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Maternal smoking during pregnancy and testicular cancer in the sons: A nested case–control study and a meta-analysis

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ABSTRACT

Some large ecological studies have noted a significant association of testicular cancer (TC) with maternal smoking during pregnancy, while several more controlled studies have been negative. It has been difficult to obtain reliable data on exposure because of the long lag time to cancer diagnosis. We performed a case–control study nested within Finnish, Swedish and Icelandic maternity cohorts exploiting early pregnancy serum samples to evaluate the role of maternal smoking in the risk of TC in the offspring. After reviewing the literature, we also performed a meta-analysis of published studies. For each index mother of the TC patient, three to nine matched control mothers with a cancer-free son born at the same time as the TC case were identified within each cohort. First trimester sera were retrieved from the 70 index mothers and 519 control mothers and were tested for cotinine level by a novel HPLC–MS–MS method developed. No statistically significant association between maternal cotinine level and risk of TC in the offspring was found (OR 0.68; 95% CI 0.35, 1.34). This is the first study based on individual exposure measurements. Its results agree with our meta-analysis of seven previous epidemiological studies (total number of 2149 cases, 2762 controls) using indirect exposure assessment (OR 1.0; 95% CI 0.88, 1.12).

1. Introduction

Incidence of testicular cancer (TC) has been increasing during the last decades in many countries,[1] and it also varies remarkably between countries.[2–4] A variety of factors have been suggested as causes of this cancer, but none explain the increasing incidence. There is a hereditary susceptibility.[5,6] As the tumour typically occurs at early age, major aeti-

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ological factors must operate early in life and possibly already *in utero*.[7,8] Cryptorchidism[9–11] and low birth weight[12,13] are established risk factors of TC. Both cryptorchidism[14,15] and low birth weight[16] have been associated with maternal smoking. TC has been associated with oestrogen treatment[17] or both low and high endogenous oestrogen levels during pregnancy, but these explanations have also been disputed.[18] TC has also been implied as one manifestation of a broader testicular dysgenesis syndrome (TDS) potentially associating with various hormonal and environmental stressors during pregnancy.[19]

Clemmesen[20] noted parallel trends of TC, and female cancers associated with smoking, such as lung cancer and bladder cancer, and this encouraged him to present a hypothesis that maternal smoking during pregnancy is an important risk factor for TC. A similar association was observed in a Swedish study.[21] The hypothesis was put to test in an ecological study from four Nordic countries.[22] There was a highly significant association between female smoking prevalence at 25–29 years of age among 5-year birth cohorts 1910–1940 and TC incidences in 5-year cohorts 28 years later in four Nordic countries pooled. Even within three of the single countries, there was a statistically significant association between female smoking and TC incidence. On the other hand, several studies with sufficient information on exposure, outcome and possible confounding factors did not show such an association.[10,23–28]

Ecological fallacy is an important source of potential error, and we therefore wanted to find a different study strategy to confirm or refute the hypothesis of maternal smoking. There is a unique possibility in the Nordic countries of attempting this. Besides a long tradition of maintaining cancer registries with practically 100% coverage,[29] there is also a maternity care system maintained by the local government (city etc.), including taking blood samples during pregnancy. Participation is a requirement for some economical benefits at childbirth, and therefore the coverage among pregnant women is close to 100%.

The samples obtained from maternity archives were used for cotinine analysis. This metabolite of nicotine is a good indicator of smoking and passive smoking, because its half-life is 14–20 h,[30] much longer than that of nicotine. Even though there are data that the half-life of cotinine may be shorter in pregnant smokers, about 9 h,[31] cotinine still reveals smoking even after 1–2 days' abstinence. Cotinine concentrations were assayed in the present study from the blood samples of mothers of boys with TC and referent mothers of boys born at the same time as the case. Validity of cotinine samples stored up to 20 years has been demonstrated.[32]

The results of the case–control study were compared with existing information by performing a meta-analysis of seven epidemiological studies using smoking information from questionnaires.

