Eur J Pediatr (2007) 166:671–678
DOI 10.1007/s00431-006-0304-z

ORIGINAL PAPER

Risk factors for hypospadias

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Received: 26 May 2006 / Accepted: 31 August 2006 / Published online: 14 November 2006

Abstract Despite being one of the most common congenital defects in boys, the etiology of hypospadias remains largely unknown. In this case-referent study, we evaluated a wide spectrum of potential risk factors for hypospadias. Cases were identified from the hospital information system, and referents were recruited through the parents of the cases. Both parents of cases and referents completed written questionnaires that they received through the mail. Logistic regression analyses were used to assess the independent contribution of different factors to the risk of hypospadias. The final database included 583 cases and 251 referents. Hypospadias more often occurred in children whose father had hypospadias (OR=9.7; 95%CI: 1.3–74.0) and in children with a low birth weight (OR=2.3; 95%CI: 1.2–4.2). Indications for elevated risks were found when mothers were DES-daughters (OR=3.5; 95%CI: 0.8–15.6), fathers were subfertile (OR=1.8; 95%CI: 0.7–4.5), the parents had undergone fertility treatment (OR=2.3; 95%CI: 0.9–5.8), and in twin or triplet pregnancies (OR=2.0; 95%CI: 0.8–5.1). Maternal use of iron supplements (OR=2.2; 95%CI: 0.8–6.0), maternal smoking (OR=1.5; 95%CI: 1.0–2.4), paternal prescriptive drug use (OR=2.6; 95%CI: 1.1–6.6), and paternal exposure to pesticides (OR=2.1; 95%CI: 0.6–7.1) during the 3 months immediately prior to conception or in the first trimester of pregnancy also appeared to increase the risk of hypospadias. The associations found in this study support the hypothesis that genetic predisposition, placental insufficiency, and substances that interfere with natural hormones play a role in the etiology of hypospadias.

Keywords Hypospadias · Maternal exposures · Paternal exposures · Pregnancy · Risk factors

Abbreviations

ART	Assisted reproductive techniques
CI	Confidence interval
DES	Diethylstilbestrol
DHT	Dihydrotestosterone
HCG	Human chorionic gonadotropin
ICSI	Intracytoplasmic sperm injection
IDA	Iron deficiency anemia
IVF	In vitro fertilization
OR	Odds ratio

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Introduction

Hypospadias is one of the most common congenital defects in boys, affecting approximately 30–40 children out of 10,000 live births [16, 25]. According to some studies, the birth prevalence increased rapidly during the 1970s and 1980s, but these trends do not appear to persist in recent evaluations [8, 10, 22, 23]. Hypospadias

results from an incomplete fusion of the urethral folds between the seventh and fourteenth week of gestation [19], but the exact etiology of the disorder remains largely unknown.

The association between growth retardation and hypospadias is well established. One possible explanation is that placental insufficiency in providing the fetus with nutrients and gonadotropins may lead to both growth retardation and hypospadias [13, 14, 17, 34]. Placental insufficiency may also explain the increased occurrence of hypospadias in twins [13, 14, 34]. However, familial clustering suggests a genetic component in the etiology of hypospadias [12]. Higher maternal age [11, 27], assisted reproductive techniques (ART) [20, 30, 35], paternal subfertility [15], maternal exposure to diethylstilbestrol (DES) in utero [6, 18, 26], maternal vegetarian diet and iron supplementation [21], and paternal smoking and exposure to solvents and pesticides [5, 7, 24] have also been put forward as risk factors for hypospadias. As male sexual differentiation in general depends on testosterone, its metabolite dihydrotestosterone (DHT), and the expression of androgen receptors by target cells [19], disturbances in the balance of this endocrine system by either endogenous or exogenous factors may lead to hypospadias.

In the past two decades, attention has focused on the potential adverse effects of environmental endocrine disruptors on male reproductive health. These substances include numerous industrial chemicals as well as dietary phyto-estrogens. Exposure during early gestation has been hypothesized to affect the developing Sertoli cells and Leydig cells, thereby increasing the risk of hypospadias, cryptorchidism, testicular cancer, and reduced semen quality later in life; all of these are conditions that have been described as different expressions of the Testicular Dysgenesis Syndrome [2, 4, 31]. Although the evidence for adverse effects of endocrine disruptors on human reproduction is limited, data from animal studies as well as observations on wildlife following environmental pollution are convincing [3, 29].

