Prenatal dexamethasone treatment for classic 21-hydroxylase deficiency in Europe

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European Journal of Endocrinology

https://eje.bioscientifica.com
https://doi.org/10.1530/EJE-21-0554

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Printed in Great Britain

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Abstract

Objective: To assess the current medical practice in Europe regarding prenatal dexamethasone (Pdex) treatment of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency.
Design and methods: A questionnaire was designed and distributed, including 17 questions collecting quantitative and qualitative data. Thirty-six medical centres from 14 European countries responded and 30 out of 36 centres were reference centres of the European Reference Network on Rare Endocrine Conditions, EndoERN.
Results: Pdex treatment is currently provided by 36% of the surveyed centres. The treatment is initiated by different specialties, that is paediatricians, endocrinologists, gynaecologists or geneticists. Regarding the starting point of Pdex, 23% stated to initiate therapy at 4–5 weeks postconception (wpc), 31% at 6 wpc and 46 % as early as pregnancy is confirmed and before 7 wpc at the latest. A dose of 20 µg/kg/day is used. Dose distribution among the centres varies from once to thrice daily. Prenatal diagnostics for treated cases are conducted in 72% of the responding centres. Cases treated per country and year vary between 0.5 and 8.25. Registries for long-term follow-up are only available at 46% of the centres that are using Pdex treatment. National registries are only available in Sweden and France.
Conclusions: This study reveals a high international variability and discrepancy in the use of Pdex treatment across Europe. It highlights the importance of a European cooperation initiative for a joint international prospective trial to establish evidence-based guidelines on prenatal diagnostics, treatment and follow-up of pregnancies at risk for CAH.

European Journal of Endocrinology
(2022) **186**, K17–K24

Introduction

Androgen excess in girls with congenital adrenal hyperplasia (CAH) results in virilization of the external genitalia of varying degree (1, 2, 3, 4, 5, 6). Surgeries, such as correction of the urogenital sinus (7, 8, 9), may lead to psychological and psychosexual issues in adult life, such as impaired genital sensitivity, sexual dysfunction and urinary incontinence (10, 11, 12, 13, 14). Dexamethasone (15) at a dose of 20 µg/kg/day initiated before 6–7 weeks postconception (wpc), that is the critical window of sexual differentiation, traverses the placenta, is able to suppress fetal androgen production and hence has been shown to effectively prevent or reduce prenatal virilization (6, 16, 17, 18, 19). It has been in use since 1984 but its use is highly debated for several reasons (20).

First, it holds an ethical dilemma since unaffected fetuses currently are treated unnecessarily during the first trimester of fetal life. Genetic diagnosis can in most countries only be established at 1012 weeks wpc by chorionic villous sampling (21, 22, 23). This translates to a risk of only one in eight fetuses benefitting from prenatal dex treatment (Pdex) or at least one in four if non-invasive sex determination is performed (18).

Secondly, and most importantly, there is not enough evidence for the safety of treated fetuses. Aberrant fetal programming with effects on the cardiovascular system, metabolism and cognitive performance have been described in both animal studies and in humans (24, 25, 26). The clinical outcome studies of Pdex show conflicting results. Some studies show no negative effect on neuropsychological functions and behaviour in non-CAH patients who have been exposed to Pdex treatment in the first trimester of fetal life (27, 28, 29, 30). A survey based on parental questionnaires of prenatally Pdex-exposed children with and without CAH compared to unexposed children did not indicate any adverse effects regarding motor and cognitive development (31). Other studies show that early Pdex treatment in individuals without CAH affects cognition and behaviour during childhood (32, 33, 34, 35, 36, 37) as well as the methylation pattern of the genome (38) and insulin secretion (26) and that the effects are stronger in girls (26, 33). Altered brain structures (39) and insulin secretion (25, 26) have also been identified during adulthood. In a small cohort of women with CAH treated with dex during the entire gestational period a negative effect on cognition was observed compared to an untreated female CAH cohort (40). During childhood, lower intellectual ability was observed in girls with CAH when treated with Pdex (36, 41). Another study showed improved cognitive development in CAH-affected girls treated with

Pdex, however, unfavorable cognitive functions in female CAH-unaffected patients were identified in the same report (37).

