Twinship influence on morbidity and mortality across the lifespan

Sara Öberg,1,2* Sven Cnattingius,3 Sven Sandin,1 Paul Lichtenstein,1 Ruth Morley4 and Anastasia N Iliadou1

1Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, 2Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA, 3Clinical Epidemiology Unit, Department of Medicine, Karolinska Institutet, Stockholm, Sweden and 4Clinical Epidemiology & Biostatistics Unit, Murdoch Childrens Research Institute, Melbourne, Australia

*Corresponding author. Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA. Email: oberg@hsph.harvard.edu

Background
Studies in twins may be questioned with respect to their representativeness of the general population, not least considering the potential importance of the fetal environment for future health and disease. To better understand the influence twinning may have on health, we investigated long-term health outcomes of twins, their singleton siblings and singletons from the population.

Methods
Morbidity and mortality in twins was contrasted to that of their singleton siblings. These singletons from families with twins were then compared with singletons of the population to further reveal potential twin family influences on health. Familial relations were identified through the Swedish Multi-Generation Register. Among individuals born between 1932 and 1958, the number of twins and their singleton siblings identified were 49,156 and 35,277, respectively. Outcomes were incident overall cancer, cardiovascular disease (CVD) and death, identified in national registers. Standardized survival functions were estimated using Cox proportional hazards regression and the corresponding cumulative risks plotted against age.

Results
Cumulative risks of cancer, CVD and death in twins did not differ from singletons of families with twins, who in turn were found to be similar to singletons of families without twins. As could be expected from these findings, no differences in risks were found when twins were compared with singletons of the population.

Conclusions
Despite their adverse intrauterine experience, twins do not seem to fare worse than singletons with respect to adult morbidity and mortality. The findings indicate that the unique experience of twinning does not lead to adverse long-term health outcomes.

Keywords
Twins, singletons, cardiovascular disease, cancer, mortality

Introduction
More than 1 in 100 pregnancies result in twins, and with advancing maternal age and use of assisted reproductive technologies [dizygotic (DZ)], twinning is increasing. In research, it has long been acknowledged that twins may offer unique opportunities to study the influence of genes and environment on
common traits or diseases, and twins are also commonly used in epidemiological studies of exposure–disease associations. The validity and interpretation of these studies, however, depends on the ability to generalize the findings to the general population.

The past two decades have seen the emergence of a new research field dedicated to early life influence on adult health and disease, originating from observations of associations between low birthweight and risks of cardiovascular diseases (CVDs) in adulthood. Increasing evidence also suggests that complications following premature birth may include long-term cardiovascular pathology. Due to the unique intrauterine conditions of twinning—sharing space and maternal supply line—twins are generally of shorter gestational age and smaller at birth than singletons. Still, previous studies have failed to show any difference between twins and singletons with respect to gestational age and smaller at birth than singletons. 

The intrauterine environment has also been implicated in cancer development (e.g. breast cancer), and it has been proposed that (predominantly DZ) twins may be exposed to higher levels of hormones in utero and thus at increased risk of breast cancer, a notion supported by some studies, but not all. For overall cancer risk in twins, the findings of previous reports are inconsistent.

A lack of differences in adult morbidity and mortality between twins and the general population has been taken to indicate that the general growth constraint of twinning has no relation to the mechanisms behind fetal programming of adult disease. Alternatively, there may be common causes of twinning and long-term health, which if not accounted for could bias comparisons of twins and the population. Under the assumption that all full siblings share early environment and genetic descent, singletons of families with twins are exposed to the same socioeconomic and genetic background as twins. In order to elucidate the potential influence of twinning on health, we therefore compared the overall morbidity and mortality of twins with those of singleton siblings of twins. By accounting for factors shared by families with twins, these comparisons may better capture the effect of the unique experience of being a twin on morbidity and mortality. Comparing the singleton siblings of twins with singletons from families without twins may further allow insight into whether families with twins share factors that influence health.

**Materials and methods**

**Setting**

Swedish residents can be traced in national registers through the unique personal registration number. In the Multi-Generation Register (MGR), individuals are recorded along with information about their parents (biological and/or adoptive), enabling identification of family structures. The register includes all individuals born after 1932 and registered as residents in Sweden any time after 1961. For individuals born in Sweden, parental coverage is >90% from 1938 for maternal information and 1943 for paternal information.

The population-based Swedish Twin Register (STR) has been created through identification of twin births in official birth registrations, and the cohort born 1926–58 includes all pairs in which both twins were alive and traceable at the time of compilation in 1970. Determination of zygosity in like-sexed twins has further required active participation in data collection, predominantly by responding to questions concerning physical similarity in a mailed questionnaire in 1972–73 (83% response rate) and/or a telephone interview in 1998–02 (74% response rate). The methods used have been found to accurately determine zygosity in 95–99%. Since active participation may lead to volunteer selection, we were interested to see whether this cohort of the STR appear representative of all twins in the population. To allow identification of familial relations through the MGR, the cohort was restricted to those born between 1932 and 1958.

