available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Pediatric Urology
Editorial by XXX on pp. x–y of this issue

Is Hypospadias Associated with Prenatal Exposure to Endocrine Disruptors? A French Collaborative Controlled Study of a Cohort of 300 Consecutive Children Without Genetic Defect

Nicolas Kalfa [a,b,*], *Françoise Paris* [b,c], *Pascal Philibert* [b], *Mattea Orsini* [d], *Sylvie Broussous* [a], *Nadège Fauconnet-Servant* [b], *Françoise Audran* [b], *Laura Gaspari* [b], *Hélène Lehors* [e], *Myriam Haddad* [e], *Jean-Michel Guys* [e], *Rachel Reynaud* [f], *Pierre Alessandrini* [g], *Thierry Merrot* [g], *Kathy Wagner* [h], *Jean-Yves Kurzenne* [i], *Florence Bastiani* [i], *Jean Bréaud* [i], *Jean-Stéphane Valla* [i], *Gérard Morisson Lacombe* [j], *Eric Dobremez* [k], *Amel Zahhaf* [d], *Jean-Pierre Daures* [d], *Charles Sultan* [b,c]

[a] *Service de Chirurgie et Urologie Pédiatrique, Hôpital Lapeyronie, CHU de Montpellier et Université Montpellier 1, Montpellier, France;* [b] *Service d'Hormonologie, Hôpital Lapeyronie, CHU de Montpellier et Université Montpellier 1, Montpellier, France;* [c] *Unité d'Endocrinologie et Gynécologie Pédiatriques, Service de Pédiatrie, Hôpital Arnaud de Villeneuve et Université Montpellier 1, CHU de Montpellier, Montpellier, France;* [d] *Institut Universitaire de Recherche Clinique, Laboratoire de Biostatistiques et d'Epidémiologie, Université Montpellier 1, Montpellier, France;* [e] *Service de Chirurgie et Urologie Pédiatrique, Hôpital la Timone, Marseille, France;* [f] *Unité d'Endocrinologie et Diabétologie Pédiatriques, Hôpital la Timone, Marseille, France;* [g] *Service de Chirurgie et Urologie Pédiatrique, Hôpital Nord, Marseille, France;* [h] *Service de Pédiatrie, Hôpital Lenval, CHU de Nice, France;* [i] *Service de Chirurgie et Urologie Pédiatrique, Hôpital Lenval, Nice, France;* [j] *Service de Chirurgie et Urologie Pédiatrique, Hôpital Saint-Joseph, Marseille, France;* [k] *Service de Chirurgie Pédiatrique, Hôpital Pellegrin Enfants, Bordeaux, France*

Article info

Article history:
Accepted May 5, 2015

Associate Editor:
Christian Gratzke

Keywords:
Hypospadias
Disorder of sex determination
Environment
Endocrine-disrupting chemicals
Pesticides
Occupation
Birth defect

Abstract

Background: Numerous studies have focused on the association between endocrine-disrupting chemicals (EDCs) and hypospadias. Phenotype variability, the absence of representative comparison groups and concomitant genetic testing prevent any definitive conclusions.
Objective: To identify the role of occupational and environmental exposures to EDCs in nongenetic isolated hypospadias.
Design, setting, and participants: A total of 408 consecutive children with isolated hypospadias and 302 normal boys were prospectively included (2009–2014) in a multi-institutional study in the south of France, the area of the country with the highest prevalence of hypospadias surgery.
Outcome measurements and statistical analysis: In patients without *AR*, *SRD5A2*, and *MAMLD1* mutations, parental occupational and professional exposures to EDCs were evaluated based on European questionnaire QLK4-1999-01422 and a validated job-exposure matrix for EDCs. Environmental exposure was estimated using the zip code, the type of surrounding hazards, and distance from these hazards. Multivariate analysis was performed.
Results: Fetal exposure to EDCs around the window of genital differentiation was more frequent in the case of hypospadias (40.00% vs 17.55%, odds ratio 3.13, 95% confidence interval 2.11–4.65). The substances were paints/solvents/adhesives (16.0%), detergents

* Corresponding author: Service de Chirurgie et Urologie Pédiatrique, Hôpital Lapeyronie, 171 Avenue Giraud, Montpellier 34295, France. Tel. +33 4 67338784; fax: +33 4 67339512.
E-mail address: nicolaskalfa@gmail.com (N. Kalfa).

http://dx.doi.org/10.1016/j.eururo.2015.05.008

(11.0%), pesticides (9.0%), cosmetics (5.6%), and industrial chemicals (4.0%). Jobs with exposure were more frequent in mothers of hypospadiac boys (19.73% vs 10.26%, $p = 0.0019$), especially cleaners, hairdressers, beauticians, and laboratory workers. Paternal job exposure was more frequent in the cases of hypospadias (40.13% vs 27.48%, $p = 0.02$). Industrial areas, incinerators, and waste areas were more frequent within a 3-km radius for mothers of hypospadiac boys (13.29% vs. 6.64%, $p < 0.00005$). Association of occupational and environmental exposures increases this risk.

Conclusions: This multicenter prospective controlled study with a homogeneous cohort of hypospadiac boys without genetic defects strongly suggests that EDCs are a risk factor for hypospadias through occupational and environmental exposure during fetal life. The association of various types of exposures may increase this risk.

Patient summary: Our multi-institutional study showed that parental professional, occupational, and environmental exposures to chemical products increase the risk of hypospadias in children.