2. Material and methods

2.1. Serum banks and cancer registries

Details about Nordic maternity cohorts have been previously reported.[33] In brief, Finnish Maternity Cohort (FMC) of the National Public Health Institute possesses serum samples from almost all pregnant Finnish women (~98%). The blood samples have been collected from women during 10 to 14 weeks of pregnancy following an informed consent for screening of congenital infections. Since 1983, the left-over sample volumes of 1–3 ml of the separated sera have been stored at –25 °C. There is a provision in the Law on the National Public Health Institute that these samples may later be used for scientific studies.

The Northern Sweden Maternity Cohort is based at the Umeå University Hospital, Umeå, including residents of the four northernmost counties of Sweden. Blood samples are drawn from pregnant women during the first trimester or the early weeks of the second trimester (weeks 7–18) as a part of screening for infectious diseases. Since 1975 the serum samples have been stored at –20 °C.

The Rubella Screening Serum Bank at the Department of Virology, University of Iceland, contains serum samples collected since 1980 from more than 95% of pregnant women in Iceland at 12–14 weeks of gestation. The samples are stored at –20 °C.

TC cases were identified in the nationwide Finnish and Icelandic cancer registries and in the regional cancer registry at the Oncological Centre in Umeå that covers the four northernmost counties in Sweden. Cancer registries receive notifications from hospitals, pathology laboratories and physicians, achieving almost 100% reporting coverage. Cancer registries in Finland and Iceland also utilise death certificate information as an additional source of information.

Over-generation linkage (son–mother) of the population census registry, cancer registry and the maternity cohort data enabled identification of women with offspring who have been diagnosed with testicular cancer. Permission for linkage information between Finnish Maternity Cohort, Population Registry and Cancer Registry has been obtained from the Ministry of Health and the Population Census Register of Finland (#1422/54/94). Relevant permissions were obtained from the Icelandic National Bioethics Committee (#03-013) and the Icelandic Data Protection Authority (#2003/308). Similar approvals were also available in Sweden from research ethical committees.

Research protocol of this study was accepted by the Institutional Review Board of the Finnish National Public Health Institute (7/2006).

2.2. Study subjects

The study was conducted as a pair-matched case–control study nested within Finnish, Swedish and Icelandic maternity cohorts. TC cases diagnosed between 1985 and 2003, between 1976 and 2006 and between 1979 and 2006 were identified from the Finnish, Swedish and Icelandic cancer registries, respectively. Initially 68 TC cases in Finland, 34 TC cases in Northern Sweden and 13 TC cases in Iceland were diagnosed with a histologically verified testicular germ cell tumour (including embryonal carcinoma, seminoma, teratoma and other histological types). Altogether 45 TC cases were excluded from the study. The main reasons for exclusion were absence of maternal serum sample in one of the maternity cohorts (36 cases), or that the pregnancy took place before

the maternity cohort was established (two cases). Among these there were three teratomas, five embryonal carcinomas, one yolk sac tumour, two mixed germ cell tumours, one seminoma, and 26 cases (from Sweden) had no available information on histology. In addition, there were four cases with a somatic malignancy of the testis (i.e. embryonal rhabdomyosarcoma) and three cases had no information on histology and age at diagnosis. Seven TC cases were diagnosed under 10 years of age (infantile/prepubertal cases) and 35 TC cases were diagnosed between 15 and 26 years of age (postpubertal cases). Seventy cases, Caucasian male offspring, whose index mothers had donated serum sample to one of the maternity cohorts, were eligible for the study. Fifty-six TC cases were diagnosed in Finland, eight TC cases in Sweden and six TC cases in Iceland. Approximately nine control mothers in Finland, four control mothers in Sweden and three control mothers in Iceland with Caucasian male offspring, free of TC at the time when diagnosis was made, were matched with the index mothers. The matching criterion was date of birth of the son (±1 month). The control group comprised 519 women.

