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Cancer risks in twins and singletons from twin and non-twin families

Lingjing Chen[1], Sven Cnattingius[1], Anastasia Nyman Iliadou[2] and Anna Sara Oberg[2,3]

[1] Department of Medicine Solna, Clinical Epidemiology Unit, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[2] Department of Medical Epidemiology & Biostatistics, Karolinska Institutet, Stockholm, Sweden
[3] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

The unique intrauterine environment has been proposed to put twins at increased risk of certain cancers compared to singletons, still large population comparisons have generally indicated lower risks in twins. To improve the understanding of potential twin influence on cancer we compared twins to their singletons siblings, to target a unique twinning influence. Singletons from twin families were contrasted to singletons from non-twin families to further capture potential twin family influence on risk of cancer. Family relations were identified using the Swedish Multi-Generation Register. Among individuals born between 1932 and 1958, 49,156 twins and $N = 35,227$ singletons were identified from 18,098 unique twin families. All incident cases of specific cancer types were identified in the National Cancer Register up to the end of 2007. Standardized survival functions were estimated using weighted Cox proportional hazard regression and the corresponding cumulative risks plotted against age. Overall, primary cancers were identified in 9% and 18% of all male and female twins, compared to 11% and 19% of their male and female singleton siblings. When specific cancer sites were compared using standardized cumulative risk plots, no consistent statistically significant differences were noted either between twins and singletons of twin families or between singletons of twin and non-twin families. Despite a different intrauterine experience, twinning does not seem to have any greater negative influence on life-time risks of cancer. The findings also indicate that twin family membership has no substantial influence on cancer risks.

Twins are commonly used in epidemiological studies to explore the genetic and environmental influence on health traits. Meanwhile, the generalizability of twin studies has been questioned, not least following the increasing appreciation of early life influence on later disease development.[1,2] As a result of the intrauterine sharing of intrauterine space and fetal nutrient supply line, twins are on average born earlier (shorter gestational age) and have lower birth weight for gestational age (are more often growth restricted) than singletons.[3] Fetal programming theory originates from widely established inverse associations between birth weight and risk of cardiovascular disease (CVD),[4,5] which if causal, would create an expectation of increased risk of CVD in twins compared to singletons. However, several population-based studies have failed to show differing CVD morbidity and mortality in twins compared to singletons.[6–8]

The role of early (intrauterine) environment has also been stressed in cancer development. Risk of testicular cancer has been found inversely associated with birth weight and gestational age.[9,10] Moreover, hormone-related cancers have been postulated to originate from prenatal exposure to high levels of endogenous hormones, notably estrogens.[11–15] The observation that twin pregnancies (particularly dizygotic) are associated with higher *maternal* blood levels of estrogen,[16–18] has led to the proposition that twins are exposed to higher levels of hormones *in utero*, possibly due to the presence of two placentae (or a large shared placenta).[19] The first reports of higher cancer risks in twins were underpowered case-control studies of breast cancer;[15,19,20] and a higher risk of testicular cancer in dizygotic compared to monozygotic twins.[21] When cancer risks have been evaluated in large, population-based Scandinavian twin samples, increased risks of breast and testicular cancer have been noted in Swedish,[22,23] but not Norwegian[24] or Finnish[25] twins. These large studies found that compared to the general population, twins were either no different or at a lower overall cancer risk. The specific origins of lower risk can be traced as indications—some only with borderline statistical significance or in certain subgroups of twins (defined by sex/zygosity)—most consistently reported for cancers of the blood/lymphoid system,[22–24] colorectum,[22–24] lung,[22,24,25] and skin (melanoma).[23,24]

Previous efforts to explore the influence of twinning on cancer risk were achieved by comparing twins to singletons and/or the general population. However, if twins as a group

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Correspondence to: Anna Sara Oberg, Department of Epidemiology, Harvard T.H. Chan School of Public Health, 677 Huntington Avenue, Boston 02115, MA, USA, Tel.: +1-857-756-0770, Fax: +1-617-566-7805, E-mail: oberg@hsph.harvard.edu

What's new?
Some studies have indicated that there may be differences in cancer risk in twins as compared to non-twins. In this study, the authors compared the incidence of various types of cancer between twins and their non-twin siblings, as well as between singletons from families with and without twins. Overall, no consistent significant differences in cancer were observed either for twins and their non-twin siblings or for singletons of twin and non-twin families. Reassuringly, with few exceptions, twins thus do not appear to have an increased risk of cancer.

are systematically different from the population with respect to genetic and socioeconomic factors, such comparisons may fail to capture the unique influence of twinning.[24] Assuming all full siblings share early environment and genetic origin, singleton siblings of twins will be exposed to the same socioeconomic and genetic background as twins. Therefore, comparing twins to singletons from twin families may better capture the unique experience of being a twin and how this relates to adult morbidity. Insight into whether twin-families share factors that influence health could further be obtained from comparing singletons from families with and without twins.

We have previously used this design to show that there is no unique twinning, or twin family influence on overall mortality or morbidity from CVD and cancer.[7] Since cancers are heterogeneous diseases with unique etiologies and characteristics, we now aim to expand our investigation to specific cancer sites.

Material and Methods

Data source and study populations

The current study was based on several Swedish national registers, the linkage of which was enabled by the unique personal identity number assigned to all Swedish residents.[26] Study populations were identified through family information available in the Multi-Generation Register (MGR), which includes individuals born in Sweden since 1932 who were also alive and residing in Sweden at any point after 1961.[27] For the present study, individuals born from 1932 up to 1958 were selected. Information on migration and death, used to estimate time at-risk, was obtained from the Total Population Register and the Cause of Death Register, respectively.

