**human reproduction**    **OPINION**

# Does a testicular dysgenesis syndrome exist?

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The concept of an increasingly common Testicular Dysgenesis Syndrome (TDS) has been widely adopted with little epidemiological appraisal. In this paper we critically review the epidemiologic evidence of the existence of a non-genetic TDS. We systematically assess and discuss the evidence of all six possible associations between the four defining conditions of TDS: impaired spermatogenesis, undescended testis, hypospadia and testicular cancer. We also evaluate whether there are common risk factors for these four conditions. We conclude that epidemiologic studies provide little support for existence of a widespread TDS because there are no consistent non-causal associations between its different manifestations. There is furthermore little evidence of shared causes between the alleged components of the syndrome.

**Key words:** testicular dysgenesis syndrome / testicular cancer / hypospadias / cryptorchidism / undescended testis

## Introduction

The occurrence of germ-cell testicular cancer has doubled every 20–30 years in many populations for as long as cancer incidence has been measured. Sadly, despite intriguing clues from heterogeneous and salient occurrence patterns and numerous analytic studies, little is known about the causes of testicular cancer (Richiardi et al., 2008). In the early 1990s reports on endocrine disruption in animal wildlife reproduction as well as parallel temporal trends in testicular cancer and other male reproductive disorders led to the hypothesis of a common environmental cause for several diseases of the male reproductive system (Sharpe and Skakkebaek, 1993). In 2001, a Testicular Dysgenesis Syndrome (TDS) was first described encompassing poor semen quality, undescended testis, hypospadia and testicular cancer (Skakkebaek et al., 2001). Now proposed to share environmental causes, these four conditions were previously known to be associated with rare genetic traits such as 45,X/46XY and androgen insensitivity (Muller et al., 1985).

With no operative definition of the syndrome, TDS was the hypothesized result from disrupted embryonal programming and gonadal development. As reflected in a growing number of scientific publications (Fig. 1) the hypothetical TDS has greatly influenced research in the field of male reproductive health. It has indeed been claimed (Muller et al., 1985; Skakkebaek et al., 2001; Boisen et al., 2005) that etiologic research on any of the TDS conditions should take all its four components into account in order to not lose important biological information. Moreover, it has been argued that the TDS concept calls for changes in clinical practice with respect to diagnostic

and follow-up procedures among patients with cryptorchidism and infertility.

The concept of the TDS has been widely adopted and investigated. With little critical appraisal, some studies evaluated if the defining conditions of the syndrome share risk factors, temporal or spatial occurrence patterns, and some of them indeed found signs of congruence (Jorgensen et al., 2001; Boisen et al., 2004, 2005; Richiardi et al., 2004b), although others, such as studies on time trends in population fecundability, did not (Joffe, 2000; Scheike et al., 2008). Ecological and risk factor compatibility can, however, only provide indirect evidence for the existence of a syndrome. It would be far more convincing to see evidence of associations between the conditions in individuals because lack of such association would argue against the syndrome concept (see for example the definition of a syndrome at http://www.nlm.nih.gov/medlineplus/mplusdictionary.html).

In this review, we systematically assess the epidemiologic evidence of all six possible associations (outlined in Fig. 2) between the four defining conditions of TDS: impaired spermatogenesis, undescended testis, hypospadia and testicular cancer. Because the syndrome concept inherently assumes shared etiology rather than causal relations between the included conditions, we also discuss whether these four conditions seem to be different manifestations of a shared pathogenic mechanism, or if there may rather be causal relations between some or several of them (such as cryptorchidism as a cause of testicular cancer). The discussion about the TDS has been often intertwined with the discussion on possible health effects of endocrine disruptors, mixing up two different concepts and hampering a correct evaluation of the evidence (Martin et al., 2008;
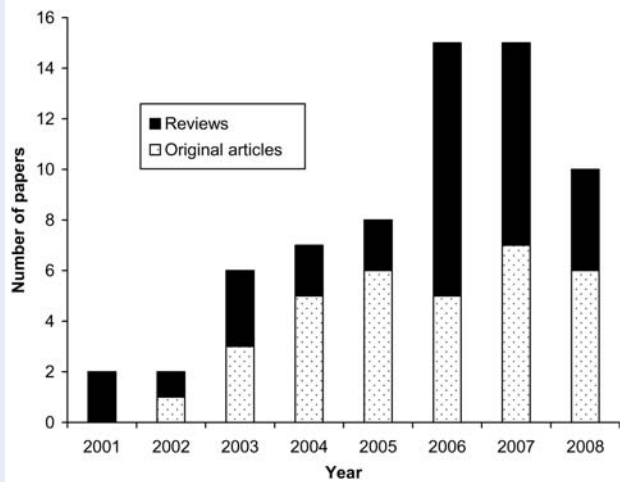
**Figure 1** Number of articles in PubMed [searched for the phrase 'testicular dysgenesis syndrome (TDS)'] to 2008, stratified into original research and reviews, since the TDS hypothesis was put forward in 2001 (Skakkebaek *et al.*, 2001).

