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PHARMACOVIGILANCE

Birth defects in children of men exposed in utero to diethylstilbestrol (DES)

Michel Tournaire[a,b,*], Emmanuel Devouche[b,c], Sylvie Epelboin[d,e], Anne Cabau[a], Annabel Dunbavand[f], Anne Levadou[g]

[a] *Assistance publique—Hôpitaux de Paris, 75014 Paris, France*
[b] *Université Paris Descartes, 75006 Paris, France*
[c] *Laboratoire psychopathologie et processus de santé EA4057, 92100 Boulogne-Billancourt, France*
[d] *Assistance publique—Hôpitaux de Paris, hôpital Bichat-Claude Bernard, 75018 Paris, France*
[e] *Université Paris 7 Diderot, 75013 Paris, France*
[f] *Mutualité française, 75015 Paris, France*
[g] *Association réseau DES France, 40000 Mont-de-Marsan, France*

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Pregnancy

Summary
Objective. — Prenatal exposure to diethylstilbestrol (DES) is associated with adverse effects, including genital anomalies and cancers in men and women. Animal studies showed birth defects and tumors in the offspring of mice prenatally exposed to DES. In humans, birth defects, such as hypospadias were observed in children of prenatally exposed women. The aim of this research was to assess the birth defects in children of prenatally exposed men.
Methods. — In a retrospective study conceived by a patients' association (*Réseau DES France*), the reports of men prenatally exposed to DES on adverse health effects in their children were compared with those of unexposed controls and general population.
Results. — An increased incidence of two genital anomalies, cryptorchidism (OR = 5.72; 95% CI 1.51—21.71), and hypoplasia of the penis (OR = 22.92; 95% CI 3.81—137.90), was observed in the 209 sons of prenatally exposed men compared with controls, but hypospadias incidence was not increased in comparison with either the controls or the general population. No increase of genital anomalies was observed in daughters.

* Corresponding author Association Réseau DES France, 1052, rue de la Ferme-du-Carboué, 40000 Mont-de-Marsan, France.
E-mail address: mftournaire@gmail.com (M. Tournaire).

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Conclusion. — With caution due to the methods and to the small numbers of defects observed, this work suggests an increased incidence of two male genital tract defects in sons of men prenatally exposed to DES. This transgenerational effect, already observed in animals and in the offspring of women prenatally exposed to DES, could be the result of epigenetic changes transmitted to the subsequent generation through men.

Abbreviations

ANSM	French national agency for the safety of medicines and health products (*Agence nationale de sécuritédu médicament et des produits de santé*)
CCA	clear-cell adenocarcinoma of vagina or cervix
CI	confident interval
ICD-10	international classification of diseases no. 10
DES	diethylstilbestrol
EUROCAT	European concerted action on congenital anomalies and twins
INSERM	French national institute of health and medical research (*Institut national de la santéet de la recherche médicale*)
NA	non applicable
NCI	National cancer institute
OR	odds ratio
SIR	standardized incidence rate

Introduction

Diethylstilbestrol (DES) is a synthetic estrogen prescribed to prevent miscarriages and other complications of pregnancy. In women prenatally exposed to DES, adverse health effects have been observed: reproductive tract anomalies, infertility, pregnancy complications, clear-cell adenocarcinoma of the vagina or cervix (CCA) and a possible increased incidence of breast cancer [1—9]. In prenatally exposed men, adverse effects included genital anomalies: cryptorchidism, hypospadias, epididymal cyst, testicle hypoplasia and genital tract inflammation [10]. Possible increased risks of infertility and testicular cancer have also been reported [11,12]. Several million persons were prenatally exposed worldwide, including approximately 80,000 women and 80,000 men in France [8,13,14].

Offspring of females prenatally exposed to DES

In mice, the female descendants of the females prenatally exposed to DES showed an increased incidence of reproductive tract defects [15—18].

In humans, an increase of overall birth defects was observed in children of prenatally exposed women [19,20]. An increased incidence of hypospadias has been observed since 2002 [20—26]. A Dutch study revealed a significant increase of esophageal atresia and tracheoesophageal fistula, observed again in a French study [20,27]. However, in an American report, the increase of this pathology was not significant [19]. Furthermore, two studies raised questions about an increase of circulatory system defects [19,20].

Offspring of males prenatally exposed to DES

In prenatally exposed male mice, an increased incidence of hypospadias was observed ranging from 18% to 100%, according to the mice strains [15]. In their offspring, malformations of external genital tract were observed in both genders: 20% hypospadias in males and 12% urethral-vaginal fistula in females. In another experimental study, a significant increased incidence of tumors was reported in the female descendants of males prenatally exposed, in particular uterus sarcoma, benign ovarian tumors and lymphomas [16].