Using the family information of the MGR we first established a twin cohort (cohort 1) consisting of all pairs of individuals born to the same mother within 3 days of each other. The method identified $N = 24{,}578$ twin pairs from a total of $N = 24{,}150$ families. Next we identified all singleton full siblings in these families (cohort 2); for each twin family an average of 1.6 full siblings were identified of which $N = 35{,}227$ were born between 1932 and 1958. The main contrast of our analyses was between these two cohorts, who share the characteristics of twin family membership, but differ with respect to the unique experience of twinning. In order to further explore the potential influence of twin family membership on cancer risk, we compared the singletons from twin families (cohort 2) to singletons from non-twin families. These (cohort 3) were obtained by matching 10 singletons from non-twin families to each singleton in cohort 2, based on sex, birth year and sibling constellation (sex and birth year of siblings). Due to the matching criteria, the average number of matches in this cohort was 4.5 (range from 0 to 10). Lastly, in order to obtain a more representative sample of singletons from the general population, we also selected singletons from non-twin families matched to the singletons in cohort 2 merely with respect to sex and birth year. This sample (cohort 4) further allowed for an overall comparison of twins to singletons of the population, similar to that obtained in previous studies. The final study populations after exclusion of left-censored individuals (due to event or migration) and their basic characteristics are presented in Table 1.

Outcome definitions

Outcome information was retrieved through linkage with the Swedish Cancer Register. This registry first started recording incident cancer cases in 1958, using diagnostic codes of the seventh revision of the International Classification of Diseases (ICD-7). It now also includes the tenth revision (ICD-10, implemented in Sweden in 1997). The completeness of the register is high, with underreporting not exceeding 4%, according to the most recent quality study done in 2008.[28] For this study, all codes within 140 to 206 in ICD-7 and C00 to C97 in ICD-10 were considered and in (rare) disputes between ICD-revisions, the ICD-10 code took precedence. The classification of sites and their corresponding ICD-7 codes are shown in detail in Table 2.

Statistical analyses

Following the inclusion criteria for the MGR, follow-up started in 1961, when the study subjects were between the ages of 3 and 29 years. Subjects became cases at the time of diagnosis of the first incident primary cancer, or censored at the time of emigration from Sweden, death or end of follow-up on December 31st 2007 (available at the time data linkage was performed), whichever happened first. When evaluating each specific cancer site, individuals whose first incident cancer was of another type were censored at the time of their first cancer diagnosis.

In order to describe and compare cancer morbidity across the lifespan in the different cohorts, cumulative risks of each specific cancer type were estimated and plotted against attained age. Survival functions were estimated through Cox proportional hazards regression with age as the underlying

Table 1. Characteristics of the study population, stratified by cohort

		Singletons		
	Twins	Twin family	Non-twin family	
	Cohort	Cohort 2	Cohort 3	Cohort 4
Characteristics	**(*N* = 47,585)**	**(*N* = 34,119)**	**(*N* = 150,727)**	**(*N* = 256,306)**
Sex, *N* (%)				
Male	23,715 (50)	17,526 (51)	76,697 (51)	130,006 (51)
Female	23,870 (50)	16,593 (49)	74,030 (49)	126,300 (49)
Birth period, *N* (%)				
1932–1937	6,117 (13)	5,617 (16)	23,787 (16)	35,939 (14)
1938–1942	8,083 (17)	7,695 (23)	32,852 (22)	56,764 (22)
1943–1947	11,537 (24)	9,159 (27)	40,830 (27)	72,322 (28)
1948–1952	10,410 (22)	7,086 (21)	32,930 (22)	55,632 (22)
1953–1958	11,438 (24)	4,562 (13)	20,328 (13)	35,649 (14)
Family size, *N* (%)				
Two siblings	11,497 (24)	0 (0)[1]	69,586 (46)	N/A
Three siblings	15,117 (32)	7,028 (21)	73,746 (49)	N/A
Four siblings	10,139 (21)	8,937 (26)	6,830 (4.5)	N/A
Five siblings	5,235 (1,1)	6,642 (19)	448 (0.3)	N/A
>5 siblings	5,597 (12)	11,512 (34)	117 (0.1)	N/A
Age median				
At entry (yr)	13	16	15	15
At exit (yr)	58	60	60	60

Final numbers included in each cohort after exclusion of left-censored individuals.
[1]The smallest number of siblings per family is three by default (the singleton and 1 twin pair).
Abbreviations: N/A is not applicable (cohort was matched on sex and birth year, family size was not considered).

time scale and allowing non-proportional hazards for the main contrast groups using the SAS PHREG procedure. Analyses were performed in all, as well as stratified by sex. To mitigate any concern for bias due to systematic differences in birth year and family size between cohorts (distributions shown in Table 1), survival functions were standardized to the joint distribution of these potential confounders. Direct standardization requires specification of Cox regression models of the outcome given exposure and confounders to obtain estimates of stratum-specific survival functions. Instead, we performed standardization based on models of the exposure, in order to avoid potential violations to outcome modeling assumptions (in particular proportional hazards). To achieve this inverse probability weighting (IPW), logistic regression models of the exposure given confounders are used to estimate the probability of exposure. The inverse of each individual's probability of experiencing the exposure they factually experienced is then used to weight a marginal Cox regression model of the outcome.