**Study populations**

To identify twins through the MGR, any pair of individuals born to the same mother within 3 days of each other was considered eligible. This method identified 24 578 twin pairs born between 1932 and 1958, with 771 being born within 1–3 days of each other. To allow comparison across birth years, the cohort was restricted to those born between 1932 and 1958.

The twin pairs represented a total of 24 150 families, some families containing two (N = 408) or three (N = 10) twin pairs. The twin cohort identified in the MGR is illustrated in Figure 1 (Cohort 1) and comprised 7631 like-sexed male, 7757 like-sexed female and 9190 opposite-sex twin pairs. The cohort from the STR consisted of 14 024 twin pairs [2381 monozygotic (MZ) male; 2935 MZ female; 4216 DZ male and 4292 (DZ) female] and 58 single occurring twins. In the 12 868 pairs that had parental information, the MGR-method verified over 99% as twins.

Wishing to separate the unique effects of twinship from those of belonging to a family with twins, twins were compared with their singleton siblings (Figure 1, Cohort 2). In 18 098 of the families with twins, 1 or more singleton full siblings were identified (averaging at 1.6 per family). To allow comparison across birth cohorts, only those born between 1932 and 1958 were considered (N = 35 227).

To further study the potential effects of belonging to a family with twins, two samples of singletons (Cohorts 3 and 4) from the population were matched to the singleton siblings of twins. In the first, the aim was to ensure that the comparison group was truly unexposed to twinning (no twins in the family). For each individual in Cohort 2, up
to 10 individuals were retrieved from the MGR matched for sex, birth year and family structure (i.e. matching also on the sex and birth year of all full siblings, shown in Figure 1; Cohorts 2 and 3). For some unique or rare family structures, the number of eligible individuals in the MGR was not enough, and as a consequence, the average number of matches in Cohort 3 was 4.5 (range 0–10). The identification of family structure also enabled future handling of potential influence from family size on the respective outcomes. The aim of the second sample was to obtain a comparison group to represent the population, and therefore all individuals of the MGR were eligible for selection (irrespective of parental information availability). Up to 10 individuals from the MGR were matched to the singletons in Cohort 2 with respect to birth year and sex (Figure 1; Cohort 4). To enable time-at-risk evaluation, dates of migration to or from Sweden were also retrieved from the Register of the Total Population at Statistics Sweden.25

Table 1 presents characteristics of the cohorts. There was a higher representation of younger birth cohorts among the twins compared with the singletons of families with twins (Cohort 2), and the samples matched to them (Cohorts 3 and 4). The birth cohort and sex distributions of the latter did not perfectly follow that of Cohort 2 as a result of incomplete matching and the exclusion of migrants before 1961. Information on family size (number of full siblings identified in the MGR) was available for Cohorts 1–3 (Table 1). Larger family sizes tended to be over-represented in Cohort 2, a result of there being more singletons than twins to represent the families with twins and more than two singletons. For Cohort 3, the number of matches per individual in Cohort 2 decreased as family size increased.

Outcome definitions
Disease status was retrieved by linkage with the Cancer, Inpatient and Cause of Death Registries. The Cancer Register was started in 195826 and completeness is high (<4% missing cases during 1998).27 The Inpatient Register spans from 1964, with 85% coverage from 1983 and full coverage of hospitalizations in Sweden from 1987.28 Information includes dates of admission and discharge and up to eight discharge diagnoses. The Cause of Death Register (computerized from 1961) contains all deaths of Swedish residents, occurring inside and outside Sweden.29 The outcomes considered were CVD, overall cancer and death, according to the diagnostic codes of the International Classification of Diseases (ICD; revisions 7–10). CVD was defined as coronary heart disease (including angina pectoris) or cerebrovascular disease (ICD-7 codes 330–334, 420–422; ICD-8 and ICD-9 codes 410–414, 430–438; and ICD-10 codes G45, I20–25, I60–69). The diagnoses used to identify overall cancer were ICD-7 codes 140–206 and ICD-10 codes C00–C97.

Statistical analyses
All individuals were followed with respect to incident case of CVD, cancer and death, respectively. Due to the inclusion criteria of the MGR, the earliest start of follow-up was in 1961, when individuals were 3–29 years old. For each outcome, time at risk extended until incident event, emigration from Sweden, death or end of follow-up on 31 December 2007.