2.3. *Laboratory methods*

Model compounds (–)-cotinine and the deuterated analogue, (±)-cotinine-d3, were obtained from Cerilliant (Round Rock, Texas, US). HPLC gradient grade methanol and analytical grade ammonium hydroxide were from J.T. Baker (Deventer, The Netherlands).

A 50 μl aliquot of serum was pipetted to a 1.5 ml plastic centrifuge tube, coded and frozen again, and sent to the Laboratory of Chemistry of the National Public Health Institute in Kuopio. Laboratory of Chemistry had no other source information of the serum samples except for the code number. In the Laboratory of Chemistry, a novel method was developed for cotinine analysis from very small samples. In brief, 50 μl of methanol containing 200 pg/μl of cotinine-d3 as an internal standard was added to the tube. Methanol precipitated sample proteins, and the sample was then mixed and extracted for 2 min in an ultrasonic bath. Ultrasonic bath was followed by centrifugation at 12,000 rpm for 10 min. A hard pellet was separated at the bottom of the tube and clear supernatant was obtained on the top. Fifty micro litres of supernatant and 100 μl of 0.1% NH_4OH in H_2O (HPLC eluent B) were pipetted to an insert of an autosampler vial that was stoppered and shaken gently. Ten micro litres of this solution was injected to HPLC–MS–MS-instrument.

For instrumental analysis, a Finnigan TSQ Quantum Discovery Max triple quadrupole system (San Jose, CA, USA) in the Atmospheric Pressure Chemical Ionisation-mode (APCI+) and a Waters 2695 Separations System (Milford, MA, USA) pump and autosampler were used. Separations were performed at 25 °C under gradient conditions using a Waters XTerra C18 column (50 mm length, 2.1 mm diameter, 3.5 μm particles size) and a similar 10 mm pre-column.

For quantification, the most intensive MS/MS ions of cotinine and cotinine-d3 were monitored. MS/MS acquisition parameters were optimised by 20vμl/min injection of 1 ng/μl standard solutions. Optimised conditions were: needle discharge current 4 kV, vaporiser temperature 400 °C, source CID –10 V, transfer capillary temperature 300 °C, Q2 collision energy 20 V and pressure of argon collision gas in Q2 of 1.3 mTorr. HPLC programme and other MS/MS conditions are given in Table 1.

In each batch of samples, 50 μl of ultrapure water was treated exactly as real samples, to control laboratory blank. Blank result was subtracted from the results of real samples. A larger amount of serum was spiked with 100 ng/ml of cotinine and divided in 50 μl aliquots to serve as control samples. Two control samples were analysed in each batch of samples. During the whole project, average spiking recovery of these control samples was 99.3% and between-batch relative standard deviation was 15.5%. Limit of quantitation (LOQ) was 2.0 ng/ml, calculated as six times standard deviation of blank, linear range of the method was from LOQ to 1000 ng/ml, and expanded measurement uncertainty was estimated to be 35%. Virtually no interfering peaks were observed due to highly specific MS/MS-detection, and cotinine was easily identified because it had the same retention time as the internal standard cotinine-d3.

2.4. *Statistical methods*

Relative risks, expressed as odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated by conditional logistic regression. Pregnant women who had serum cotinine level of $\geqslant$15 ng/ml were considered smokers, whereas those who had <15 ng/ml were considered non-smokers in the main analysis.

Separate analyses were performed for the two main histologic sub-groups of TC, seminomas and non-seminomas further divided in teratomas, yolk sac tumours, embryonal carcinomas and other tumours; for two groups according to age at diagnosis ($\leqslant$8 years of age and $\geqslant$15 years of age); for the two groups of maternal age (17–28, 29–47 years of age); and for the four groups of cotinine level: non-smokers (<5 ng/ml), passive smokers (5–14.9 ng/ml), light smokers (15–99.9 ng/ml) and heavy smokers($\geqslant$100 ng/ml).

