

**BRITISH SOCIETY FOR PAEDIATRIC**

**ENDOCRINOLOGY**

**AND**

**DIABETES**

**Protocol**

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**The antenatal assessment and treatment of women with a previously affected child with Congenital Adrenal Hyperplasia (CYP21) and the subsequent monitoring of outcome parameters post delivery**

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## **1. BACKGROUND**

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (CYP21) shows a recessive pattern of inheritance. The incidence of the disease in the Caucasian population is 1 in 10 000 - 1 in 12 000 live births. In 3 of 4 cases the child has a severe form of the disease with prenatal virilisation of affected females due to excessive secretion of adrenal androgens. In these cases salt-wasting may also occur in the neonatal period in both sexes. Although genotype-phenotype correlations are less-than-complete it is well accepted that the condition breeds true within a family so that the chances of subsequent affected females is close to 100%.

Having a child with ambiguous genitalia is a major psychological trauma for the parents and, in addition, the virilised girl will be subjected to reconstructive surgery at several times during childhood and adolescence. In all virilising forms of CAH severely affected female fetuses would benefit from treatment which suppresses the fetal adrenal cortex, thereby reducing levels of adrenal androgens. The first attempts of prenatal therapy of CYP21 deficiency, in order to avoid or reduce virilisation of external genitalia in female fetuses, were presented in 1984 (David and Forest 1984). Dexamethasone (DEX) (which is not metabolised by the placenta and therefore accesses the fetus) was administered to a mother that previously had given birth to a child with severe CAH. The affected offspring was born with normal female genitalia.

The identification of the molecular defects in CYP21 deficiency has allowed for a clearer definition of the condition and opened the way for antenatal diagnosis of the condition. There has been a natural move, as a consequence, towards the concept of antenatal treatment of the mother and affected fetus with dexamethasone in order to limit /abolish the virilisation of affected females. In order to be fully effective, treatment has to be started before the 7<sup>th</sup> week of gestation and continued until term in those cases (1 of 8) when the fetus is an affected female. Since prenatal diagnosis can be performed at earliest from the 10<sup>th</sup> week of pregnancy on DNA from chorionic villous biopsies, 7 of 8 cases will be treated unnecessarily during several weeks of early embryonic and fetal life. Thus, it is of extreme importance that desired effects for minorities of the treated fetuses are not accompanied by side effects for the majority.

Reports in the literature of uncontrolled interventions suggest that the cosmetic appearances can be improved in the majority of individuals but very little attention has been paid to the potential complications that might ensue from prenatal exposure of the fetus to dexamethasone. The safety profile of dexamethasone in pregnancy largely stems from its acute use to manage the neonatal lung problems associated with premature birth but such treatment is usually administered in the late second and early to mid third trimesters. During the latest decade more than 300 cases have been treated with DEX and approximately 50 CYP21-affected girls have received this therapy (David and Forest 1984, Dörr and Sippell 1993, Forest et al 1993, Levine and Pang 1994, Mercado et al 1995, Lajic et al 1998). Approximately 70% of prenatally treated females are born with normal or only slightly virilised genitalia. The reasons for treatment “failures” are unclear

but probably include non-compliance, sub-optimal dosing, late onset of treatment or cessation in midgestation (Lajic et al, 1998). Overall, treated children seem to grow and develop normally compared to untreated peers and siblings. However, several adverse events such as failure to thrive and delayed psychomotor development, were reported among the treated infants.

Maternal complications appeared higher (10%) than in controls. Predominant effects related to rapid weight increase and striae during early pregnancy and oedema (Forest et al, 1998). It should be noted that DEX did not seem to increase the incidence of hyperglycemia or hypertension in treated mothers during pregnancy. Effects were dose dependent and have been reduced more recently by the use of dosing schedules not exceeding 20mcg/kg/day.

Studies concerning the psychological development of treated children are sparse. At present only a single case report exists indicating a normal outcome in late adolescence (Forest et al, 1998). One preliminary report suggests that DEX could have an effect on behavioral development, reflected by increased shyness and avoidance, but does not seem to have any impact on cognition (Trautman et al 1995).

