chromosome containing the HLA haplotype A2, B-, C- in the father and the chromosome containing the HLA haplotype A2, Bw62, Cw4 in the mother.

Analysing the HLA haplotypes of amniotic cells, the fetus received one chromosome from the mother containing HLA haplotype A2, Bw62, Cw4 in which 21-OH deficiency mutant genes are located and one chromosome from the father containing HLA haplotype A24, B40, Cw3 in which normal 21-OH genes are located. Therefore, we can infer the fetus as heterozygote. By this genetic linkage analysis, prenatal diagnosis of CAH due to 21-OH deficiency was sucessful (Fig 2).

Cytogenetic studies

Chromosome analysis with cultured amniotic cells revealed normal 46, XY consititution.

DISCUSSION

CAH is caused by an inborn error of adrenal steroidogenesis due to deficient activity of one of the various enzymes necessary for cortisol synthesis. The most common form of CAH (approximately 95 percent) is caused by 21-OH deficiency. It is well known

Table 1. 17-OH P levels of obligate carrier parents in response to ACTH and 17-OH P levels in amniotic fluid

Sample	Subjects	17-OH P levels in response to ACTH (ng/ml)		
		Baseline	30 Minutes	60 Minutes
Serum	Father (I-1)	3.3	4.7	4.5
	Mother (I-2)	5.1	7.0	6.0
	Control (M±S.D)	1.45±0.6	3.41±0.78	3.25±0.78
Amniotic fluid	Fetus* (II-3)	5.7	—	—
	CAH fetus**	>12.0	—	—
	Normal control	< 3.0	—	—

* 17-OH P levels in amniotic fluid at 16 weeks gestation

** Pang's et al., report (J Clin Endocrinol Metab. 5:223, 1980)

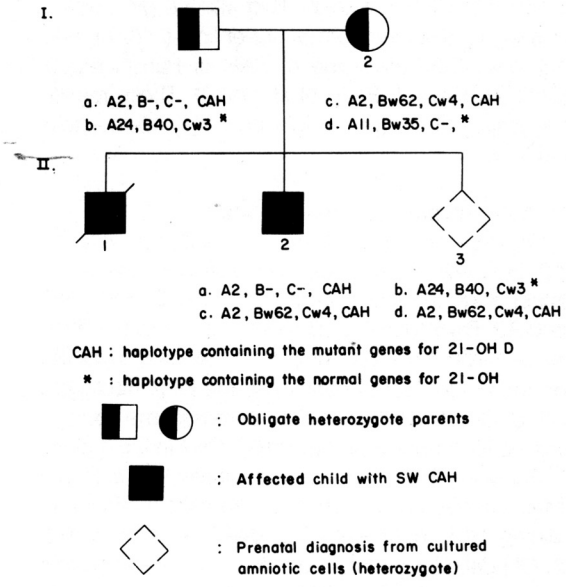

Fig. 2. Pedigree of the family of patients with SW CAH

that the clinical symptoms, biological test values and hormone levels were different by the types of the deficient enzymes causing CAH (Bongiovanni, 1981). Various forms of CAH due to 21-OH deficiency are inherited in an autosomal recessive fashion and close genetic linkage to the HLA complex has been demonstrated (Dupont et al., 1977). Futhermore, the genes coding the 21-OH have been located to the chromosome No, 6 between the HLA B and C4 loci within the HLA complex (Amor et al., 1985; Levine et al., 1978; Caroll et al., 1985).

In the past, 17-KS, pregnanetriol, 17-OHP, testosterone, androstenedione and dehydroepiandrosterone (DHEA) were frequently determined for the purpose of the diagnosis of CAH (New & Levine., 1973). Recently, measurement of 17-OHP concentration in blood by ACTH stimulation was recommended as the most useful screening test even though it is difficult to diagnose CAH by hormone studies alone because the hormone levels might be different according to the types and degree of deficiency of 21-OH enzymes (Pang et al., 1980).

In this study, 17-OHP level of the obligate carrier father was in normal range and this fact indicate the discrimination of heterozygote was only partially successful by the ACTH stimulation alone. In addition, it has been recognized that the prenatal diagnosis of CAH due to 21-OH deficiency by the hormone studies were only possible in case of SW CAH. Therefore, the necessity of genetic linkage analysis by HLA genotyping was increased for the detection of heterozygote and prenatal diagnosis of CAH due to 21-OH deficiency. We have attempted the genetic linkage analysis by HLA genotyping with cultured amniotic cells for prenatal diagnosis of CAH, and clarified some limitations as well as availability of the HLA genotyping. Regarding the some limitation of HLA genotyping, it is reported that there is some cross reaction among the HLA B antigen group (Couillin et al., 1980). Thus, antibody absorption test may be required and the complement should be preabsorbed with amniotic cells for the prevention of the non-specific cytolysis, In the procedure of HLA genotyping with cultured amniotic cells, we observed that the reaction pattern was not so clear cut as HLA genotyping with lymphocytes and it will be solved by the improvement of cell culture technique and adjustment of the reaction time. Although HLA genotyping with amniotic cells have above mentioned limitation, a positive or negative reactions was clearly shown.

As a results of HLA genotyping with cultured amniotic cells, the fetus received one chromosome from the mother containing HLA haplotype A2, Bw62, Cw4 in which mutant genes for 21-OH are located and one chromosome from the father containing HLA haplotype A24, B40, Cw3 in which normal 21-OH genes are located. Therefore, we can infer the fetus as CAH carrier. Of couse, the possibility of misdiagnosis can not be completly excluded due to the recombination event during the meiosis I, but it is reported that the probability of recombination by crossing over does not exceed 1 percent (Couillin et al., 1980).

To our knowlege, prenatal diagnosis of CAH by the genetic linkage analysis using the HLA genotyping was not yet reported in Korea. This is the first case of successful prenatal diagnosis of CAH by genetic linkage analysis in Korea.

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