Homogeneity of ORs across age at primary diagnosis, maternal age, cotinine level and histologic sub-groups was tested using the methods of Breslow and Day. Two-sided $p < 0.05$ was considered statistically significant. The statistical analyses were performed using SPSS for Windows 15.1 (SPSS, Inc., Chicago, Illinois).

2.5. *Methods used for meta-analyses*

Literature search was done in PubMed up to January 2008 using search terms 'maternal', 'smoking', 'epidemiology', 'offspring', 'testi*' ('cancer' or 'tumour' or 'malignancy') and with no language restrictions. Additionally, we checked references from relevant publications and review articles. Out of a total of 17 publications, 7 publications were eligible for further analysis fulfilling the selection criteria of sufficient information on exposure (maternal smoking), outcome (TC in the offspring) and study design. To be included in meta-analysis, studies had to present appropriate information both on exposure (obtained via questionnaires/interviews with answer 'Yes/No' or on number of cigarettes smoked per day) and outcome (obtained through hospital or population-based registries). The studies had to be conducted with similar study

Table 1 – HPLC–MS/MS instrumental parameters for chemical analysis of cotinine from serum samples. HPCL used was Waters 2695 Separations System connected to Finnigan TSQ Quantum Discovery Max triple quadrupole operated in the atmospheric pressure chemical ionisation-mode. In the HPLC system Waters XTerra C18 column was used (50 mm length, 2.1 mm diameter, 3.5 μm particles size).

HPLC			
Time (min)	Flow rate (ml/min)	Eluent A: methanol 0.1% NH_4OH (%)	Eluent B: water 0.1% NH_4OH (%)
0.00	0.200	2.0	98.0
8.00	0.200	72.0	28.0
10.00	0.200	72.0	28.0
11.00	0.200	2.0	98.0
20.00	0.200	2.0	98.0
MS/MS			
Compound	Mother ion	Daughter ion	Scan width
Cotinine	177.2	98.0	0.6
Cotinine-d3	180.2	101.0	0.6

Table 2 – Nested case–control study: Histological types of testicular cancer cases diagnosed in infantile/prepubertal period (under 8 years of age) and postpubertal period (between 15 and 25 years of age).

Histology	Age at diagnosis, years				Total
	Infantile/prepubertal period (⩽8)		Postpubertal period (15–25)		
	Number	Median age (range), years	Number	Median age (range), years	Number (%)
Seminoma	0	–	11	19 (17–25)	11 (15.7)
Teratoma	15	1 (0–8)	9	17 (15–21)	24 (34.3)
Embryonal carcinoma	2	1 (0–3)	14	18 (17–25)	16 (22.9)
Yolk sac tumour	14	1 (0–2)	0	–	14 (20)
Mixed germ cell tumour	0	–	4	17 (15–25)	4 (5.7)
Choriocarcinoma	0	–	1	18 (–)	1 (1.4)
Total	31		39		70 (100)

Table 3 – Nested case–control study: odds ratio (OR) (95% confidence interval [CI]) of testicular cancer associated with maternal smoking, stratified by histological type and age at diagnosis.

Category	Number of cases (smokers/total)	Number of controls (smokers/total)	Maternal smoking	
			OR[a]	95% CI
Age at diagnosis, years[b]				
Infantile/prepubertal period (⩽8)	4/31	51/260	0.60	0.20, 1.79
Postpubertal period (⩾15; ⩽26)	9/39	62/259	0.74	0.32, 1.76
Histology				
Non-seminoma	11/59	96/445	0.71	0.35, 1.47
Infantile/prepubertal period (⩽8)	4/31	51/260	0.60	0.20, 1.79
Postpubertal period (⩾15; ⩽26)	7/28	45/185	0.83	0.31, 2.22
Seminoma	2/11	17/74	0.55	0.09, 3.13
Postpubertal period (⩾15; ⩽26)	2/11	17/74	0.55	0.09, 3.13
Total	13/70	113/519	0.68	0.35, 1.34

Note: There were no TC cases between 8 and 15 years of age.
a 15 ng/ml as a cut off level for smokers and non-smokers used in the model.
b Included cases with all histological types.

design (case–control or nested case–control studies), present information on number of cases and controls and on measures of relative risk (unadjusted and/or adjusted odds ratios). Data concerning study design, population characteristics, matching factors for cases and controls and adjusted factors for the multivariate analysis were extracted from these studies using a standardised data abstraction form (Table 5).

