Risk of Sex-Specific Cancers in Opposite-Sex and Same-Sex Twins in Denmark and Sweden

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Abstract

Background: Increasing evidence shows that some cancers originate in utero. It is hypothesized that elevated exposure to some steroid hormones might increase cancer risk and that hormone transfer between twin fetuses could result in different prenatal exposure to testosterone.

Methods: This large-scale prospective twin study compared opposite-sex (OS) and same-sex (SS) twins to test the impact of intrauterine exposures on cancer risk. On the basis of the Danish and Swedish twin and cancer registries, we calculated incidence rate ratios for OS and SS twins, whereas standardized incidence ratios (SIR) with 95% confidence intervals (CI) were calculated for OS/SS twins compared with the general population.

Results: A total of 18,001 cancers were identified during 1943–2009. No significant differences were observed between OS and SS twins, neither for the sex-specific cancers nor for cancer at all sites. All-cause cancer was slightly reduced for OS and SS twins compared with the general population, significant for OS males (SIR, 0.95; 95% CI, 0.92–0.98) and for SS males and females (SIR, 0.97; 95% CI, 0.94–0.99).

Conclusions: Our data suggest that having a male co-twin—which may entail higher exposure to prenatal testosterone—does not increase the risk of sex-specific cancers in OS females. Furthermore, the study supports that twinning per se is not a risk factor of cancer.

Impact: Findings are reassuring, as they fail to provide evidence for the hypothesis that endocrine or other difference in the in utero milieu affects the risk of sex-specific cancers. Cancer Epidemiol Biomarkers Prev; 24(10): 1622–8. © 2015 AACR.

Introduction

Studies of periods in human life that are associated with higher risk of cancer development have in recent years extended to the intrauterine life (1–3). Studies in rodents have consistently demonstrated that exposure to sex hormones influences the intrauterine fetal position and the sex of littermates (4, 5). Female rodents that develop between male fetuses show a less typically female pattern of anatomy and behavior in adulthood compared with those that gestate between females (4, 5). On the other hand, female fetuses developing between females show more feminized traits as adults, including altered hormone levels, reproductive organs, and aggressive behaviors (4). This intrauterine effect is hypothesized to occur through the testosterone transfer from male fetuses to adjacent fetuses (4). However, regardless of sex, a fetus located between 2 males have higher blood concentrations of testosterone and lower concentrations of estradiol than fetuses located between 2 females, and some features have been more sex-specific by gestating next to littermates of the same sex (for review, see refs. 4, 5).

According to the “twin testosterone transfer” (TTT) hypothesis (6), a similar effect may occur in human twins. Especially opposite-sex (OS) twin females are assumed to be exposed to higher levels of testosterone during prenatal development compared with same-sex (SS) females (6, 7). In addition, SS males may be exposed to higher levels of testosterone than OS males (7); however, because males are already exposed to high levels of testosterone, the effect of testosterone exposure from male co-twins is likely to be more pronounced in females than in males (6). The literature investigating the TTT hypothesis remains inconsistent (for review, see refs. 6, 7). Increased risk of breast cancer (8, 9), endometrium cancer (10), and ovary cancer (11) with exposure to elevated levels of androgens has been suggested in, for instance, laboratory studies (8, 9), and androgens are also suggested to be an important etiologic factor for prostate (12) and testicular (13) cancer. In addition, it has been suggested that raised levels of estrogen during pregnancy may increase the risk of later development of cancers, for instance, the sex-specific cancers (3, 11, 12, 14–16), and twin pregnancies are characterized by elevated levels of maternal serum estrogens compared with singleton pregnancies (17).

Few studies have compared the incidence of cancers among OS and SS twins, but some evidence exists, for example, from two
Swedish cohort twin studies (18, 19). The larger of the studies found a significantly increased risk of testicular cancer for SS males as well as for all twin males compared with the general population, but this may be a chance finding due to multiple testing (18).

Except from differences in the fetal hormonal environment, cancer risks in twins may be altered for other reasons, such as low birth weight, fast postnatal growth period, and space limitations (18). Results from studies of all-cause cancer risk in adult twins have been inconsistent. A reduced risk for twins compared with singletons for both sexes has been observed (20–22). In addition, a somewhat lower cancer risk for SS male twins was suggested in the larger Swedish cohort study (18), whereas the standardized incidence ratio (SIR) for all twins was at the same level as for the general population. In the Utah Genealogy study, OS female twins had an increased risk of all adult cancers compared with singleton females (23).