All standardized survival estimates were converted into cumulative risks of failure ($f(t) = 1 - s(t)$) and plotted against age. To facilitate comparison between the two groups contrasted, we also estimated and plotted the risk difference and its 95% confidence interval (CI) obtained from 1,000 bootstrap samples. All data management and statistical analyses were done with SAS software version 9.3, and graphs with R software version 2.15.0. Registers, linkage, cohort selection and statistical procedures have been described in more detail elsewhere.[7]

Results

Table 1 shows the baseline characteristics of the four principal study cohorts. Because the number of singleton full siblings to twins decreased over calendar time, twins were overrepresented in the youngest birth cohorts compared to singletons from twin families (cohort 2) and the samples of singletons from non-twin families matched to them (cohorts 3 and 4). The birth cohort as well as sex distributions of the latter did not perfectly follow that of cohort 2 due to incomplete matching and exclusions (migration before 1961). Information on family size (number of full siblings identified in the MGR) was available for cohorts 1, 2 and 3. Twin families with more than two singletons were by default represented by more singletons (cohort 2) than twins (cohort 1). Among the singletons from non-twin families in cohort 3, the number of matches (0–10) per individual in cohort 2 decreased as family size increased.

We identified primary cancers in 2210 male and 4263 female twins, representing 9.3% and 17.9% of all male and

Table 2. Number of cancer cases (and cases per person-years at risk) in twins and singletons of the Swedish population, born between 1932 and 1958

		Males				Females			
		Twins	Singletons			Twins	Singletons		
		Cohort 1 (N = 23,715)	Cohort 2 (N = 17,526)	Cohort 3 (N = 76,697)	Cohort 4 (N = 130,006)	Cohort 1 (N = 23,870)	Cohort 2 (N = 16,593)	Cohort 3 (N = 74,030)	Cohort 4 (N = 126,300)
Cancer site	**ICD-7**	***N*** **(rate)**	***N*** **(rate)**	***N*** **(rate)**	***N*** **(rate)**	***N*** **(rate)**	***N*** **(rate)**	***N*** **(rate)**	***N*** **(rate)**
Upper aerodigestive tract	140–141; 143–148; 161	78 (7.4)	59 (7.7)	265 (7.9)	482 (8.4)	36 (3.6)	23 (3.3)	92 (3.0)	160 (3.0)
Stomach	151	58 (5.5)	42 (5.5)	168 (5.0)	271 (4.7)	36 (3.6)	29 (4.1)	72 (2.3)	143 (2.7)
Colorectal	153–154	233 (22)	194 (25)	933 (28)	1,544 (27)	183 (18)	163 (23)	751 (24)	1,253 (24)
Lung	163	124 (12)	122 (16)	421 (13)	777 (14)	127 (13)	88 (13)	412 (13)	660 (12)
Breast	170					1,033 (102)	747 (107)	3,541 (114)	5,919 (111)
Cervix	171					1,577 (156)	1,118 (160)	4,667 (150)	8,197 (154)
Uterus	172					148 (15)	118 (17)	525 (17)	915 (17)
Ovary	175					152 (15)	137 (20)	597 (19)	946 (18)
Prostate	177	635 (60)	549 (71)	2,347 (70)	3,867 (68)				
Testis	178	77 (7.3)	43 (5.6)	173 (5.1)	255 (4.5)				
Kidney and Urinary	180–181	180 (17)	147 (19)	703 (21)	1,176 (21)	63 (6.2)	67 (9.6)	288 (9.3)	396 (7.5)
Melanoma	190	144 (14)	117 (15)	616 (18)	965 (17)	169 (17)	127 (18)	656 (21)	1,106 (21)
Non-melanoma skin	191	106 (10)	109 (14)	532 (16)	768 (13)	112 (11)	87 (12)	426 (14)	669 (13)
CNS incl. eyes	192–193	106 (10)	86 (11)	351 (10)	640 (11)	136 (13)	90 (13)	362 (12)	644 (12)
Thyroid	194	20 (1.9)	9 (1.2)	54 (1.6)	98 (1.7)	37 (3.7)	20 (2.9)	129 (4.1)	248 (4.7)
Endocrine	195	47 (4.5)	45 (5.9)	147 (4.4)	257 (4.5)	82 (8.1)	66 (9.4)	243 (7.8)	357 (6.7)
Bone and Mesothelium	196–197	31 (3.0)	29 (3.8)	105 (3.1)	183 (3.2)	27 (2.7)	17 (2.4)	89 (2.9)	136 (2.6)
Blood/lymphoid	198; 200–206	193 (18)	179 (23)	787(23)	1,267 (22.1)	125 (12)	109 (16)	502 (16)	833 (16)
Hodgkin	201	20 (1.9)	12 (1.6)	58 (1.7)	108 (1.9)	3 (0.3)	9 (1.3)	41 (1.3)	71 (1.3)
Non-Hodgkin	200, 202	111 (11)	101 (13)	419 (13)	727 (13)	69 (6.8)	57 (8.1)	284 (9.1)	463 (8.7)
Myeloma	203	21 (2.0)	27 (3.5)	110 (3.3)	152 (2.7)	20 (2.0)	18 (2.6)	65 (2.1)	107 (2.0)
Leukemia	204–206	24 (2.3)	23 (3.0)	116 (3.4)	179 (3.1)	18 (1.8)	17 (2.4)	49 (1.6)	98 (1.8)
All sites	140–207	2,213 (211)	1,909 (248)	8,281 (246)	13 782 (241)	4,264 (421)	3,181 (454)	14 012 (451)	23 681 (446)