**Table I** Malformations statistically significantly associated with cryptorchidism in a population-based case–control study of 2395 cases of cryptorchidism and 9580 controls (Biggs *et al.*, 2002)

| Congenital malformation | No. of exposed cases | OR | 95% CI[a] |
|---|---|---|---|
| Digestive | 27 | 6.8 | 3.7–13 |
| Eye | 6 | 6.0 | 1.7–21 |
| Central nervous system | 13 | 3.3 | 1.6–6.8 |
| *Hypospadias/epispadias* | *41* | *3.2* | *2.1–4.8* |
| Muskoloskeletal | 79 | 2.9 | 2.2–3.9 |
| Cleft lip/palate | 11 | 2.8 | 1.3–6.0 |
| Ear/face/neck | 12 | 2.5 | 1.2–5.2 |
| Heart | 24 | 2.0 | 1.2–3.2 |
| Skin | 60 | 1.4 | 1.1–1.9 |

Malformations are ranked according to the odds ratio (OR) estimate.
[a]CI, confidence intervals.

Sharpe and Skakkebaek, 2008). For sake of clarity and simplicity, this review will focus exclusively on TDS.

# Are the manifestations of TDS associated?

## Undescended testis and hypospadia

An association between cryptorchidism and hypospadia has been demonstrated in a number of investigations, mainly case–control studies on risk factors for cryptorchidism (Hjertkvist *et al.*, 1989; Berkowitz *et al.*, 1995; Akre *et al.*, 1999; Weidner *et al.*, 1999; Biggs *et al.*, 2002; McGlynn *et al.*, 2006). The two birth defects are, however, associated not only with each other but also with other major malformations. In one population-based study among more than 2300 cases of cryptorchidism (summarized in Table I) undescended testis was more strongly associated with the prevalence of digestive, eye and central nervous system malformations than with hypospadia (Biggs *et al.*, 2002). Likewise, in another large population-based study, hypospadia was associated with 3-fold excess risk of cleft lip/palate (95% CI: 1.7–5.4), 19.8-fold excess of anorectal atresia (95% CI: 9.3–42.4) and 6.9-fold excess of esophageal atresia (95% CI: 2.1–22.3) (Aschim *et al.*, 2004).

Cryptorchidism and hypospadias are also associated as components of different genetic syndromes with multiple malformations (Virtanen *et al.*, 2007; Kalfa *et al.*, 2008). The proportion of hypospadias attributable to defined genetic traits is, however, small.

## Undescended testis and subsequent fertility

Undescended testis is strongly associated with impaired spermatogenesis. Although boys with cryptorchidism are born with germ cells, the number of such cells may be reduced and they seem to gradually acquire a lack of germ cells starting around 18 months of age (Cortes *et al.*, 2001). Nearly all men with uncorrected bilateral maldescent testes have azoospermia (Ritzen *et al.*, 2007). Hence, infertility may be due largely to changes that occur if the maldescent testis is left untreated. Supporting this theory, surgical correction in childhood strongly improves sperm concentration, although not all men reach normal levels. Also among men with unilateral cryptorchidism, early treatment improves sperm count, but their overall fertility depends less on proper treatment (Ritzen *et al.*, 2007); reduced paternity rates have been reported after treatment for bilateral (Lee and Coughlin, 2001), but not unilateral (Miller *et al.*, 2001; Lee and Coughlin, 2002), maldescent.

