Clomiphene and Hypospadias on a Detailed Level: Signal or Chance?

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BACKGROUND: Clomiphene, a drug used to induce ovulation, is chemically related to diethylstilbestrol (DES). DES is associated with vaginal cancer and infertility among daughters and with hypospadias among second-generation male offspring. Because clomiphene has a long half-life and metabolites have been found in feces up to 6 weeks after administration, fetal exposure is possible if the mother took this drug prior to becoming pregnant. **METHODS:** Case-control analyses were performed to investigate the association between clomiphene exposure and hypospadias. Cases were all male subjects registered in the European Concerted Action on Congenital Anomalies and Twins (EUROCAT) Northern Netherlands registry for congenital anomalies with nonsyndromal hypospadias. Controls were all male children born without hypospadias, including those with chromosomal and monogenic defects. Logistic regression analyses were performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs). **RESULTS:** Of 392 cases, 7 (1.8%) were exposed to clomiphene compared with 64 of 4538 controls (1.4%). For penoscrotal hypospadias, we found that the OR was significantly increased (6.08; 95% CI, 1.40–26.33); for the mild and moderate forms of hypospadias, the ORs were not increased. **CONCLUSIONS:** Because penoscrotal hypospadias is rare, the effect is diluted when all forms of hypospadias are studied as a group. Therefore, our study stresses the importance of studying birth defects on as detailed a level as possible. Other studies should be conducted to confirm our findings. *Birth Defects Research (Part A) 76:249–252, 2006.* © 2006 Wiley-Liss, Inc.

Key words: hypospadias; clomiphene; birth defects

INTRODUCTION

Hypospadias is a congenital anomaly of the male genitals characterized by an abnormal location of the urethral opening, varying from the ventral surface of the penis to the scrotum or even the perineum. The prevalence in Europe is estimated as 2–5 per 1000 male births (Dolk et al., 2004). The cause of hypospadias is likely to be multifactorial. Sexual differentiation is determined by testosterone and its metabolites. Changes in concentrations of sex hormones during the critical period of development (up to week 12) may play a role in the development of hypospadias (Baskin, 2000).

Clomiphene, widely used to induce ovulation, has a long half-life and its metabolites have been found in blood samples after 22 days and in feces up to 6 weeks after administration (Geier et al., 1987). Therefore, exposure to the fetus in the first weeks of pregnancy is possible.

Clomiphene is chemically related to diethylstilbestrol (DES), a drug associated with vaginal adenocarcinoma and infertility among women exposed in utero (DES daughters). Klip et al. (2002) reported an increased risk of hypospadias among sons of DES daughters (DES grandsons).

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Table 1
Characteristics by Exposure Groups and Outcome†

Characteristics	Unexposed (n = 4859) no. (%)	Exposed (n = 71) no. (%)	P*	Cases (n = 392) no. (%)	Controls (n = 4538) no. (%)	P*
Is the child part of a multiple pregnancy?						
Yes, twin	188 (4.0)	6 (8.5)	0.17	15 (3.9)	179 (4.1)	.61
Yes, triplet or more	5 (0.1)	0 (0)		1 (0.3)	4 (0.1)	
Is the mother a DES-daughter?						
Yes	11 (0.2)	0 (0)	0.69	2 (0.5)	9 (0.2)	.21
Did the mother use folic acid?						
Yes, periconceptional	600 (15.7)	18 (25.7)	0.015	65 (19.7)	553 (14.6)	.002
Yes, other period	498 (13.0)	13 (18.6)		71 (21.5)	650 (17.2)	
Exposed	—	—		7 (1.8)	64 (1.4)	.55

†Percentages can vary because of missing data.
*By 2-tailed χ^2 test.

Furthermore, although the numbers were small, Klip et al. (2002) found more severe cases of hypospadias among these DES grandsons than in the unexposed group.

Because clomiphene could be present in the mother's body after conception and because of the chemical similarity of clomiphene to DES, we investigated the association between clomiphene use by the mother and hypospadias among male offspring.

MATERIALS AND METHODS

Case-control analyses were performed using all live births, stillbirths, and terminated pregnancies from 1981 to 2003 from the European Registration of Congenital Anomalies and Twins (EUROCAT) Northern Netherlands. This population-based registry monitors ~20,000 births per year. Information about the anomalies is collected through physicians, midwives, clinical geneticists, and pathologists. Methods for case ascertainment have not changed over time and are in accordance with EUROCAT Central Registry guidelines (www.eurocat.ulster.ac.uk). Until 1996, information about the mothers' conditions was collected through these same health care providers. Pharmacy data have been routinely collected since 1997, and the actual use of the reported drugs is verified with the mother.

