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HORMONAL RISK FACTORS IN TESTICULAR CANCER

A CASE-CONTROL STUDY

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The authors interviewed 273 northern California testicular cancer cases aged 40 and under diagnosed between 1976 and 1981, their mothers, and matched peer controls and their mothers on prenatal hormone exposure and other variables. Included was a population-based substudy (1979–1981) of all interviewable cases reported to the San Francisco Bay Area Surveillance, Epidemiology, and End Results registry. They found odds ratios (OR) of from 8.3 (sons' report) to 4.5 (mothers' report) associated with cryptorchidism, but found no association with mothers' hormone exposure or diethylstilbestrol exposure in pregnancy. They also found a significant association with lower age at puberty (OR = 2.0); a marginally significant association with mothers' breast cancer (OR = 2.9, p = 0.054); and a significant protective effect of reported mononucleosis (OR = 0.6). These associations remained strong in the population-based substudy. When cases were divided by histology, strong and specific associations of earlier puberty (OR = 2.3) and mothers' breast cancer (OR = 4.4) with nonseminomatous cancer, and of reported mononucleosis (OR = 0.3) with seminomatous cancer, were found. These observations suggest that 1) prenatal exogenous hormone exposure does not account for a significant fraction of testicular cancer, 2) a cluster of "breast-cancer-like" risk factors are associated with nonseminomas, and 3) there is some genetic risk of nonseminomas.

seminoma; testicular neoplasms

The age-specific incidence curve for testicular cancer has shifted over the past half-century so that the peak is now among men in their thirties, suggesting that gestational or early childhood events are important in the genesis of this tumor. Furthermore, cryptorchidism, the primary risk factor for testicular cancer, is believed to be associated with hormonal imbalance during gestation (1–5). Since cryptorchidism is associated with a risk of cancer in the contralateral testicle as well as in the cryptorchid testicle, it has been proposed that a single underlying hormonal abnormality may be responsible for both the cryptorchidism and the cancer (3). Recently, Henderson et al. (6) have suggested that this abnormality may be, or may be associated with, a rise in maternal estrogen production in early pregnancy. In a case-control study, Henderson et al. (1) found excessive nausea and vomiting during pregnancy, both of which

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are believed to follow a rapid rise in estrogen production, to be associated with subsequent testicular cancer.

Henderson and others have also reported associations between *exogenous* hormone exposure during pregnancy and subsequent testicular cancer (1–3). Prenatal estrogen exposure is known to produce neoplasms in mice, including testicular neoplasms in male offspring (7). As is well known, diethylstilbestrol, a synthetic estrogen, was given to several million mothers in 1943–1970 to prevent miscarriage. It has been estimated that about one million sons and one million daughters were exposed in utero (8, 9). Daughters exposed in utero have been found in case-control studies to be at an excess risk for clear-cell adenocarcinoma of the vagina (estimated to be between 0.14 and 1.4 per 1,000 through age 24 in the highest-risk birth cohort (10)) and in prospective studies (11–14) to be at risk for various reproductive defects. Prospective studies of diethylstilbestrol-exposed sons have shown approximately tripled risks for cryptorchidism, hypoplastic testes, and capsular induration, as well as spermatozoal abnormalities (14–17). Although there is at least one case report of testicular cancer in a diethylstilbestrol-exposed son (18), no neoplasms have been reported in prospective studies.

Two case-control studies have reported associations between prenatal exogenous hormone exposure and testicular cancer. Schottenfeld et al. (3) found odds ratios of 1.96 and 1.80 when testicular cancer cases were compared with neighborhood and hospital controls, respectively, for diethylstilbestrol or other hormone exposure during pregnancy. Depue et al. (2) found an odds ratio of 8.0 for any hormone exposure during the first trimester of pregnancy when testicular cancer cases aged 30 and under were compared with matched neighborhood controls. These results, together with those of the animal studies, are suggestive of an association, although clearly preliminary. Here, we report on a third case-control study of the association between hormone exposure during pregnancy, and other hormone-associated risk factors, and testicular cancer.

METHODS

Eligible cases were men diagnosed with germ-cell carcinoma of the testis between 1976 and 1981, residing in northern California or northwestern Nevada, born in 1940 or later, and at least 18 years of age at time of diagnosis. Deceased patients were not included in the study. All testicular cancer patients reported to the California Tumor Registry (the population-based Surveillance, Epidemiology, and End Results registry for the San Francisco-Oakland standard metropolitan statistical area) in 1979–1981 were contacted for interview. Of 171 eligible cases, 131 (77 per cent) were interviewed. In addition, 247 cases from outside the population-based subset were contacted as clinical referrals from Stanford University Medical Center, the University of California Medical Centers at San Francisco and Davis, Letterman and Oaknoll military hospitals, and other hospitals and private practices outside the registry area. Of these 247 cases, 193 (78 per cent) were interviewed. Of the clinical referral cases interviewed, 76 per cent were diagnosed in 1976–1979. In total, 324 eligible cases were interviewed, of whom 40 per cent were from the population-based subset.