Rate is number of cases per 100,000 person-years.
[1]Including cervix carcinoma *in situ*.
Abbreviations: CNS is central nervous system.

female twins identified through the MGR (Table 2). Among the singleton siblings of twins, 1,909 (10.9%) males and 3,181 (19.2%) females with cancer were identified. Overall, twins had lower risk of cancer compared to either of the singleton samples. Consistently lower crude incidence rates in twins were noted for cancer of the colorectum, kidney and urinary, prostate, breast, ovaries, skin, and blood/lymphoid system. In contrast, male twins had a higher crude incidence rate of testicular cancer compared to all cohorts of singletons.

To account for the systematic differences in birth year and family size, cumulative risks were standardized according to the joint distribution of these characteristics. For each cancer site, risks were plotted for males and females separately and for the three principal contrasts; (*i*) twins and singletons of twin families, (*ii*) singletons from twin and non-twin families, and (*iii*) twins and population (non-twin family) singletons. Each individual plot conveyed the cumulative risk curves for the two groups contrasted and the estimated risk difference between them, with a shaded area indicating its 95% confidence interval (CI).

Each contrast was evaluated separately and conservatively, starting with the main comparison of twins and singletons of twin families (cohort 1 *vs*. cohort 2; Supporting Information Fig. 1); reflecting potential unique twin influence on risk of cancer. Once differences in the distribution of sex, birth year and family size were accounted for, there was little indication of any consistent and statistically significant differences. Similarly, when singletons from twin families were compared to singletons from non-twin families (cohort 2 *vs*. cohort 3: Supporting Information Fig. 2) to capture a potential twin family influence on risk of cancer, no consistent and statistically significant differences could be discerned for either of the cancer sites. Lastly, to assess the total influence of being a twin (unique twin and twin family membership), the twin cohort was also compared to singletons from non-twin families (cohort 1 *vs*. cohort 4; Supporting Information Fig. 3). This contrast showed several consistent trends (with partial or borderline statistical significance) of twins having lower cumulative risk of cancer in the skin, blood/lymphoid system and colorectum (females). Similarly twins appeared at higher risk of testicular cancer, stomach cancer and cancers of the CNS (females).

Having identified these differences in the overall comparison, we revisited the specific contrasts to possibly trace the nature of the twin influence (unique twin or twin family) *post hoc*. The contrasts for these cancer sites are presented in Figure 1, each panel showing the (*i*) unique twin, (*ii*) twin family, and (*iii*) total twin influence. With the exception of female gastrointestinal cancers, twins' risk deviations were noted as *tendencies* (some with borderline significance), also in the comparison to singletons from twin families. For females' risk of gastrointestinal cancers, the trends from the overall comparison were instead noted (as *tendencies*) in singletons from twin families compared to singletons from non-twin families. Lastly, when more carefully considering clear *tendencies* of risk differences, a consistent and partially significant lower risk of ovarian cancer was noted in the twins' comparison to singletons from twin families (twinning influence; Supporting Information Fig. 1).

Discussion

Overall, the findings of this large population-based study indicate that twins are not at any substantially elevated risk of cancer compared to singletons from twin families, who in turn have similar cancer risks as singletons of non-twin families.

This is in agreement with conclusions of previous large register-based studies comparing twins to the general population, from which the most salient finding has been of no overall increased cancer risk in twins. The only exceptions occur in two prior Swedish studies, reporting some increased risk of cancer of the stomach, breast and testes in twins.[22,23] Otherwise, twins have if anything shown indications of lower risk of cancer compared to the general population, with tendencies (some only with borderline statistical significance, or in subgroups defined by sex/zygosity) most consistently reported for the blood/lymphoid system,[22–24] colorectum,[22–24] lung,[22,24,25] and skin (melanoma).[23,24]

In the present study, the contrast between twins and singletons from non-twin families most closely resembles previous study designs, and these results did indeed replicate previously reported differences (with the exception of cancer of the lung and breast). In addition, it also indicated a not previously reported higher risk of CNS cancer in female twins. These findings reflect the overall difference between twins and the population, and in contrast to previous studies, our design allowed for further, more detailed evaluation by separating the unique influence of twinning from that of twin family membership.

When twins were compared to singletons of twin families, to target a unique twinning influence, we did not identify any substantial differences that were consistent with respect to both direction and statistical significance. However, in *post hoc* evaluation of the cancer types for which differences were noted in the overall comparison (and in previous studies), it was possible to trace similar *tendencies* in either the contrast between twins and singletons of twin-families (unique twinning influence), or between singletons from twin and non-twin families (twin family influence).