Our primary objective was to compare the risk of sex-specific and all-cause cancers among female and male twins from OS and SS pairs, including both monozygotic (MZ) and dizygotic (DZ) twins. If such differences exist, they would support the hypothesis of an intrauterine origin of certain malignancies. Also, we attempted to elucidate whether the associations between OS and SS twin groups with cancer risks differed from the general population.

Materials and Methods

Data sources

This study is a part of the Nordic Twin Study of Cancer (NorTwinCan), which has been described in detail previously (24). For this analysis, we limited data to the population-based Danish (25) and Swedish (26) twin registries that contain a sizeable number of OS twins. The data collection by these registries has been described in detail previously (25, 27). In both registries, the zygosity determination of SS twin pairs was based on answers to standard questionnaires known to be more than 95% precise (28, 29).

Both twin registries are linked to national health registers to obtain information on immigration, cancer diagnoses, and death through a national registration number (27, 30). Cancer registration methods have been almost similar in the Nordic countries and have a high degree of comparability and validity (31). Incidence rates for the general population were retrieved from the NORDCAN database (31). NORDCAN is a collaborative project between the Nordic countries with registration of cancer incidences and cancer mortality. For this analysis, we focused on all-cause cancers, including all cancer sites except nonmelanoma skin, and on the sex-specific cancers: breast, ovary, corpus uteri, cervix uteri and other genital organs among women, and prostate and testis among men. The cancer groups "uterus, other" and "penis and other male genital organs" were excluded because of too few cases to stratify on OS/SS twin status.

Study population and follow-up

The study population comprised twins born in Denmark from 1870–1910 and from 1931–2004. The reason for omitting twins born between 1911 and 1930 is that in these birth cohorts only SS twin pairs are included in the Danish Twin Registry (25). In Sweden, the starting date of follow-up was not the same for OS and SS twins until 1926; therefore, only the birth cohorts 1926–1999 were included in this study. We analyzed 44,650 OS and 84,721 SS twin females and 44,660 OS and 88,261 SS twin males (Table 1). Beginning of follow-up was dependent on birth cohort and country (further details are presented in Supplementary Table S1). We included all birth cohorts to cover even the earliest occurrences of the cancers. Twins were followed until cancer diagnosis, or censored at death, emigration, or loss to follow-up as defined in the population registry or at the end of the study on December 31, 2009. Persons with one cancer diagnosis were followed for additional cancer diagnoses.

Statistical analysis

All analyses were stratified by sex. We analyzed data from Sweden and Denmark combined to increase power, as separate analyses yielded close to identical results (Table 2). SIRs were used to measure cancer risks for OS and SS twins, respectively, compared with the general background population and based on country-standardized rates stratified by sex, cancer type, 5-year age groups, and 5-year periods. Incidence rate ratios (IRR) were calculated for OS and SS twins using Poisson regression adjusted for dependence within twin pairs. Moreover, we tested the interaction between country and having an OS co-twin. In addition to the Poisson regression, we applied a Fine–Gray survival model to the data with the different cancers as events taking competing risk of deaths and dependence within twin pairs as well as left truncation into account. We fitted both a model with only OS and SS twins and a model adjusted for country and birth cohorts in 20-year intervals. Moreover, we checked for interactions between country and having an OS co-twin. We calculated sub-HRs (SHR) with 95% CIs and checked the proportional hazards assumption by graphically investigating the Schoenfeld residuals, if possible violations were observed, we investigated time varying hazards. In a separate analysis, we distinguished between breast cancer diagnosed before and after the age of 50.

In this study, both MZ and SS dizygotic (sDZ) twins were included in the SS twin group. This was done because of the rather large proportion (~16%) of twins with unknown zygosity (sUZ), which made it impossible to allocate all the SS to zygosity groups. To check the robustness of the results, we repeated all analyses.