Glucocorticoids (GC) administered *in utero* to primates and rodents have been shown to have severe effects on neuronal architecture, fetal growth and behavior (Reinisch et al 1980, Uno et al 1990, Edwards et al 1996). The hippocampus is especially vulnerable to GC due to the high concentration of GC receptors (Sousa et al 1989). The hippocampus is part of the limbic system and as such plays an important role in memory, mood, and behavior. However, it is important to remember that these studies on monkeys and rodents have been performed with doses several hundred times higher than those used for prenatal therapy of CAH.

In the latest decade data suggest that events *in utero* and early neonatal environment may influence the development of adult diseases. There is an increasing body of evidence in animals and man to suggest that prenatal exposure to GC can lead to long term problems. This is particularly the case in the development of cardiovascular risk factors namely raised blood pressure and type 2 Diabetes Mellitus, as well as suggestions of alterations in the functioning of the hippocampus, thymic development and mild left ventricular hypertrophy in preterm neonates. It is clear that species differences in DEX responsivity operate as intrauterine growth restriction occurred in 14% of rodent pregnancies compared to 2% of human DEX treated ones (Benediktsson et al, 1993; Forest et al, 1998). These observations are pertinent as the DEX dosing schedule is the same as that used in humans. Extrapolation from these animal studies to those in humans may not be warranted or at least need to be undertaken with caution.

Since 1984 there have been 8 publications on antenatal treatment of CYP21 with DEX. The information on outcome has been widely disseminated and communicated to parents with affected offspring. The concept has become enshrined in Paediatric Endocrine and Genetic practice that it is almost impossible to conduct a randomised clinical study to

address these issues. The best that can be achieved would be a careful follow-up of all pregnancies along with the outcome of all pregnancies managed for antenatal diagnosis and treatment of CYP21. Given that an effect has been demonstrated in over 70% of cases a position of equipoise does not exist in the Endocrine Community although concerns are voiced about the potential for long term side effects (Seckl and Miller, 1997). It is generally felt that this area needs ongoing surveillance although not all are agreed that review by Ethics panels is required (Miller, 1998; White and Speiser, 2000).

The following protocol has been drawn up following extensive consultation with Paediatric Endocrinologists, Geneticists, Molecular Diagnostic Groups and Obstetric and Endocrine colleagues. The numbers needed for the study in order to determine effects on birth size and blood pressure at 5 years of age are in excess of those likely to present over a 5 year period in the United Kingdom. For this reason the CYP21 Working Group has developed this study jointly with colleagues in Sweden, France, Finland, Norway, Poland, Spain and Germany (PREDEX, a joint Paediatric Endocrinology Study of antenatal DEX treatment in CYP21) and all members will work to a common protocol.

**2. TABLE 1. Actual and Theoretical Advantages and Disadvantages of Ante-natal Treatment with Dexamethasone in CYP21 Deficiency**

Advantages	Disadvantages
<p>1. Avoidance of genital ambiguity and need for further surgery during childhood and adolescence (A).</p> <p>2. Avoid “masculinisation” of the female brain by antenatal androgen exposure (T).</p> <p>3. Post-natal suppression of adrenal androgens may be easier (A).</p>	<p>1. Effect observed in 70% of pregnancies. Reasons for treatment failures unclear. Need to treat 7 “unaffected” individuals in order to prevent event in one affected female (A).</p> <p>2. Unknown effects on hippocampal function in particular (T).</p> <p>3. Potential effect on fetal growth and “programming” of higher blood pressure and Type 2 Diabetes Mellitus in later life (T).</p> <p>4. Maternal weight gain, striae (10%) and raised blood pressure in pregnancy (A).</p>

A = Actual

T = Theoretical or based on Animal Experiments

### **3. STUDY OBJECTIVES**

The study aims to document in a prospective fashion and intention to treat basis all mothers presenting for antenatal diagnosis and treatment of CYP21.