Higher risks (tendencies) in twins

The increased risk of testicular cancer, in line with previous findings in Swedish,[22,23] but not Norwegian[24] or Finnish[25] data, appeared if anything to be due to a unique twinning influence. This would be consistent with existing etiological theories of twins being at greater risk of perinatal risk factors for testicular cancer, whether it concerns hormonal exposure[19] or growth restriction.[9,10] As for stomach cancer, the two previous Swedish reports found an increased risk restricted to male twins, and female twins respectively.[22,23] Interestingly, while our overall comparison indicated increased risk in both sexes the putative mechanism behind it differed; in males, the risk

appeared due to unique twinning and in females due to twin family influence. Seemingly in line with this finding, the lower risk of colorectal cancer in female twins also appeared due to twin family influence. We may only speculate that twin family influence might reflect the role of diet in these gastrointestinal cancers. A study on young twins found that the magnitude of genetic and environmental influences on children's 24-hr food and beverage intake differed for boys and girls, which suggests sex differences in the development of eating patterns.[29]

Our comparisons found no support for the previously reported increased risk of breast cancer in twins compared to singletons, instead, it showed female twins at a not previously reported increased risk of CNS cancer, seemingly independent of twin family influence. There are studies to suggest a role for female sex hormone exposure (specifically through estrogen-only hormone replacement therapy) in the risk of developing cancer of the CNS,[30] but whether this could be connected to the increased risk in twins compared to singletons is unknown.

Lower risk (tendencies) in twins

With the exception of the already mentioned twin family influence on female colorectal cancer, all noted lower risks in twins were most likely due to a unique twinning influence. We note that a previous speculation that socioeconomic constraints could explain twins' lower risk of skin cancer, by limiting the amount of sun exposure,[23] seems less likely in light of our findings, as we would have rather expected the influence to be at the twin family, and not unique twinning, level.

Lastly, when more carefully considering clear *tendencies* of risk differences, we also noted a consistent and borderline

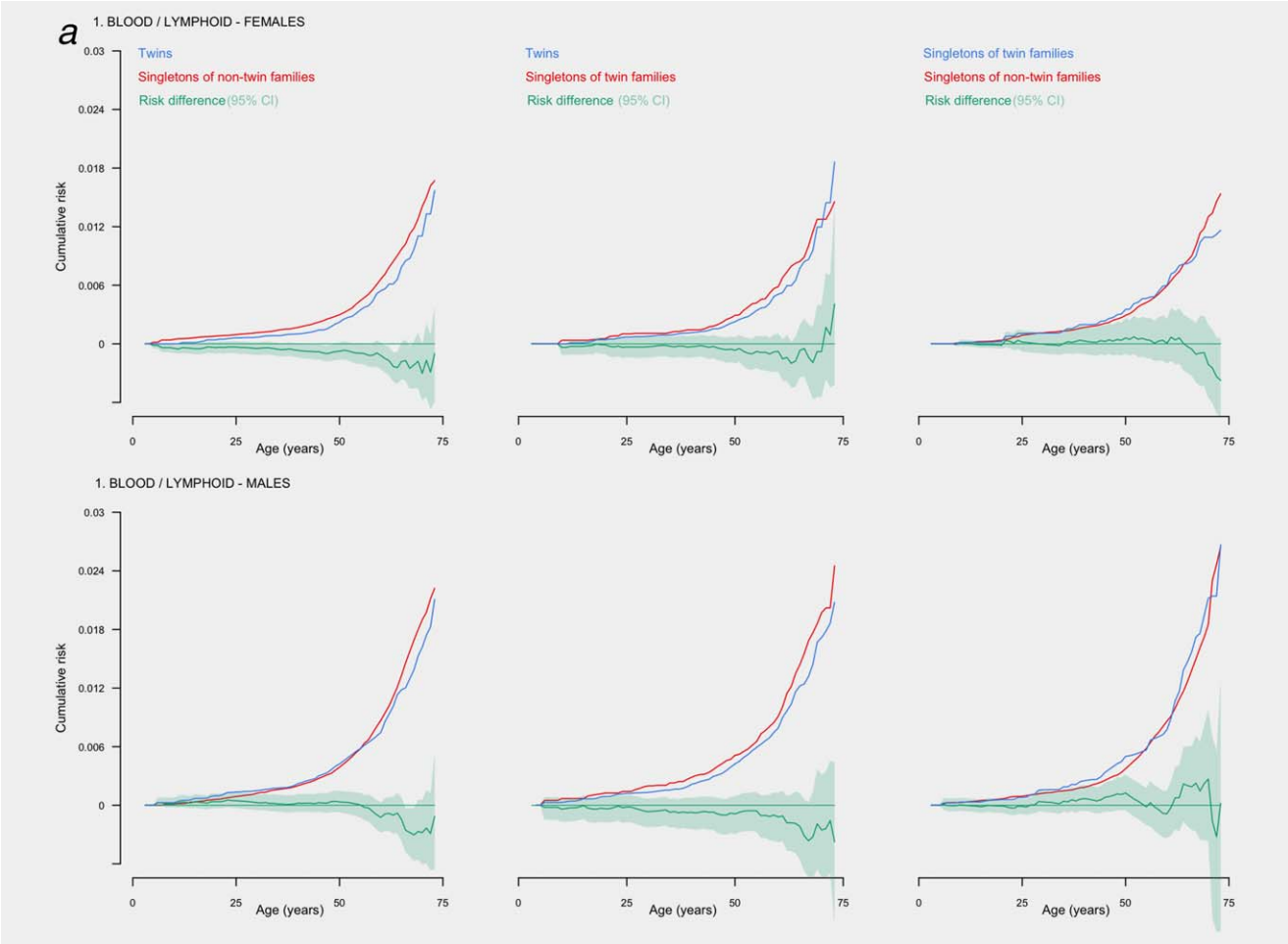

Figure 1. Cumulative risk plots for specific cancer sites in males and females separately. Each plot also includes a graphical representation of the difference in risk between the two groups contrasted, along with its confidence interval (shaded area) obtained from bootstrap. When the shadow does not include the reference line (risk difference = 0) this indicates a statistically significant difference in risk between the two groups and conversely, when the shadow covers the reference line, there is no statistically significant difference. Each panel shows the contrast between (1) twins and singletons of non-twin families, (2) twins and singletons of twin families and (3) singletons of twin and non-twin families. Panels 1 to 3 show sex-specific risks of blood/lymphoid, skin and stomach cancer respectively; panels 4 and 5 show females' risks of colorectal and CNS cancer; and panel 6 shows risks of testicular cancer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