The origin of hypospadias thus appears to be multifactorial. Although previous research has provided evidence for some risk factors, more knowledge is required in order to prevent future cases. The majority of studies carried out to date have focused on distinct components in the etiology of hypospadias; the result has been a fragmented body of knowledge on risk factors. Consequently, there is an urgent need to study the full spectrum of potential risk factors and assess the independent contribution of each factor as this may lead to more insight into the etiology. The aim of the investigation reported here was to identify a wide range of risk factors for hypospadias in a large case-referent study in which we focused on both maternal and paternal exposure.

Materials and methods

Recruitment of cases and referents

Data on 937 boys who were treated for hypospadias at the Pediatric Urology Centre of the Radboud University Nijmegen Medical Centre (The Netherlands) were retrieved from the hospital information system. Approximately 80 hypospadias corrections are performed each year at the Pediatric Urology Centre, with most of these patients presenting with a mild defect (glandular or coronal hypospadias, 60%). Intermediate (penile) and severe (scrotal or perineal) manifestations of hypospadias are seen in approximately 25 and 15% of the patients, respectively. The selection of cases was restricted to children who were born in the period 1987 through 1997 and who were residents of The Netherlands. Among the cases were 14 boys of whom an older brother was also included in the study population; these boys were excluded in order to avoid bias in the effect parameters. In addition, only one child was included from four twins and one triplet. In October 2002, written questionnaires were mailed to the parents of all selected cases. Due to unknown address changes, however, the parents of at least 98 boys did not receive the questionnaires. Two boys had foster parents and were therefore not eligible to participate in the study. As a result, the total number of eligible boys with hypospadias was 817. In order to recruit a referent group, we mailed an extra set of questionnaires to the parents of the eligible boys together with the request that they give these questionnaires to the parents of a boy of approximately the same age as their son – for example, a school friend – who was then included in the referent group. The aim of this recruitment strategy was to realize a referent group that reflects the hospital's catchment area from which the cases originated. Consequently, the final study population consisted of a mixture of 'case-referent pairs' and 'single' cases for whom no referent was recruited.

Data collection

Both parents of cases and referents were asked to fill out the same written questionnaires. First and second reminders were sent to the parents of the cases in order to increase the response rate. The questionnaires for both sets of parents contained questions on age, ethnicity, education, prenatal exposure to DES, and medical history. Information was requested on the 3 months immediately prior to conception and the first trimester of pregnancy with respect to illnesses, medication, life style, and occupational exposure to various agents. Additionally, mothers were questioned about oral contraceptive use, assisted reproductive techniques, the course of pregnancy, their son's birth

weight, and the diagnosis of hypospadias or other congenital defects. Finally, the mothers were asked to provide information on their other children and pregnancies. The study was approved by the Regional Committee on Research Involving Human Subjects.

Statistical analyses

Data were entered into an MS Access database and converted to SPSS ver. 12.0.1 (SPSS, Chicago, Ill.) and SAS V8.2 for statistical analyses (SAS, Raleigh, N.C.). All potential risk factors, with the exception of birth weight and maternal age, were dichotomous (yes vs. no). Crude associations with hypospadias were estimated by odds ratios (OR) with 95% confidence intervals (95%CI) in univariable analyses. Odds ratios greater than one indicate increased risks for hypospadias, especially when the lower bound of the 95%CI excludes unity. Educational level (three levels) and native country (Netherlands vs. other) of both parents were considered to be potential confounders. Educational level was defined as low (elementary school or lower vocational education), intermediate (intermediate secondary school or intermediate vocational education), and high (higher secondary school, higher vocational education, or university). Effect-measure modification was assessed through bivariable stratified analyses that involved all combinations between risk factors as well as combinations with educational level and native country of the parents and was evaluated using both the Breslow-Day test for homogeneity across strata and strata-specific point estimates. Subsequently, all potential risk factors and confounders were simultaneously included in logistic regression models in order to assess their independent contribution to the risk of hypospadias. In a stepwise manner, risk factors with minimal effect estimates (OR<1.5 and/or lower bound of 95%CI<0.7) were manually excluded from the model if exclusion did not have a substantial effect on the coefficients of other risk factors (<10%). Additionally, conditional logistic regression analyses were performed on the subset of parents of cases for whom referent parents were available ('case-referent pairs') to control for potential confounding by unmeasured factors as a result of the recruitment strategy. The same inclusion and exclusion criteria were applied as in the analyses on the complete study population.

