



Review

Testicular dysgenesis syndrome and the development and occurrence of male reproductive disorders

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Abstract

Patients with 45,X0/46XY karyotype often present with intersex phenotype and testicular dysgenesis. These patients may also have undescended testes (cryptorchidism), hypospadias and their spermatogenesis is severely disrupted. They have a high risk for testicular cancer. These patients have the most severe form of testicular dysgenesis syndrome (TDS). We have hypothesized that testicular cancer, cryptorchidism, hypospadias and poor spermatogenesis are all signs of a developmental disturbance that was named as testicular dysgenesis syndrome. The hypothesis is based on clinical and epidemiological findings and on biological and experimental evidence. Signs of TDS share several risk factors, such as small birth weight (particularly being small for gestational age), and they are risk factors for each other. All of them have background in fetal development. They show strong epidemiological links so that countries with high incidence of testicular cancer, such as Denmark, tend to also have high prevalence rates of cryptorchidism and hypospadias and poor semen quality. Vice versa, in countries with good male reproductive health, e.g., in Finland, all these aspects are better than in Denmark. Although genetic abnormalities can cause these disorders, in the majority of cases, the reasons remain unclear. Adverse trends in the incidence of male reproductive disorders suggest that environmental and life style factors contribute to the problem. Endocrine disrupters are considered as prime candidates for environmental influence. Fetal exposure to high doses of dibutyl phthalate was shown to cause a TDS-like phenotype in the rats. Studies are underway to assess whether there is any exposure–outcome relation with selected chemicals (persistent organic pollutants, pesticides, phthalates) and cryptorchidism.

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Introduction

Several investigators have reported adverse trends in male reproductive health, including increasing incidence of testicular cancer (Adami et al., 1994; Forman and

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Moller, 1994), low and probably declining semen quality (Carlsen et al., 1992; Swan et al., 1997; Andersen et al., 2000) and increasing incidences of cryptorchidism, i.e., undescended testis and hypospadias (Chilvers et al., 1984; Matlai and Beral, 1985; Toppari et al., 1996; Paulozzi et al., 1997). Both the increase of the incidence of testicular cancer and decrease in sperm quality have shown a birth-cohort effect (Irvine, 1994; Bergstrom et al., 1996), meaning that there are more problems in younger generations. It has been proposed that all these disorders have common origin in fetal life and thus they all represent different symptoms of the same underlying entity called the testicular dysgenesis syndrome (TDS) (Skakkebaek et al., 2001; Sharpe, 2003; Asklund et al., 2004).

Clinical evidence for the existence of testicular dysgenesis in male reproductive disorders comes from the observation that patients with rare genetic abnormalities that cause testicular dysgenesis (like 45X0/46XY and androgen insensitivity) are associated with high risk of testicular cancer, often combined with cryptorchidism and hypospadias (Aarskog, 1970; Scully, 1981; Savage and Lowe, 1990). Testicular germ cell cancer arises from carcinoma in situ (CIS) cells, which are presumed to derive from primordial germ cells that escaped normal fetal differentiation (Skakkebaek et al., 1987; Rajpert-De Meyts et al., 1998). Furthermore, in patients with testicular cancer, subfertility or cryptorchidism, dysgenetic changes have been found in the histology of testis (Sohval, 1954, 1956; Berthelsen and Skakkebaek, 1983; Huff et al., 1993; Hoei-Hansen et al., 2003; Skakkebaek et al., 2003).