The age at surgical treatment has been gradually decreasing over time and operation is now recommended if spontaneous descent has not occurred at 6 months of age (Ritzen *et al.*, 2007). No studies with long enough follow-up have evaluated whether early intervention can entirely preserve normal testicular function and sperm count. A study measuring testicular growth as an intermediate end-point, however, indicated that further improvement is to be expected by very early surgical treatment (Kollin *et al.*, 2007). Collectively, this evidence does not support that cryptorchidism and subfertility are independent manifestations of a syndrome but rather that the latter is a consequence of the former.

## Undescended testis and testicular cancer

The established association between cryptorchidism and increased risk for testicular cancer has been a back bone in the concept of a TDS. Overall, the risk of testicular cancer among cryptorchid men is increased two to eight times, and 5–10% of all men with testicular cancer have a history of cryptorchidism (Toppari and Kaleva, 1999; Dieckmann and Pichlmeier, 2004). Two models, that are not mutually exclusive, may explain the association: the ectopic position of the testis increases the risk of testicular cancer, or cryptorchidism and testicular cancer share intrauterine and/or genetic causes.

Surgical treatment of undescended testis before puberty decreases the excess risk of testicular cancer among cryptorchid men from 5-fold to 2-fold (Pettersson *et al.*, 2007b) compared with men without
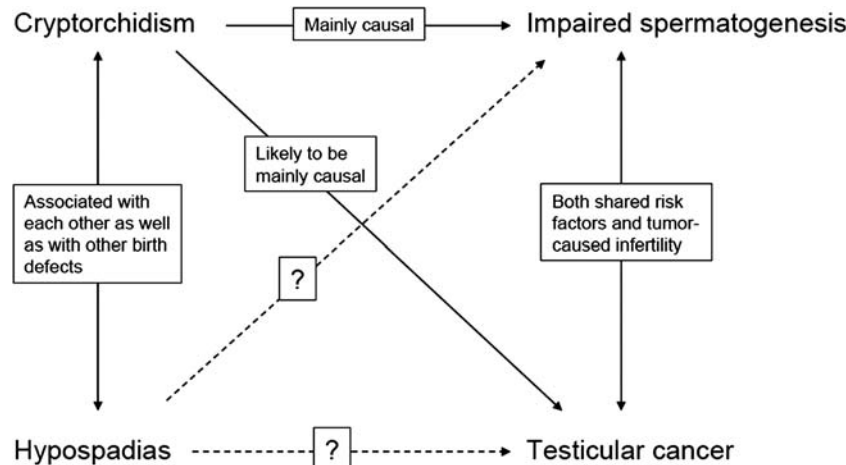
**Figure 2** Summary of the current knowledge of the associations between the four conditions included in the TDS hypothesis. Dashed arrows indicate a lack of studies of the association. Unidirectional solid arrows indicate causal associations. Bidirectional solid arrows indicate associations explained by shared risk factors.

**Table II** Studies on hypospadias and risk of testicular cancer

| Study | Cases (exposed/ unexposed) | Controls (exposed/ unexposed) | Adjusted OR (95% CI)[a] |
|---|---|---|---|
| Swerdlow et al. (1987) | 1/259 | 2/497 | 0.96 (0.02–18.5)[b] |
| UK TC Study Group (1994) | 1/793 | 1/793 | 0.54 (0.03–9.98) |
| Moller et al. (1996) | 2/514 | 0/720 | ∞[b] |
| Prener et al. (1996) | 3/180 | 1/365 | 4.2 (0.4–42.7) |
| Pooled estimate[c] | 7/1746 | 4/2375 | 2.25 (0.48–10.68) |

[a]OR, odds ratio; CI, confidence intervals.
[b]No measure of association, adjusted or crude, was given in these reports. Both studies contribute to the pooled OR with a crude estimate calculated by us. In the study by (Moller et al., 1996) an approximate exact OR was calculated by imputation of 0.1 exposed controls.
[c]We calculated the pooled OR and 95% CIs using fixed-effects meta-analysis (P for homogeneity 0.47).