Each interviewed case who had a living mother was asked for permission to contact her and was asked to name two "peer controls." The algorithm for identifying peer controls was introduced by first asking about male friends from grammar school and then about those from high school with whom the subject had stayed in touch. If more than one male friend was named, the subject was asked for the person to whom he was closest. Subjects were also asked to name at least two friends made since high school. Later in the interview, permission was asked to contact two friends as controls, beginning with the earliest friend named and progressing to high school and

post-high school friends, if necessary. Each potential control was matched on race and age (±5 years) and was excluded if he had a history of cancer. Of 324 interviewed cases, 19 could not or would not name a control; in addition, five controls refused, seven were not locatable, and five were ineligible. If the first control who was named or his mother could not be interviewed, the second control was contacted and interviewed. In those instances in which the first control (initially difficult to locate) was later interviewed, two controls with living mothers were interviewed. Thus, two controls were obtained for about 10 per cent of the cases. The analysis is based on 273 pairs of cases and controls and their mothers. For those cases with more than one control, the control for this analysis was chosen by selecting a childhood friend in preference to a high school friend and a high school friend in preference to a post-high school friend. Peer controls were used to match for social class at birth since diethylstilbestrol exposure is known to be associated with mother's social class. This matching procedure may overmatch for other variables possibly associated with risk of testicular cancer, such as birth order or access to medical centers that were particularly likely to have prescribed hormones during pregnancy.

The percentages of case and control mothers alive at the time of interview were 87 per cent and 89 per cent, respectively. For the 273 matched pairs, 222 (93 per cent of living mothers) case mothers and 224 (92 per cent of living mothers) control mothers were interviewed (table 1).

All interviews were conducted by telephone by trained interviewers. Information was obtained from cases and controls on demographics, medical history, job history, and risk factors identified by previous studies. Information was also collected on age at puberty as reflected by first emission and appearance of pubic hair; however, the emission data were found to be unreliable.

Each mother was questioned about her son's medical history and her own reproductive history—including abortions, stillbirths, terms of each pregnancy, birth weight, drug use during pregnancy, use of fertility drugs to become pregnant, x-rays, bleeding and spotting, and other complications. Information was also sought on mothers' medical history and family history of hernia and cryptorchidism. For all positive reports of hormone use during pregnancy, permission for medical records was

TABLE 1

Accrual and completion of cases, controls, case and control mothers, and matched pairs, northern California, 1976–1981

Study population characteristic	Matched pairs (n = 273)	Case mothers (n = 222)	Control mothers (n = 224)	Matched quadruples (n = 193)
Age (years)				
≤30	158	135	136	122
>30	115	87	88	71
Histology				
Seminoma	93	75	71	60
Mixed histology	42	30	35	28
Nonseminoma	138	117	118	105
Method of accrual				
Cases diagnosed in San Francisco-Oakland SMSA,* 1979–1981	111	90	88	75
Cases not diagnosed in San Francisco-Oakland SMSA, 1979–1981	162	132	136	118

* SMSA, standard metropolitan statistical area.

obtained and an attempt was made to get a copy of the hospital birth record and to locate the attending physician for confirmation of drug use. Many of the attending physicians were deceased at the time of inquiry, however, and some of the hospital records had been destroyed. Hormone use was reported by 19 mothers, two of whom were mothers of second controls not chosen for this analysis, and was confirmed by physician's or hospital record in four of the 19 cases. (Comparable proportions of verified exposures have been reported by other investigators (3, 19).)

Medical record information was abstracted for all interviewed cases on histology, date of diagnosis, laterality, and treatment.

The analyses which follow were carried out on the set of 273 matched, case-control pairs and the set of 222 case mothers and 224 control mothers. Both paired and unpaired odds ratio estimates and confidence intervals were estimated, the paired-comparisons for the mothers being carried out on the subset of 193 matched case-mother-control-mother quadruples. Odds ratios estimated by paired and unpaired methods were generally in close agreement, and confidence intervals estimated by unpaired methods were generally narrower. Since there was no evidence of bias when we compared the paired and unpaired estimates, we have taken advantage of the extra number of case and control mothers in the unpaired comparison and report the unpaired odds ratios and confidence intervals throughout. Paired estimates for the most important variables are given under Results for comparison.