1. Introduction

Hypospadias is the second most common malformation of the male genitalia. It consists of congenital hypoplasia of the ventral face of the penis, with displacement of the urethral opening, disjunction of the corpus spongiosum, a dorsal hooded foreskin, and, in some cases, ventral chordee. A multifactorial pathophysiology has been proposed, including genetic and environmental causes [1,2], to explain this undermasculinization of the fetus. Mutations of the many genes implicated in male sex development have been described, regardless of phenotype severity [3–5]. However, the majority of hypospadias cases do not exhibit genetic variants with functional consequences, and large studies have called into question the impact of these variants [6].

The increasing incidence of hypospadias in certain regions or time periods [7,8] has led to the suspicion that environmental chemicals may be detrimental to male genital development during fetal life, even though the findings are not generalizable [9]. According to the theory of testicular dysgenesis syndrome [10], fetal exposure to xenoestrogens suppresses testosterone production and action and/or androgen receptor (AR) expression [11], thereby causing neonatal genital malformation and long-term effects, including decreased spermatogenesis. Wildlife observations [12], experimental exposure to diethylstilbestrol (DES), which is a xenoestrogen model in mammals, and experimental in vitro data have suggested that manmade chemicals may interfere with androgen-dependent sex differentiation of the male fetus [13]. Some 8% of all known chemicals exhibit antiandrogen activity [14] and their widespread use has prompted the suspicion that endocrine-disrupting chemicals (EDCs) are a potential cause of hypospadias. Whether results from in vitro and animal experiments can be transposed to human pathology is questionable, and the key issue to be resolved is whether the real level of EDC exposure is sufficient to induce hypospadias in boys [15].

Recent studies by our group reported an association between genital disorders and environmental exposure to EDCs. For example, it was found that the prevalence of micropenis was higher in French regions with intensive pesticide use [16] and that male disorders of sexual development were more frequent in boys born to parents with occupational exposure to pesticides [17]. Increased serum estrogenic bioactivity may be a marker of exposure to pollutants [18], with clinical repercussions for both genital development [19] and puberty [20]. Exposure to medications with estrogenic activity such as DES can also lead to genital malformation [21].

Discrepancies among epidemiologic studies should nevertheless be noted [22,23]. For example, studies of occupations that expose workers to pesticides—one of the EDC classes most investigated in hypospadias—show contradictory results. Some authors have shown that farmers have a higher risk of giving birth to a boy with hypospadias [24], whereas others show no association with maternal exposure to pesticides [25]. Measurement of pesticide concentrations in maternal serum [26] and investigation of residential proximity to pesticide applications [27] have not helped to resolve these discrepancies. Many studies have focused on other professions, including the leather, automotive, and metal industries, as well as hairdressing, and have shown potential confounding factors at best or even completely contradictory results. The surrounding home environment has been also evaluated [28]. The prevalence of hypospadias seems to be higher in areas with intensive agriculture [29], but the increasing level of potential contaminants in maternal serum has not reached significance [30,31]. These results may be explained by the complexity of the mechanisms of action of xenoestrogens, the highly effective metabolites of EDCs, or the cocktail effects of hundreds of EDCs.

Most studies have also been retrospective and based on data banks with limited clinical details and sometimes wide variability in phenotypes and nonhomogeneous cohorts. Ethnic pair-based comparison groups that are representative of the population from which cases are derived are often missing, as is concomitant genetic testing to rule out genetic causes, making it difficult to draw conclusions. To address these issues, this multicenter prospective phenotype-specific study was performed in an area of France with the highest national rate of hypospadias surgery [32]. The study focused on patients with strictly isolated hypospadias after excluding the most frequent genetic defects.

Please cite this article in press as: Kalfa N, et al. Is Hypospadias Associated with Prenatal Exposure to Endocrine Disruptors? A French Collaborative Controlled Study of a Cohort of 300 Consecutive Children Without Genetic Defect. Eur Urol (2015), http://dx.doi.org/10.1016/j.eururo.2015.05.008

2. Patients and methods

2.1. Patients

A total of 408 boys presenting with isolated hypospadias (no micropenis, no cryptorchidism) were included prospectively (newborn to 12 yr). Clinical diagnosis was made via direct clinical examination by a pediatric urologist and/or pediatric endocrinologist. The location of the urethral meatus ranged from glandular to perineal hypospadias (glandular and penile anterior $n = 283$, midshaft $n = 91$, penile posterior $n = 26$, penoscrotal and perineal $n = 8$). The level of division of the corpus spongiosum—which is more reliable for assessing hypospadias severity and is assessed only during degloving of the penis at the time of surgical correction—was not used for classification because some of the patients with an anterior or glandular meatus had not undergone surgery. The length of the penis was measured on the dorsal face from the base of the corpus cavernosum to the top of the stretched penis.

A total of 302 normal boys (no congenital malformation; no urological, genital, or nephrological pathology; no inguinal hernia; no endocrine disease) were included. The reasons for hospitalization were mainly acute appendicitis, idiopathic intussusception, minor abdominal trauma, and pyloric stenosis. Cases and controls were matched for ethnic origin, with Caucasians accounting for 75.9% of the cases, Arabs 19%, Africans 2.2%, and others 2.9%.

The study was approved by the Institutional Review Board (Centre de Protection des Personnes Sud Méditerannée 4, CPPSMIV, ID RCB 2008-A00781-54) and written consent was obtained from all parents.