cryptorchidism. These results, not supported by a similar analysis of Danish data (Myrup et al., 2007), suggested that the ectopic position of the testis at puberty is an important factor in the development of testicular cancer. The 2-fold increase in risk that remained regardless of age at surgery indicates, however, that both shared risk factors and post-natal location of the testicle contribute to tumor development. Because an undescended testis can descend spontaneously during puberty (Sijstermans et al., 2006), cryptorchidism that persists through puberty may be more strongly associated with testicular cancer independent of surgical intervention. This would imply that the ectopic position does not have an effect on testicular cancer, and that the increased risk in cryptorchid boys would be entirely

due to shared risk factors. This hypothesis has, however, not been tested.

Further clues to the role of ectopic position versus shared risk factors as part of a syndrome, are provided by studies of cancer risk in the contralateral testis among men with unilateral cryptorchidism. Although many studies have attempted to address this question (Morrison, 1976; Henderson et al., 1979; Schottenfeld et al., 1980; Coldman et al., 1982; Pottern et al., 1985; Strader et al., 1988; United Kingdom Testicular Cancer Study Group, 1994; Moller et al., 1996; Prener et al., 1996; Swerdlow et al., 1997; Stang et al., 2001; Herrinton et al., 2003) they were all hampered by low statistical power due to small number of cases. In a recent meta-analysis (Akre et al., 2009), we found distinct differences in risk between the two testes, with a pooled relative risk for testicular cancer of 6.33 (95% CI, 4.30–9.31) in the ipsilateral and of 1.74 (95% CI, 1.01–2.98) in the contralateral testis. These results indicate that some risk determinants may be shared between undescended testis and testicular cancer. Most of the excess cancer risk appears, however, caused by a local effect of the ectopic position.

## Hypospadia and fertility

Among men with a misplaced urethral orifice, only those with a proximal urethral meatus, encounter problems in fathering children (Dodds et al., 2008). In a survey of 56 adult men with uncorrected hypospadia of varying degrees, 41 out of 43 men who had attempted fertility, had been successful; the two infertile men had significant oligospermia. We have found no study assessing sperm quantity or quality among men with hypospadia. Hence, the proposed association between hypospadia and infertility as components of the TDS remains to be scientifically documented.

## Hypospadia and risk of testicular cancer

Apart from the increased risk of testicular cancer in patients with some rare genetic traits that include hypospadia, there is no evidence of a general association between hypospadia and testicular cancer. In

the four studies evaluating the hypothesis (Swerdlow *et al.*, 1987; United Kingdom Testicular Cancer Study Group, 1994; Moller *et al.*, 1996; Prener *et al.*, 1996) findings are inconclusive because none of them had more than three exposed cases or controls (Table II). Larger studies with the power to detect a possible association between hypospadia and risk of testicular cancer seem, therefore, fundamental. The evaluation of an association between an outcome with an occurrence of 5 per 100 000 person years and a malformation with a prevalence of around 5 per 1000 live births is a challenge, though, and could only be done in a large source population where both conditions have been reliably registered over many years.

## Fertility and testicular cancer

Overwhelming evidence indicates that testicular cancer patients have an impaired fertility at the time of the cancer diagnosis. However, as for cryptorchidism, there is debate on the underlying mechanism. The main alternatives are: (i) shared genetic causes, (ii) shared environmental risk factors or (iii) impaired fertility being a consequence of the malignant disease. Case–control studies assessing past fertility through questionnaires have, in general, found a positive association between reduced fertility and testicular cancer risk (Depue *et al.*, 1983; Brown *et al.*, 1986; Haughey *et al.*, 1989; Swerdlow *et al.*, 1989; United Kingdom Testicular Cancer Study Group, 1994; Baker *et al.*, 2005; Doria-Rose *et al.*, 2005). Interpretation of these studies is hampered by problems of misclassification of the exposure status that may be both differential and non-differential between cases and controls. However, large studies carried out in Scandinavia have consistently shown decreased paternity among testicular cancer patients (Moller and Skakkebaek, 1999; Fossa and Kravdal, 2000; Jacobsen *et al.*, 2000b; Richiardi *et al.*, 2004a) several years before the cancer diagnosis.