Three subset analyses were carried out, dichotomizing by age at diagnosis (≤30 years vs. >30 years), histology (seminoma only vs. nonseminoma and mixed types combined), and population-based versus nonpopulation-based ascertainment (table 1).

Odds ratios were calculated from the sons' and mothers' responses and were estimated by standard methods for matched and unmatched case-control studies (20). Because standard, approximate techniques are inaccurate for risk factors with very few positives, significance levels and confidence intervals are based on exact distributions conditional on marginal totals (20). Significance levels are one-sided against the hypothesis that the odds ratio is exactly 1.00, and confidence intervals are 95 per cent confidence intervals for the odds ratio. The adjusted odds ratios were estimated and tested for significance and homogeneity by the Mantel-Haenszel method (21).

RESULTS

Case and control matching

Since cases and controls were matched on age and race, there were no differences between the two groups on these variables (table 2). Cases and controls also agreed closely on birth order and religion, and case and control mothers agreed well on education, religion, and race. The close agreement on birth order may be in part because of a tendency for cases to pick controls of the same birth order as themselves, resulting in overmatching for this potential risk factor.

Although the matching process would be expected to produce approximate social class agreement, cases and controls were slightly different in educational level and father's occupation, with cases skewed toward the upper end of both distributions (table 2). The difference may reflect a tendency of subjects to match downward in control selection, or it may represent the increased risk of testicular cancer at the upper end of the social class spectrum (22). The possible effects of this matching bias on the major risk factors are discussed below.

Odds ratios measured from sons' and mothers' responses

Several variables were measured both from the sons' responses and the mothers' responses, including cryptorchidism, hernia, and childhood diseases. Consistent

TABLE 2

Demographic comparisons of cases and controls and case and control mothers, northern California, 1976–1981

	Cases (n = 273) (%)	Controls (n = 273) (%)	Case mothers (n = 222) (%)	Control mothers (n = 224) (%)
Education				
High school graduate	14	22	53	54
Some college	36	30	33	29
College graduate +	50	48	14	17
Religion				
Catholic	31	28	29	28
Protestant	38	37	56	53
Other	31	35	16	19
Race				
White	88	86	95	91
Black	1	1	0	1
Latin	4	5	3	5
Asian	4	4	2	2
Other	3	3	1	0
Father's occupation				
Professional/managerial/ proprietary	28	19		
Technical/clerical	40	38		
Skilled/manual	32	43		
Birth order				
1	37	35		
2	33	35		
≥3	30	30		
Mean age (years)	31.5	31.9	59.0	58.8

with earlier reports, cryptorchidism was the largest risk factor in our data. The odds ratio (OR) associated with cryptorchidism was 8.3 when measured from the sons' responses and 4.5 when measured from the mothers' responses (table 3). There was no association with hernia when measured either by the sons' responses or the mothers' responses. No association was found with infectious diseases of childhood (including mumps) when measured either by the sons' responses or the mothers' responses (odds ratios not shown).

Among the variables measured from the sons' responses only, the greatest association was with puberty at age less than 14 years, as measured by the appearance of pubic hair. (Reported appearance of pubic hair may differ from physiologic puberty by two years or more.) The odds ratio associated with earlier puberty was 2.0, and this was statistically significant (p = 0.001). Three variables appeared to have a protective effect. Exogenous hormone use by the son (excluding hormone use as part of therapy for the tumor) showed a statistically significant protective effect (OR = 0.5, p = 0.042). Reported mononucleosis was protective (OR = 0.6, p = 0.046). An odds ratio of 0.6 was also associated with reported vasectomy but did not reach statistical significance.

Of the reported hormone exposures in cases and controls, half were cortisone/prednisone, most commonly given for poison oak. Clomiphene was prescribed for two controls versus no cases; five controls were exposed to anabolic steroids versus two cases.

We found no association between testicular cancer risk and severe acne or treatment for acne when measured from the sons' reports. There was no association with reported history of venereal disease,

TABLE 3

Odds ratios calculated from subjects' and mothers' responses, northern California, 1976–1981