Table 1. Description of the Danish and Swedish twin cohorts

<table>
<thead>
<tr>
<th>Birth cohorts</th>
<th>Denmark</th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of OS/SS female twins</td>
<td>21,344/39,174</td>
<td>25,306/45,547</td>
</tr>
<tr>
<td>Number of OS/SS male twins</td>
<td>21,575/42,650</td>
<td>25,283/45,611</td>
</tr>
<tr>
<td>Cancer registration since</td>
<td>January 1, 1943</td>
<td>January 1, 1958</td>
</tr>
<tr>
<td>Age at start of follow-up, y</td>
<td>0–73 (mean, 25)</td>
<td>0–47 (mean, 24)</td>
</tr>
<tr>
<td>End of follow-up</td>
<td>December 31, 2009</td>
<td>December 31, 2009</td>
</tr>
<tr>
<td>Number of cancer cases OS/SS females</td>
<td>1,634/3,162</td>
<td>1,740/2,945</td>
</tr>
<tr>
<td>Number of cancer cases OS/SS males</td>
<td>1,400/2,922</td>
<td>1,559/2,639</td>
</tr>
</tbody>
</table>
Ahrenfeldt et al.

Table 2. Cancer risk for sex-specific and all-cause cancers in OS and SS twins as well as in OS/SS twins compared with the general population born in Denmark and Sweden during 1870 to 2004 and followed from 1943 to 2009

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Cancer numbers OS/SS twins</th>
<th>IRR (95% CI) OS twins vs. SS twins</th>
<th>SIR (95% CI) OS twins vs. the general population</th>
<th>SIR (95% CI) SS twins vs. the general population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>1,333/1,955</td>
<td>1.02 (0.95–1.10)</td>
<td>1.04 (0.98–1.10)</td>
<td>1.02 (0.97–1.06)</td>
</tr>
<tr>
<td>Ovary and uterine adnexa</td>
<td>171/322</td>
<td>0.98 (0.81–1.18)</td>
<td>0.95 (0.82–1.10)</td>
<td>0.97 (0.87–1.09)</td>
</tr>
<tr>
<td>Corpus uteri</td>
<td>182/301</td>
<td>1.11 (0.92–1.33)</td>
<td>0.95 (0.82–1.10)</td>
<td>0.86 (0.77–0.96)*</td>
</tr>
<tr>
<td>Cervix uteri</td>
<td>175/284</td>
<td>1.15 (0.95–1.39)</td>
<td>1.07 (0.92–1.24)</td>
<td>0.93 (0.83–1.04)</td>
</tr>
<tr>
<td>Other female genital organs</td>
<td>24/52</td>
<td>0.91 (0.56–1.48)</td>
<td>0.91 (0.61–1.35)</td>
<td>1.00 (0.76–1.31)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>737/1,309</td>
<td>0.99 (0.90–1.08)</td>
<td>1.06 (0.99–1.14)</td>
<td>1.08 (1.02–1.14)*</td>
</tr>
<tr>
<td>Testis</td>
<td>84/171</td>
<td>0.95 (0.73–1.23)</td>
<td>1.02 (0.82–1.26)</td>
<td>1.07 (0.92–1.25)</td>
</tr>
<tr>
<td>All sites except nonmelanoma, skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>3,374/6,107</td>
<td>1.01 (0.97–1.06)</td>
<td>0.98 (0.95–1.01)</td>
<td>0.97 (0.94–0.99)*</td>
</tr>
<tr>
<td>Males</td>
<td>2,959/5,561</td>
<td>0.98 (0.94–1.03)</td>
<td>0.95 (0.92–0.98)*</td>
<td>0.97 (0.94–0.99)*</td>
</tr>
</tbody>
</table>

*Statistically significant at the 0.05 level.
#Statistically significant at the 0.01 level.