One Danish study has prospectively evaluated the association between semen characteristics and testicular cancer risk (Jacobsen *et al.*, 2000a). In this study about 32 000 men from couples with fertility problems—who had an analysis of the semen—were followed-up for testicular cancer. In this cohort, risk of testicular cancer was 60% higher compared with the general population, with a higher excess risk among men with more than one semen abnormality. Evaluation of the relative risk with follow-up time is important in order to distinguish between an effect of shared risk factors from the possible effect of an undetected tumor on fertility. The relative risks of testicular cancer were 1.8 (95% CI, 1.1–2.7) in the first 2 years after the semen analysis, 1.5 (95% CI, 1.0–2.1) between 3 and 6 years after the analysis, 1.6 (95% CI, 1.0–2.3) between 7 and 11 years and 1.3 (95% CI, 0.7–2.3) after at least 12 years suggesting that an effect of a subclinical testicular tumor cannot entirely explain impaired semen quality. A similar study has been recently carried out in the USA (Walsh *et al.*, 2009), in which approximately 4500 men with male factor infertility had a relative risk of testicular cancer of 2.8 (95% CI, 1.5–4.8) compared with the general population. Analyses by follow-up time revealed relative risks of 4.0 (95% CI, 1.4–8.6; six observed cases among the infertile men) between 1 and 3 years after infertility evaluation, 2.8 (95% CI, 0.9–6.6; five cases) between 4 and 7 years, 1.0 (95% CI, 0.0–5.4, 1 case) between 8 and 11 years and 3.6 (95% CI, 0.1–19.8, one case) between 12 and 15 years. Because of the small number of cases in some of these

follow-up categories, we have roughly re-calculated the relative risks based on data provided in the original paper (Walsh *et al.*, 2009) and found that the relative risk of 4.0 (six observed cases) for the period between 1 and 3 years after the infertility evaluation decreased to 2.3 (seven observed cases) for the subsequent years.

# Is there a common pathogenic mechanism for the manifestations of TDS?

Identification of a common pathogenic mechanism for its four proposed components would support the existence of a TDS. Several studies have attempted to identify a shared risk factor, and some of the exposures that have been scientifically evaluated are reviewed in this section.

## Diethylstilbestrol and other exogenous estrogens

A recent comprehensive quantitative meta-analysis (Martin *et al.*, 2008) on exogenous hormones and risk for TDS, found excess risk for undescended testis, hypospadia and testicular cancer among men with prenatal exposure to estrogen diethylstilbestrol (DES). The pooled relative risk estimate for testicular cancer was 2.47 (95% CI, 0.61–10.00). The risk of hypospadia was significantly increased (2.14; 95% CI, 1.15–3.98) but three out of five studies in that analysis evaluated 3rd generation exposure rather than direct prenatal exposure, and these two exposures are fundamentally different. Finally, the risk of cryptorchidism was increased significantly in the fixed effects analyses, but not in the random effects analysis, indicating heterogeneity between studies. Moreover, some of the included studies that reported strong associations were of questionable validity. In the meta-analysis of estrogenic compounds other than DES, the pooled relative risk was 0.93 (95% CI, 0.89–1.09) for hypospadia, based on seven studies, and 1.06 (95% CI, 0.70–1.59) for cryptorchidism, based on three studies. Hence, prenatal exposure to exogenous hormones other than DES is not documented to be associated with any component of TDS (Storgaard *et al.*, 2006; Martin *et al.*, 2008).

Offspring fertility following prenatal DES exposure was not evaluated in the meta-analysis. Although there is weak evidence that high doses of DES reduce sperm count in the male offspring (Wilcox *et al.*, 1995; Storgaard *et al.*, 2006), lower doses do not affect male fertility (Handelsman, 2001; Storgaard *et al.*, 2006). DES, however, causes a plethora of other outcomes unrelated to male reproductive function, including vaginal clear-cell carcinoma (Herbst *et al.*, 1971) and breast cancer (Palmer *et al.*, 2006) in the female offspring, ectopic pregnancy, spontaneous miscarriage, premature birth and neonatal death (Kaufman *et al.*, 2000).