	Cases (No. positive/ total no.)	Controls (No positive/ total no.)	Odds ratio	Confidence interval	*p* value
		Subjects			
Cryptorchidism	29/246	4/252	8.3	2.8–32.9	<0.001
Hernia	47/272	38/271	1.3	0.8–2.1	0.18
Age at puberty <14 years	206/254	170/248	2.0	1.3–3.1	0.001
Mononucleosis	17/173	35/217	0.6	0.3–1.1	0.046
Vasectomy	15/173	30/212	0.6	0.3–1.2	0.065
Hormone use	12/238	23/230	0.5	0.2–1.0	0.042
		Mothers			
Cryptorchidism	24/217	6/223	4.5	1.7–13.7	<0.001
Hernia	27/219	25/223	1.1	0.6–2.1	0.41
Breast cancer	11/219	4/221	2.9	0.8–12.5	0.054
Exogenous hormones in pregnancy	9/211	10/214	0.9	0.3–2.6	0.52
10-month pregnancy	15/218	8/224	2.0	0.8–5.6	0.088
Medication for nausea in pregnancy	17/220	12/224	1.5	0.7–3.5	0.21
Nausea (first born)	7/221	4/224	1.8	0.5–8.5	0.26
Low birth weight (<2.7 kg)	19/213	19/200	1.0	0.5–2.0	0.52

years of rural residence versus urban residence, or use of jockey shorts (odds ratios not shown).

Among variables measured from the mothers' responses only, we found that an odds ratio of 2.9 was associated with history of mothers' breast cancer. Aside from cryptorchidism, mother's breast cancer was the largest risk factor in either the sons' or the mothers' responses, but it was marginally nonsignificant ($p = 0.054$). When mothers' reported cancer was examined in detail, there were 28 cancer cases reported among case mothers and 23 among control mothers; however, there were 11 cases of breast cancer in case mothers as opposed to four cases of breast cancer in control mothers. The birth of the index case preceded the mother's cancer diagnosis in all cases; thus, in no instance could hormonal events associated with breast cancer treatment have affected the birth of the son. The son's diagnosis followed the mother's diagnosis in eight of 11 cases, suggesting that oversurveillance as a result of the sons' cancers was not responsible for the excess of breast cancers in the mothers.

The mothers' responses showed a doubled risk for 10-month pregnancy and a risk of 1.5 associated with reported medication for nausea in pregnancy, but these were not statistically significant. When nausea in pregnancy was examined in first pregnancies only, the odds ratio was 1.8. (Nausea in pregnancy was reported only if medication was prescribed for nausea.) There was no association with low birth weight (OR = 1.0). We found no association with order of birth, bleeding or spotting during pregnancy, or x-rays of the womb during pregnancy (odds ratios not shown).

There was no association between testicular cancer risk and exogenous hormone exposure during pregnancy as reported by the mother (OR = 0.9). Examining hormone exposures during pregnancy in detail, we found that a total of 19 were reported by case and control mothers, of which two were diethylstilbestrol exposures in second controls (not included in the analysis (table 4)). Of the remaining 17 exposures, eight were in cases, including four diethylstilbestrol exposures, and nine were in controls, including two diethylstilbestrol exposures.

TABLE 4

Exogenous hormone exposure during pregnancy, northern California, 1976–1981

	No. of exposed mothers	
	Cases	Controls*
Diethylstilbestrol	4	2
Progestin	1	3
Pregnancy test	2	2
Fertility drug	1	2
All reasons	8	9

* Two diethylstilbestrol exposures in extra controls not included in this analysis.

There was an odds ratio of 2.0 associated with prenatal diethylstilbestrol exposure, but this was not statistically significant.

Odds ratios by age of case

The variables reported above were examined further in three subset analyses, dichotomizing by age, tumor type, and population-based versus nonpopulation-based ascertainment (table 5). First, for comparison with earlier studies, cases were divided into those aged 30 years or less at diagnosis and those aged more than 30 years at diagnosis (table 5). Of the variables that were statistically significant in the overall analysis, cryptorchidism, measured both by the sons' and the mothers' reports, remained the principal risk factor in both age groups.

The associations with age at puberty and with mothers' breast cancer were confined to younger cases, with nine of 135 mothers of younger cases and one control mother reporting breast cancer. There was no association with exogenous hormones during pregnancy in either age group.

Odds ratios by histologic subtype

Next, we divided the cases into two broad histologic subtypes, seminomas ($n = 93$) and all others ($n = 180$), including nonseminomatous tumors ($n = 138$) and tumors of mixed histology ($n = 42$). Again, the risk associated with cryptorchidism was comparable in the two groups (table 5). The risk associated with mothers' breast cancer was confined to nonseminomatous tumors and was comparable in magnitude to the risk associated with cryptorchidism (OR = 4.4). The risk associated with puberty at age less than 14 years also retained statistical significance only in the nonseminomatous group. The protective effects of mononucleosis and of sons' use of exogenous hormones were confined to seminomas only, with mononucleosis reaching statistical significance. The risk associated with vasectomy was not statistically significant in either group. There was no association with mothers' use of exogenous hormones during pregnancy in either group.