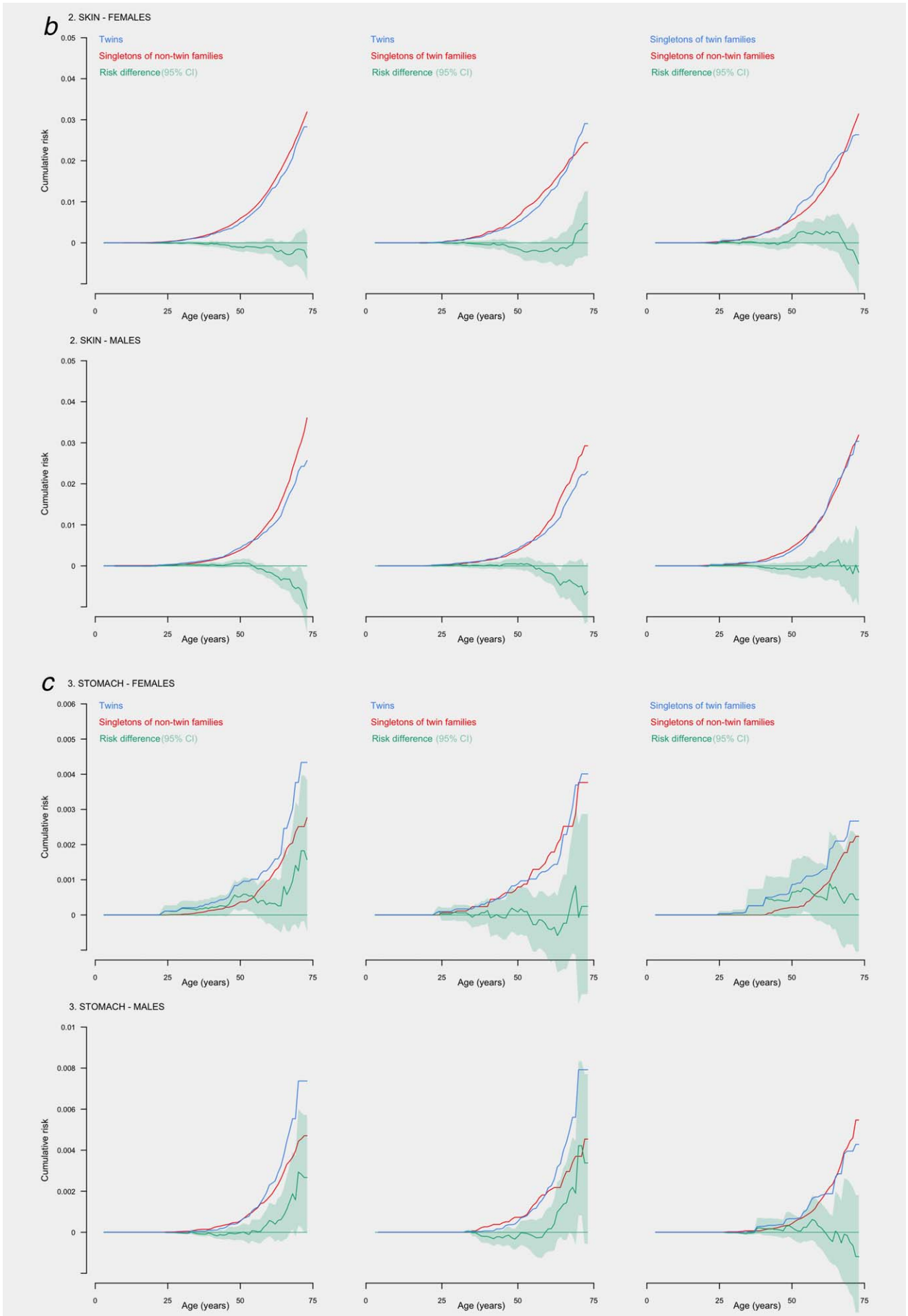

Figure 1. Continued

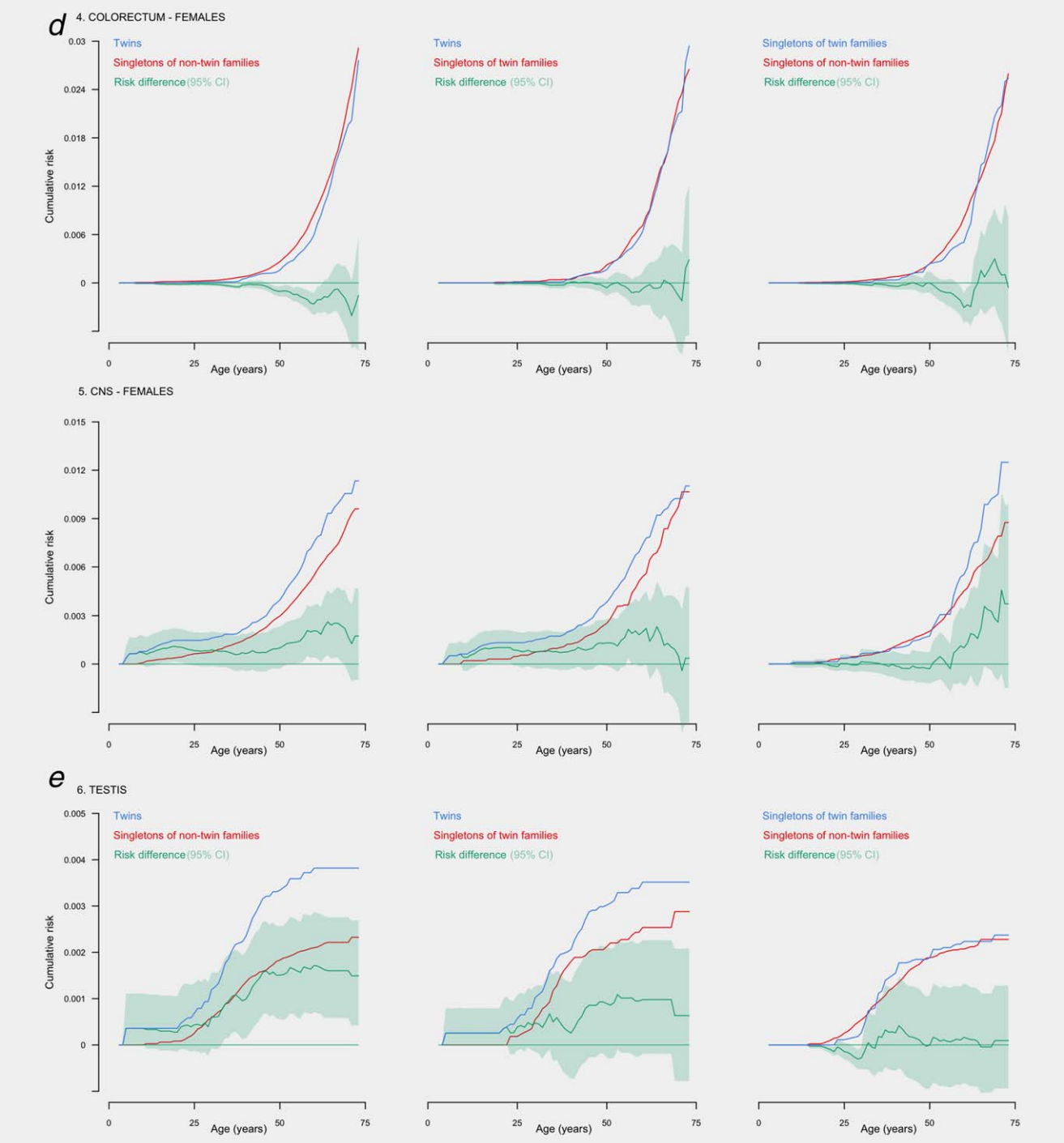

Figure 1. Continued

significant lower risk of ovarian cancer in twins compared to singletons from twin families (twinning influence; Supporting Information Fig. 1). This has not been noted in Swedish and Finnish twins, whereas in Norway twins were in fact at increased risk of ovarian cancer (SIR 1.21, 95% CI 1.02–1.43) when compared to the population. Hence this appears a novel finding, possibly allowed by our unique ability to account for twin family membership. We note that in an earlier report of cancer risks in twins of the Swedish Twin Register, dizygotic like-sexed twins had a borderline significant lower risk of ovarian cancer compared to the population (SIR 0.8, 95% CI 0.7–1.0).

The unique twinning influence captured and discussed in our study may not solely refer to the unique intrauterine experience of twins, but also to a unique sharing of experiences throughout life. For instance, lower suicide rates in twins

than in the general population has been proposed to stem from a strong family bond between twins.[31] Whether this bond is truly unique to twins, or could extend to singleton siblings (and/or singleton siblings of twins) is not known, nor are the potential long-term health influences of such a bond. For a difference (between twin and singleton sibling sharing) that also has potential consequence for later somatic health, the *in utero* experience is still the most considerable part of the unique twinning effect.