## Low birthweight

Low birthweight and fetal growth retardation are both strongly associated with cryptorchidism (Berkowitz *et al.*, 1993; Thong *et al.*, 1998; Akre *et al.*, 1999; Weidner *et al.*, 1999; Boisen *et al.*, 2004; Preiksa *et al.*, 2005; Virtanen and Toppari, 2008) and hypospadia (Akre *et al.*, 1999; Aschim *et al.*, 2004; Brouwers *et al.*, 2007; Chong *et al.*, 2006; Hussain *et al.*, 2002). The joint effect of being

small-for-gestational-age (SGA) and born preterm is strong; boys born SGA in gestational week 32 or earlier were at a 7-fold excess risk of cryptorchidism and a 5-fold excess risk of hypospadia compared with those born at term with a normal weight (Akre *et al.*, 1999).

Although several investigators consider low birthweight a risk factor for testicular cancer (Virtanen *et al.*, 2005; Main *et al.*, 2006; Nori *et al.*, 2006), the epidemiologic evidence is weak (Richiardi *et al.*, 2007). A pooled analysis of 12 studies found an overall odds ratio (OR) (comparing <2500 versus >2500 g in most studies) of 1.28 (95% CI, 0.99–1.65). The pooled estimate from questionnaire-based studies was 1.58 (95% CI, 1.12–2.23) (Depue *et al.*, 1983; Brown *et al.*, 1986; Moss *et al.*, 1986; Moller and Skakkebaek, 1997; Petridou *et al.*, 1997; Weir *et al.*, 2000; Coupland *et al.*, 2004), whereas the estimate from studies with more reliable register-based birthweight data was 1.01 (95% CI, 0.73–1.40) (Malone and Daling, 1986; Sabroe and Olsen, 1998; Richiardi *et al.*, 2002; English *et al.*, 2003; Aschim *et al.*, 2005). In four subsequent studies of low birthweight and testicular cancer, two found ORs of 1.31 (95% CI, 0.82–2.10) (Cook *et al.*, 2008) and 1.54 (no CI reported) (Ahlgren *et al.*, 2007) for birthweights below 2500 g. We found a non-significantly decreased OR for the lowest compared with the middle tertile (OR, 0.73; 95% CI, 0.48–1.11) (Pettersson *et al.*, 2007a). Finally, Sonke *et al.* (2007) compared subjects with a birthweight of less than 3000 versus 3000–4000 g, and found an OR of 2.4 (95% CI, 0.7–8.1). No interaction between birthweight and short gestational duration has been reported from studies of testicular cancer.

Three studies assessed birthweight in relation to subsequent sperm count (Francois *et al.*, 1997; Olsen *et al.*, 2000; Auger *et al.*, 2001), and in the two studies with more valid designs (Olsen *et al.*, 2000; Auger *et al.*, 2001) sperm parameters were slightly better among those with a low than those with a normal birthweight.

In conclusion, there is no consistent association between birthweight and the four components of the hypothesized TDS.

## Pre-eclampsia

Boys exposed *in utero* to pre-eclampsia (Jones *et al.*, 1998; Akre *et al.*, 1999; McGlynn *et al.*, 2006) are not at increased risk of cryptorchidism. There is, however, consistent evidence of a strong positive association between pre-eclampsia or preeclamptic symptoms and hypospadia (Akre *et al.*, 1999; Aschim *et al.*, 2004; Chong *et al.*, 2006; Akre *et al.*, 2008) with an about 80% increased risk in two Scandinavian studies (Aschim *et al.*, 2004; Akre *et al.*, 1999). The Swedish study also found a dose–response relation between severity of pre-eclampsia and risk (Akre *et al.*, 1999), while the Norwegian study reported an association between hypospadia and retained placenta (Aschim *et al.*, 2004). Moreover, Fujimoto *et al.* (2008) found a significantly higher prevalence of placental abnormalities among cases with hypospadia than among healthy controls.