Thirdly, only few data on the mothers' safety are available so far. The available literature has highlighted the adverse effects of glucocorticoid excess (16, 21, 42, 43). Strong opinions on this controversial experimental treatment (44, 45) have been raised, yet only a few countries have evaluated the effect and plausible side effects of Pdex in a systematic way in their own population, but still, centres in Europe do use Pdex. According to the Endocrine Society its use is to be restricted to institutional review board-approved research settings (5, 46). In Sweden, the practice of Pdex has been put on hold due to the negative findings in non-CAH first trimester treated cases (47).

The aim of this study is to offer a current reflection of the international views on and practice of Pdex in CAH across Europe and to highlight the importance of longitudinal follow-up of treated cases and prospective clinical trials that investigate different aspects of this therapy.

Methods

This study/survey did not involve patients, thus no patient consent was necessary. The study was approved by the ethics committee of the Medical Faculty of the Ludwig-Maximilians-Universität München, Munich, Germany (project no 21-0760).

Questionnaire design

A questionnaire was designed and distributed using Microsoft Forms (Microsoft Office 365, Windows 10, Microsoft). Seventeen questions were designed including a mixture of open questions and dichotomous or multiple-choice questions with either a single or multiple answer possibilities.

Study group

A total of 45 centres were contacted for completion of the questionnaire. These comprised all European Reference Network on Rare Endocrine Conditions (EndoERN) healthcare centres part of the main thematic groups 'adrenal' and/or 'sex development and maturation'. Additionally, we included a few (n= 6) further tertiary care centres with specific expertise in the field but not yet certified as an EndoERN reference centre. The questionnaire was completed by 80% (36/45) of these endocrine tertiary care centres across Europe, of which 83% (30/36) are reference centres of EndoERN and all are certified centres for adrenal conditions.

Data extraction and analysis

Data extraction was performed using Microsoft Excel. Prism version 8 (GraphPad Software) and Adobe Illustrator 24.3 2020 (Adobe) were used for statistical analysis and graphical presentation of the results.

Results

Characteristics of study cohort

The questionnaire entitled 'Prenatal dexamethasone treatment in CAH across Europe' was completed by a total of 36 medical centres across 14 different European countries (Table 1). Currently, 36% (13/36) of the listed medical centres provide Pdex (Fig. 1). As depicted in Table 1, Pdex is applied in 0.5–8.25 cases per country and year. This data mostly depended on individual estimation and is only in the minority of cases generated by hospital or disease registries. The median number of pregnant women who received Pdex during the first trimester of pregnancy per centre was ten (n= 197) and a lower median amount of five women per centre received Pdex for the entire gestational period (n= 72).

Current use of Pdex across Europe

The majority of countries listed Pdex being provided not by a single, but different specialties (paediatric endocrinologists, endocrinologists and gynaecologists/obstetricians and in rare cases also by geneticists; Fig. 1B). Of all centres using Pdex, there was 100% congruency on the recommended dose of 20 µg/kg/day; however, the daily dose distribution varied. Most medical centres (9/13, 69%) use thrice daily (TID) application of dex. Twice daily (BID) application is used by 23% (3/13) of centres and once daily (QD) dex application is used by one of the surveyed medical centres (Fig. 1C). Regarding the starting point of Pdex responses showed at least some congruency with 23% of centres initiating therapy at 4 to 5 wpc, 31% at 6 wpc, and 46% as early as pregnancy is confirmed and before 7 wpc at the latest (Fig. 1E). Prenatal diagnostics for CAH in treated cases are conducted at 72% (26/36) of recruited centres. For the question regarding the types of prenatal diagnostics used at each centre, multiple answers were possible. The

Table 1 Countries and centres included in the questionnaire 'Prenatal dexamethasone treatment in CAH across Europe' and numbers of treated pregnancies per year and per total time since initiation of treatment.