2.2. Evaluation of environmental exposure

Maternal and paternal occupational and professional exposures to EDCs were evaluated using a standardized questionnaire based on the previously used European questionnaire QLK4-1999-01422 in simplified form and a previously validated job-exposure matrix for EDCs [33,34]. The questionnaire was filled out directly by the surgeon or endocrinologist and not by the parents themselves to limit bias. We collected information on the type of exposure, the type of products, the timing of exposure during pregnancy, the frequency of product use, any concomitant exposure through medication during pregnancy, and previous exposure to DES for mothers and grandmothers. Only repeated exposure was taken into account. The jobs of both parents were recorded. For the mother, exposure data were detailed for the three trimesters of pregnancy. For the father, jobs around the time of fertilization and 1 yr before were noted. Environmental exposure was estimated by geocoding through the zip code at the time of pregnancy, and the types of surrounding hazards and distance to them were determined.

Additional methods, including sequencing methods for *AR*, *SRD5A2*, and *MAMLD1* genes, are presented in the Supplementary material.

3. Results

A total of 108 familial forms with vertical transmission or mutations were identified and these patients were excluded from the study. Thus, 300 patients and 302 controls were finally compared. Missing data were less than 3.3%.

3.1. Overall description of the population

Regarding the severity of phenotype, hypospadias was isolated in all cases, with a glandular or penile anterior meatus in 70% ($n = 210$), a penile midshaft meatus in 21.7% ($n = 65$), a penile posterior meatus in 6% ($n = 18$), and a scrotal meatus in 2.3% ($n = 7$) of cases.

Most of the children, both cases and controls, were born after 37 wk of amenorrhea, but prematurity was more frequent in hypospadiac boys (21% vs 12%, $p = 0.006$). Intrauterine growth retardation (defined as birth weight less than two standard deviations) was not significantly different between the two groups (14% vs 12%) when adjusted by gestation weeks. The rates of twin pregnancy (5.3% vs 6%, $p = 1$) was similar.

3.2. Fetal exposure to EDCs during pregnancy

Fetal exposure to EDCs was defined as exposures for which the fetus was exposed through the mother across the three trimesters of pregnancy. This included occupational and/or professional exposure. The results are presented in Table 1. Overall, fetal exposure to EDCs was more frequent in pregnancies that led to the birth of hypospadiac sons (40.00% vs 17.55%), with an odds ratio (OR) of 3.13 (95% confidence interval [CI] 2.11–4.65). The substances to which fetuses were exposed were paints/solvents/adhesives (16.00%), detergents (11.00%), pesticides (9.00%), cosmetics (5.67%), and other industrial chemicals (4.00%) including metals, polycyclic aromatic hydrocarbons, and herbicides (0.67%). It is notable that most of these exposures occurred around the window of genital differentiation, mainly in the first trimester (78% of all exposures).

3.3. Maternal and paternal professions

Maternal and paternal professions with potential for EDC exposure were taken from the validated job-exposure matrix published by Van Tongeren et al [33,34]. The list of jobs encountered in our series is presented in the

Table 1 – Fetal exposure to endocrine-disrupting chemicals (EDCs) during pregnancy

Exposure	Group, *n* (%)		Odds ratio
	Hypospadias ($n = 300$)	Control ($n = 302$)	(95% CI)
Any EDC			
Exposure	120 (40.00)	53 (17.55)	3.13 (2.11–4.65)
No exposure	180 (60.00)	249 (82.45)	
Paints/solvents			
Exposure	48 (16.00)	15 (4.97)	3.63 (1.94–7.17)
No exposure	252 (84.00)	287 (95.03)	
Detergents			
Exposure	33 (11.00)	17 (5.63)	2.05 (1.08–4.02)
No exposure	267 (89.00)	285 (94.37)	
Pesticides			
Exposure	27 (9.00)	13 (4.30)	2.20 (1.07–4.74)
No exposure	273 (91.00)	289 (95.70)	
Cosmetics			
Exposure	17 (5.67)	7 (2.32)	2.53 (0.98–7.32)
No exposure	283 (94.33)	295 (97.68)	
Other industrial chemicals			
Exposure	12 (4.00)	6 (1.99)	2.05 (0.70–6.76)
No exposure	288 (96.00)	296 (98.01)	
Herbicides			
Exposure	2 (0.67)	2 (0.66)	1.00 (0.07–13.97)
No exposure	298 (99.33)	300 (99.34)	

Please cite this article in press as: Kalfa N, et al. Is Hypospadias Associated with Prenatal Exposure to Endocrine Disruptors? A French Collaborative Controlled Study of a Cohort of 300 Consecutive Children Without Genetic Defect. Eur Urol (2015), http://dx.doi.org/10.1016/j.eururo.2015.05.008

Supplementary material. Jobs with EDC exposure were more frequently performed by the mothers of hypospadiac boys than control boys (19.73% vs 10.26%, $p = 0.0019$; Fig. 1). In descending order of frequency, the mothers of hypospadiac boys were cleaners, hairdressers, beauticians, and laboratory workers. Similarly, paternal job exposure around the time of fertilization was more frequent in the cases of hypospadias (40.13% vs 27.48%, $p = 0.02$; Fig. 1).