Results

The parents of 613 of the 817 hypospadias cases completed the questionnaires, resulting in a response rate of 75%. Additionally, completed questionnaires were received from the parents of 264 referents. For 30 cases and 13 referents information could only be obtained from one parent; these data were excluded from further analysis. The final database included 583 cases and 251 referents, in which 232 'case-referent pairs' were identified. Cases and referents resided throughout the country and showed a similar spatial distribution based on the 61 regional postal area codes that were reported. Descriptive statistics of the cases and referents in the complete study population and in the case-referent pairs are shown in Table 1. Cases and referents were all approximately 10 years of age at the time of the study. In the complete study population, parents of cases tended to have a lower educational level than those of the referents. They also reported more often to have been born in a foreign country, which was a European country in about 50% of the responses. Among the case-referent pairs, parents' educational level and native country were more alike.

The crude odds ratios and 95% confidence interval for the analyses in the complete study population are presented in Tables 2 and 3. Table 2 shows potential risk factors for hypospadias that are associated with the parent's health and fertility status and pregnancy characteristics. Our data did not support an association with increased maternal age (>35 years). An assessment of the effect of maternal age on a continuous scale or in multiple age categories did not lead to different findings. The most profound result was the increased risk of hypospadias for boys whose fathers had hypospadias (*n*=23; OR=9.8; 95%CI: 1.3–73.0). Strong indications for an increased risk of hypospadias were also found when mothers were exposed to DES in utero

Table 1 Descriptive statistics of cases and referents in the complete study population and in the case-referent pairs

		Cases in complete population (*n*=583)[a]	Cases in case-referent pairs (*n*=232)[a]	Referents[b] (*n*=251)[a]
Age of cases/ referent – mean (SD)		10.2 (3.6)	9.7 (3.5)	9.6 (3.5)
Educational level of parents – frequency (%)				
Mother	Low	143 (24.6)	32 (13.8)	28 (11.2)
	Intermediate	243 (41.8)	105 (45.3)	101 (40.6)
	High	195 (33.6)	95 (40.9)	120 (48.2)
Father	Low	173 (29.8)	48 (20.7)	50 (20.2)
	Intermediate	192 (33.1)	84 (36.2)	80 (32.3)
	High	215 (37.1)	100 (43.1)	118 (47.6)
Native country of parents – frequency (%)				
Mother	Netherlands	545 (93.5)	228 (98.3)	244 (97.6)
	Other	38 (6.5)	4 (1.7)	6 (2.4)
Father	Netherlands	545 (93.6)	226 (97.4)	243 (96.8)
	Other	37 (6.4)	6 (2.6)	8 (3.2)

[a] Numbers do not add up to total group size due to missing values.
[b] Descriptive statistics of the 232 referents in the case-referent pairs are similar to the statistics of all referents that are presented here.

Table 2 Associations between hypospadias and potential risk factors related to parent's health and fertility status and pregnancy characteristics

	Number of cases (n=583)[a]	Number of referents (n=251)[a]	Crude OR (95%CI)
Increased maternal age at time of delivery (>35 years)	58	21	1.2 (0.7–2.1)
Hypospadias in father	22	1	9.8 (1.3–73.0)
Intra-uterine DES exposure			
Mother	18	3	2.6 (0.8–9.0)
Father	8	3	1.2 (0.3–4.4)
Subfertility			
Mother	43	10	1.9 (1.0–3.9)
Father	30	6	2.2 (0.9–5.4)
Pregnancy achieved with fertility treatment[b]	35	6	2.6 (1.1–6.3)
Twin or triplet pregnancy	34	6	2.5 (1.1–6.1)
Low birth weight (<2500 g)[c]	114	19	2.3 (1.2–4.2)

[a] Maximum numbers of missing values per risk factor were 27 and 10 for cases and referents, respectively.
[b] Including Assisted Reproductive Techniques
[c] Adjusted for duration of gestation (in weeks)

(OR=2.6; 95%CI: 0.8–9.0) and when subfertility was reported by mothers (OR=1.9; 95%CI: 1.0–3.9) or fathers (OR=2.2; 95%CI: 0.9–5.4). In addition, parents of cases more often achieved pregnancy following fertility treatment (OR=2.6; 95%CI: 1.1–6.3), which mostly involved ovulation stimulants (19 cases and three referents). Three fathers and one mother of cases used other fertility-enhancing drugs prior to pregnancy. Ten cases and one referent were born following in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatments, and two cases and one referent were born following intra-uterine insemination. Associations with twin or triplet pregnancies (OR=2.5; 95% CI: 1.1–6.1) and low birth weight (OR=2.3; 95%CI: 1.2–4.2) were also found.