Country (*n* = 14)	Centers/Country (*n* = 36)	Use of pdex (N/N centres)	Number of PDEX cases/year		Total number of pregnancies	
			Estimated	Reported	First trimester	Entire pregnancy
Austria	1	0				
Belgium	1	0				
Cyprus	1	0				
Denmark	2	0				
France	5	3	4	2	64	21
Germany	10	5	8.25		38	27
Italy	7	2	3.5		60	10
Netherlands	1	1	1		10	5
Norway	1	0				
Slovakia	1	0				
Slovenia	1	0				
Spain	1	1		0.5	18	3
Sweden	1	0				
UK	3	1	2		7	6

majority (65 % of centres, 41 % of answers) uses chorionic villus sampling (CVS) including *CYP21A2* genotyping (*CYP21A2* GT) and sex typing between the gestational week (GW) 1012, whereas 38% of centres (24% of answers) uses amniocentesis (AC) including *CYP21A2* GT and sex typing in GW 1516. Genotyping of the sex-determining region Y (*SRY*typing) from maternal blood (GW 5–7) combined with CVS+*CYP21A2* GT at GW 10–12 is used by 46% of centres (29% of answers). Early non-invasive prenatal diagnostics (NIPD) using the combination of *SRY*typing and *CYP21A2* GT by massively parallel sequencing of cell-free fetal DNA in maternal blood (cfDNA) is offered by only one of the surveyed centres (Birmingham, UK). Of the 13 centres providing Pdex treatment, 11/13 centres offer *SRY*typing+CVS and *CYP21A2* GT (Fig. 1D).

Discussion

This study offers a current cross-sectional status quo of Pdex in CAH in different tertiary care centres across Europe.

The data obtained demonstrate that only approximately a third of centres included in the study are actually providing Pdex (Fig. 1A). Follow-up on treated Pdex cases for both 21OHD and treated unaffected children with a rate of only 42% (15/36) and availability of registries for prenatally treated cases at only 46% (6/13) of the centres applying experimental Pdex is unacceptable. Moreover, only a small number of cases are treated at each centre per year with a minimum of approximately one case per every 4 years to three cases per year (Table 1). National registries were reported to be available in Sweden, Italy, France and Germany, however, only in Sweden and France they are population based. The total number of treated cases in other European countries can only be estimated. The Swedish PREDEX database registered 276 treated cases and untreated controls in Sweden and Italy within 10 years. In Germany, in a period of 10 years, 148 Pdex treated cases have been documented based on voluntary reporting of the treating physicians; however, the data has not been published due to incomplete documentation. In France, over a period of 9 years (2002–2011), a total of 258 fetuses at risk of CAH were subjected to early non-invasive sex determination (18). After the exclusion of male fetuses, 154 of them were subjected to Pdex. Currently, in France, a multicentric study is investigating somatic, neurocognitive and metabolic outcomes of Pdex-treated subjects. Based on these data at least 10 prenatal treatments per year can be expected in Germany and France. This highlights the strong need and additional benefit of a European collaborative initiative, which was expressed by 75% (27/36) of research centres.

Our data show that a substantial number of cases are treated outside the adrenal endocrine tertiary care centres that were approached in this questionnaire and that treatment is initiated by various disciplines. In such an ultra-rare and complex condition, this is alarming. We propose to channel this experimental treatment by international study protocols to endocrine expert centres with adequate long-term follow-up in order to disclose potential side effects.

The critical time point of differentiation of external genitalia is between 8 and 12 wpc or even from 6 wpc onwards (6, 7, 48). To safeguard the development of female genitalia and prevent virilization, dex administration is required as early as from 6 wpc onwards up to at least 16 wpc (49). Analysis of current practice in Europe indicates