In descending order of frequency, the fathers exposed to EDCs having hypospadiac boys were agricultural workers, laboratory technicians, domestic cleaners, mechanics and painters. When we combined the data to examine the

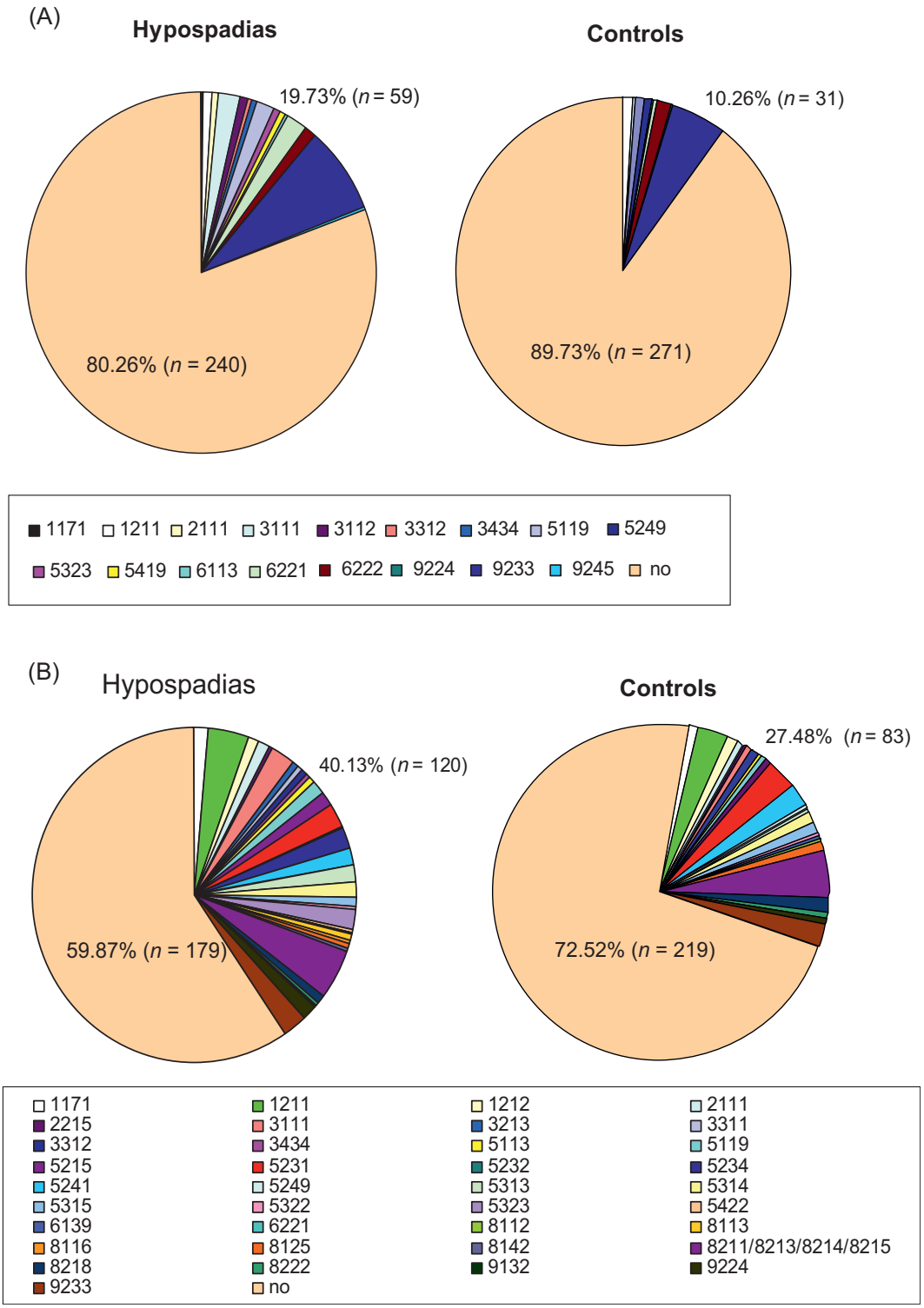

Fig. 1 – Distribution of (A) maternal and (B) paternal professions for the hypospadias and control groups. The light orange area represents jobs with no exposure. Colored areas represent jobs with exposure. Numbers refer to the matrix of Van Tongeren et al [33,34]. Mother's job, $p = 0.0019$; father's job, $p = 0.02$.

Table 2 – Substances corresponding to jobs with exposure for mothers and fathers [a]

	Professional exposure, *n* (%)			
	Maternal		Paternal	
	Hypospadias	Control	Hypospadias	Control
Polyaromatic hydrocarbons	–	1 (0.33)	23 (7.67)	21 (7.00)
Polychlorinated compounds	–	–	–	–
Pesticides	7 (2.33)	6 (1.99)	24 (8.00)	19 (6.33)
Phthalates	11 (3.67)	8 (2.65)	33 (11.00)	14 (4.67)
Organic solvents	47 (15.67)	25 (8.28)	65 (21.67)	39 (13.00)
Bisphenol A	–	–	1 (0.33)	–
Alkylphenolic compounds	38 (64.41)	24 (7.95)	26 (8.67)	17 (5.67)
Flame retardants	–	–	2 (0.67)	–
Metals	7 (12.67)	5 (1.66)	52 (17.33)	44 (14.67)
Miscellaneous	10 (3.33)	5 (1.66)	2 (0.67)	–

[a] Sums may exceed 100% since a job may involve exposure to several chemicals. Correspondence between the type of job and the exposure was established using the data reported by Van Tongeren et al [33,34].

concomitant job exposure of both parents, we found that maternal exposure was the main risk factor, with no clear cumulative effect (Supplementary material).

Each profession exposed the parent to a specific cocktail of chemical substances. On the basis of previous studies, we determined the types of chemicals to which the mothers and fathers were exposed. The main substances were organic solvents and alkylphenolic compounds for mothers, and organic solvents, alkylphenolic compounds, and phthalates for fathers (Table 2).