There were eight breast cancers in mothers of sons with nonseminomas and mixed tumors versus two in control mothers (table 6). Six of the eight breast cancers in case mothers and neither of the two in control mothers was diagnosed at age 49 or less.

Odds ratios by method of ascertainment

The third subgroup analysis compared those cases who were identified through the population-based tumor registry for the San Francisco-Oakland standard metropolitan statistical area with those identified outside the population-based referral mechanism. This analysis was performed as a check on referral bias in the cases contacted through clinical sources. First, the population-based cases were compared with the nonpopulation-based cases on the demographic variables. The two groups were similar on race, religion, and father's occupation. Population-based cases were about one year older at diagnosis (29 years vs. 28 years), and a higher percentage of population-based cases had graduate degrees (21 per cent vs. 14 per cent). The proportion of seminomas among all tumors was lower in the population-based subset (23 per cent vs. 34 per cent).

Cryptorchidism, mothers' breast cancer, and earlier puberty remained among the principal risk factors when the study was restricted to population-based cases (table 5). The odds ratios associated with cryptorchidism and earlier puberty were comparable to or greater than those in the

TABLE 5

Odds ratios calculated from subjects' and mothers' responses, by age, histology, and method of ascertainment, northern California, 1976–1981

	Subject's age (years)				Histology				Method of ascertainment			
	≤30		>30		Seminomas		Nonseminomas and mixed tumors		Population-based		Nonpopulation-based	
	Odds ratio	*p*	Odds ratio	*p*	Odds ratio	*p*	Odds ratio	*p*	Odds ratio	*p*	Odds ratio	*p*
					Subjects							
Cryptorchidism	6.0	0.002	15.3	0.001	10.2	0.008	7.7	<0.001	5.0	0.007	18.1	<0.001
Age at puberty <14 years	2.4	0.001	1.5	0.13	1.5	0.2	2.3	0.001	2.3	0.009	1.8	0.024
Mononucleosis	0.5	0.067	0.7	0.26	0.3	0.009	0.9	0.49	0.4	0.041	0.8	0.34
Vasectomy	0.7	0.42	0.5	0.053	0.6	0.24	0.5	0.12	0.8	0.44	0.5	0.066
Hormone use	0.5	0.15	0.5	0.13	0.2	0.054	0.7	0.22	0.8	0.41	0.3	0.028
					Mothers							
Cryptorchidism	6.6	0.001	2.5	0.16	3.1	0.15	5.3	0.001	6.1	0.01	3.7	0.016
Breast cancer	9.6	0.01	0.7	0.51	1.4	0.53	4.4	0.045	6.2	0.062	1.8	0.34
Exogenous hormones in pregnancy	1.2	0.48	0.5	0.35	1.0	0.74	0.9	0.54	0.7	0.49	1.0	0.59
10-month pregnancy	1.8	0.2	2.7	0.21	1.4	0.53	2.2	0.086	1.5	0.37	2.5	0.11
Medication for nausea in pregnancy	1.4	0.3	1.6	0.37	1.3	0.53	1.6	0.21	7.3	0.034	1.0	0.63
Low birth weight	0.6	0.18	2.8	0.11	1.4	0.53	0.9	0.47	0.7	0.29	1.3	0.36

TABLE 6

Cancer reported by case and control mothers, northern California, 1976–1981

	Total		Son's histology			
			Seminomas only		Nonseminomas and mixed	
	Case mothers (n = 222)	Control mothers (n = 224)	Case mothers (n = 75)	Control mothers (n = 71)	Case mothers (n = 147)	Control mothers (n = 153)
Mothers' cancer by site						
Breast	11	4	3	2	8	2
Other	18	19	6	10	12	9
Total reporting cancer	29	23	9	12	20	11

overall study (table 3). The odds ratio associated with mothers' breast cancer in the population-based subset (OR = 6.2) was more than twice that in the overall study, but failed to reach statistical significance ($p = 0.062$). The protective effect of son's hormone use was not apparent in the population-based subset and may have been due to a referral bias, nor was there a protective effect of vasectomy. The protective effect of reported mononucleosis remained statistically significant in the population-based subset (OR = 0.4, $p = 0.041$). Nausea in pregnancy showed a greatly increased odds ratio of 7.3 in the population-based subset, and this was statistically significant ($p = 0.034$). There was no association with mothers' use of exogenous hormones during pregnancy in either group.

Multivariate analysis

Next, we examined the effect of the paternal occupation difference between cases and controls. To adjust for paternal occupation, within-stratum and adjusted odds ratios were calculated and tested for homogeneity by the Mantel-Haenszel method (21). Because the numbers of observations within strata were small, paternal occupation was recoded into two categories only: professional, managerial, technical, and proprietary versus clerical, skilled, and manual. Because the numbers of observations within strata were still small, however, large-sample assumptions may not be met. Thus, the p values associated with the chi-squared tests may not be accurate.