Potential risk factors concerning life style, drug use, and occupational exposures in the 3 months immediately prior to conception and/or during pregnancy are shown in Table 3. Increased risks were found when mothers smoked during pregnancy (OR=1.8; 95%CI: 1.2–2.8) or fathers smoked before pregnancy (OR=1.4; 95%CI: 1.0–1.9). No associations were observed between hypospadias and maternal alcohol consumption, vegetarian diet, and the use of folic acid or other vitamin supplements. However, we did find some indication that oral contraceptive use after conception might be associated with hypospadias (OR=2.0; 95%CI: 0.7–5.9). Also, there appeared to be an increased risk when mothers used iron supplements in the 3 months immediately prior to conception and/or during the first trimester of pregnancy (OR=2.1; 95%CI: 0.9–5.2). The use of other prescriptive drugs by mothers did not seem to be associated with hypospadias, but our findings do suggest an increased risk when fathers used prescriptive drugs (OR=1.9; 95%CI: 0.8–4.4). A wide variety of drugs were listed, of which the five mostly used were anti-inflammatory drugs, antihypertensives, antipsychotics, respiratory drugs, and drugs for gastritis. With respect to occupational exposures, the parents were asked about pesticides, paint, varnish or thinners, cleaning agents, heavy metals, and exhaust fumes. We found no associations between hypospadias and any of these exposures.

We also attempted to identify whether the retrospective assessment of risk factors affected the results by comparing the univariable effect estimates presented in Tables 2 and 3 between boys who were younger and older than 10 years of age at time of data collection, respectively. The population size did not allow evaluation of other cut-off points yielding unequally sized groups. The association

Table 3 Associations between hypospadias and potential risk factors concerning parent's life style, drug use, and occupational exposures[a]

	Number of cases (n=583)[b]	Number of referents (n=251)[b]	Crude OR (95%CI)
Smoking			
Mother[c]	133	35	1.8 (1.2–2.8)
Father	234	81	1.4 (1.0–1.9)
Mother drank alcoholic beverages[c]	117	63	0.8 (0.5–1.1)
Mother had a vegetarian diet	10	7	0.6 (0.3–1.6)
Mother used folic acid	56	24	1.0 (0.6–1.7)
Oral contraceptive use after conception	18	4	2.0 (0.7–5.9)
Mother used iron supplements	29	6	2.1 (0.9–5.2)
Prescriptive drug use			
Mother	42	14	1.3 (0.7–2.5)
Father	30	7	1.9 (0.8–4.4)
Occupational exposure to pesticides			
Mother	6	3	0.9 (0.2–3.5)
Father	24	8	1.3 (0.6–2.9)
Occupational exposure to paint, varnish or thinners			
Mother	35	12	1.3 (0.7–2.5)
Father	78	42	0.8 (0.5–1.2)

[a] Maternal exposures concern the 3 months immediately prior to conception and/or the first trimester of pregnancy, unless otherwise specified. Paternal exposures concern the 3 months immediately prior to conception.
[b] Maximum numbers of missing values per risk factor were ten and six for cases and referents, respectively.
[c] Specified as smoking or drinking alcohol during pregnancy only.

with subfertility of the mother appeared to be only present for boys under 10 years of age (OR=3.5; 95%CI: 1.2–10.3) but not among older boys (OR=1.1; 95%CI: 0.4–2.7). Likewise, a much larger effect estimate was found for the maternal use of iron supplements among younger boys (OR=4.0; 95%CI: 0.9–17.5 vs. OR=1.3; 95%CI: 0.4–4.1). For older boys, however, an increased risk of hypospadias was found when mothers were occupationally exposed to paint, varnish, or thinners [OR=4.1; 95%CI: 0.9–17.7 (older boys) vs. OR=0.7; 95%CI: 0.3–1.6 (younger boys)]. The effect estimates of other risk factors did not substantially differ between the two age groups.

