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Original article

Early-life mortality risks in opposite-sex and same-sex twins: a Danish cohort study of the twin testosterone transfer hypothesis

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ABSTRACT

Purpose: To investigate the twin testosterone transfer (TTT) hypothesis by comparing early-life mortality risks of opposite-sex (OS) and same-sex (SS) twins during the first 15 years of life.
Methods: We performed a population-based cohort study to compare mortality in OS and SS twins. We included 68,629 live-born Danish twins from 1973 to 2009 identified through the Danish Twin Registry and performed piecewise stratified Cox regression and log-binomial regression.
Results: Among 1933 deaths, we found significantly higher mortality for twin boys than for twin girls. For both sexes, OS twins had lower mortality than SS twins; the difference persisted for the first year of life for boys and for the first week of life for girls.
Conclusions: Although the mortality risk for OS boys was in the expected direction according to the TTT hypothesis, the results for OS girls pointed in the opposite direction, providing no clear evidence for the TTT hypothesis.

Introduction

It is well known that boys have higher risk of infant mortality and morbidity than girls [1,2]. Sex differences in mortality have been explained by a combination of biological, social, and environmental factors, but lifestyle and behavioral factors are less likely to explain sex difference in infant mortality [3]. Studies of animal models find that males exhibit reduced immune responses and increased intensity and prevalence of infections compared with females [4]. These sex differences may reflect the immunosuppressive effects of testosterone, as well as the positive effects of progesterone and estradiol on immune responses [4,5]. Whether these findings apply to humans is unknown [1], but there is evidence that girl infants have lower mortality from infections [6] and respiratory conditions [7] compared with boys. It is hypothesized that the male disadvantage begins *in utero* [8], where the gonadal steroid production differs between the sexes [3].

Twin studies offer unique opportunities to investigate mechanisms underlying sex differences. There is evidence suggesting that male sex hormones could influence the female fetus in opposite-sex (OS) twins. For instance, studies in mammals have found that female fetuses positioned between two males are likely to express masculinization of anatomical, physiological, and behavioral traits in adult life [9]. Sex hormones are lipid soluble steroids capable of crossing fetal membranes [10]. The twin testosterone transfer (TTT) hypothesis states that human fetuses gestated with a male co-twin are masculinized in development, which may be due to the exposure of prenatal androgens [11]; however, the evidence remains inconclusive (for reviews, see [11,12]).

Hence, early-life mortality risks in OS and same-sex (SS) twins may also differ due to zygosity differences. OS twins are always dizygotic (DZ), whereas SS twins are either monozygotic (MZ) or DZ. A large part of MZ twins (70%–75%) are monochorionic (MC), sharing only one placenta, and the remaining part has completely separate placentas and membranes (dichorionic [DC]) [13]. MC twins are at increased risk for perinatal mortality and morbidity compared with DC twins [14].

The authors have no competing interests to declare.

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The literature comparing OS and SS twins with regard to early-life mortality risks is limited and inconsistent, but higher infant mortality for SS boys than for OS boys [15–17] and adverse outcomes including increased respiratory morbidity and higher mortality for OS twin girls in agreement with the TTT hypothesis have been reported [18–20]. Our study using data on a sample of 68,629 Danish twins aims to compare mortality in twin boys and girls and in OS and SS twins by age and over time within the first 15 years of life.

Materials and methods

Material

This study consists of all twins born in Denmark during the period 1973–2009. The twins were identified through the Danish Twin Registry [21,22]. The Danish Twin Registry has collected data on twins including zygosity of SS twin pairs born up to 2000, which is based on questions about the degree of co-twin similarity [23]. However, both twins have to be alive beyond infancy to have their zygosity assessed by questionnaire and therefore zygosity is generally available only for twin pairs surviving infancy [21]. All twins both live-born and stillborn have been identified through the Danish Medical Birth Registry since 1973 [24]. For these twins, information about the personal identification numbers for the infant and the mother, information on deaths among live-born twins, as well as information on emigrations came from the Danish Civil Registration System [25]. Information on causes of death was obtained from the Danish Registry of Causes of Death [26]. Information on maternal education came from the Danish Education Registers [27], and information about birth weight was obtained from the Medical Birth Registry [28]. In total, 68,629 live-born twins were eligible for analyses (Table 1). For the birth cohorts 1973–1996, information about stillborn twins was available for 34,736 twins.