The aims of the study are to:

1. Document in a systematic manner the effects of DEX therapy in the mother whether given for a short or long period of time with reference to the well-being of the mother during treatment and glucose tolerance and blood pressure during pregnancy.
2. Determine the perinatal outcome of the intervention in terms of the genital appearance compared to previous pregnancies and fetal growth and duration of gestation.
3. The longterm effect of short or long term DEX therapy on blood pressure, glucose tolerance, growth and cognitive function compared to none DEX treated individuals with CYP21.

### **4. STUDY DESIGN**

Open, controlled, nonrandomized, multicentre study. No random allocation to treatment or non-treatment is made, as equipoise does not exist in the Paediatric Endocrine and Genetics communities. Women who had previously given birth to a child with a severe form of CYP21 deficiency are informed about the possibility of receiving prenatal treatment during their next pregnancy. The woman decides whether she will undergo therapy with DEX or not within the frames of the study.

### **5. DURATION OF THE STUDY**

Recruitment of patients will continue for 5 years, starting during 2002. The children are followed until the age of 5 years.

### **6. CRITERIA FOR ENTRANCE INTO THE STUDY**

All women who have previously given birth to a child with CYP21 will be eligible to participate in the study. The minimum numbers required will consist of 80 treated and untreated females in order to fulfill the sample size criteria detailed below.

Women receiving therapy with DEX who have produced a live female affected offspring along with those pregnancies where the therapy is discontinued due to unaffected fetus or male affected fetus.

In order to achieve these numbers 640 will need to be studied and the population from this will act as a short term dexamethasone treatment group. It will consist of affected males, normal males and females and heterozygote males and females.

**a) Inclusion criteria**

- i) Pregnancies at risk of having a fetus with virilizing 21-hydroxylase deficiency in which careful documentation of the condition in terms of the proband and the carrier status of the parents has been undertaken.
- ii) Both parents must have given their written, informed consent regarding the study protocol.

**b) Exclusion criteria**

- i) Mothers will be excluded if documentation of number 1 of the inclusion criteria is absent.
- ii) Maternal administration of glucocorticoids for chronic medical conditions.
- iii) Women with IDDM or NIDDM.
- iv) Women that have had severe gestational diabetes mellitus during their previous pregnancy.
- v) Women with hypertension.
- vi) Women with osteoporosis (multiple previous fractures).
- vii) Women with a history of psychosis or severe depression during glucocorticoid therapy.
- viii) Non-compliance to therapy or long-term follow-up.

**7. SAMPLE SIZE CONSIDERATIONS**

The prevalence of CYP21 in the United Kingdom is estimated to be 1 in 12,000. With an annual birth rate of 600,000 there are approximately 50 new cases per annum half of which will be virilised females. In a subsequent pregnancy there is a 1 in 8 chance that the fetus will be an affected female and likewise an affected male. Uptake for antenatal treatment amounts to some 80-90% so that we can expect a 40-45 DEX exposed individuals per annum assuming that each family contributes a further pregnancy. In this population of 40-45 per annum there will be 5 affected females treated throughout the pregnancy, 5 affected males treated for the first trimester and about 30 carriers/unaffected males and females. Over a 5 year period of recruitment this will generate 25 DEX treated affected females, 25 DEX treated affected males and 150 carriers/unaffected males and

females. Sample size has been determined using the equation (Armitage and Berry, 1994):-

$$U_{2\beta} = [(d_0 / s) \sqrt{(N/2)}] - U_{2\alpha}$$

where

N	=	sample size of each group
$d_0$	=	difference to be measured between groups
s	=	standard deviation of measure in the population
$U_{2\alpha}$	=	the standardized normal deviate defining the two-tail type I error probability $2\alpha$ (eg. for $2\alpha = 0.05$ , $U_{2\alpha} = 1.96$ ).
$U_{2\beta}$	=	the standardized normal deviate defining the type II error probability $2\beta$ (eg. For power $1 - \beta = 0.8$ , $U_{2\beta} = 0.84$ ).