Table 4 shows the independent effect estimates for the risk factors that were found to contribute to an increased risk of hypospadias as obtained from the multivariable analyses on the complete study population and on the population of case-referent pairs. The latter analysis was performed using a conditional logistic regression model in which the tie between the cases and referents was included as a matching variable. In both analyses, the full models contained all risk factors that showed an indication for an association with hypospadias in the previous analyses (see Tables 2 and 3), with the exception of low birth weight as it shares certain causal factors with hypospadias. Educational level and native country of the parents were included as potential confounders. Increased maternal age, maternal vegetarian diet, and paternal exposure to pesticides or solvents were added as well because these are potential risk factors according to previous studies. Following the exclusion of factors that did not contribute to the risk of hypospadias, the final models contained the risk factors that are shown in Table 4. Some risk factors that should be excluded from one of the models according to our criteria were maintained, so both models contained the same risk factors and their effect estimates can be compared.

Table 4 Multivariable analyses of risk factors for hypospadias using logistic regression models for the complete study population and the case-referent pairs separately

Risk factors	Complete population OR (95%Cl), n=769[a]	Case-referent pairs[b] OR (95%Cl), n=440[a]
Education level of mother[c]		
Low vs. high	2.9 (1.7–4.7)	2.0 (0.9–4.4)
Intermediate vs. high	1.5 (1.1–2.1)	1.8 (1.1–3.1)
Native country of mother[c]		
Other vs. Netherlands	2.9 (1.2–7.1)	0.5 (0.1–2.9)[f]
Hypospadias in father	9.7 (1.3–74.0)	9.2 (1.0–84.1)
Fertility treatments [d]	2.3 (0.9–5.8)	1.3 (0.4–4.2)[f]
Twin or triplet pregnancy	2.0 (0.8–5.1)	1.7 (0.5–5.6)
Mother exposed to DES in utero	3.5 (0.8–15.6)	3.7 (0.8–17.4)
Mother smoked during pregnancy [e]	1.5 (1.0–2.4)	1.5 (0.8–2.8)
Mother used iron supplements	2.2 (0.8–6.0)	2.6 (0.7–9.7)
Father used prescriptive drugs	2.6 (1.1–6.6)	2.7 (0.8–9.0)
Father exposed to pesticides	1.2 (0.5–2.7)[f]	2.1 (0.6–7.1)

[a] Numbers do not add up to total population sizes (n=834 and n=464, respectively), due to missing values.
[b] Including cases for whom a referent was available. This analysis involved a conditional logistic regression model in which the tie between the cases and referents was treated as a matching variable and included in the model.
[c] Educational level and native country of mother were considered to be confounders in both models.
[d] Including Assisted Reproductive Techniques
[e] Specified as smoking during pregnancy only
[f] These risk estimates met the criteria to be excluded from the model (OR <1.5 and/or lower bound of 95%CI<0.7), but were included in order to obtain two consistent models.

Overall, the effect estimates for the complete study population corresponded with the univariable analyses, although some risk factors did appear to be somewhat stronger (maternal DES exposure, paternal drug use) or weaker (fertility treatments, twin or triplet pregnancy) in the multivariable analysis. Oral contraceptive use and paternal smoking dropped out of the model completely, whereas subfertility of mother or father could not be included in the multivariable model because of strong correlations with fertility treatment. An alternative model which included subfertility instead of fertility treatments showed that maternal subfertility did not contribute substantially to the risk of hypospadias, but the effect estimate for paternal subfertility was OR=1.8 (95%CI: 0.7–4.5) in the complete study population. An additional analysis excluding children who were born following fertility treatment did not yield essentially different findings. The risk estimates for the case-referent pairs (see Table 4, right-hand column) deviate from those for the complete study population in a few respects. The indications for increased risks of hypospadias in twin or triplet pregnancies or following fertility treatment were much less convincing: OR=1.7 (95%CI: 0.5–5.6) and OR=1.3 (95%CI: 0.4–4.2), respectively. In contrast, the risk estimate for paternal subfertility from the alternative model was stronger (OR=2.6; 95%CI: 0.9–7.2). Surprisingly, some indication for an association between hypospadias and paternal pesticide exposure was revealed among case-referent pairs (OR=2.1; 95%CI: 0.6–7.1). Sub-analyses in which cases and referents whose father had hypospadias were excluded did not lead to different findings in either model.