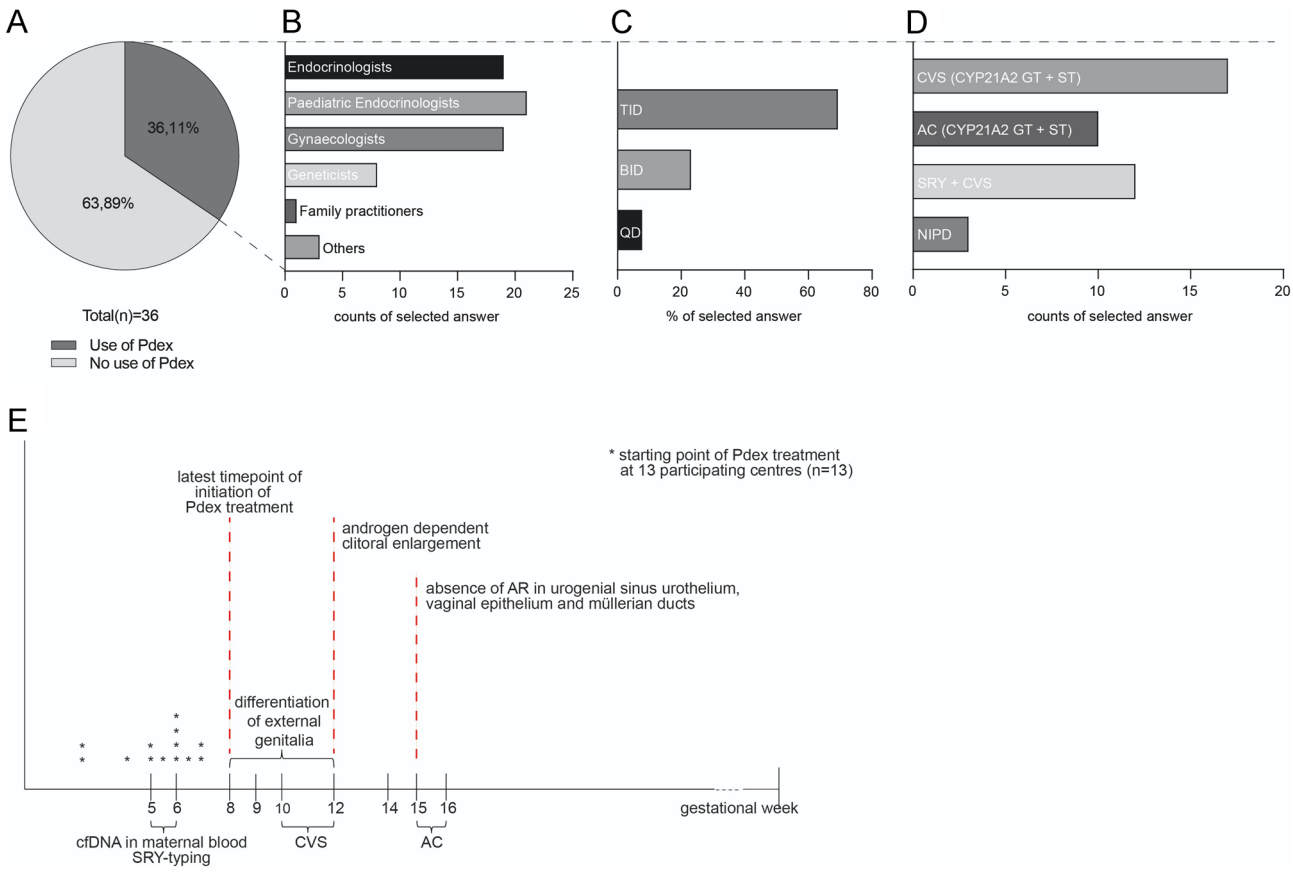

Figure 1

Use of Pdex treatment and prenatal diagnostics to prevent virilization in girls with CAH. (A) Pie chart depicting the percentage of included centres using or not using Pdex treatment (*n* = 36). (B) Selected disciplines providing Pdex treatment in the corresponding country. Multiple selections were possible (*n* = 31; 5 NA). (C) Daily dosing distribution of Pdex. Only centres using Pdex treatment were included (*n* = 13). (D) Types of prenatal diagnostics for CAH used in each corresponding country (*n* = 25). CVS (*CYP21A2* GT + ST): *CYP21A2* genotyping and sextyping between 1012 wpc; treatment is discontinued for male foetuses or notaffected females. AC (*CYP21A2* GT + ST): *CYP21A2* genotyping and sextyping between 1516 wpc; treatment is discontinued for male foetuses or notaffected females. *SRY* + CVS: *SRY*typing from maternal blood (cfDNA); treatment of females only; CVS for *CYP21A2* GT between 1012 wpc; treatment only continued for affected females. NIPD: massively parallel sequencing using cfDNA from maternal blood; only affected females are treated. (E) Overview of important timepoints of development of external genitalia in females. Stars are marking the starting point of Pdex treatment as indicated by each centre (*n* = 13).

that centres conducting Pdex usually start treatment as early as pregnancy is confirmed or before 6/7 wpc at the latest (Fig. 1E). Of the estimated 269 cases treated at all included centres since starting point of Pdex at each individual centre, most cases were only treated for the time period of the first trimester of pregnancy (*n* = 197; 73 %), whereas 72 cases (27 %) received full-term treatment (Table 1). Currently, a dose of 20 µg/kg/day is used by all centres surveyed, however, distribution regarding multiple doses during the day varies (Fig. 1C). In 2006, the idea was promoted of reducing dex dosage after 16 wpc in order to decrease adverse maternal effects of glucocorticoid therapy without compromising treatment efficacy (49). A recent publication even stated the traditional Pdex dose being three-fold higher than actually needed and suggested TID administration to allow for more stable plasma concentrations (50).

Another important aspect refers to prenatal diagnostics in fetuses potentially affected with classic CAH. Despite the improvement of CVS and AC, which is used in most cases, there is still a small risk of miscarriage in the first 23 weeks of pregnancy due to diagnostic-related complications (51). A combinational approach of early NIPD *SRY*typing from maternal plasma (GW5-6) and