The observation that the occurrence of one disorder increases the risk of the occurrence of another disorder gives epidemiological evidence for the existence of TDS, e.g., the incidence of undescended testis and /or hypospadias is increased among patients having testicular cancer (Skakkebaek et al., 2001). Furthermore, the disorders of male reproductive health discussed in this paper have shared common risk factors. For example low birth weight compared to the duration of pregnancy, i.e., being born as small for gestational age (SGA) increases the risk of testicular cancer, cryptorchidism and hypospadias (Moller and Weidner, 1999; English et al., 2003). Also, reduced testicular size and low inhibin B levels in postpubertal boys have been linked to SGA (Cicognani et al., 2002), which supports a link between low birth weight and lower fertility in adulthood. However, results of the effect of low birth weight on sperm counts in adult men have been contradictory (Francois et al., 1997; Olsen et al., 2000; Ozturk et al., 2001; Sharpe and Franks, 2002).

In the following we present comparison of the incidence of TDS linked diseases in two Nordic countries, Denmark and Finland.

Cryptorchidism and hypospadias

Comparison of registry-based information on cryptorchidism and hypospadias may have pitfalls because of underreporting, differences in registration systems, and inclusion criteria (Toppari et al., 2001). Therefore, prospectively designed cohort studies have been performed in Denmark and Finland to assess the incidence of these disorders during the years 1997–2001. The results of these studies showed that at birth, the rate of congenital cryptorchidism was significantly higher in Denmark as compared to Finland (9.0% in Denmark and 2.4% in Finland, Fig. 1) (Boisen et al., 2004). The geographical difference was still present at the age of three months even though the rates had declined in both countries (rate was 1.9% in Denmark and 1.0% in Finland) (Boisen et al., 2004). In Denmark, the rate has significantly increased as compared to the rate reported in the 1960s (Buemann et al., 1961). In addition, the present Danish cryptorchidism rate at birth is approximately two-fold as compared to rates given by cohort studies performed in other countries in the 1980s and 1990s (Group, 1992; Berkowitz et al., 1993; Thong et al., 1998; Boisen et al., 2004). In Finland, temporal trend analysis was not possible because no previous clinical studies have been performed in this area.

The birth rate of hypospadias in Finland was 17.0 per 10 000 total live births, which indicates that 0.33% of live-born boys had hypospadias (Virtanen et al., 2001). During the study period, only one serious hypospadias was detected, which indicated that the rate of serious hypospadias is low in Finland (Virtanen et al., 2001). No indication of an increase in the birth rate of hypospadias in Finland was found when the results of this cohort study were compared to data including previously operated hypospadias cases (Aho et al., 2000). In Denmark also the rate of hypospadias was higher as compared to Finland (Boisen et al., in press).

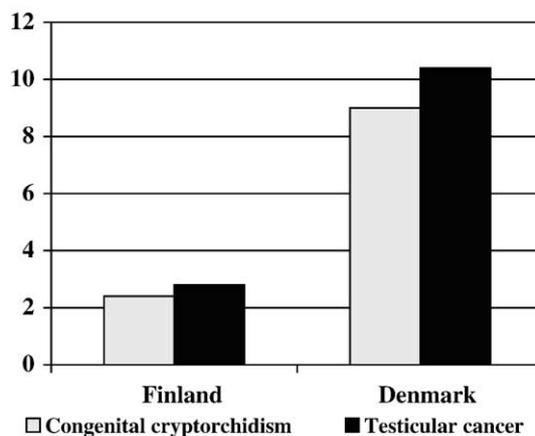


Fig. 1. Incidence of congenital cryptorchidism (% of newborn boys) and testicular cancer (age-standardized (world standard population) incidence per 1000 person-years) in Finland and Denmark.

Recently published cross-sectional study showed that in the Netherlands, the hypospadias rate was over two-fold as compared to Finland (Pierik et al., 2002).

Testicular cancer

The first large European collaborative study on testicular cancer incidence was based on cancer registries, which in Nordic countries have almost 100% coverage of incident cancers. This study showed that the incidence of testicular cancer has increased in both countries, and that since the 1950s, when the Finnish cancer registry started, the rates in Denmark have been higher than those in Finland (Adami et al., 1994). The age-standardized (world standard population) incidence in 1980 reported in this study was 7.8 per 100 000 person-years in Denmark and 1.3 per 100 000 person-years in Finland. The same increasing trend in both countries and the existence geographical difference seem to have continued, since in Denmark, the incidence was 10.4 per 100 000 person-years in 1996 and in Finland, it was 2.8 per 100 000 person-years in 1997 (Fig. 1) (Ferlay et al., 2001).

