A Family at Risk of Congenital Adrenal Hyperplasia: A Molecular Approach for Prenatal Diagnosis

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Abstract

The molecular method for prenatal diagnosis in the first trimester was carried out on the second and third pregnancies of a family at risk of congenital adrenal hyperplasia (CAH). The first child, an 8-year-old daughter, was affected. The molecular and cytogenetic prenatal diagnosis on the second pregnancy revealed that the fetus which was a female had been affected. The pregnancy was then terminated. The couple presented with the third pregnancy at 8 weeks' gestation. The same approach revealed that the fetus, a male, was affected. The couple opted for continuation of pregnancy which was on-going at the time of the manuscript preparation. To our knowledge, this is the first family in Thailand who had molecular approach for prenatal diagnosis of CAH. This approach allows early information about the fetal status of the disease and, together with the result of fetal gender, will help early decision making in pregnancy management.

Key word : Congenital Adrenal Hyperplasia, Prenatal Diagnosis

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Congenital adrenal hyperplasia (CAH) is a group of diseases resulting from an enzyme deficiency in the steroidogenesis pathway from cholesterol. This deficiency causes reduction or absence of cortisol levels which in turn causes overproduction of adrenocorticotrophic hormone (ACTH) from the pituitary gland. Adrenal cortex is therefore overstimulated and, as a result, the precursors proximal to the enzyme blockage accumulate. In 21-hydroxylase (21-OH) deficiency, which comprises 95 per

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cent of CAH cases[1], cortisol and aldosterone are depleted or deficient and there is an accumulation of 17 α-hydroxyprogesterone (17-OHP) and progesterone and their precursors. These precursors are diverted to androgenesis pathway, causing clinical manifestations of androgen excess. Prenatally, this results in masculinization of the external genitalia if the fetus is female. Postnatal manifestations include further virilization in females or precocious puberty in males and salt-wasting episodes in two-thirds to 75 per cent of cases. Although the patients are tall in childhood due to advanced bone age caused by androgens, they are short in adulthood due to premature closure of the epiphyses[1]. Patients may be infertile. Untreated females may have secondary amenorrhea, oligomenorrhea or polycystic ovarian syndrome. Increased estrogen levels from peripheral aromatization of androgens in untreated males may suppress gonadotropin secretion, causing them to have small testes.

There are 3 types of 21-OH deficiency according to the manifestations. They are salt-wasting (SW), simple virilizing (SV) and non-classic (NC) groups. In fact these represent a spectrum of severity of the disease due to different mutations resulting in different levels of enzyme activity. About 75 per cent of classic cases are salt-wasting[1]. In this group, in addition to androgen excess, there is a deficiency in aldosterone synthesis causing hyponatremia, hyperkalemia, hypervolemia, metabolic acidosis, shock and sometimes fatality. In the non-classic group, signs of androgen excess may develop at any stage after birth.

Prenatal diagnosis can be performed by amniocentesis in the second trimester for the levels of 17-OHP, or HLA typing. However, currently, molecular genetic evaluation can be performed and first trimester diagnosis can be achieved by chorionic villus sampling (CVS)[2,3]. Here we report a case history of a family at risk of having an affected pregnancy who had prenatal diagnoses performed on two pregnancies using molecular diagnosis. To our knowledge, this is the first family in Thailand to use this approach.