3.4. *Environmental exposure during pregnancy*

Industrial areas, incinerators, and waste areas were more frequently encountered within a 3-km radius for mothers of hypospadiac boys (13.29% vs 6.64%, $p < 0.00005$; Table 3). The south of France is an area of extensive agriculture, at the crossroads of wine, rice, and fruit production. This type of exposure was thus tested after exclusion of agriculture workers. An area with intensive agriculture was more frequently present within a 3-km radius for mothers of hypospadiac boys (19.44% vs 14.78%, $p = 0.0137$). We tested whether proximity to environmental hazards of any type differed between cases and controls. In addition to being more frequent, the source of potentially contaminated areas was closer for hypospadiac boys than for controls: the mean distance from the source was 1.29 km for cases (variance 1.6) and 1.63 km for controls (variance 1.65; $p = 0.0026$, Supplementary material).

Table 3 – Comparison of the presence of an industrial area, incinerator, waste area, or intensive agriculture within a 3-km radius around the residence of pregnant women

	Group, *n* (%)		*p* value
	Hypospadias	Control	
Industrial area, incinerator, or waste area			
No	220 (36.54)	262 (43.52)	
Yes	80 (13.29)	40 (6.64)	<0.00005
Intensive agriculture			
No	183 (30.40)	213 (35.38)	
Yes	117 (19.44)	89 (14.78)	0.0137
Industrial area, incinerator, or waste area + agriculture			
No	263 (43.69)	286 (47.51)	
Yes	37 (6.15)	16 (2.66)	<0.0023

3.5. *Combination of all exposures*

Although all types of EDC exposure are not comparable, they may have cumulative dose- and time-related effects. Figure 2 summarizes ORs for cases of accumulated types of exposure. The association of maternal occupational and environmental exposures showed the highest risk of hypospadias occurrence.

We did not identify a significant seasonal variation in birth for hypospadias. The severity of phenotypes did not differ according to EDC exposures (Supplementary material). ART was not more frequent for parents of hypospadiac boys than for control parents (6% vs 6.9%, $p = 0.66$). It is suspected that ART, especially in vitro fertilization, contributes to the

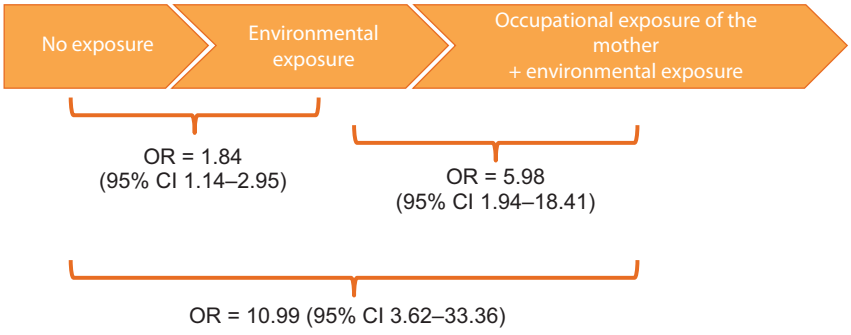

Fig. 2 – Effect of cumulative exposures according to multivariate analysis. OR = odds ratio; CI = confidence interval.

occurrence of hypospadias, but the size of our series did not allow us to confirm these data or to evaluate precisely the effects of each type of ART.

4. Discussion

Despite some experimental evidence, demonstration that EDCs cause hypospadias remains a great challenge since EDCs are ubiquitous and are present in variable mixtures, and their effects are time-dependent and sometimes tenuous or hard to detect. Only rigorous epidemiologic studies can overcome such problems. In most of the previous studies, the series included variable phenotypes with patients extracted from registers, patient inclusion was not prospective, the focus was on one or a limited number of EDCs, control groups were not always included, and genetic causes of hypospadias were not excluded.

The present study was designed to limit these biases. (1) The phenotype was limited to isolated hypospadias without micropenis or cryptorchidism to ensure the most homogeneous group possible. (2) The population was prospectively enrolled; patients were included after clinical examination by a pediatric urologist and/or a pediatric endocrinologist. (3) The main genetic causes, as well as familial cases with vertical transmission, were excluded after systematic sequencing of the candidate genes reported to be potentially mutated in hypospadias (*AR*, *SRD5A2*, and *MAMLD1*). It should be noted that this selection removed more than 25% of the patients initially included that would have easily falsified the results of the study. Nevertheless, the presence of some exceptional genetic defects such as *NR5A1* mutation cannot be totally excluded [35]. (4) The quality of the control group was also a key point. Control group members were not drawn from a data bank but were enrolled after a clinical examination of the genitals and were excluded if there was any endocrine or urinary disease or hernia that could be associated with hypospadias. Given the variation in hypospadias prevalence over time and space [36], matching for ethnic background and sampling from the same geographic areas ensured that the controls were representative of the population from which the cases were derived. (5) All potential sources of exposure were sought and we did not focus on any one candidate job, occupation, or substance.

We found that fetal exposure to EDCs was a significant risk factor for hypospadias in our series (OR 3.8). The types of substance having an impact on the phenotype were heterogeneous, but detergents, pesticides, and cosmetics accounted for 75% of the cases. This finding agrees with previous studies, and our OR is in the high range of data from the literature [11]. This may be explained by the cumulative effect of various substances, as our questionnaire did not focus on a limited number of EDCs or professional activities.

In addition to confirming fetal EDC exposure as a risk factor, the study yields other results of clinical interest. Painters, farmers, and chemists are thought to be highly exposed to EDCs and numerous studies have focused on these professions. However, other jobs that may appear to be benign in fact carry risks. For instance, domestic cleaning seems to have been overlooked in the literature, yet it was the job with an exposure risk most frequently held by the mothers of hypospadiac boys in our series. Moreover, domestic cleaning seems likely to expose workers to a number of aggravating factors. The domestic cleaners in this study often did not protect themselves with gloves and a mask, had no occupational physician assigned for medical follow-up, and were exposed to multiple EDCs as opposed to a single one over entire work sessions. This observation suggests that future studies should widen the scope of what constitutes an at-risk profession.