CVS in GW10-12 is offered by 11/13 centres providing Pdex and therefore preventing boys from unnecessary prenatal treatment. In France, boys have not been treated anymore for several years. Thus SRY-typing is recommended to reduce the number of treated fetuses but does not solve the entire problem. Targeted massively parallel sequencing of cell-free DNA from plasma drawn from an expectant mother (52, 53) and *SRY*typing (18) to determine sex and *CYP21A2* GT as early as 6 wpc; however, is only provided by one of the included centres. This strategy could efficiently prevent needless treatment of unaffected children and discard the risk of miscarriage.

Conclusion

Current medical standards regarding Pdex are lacking evidence-based guidelines on the optimal starting point, optimal duration and optimal dosing as well as standardized surveillance and follow-up at specialized centres. An international collaborative initiative on a prospective randomized trial is needed to allow for sufficient sample sizes in order to answer the key questions of this therapy to allow its future use or to ban it.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this article.

Funding
This work was supported by the Deutsche Forschungsgemeinschaft (Heisenberg Professorship, 325768017 to N R and 314061271-TRR205 to N R and A H), the European Commission for funding EndoERN CHAFEA FPA grant no. 739527, the Eva Luise und Horst Köhler Stiftung & Else Kröner-Fresenius-Stiftung (2019_KollegSE.03 to H N) and the Stockholm County Council (Senior clinical research fellowship dnr RS 2019-1140 to S L), Stiftelsen Frimurare Barnhuset i Stockholm and Lisa and Johan Grönbergs Stiftelse.

Author contribution statement
S Lajic and N Reisch contributed equally to this work.

Acknowledgements
This work was supported by the European Reference Network on Rare Endocrine Conditions. We acknowledge the entire French working group on PDex for fruitful discussions and comments on this study and manuscript: Dr Véronique Tardy-Guidollet, Dr Daniela Gorduza, Dr Rita Menassa, Dr Claire-Lise Gay, Dr Patricia Bretones, Dr Aude Brac de la Perrière, Dr Claire Bouvattier, Dr Lise Duranteau, Dr Catherine Pienkowski, Pr Anne Bachelot and Dr Audrey Cartault.

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Received 29 September 2021
Revised version received 1 February 2022
Accepted 2 March 2022