Statistical analysis for meta-analysis was performed with R version 2.5–1 (R Development Core Team). Mantel–Haenszel fixed-effects model was used to calculate the summary estimate. Two-sided $p < 0.05$ was considered statistically significant. Chi-square test for heterogeneity was evaluated ($p < 0.1$ was considered representative of significant statistical heterogeneity).

Table 4 – Nested case–control study: Odds ratio (OR) (95% confidence interval [CI]) of testicular cancer associated with non-smoking, passive smoking, light smoking and heavy maternal smoking during pregnancy, stratified by maternal age.

Category	Number (cases/controls)	Non-smokers*		Number (cases/controls)	Passive smokers[a]		Number (cases/controls)	Light smokers[b]		Number (cases/controls)	Heavy smokers[c]		Number (cases/controls)	Total smokers[d]	
		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI
Maternal age, years															
17–28	26/215	1.0 (Referent)		1/8	0.70	0.07, 6.78	5/39	0.89	0.26, 3.08	1/32	n.a.	n.a.	7/79	0.48	0.16, 1.48
29–47	28/177	1.0 (Referent)		2/3	3.40	0.46, 25.14	2/18	0.44	0.09, 2.09	5/24	1.23	0.40, 3.75	9/45	1.01	0.42, 2.40
Total	54/395	1.0 (Referent)		3/11	1.59	0.47, 6.10	7/57	0.78	0.32, 1.89	6/56	0.64	0.25, 1.63	16/124	0.78	0.41, 1.49

* Cotinine serum level <5 ng/ml.
a Cotinine serum level 5–14.9 ng/ml.
b Cotinine serum level 15–99.9 ng/ml.
c Cotinine serum level ⩾100 ng/ml.
d 5 ng/ml as a cut off level for smokers and non-smokers used in the model.

3. Results

Both mean and median age of the index and control mothers at sample withdrawal were 28 years (range 17–47 years). The mean and median ages at diagnosis of TC were 10 and 15 years (range 0–25 years). The group of infants and children diagnosed under 8 years of age (infantile/prepubertal period) comprised 31 TC cases (44%) and the group of adolescents and young adults diagnosed between 15 and 26 years of age (postpubertal period) comprised 39 cases (56%). There were no TC cases between 8 and 15 years of age. All seminoma cases were diagnosed over 15 years of age.

Eleven cases (16%) had seminomas, 24 cases (34%) had teratomas, 16 cases (23%) had embryonal carcinomas, 14 cases (20%) had yolk sac tumours, 4 cases (5.7%) had mixed germ cell tumours, and 1 case (1.4%) had choriocarcinoma (Table 2).

The mean serum concentration of cotinine in index mothers was 21.1 ± 6.7 ng/ml (mean ± SEM), and in control mothers was 25.5 ± 2.7 ng/ml. Medians were zero in both groups, because most samples were below the limit of quantitation. Thirteen (21.8%) index mothers and 113 (18.6%) control mothers were smokers with mean serum cotinine concentrations of 111.9 ± 82.9 ng/ml and 116.3 ± 85.8 ng/ml, respectively. No statistically significant association between maternal cotinine concentration and risk of TC in the offspring was revealed (OR, 0.68; 95% CI, 0.35, 1.34). Subdivision of the groups results in small numbers, but trends towards increased risk were seen neither in infantile/prepubertal or postpubertal age, nor in the seminoma and non-seminoma cases in the results (Table 3). No obvious trends were revealed when dividing index and control mothers to passive smokers, light smokers or heavy smokers (Table 4), nor when related to maternal age (Table 4).