Another clinical point is the finding that no specific phenotype indicated environmental exposure. The suspicion has been that genetic defects are more frequently found in patients with severe hypospadias and that environmental EDCs induce only minor hypospadias. Our study does not confirm this hypothesis, and environmental exposure to EDCs was associated with a wide range of severity for malformations. EDCs do not show a linear dose-effect relationship and their effect may depend on many factors [37]: the window of exposure during fetal life, the half-life and kinetics of the contaminant, its storage in the placenta and the mother's body fat, the effects of its metabolites, individual genetic susceptibility, and the various mechanisms of genital differentiation that are disturbed, sometimes at multiple levels. Therefore, neither the severity of hypospadias nor the size of the penis according to age differed between exposed and nonexposed cases. A dose-response effect cannot necessarily be assumed: low doses may exert even more potent effects than higher doses [38] and EDCs may result in nontraditional dose-response curves [39].

Our results raise two points of pathophysiologic interest. The first concerns the cumulative effect of various types of exposure. Environmental exposure alone was a risk factor (OR 1.8), but concomitant exposure to professional, occupational, and environmental EDCs significantly increased the OR. To the best of our knowledge, such a cumulative effect has never been reported for hypospadias, but it might correspond to the known experimental effect of a substance cocktail. Low-dose chemical mixtures have an effect in animals [40] and even though some substances alone do not produce an adverse effect, a cocktail of these substances results in a high frequency of genital malformations in offspring [41]. Similarly, a cocktail effect has been suspected in the occurrence of cryptorchidism in humans [42–44]. Contamination of environments is rarely due to a single compound according to the Endocrine Society Scientific Statement on EDCs [38], and the effects of different classes of EDCs may be additive or even synergistic [45]. Assessing the risk of EDCs should no longer be done on a chemical-by-chemical basis, and our work does not support such an approach. The joint actions of multiple substances at different levels of the androgen signaling pathway may be more harmful than chemicals that disrupt a narrowly defined molecular mechanism.

The second pathophysiologic point highlighted by our results is the role of paternal exposure. The notion that paternal EDC exposure is a risk factor for hypospadias has already been discussed, and a meta-analysis demonstrated that occupational exposure to pesticides moderately

Please cite this article in press as: Kalfa N, et al. Is Hypospadias Associated with Prenatal Exposure to Endocrine Disruptors? A French Collaborative Controlled Study of a Cohort of 300 Consecutive Children Without Genetic Defect. Eur Urol (2015), http://dx.doi.org/10.1016/j.eururo.2015.05.008

increases the risk (OR 1.2) [46]. Paternal exposure to biphenolic compounds has also been suspected as a risk factor, but the effect did not reach significance [46]. In the present study, paternal exposure to EDCs was a weaker risk factor than maternal exposure. Nevertheless, paternal work-related exposure around the time of fertilization was more frequent in cases of hypospadias. The most frequent jobs of fathers of hypospadiac boys around the time of fertilization (agricultural workers, laboratory technicians, domestic cleaners, car mechanics, and painters) exposed them to solvents, detergents, and pesticides, and ultimately sum up with exposure due to the mothers' jobs. The mechanism by which paternal exposure plays a role remains to be elucidated. It could be maternal cross-exposure through seminal fluid [46] or an epigenetic mechanism; modifications in DNA methylation and histone acetylation for paternal gametes may modify the regulation of gene expression [47].

Quantification of exposure to all EDCs in our daily environment remains challenging. For instance, it is well known that substantial contamination can arise from cleaning products (parabens), wall and table paints (pesticides), and rugs, carpeting, curtains, and armchairs (flame retardants). Such contamination may not be negligible and these sources should be considered as secondary risk factors for higher environmental risk. Unfortunately, quantification of exposure to these ubiquitous EDCs is particularly difficult and the use of questionnaires may not be reliable enough. Exhaustive dosage calculations for thousands of EDCs at the time of the exposure (during the first trimester of gestation) would provide more objective information about the level of this hidden contamination, but this approach is unrealistic.

5. Conclusions

This multicenter prospective controlled study with a very homogeneous cohort of hypospadiac boys screened for *AR*, *SRD5A2*, and *MAMLD1* mutations strongly suggests that EDCs through occupational, professional, and environmental exposure during fetal life are a risk factor for hypospadias. It further suggests that the association of various exposures of both the mother and father may interfere with the genital development of the male fetus. The effect may not be a simple on/off mechanism but rather a continuum leading to more or less severe malformation. At the individual level, the exact part due to EDC in the occurrence of the malformation remains imprecise: individual differences in exposure to known and unknown substances, metabolism, body composition and genetic susceptibility make the half-life and effects of EDCs highly variable. The potential latency between EDC exposure and the occurrence of hypospadias and the role of chronic exposure to EDCs mean that establishing a direct causal relationship in a given individual is highly complex.

This work was previously presented at the American Urological Association annual meeting, San Diego, May 2013, and the European Society of Pediatric Endocrinology conference, Dublin, September 2014.