Semen quality

Co-ordinated cross-sectional studies have shown differences in semen quality between Finnish and Danish men: In fertile men sperm concentration and total sperm counts were higher in Finland as compared to Denmark (Jorgensen et al., 2001). Also, young men not selected due to fertility have higher sperm concentrations in Finland than in Denmark (medians $54 \times 10^6/\text{ml}$ and $41 \times 10^6/\text{ml}$, respectively) (Jorgensen et al., 2002). Furthermore, as many as 40% of young adult Danish men not selected for fertility have been shown to have sperm concentration below the level of $40 \times 10^6/\text{ml}$ (Andersen et al., 2000), which has been associated with decreased fertility (Bonde et al., 1998). According to a retrospective analysis, the sperm density and total sperm counts of infertile men in Finland have been high (over $80 \times 10^6/\text{ml}$) and have not changed significantly between the years 1967–1994 (Vierula et al., 1996). The sperm concentrations of normal men reported in this study were higher than those recently reported of young Finnish men (Jorgensen et al., 2002). However, reliable indication of possible time trends in semen qualities requires prospective studies (Irvine, 1996).

The consistent higher incidence of all disorders in Denmark gives a strong evidence for the existence of TDS as a common cause to these disorders. In addition to this geographical difference, increases in the incidences of testicular cancer in both countries and cryptorchidism in Denmark were observed. However, evaluation of possible changes in hypospadias rates or sperm concentrations has to

Table 1

Examples of endocrine disrupting chemicals, which have been shown to cause TDS-linked disorders in animal exposure studies

Chemicals with oestrogenic activity
Diethylstilbestrol (pharmaceutical)
Ethinyl oestradiol (pharmaceutical)
Bisphenol A (industrial chemical)
Chemicals with antiandrogenic activity
Flutamide (pharmaceutical)
Vinclozolin (fungicide)
Procymidone (fungicide)
1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) (congener of DDT pesticide)
Dibutyl phthalate (DBP) (industrial chemical)
Diethylhexyl phthalate (DEHP) (industrial chemical)

be postponed until the appearance of comparable prospective studies in the future.

The rapid increase of the incidence of reproductive disorders and geographical clustering of the symptoms indicate that environmental or life-style factors, rather than an accumulation of genomic structural defects, are the most likely causes of TDS. Initially, it was hypothesized that increased oestrogen exposure in utero might be related to the increase in the incidence of male reproductive disorders in humans (Sharpe and Skakkebaek, 1993). This oestrogen hypothesis has been expanded to include also environmental chemicals that disrupt the function of the endocrine system also by acting as anti-androgens (Toppari et al., 1996). Animals experimentally exposed to endocrine disrupting chemicals have similar reproductive disorders which in humans have been linked to TDS, except for the germ cell cancer (Table 1) (Skakkebaek et al., 2001; Damgaard et al., 2002; Fisher, 2004). Recently, in utero, exposure of the rat to dibutyl phthalate (DBP) has been proposed to be a possible model to human testicular dysgenesis syndrome, since the testicular and other changes in DBP-exposed rats have been reported in human TDS (Fisher et al., 2003).

Fat tissue samples of cryptorchid boys have been shown to have higher bioaccumulation of heptachloroepoxide and hexachlorobenzene as compared to controls (Hosie et al., 2000). It remains to be seen whether any exposure–outcome relation with selected chemicals (persistent organic pollutants, pesticides, phthalates) and cryptorchidism will be seen in Finnish and Danish cryptorchidism studies.