We found no significant differences for the sex-specific cancers between OS twins and the general population, neither for females nor for males (Table 2). However, in Denmark, a significantly higher risk of prostate cancer was found for OS compared with general population males (SIR, 1.17; 95% CI, 1.03–1.33, results not shown). For prostate cancer, there was a significant interaction between country and having an OS co-twin (P = 0.04). A higher risk was suggested for OS than for SS males in Denmark (IRR, 1.14; 95% CI, 0.96–1.34), whereas the opposite was indicated in Sweden (IRR, 0.93; 95% CI, 0.83–1.03; results not shown). In the combined analysis, no significantly increased risk of prostate cancer was found for OS males compared with the general population (SIR, 1.06; 95% CI, 0.99–1.14). For SS males, the risk was, however, significantly increased also at the one percent level (SIR, 1.08; 95% CI, 1.02–1.14; Table 2). For the female sex-specific cancers, the only significant difference was a lower risk of corpus uteri cancer in SS female twins than the general population (IRR, 0.86; 95% CI, 0.77–0.96). The difference became borderline significant when we excluded the known MZ twins (Table 3).

The SIRs for all-cause cancer comparing twins and the general population were close to unity, but with a borderline significantly excluding twins with known MZ status (Table 3). Moreover, we compared OS versus ssDZ twins excluding both the MZ and the ssUZ twins (Supplementary Table S2).

Results

From the earliest cancer registration in 1943 through latest follow-up in 2009, a total of 18,001 cancers of all sites except nonmelanoma skin were observed in 16,779 twins. This corresponded to 15,623 individuals with 1 cancer diagnosis, 1,092 individuals with 2 cancers, 62 individuals with 3 cancers, and 2 individuals with 4 cancer diagnoses. OS females had 3,374 and SS females had 6,107 cancers. OS males had 2,959 and SS male individuals with 4 cancer diagnoses. OS males had 3,374 and SS male twins had 6,107 cancers. OS males had 2,959 and SS male twins had 5,561 cancers (Table 1).

For both sexes, no significant differences in risk were observed between OS and SS twins, neither for the sex-specific cancers nor for cancer at all sites (Table 2). After repeating the analyses excluding twins with known MZ status (Table 3) or comparing OS and ssDZ twins (Supplementary Table S2), the results for OS and SS twins were not changed appreciably, confirming the lack of differences. The largest deviation from unity was an indication of a higher incidence of cervical cancer in OS than in SS female twins (IRR, 1.15; 95% CI, 0.95–1.39; Table 1). When we compared OS and ssDZ twins only (Supplementary Table S2), the risk-ratio was significantly increased to an IRR of 1.25 (95% CI, 1.00–1.57).

We found no significant differences for the sex-specific cancers between OS twins and the general population, neither for females nor for males (Table 2). However, in Denmark, a significantly higher incidence of cervical cancer in OS than in SS female twins (IRR, 1.14; 95% CI, 0.96–1.39) was observed in females. Tests for statistical significance were performed using a log-rank test for survival analysis. The SIRs for all-cause cancer comparing twins and the general population were close to unity, but with a borderline significantly
lower risk for OS (SIR, 0.98; 95% CI, 0.95–1.01) and a significantly lower risk for SS (SIR, 0.97; 95% CI, 0.94–0.99) female twins. Also for males, the risk was lower for both OS (SIR, 0.95; 95% CI, 0.92–0.98) and SS (SIR, 0.97; 95% CI, 0.94–0.99) twins (Table 2). The significantly lower risk for OS male twins persisted at the one percent level also when excluding the MZ twin males (Table 3) or investigating only the differences between OS and SSZ males (Supplementary Table S2).

When we repeated the analyses comparing OS and SS twins with regard to first cancer diagnosis, taking competing risk of death into account, the results for OS and SS twins were not changed to any substantial degree (Table 4). Also in the age-stratified analyses of breast cancer, we found similar cancer risks for OS and SS twins both before the age of 50 (adjusted SHR, 0.98; 95% CI, 0.85–1.12) and after the age of 50 (adjusted SHR, 1.03; 95% CI, 0.94–1.13). For cases diagnosed after the age of 50, a significant interaction between country and having an OS co-twin was present (P = 0.02). The adjusted HR taking this interaction into account indicated a lower risk (although nonsignificant) of breast cancer for OS compared with SS (SHR, 0.91; 95% CI, 0.79–1.05). A significant interaction between country and having an OS co-twin (P = 0.02) was also found for females when all cancers were combined. However, after making adjustments and taking the interaction into account, the difference in risk was still nonsignificant (SHR, 0.95; 95% CI, 0.89–1.01). We did not detect any violations of the proportional hazards assumption apart from a slight violation in the prostate cancer analyses and in the adjusted analysis of testicular cancer.