Author contributions: Nicolas Kalfa had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Kalfa, Paris, Philibert, Orsini, Broussous, Fauconnet-Servant, Audran, Gaspari, Zahhaf, Daures, Sultan.
Acquisition of data: Kalfa, Paris, Philibert, Orsini, Broussous, Fauconnet-Servant, Audran, Gaspari, Lehors, Haddad, Guys, Reynaud, Alessandrini, Merrot, Wagner, Kurzenne, Bastiani, Bréaud, Valla, Morisson Lacombe, Dobremez, Zahhaf, Daures, Sultan.
Analysis and interpretation of data: Kalfa, Paris, Philibert, Orsini, Broussous, Fauconnet-Servant, Audran, Gaspari, Zahhaf, Daures, Sultan.
Drafting of the manuscript: Kalfa, Paris, Philibert, Orsini, Broussous, Fauconnet-Servant, Audran, Gaspari, Zahhaf, Daures, Sultan.
Critical revision of the manuscript for important intellectual content: Kalfa, Paris, Philibert, Orsini, Broussous, Fauconnet-Servant, Audran, Gaspari, Lehors, Haddad, Guys, Reynaud, Alessandrini, Merrot, Wagner, Kurzenne, Bastiani, Bréaud, Valla, Morisson Lacombe, Dobremez, Zahhaf, Daures, Sultan.
Statistical analysis: Kalfa, Orsini, Broussous, Zahhaf, Daures, Sultan.
Obtaining funding: Kalfa, Paris, Philibert, Sultan.
Administrative, technical, or material support: Broussous, Fauconnet-Servant, Audran.
Supervision: Kalfa, Sultan.
Other: None.

Financial disclosures: Nicolas Kalfa certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: This work was supported by Programme Hospitalier de Rercherche Clinique PHRC UF 8270 and Contrat Fort Chercheur CHUMPT2012. The sponsors did not play a role in the design and conduct of the study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2015.05.008.

References

[1] Kalfa N, Philibert P, Baskin LS, Sultan C. Hypospadias: interactions between environment and genetics. Mol Cell Endocrinol 2011;335:89–95.
[2] Thorup J, Nordenskjold A, Hutson JM. Genetic and environmental origins of hypospadias. Curr Opin Endocrinol Diabetes Obes 2014;21:227–32.
[3] Kalfa N, Cassorla F, Audran F, et al. Polymorphisms of MAMLD1 gene in hypospadias. J Pediatr Urol 2011;7:585–91.
[4] Kalfa N, Fukami M, Philibert P, et al. Screening of MAMLD1 mutations in 70 children with 46,XY DSD: identification and functional analysis of two new mutations. PLoS One 2012;7:e32505.
[5] Kalfa N, Philibert P, Werner R, et al. Minor hypospadias: the "tip of the iceberg" of the partial androgen insensitivity syndrome. PLoS One 2013;8:e61824.
[6] van der Zanden LF, van Rooij IA, Feitz WF, Franke B, Knoers NV, Roeleveld N. Aetiology of hypospadias: a systematic review of genes and environment. Hum Reprod Update 2012;18:260–83.
[7] Berman T, Levine H, Gamzu R, Grotto I. Trends in reproductive health in Israel: implications for environmental health policy. Isr J Health Policy Res 2012;1:34.