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References

- Aarskog, D., 1970. Clinical and cytogenetic studies in hypospadias. *Acta Paediatr. Scand. (Suppl. 203)*, 201+.
- Adami, H.O., Bergstrom, R., Mohner, M., Zatonski, W., Storm, H., Ekblom, A., Tretli, S., Teppo, L., Ziegler, H., Rahu, M., et al., 1994. Testicular cancer in nine northern European countries. *Int. J. Cancer* 59, 33–38.
- Aho, M., Koivisto, A.M., Tammela, T.L., Auvinen, A., 2000. Is the incidence of hypospadias increasing? Analysis of Finnish hospital discharge data 1970–1994. *Environ. Health Perspect.* 108, 463–465.
- Andersen, A.G., Jensen, T.K., Carlsen, E., Jorgensen, N., Andersson, A.M., Krarup, T., Keiding, N., Skakkebaek, N.E., 2000. High frequency of sub-optimal semen quality in an unselected population of young men. *Hum. Reprod.* 15, 366–372.
- Asklund, C., Jorgensen, N., Kold Jensen, T., Skakkebaek, N.E., 2004. Biology and epidemiology of testicular dysgenesis syndrome. *BJU Int.* 93 (Suppl. 3), 6–11.
- Bergstrom, R., Adami, H.O., Mohner, M., Zatonski, W., Storm, H., Ekblom, A., Tretli, S., Teppo, L., Akre, O., Hakulinen, T., 1996. Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. *J. Natl. Cancer Inst.* 88, 727–733.
- Berkowitz, G.S., Lapinski, R.H., Dolgin, S.E., Gazella, J.G., Bodian, C.A., Holzman, I.R., 1993. Prevalence and natural history of cryptorchidism. *Pediatrics* 92, 44–49.
- Berthelsen, J.G., Skakkebaek, N.E., 1983. Gonadal function in men with testis cancer. *Fertil. Steril.* 39, 68–75.
- Boisen, K.A., Kaleva, M., Main, K.M., Virtanen, H.E., Haavisto, A.M., Schmidt, I.M., Chellakooty, M., Damgaard, I.N., Mau, C., Reunanen, M., Skakkebaek, N.E., Toppari, J., 2004. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet* 363, 1264–1269.
- Bonde, J.P., Ernst, E., Jensen, T.K., Hjollund, N.H., Kolstad, H., Henriksen, T.B., Scheike, T., Giwercman, A., Olsen, J., Skakkebaek, N.E., 1998. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet* 352, 1172–1177.
- Buemann, B., Henriksen, H., Villumsen, A.L., Westh, A., Zachau-Christiansen, B., 1961. Incidence of undescended testis in the newborn. *Acta Chir. Scand. (Suppl. 283)*, 289–293.
- Carlsen, E., Giwercman, A., Keiding, N., Skakkebaek, N.E., 1992. Evidence for decreasing quality of semen during past 50 years. *BMJ* 305, 609–613.
- Chilvers, C., Pike, M.C., Forman, D., Fogelman, K., Wadsworth, M.E., 1984. Apparent doubling of frequency of undescended testis in England and Wales in 1962–81. *Lancet* 2, 330–332.
- Cicognani, A., Alessandroni, R., Pasini, A., Pirazzoli, P., Cassio, A., Barbieri, E., Cacciari, E., 2002. Low birth weight for gestational age and subsequent male gonadal function. *J. Pediatr.* 141, 376–379.
- Damgaard, I.N., Main, K.M., Toppari, J., Skakkebaek, N.E., 2002. Impact of exposure to endocrine disruptors in utero and in childhood on adult reproduction. *Best Pract. Res., Clin. Endocrinol. Metab.* 16, 289–309.
- English, P.B., Goldberg, D.E., Wolff, C., Smith, D., 2003. Parental and birth characteristics in relation to testicular cancer risk among males born between 1960 and 1995 in California (United States). *Cancer Causes Control* 14, 815–825.
- Ferlay, J., Bray, F., Pisani, P., Parkin, D.M., 2001. GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. IARC Cancerbase, Vol. 5. IARC Press, Lyon.
- Fisher, J.S., 2004. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. *Reproduction* 127, 305–315.
- Fisher, J.S., Macpherson, S., Marchetti, N., Sharpe, R.M., 2003. Human ‘testicular dysgenesis syndrome’: a possible model using in-utero exposure of the rat to dibutyl phthalate. *Hum. Reprod.* 18, 1383–1394.