Discussion

In this large, prospective, population-based twin study with up to 66 years of complete follow-up, we found a similar incidence of sex-specific cancers among OS and SS twins and among OS female twins and females in the general population. Similar cancer incidence was also found for OS and SS male twins. Thus, our results fail to provide evidence for the hypothesis that endocrine or other difference in the in utero milieu affects the risk of sex-specific cancers in adulthood. Moreover, our study confirms that being a twin is not a risk factor for all-cause cancer, independent of co-twin sex.

In 1990, it was proposed that some breast cancers originate in utero [16], and since then, several studies have given support for this hypothesis. Direct evidence comes from the daughters of women who used a synthetic estrogen diethylstilbestrol (DES) during pregnancy to prevent miscarriage and who experienced an increased breast cancer risk after the age of 40 years [32, 33]. These findings indicate that a causal association exists between in utero exposure to DES and later breast cancer risk, provided that doses are sufficiently high and the duration of treatment sufficiently long. One corollary of the DES findings is that studies among twins might be informative if they show convincing associations. However, they cannot exclude intrauterine effects because the range of exposure (average difference in hormonal levels between OS and SS twins or between twin and non-twin pregnancies) may not be large enough to give measurable effects on cancer incidence. Thus, this study cannot exclude an association between potential, excessive exposure to prenatal testosterone and cancer risk.

Our results could also be interpreted as not providing supporting evidence of the TTT hypothesis. Transfer of testosterone is assumed on the basis of animal studies of placentation patterns, which are markedly different from those of human twin pregnancies [4]. In humans, there are two possible routes for prenatal testosterone transfer between fetuses: the maternal–fetal and the fetal–fetal route [34]. One study of pregnant mothers of twins suggested that maternal serum testosterone levels were not different in pregnancies with OS and SS twin pairs [35]; however, the study measured maternal testosterone levels at 24 and 32 weeks of gestation, although the highest prenatal testosterone production in male fetuses is occurring from 8 to 24 gestational weeks [36]. The other suggested route goes directly from fetus to fetus. Amniotic fluid can permeate the fetal skin and the placenta until 18 weeks of gestation [37], and testosterone production in males is at highest before that gestational age [38, 39]. Unfortunately, there are no studies of amniotic fluid testosterone levels from OS and SS twin pairs [40], and the literature in the field is inconsistent [6]. The present study suggests that if prenatal testosterone

Table 4. Cancer risk for sex-specific and all-cause cancers in OS and SS twins born in Denmark and Sweden during 1870 to 2004 and followed from 1943 to 2009 taking competing risk of death into account.

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Cancer numbers</th>
<th>Crude SHR (95% CI) for OS vs. SS twins</th>
<th>Adjusted* SHR (95% CI) for OS vs. SS twins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>1083/1881</td>
<td>1.05 (0.97–1.13)</td>
<td>1.01 (0.94–1.09)</td>
</tr>
<tr>
<td>&lt;50 y</td>
<td>334/569</td>
<td>1.00 (0.87–1.15)</td>
<td>0.98 (0.85–1.12)</td>
</tr>
<tr>
<td>&gt;50 y</td>
<td>749/1313</td>
<td>1.07 (0.97–1.17)</td>
<td>1.03 (0.94–1.13)</td>
</tr>
<tr>
<td>Ovary and uterine adnexa</td>
<td>277/653</td>
<td>0.99 (0.82–1.20)</td>
<td>1.00 (0.82–1.22)</td>
</tr>
<tr>
<td>Corpus uteri</td>
<td>160/298</td>
<td>1.11 (0.91–1.34)</td>
<td>1.08 (0.89–1.32)</td>
</tr>
<tr>
<td>Cervix uteri</td>
<td>170/271</td>
<td>1.12 (0.92–1.36)</td>
<td>1.18 (0.97–1.43)</td>
</tr>
<tr>
<td>Other female genital organs</td>
<td>21/47</td>
<td>0.85 (0.51–1.43)</td>
<td>0.84 (0.50–1.41)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>693/1227</td>
<td>1.07 (0.97–1.17)</td>
<td>0.98 (0.89–1.08)</td>
</tr>
<tr>
<td>Testis</td>
<td>84/165</td>
<td>0.95 (0.73–1.24)</td>
<td>0.97 (0.74–1.26)</td>
</tr>
<tr>
<td>All sites except nonmelanoma, skin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>3,108/5,669</td>
<td>1.02 (0.98–1.07)</td>
<td>1.00 (0.96–1.04)</td>
</tr>
<tr>
<td>Males</td>
<td>2,752/5,164</td>
<td>1.00 (0.95–1.05)</td>
<td>0.98 (0.94–1.03)</td>
</tr>
</tbody>
</table>