The characteristics of the studies included in meta-analysis are listed in Table 5. Seven studies were published between 1987 and 2007 and included a total of 2149 TC cases. The summary estimate of meta-analysis supported our results and indicated no association between maternal smoking and risk of TC in the offspring (OR, 1.0; 95% CI, 0.88, 1.12). Effect estimates were homogeneous across reviewed studies (p for heterogeneity, 0.80).

4. Discussion

The present results provided no support for the hypothesis that maternal smoking during pregnancy is a risk factor for the testicular cancer in the offspring. Based on the 95% confidence interval, the power of this study allowed us to exclude relative risks of 1.34 and higher.

However, some limitations to this approach should be appreciated. The serum sample for cotinine analysis had been taken during the first or early second trimester of pregnancy. This time period is well suited for detecting the risk factors of structural anomalies later seen as terata with a critical period peaking in weeks 3–10.[34] A typical example is phocomelia caused by thalidomide.[35] It may be argued to indicate risks of foetus less effectively towards the end of pregnancy. Nicotine addiction is quite strong; however, and indication

Table 5 – Meta-analysis of seven published studies. Selected characteristics of studies on the association between maternal smoking and TC in the offspring.

Author, year	Study design	Source of cases/controls	Source of smoking data	Case recruitment period	Matching factors	Adjusting factors	Cases (No)[b]	Controls (No)[b]	OR	95% CI (lower)	95% CI (upper)
Sonke, 2007[28]	CCS[*]	Registry, hospital-based/ case nominated	Interview, self-administered questionnaire	1990–1996, 18–50 years of age	Race, age (±5 years), state of residence	Mother's race, education, body mass index, son's birth weight, age, history of cryptorchidism, nausea, length of pregnancy	144	86	1.1	0.5	2.2
McGlynn, 2006[26]	CCS	Military medical databases/Defense Serum Repository	Interview, questionnaire	2002–2005, 0-46 years of age	Age (within 1 year), race, date of serum sample drawn (within 30 days)	Son's age, race, family history of TC	514	560	1.01	0.80	1.29
Weir, 2000[10]	CCS	Cancer Registry/ Ministry of Revenue's Enumeration Composite Records	Interview, self-administered questionnaire	1987–1989, 16-59 years of age	Age (the same within 5-years age groups)	Age, exogenous hormone use during pregnancy, bleeding/threaten miscarriage, pregnancy length, treatment for undescended testicle	339	511	0.86	0.64	1.17
Pettersson, 2007[27]	NCCS[a]	Cancer Registry/ hospital controls	Birth registry	1973–2002, ⩾15 years of age	Date of birth (first three control children born after case child)	Age, histology, maternal age, birth order, gestational duration	192	494	0.90	0.64	1.26
Coupland, 2004[25]	CCS	Cancer treatment centres or regional cancer registries/ hospital controls	Interview, Questionnaire	1984–1987, 15-49 years of age	Date of birth (within 1 year)	Age, place of residence	446	420	1.22	0.91	1.63
Moller, 1996[24]	CCS	Cancer Registry/ Central Person Register	Interview, questionnaire	1986–1988	Year of birth	–	296	287	0.97	0.69	1.36
Swerdlow, 1987[23]	CCS	Radiotherapy centres/hospital controls	Interview, case notes	1977–1981	Age (the same)	–	218	404	1.01	0.71	1.45
Summary estimate, Mantel–Haenszel OR: 1.0; 95% CI: (0.88, 1.12) Test for heterogeneity: Chi-2 = 3.02; (*p*-value, 0.80)											

[*] Case–control study.
a Nested case–control study.
b Number of cases and controls eligible for the smoking exposure data.

of smoking at any time during pregnancy is likely to be representative of the whole pregnancy.[36,37]

Tobacco smoke contains many aromatic hydrocarbons that influence, usually induce, oxidative enzymes important in steroid metabolism,[38] although effects on hormone levels seem to be moderate.[39] Tobacco smoke has many detrimental effects, such as reducing placental blood flow and interfering with pregnancy oestrogens that could disturb normal testicular cell differentiation.[40]

Another possible mechanism of smoking causing TC could be mutations in germ cells caused by the many mutagenic compounds in tobacco smoke.[41] Such cancer initiation might be possible at any time after differentiation of the cells to form the testes. Because this takes place very early during development,[42] the timing of sample in this study is appropriate for the detection of exposure.