- Forman, D., Moller, H., 1994. Testicular cancer. *Cancer Surv.* 19–20, 323–341.
- Francois, I., de Zegher, F., Spiessens, C., D’Hooghe, T., Vanderschueren, D., 1997. Low birth weight and subsequent male subfertility. *Pediatr. Res.* 42, 899–901.
- Group, J.R.H.C.S., 1992. Cryptorchidism: a prospective study of 7500 consecutive male births, 1984–8. John Radcliffe Hospital Cryptorchidism Study Group. *Arch. Dis. Child.* 67, 892–899.
- Hoei-Hansen, C.E., Holm, M., Rajpert-De Meyts, E., Skakkebaek, N.E., 2003. Histological evidence of testicular dysgenesis in contralateral biopsies from 218 patients with testicular germ cell cancer. *J. Pathol.* 200, 370–374.
- Hosie, S., Loff, S., Witt, K., Niessen, K., Waag, K.L., 2000. Is there a correlation between organochlorine compounds and undescended testes? *Eur. J. Pediatr. Surg.* 10, 304–309.
- Huff, D.S., Hadziselimovic, F., Snyder III, H.M., Blythe, B., Duckett, J.W., 1993. Histologic maldevelopment of unilaterally cryptorchid testes and their descended partners. *Eur. J. Pediatr.* 152 (Suppl. 2), S11–S14.
- Irvine, D.S., 1994. Falling sperm quality. *BMJ* 309, 476.
- Irvine, S., 1996. Is the human testis still an organ at risk? *BMJ* 312, 1557–1558.
- Jorgensen, N., Andersen, A.G., Eustache, F., Irvine, D.S., Suominen, J., Petersen, J.H., Andersen, A.N., Auger, J., Cawood, E.H., Horte, A., Jensen, T.K., Jouannet, P., Keiding, N., Vierula, M., Toppari, J., Skakkebaek, N.E., 2001. Regional differences in semen quality in Europe. *Hum. Reprod.* 16, 1012–1019.
- Jorgensen, N., Carlsen, E., Neramo, I., Punab, M., Suominen, J., Andersen, A.G., Andersson, A.M., Haugen, T.B., Horte, A., Jensen, T.K., Magnus, O., Petersen, J.H., Vierula, M., Toppari, J., Skakkebaek, N.E., 2002. East–West gradient in semen quality in the Nordic–Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. *Hum. Reprod.* 17, 2199–2208.
- Matlai, P., Beral, V., 1985. Trends in congenital malformations of external genitalia. *Lancet* 1, 108.
- Moller, H., Weidner, I.S., 1999. Epidemiology of cryptorchidism and hypospadias. *Epidemiology* 10, 352–354.
- Olsen, J., Bonde, J.P., Basso, O., Hjollund, N.H., Sorensen, H.T., Abell, A., 2000. Birthweight and semen characteristics. *Int. J. Androl.* 23, 230–235.
- Ozturk, O., Armstrong, K., Bhattacharya, S., Templeton, A., 2001. Fetal antecedents of male factor sub-fertility: how important is birthweight? *Hum. Reprod.* 16, 2238–2241.
- Paulozzi, L.J., Erickson, J.D., Jackson, R.J., 1997. Hypospadias trends in two US surveillance systems. *Pediatrics* 100, 831–834.
- Pierik, F.H., Burdorf, A., Nijman, J.M., de Muinck Keizer-Schrama, S.M., Juttman, R.E., Weber, R.F., 2002. A high hypospadias rate in The Netherlands. *Hum. Reprod.* 17, 1112–1115.
- Rajpert-De Meyts, E., Jorgensen, N., Brondum-Nielsen, K., Muller, J., Skakkebaek, N.E., 1998. Developmental arrest of germ cells in the pathogenesis of germ cell neoplasia. *APMIS* 106, 198–204 (discussion 204–206).
- Savage, M.O., Lowe, D.G., 1990. Gonadal neoplasia and abnormal sexual differentiation. *Clin. Endocrinol. (Oxf.)* 32, 519–533.
- Scully, R.E., 1981. Neoplasia associated with anomalous sexual development and abnormal sex chromosomes. *Pediatr. Adolesc. Endocrinol.* 8, 203–217.
- Sharpe, R.M., 2003. The ‘oestrogen hypothesis’—Where do we stand now? *Int. J. Androl.* 26, 2–15.
- Sharpe, R.M., Franks, S., 2002. Environment, lifestyle and infertility—An inter-generational issue. *Nat. Cell Biol.* 4, s33–s40 (Suppl.).
- Sharpe, R.M., Skakkebaek, N.E., 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 341, 1392–1395.
- Skakkebaek, N.E., Berthelsen, J.G., Giwercman, A., Muller, J., 1987. Carcinoma-in-situ of the testis: possible origin from gonocytes and