Cases were male subjects with hypospadias, either glandular/coronary, penile, penoscrotal, or perineal. The hypospadias could be isolated or present in combination with other defects. Subjects with hypospadias recognized as part of a syndrome (n = 30) were excluded from this study, as well as subjects with epispadias (n = 12). The mildest form, glandular hypospadias, is registered only if other defects are present, regardless of whether these defects are associated with hypospadias. Because the database has no information on nonmalformed births and no comparable database can be used to validly extract nonmalformed births, all male subjects without hypospadias were considered controls, including those with chromosomal or monogenic defects and other syndromes. This method corresponds with the one used in the Malformation Drug Exposure (MADRE) project from the International Clearinghouse for Birth Defects Surveillance and Research (Robert et al., 1994).

During the study years 1981–1996, exposure to clomiphene was defined as use of clomiphene by the mother prior to the index pregnancy as recorded by the health care provider who was explicitly asked whether the pregnancy resulted from clomiphene treatment. During the study years 1997–2003, exposure to clomiphene was defined as evidence of any clomiphene prescription dispensed to the mother 0–3 months prior to the index pregnancy as recorded by the pharmacy. This period was chosen because date of issue of the pharmacy is not necessarily date of use by the mother; she has to adapt her use to her menstrual cycle. Furthermore, clomiphene can be issued for >1 menstrual cycle. Actual use of clomiphene had to be confirmed

Table 2
Detailed Descriptions of 7 Clomiphene-Exposed Cases in The Netherlands*

Case no.	Year of birth	Periconceptional folic acid use[a]	Description of anomalies
1	1989	No	Isolated coronary hypospadias
2	1991	No	Isolated penile hypospadias
3	1994	No	Penoscrotal hypospadias and craniosynostosis
4	1998	Advised period	Glandular hypospadias and penile chordee
5	1998	Advised period	Isolated penoscrotal hypospadias
6	2000	Advised period	Isolated penile hypospadias
7	2002	Advised period	Isolated coronary hypospadias

*None of the cases was a multiple birth. All of the mothers were treated with clomiphene only for fertility problems.
[a]In the Netherlands, women planning a pregnancy are advised to take folic acid supplements from 4 weeks before until 8 weeks after conception.

Table 3
Total Number of Cases of Hypospadias and Number of Isolated and Exposed Cases

Anomaly	*n*	No. (%) of isolated cases	No. exposed	OR	95% CI
Glandular/coronary hypospadias	186	161 (87%)	3	1.15	0.36–3.68
Penile hypospadias	143	131 (92%)	2	0.99	0.24–4.09
Penoscrotal hypospadias	25	19 (76%)	2	6.08	1.40–26.33
Perineal hypospadias	0				
Hypospadias NOS	38	10 (26%)	0		

OR, odds ratio; CI, confidence interval; NOS, not otherwise specified.

by the mother by asking whether the conception was indeed the result of clomiphene use.

Variables that could influence the results, such as multiple birth, exposure to DES, periconceptional use of folic acid, and maternal age, were investigated as well. Folic acid use around the time of conception has been requested in the mother's questionnaire since 1997 and is often missing for the previous years. The other variables, multiple pregnancy, intrauterine DES exposure of the mother, and maternal age, were collected through the health care providers by asking for these variables specifically (until 1996) and through the mother's questionnaire since 1997. Logistic regression was performed to produce odds ratios (ORs) and 95% confidence intervals (CIs).

RESULTS

We identified 392 subjects with hypospadias. Distributions of some characteristics for cases and controls as well as for exposed and unexposed births are shown in Table 1. Of all 4930 male subjects included in the analyses, 71 (1.4%) were exposed to clomiphene. Exposure was 1.8% ($n = 7$) among cases and 1.4% ($n = 64$) among controls. More twins were born in the exposed group. Of the 11 sons of DES daughters, 2 had hypospadias (both penile) and none of these 11 DES grandsons were exposed to clomiphene. Use of folic acid differed significantly between exposed and unexposed births as well as between cases and controls.

A detailed description of the 7 exposed cases and their characteristics is shown in Table 2. These 7 cases were born between 1989 and 2002; all were singletons and all resulted from other clomiphene-only treatment. Of these 7 cases, 5 had isolated hypospadias. The percentages of isolated cases among all cases are presented in Table 3.

For hypospadias in general we found an OR of 1.27 (95% CI, 0.58–2.79). The results for the different types of hypospadias are given in Table 3. No subject with perineal hypospadias was found. Penoscrotal hypospadias had a significantly increased OR of 6, but the milder forms had no increased OR. Of the 38 cases without further information about the severity of the hypospadias, none were exposed to clomiphene. Because the numbers were too small, we could not adjust for possible confounders or effect modifiers.

DISCUSSION

In our study, we determined different ORs for different forms of hypospadias in relation to preconceptional exposure to clomiphene: we found no association for the milder forms, but we did find a significantly increased OR for penoscrotal hypospadias.