The adjusted odds ratios were 7.5 (sons' report) and 4.6 (mothers' report) for undescended testis, 2.6 for mother's breast cancer, 2.2 for earlier age at puberty, and 0.5 for mononucleosis. These ratios were closely comparable to the unadjusted ratios (table 3). In no instance were the odds ratios heterogeneous between strata according to the Mantel-Haenszel test. The five adjusted odds ratios were significantly different from 1.0 except the odds ratio associated with breast cancer. One breast cancer case was excluded because the father's occupation was unreported.

When the adjusted odd ratios were examined in the nonseminoma group only, the odds ratio associated with mother's breast cancer was 3.8, and the odds ratio associated with lower age at puberty was 2.5. In the seminoma group, the odds ratios associated with reported mononucleosis and hormone use were 0.3 and 0.1, respectively. The chi-squared statistics for homogeneity were nonsignificant, and the odds ratios were significantly different from 1.0.

In a second multivariate analysis, we examined the odds ratios associated with the major risk factors in combination. In a stepwise multiple logistic analysis of the variables reported by the sons only, cryptorchidism was selected first (OR = 5.3, p = 0.005), followed by age at puberty (OR =

2.2, $p = 0.006$). When the analysis was restricted to those aged 30 years or less, only age at puberty was selected, and when it was restricted to those over 30, only cryptorchidism was selected. When the analysis was restricted to nonseminomas and mixed tumors only, age at puberty was selected first (OR = 3.4), followed by cryptorchidism (OR = 4.6). When the analysis was restricted to the population-based subset only, cryptorchidism and age at puberty were selected (OR = 5.2 and 2.5, respectively).

Because multiple logistic analysis programs require that no variable has missing values, the data became too sparse when mothers' variables were analyzed along with the sons' variables. We therefore examined the odds ratios associated with each risk factor when stratified by the other major risk factors. When stratified by age at puberty, the odds ratios associated with undescended testis were 8.9 in the lower age stratum and 5.4 in the higher age stratum. The odds ratios associated with mother's breast cancer were 2.6 in both strata. When stratified by undescended testis, the ratios associated with mother's breast cancer were infinity and 4.9 in the cryptorchid and noncryptorchid groups, respectively. (Two breast cancer cases were excluded because cryptorchid status was unknown.) Because there were small numbers of exposures within strata, the chi-squared statistics for homogeneity were zero in all three analyses. These data, although scanty, suggest that there is some degree of independence among the three principal risk factors.

Finally, we examined the odds ratios associated with different combinations of risk factors (table 7). Here, the analysis was confined to subjects for whom complete data were available on all three major risk factors. Some strata were almost depleted by this requirement. The odds ratio associated with any one of the major risk factors singly was 1.9. The odds ratio associated with any two was 11.5. It should be noted that the odds ratio associated with cryptorchidism alone was 3.6, lower than the estimates on table 3. The higher risk usually associated with cryptorchidism may reflect the fact that many cryptorchid cases are also positive on other risk factors. There were no subjects with complete data who were positive on all three major risk factors.

Paired and unpaired analysis

Paired and unpaired odds ratios were estimated for the principal variables investigated, taking advantage of the additional information in the unpaired mothers' data

TABLE 7

Odds ratios associated with combinations of the major risks, northern California, 1976–1981

	Odds ratio	95% confidence interval	*p* value
Cryptorchidism	3.6	0.18–218	0.3
Mother's breast cancer	3.6	0.18–218	0.3
Age at puberty <14 years	1.9	1.1–3.2	0.009
Any single risk factor	1.9	1.1–3.2	0.007
Crypotorchidism and mother's breast cancer	∞	0.09–∞	0.36
Cryptorchidism and age at puberty <14 years	11.8	2.4–112	<0.001
Mother's breast cancer and age at puberty <14 years	9.1	0.9–436	0.03
Any two risk factors	11.5	3.0–63.8	<0.001