It is an underlying limitation of our study that we were unable to evaluate childhood cancers for a majority of the individuals. This is in part because the Cancer Register was established in 1958, but also because identification via the Multi-Generation Register required individuals to be alive and resident in Sweden at some point after 1961. Childhood cancer could influence likelihood of later cancer, so that e.g. those who were unaffected may be on average more resilient to carcinogenesis, and those affected may have a different risk due to their experience. A strong (positive or negative) twinning influence on risk of childhood cancer could therefore at least theoretically influence the risk contrasts between twins and singletons down the line. Still, the burden of cancer is small in childhood compared to later in life, and among those followed from an early age we saw no greater differences between twins and singletons. Moreover, there is little previous evidence of twin influence on childhood cancer to support any serious concern.

The advantage of using the population-based Multi-Generation Register was that we were able to establish the exposure, i.e. twinship, independently of outcome status, and without requiring active or passive participation in specific twin registers. In contrast to previous comparisons of twins and the general population (and/or unrelated singletons), the family linkage also allowed us to identify singletons from twin and non-twin families to enable a comparison independent of all factors shared by twin families (as well as evaluate the influence of such factors on the risk of cancer). Despite large numbers of twins and singletons, our investigation of specific cancer sites rendered relatively few cases and with the additional stratification on sex, several of the comparisons were hampered by limited power.