We found no increased risk of hypospadias in general, which corresponds with the findings of Sørensen et al. (2005). In that study, no discrimination in severity of hypospadias was made. However, in our study, by investigating all hypospadias as a group, the results for penoscrotal hypospadias were diluted. This stresses the importance of studying any possible association at the most detailed level possible, which is the strength of this study.

Is the association with penoscrotal hypospadias found for only 2 exposed cases based on chance or is there a biologically plausible explanation for our findings? Clomiphene has estrogenic and antiestrogenic characteristics (Parfitt, 1999). Kim et al. (2004) studied the effect of estrogen exposure on hypospadias among mice. Hypospadias, not specified, occurred in ~50% of the male fetuses. Disturbed concentrations of sex hormones during the critical period of penile and urethral development may play a part in the development of hypospadias. Because clomiphene can still circulate in the mother's body after conception due to its long half-life, it might influence the first weeks of fetal development, leading to severe cases of hypospadias. In the latter weeks of development, when the milder forms of hypospadias appear, the time window between exposure and development of the defect might be too large. This could explain the lack of association between clomiphene and the milder forms of hypospadias.

How does the study published by Klip et al. (2002), which reported an increased risk of hypospadias among sons of DES daughters, relate to ours? In their study, 4 of the 250 DES grandsons had hypospadias, of whom 3 had penoscrotal hypospadias and 1 had the urethral opening located on the distal shaft. Of the 8729 non–DES grandsons, 8 had hypospadias. Of these only 1 had penoscrotal hypospadias, 3 had the urethral opening at the penile shaft, 2 were described as having penile hypospadias and 2 as (sub)coronal. Although we acknowledge the differences in exposure between both studies, we feel that the comparable findings might support the likelihood of our findings because of the chemical relationship between DES and clomiphene. In many cases, actual mechanisms of teratogens are often unknown, which forces us to be speculative. Because DES daughters often have fertility problems, this could be a mutual cause in our and the Klip et al. (2002) study. They investigated this possibility in their study and concluded that fertility treatment did not affect the risk they found. In our study, we cannot establish whether the underlying subfertility may account for the observed asso-

ciation because no alternative medicines are available. The increased risk might be associated with clomiphene use (as a marker of subfertility) but not caused by it.

Although the findings of our study might be biologically plausible and support a possible causal relation, the small numbers are a problem. The OR calculated for penoscrotal hypospadias, although statistically significant, has a wide confidence interval, indicating uncertainty of the actual effect. Nevertheless, if our findings are real and clomiphene use increases the risk of penoscrotal hypospadias, it is a rare condition and therefore the absolute risk remains low.

Although most cases of hypospadias are isolated (Table 3), we did not have the power to limit the study to these isolated cases. Also, adjusting for the relevant variables shown in Table 1 was impossible. Although information on periconceptional folic acid use is missing for most births before 1997, we did find that folic acid use differed between cases and controls and between exposed and unexposed births. Not being able to adjust for this possibly important confounder is a limitation.

Another problem in our study is the lack of pharmacy data and information from the parents before 1997. On the other hand, we explicitly asked about the use of fertility treatment before 1997; therefore, exposure misclassification is likely to be negligible. Also, in the literature clomiphene was not associated with hypospadias. Therefore, if misclassification occurred, it is likely to be nondifferential and will bias the estimates towards null and thus underestimate the real effect. Any possible association between clomiphene and anomalies in the control group would also bias the estimates towards the null. Because these associations are uncertain and the selection of specific anomalies as controls might introduce selection bias, we felt strongly that our study would be better if it included all non-cases as controls. By using malformed controls, one assumes that exposure of clomiphene among the controls is an estimation of exposure in a general population. It is unlikely that exposure in our control group is lower than in the general population because that would mean clomiphene would decrease the chance of birth defects, which is highly unlikely. Also, information about infertility treatment is consistently requested for all malformed births in the registry. If exposure in our control group is higher than in the general population, the differences between exposure among cases and among controls is underestimated in our analysis, and the association found might also be an underestimation.

More multiple pregnancies were found among clomiphene-exposed births than among unexposed births, as would be expected with ovulation induction. Although hypospadias is associated with multiple births in the literature (Fredell et al., 1998), cases and controls did not differ with respect to this variable (Table 1). Defects other than hypospadias are probably associated with multiple pregnancy as well; as a result, our total population may include more multiple pregnancies than does a general population. Again, we could not adjust for this variable.

Despite the statistical limitations of small numbers, our study stresses the importance of collecting and analyzing data about congenital anomalies at the most detailed level possible. Therefore, we encourage birth defects monitoring registries to pursue their work and to collaborate to enhance the power of future studies. We highly recommend evaluating the relation between clomiphene and hypospadias in other databases to confirm our findings.

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