TABLE 8

Hormone exposure during pregnancy, northern California, 1976–1981

Mothers' reported exposure	Current study		Depue et al. (2)		Schottenfeld et al. (3)		Combined	
	n	%	*n*	%	*n*	%	*n*	%
All hormones								
Cases	8/222	3.6	9/97	9.3	11/190	5.8	28/509	5.5
Controls (group 1)	9/224	4.0	2/105	1.9	3/141	2.1	14/470	3.0
Controls (group 2)					4/163	2.5		
Diethylstilbestrol								
Cases	4/222	1.8	2/97	2.1			6/319	1.9
Controls	2/224	0.9	1/105	1.0			3/329	0.9

set. For variables measured from sons' responses, the paired odds ratio estimates and 95 per cent confidence intervals corresponding to the main comparisons discussed above are cryptorchidism 8.3 (2.5–43.2); age at puberty less than 14 years 1.9 (1.2–3.2); mononucleosis 0.6 (0.3–1.2); and hormone use 0.5 (0.2–1.0). For variables measured from mothers' responses, the odds ratios and 95 per cent confidence intervals are cryptorchidism 6.7 (2.0–35.1); breast cancer 2.7 (0.6–15.6); and nausea in pregnancy 1.9 (0.8–5.1). These odds ratio estimates are closely comparable to those of table 3, but the confidence intervals are generally wider.

DISCUSSION

Our findings confirm that cryptorchidism is the primary risk factor for testicular cancer, with an odds ratio of 4.5 by mothers' reports and 8.3 by sons' reports. These results are comparable to those reported by Morrison (4), Henderson et al. (1), and Depue et al. (2) and to a series reported by Schottenfeld et al. (3). In our study, the odds ratio for hernia was 1.1 by mothers' reports and 1.3 by sons' reports, comparable to the risk reported by Schottenfeld et al., although somewhat smaller than that found in the studies reviewed by Depue et al. Like these authors, we did not find hernia to be a significant risk factor for testicular cancer.

No association was found between exogenous hormone exposure during pregnancy and testicular cancer. There were eight reported hormone exposures in case mothers versus nine in control mothers, for an odds ratio of 0.9. Here, our results do not agree with those of Depue et al., who found a fourfold difference in exposures between cases and controls (table 8). In that study, however, the excess of reported hormone exposure in cases was almost completely due to cases reporting one-shot pregnancy tests. Schottenfeld et al. did not report hormone exposure by category.

We found four confirmed or probable diethylstilbestrol exposures in case mothers against two in control mothers. These numbers are too small for the odds ratio of 2.0 to approach significance. The percentage of exposures in our controls is similar to that reported by Depue et al. (2) (table 8) and accords well with Heinonen's estimate that diethylstilbestrol was prescribed in 1.68 per 100 live births in the western United States during 1960–1970 (8). Given the difficulty of confirming diethylstilbestrol exposures, the possibility of different recall biases in case and control mothers, and the presence of two additional diethylstilbestrol exposures in extra controls (not included in the analysis), it cannot be said that our results lean strongly in the direction of an association between diethylstilbestrol exposure and testicular cancer. When our results are combined with those of Depue et al. for diethylstilbestrol, the results still do not reach statistical significance. Given the low prevalence of diethylstilbestrol exposures in controls and the uncertainties associated with verification of exposure, a much larger study than ours would be necessary to resolve this issue.

Aside from cryptorchidism, the two strongest risk factors in our study were puberty at age less than 14 years (OR = 2.0), as measured by the reported appearance of pubic hair, and breast cancer (OR = 2.9), as reported by the mothers. Both risk factors were essentially confined to younger cases and to the nonseminomatous group. Eight of 11 breast cancer cases reported by case mothers were in the nonseminomatous group, and in this group the odds ratio associated with mother's breast cancer was 4.4, approaching the risk associated with cryptorchidism. Both associations persisted in the population-based subset.

Age at puberty was not a primary focus of the study and was measured only by the appearance of pubic hair. We attempted to collect first emission data as well, but the data proved unreliable. Thus, we have only the reported appearance of pubic hair to measure the onset of physiologic puberty, an event which may precede the appearance of pubic hair by two years or more. Following Ross et al. (23), who found that age at puberty as measured by appearance of pubic hair, emission, and voice change averaged 13.8 years in diethylstilbestrol-exposed boys and 13.9 years in controls, we chose to dichotomize at age 14 to distinguish earlier and later puberty. (The odds ratio associated with age at puberty at 13 years or less was smaller at 1.5, but still statistically significant ($p = 0.014$).) Although the measure we used is weak, the specificity of the association, and its persistence in the population-based subset and in multivariate analyses of the sons' responses, suggests that this association should be explored further.

We found a doubled, but not statistically significant, risk associated with nausea in pregnancy, which changed little when restricted to first pregnancies. Among the population-based cases, however, the odds ratio associated with nausea in pregnancy was 7.3 and was highly statistically significant. In the current study, nausea in pregnancy was the only major variable showing referral bias, as measured by the difference between the population-based and non-population-based subsets. The odds ratio associated with nausea in pregnancy was homogeneous within paternal occupation strata; thus, the bias in question was not a social class bias.