#### **a) Size at Birth**

Mean birth weight in the UK is 3.48 (SD 0.47) kg. A difference of 0.5 of a SD or 230g would be considered the minimum that would cause concern. In fact in order to detect a shift to intrauterine growth restriction then differences in SD far greater than this would be easily detected. A difference of 0.5 of a SD could be detected at the 5% level of significance with 90 % statistical power with 80 treated and 80 untreated female patients. There is no evidence to suggest that CYP21 itself is associated with any alteration in birth size.

#### **b) Blood Pressure at 5 years of age**

In the rodent model using a similar DEX dosing schedule a rise in systolic blood pressure of approximately 15% was observed (Benediktson et al, 1993). We present two scenarios in terms of sample size, given that mean systolic blood pressure at 5 years is 94.3 (SD 10.9) mm Hg. With a total study population of 400 a 5 mm Hg difference in blood pressure between treated and untreated groups could be detected at the 1% level of significance and 95% statistical power. For a 10mm Hg difference in blood pressure (similar to the rodent data) a total study population of 60 patients will be required. Given the joint collaboration proposed a 10 mm Hg difference should be easily detected. As both DEX and CYP21 postnatal treatment may be associated with raised BP it is important that in this comparison a control group of non-CYP21 subjects is used as a reference. Fortunately, good BP standards are available so an actual normal control group is not essential. The proposal would be that DEX heightens the tendency to raised BP in patients with CYP21.

These numbers are in excess of those likely to be recruited. For this reason it is proposed that study collaboration is undertaken with colleagues in Sweden, France, Finland, Norway, Poland, Spain and Germany as part of PREDEX, a joint Paediatric Endocrinology Study of DEX treatment in CYP21.

## **8. ANTENATAL ASSESSMENT OF THE FAMILY**

### **i) On presentation of the index case (with documented CYP21 deficiency)**

- Prader staging with genital photography should be obtained for later comparison with subsequent affected siblings.
- Genetic counseling should automatically be offered to the family regardless of whether further pregnancies are planned.
- Genetic studies to confirm the defect should be performed in the index case and in both parents.
- DNA should be sent for analysis to one of two central laboratories (London or Manchester {L, M}) with an agreed screening protocol.
- If the family is informative for a mutation in the CYP21 gene then antenatal diagnosis and treatment should be offered. If accepted by the family then the following protocol should be followed within a recognized center for Paediatric Endocrinology and with close liaison with Genetics & Obstetrics/Fetal medicine.
- Entrance into the long-term follow-up study after consent to the study.
- Obstetrician to advise re: any absolute contra-indications in the mother to receiving dexamethasone at all ( i.e. gestational diabetes in first pregnancy)

### **ii) Onset & duration of Dexamethasone treatment**

- Start dexamethasone *as soon as pregnancy is confirmed*
- Review with the results of antenatal genetic studies (*see iii*)
- Continue treatment to term in pregnancies where fetus is an affected female.
- Discontinue dexamethasone in pregnancies where fetus is unaffected *or* affected male
- It is *not* recommended to treat a pregnancy "blind", i.e. without diagnostic CVS.

### **iii) Genetic studies**

- CVS at 11 weeks.  
Sample to be split:
  - Local lab to perform Karyotype (48hrs)
  - Central lab {L, M} to perform mutational analysis (2weeks)
- Karyotype 46XY: treatment can be stopped
- Karyotype 46XX: treatment continued until mutational analysis available
  - Negative for CYP21 mutation: stop treatment
  - Positive for CYP21 mutation: continue treatment to term.

### **iv) Dexamethasone dosage regimen**

- 20 mcg/kg/day tds regimen (on waking, on retiring, third dose in between)
- Dose may be increased if there is evidence of inadequate suppression not thought to be due to non-compliance
- Dose will be decreased/stopped at any stage at the discretion of the Obstetrician if unacceptable side effects occur in the mother.