In humans, two studies showed effects of paternal exposure to DES. In a case-control retrospective study, prenatal exposure of men was not associated with an increased risk of hypospadias in their sons but this risk was increased when mothers were prenatally exposed to DES: (OR = 4.9; 95% CI 2.1—22.3) [24].

Another retrospective study showed the same tendency: no increase of hypospadias when the prenatal exposure was paternal, but increase in the case of maternal exposure [26].

Human and animal studies raise the question of transgenerational transmission of epigenetic alterations, through the female, but also through males prenatally exposed, to their offspring of third generation.

The aim of this report is to evaluate birth defects in children of third generation, descendants of men prenatally exposed to DES.

Methods

Participants

This retrospective study was conceived by a patients association, *Réseau DES France*, funded by the French drug agency, (*Agence nationale de sécuritédu medicament et des produits de santé* [ANSM]), and supported by a national health insurance, ''*Mutualitéfrançaise*''. This investigation

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was retrospective, due to the absence of any sufficient prospective cohort of DES exposed subjects available in France. Questionnaires were designed to assess adverse events associated with DES exposure for three generations: first, women treated during their pregnancies, second, women and men prenatally exposed and third, daughters and sons of prenatally exposed individuals. A questionnaire was also sent to unexposed women, constituting a control population of parents. These unexposed women were defined by their date of birth, 1950 to 1977, corresponding to ages of DES exposure in France [20]. This control population was used in a previous publication on adverse effects in children of women prenatally exposed to DES. The children of these unexposed parents are used again as control group for children of men exposed [20]. The exposed/unexposed populations were therefore classified at first, prior to the questions on birth defects. The questionnaires were distributed nationwide through patients' associations, health insurance, media (generalist and medical), family, and acquaintances. They were answered on a voluntary basis and returned through the Internet or by post from April to September 2013.

We used the questionnaires of prenatally exposed men, of second generation, reporting adverse health effects in their children, of third generation.

Populations compared

Children of prenatally exposed men were compared with children of unexposed controls and with the general population of European concerted action on congenital anomalies and twins (EUROCAT) reporting congenital anomalies in Europe [28]. These children of exposed men were also compared with children of prenatally exposed women reported in a previous publication and with the literature data [19,20,24,26].

DES exposure

The prequestionnaire section dedicated to DES exposure proposed three possible answers: (a) certain with medical documents, (b) certain without documents and (c) probable. To reduce the selection bias, men who gave the ''probable'' answer were excluded and called ''uncertain'' in this text and Fig. 1.

Diagnosis of birth defects

By definition, birth defects include anatomical abnormalities discovered prenatally or in newborns. To improve information on these defects, details obtained from