CASE REPORT

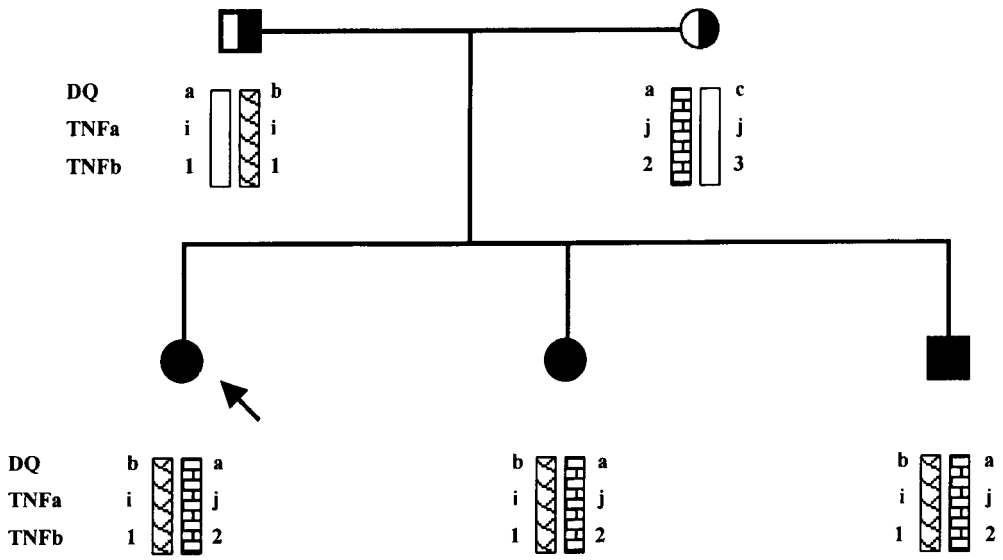
A 38-year-old Thai woman of Chinese ethnicity, G3P1A1, came to see us at 8 weeks' gestation of her third pregnancy. Her first child, an 8-year-old daughter, was affected by 21-OH deficiency and was now on hormonal treatment. Her second pregnancy took place 3 years previously and she came to the antenatal clinic at 8 weeks' gestation when dexamethasone was started at 0.5 mg daily. CVS performed a week later revealed that the fetus was a female. Due to some difficulties in arranging DNA diagnosis for 21-OH deficiency, the results came when she was 20 weeks pregnant, revealing that the fetus was affected (see *Molecular Diagnosis* below). Despite being on dexamethasone throughout while waiting for the results, the couple opted for termination of pregnancy at 21 weeks' gestation. The fetus, weighing 500 g, had male apparent genitalia. The couple then tried sex selection by sperm preparation but later gave up.

The current pregnancy, conceived naturally, was at 8 weeks. The patient and her husband were counceled and again agreed to do prenatal diagnosis for sexing and DNA analysis. Also, the patient started dexamethasone 0.5 mg twice daily. CVS was performed at 10 weeks' gestation. Direct preparation, with some modifications from Simoni et al[4] revealed 3 days later that the fetus was a male and dexamethasone was discontinued. Two weeks later, DNA analysis revealed that the fetus was affected (see *Molecular Diagnosis*). The couple opted to continue pregnancy which was still ongoing at the time of the manuscript preparation. They also notified the pediatrician who looked after their first child.

Molecular Diagnosis

In the first prenatal diagnosis, blood samples from the couple and their first child were tested along with the CVS sample from the second pregnancy. The method used was linkage analysis with four different markers. For clarity, only three markers are shown in Fig. 1. The results obtained later from the third pregnancy were also included in this diagram.

Results from the index case and her parents showed that, paternal haplotype with alleles a, i, 1 for DQ, TNFa and TNFb markers and maternal haplotype with alleles a, j, 2 for the same markers linked with the mutant genes for 21-OH. Unfortunately, the father was homozygous for TNFa and TNFb, therefore these markers were uninformative for prenatal diagnosis (Fig. 2). However, these markers were useful in the demonstration of the paternal alleles in the obtained samples, ensuring that they contained cells of fetal origin. Both CVS samples from the second and third pregnancies had