Only few studies have assessed pre-eclampsia and risk of testicular cancer in the offspring. In a prospective study, we recently (Pettersson *et al.*, 2008), found a substantial decrease in risk associated with severe gestational hypertension (OR, 0.29; 95% CI, 0.12–0.74), and a similarly negative but non-significant association with pre-eclampsia. We also found an increased risk associated with mild gestational hypertension suggesting pathophysiological differences between severe and mild hypertension. Based on these data, it seems unlikely that testicular cancer is strongly positively associated with pre-eclampsia. And the risk patterns associated with pre-eclampsia and placental abnormalities seem heterogeneous among the TDS components.

# Summary, discussion and conclusions

Associations between the components in the hypothesized TDS and shared risk factors are essential for the syndrome. After having reviewed the evidence, we conclude the following (see also Fig. 2):

  (i) Cryptorchidism and hypospadia are associated, but the association is equally strong or stronger with other non-genital birth defects.

  (ii) Cryptorchidism causes infertility, and it remains elusive whether any part of the association is due to shared causes.

  (iii) Cryptorchidism causes testicular cancer. Part of the association may, however, be due to shared causes.

  (iv) It is unknown whether hypospadia is associated with infertility or testicular cancer.

  (v) Infertility or subfertility and testicular cancer are associated, and it seems unlikely that the association is due entirely to reverse causality.

  (vi) There are no known common risk factors for the four components of the TDS apart from genetic traits.

Thus, epidemiologic studies provide little support for existence of a widespread TDS because there are no consistent non-causal associations between its manifestations.

When the TDS hypothesis was put forward in 2001 (Skakkebaek *et al.*, 2001), it was proposed that etiologic studies should attempt to study components of the syndrome together instead of separately. Several investigators, including us, have analyzed and interpreted data accordingly (Moller *et al.*, 1996; Moller and Skakkebaek, 1996; Akre *et al.*, 1999; Aschim *et al.*, 2004; Carbone *et al.*, 2007). In the light of current knowledge, we now argue that this approach may in fact be counterproductive. The search for causes of undescended testis, hypospadia, infertility and testicular cancer must remain unprejudiced, to allow for heterogeneity in their etiologies. In particular, pooling these outcomes together in studies will most certainly conceal important biological and etiologic information.

The TDS concept seems also to have a limited clinical utility. First, the search for other birth defects among children with a diagnosed malformation is part of clinical routine regardless of the TDS notion. Second, the treatment of cryptorchidism is entirely accounted for by the concern that cryptorchidism seems to cause infertility and testicular cancer (Ritzen *et al.*, 2007). Third, clinical examination and ultrasonography of the testicles may be merited in the diagnostic work up of men seeking help for fertility problems regardless of the existence of a TDS.

Geographical and temporal correlations between components of the TDS have frequently been invoked as evidence of the existence of the syndrome (Olesen *et al.*, 2007; Skakkebaek *et al.*, 2007; Sharpe and Skakkebaek, 2008; Sonne *et al.*, 2008). It is beyond the scope of this review to scrutinize such correlations; while ecological correlations can be both appealing and effective in generating new

hypotheses, they cannot document the existence of a syndrome. In addition, a clear operative definition of the TDS has, to our knowledge, never been put forward. Apart from a definition based on the association between the components, different views of TDS prevail in the literature. It has been indicated (Skakkebaek *et al.*, 2003), for instance, that a man has the syndrome when he has one of the four conditions in combination with certain pathological changes in the testicular histology, such as microliths, Sertoli-cell only, or undifferentiated Sertoli cells (Skakkebaek *et al.*, 2003). Notwithstanding the need for a clear definition in order to test the TDS hypothesis, associations between all four component conditions (undescended testis, hypospadias, impaired sperm production and testicular cancer) seem to be essential for the TDS concept.

In conclusion, the TDS hypothesis has stimulated research in the field of male reproduction, and provided a framework for the design of new studies and interpretation of results. This framework appears premature and hampers flexibility of research. Thus, we suggest that clinical and etiological research prioritize the understanding of the four component diseases separately. Assessing ecological correlations between the TDS conditions are unlikely to advance biologic understanding. We would find it more productive to design studies to challenge the TDS hypothesis with more truly hypothesis-testing designs.

# Authors' role

Both authors have equally contributed to the review of the literature, its interpretation and manuscript writing.

# Acknowledgements

# Funding

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