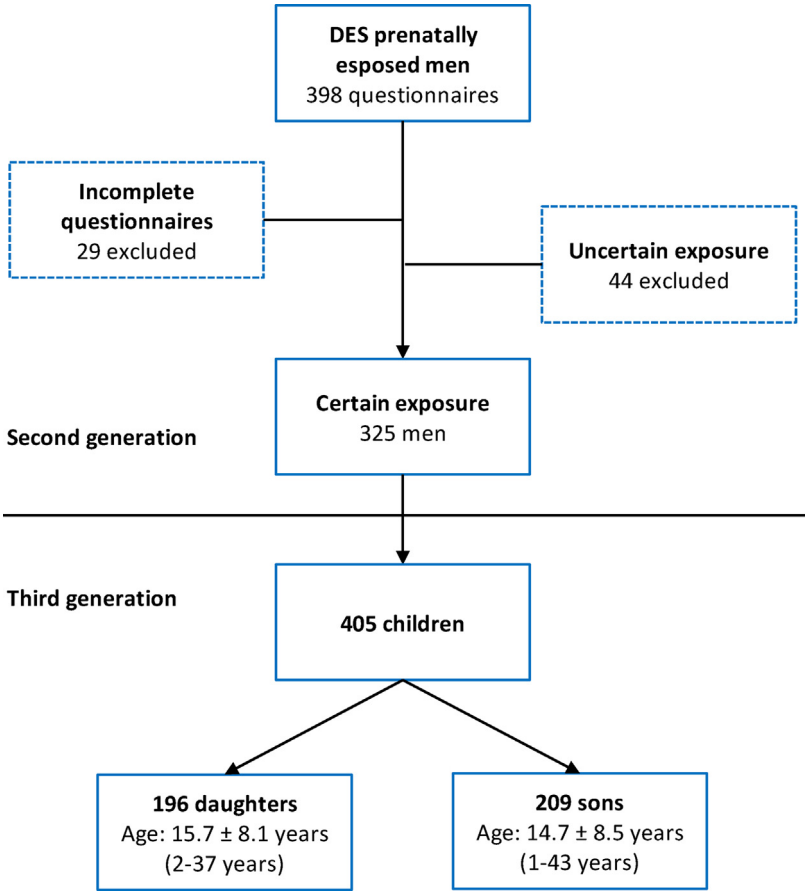

Figure 1. Populations according to reports of men prenatally exposed to diethylstilbestrol (DES).

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semi-opened questions on anatomical changes, treatments and progress allowed these anomalies to be coded according to international classification of diseases no. 10 (ICD-10) [29]. To select the major defects, we then used the EUROCAT classification for each category except for genital tract in which minor anomalies were kept. We have to specify that the diagnosis reported by the families could not be controlled in medical documents.

Statistical methods

Participants' characteristics were detailed in a previous publication, using the same populations [9]. Odds ratio (OR), *P* and standardized incidence rate (SIR) were calculated to compare observed cases with controls and with expected incidences in the general population according to EUROCAT registers. The categorical variables were compared using the Chi^2 test or the Fisher exact test. All analyses were performed using SAS® software, version 9.4 (SAS Institute Inc., Cary. NC, USA).

Results

Population

From the questionnaires of 398 prenatally exposed men (second generation), we excluded 29 of them, incomplete, and 44 for uncertain exposure to DES. We finally used 325 questionnaires of prenatally exposed men to assess birth defects of their 405 third generation children, 196 daughters and 209 sons (Fig. 1).

Birth defects in children of men prenatally exposed to DES

Prenatally exposed men reported 14 birth defects in their 405 children (3.45%, 95% CI 2.07—5.72), including 1 circulatory system defect (absence of portal venous), 1 orofacial cleft and 4 urinary defects (3 ureter, 1 kidney). There were 8 genital defects in their sons (3 cryptorchidism, 2 hypospadias, 3 hypoplasia of the penis). No genital defect was observed in their daughters (Tables 1 [A] and 2 [A]).

Comparison with controls

Table 1 showed that globally, children of men exposed (A) did not present more defects than controls (B). Compared separately, the incidence of each birth defect in children of men exposed was not different from controls for circulatory, orofacial and urinary defects. There was a higher incidence of genital defects in comparison with controls (OR = 4.98; 95% CI 2.23—11.11, $P < 0.0001$).

Table 2 compares four types of genital defects in sons of exposed men (A) and sons of controls (B). A significant increase was found for cryptorchidism (OR = 5.72; 95% CI 1.51—21.71; $P = 0.03$), and hypoplasia of the penis (OR = 22.92; 95% CI 3.81—137.90; $P = 0.002$). There was no increase of incidence for hypospadias (OR = 2.53; 95% CI 0.56—11.36; $P = 0.22$) and for absence or hypoplasia of testicle [30].

Comparison with the general population of EUROCAT

Globally and separately for circulatory, orofacial and urinary defects there was no significant difference. For hypospadias, the only male defect reported in EUROCAT register, the difference between sons of exposed men (0.96%) and the general population (0.16%) was not statistically significant (SIR = 6.15; 95% CI 0.69—22.19).

To summarize, cryptorchidism and hypoplasia of the penis were increased when compared with controls (OR = 5.72; 95% CI 1.51—21.71 and OR = 22.92; 95% CI 3.81—137.90). Hypospadias was not increased when compared with controls or the general population. However, we have cautiously to notice that the small number of defects limited the possibility to conduct reliable analyses.

Discussion

Methods

Recruitment bias

We acknowledge that, with the retrospective method based on voluntary answers to questionnaires, recruitment bias may be present, which is difficult to estimate. It can be observed Table 1 that the incidence of birth defects in controls (B: 2.67%) and the general population (D: 2.51%) are close. This is not in favor of a recruitment bias for the controls.

Reporting bias

Data on pregnancies and their outcome should probably be less accurate when reported by prenatally exposed men than by exposed or unexposed women. In particular, men may report information mostly on their living children when women should also consider pregnancy complications including perinatal death with possible malformations.

The diagnosis of birth defects was first given by obstetricians and pediatricians to the parents in the same way in exposed or unexposed group. It was reported by the parents answering their questionnaires with the possible bias associated.