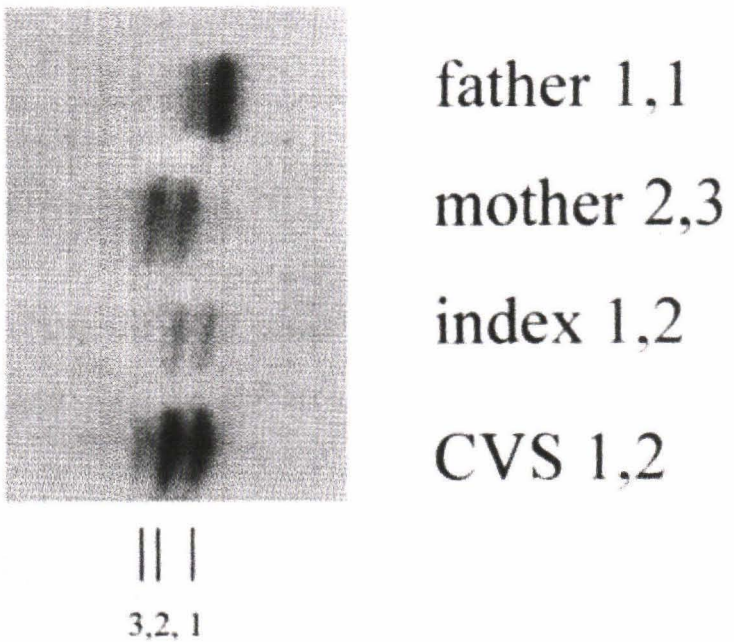
Deduced from the results in the index case (arrow), paternal haplotype b, i, 1 and maternal haplotype a, j, 2 linked with the mutant genes for 21-OH. The informative marker (DQ) shows that the second and third pregnancies are affected.

Fig. 1. The haplotype results from the family.

both paternal and maternal alleles that linked with the mutant genes for 21-OH and were diagnosed as affected.

DISCUSSION

Normally, external genitalia at 6 weeks' gestation are at bipotential state, being able to differentiate to either sex. Androgen exposure between 6-14 weeks' gestation causes masculinization of the external genitalia[5]. The genital tubercle becomes the penis, the labioscrotal folds fuse to become scrotum and folds of urogenital sinus become penile urethra. In normal male fetuses, this masculinization is completed by 14 weeks' gestation. Without androgens, the genital tubercle develops into clitoris, the labioscrotal folds become labia majora, the folds of urogenital sinus become labia minora and the urogenital sinus develops into the vagina and urethra. Exposure to androgens at this stage causes the female genitalia to be masculinized, resulting in ambiguous or male apparent genitalia. Steroid given to a pregnancy affected by 21-OH deficiency at this stage reduces the oversecretion of ACTH, thereby reduces the adrenal cortex overstimulation, resulting in less accumulation of androgen precursors along the pathways. This can help ameliorate the effect of androgens on female external genitalia. Therefore, it is suggested that steroid be started as soon as a pregnancy is recognized in families at risk for 21-OH deficiency, usually in the form of oral dexamethasone to the mother at 20 μg/kg/day prepregnancy weight divided in three equal doses[1,2]. Because dexamethasone crosses the placenta and is not a substrate of placental 11β-hydroxysteroid dehydrogenase, it will suppress ACTH in the fetus. Prenatal diagnosis for fetal sex and DNA analysis is recommended to determine the diagnosis of the fetus and also the length of dexamethasone treatment[1,2]. If the fetus is male, the effect of the disease on the genitalia will be unremarkable and the treatment can be stopped whether the fetus is affected with the disease or not. If the fetus is an unaffected female, dexamethasone can also be stopped. However, if the fetus is an affected female, dexamethasone should be administered throughout pregnancy. This therapy reduces the severity of



The father was homozygous (alleles 1,1). The mother was heterozygous (alleles 2,3). The index was heterozygous (alleles 1,2). It was not possible to determine if this paternal allele 1 in the index linked with the mutant gene or normal gene for 21-OH. The same applied to the results of CVS. The faint band in CVS at the position of allele 3 could be the small amount of maternal cell contamination in the sample.

Fig. 2. TNFb microsatellite marker results in the first prenatal diagnosis.

masculinization of external female genitalia and about half of affected female infants who have prenatal treatment require no genital surgery and the extent of the surgical repair is reduced in those who do require surgery. This has tremendous psychological benefits to the patients and families. It has been suggested that long-term antenatal dexamethasone therapy is safe for the fetus and no teratogenic effects have been reported[6-8]. However, some adverse effects of steroids on the mother can be found. The couple in this report presented with a pregnancy at risk for 21-OH deficiency at the gestational age of 8 weeks in the second gravidity with the starting of dexamethasone 0.5 mg per day which was somewhat undertreatment. Prenatal diagnosis showed that the fetus was an affected female. At the termination of pregnancy, the fetus still had masculinization of the external genitalia. In the current pregnancy, dexamethasone treatment was started at 8 weeks' gestation with the dose of 0.5 mg twice daily and was discontinued as soon as it was known that the fetus was a male. Although DNA analysis revealed that the fetus was affected with the disease, the couple chose to continue pregnancy. At the time of the preparation of the manuscript, the pregnancy was still on-going.