## **9. MATERNAL MONITORING**

- Obstetrician coordinated and occurs throughout every pregnancy  
**All mothers will be monitored regardless of whether or not they received any dexamethasone & even if the DEX has been discontinued**
- At booking, urine screen for CMV
- Baseline urine at CVS for steroid biochemistry (13wks) {see below}
- Review monthly until 28 weeks then 2 weekly to term
- Monitor on each visit for
  - Weight gain (excessive)
  - Striae
  - Blood Pressure
  - Oedema /proteinuria/pre-eclampsia
  - Glucose intolerance. Oral GTT at 28 weeks. HBA1c & urinalysis at other time points
  - Maternal plasma unconjugated oestriol (fetal suppression) (Samples to Dr J Honour, Cobbold Laboratories, Middlesex Hospital, Mortimer Street, London W1N 8AA) DHEAS and Cortisol (maternal suppression). The latter to be maintained between 100-200 nmols/L).
  - Psychological screening (Questionnaire) covering in particular depression, emotional lability and insomnia.

## **10. Fetal monitoring**

Occurs in every pregnancy even where the DEX has been discontinued and regardless of whether or not the fetus is affected.

- **Fetal medicine ultrasound scan for fetal growth monitoring at :**
  - Booking
  - 20 weeks
  - 28 weeks
  - 34 weeks
- **Fetal adrenal suppression (maternal unconjugated oestriol.**
  - As above

▪ **Post delivery**      **All Infants**

Birth weight, birth length and head circumference.

Gestation

Placental weight (and stereology in selected instances)

Cord blood for confirmation of CYP21 genetics, osteocalcin, IGF-1, IGF-2, IGFBP-1 and 3, Insulin

Monitor blood glucose (pre feed) & electrolytes (daily)

▪ **Day 3**                      **All Infants**

17 OH Progesterone, urine steroid profile & Plasma Renin Activity

Short synacthen (Dose: 1mcg/kg) and measure cortisol response.

▪ **Day 3**                      **Affected Infants**

**NB:** Start treatment **only** when diagnosis is confirmed

Prader stage (comparison with index case)

Genital photography

Need for and extent of genital surgery documented

**11. Assessment of offspring at 5 years of age**

▪ **Growth**

Measures will include height, weight, head circumference at 3 monthly intervals for the first two years and 6 monthly thereafter. Bone age assessments will take place on a yearly basis after 3 years of age.

▪ **Neurodevelopmental Outcome**

Psychological and behavioral outcomes will be assessed in DEX-treated participants and controls. The aim will be to determine if DEX-treatment alters: 1. general psychological or behavioral development; and 2. Psychological sexual differentiation. Measures of general development will include the Child Behavior Checklist and the Wechsler scales of general intelligence. Measures related to sexual differentiation will focus on psychological and behavioral characteristics that have been found to differ in children with CAH. Childhood gender role behavior will be assessed by three methods: 1. Observation of each child's toy choices in a playroom; 2. An interview with each child regarding their favorite toys, playmates and activities; and 3. A standardised questionnaire (the Pre-School Activities Inventory (PSAI)) that is completed by a parent. Parents will also complete questionnaires assessing other sex-typed characteristics, including aggression, shyness, anxiety and social cognition, in their child. Finally, tests of cognitive and motor abilities that show sex differences (visuospatial abilities, verbal fluency and targeting) will be

administered to each child. The behavioral protocol will require approximately 2½ hours for each child and 1 hour for each parent.

- Glucose tolerance

Oral GTT with glucose and insulin measures.

- Blood pressure 24hr ambulatory Blood Pressure

- Blood lipids Cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol.

## **12. CYP21 Working Group of the United Kingdom**

Dr Caroline Brain (Paediatric Endocrinologist, Great Ormond Street Hospital for Sick Children, London)

Dr E Crowne (Paediatric Endocrinologist, Bristol Children's Hospital)

Prof CRW Edwards (Endocrinologist and Principal Imperial College, London)

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Dr P Midgeley (Neonatologist, Royal Hospital for Sick Children, Edinburgh)

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Dr G Rumsby (Molecular Diagnostics, University College London Hospitals, London)

## **13. Data Handling**

All data will be recorded on a standard Proforma for each clinic visit. Completed forms will be processed centrally through the BSPED Clinical Trials Office in Cambridge.

## **14. Time Period**

It is envisaged that recruitment will take place over a 5-year period in collaboration with our European colleagues

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