To evaluate the reporting bias in the controls, we compared the birth defects that are not supposed to be associated with DES, lines 1,2,3,5,9 and 11 of Table 1. The total incidence was 1.36% in controls (B) and 1.11% in EUROCAT (D), which is not pleading for an important bias.

Comparison of birth defects in children of men with other studies

Non-genital defects

In our previous publication on children of women exposed prenatally to DES, incidences of several defects were statistically increased when compared with controls and the general population: digestive defects, in particular esophageal atresia, orofacial cleft and circulatory defects [20]. In the DES follow-up study, an increased incidence of circulatory defects was suggested in the offspring of exposed

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Table 1 Incidence of birth defects in the offspring of: A: men prenatally exposed to diethylstilbestrol (DES); B: unexposed population, C: women prenatally exposed to DES [20] and D: general population European concerted action on congenital anomalies and twins (EUROCAT) [28].

Birth defects (EUROCAT label/ICD-10 label)	A Children of men DES+ $n = 405$ n (%)	B Children of controls $n = 6230$ n (%)	A vs. B		C Children of women DES+ $n = 4409$ n (%)	A vs. C		D Gen. pop. EUROCAT 2010–2014 (%)	A vs. D
			OR 95% CI	P level[d]		OR 95% CI	P level[d]		SIR 95% CI
All anomalies[a]	14 (3.45)	163 (2.62)	1.33 (0.76 2.31)	.32	275 (6.23)	0.54 (0.31 0.93)	.02	2.51	1.37 (0.75 2.31)
1 Nervous system	0 (0.00)	15 (0.24)	–		14 (0.31)	–		0.26	–
2 Eye	0 (0.00)	10 (0.16)	–		12 (0.27)	–		0.04	–
3 Ear, face and neck	0 (0.00)	5 (0.08)	–		6 (0.13)	–		0.02	–
4 Circulatory	1 (0.25)	21 (0.33)	0.73 (0.10 5.43)	1.00	33 (0.75)	0.33 (0.04 2.41)	.36	0.76	0.32 (0.004 1.81)
5 Respiratory	0 (0.00)	2 (0.03)	–		5 (0.11)	–		0.04	–
6 Oro-facial clefts	1 (0.25)	4 (0.06)	3.84 (0.43 34.4)	.27	12 (0.27)	0.91 (0.12 6.99)	1.00	0.14	1.73 (0.02 9.63)
7 Digestive	0 (0.00)	(0.08)	–		32 (0.72)	–		0.18	–

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Table 1 (*Continued*)

Birth defects (EUROCAT label/ICD-10 label)	A Children of men DES+ *n* = 405 *n* (%)	B Children of controls *n* = 6230 *n* (%)	A vs. B		C Children of women DES+ *n* = 4409 *n* (%)	A vs. C		D Gen. pop. EUROCAT 2010—2014 (%)	A vs. D
			OR 95% CI	*P* level[d]		OR 95% CI	*P* level[d]		SIR 95% CI
8 Genital[b]	8 (1.98	25 (0.40)	4.98 (2.23 11.11)	<.0001	75 1 .70)	1.16 (0.56 2.43)	.68	0.22[c]	NA
9 Urinary	4 (0.99)	35 (0.56)	1.76 (0.62 4.97)	.30	40 (0.90)	1.09 (0.39 3.06)	.78	0.33	3.01 (0.81 7.72)
10 Musculo-skeletal	0 (0.00)	21 (0.33)	—		51 (1.15)	—		—	—
11 Chromosomal	0 (0.00)	18 (0.29)	—		9 (0.20)	—		0.42	—

EUROCAT: European concerted action on congenital anomalies and twins; ICD-10: international classification of diseases no. 10.
[a] All anomalies = all cases of congenital anomaly, excluding cases with minor anomalies. Cases with more than one anomaly are only counted once in the ''All anomalies'' subgroup.
[b] Including minor anomalies according to ICD-10 label
[c] Genital defects EUROCAT are limited to hypospadias and undeterminated se.(28)
[d] The categorical variables were compared using the Chi^2 test or the Fisher exact test.

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Table 2 Description of genital malformations in the offspring of: A: men prenatally exposed to diethylstilbestrol (DES); B: unexposed population; and C: women prenatally exposed to DES [20].