Since the incidence rate of TC in Finland, Sweden and Iceland is similar (age-standardised rates per 100.000 men 3.7, 5.1, and 5.3, respectively) we pooled data from these countries. A possible limitation of our study was that we were not able to control for some pregnancy-related characteristics (cryptorchidism, maternal weight, birth order, birth weight, etc.), which might confound the disease-exposure association.

Because serum samples have been stored in Finland only from the year 1983, in Sweden from 1975 and in Iceland from 1980, young age TC cases dominate the analysis. This shows in the low number of seminomas (15%), because its peak incidence is at the age of over 30 years.[1] Within this limitation, we were not able to see any trend among seminomas towards a positive association with maternal smoking. The point estimates of odds ratios of different tumour types were quite consistent but the confidence intervals were wide.

Testicular tumours include prepubertal TC, postpubertal TC and spermatocytic seminoma with some evidence of different origin and biology. Tumours arising in prepubertal period are either teratomas or yolk sac tumours, whereas postpubertal tumours are often seminomas, embryonal carcinomas or mixed germ cell tumours. Prepubertal TC may be initiated during germ cell migration to the genital ridge, but postpubertal TC at a later stage.[43] There is a strong association between postpubertal TC and carcinoma in situ, but not regarding prepubertal TC. While prepubertal TC is rare with no apparent change in incidence rates over time,[44] the incidence of postpubertal TC has more than doubled over the last 40 years.[1,45] Due to the excess of young offspring of the maternity cohorts prepubertal TC cases dominate in our study population. Especially the low numbers of seminomas preclude the extrapolation to the population of TC cases between 20 and 40 years of age.

Sensitivity of the HPLC/MS/MS method to analyse cotinine was adequate for the purposes of this study. Usually the limit of active smoking is set at 10–25 ng/ml, and the limit of quantification (LOQ) was 2.0 ng/ml with our method. Variation of the cotinine levels in the analysed serum samples was large, from less than the LOQ to 538 ng/ml. Among the whole study population, 21% exceeded 15 ng/ml, the criterion set for active smoking, and 11% exceeded 100 ng/ml, which indicates heavy smoking. These numbers are consistent with smoking prevalence among young women in Finland (26%, self-reported)[37] and in Sweden (21%, self-reported).[46]

Although the risk of TC was associated with maternal smoking in some studies,[20–22] no consistent association between maternal smoking and risk of TC in the offspring have been shown in other epidemiological studies.[10,23–28,47,48] This is supported by our meta-analysis of seven studies (Table 5) resulting in a summary estimate of odds ratio between maternal smoking and risk of TC (OR, 1.0; 95% CI, 0.88, 1.12).

Maternal smoking is notorious in being unreliably reported in questionnaire studies. In a recent Estonian study, 73% (122 of 168) of heavy smokers (serum cotinine >100 ng/ml) reported being non-smokers when questioned during pregnancy.[49] In a Swedish study, 6% of women reporting to be non-smokers were most likely smokers.[50] The present study provides no evidence for an association between maternal smoking and risk of TC, when smoking status was based on a biological measure, serum cotinine levels. To the best of our knowledge this is the first study on TC risk in the offspring and maternal serum cotinine levels.