In order to describe and compare the overall morbidity and mortality across the lifespan in the different cohorts, cumulative risks of each of the three main outcomes were estimated and plotted against attained age. Survival functions were estimated through Cox proportional hazards regression with age as underlying time scale and allowing non-proportional hazards for the two groups being contrasted (the SAS PHREG procedure). To account for potential systematic differences in the distribution of predictors of the outcome (e.g. birth year, sex and family size) between groups, the survival functions were standardized to the distribution of these covariates in the contrast sample under study. From a model predicting the log hazard given the covariates, survival estimates were predicted for each individual given their covariate pattern had they been exposed and had they not been exposed, respectively. By separately averaging these estimates at each failure time and for each of the two groups, standardized estimates were obtained. To address potential model misspecification (for the outcome), we also performed standardization based on a model for the exposure.
From a model predicting the log-odds of exposure (group membership) given the covariates, each individual was assigned a weight equal to the inverse of the predicted probability of belonging to the group they actually belonged to (the SAS LOGISTIC procedure). Standardized survival estimates were obtained from weighting a marginal Cox regression model (allowing non-proportional hazards for the groups contrasted) by these inverse probabilities of exposure. All standardized survival estimates were converted into cumulative risks of failure ($F(t) = 1 - S(t)$) and plotted against age. As the method of standardization had little influence on the comparisons, we present results based on inverse probability weighting only.

To enable evaluation of potential differences between groups, the standardization procedures were repeated in random samples drawn with replacement from the contrast sample under study. The median, 2.5th and 97.5th percentiles of the risk difference at each failure time were then extracted from these bootstrap samples and plotted against age. All statistical analyses were performed in SAS software version 9.22, and graphs produced in R software version 2.10.1.

### Results

Crude incidence rates of CVD, overall cancer and mortality did not appear increased in twins compared with singletons (Table 1). Singleton from families with twins had higher incidence rates of CVD (2.6 cases/1000 person-years (PY) for Cohort 2) than twins (2.1) as well as singletons of the population.
(2.3 and 2.4 for Cohort 3 and 4, respectively). A similar, although more modest, indication was noted also for overall mortality.

To allow fair comparison between twins and singleton siblings of twins, cumulative risks were standardized according to sex, birth cohort and family size (thus excluding twins for whom no siblings had been identified). Figure 2 displays the standardized cumulative risks of CVD, cancer and death for twins (Cohort 1) compared with singletons from families with twins (Cohort 2). Each graph in the figure also shows estimates of the risk difference plotted against age surrounded by a shaded field representing the 95% confidence interval (CI). Compared with their singleton siblings, twins did not appear to experience any increased risk of any of the outcomes. In fact, when differences in sex, birth cohort and family size distributions were accounted for, there were no discernible differences between the two groups (Figure 2).

Figure 3 is similarly composed to contrast the experience of singletons from families with and without twins (Cohorts 2 and 3, respectively). Overall, the groups experienced similar risks. If anything, singletons of families with twins had slightly increased risks of CVD and death, but the risk differences appeared modest once differences in sex, birth cohort and family size distributions had been accounted for.

As could be expected, we found no considerable differences in risk between the entire twin cohort and the sample of singletons representing the population (Cohort 4) (Supplementary Figure 1 available at IJE online).

Since the MGR identification of twins required family information, we imposed the same criteria in the evaluation of the STR cohort of like-sexed twins with known zygosity (i.e. excluding the subset of STR twins for whom family information was not available in MGR). Although there were no differences in cumulative morbidity in CVD and Cancer, STR twins were found to have a slight survival advantage (Supplementary Figure 2 available at IJE online). With the inclusion of twins with unknown zygosity in the STR (largely non-responders; 11% of all like-sexed STR twins), the two groups of twins (STR and MGR) were indistinguishable for all outcomes (results not shown).

Finally, sub-analyses comparing like-sexed and opposite-sexed twins within the MGR cohort, and MZ and DZ twins within the STR cohort, did not reveal any substantial differences in cumulative morbidity in CVD and cancer (results not shown).

Discussion

Past the first years of life, twins do not appear to have any survival disadvantage compared with singleton individuals. Twins do not fare worse than their singleton siblings with respect to risk of common diseases such as cancer and CVD, or with respect to all-cause mortality. Our results further find no indication that membership in a family with twins would confer better health, as reflected by overall morbidity and mortality.