*Adjusted for country and birth cohorts in 20-year intervals.
*Proportional hazards slightly violated.
*A significant interaction between country and having an OS co-twin (P = 0.02).
exposure from male co-twins exists, it is not large enough to affect cancer risk in females to any substantial degree later in life. Previous, large studies comparing the incidence of breast cancer for OS/SS twins and singletons show conflicting results (41, 42). A study including 1,230 breast cancer cases found a significantly increased risk of postmenopausal breast cancer in SS compared with singleton females, whereas the elevated risk in OS females was nonsignificant (42). Conversely, a study of 2,202 cancer cases among premenopausal females found higher breast cancer risk among OS than among singleton females compared with a slightly increased, but nonsignificant, risk among SS female twins (41). Consistent with the Swedish cohort studies (18, 19), we found that the risk of breast cancer for OS and SS twins is close to that of the general population. Also, in line with a nested case-control study, including 543 cases and 2,715 controls, investigating the influence of birth weight and co-twin birth weight on the risk of breast cancer in OS female twins (43), our results do not suggest that elevated hormone exposure in utero influences breast cancer risk.

Few epidemiologic studies have compared OS and SS twins with regard to ovary and endometrial cancers. Endometrial cancer is often included under the category "corpus uteri cancer," which includes sarcomas that arise from the endometrial stroma or from the smooth muscle of the uterus (44). In line with the larger Swedish study, which indicates a higher but nonsignificantly increased risk of cancer in endometrium for OS female twins (18), our results suggest a slightly elevated risk of corpus uteri cancer in OS compared with SS females. However, this difference was caused by a significantly 14% lower risk of corpus uteri cancer in SS twins compared with the general population, whereas the risk in OS female twins was only slightly decreased and thus provided no evidence of an influence of potential, excessive testosterone exposure in utero on the risk of corpus uteri cancer.

We found no significant differences between OS and SS twin males for either prostate cancer or testicular cancer. The causes of prostate cancer are poorly understood compared with other common cancers such as breast cancer (45), but a recent review suggests that the risk of prostate cancer could be determined in pre- and perinatal life and that estrogen alone or in synergy with androgen causes the pathogenesis of prostate cancer in later life (12). Epidemiologic studies support an association between conditions in utero and later risk of prostate cancer, but the results are inconclusive (1, 2). Our findings of a slightly increased risk of prostate cancer among SS and OS males compared with males in the general population may support this hypothesis; however, the similar incidence between OS and SS males provides no support for an effect of excessive testosterone exposure in utero on prostate cancer risk among males with SS co-twins.

In a modification of the estrogen hypothesis, Sharpe suggested that the balance between estrogen and androgen may be etiologically important for testicular cancer (13), and increases in risk of testicular cancer for twins have been suggested (46). Hence, a higher risk of testicular cancer for SS than OS males is to be expected, as also found in the Swedish cohort studies (18, 19). The results from the Swedish studies are in agreement with a recent Danish study that found an IRR of 0.5 (95% CI, 0.2–1.1) for OS compared with SS twins and concluded that having a twin sister was associated with a reduced risk of testicular cancer. However, the results were limited by the low number of cases (n = 26; ref. 47). Our estimates comparing OS and SS twins with regard to testicular cancer were nonsignificant, although they pointed in the same direction as the previous studies (18, 19, 47). Thus, our data did not contradict the modified hypothesis (13) but neither did it provide evidence of it.