We found two associations which appear to be specific to seminomatous tumors, namely the protective effects of reported mononucleosis (OR = 0.3 in the seminomatous group) and sons' reported hormone use (OR = 0.2). The association with hormone use may be the result of referral bias; the protective effect of mononucleosis was confirmed in the population-based substudy. Henderson et al. (1) reported an odds ratio of 0.7 for mononucleosis, but mononucleosis is not reported in the follow-up study by Depue et al. (2).

Since mononucleosis is usually caused by a first infection with Epstein-Barr virus after childhood, a protective effect associated with reported mononucleosis would correspond to an increased risk associated with primary Epstein-Barr virus infection early in life, e.g., neonatally. Epstein-Barr virus is known to be an oncogenic virus, and early infection is believed to be associated with Burkitt's lymphoma in African children and perhaps with nasopharyngeal carcinoma. High titers of antibodies to the viral capsid antigen have also been shown in a series of studies comparing Hodgkin's disease patients with controls (24, 25). Newell et al. (26) have explored the similarities between the epidemiology of Hodgkin's disease and of testicular cancer in detail and have suggested an early infectious disease exposure as an explanation for the similarities. Although these authors do not suggest Epstein-Barr virus as the agent in question, our data support the proposition that early infectious disease exposures may be of interest in testicular cancer and suggest that Epstein-Barr virus antibody studies would be useful in future epidemiologic investigations.

The hypothesis with which this study

began was that dysgenesis of the testis during pregnancy may be caused by excessive free estrogen present in the mother, leading both to cryptorchidism and to increased risk of testicular cancer. We found some support for this proposition in the high risk associated with (medicated) nausea in pregnancy in the population-based substudy. We found no association, however, between testicular cancer risk and exogenous estrogen use in pregnancy. We did find two hormone-associated risk factors which appear to be specific for tumors of nonseminomatous and mixed histology: mother's reported breast cancer and son's reported early puberty. The protective effect of reported mononucleosis on seminomas was equally specific. These results suggest that future studies of testicular cancer should distinguish cases by histology. Furthermore, the two type-specific hormonal risk factors should clearly be distinguished from cryptorchidism and (perhaps) nausea in pregnancy, which appear to be associated with increased risk of all types of testicular tumors.

Mother's breast cancer and son's early age at puberty appear to parallel two of the established risk factors for breast cancer—family history of breast cancer and early menarche—suggesting a hormonal etiology paralleling that of breast cancer for nonseminomatous tumors (in addition to those hormonal factors independent of histology). Furthermore, the excess of breast cancer in case mothers in the present study was strongest in the premenopausal age range, with a median age of 49 at diagnosis. The genetic effect of breast cancer is generally believed to be strongest in the premenopausal group (27, 28). (A recent Swedish study, however, has failed to confirm this finding (29).) The restriction of the association to younger mothers strengthens the possibility of a familial risk for nonseminomatous testicular tumors in sons of mothers with breast cancer, comparable to the risk of breast cancer in daughters. No such association has previously been reported, although Depue et al. (2) found an association with mother's body weight, also a breast cancer risk factor. Lynch et al. (28), reporting familial breast cancer in males, suggest that hereditary breast cancer has an autosomal dominant genetic etiology. This would be consistent with an excess risk of testicular cancer in sons if the same inherited hormonal defect were associated with both male breast cancer and testicular cancer. No hereditable hormonal pattern, however, has yet been demonstrated for breast cancer in either males or females (30).

The shift in this century toward an earlier age for the peak incidence of testicular cancer in Western countries parallels the increase in breast cancer, particularly among younger women (30). Furthermore, younger cases of testicular cancer are more likely to have nonseminomatous tumors. In the current study, 87 per cent of cases aged less than 25 years had nonseminomatous tumors versus 75 per cent of cases aged 25–29 years and 47 per cent of cases aged 30–40 years. The existence of a cluster of risk factors commonly associated with breast cancer itself, such as mother's breast cancer and early puberty, would attribute at least part of the shift to early testicular cancer to the same hormonally mediated social change that has produced the modern pattern of breast cancer (diet is a frequent suggestion) and would also be consistent with the similar social class distributions of the two tumors. Also, the existence of such a cluster would predict that the increased incidence of testicular cancer among young men over the past century is in tumors of nonseminomatous histology only. Unfortunately, no data exist to test this proposition. One English review of cohort mortality rates from testicular cancer notes that the change in rates over the century appears to have begun among men of higher social class (31), suggesting that the risk factors associated with the modern increase are probably social class-dependent. Since cryptorchidism is not associ-

ated with social class, this mortality rate analysis therefore suggests that there are two independent sets of hormone-associated risk factors for testicular cancer, as in the present study.

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