Genital malformations (ICD-10 label)	A Children of men DES+ *n* = 405 *n* (%)	B Children of controls *n* = 6230 *n* (%)	C Children of women DES+ *n* = 4409 *n* (%)
All anomalies[a]	8 (1.98)	25 (0.40)	75 (1.70)
Male, *n*	209	3149	2181
All males	8 (3.83)	24 (0.76)	73 (3.35)
Cryptorchidism	3 (1.44)	8 (0.25)	22 (1.01)
Hypospadias	2 (0.96)[b]	12 (0.38)	39 (1.79)
Hypoplasia of penis	3 (1.44)	2 (0.06)	9 (0.41)
Absence or hypoplasia of testicle	0 (0.00)	2 (0.06)	3 (0.14)
Female, *n*	196	3054	2228
All females	0 (0.00)	1 (0.03)	2 (0.09)
Congenital malformations of uterus and cervix	0 (0.00)	1 (0.03)	2 (0.09)

EUROCAT: European concerted action on congenital anomalies and twins; ICD-10: international classification of diseases no. 10; SIR: standardized incidence rate.
[a] Cases with more than one anomaly are only counted once in the ''All anomalies'' subgroup.
[b] Hypospadias = 0.16% in EUROCAT [28]: SIR (95% CI) = 6.15 (0.69—22.19).

women [19]. None of these defects were increased in children of men in this study.

Male genital defects (Table 2)

Sons of DES exposed males

DES being an endocrine disruptor, genital defects occurred to be a preliminary hypothesis of this study. As seen earlier, the overall incidence of 8 male genital defects in the sons of prenatally exposed men was significantly higher than in controls. Individually, cryptorchidism and hypoplasia of the penis were increased when compared with controls but hypospadias was not increased, in comparison with controls or the general population.

In mice, the male descendants of prenatally exposed males presented a high incidence of hypospadias (20%) [15].

Two publications evaluated the incidence of hypospadias in the sons of men prenatally exposed to DES. In a case-control retrospective study comparing 583 cases of hypospadias and 251 referents, there was no association between hypospadias and prenatal exposure of the fathers (OR = 1.1; 95% CI 0.3—4.2) [24]. In a retrospective study, there was no hypospadias in the sons of 448 prenatally exposed men [26]. The results of these three studies were in agreement with an absence of increase of hypospadias.

Sons of DES exposed women

Two genital defects were more frequent in the sons of exposed men than in the sons of exposed women: cryptorchidism and hypoplasia of the penis. Conversely, hypospadias were more frequent in sons of exposed women than in sons of exposed men [20,30]. However, these results should be interpreted cautiously, the numbers of genital anomalies in men's sons being small.

Most of the studies in humans taking into account third generation anomalies reported data on children of exposed women. Of seven studies evaluating the incidence of hypospadias six reported a significant increase with large differences in OR, from 4.5 to 21, three of them close to 5 and one with non-significant difference [21—26]. In our previous study an increased risk of hypospadias was observed when mothers had been exposed prenatally to DES (OR = 4.9; 95% CI 1.1—22.3) [20].

To summarize, genital tract anomalies were observed in the male offspring of prenatally exposed humans and animals while these subjects had not been in contact with DES. In humans, cryptorchidism, hypospadias and hypoplasia of the penis are partly under hormonal control. The hypothesis to explain this adverse effect in the third generation is that DES, considered as model of endocrine disruptors, may cause epigenetic changes transmitted to the subsequent generation through men as well as through women prenatally exposed. However, the defects observed, if confirmed, seem to be different: cryptorchidism and hypoplasia of the penis through men, hypospadias through women. [15,19,20,24,31—33].

Female genital defects

We did not find any increase of female genital defects in the daughters of prenatally exposed men. This normal incidence of birth defects had already been observed in the daughters of exposed women [19,20,34]. These results are encouraging because in mice, 12% of genital defects (urethral-vaginal fistulas) were observed in females' descendants of prenatally exposed males [15].

Conclusion

With caution attached to the possible bias associated to the retrospective method, and to the small numbers of adverse

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health effects in children of prenatally exposed men, our study suggests an increased incidence of two male genital defects, cryptorchidism and hypoplasia of the penis in sons of men prenatally exposed to DES. This result, if confirmed by other studies, suggests a transgenerational transmission of genital defects to the third generation, through prenatally exposed men. This transmission has already been observed through prenatally exposed women in humans and, in animals, through exposed male and female. An encouraging point is the normal incidence, in humans, of female genital defects.

Ethical standards

This study was submitted to the Ethics Review Committee ''*Comitédeprotection despersonnesÎle-de-France III*'' which testified that ''the study appears to be in accordance with the scientific principles generally accepted, and to the ethical standards of research; the study was performed in the respect of the French law and regulation''.

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Disclosure of interest

The authors declare that they have no competing interest.

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