Discussion

In this large case-referent study we assessed the contribution of a wide spectrum of potential risk factors in the development of hypospadias, including the parents' health and fertility status, fertility treatments, DES exposure, and pregnancy characteristics as well as the parents' life style,

drug use, and occupational exposures to various agents during the 3 months immediately prior to conception and during the first trimester of pregnancy. By simultaneously including all potential risk factors in the multivariable models, our aim was to identify the independent contribution of each factor to the risk of hypospadias. Before these results can be reliably interpreted, however, it is necessary to consider the potential shortcomings in study design and analysis that may have affected the findings.

By recruiting referents through the parents of the cases, we attained a referent population that properly reflected the hospital's catchment area from which the cases originated, as shown by the similar distribution of postal area codes. However, this recruitment strategy may also raise concerns about selection bias, especially because our data suggest that the willingness of the parents of the cases to approach other parents was associated with their educational level and native country. Therefore, we performed additional analyses focusing only on those cases for whom a referent was available. The risk estimates for these case-referent pairs may be considered to be the most valid. However, somewhat different findings may also result from a loss of precision due to the reduced population size. Therefore, we feel that both analyses are informative for the purpose of exploring risk factors for hypospadias. We treated parents' educational level and native country as confounders under the assumption that the differences between the parents of the cases and those of the referents resulted from the referent recruitment strategy. However, educational level and native country may also be associated with hypospadias through, for example, exposures related to life style or occupation. In that case, some odds ratios may be underestimated due to overcorrection. An underestimation of some risk factors may also have occurred due to the similarities in, for example, residential area between cases and referents, which would have resulted from the recruitment of referents through the parents of cases.

Potential information bias should be considered as well, especially since all exposure data were self-reported and collected retrospectively as far back as an average of 10 years prior to the study. Non-differential misclassification of exposure may have led to an underestimation of effects. However, risk estimates may also have been overestimated due to recall bias, as parents of cases may have tried to find an explanation for their child's defect. A comparison of the effect estimates between boys younger and older than 10 years of age at the time of data collection revealed a weaker association between hypospadias and maternal use of iron supplements in the older age group. This may be due to recall difficulties with respect to the use of iron supplements among the mothers of both cases and referents. The lack of an association of hypospadias with maternal subfertility in older boys may be explained by changes in medical practice regarding subfertility (e.g., availability of fertility treatment) in the past 10–20 years. A possible explanation for the increased risk of hypospadias found in this study when mothers of older children were occupationally exposed to paint, varnish, or thinners is that 10 years ago paints more often contained high levels of organic solvents. However, overestimation due to differential recall cannot be ruled out either, although the effect estimates of other risk factors did not differ substantially between older and younger boys. Furthermore, many of our findings are consistent with those reported in earlier studies, which adds support to the validity of our results.

The most profound association revealed in this study was the increased risk of hypospadias among boys whose father had hypospadias himself. Among the fathers of the cases in our study, 4% reported hypospadias, which seems to be compatible with previous studies which reported that hypospadias affects about 7% of first-, second-, and third-degree relatives of cases [12]. The familial occurrence of hypospadias indicates an important genetic component in the etiology of hypospadias, although family members may share environmental risk factors as well. Also consistent with previous literature, we found an increased occurrence of hypospadias in children with a low birth weight or born out of a multiple pregnancy. In these pregnancies, the placenta may have been insufficient in providing the fetus with nutrients and gonadotropins, of which Human Chorionic Gonadotropin (HCG) appears to play a specific role in male sexual differentiation. This may have led to both growth restriction, to which twins and triplets are more susceptible, and hypospadias [13].

A strong indication for an increased risk of hypospadias was found among boys whose mothers were exposed to DES in utero – 'DES-daughters' – an association reported in a previous article by our group [6]. In 2002, Klip et al. reported the prevalence ratio for hypospadias in sons of DES-daughters to be 21.3 (95%CI: 6.5–70.1), which was the first suggestion of a transgenerational effect of DES in humans [18]. However, the association between intrauterine DES exposure and hypospadias was assessed in a cohort of women with fertility problems, who do not reflect DES-daughters in general. According to our findings, the excess risk of hypospadias appears to be of a much smaller magnitude. This may be explained by the differences in study design, and in the study population in particular, and probably results in a more valid risk estimate that is concordant with findings from a recent study in France [26]. It is possible that DES-related pathology of reproductive structures in DES-daughters interferes with normal fetal development during pregnancy, but other explanations

have been suggested as well [18]. We found no indication that DES-sons 'transmit' a predisposition to hypospadias to their sons.