[8] Lund L, Engebjerg MC, Pedersen L, Ehrenstein V, Nørgaard M, Sørensen HT. Prevalence of hypospadias in Danish boys: a longitudinal study, 1977–2005. Eur Urol 2009;55:1022–6, Corrigendum. Eur Urol 2009;56:e41.
[9] Fisch H, Lambert SM, Hensle TW, Hyun G. Hypospadias rates in New York state are not increasing. J Urol 2009;181:2291–4.
[10] Sharpe RM, Skakkebaek NE. Testicular dysgenesis syndrome: mechanistic insights and potential new downstream effects. Fertil Steril 2008;89(2 Suppl):e33–8.
[11] Sultan C, Philibert P, Kalfa N. Environment and hypospadias. Dialogues Pediatr Urol 2007;28:8–9.
[12] Edwards TM, Moore BC, Guillette Jr LJ. Reproductive dysgenesis in wildlife: a comparative view. Int J Androl 2006;29:109–21.
[13] Toppari J, Larsen JC, Christiansen P, et al. Male reproductive health and environmental xenoestrogens. Environ Health Perspect 1996; 104(Suppl 4):741–803.
[14] Vinggaard AM, Niemela J, Wedebye EB, Jensen GE. Screening of 397 chemicals and development of a quantitative structure-activity relationship model for androgen receptor antagonism. Chem Res Toxicol 2008;21:813–23.
[15] Fisher JS. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. Reproduction 2004;127:305–15.
[16] Gaspari L, Sampaio DR, Paris F, et al. High prevalence of micropenis in 2710 male newborns from an intensive-use pesticide area of Northeastern Brazil. Int J Androl 2012;35:253–64.
[17] Gaspari L, Paris F, Jandel C, et al. Prenatal environmental risk factors for genital malformations in a population of 1442 French male newborns: a nested case-control study. Hum Reprod 2011;26: 3155–62.
[18] Paris F, Gaspari L, Servant N, Philibert P, Sultan C. Increased serum estrogenic bioactivity in girls with premature thelarche: a marker of environmental pollutant exposure? Gynecol Endocrinol 2013;29: 788–92.
[19] Gaspari L, Paris F, Philibert P, et al. "Idiopathic" partial androgen insensitivity syndrome in 28 newborn and infant males: impact of prenatal exposure to environmental endocrine disruptor chemicals? Eur J Endocrinol 2011;165:579–87.
[20] Gaspari L, Paris F, Jeandel C, Sultan C. Peripheral precocious puberty in a 4-month-old girl: role of pesticides? Gynecol Endocrinol 2011; 27:721–4.
[21] Kalfa N, Paris F, Soyer-Gobillard MO, Daures JP, Sultan C. Prevalence of hypospadias in grandsons of women exposed to diethylstilbestrol during pregnancy: a multigenerational national cohort study. Fertil Steril 2011;95:2574–7.
[22] Nordkap L, Joensen UN, Blomberg Jensen M, Jorgensen N. Regional differences and temporal trends in male reproductive health disorders: semen quality may be a sensitive marker of environmental exposures. Mol Cell Endocrinol 2012;355:221–30.
[23] van der Zanden LF, Galesloot TE, Feitz WF, et al. Exploration of gene-environment interactions, maternal effects and parent of origin effects in the etiology of hypospadias. J Urol 2012;188:2354–60.
[24] Kristensen P, Irgens LM, Andersen A, Bye AS, Sundheim L. Birth defects among offspring of Norwegian farmers, 1967–1991. Epidemiology 1997;8:537–44.
[25] Morales-Suarez-Varela M, Ibanez-Cabanell P, Gimeno-Clemente N, Roig-Garcia JM, Nieto-Garcia MA, Llopis-Gonzalez A. Oral and dental health of non-institutionalized elderly people in Spain. Arch Gerontol Geriatr 2011;52:159–63.
[26] Giordano F, Abballe A, De Felip E, et al. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth Defects Res A Clin Mol Teratol 2010;88:241–50.
[27] Carmichael SL, Yang W, Roberts EM, et al. Hypospadias and residential proximity to pesticide applications. Pediatrics 2013;132: e1216–26.
[28] Dolk H, Vrijheid M, Armstrong B, et al. Risk of congenital anomalies near hazardous-waste landfill sites in Europe: the EUROHAZCON study. Lancet 1998;352:423–7.
[29] Morera AM, Valmalle AF, Asensio MJ, et al. A study of risk factors for hypospadias in the Rhone-Alpes region (France). J Pediatr Urol 2006;2:169–77.
[30] McGlynn KA, Guo X, Graubard BI, Brock JW, Klebanoff MA, Longnecker MP. Maternal pregnancy levels of polychlorinated biphenyls and risk of hypospadias and cryptorchidism in male offspring. Environ Health Perspect 2009;117:1472–6.
[31] Carmichael SL, Herring AH, Sjodin A, et al. Hypospadias and halogenated organic pollutant levels in maternal mid-pregnancy serum samples. Chemosphere 2010;80:641–6.
[32] Paty AC, Gomes do Espirito Santo E, Suzan F. Étude des cryptorchidies et hypospadias opérés en France de 1998 à 2008 chez le petit garçon de moins de 7 ans à partir des données du Programme de médicalisation des systèmes d'information (PMSI). Saint-Maurice, France: Institut de Veille Sanitaire; 2011.
[33] Brouwers MM, Van Tongeren M, Hirst AA, Bretveld RW, Roeleveld N. Occupational exposure to potential endocrine disruptors: further development of a job exposure matrix. Occup Environ Med 2010; 66:607–14.
[34] Van Tongeren M, Nieuwenhuijsen MJ, Gardiner K, et al. A job-exposure matrix for potential endocrine-disrupting chemicals developed for a study into the association between maternal occupational exposure and hypospadias. Ann Occup Hyg 2002;46:465–77.
[35] Philibert P, Paris F, Audran F, et al. Phenotypic variation of SF1 gene mutations. Adv Exp Med Biol 2011;707:67–72.
[36] Fisch H, Hyun G, Hensle TW. Rising hypospadias rates: disproving a myth. J Pediatr Urol 2010;6:37–9.
[37] De Coster S, van Larebeke N. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. J Environ Public Health 2012;2012:713696.
[38] Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocrine Rev 2009;30:293–342.
[39] Vom Saal FS, Akingbemi BT, Belcher SM, et al. Chapel Hill Bisphenol A Expert Panel Consensus Statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod Toxicol 2007;24:131–8.
[40] Kortenkamp A. Low dose mixture effects of endocrine disrupters: implications for risk assessment and epidemiology. Int J Androl 2008;31:233–40.
[41] Christiansen S, Scholze M, Axelstad M, Boberg J, Kortenkamp A, Hass U. Combined exposure to anti-androgens causes markedly increased frequencies of hypospadias in the rat. Int J Androl 2008;31:241–8.
[42] Damgaard IN, Skakkebaek NE, Toppari J, et al. Persistent pesticides in human breast milk and cryptorchidism. Environ Health Perspect 2006;114:1133–8.
[43] Main KM, Kiviranta H, Virtanen HE, et al. Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. Environ Health Perspect 2007;115:1519–26.
[44] Swan SH, Main KM, Liu F, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect Aug 2005;113:1056–61.
[45] Crews D, Putz O, Thomas P, Hayes T, Howdeshell K. Animal models for the study of the effects of mixtures, low doses, and the embryonic environment on the action of endocrine disrupting chemicals. Pure Appl Chem 2003;75:2305–20.
[46] Rocheleau CM, Romitti PA, Dennis LK. Pesticides and hypospadias: a meta-analysis. J Pediatr Urol 2009;5:17–24.
[47] Lane M, Gardner DK. Removal of embryo-toxic ammonium from the culture medium by in situ enzymatic conversion to glutamate. J Exp Zool 1995;271:356–63.

Please cite this article in press as: Kalfa N, et al. Is Hypospadias Associated with Prenatal Exposure to Endocrine Disruptors? A French Collaborative Controlled Study of a Cohort of 300 Consecutive Children Without Genetic Defect. Eur Urol (2015), http://dx.doi.org/10.1016/j.eururo.2015.05.008