On the whole, twins are not at significantly increased risk of cancer compared to singletons. For specific cancer sites, most of the noted differences between twins and singletons from the general population appear to be due to a unique twinning influence.

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References

1. Phillips DI. Twin studies in medical research: can they tell us whether diseases are genetically determined? *Lancet* 1993; 341:1008–9.
2. Gluckman PD, Hanson MA, Pinal C. The developmental origins of adult disease. *Matern Child Nutr* 2005; 1:130–41.
3. Andrew T, Hart DJ, Snieder H, et al. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Res Hum Genet* 2001; 4:464–77.
4. Barker DJ. Fetal origins of coronary heart disease. *BMJ* 1995; 311:171–4.
5. Huxley R, Owen CG, Whincup PH, et al. Is birth weight a risk factor for ischemic heart disease in later life? *Am J Clin Nutr* 2007; 85:1244–50.
6. Christensen K, Wienke A, Skytthe A, et al. Cardiovascular mortality in twins and the fetal origins hypothesis. *Twin Res Hum Genet* 2001; 4: 344–9.
7. Öberg S, Cnattingius S, Sandin S, et al. Twinship influence on morbidity and mortality across the lifespan. *Int J Epidemiol* 2012; 41:1002–9.
8. Vagero D, Leon D. Ischaemic heart disease and low birth weight: a test of the fetal-origins hypothesis from the Swedish Twin Registry. *Lancet* 1994; 343:260–3.
9. Michos A, Xue F, Michels KB. Birth weight and the risk of testicular cancer: a meta-analysis. *Int J Cancer* 2007; 121:1123–31.
10. Cook MB, Akre O, Forman D, et al. A systematic review and meta-analysis of perinatal variables in relation to the risk of testicular cancer–experiences of the son. *Int J Epidemiol* 2010; 39:1605–18.
11. Trichopoulos D. Hypothesis: does breast cancer originate in utero? *Lancet* 1990; 335:939–40.
12. Ekbom A. Growing evidence that several human cancers may originate in utero. *Semin Cancer Biol* 1998; 8:237–44.
13. Ekbom A, Hsieh CC, Lipworth L, et al. Perinatal characteristics in relation to incidence of and mortality from prostate cancer. *BMJ* 1996; 313:337–41.
14. Shankar S, Davies S, Giller R, et al. In utero exposure to female hormones and germ cell tumors in children. *Cancer* 2006; 106:1169–77.
15. Ekbom A, Trichopoulos D, Adami HO, et al. Evidence of prenatal influences on breast cancer risk. *Lancet* 1992; 340:1015–8.
16. Kappel B, Hansen K, Moller J, et al. Human placental lactogen and dU-estrogen levels in normal twin pregnancies. *Acta Genet Med Gemellol (Roma)* 1985; 34:59–65.
17. Murphy M, Doherty J. Does breast cancer originate in utero? *Lancet* 1990; 335:1604
18. TambyRaja RL, Ratnam SS. Plasma steroid changes in twin pregnancies. *Prog Clin Biol Res* 1981; 69:189–95.
19. Hsieh CC, Lan SJ, Ekbom A, et al. Twin membership and breast-cancer risk. *Am J Epidemiol* 1992; 136:1321–6.
20. Weiss HA, Potischman NA, Brinton LA, et al. Prenatal and perinatal risk factors for breast cancer in young women. *Epidemiology* 1997; 8: 181–7.
21. Swerdlow AJ, De Stavola BL, Swanwick MA, et al. Risks of breast and testicular cancers in young adult twins in England and Wales: evidence on prenatal and genetic aetiology. *Lancet* 1997; 350:1723–8.
22. Braun MM, Ahlbom A, Floderus B, et al. Effect of twinship on incidence of cancer of the testis, breast, and other sites (Sweden). *Cancer Causes Control* 1995; 6:519–24.
23. Hemminki K, Chen BW. Are twins at risk of cancer: results from the Swedish family-cancer database. *Twin Res Hum Genet* 2005; 8: 509–14.
24. Iversen T, Tretli S, Kringlen E. An epidemiological study of cancer in adult twins born in Norway 1905-1945. *Br J Cancer* 2001; 84:1463–5.
25. Verkasalo PK, Kaprio J, Koskenvuo M, et al. Genetic predisposition, environment and cancer incidence: a nationwide twin study in Finland, 1976-1995. *Int J Cancer* 1999; 83:743–9.
26. Ludvigsson J, Otterblad-Olausson P, Pettersson B, et al. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. *Eur J Epidemiol* 2009; 24:659–67.
27. Statistics Sweden. Background Facts, Population and Welfare Statistics 2010:3, Multigeneration register 2009. A description of contents and quality. Statistics Sweden, Örebro.
28. Barlow L, Westergren K, Holmberg L, et al. The completeness of the Swedish Cancer Register – a sample survey for year 1998. *Acta Oncol* 2009; 48:27–33.
29. Faith MS, Rhea SA, Corley RP, et al. Genetic and shared environmental influences on children's 24-h food and beverage intake: sex differences at age 7 y. *Am J Clin Nutr* 2008; 87:903–11.
30. Benson VS, Kirichek O, Beral V, et al. Menopausal hormone therapy and central nervous system tumor risk: Large UK prospective study and meta-analysis. *Int J Cancer* 2014;136:2369–77.
31. Tomassini C, Juel K, Holm NV, et al. Risk of suicide in twins: 51 year follow up study. *BMJ* 2003; 327:373–4.