Our results also point towards an association between paternal subfertility and hypospadias. According to the Testicular Dysgenesis Syndrome hypothesis, hypospadias and male subfertility may share the same origin, with genetic and environmental components [31]. Hence, subfertile fathers may transmit a certain predisposition to their sons. Furthermore, we found indications that fertility treatment increased the risk of hypospadias. In previous studies, an increased occurrence of hypospadias was reported following IVF and ICSI treatments [30, 35], and these risks were reflected in our data as well. We also found that mothers of cases used ovulation stimulants relatively more often, which corresponds with the recently reported increased risk of hypospadias following clomiphene treatment [20]. One possible explanation is that hormones administered as part of fertility treatment interfere with male sexual hormones in early gestation and thereby disturb normal genital development. Progesterone used to support pregnancies achieved with ART, in particular, may impair testosterone production or its conversion to DHT [9, 30].

Our data suggest a slightly increased risk of hypospadias when mothers smoked during pregnancy. This finding should be interpreted with caution, however, as smoking behavior is probably associated with the parents' educational level and native country, and residual confounding by these cofactors cannot be ruled out. On the other hand, the suggestion that placental malfunctioning plays an important role in the origin of hypospadias supports an increased risk when mothers smoked during pregnancy. The strongly increased risk of hypospadias when fathers smoked prior to pregnancy reported by Pierik et al. [24], was not reflected in our data.

An increased risk of hypospadias was found when mothers used iron supplements immediately prior to conception and/or during the first trimester of pregnancy. Based on the results of their study, North et al. also mentioned such an association [21]. Iron Deficiency Anemia (IDA) in early pregnancy has been associated with preterm delivery, possibly due to long-term hypoxia and oxidative stress [1]. Furthermore, it has been suggested that iron supplementation in mothers who are not iron deficient may cause toxic reactions or increase blood viscosity, which subsequently impairs placental blood flow [28]. No association with hypospadias was found when mothers used other prescriptive drugs. However, we did observe an increased risk when fathers used prescriptive drugs during the 3 months immediately prior to conception. Because we pooled all drugs in this study, thereby disregarding their pharmacological characteristics, this unexpected finding is difficult to interpret. Unfortunately, the population size did not allow us to distinguish drugs that are biologically plausible to cause hypospadias. As past drug use is often hard to remember, the possibility that recall bias influenced the association between drug use and hypospadias cannot be ruled out.

The analyses on the case-referent pairs revealed an indication for an elevated risk when fathers were exposed to pesticides. Previous studies have reported inconsistent results on the association between pesticides and hypospadias, while there appears to be more agreement on an association with cryptorchidism [5, 7, 24, 32, 33]. Pierik and colleagues also reported a slightly increased risk of hypospadias when fathers were occupationally exposed to solvents; this association, however, was not reflected in our data [24]. Exposure to pesticides, solvents, and other chemicals can be work-related, but may also occur through leisure time activities, diet, or personal care products. Future research projects should take all potential sources into account (in order) to clarify the role of these endocrine-disrupting chemicals in the development of hypospadias. Some other associations have been described in the literature, but these did not appear in our data; these include the association with increased maternal age [11, 27] and the strongly increased risk of hypospadias in mothers with a vegetarian diet during pregnancy previously reported by North et al. [21]. However, the number of vegetarian mothers in our study was extremely small.

In conclusion, we found that the birth prevalence of hypospadias was higher in children whose father had hypospadias, in children with a low birth weight, and in twins or triplets. The use of iron supplements by mothers and of other prescriptive drugs by fathers also appeared to be associated with hypospadias. We found indications for an increased risk of hypospadias when mothers were DES-daughters, when fathers were subfertile, and when pregnancy was achieved by means of a fertility treatment. Some indication was found for an increased risk when mothers smoked during pregnancy and when fathers were exposed to pesticides. These risk factors support the idea that genetic predisposition, placental insufficiency, and substances that interfere with natural hormones before conception or during fetal development play a role in the etiology of hypospadias. However, the size of our study population did not allow us to identify effects of rare or weak risk factors. Larger studies could facilitate the identification of these risk factors as well and provide opportunities for the further in-depth investigation of the associations found to date.

Acknowledgements We thank Professor Gerhard Zielhuis for his advice on methodology and all of the parents for their participation in the study.

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