This study confirms previous reports that CVD morbidity and overall mortality in Swedish and Danish
twins do not differ from those in the general population. Our findings also extend on these crude comparisons by evaluating risks independent of potential twin family influence on health (shared by all families with twins). Since twins experience an adverse intrauterine environment compared with singletons, including a greater degree of intrauterine growth impairment, it was speculated that twins should be at increased risk of CVD and adult mortality. Finding no differences in risk between twins and the general population led to the hypothesis that the ‘general growth constraint’ due to twinning (sharing space and supply line) is different from the type of growth impairment (experienced by both twins and singletons) associated with diseases later in life. In a recent within-twin-pair comparison, we found that the association between birthweight and risk of CVD disappeared in the absence of genetic variation. If the well-established association between birthweight and CVD were to be the result of common causes of birthweight and CVD (such as genetic factors), there would be no reason to expect a greater risk of CVD in twins on the basis of their intrauterine experience.

With respect to overall cancer risk, the situation may be more complex. Overall, our findings do not support any effect of twinning on overall cancer risk. This is in line with previous findings from comparisons of twins and the general population in Finland and Sweden, whereas in Norway twins have been found at decreased risk of cancer overall when compared with the general population. Cancer is a heterogeneous group of diseases and it is possible that increased risks for some type(s) are washed out by decreased risks for other(s), when considered all together. There are, for example, reports of increased risks of cancer of the breast and testis in young twins along with indications of decreased risks of some skin cancers and colorectal cancer in twins compared with singletons. Comparing risks of subtypes of cancer using our study design was however beyond the scope of the current study focus.

Finding that the unique experience of twinning, including the general growth constraint in utero, does not appear to have any greater consequences for future health has important implications for the use of twins in epidemiological research. Twins are considered a great asset, not only for investigations of the influence of shared environment and common genes, but also for being a great resource of longitudinal data for traditional studies of exposures and outcomes. The ability to generalize findings in twins is of great importance for the relevance of such studies. Our findings support the hypothesis that twins are not any different from singletons with respect to common disease risk across the lifespan. Particularly, with respect to CVD and overall mortality—which have been associated with an adverse intrauterine experience—the present findings support previous claims that the twinning experience per se does not put twins at greater risk of common adverse health outcomes. Even though the present findings of no effect of twinning on overall risk of cancer may also present some comfort with respect to representativeness, generalizability should not be readily inferred until the potential influence of twinning (and possibly

**Figure 3** Plots of standardized cumulative risks of (a) CVD, (b) cancer and (c) death in singletons from families with and without twins. Each plot also shows the risk difference and its 95% CI (shaded area)
also zygosity) on different types of cancer has been further elucidated. These findings are naturally also reassuring for twins and their parents. The twins in the current study were born before more recent increases in twinning rates following advancing maternal age and use of assisted reproductive technologies. Thus, until the potential long-term health consequences for these groups are known, the present results may not be generalizable to younger cohorts.

Due to limitations of the national registers, we were unable to evaluate the experience throughout the entire life course, as all individuals were required to survive past the first years of life and some up to adulthood. Hence, we have not captured the increased risks of stillbirth and infant mortality that twins experience compared with singletons. It has been speculated that this early selection may make twins on average more resilient than singletons and at least in theory, findings of similar adult mortality in twins and singletons could be the result of such resilience perfectly outweighing any negative health effect of twinning (e.g. intrauterine programming). Previous reports of similar risks in twins and singletons across birth cohorts with different perinatal and infant mortality indicate a limited influence of such selection. Also, it is important to point out that the unique experience of twinning may not be limited to the prenatal period. Twins have been found to have lower suicide rates than the general population, and this has been attributed to the influence of strong family ties. Whether such ties could be different/stronger in twin pairs than in siblings and how this then would influence long-term health is not known. Thus, with respect to differences between twins and singletons, the intrauterine experience still remains the most substantial and well-defined distinguishing feature.

Establishment of zygosity in like-sexed twins generally requires participation in data collection (questions about similarity or DNA analysis), leading to a ‘volunteer’ selection that may threaten representativeness. In the present STR cohort, participation rates have been very high; still, the like-sexed twins with known zygosity showed a slight survival advantage compared with twins identified through the MGR (further diminished by the inclusion of twins with unknown zygosity/non-responders). However, there were no discernible differences with respect to overall morbidity in cancer and CVD.

This study is population based. The unique family information of the MGR has enabled verification of twin status without imposing any selection pressure other than that made for all comparison groups. The present design differs further from all previous comparisons of twins and singletons in having allowed the evaluation of risks independent of factors shared by families.

To conclude, we have found that the life course experience of overall cancer, CVD and all-cause mortality is similar in twins and their singleton siblings, as well as singletons from the population. The findings thus support that twins are no different from singletons with respect to mortality and morbidity throughout the life course.

Supplementary Data
Supplementary Data are available at IJE online.

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KEY MESSAGES
- Twins experience the same cumulative risks of CVD, cancer and death as their singleton siblings.
- Twin family membership does not appear to have any greater influence on health.
- Overall, adult morbidity and mortality appear to be similar in twins and singletons.

References