Variables

Possible confounders in this study were year of birth, maternal education, and maternal age. Year of birth ranged from 1973 to 2009 and was stratified into decades (birth cohorts: 1973–1979, 1980–1989, 1990–1999, 2000–2009). Maternal education refers to the highest completed education until year 2012. The categorization of education was based on the International Standard Classification of Education [29]. Three groups were distinguished: primary and lower secondary (<10 years), upper and post-secondary (10–12 years), and tertiary (>12 years). Maternal age at delivery was categorized as younger than 20 years, 20–34 years, and 35 years and older. Birth weight was considered an intermediate factor in the association between OS/SS twins and mortality. However, all analyses were repeated including birth weight, and the results were similar (not shown). Based on age-at-death, we generated four separate risk periods according to the definitions of the categories of infant deaths: early neonatal deaths (0–7 days), late neonatal deaths (8–28 days), postneonatal deaths (29–365 days), and child mortality (1–15 years) [30].

Statistical analyses

Analyses of differences in categorical baseline characteristics (emigration and maternal education) for OS and SS twins were performed by χ^2 tests. Differences in continuous background variables (maternal age and birth weight) were investigated using *t* tests. Relative risks (RRs) for child mortality (0–15 years) for each of the possible confounders were calculated stratified by sex applying log-binomial regression both crude and adjusted for the other covariate. All estimates were adjusted for decades.

To analyze the associations of mortality between girls and boys and between OS and SS twins, we used piecewise stratified Cox regression, adjusting for the nonindependence of twins in a pair [31]. The twins were followed up for 15 years or until July 1, 2013, whichever came first. All associations were expressed as hazard ratios (HRs) with 95% confidence intervals (CIs). The Cox proportional hazards assumption was fulfilled in all risk periods. Mortality HRs, taking into account emigrations as censoring, were reported including adjustment for decades, crude, and adjusted for maternal age and education. Analyses of sex differences were done within all twins as well as within intact OS twin pairs. All analyses were repeated within each decade, and analyses of OS and SS twins were conducted separately for each sex. Cause-specific mortality was

Table 1
Characteristics of live-born twins in Denmark during 1973–2009

Characteristics	Twin boys			Twin girls			All
	Opposite sex	Same sex	All	Opposite sex	Same sex	All	Total
Study population *N*	12,051	22,901	34,952	12,033	21,644	33,677	68,629
Deaths before age 15* (% of study population *N*)	271 (2.25)	825 (3.60)	1096 (3.14)	252 (2.09)	585 (2.70)	837 (2.49)	1933 (2.82)
Early neonatal deaths (0–7 d)	179 (1.49)	551 (2.41)	730 (2.09)	166 (1.38)	424 (1.96)	590 (1.75)	1320 (1.92)
Late neonatal deaths (8–28 d)	27 (0.22)	88 (0.38)	115 (0.33)	27 (0.22)	53 (0.24)	80 (0.24)	195 (0.28)
Postneonatal deaths (29–365 d)	35 (0.29)	117 (0.51)	152 (0.43)	36 (0.30)	67 (0.31)	103 (0.31)	255 (0.37)
Child mortality (1–15 y)	30 (0.25)	69 (0.30)	99 (0.28)	23 (0.19)	41 (0.19)	64 (0.19)	163 (0.24)
Emigrated*	187 (1.6)	346 (1.5)	533 (1.5)	185 (1.5)	363 (1.7)	548 (1.6)	1081 (1.6)
Mean (SD) birth weight,† *N*	2581 (603), 1913	2484 (630), 22,406	2518 (623), 34,319	2470 (584), 11,909	2416 (593), 21,178	2435 (591), 33,087	2477 (609), 67,406
Mean (SD) maternal age‡	31.05 (4.6)	30.04 (4.8)	30.39 (4.8)	31.05 (4.6)	30.00 (4.8)	30.37 (4.8)	30.38 (4.78)
−20	69 (0.6)	334 (1.5)	403 (1.2)	69 (0.6)	343 (1.6)	412 (1.2)	815 (1.2)
20–34	9561 (79.4)	18,965 (82.9)	28,526 (81.7)	9551 (79.4)	17,865 (82.6)	27,416 (81.5)	55,942 (81.6)
35+	2415 (20.1)	3591 (15.7)	6006 (17.2)	2407 (20.0)	3419 (15.8)	5826 (17.3)	11,832 (17.3)
Maternal education§							
Primary and lower secondary	2231 (18.8)	4609 (20.4)	6840 (19.9)	2226 (18.8)	4412 (20.8)	6638 (20.0)	13,478 (19.9)
Upper and post-secondary	4852 (40.8)	9272 (41.1)	14,124 (41.0)	4847 (40.9)	8702 (40.9)	13,549 (40.9)	27,673 (40.9)
Tertiary	4798 (40.4)	8694 (38.5)	13,492 (39.2)	4790 (40.4)	8153 (38.3)	12,943 (39.1)	26,435 (39.1)

Values are numbers (percentages) unless otherwise stated.
* Before 15 years or before July 1, 2013.
† In grams.
‡ In years.
§ Highest completed education during 1981–2012. Primary and lower secondary education refers to less than 10 years of education, upper and post-secondary education refers to 10–12 years education, and tertiary education refers to more than 12 years of education.