Molecular diagnosis in this couple was performed using linkage analysis. The gene encoding 21-hydroxylase is on the short arm of chromosome 6, in close linkage to human lymphocyte antigen complex. This gene which is called CYP21 and its homologue, CYP21P which is a pseudogene, are situated alternatively with two other genes, C4B and C4A, which encode the fourth component of serum complement. Mutations in the CYP21 gene occur owing to two mechanisms: gene deletion and appa-

rent gene conversion (short sequences on the pseudogene are transferred to the active coding gene) (1). Haplotypes from linkage study in this family from the index case and the parents shown in Fig. 1 revealed that the fetuses in the second and the third pregnancies were affected. These results were obtained by linkage analysis using closely linked microsatellite markers and carry a small (less than 1%) risk of recombination with the 21-hydroxylase gene. Small chances of errors can occur due to undetected recombination events. The samples were confirmed to be of fetal origin by the finding of paternally derived markers.

The parents are aware of the necessity of postnatal lifelong treatment and the possibility that hormonal replacement may not be completely effective and the child may still have poor growth. Also, the vigilance for signs of salt-wasting in the neonatal period is needed. In addition, hormonal treatment must be under careful monitoring as both undertreatment and overtreatment can cause undesirable effects. Undertreatment may not control the disease while overtreatment may result in Cushing's syndrome.

SUMMARY

A family at risk of having a pregnancy affected with congenital adrenal hyperplasia had prenatal diagnoses on two pregnancies using molecular approach. This approach enabled the information about the status of the disease early in the first trimester instead of the second trimester obtained from the conventional approach. Along with the result of fetal gender, decision making about the pregnancy management was possible.

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ครอบครัวที่มีความเสี่ยงต่อภาวะต่อมหมวกไตส่วนคอร์เทกซ์เจริญมากเกินไปแต่กำเนิด: การวินิจฉัยก่อนคลอดด้วยวิธีระดับอณู

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รายงานการใช้วิธีระดับอณูสำหรับการวินิจฉัยก่อนคลอดในไตรมาสแรกของการตั้งครรภ์ที่สองและสามของครอบครัวที่มีความเสี่ยงต่อภาวะต่อมหมวกไตส่วนคอร์เทกซ์เจริญมากเกินไปแต่กำเนิด บุตรสาวคนแรก ซึ่งอายุ 8 ปี เป็นโรค การวินิจฉัยก่อนคลอดวิธีระดับอณูและวิธีไซโตเจเนติกในครรภ์ที่สองพบว่าทารกเป็นเพศหญิงและเป็นโรคและได้ยุติการตั้งครรภ์ คู่สามีภรรยาได้มาพบแพทย์อีกเมื่อตั้งครรภ์ที่สามได้ 8 สัปดาห์ การวินิจฉัยก่อนคลอดวิธีเดียวกันพบว่าทารกเป็นเพศชายและเป็นโรค คู่สามีภรรยาเลือกที่จะตั้งครรภ์ต่อไป ซึ่งขณะที่รายงานนี้การตั้งครรภ์ยังดำเนินต่อไปอยู่ ครอบครัวนี้เป็นครอบครัวแรกในประเทศไทยที่ใช้วิธีระดับอณูช่วยในการวินิจฉัยก่อนคลอด วิธีนี้ช่วยให้ทราบผลสภาวะของทารกในครรภ์ได้เร็วขึ้น ซึ่งเมื่อประกอบกับผลเพศทารกจะช่วยให้การตัดสินใจเกี่ยวกับการดูแลการตั้งครรภ์ทำได้เร็วขึ้น

คำสำคัญ : ภาวะต่อมหมวกไตส่วนคอร์เทกซ์เจริญมากเกินไปแต่กำเนิด, การวินิจฉัยก่อนคลอด

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