- precursor of all types of germ cell tumours except spermatocytoma. *Int. J. Androl.* 10, 19–28.
- Skakkebaek, N.E., Rajpert-De Meyts, E., Main, K.M., 2001. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum. Reprod.* 16, 972–978.
- Skakkebaek, N.E., Holm, M., Hoei-Hansen, C., Jorgensen, N., Rajpert-De Meyts, E., 2003. Association between testicular dysgenesis syndrome (TDS) and testicular neoplasia: evidence from 20 adult patients with signs of maldevelopment of the testis. *APMIS* 111, 1–9 (discussion 9–11).
- Sohval, A.R., 1954. Testicular dysgenesis as an etiologic factor in cryptorchidism. *J. Urol.* 72, 693–702.
- Sohval, A.R., 1956. Testicular dysgenesis in relation to neoplasm of the testicle. *J. Urol.* 75, 285–291.
- Swan, S.H., Elkin, E.P., Fenster, L., 1997. Have sperm densities declined? A reanalysis of global trend data. *Environ. Health Perspect.* 105, 1228–1232.
- Thong, M., Lim, C., Fatimah, H., 1998. Undescended testes: incidence in 1,002 consecutive male infants and outcome at 1 year of age. *Pediatr. Surg. Int.* 13, 37–41.
- Toppiari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette Jr., L.J., Jegou, B., Jensen, T.K., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J.A., Meyer, O., Muller, J., Rajpert-De Meyts, E., Scheike, T., Sharpe, R., Sumpster, J., Skakkebaek, N.E., 1996. Male reproductive health and environmental xenoestrogens. *Environ. Health Perspect.* 104 (Suppl. 4), 741–803.
- Toppiari, J., Kaleva, M., Virtanen, H.E., 2001. Trends in the incidence of cryptorchidism and hypospadias, and methodological limitations of registry-based data. *Hum. Reprod. Updat.* 7, 282–286.
- Vierula, M., Niemi, M., Keiski, A., Saaranen, M., Saarikoski, S., Suominen, J., 1996. High and unchanged sperm counts of Finnish men. *Int. J. Androl.* 19, 11–17.
- Virtanen, H.E., Kaleva, M., Haavisto, A.M., Schmidt, I.M., Chella-kooty, M., Main, K.M., Skakkebaek, N.E., Toppiari, J., 2001. The birth rate of hypospadias in the Turku area in Finland. *APMIS* 109, 96–100.