The 2% to 5% reduced all-cause cancer risk among twins compared with the general population that we found for both sexes has previously been observed (20–22); however, other studies found no significant differences in risk of all-cause cancer between twins and the general population (18), nor between the general population and MZ and DZ twins, respectively (48, 49). The Finnish twin study, however, indicated a slightly decreased risk for both MZ (SIR, 0.96; 95% CI, 0.88–1.05) and DZ (SIR, 0.95; 95% CI, 0.89–1.01) twins (49). Findings from the Utah Genealogy showed that OS female twins have an increased risk of all adult cancers compared with singleton females [adjusted relative risk (RR), 1.46; 95% CI, 1.06–2.02; ref. 23], but this could not be confirmed in our study. The observed differences between The Utah Genealogy study and our study may be explained by differences in the study population, study design, and sample size (65 vs. 3,374 cancers among OS females), as well as by differences in adjustments. This study focused on the sex-specific cancers and on overall cancer risk and thus did not investigate which cancer sites were responsible for the small deficit in overall cancer risk for twins versus the general population. However, previous studies have suggested that twins had lower risk for cancers of the lung (20, 48, 49), skin (20), colon, and rectum (18, 20, 48). Thus, it does not seem likely that the average lower birth weight and gestational age for twins (50, 51) compared with singletons influence cancer risk, which has also been supported in previous studies (52–54).

Apart from possible differences in prenatal hormone exposure, OS twin pairs may also differ in postpregnancy effects compared with SS twins, as they are born and reared with an OS co-twin (55, 56), which may affect cancer risk through a number of possible mechanisms. In general, evidence on twin interactions is sparse. Some studies comparing OS and SS twins have used siblings as a control group for the psychosocial environment, for instance, with regard to mental rotation abilities (57) and sensation-seeking (58), but these studies failed to find evidence of socialization effects in these outcomes. However, indications of differences in socialization between OS and SS twins are present (55). In this study, we did not control for specific environmental factors that may affect the selected cancer types and thus may have attenuated or strengthened the associations. This study indicated a slightly higher incidence of cervical cancer in OS than in SS female twins (IRR, 1.15; 95% CI, 0.95–1.39). When we compared OS and ssDZ twins only, the risk ratio was significantly increased to an IRR of 1.25 (95% CI, 1.00–1.57). Risk factors for cervical cancer are related to female sexual behavior, and oncogenic HPV, a sexually transmitted agent, is accepted as a necessary but not sufficient cause (59). Whether OS females may adapt some aspects of behavior (e.g., sexual or smoking-related) from their twin brothers cannot be investigated in this study. However, recent studies comparing OS and SS female twins failed to find differences in variables that have been adjusted for in, for instance, studies of breast cancer (41), such as age at menarche (60), fertility (40), anthropometric measures (40), and alcohol dependence (61).

The group of ssUZ twins (~16%) was a limitation of this study because the unknown zygosity of these SS twins made it impossible to exclude all the MZ twins and thus make the most valid test of the TTT hypothesis, namely, a test that only compares ssDZ with OS twins to avoid possible confounding from variation in
placentation patterns for MZ and DZ twins (7). Twins of unknown zygosity have lower socioeconomic status and poorer school performance than the other twin groups (62). If differences between respondents and nonrespondents influence cancer risk, and we exclude the ssDZ twins, the SS twin group is not comparable with the OS twin group in which zygosity information is available for all the twins. However, these differences cannot be the primary drivers of a difference, at most risk indicators, for the sex-specific cancers. To check the robustness of the results, we repeated all the analyses excluding twins of known MZ status and of OS versus known ssDZ twins. However, these analyses did not change the estimates to any substantial degree. Thus, studying MZ and DZ twins together does not seem to have biased group comparisons.

Inclusion of second, third, and fourth cancers may be a concern, and in the Poisson regressions, we were not able to exclude these cancers because the cancer rates were compared with the rates for the general population, and these rates include all cancers. However, independent of which method we used, the results for OS versus SS twins were similar and the inference to be made did not depend on the method.

In conclusion, the present study, which is based on a large twin population, lends no support for an association between having a male co-twin and potential, excessive testosterone exposure in utero, and subsequent increased risk of sex-specific cancers. In addition, the study verifies that twinning per se is not a risk factor of cancer, independently of co-twin sex.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L.J. Ahrenfeldt, A. Skytte, S. Möller, I. Petersen, L.A. Mucci, K. Christensen, R. Lindahl-Jacobsen

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Ahrenfeldt et al.


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