If there is no association between maternal cotinine and TC in the offspring, why was there an association in the large Nordic ecological study?[22] One possible explanation is that smoking would associate with another potential risk factor such as alcohol. Alcohol consumption and smoking in the Nordic countries are associated especially among women, and the consumption of both has increased.[51] Alcohol consumption during pregnancy was associated with incidence of cryptorchidism in a recent study.[52] However, in two studies no association was found between childhood germ cell tumours and maternal alcohol intake.[10,53]

In conclusion, we could not find any association between maternal smoking during pregnancy as assessed by measuring cotinine around the end of the first trimester of pregnancy, and risk of TC in the offspring. The result agrees with a meta-analysis of seven epidemiological studies using indirect exposure assessment.

Conflict of interest statement

None declared.

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REFERENCES

1. Huyghe E, Matsuda T, Thonneau P. Increasing incidence of testicular cancer worldwide: a review. *J Urol* 2003;**170**:5–11.
2. Bray F, Richiardi L, Ekbom A, Pukkala E, Cuninkova M, Møller H. Trends in testicular cancer incidence and mortality in 22

European countries: continuing increases in incidence and declines in mortality. *Int J Cancer* 2006;**118**:3099–111.
3. Engholm G, Storm HH, Ferlay J, et al. *NORDCAN: cancer incidence and mortality in the Nordic countries*. Version 3.0. Association of Nordic Cancer Registries; 2007. <www.ancr.nu>.
4. Jacobsen R, Møller H, Thoresen SØ, Pukkala E, Krüger Kjær S, Johansen C. Trends in testicular cancer incidence in the Nordic countries, focusing on the recent decrease in Denmark. *Int J Andrology* 2006;**29**:199–204.
5. Nicholson PW, Harland SJ. Inheritance and testicular cancer. *Brit J Cancer* 1995;**71**:421–6.
6. Hemminki K, Li X. Familial risk in testicular cancer as a clue to heritable and environmental etiology. *Brit J Cancer* 2004;**90**:1765–70.
7. Grotmol T, Weiderpass E, Tretli S. Conditions in utero and cancer risk. *Eur J Epidemiol* 2006;**21**:561–70.
8. Ekbom A. Growing evidence that several human cancers may originate in utero. *Semin Cancer Biol* 1998;**8**:237–44.
9. Pottern LM, Brown LM, Hoover RN, et al. Testicular cancer risk among young men: role of cryptorchidism and inguinal hernia. *J Natl Cancer Inst* 1985;**74**:377–81.
10. Weir HK, Marrett LD, Kreiger N, Darlington GA, Sugar L. Pre-natal and peri-natal exposures and risk of testicular germ-cell cancer. *Int J Cancer* 2000;**87**:438–43.
11. Garner MJ, Turner MC, Ghadirian P, Krewski D. Epidemiology of testicular cancer: an overview. *Int J Cancer* 2005;**116**:331–9.
12. Akre O, Ekbom A, Hsieh CC, Trichopoulos D, Adami HO. Testicular seminoma and non-seminoma in relation to perinatal characteristics. *J Natl Cancer Inst* 1996;**88**: 883–9.
13. Møller H, Skakkebaek NE. Testicular cancer and cryptorchidism in relation to prenatal factors: case–control studies in Denmark. *Cancer Causes Contr* 1997;**8**:904–12.
14. Thorup J, Cortes D, Petersen BL. Cryptorchidism is increased and the fertility potential is reduced in sons born to mothers who have smoked during pregnancy. *J Urol* 2006;**176**:734–7.
15. Jensen MS, Toft G, Thulstrup AM, Bonde JP, Olsen J. Cryptorchidism according to maternal gestational smoking. *Epidemiology* 2007;**18**:220–5.
16. Michos A, Xue F, Michels KB. Birth weight and the risk of testicular cancer: a meta-analysis. *Int J Cancer* 2007;**121**:1123–31.
17. Depue RH, Pike MC, Henderson BE. Estrogen exposure during gestation and risk of testicular cancer. *J Natl Cancer Inst* 1983;**71**:1151–5.
18. McGlynn KA, Graubard BI, Nam J-M, Stanczyk FZ, Longnecker MP, Klebanoff MA. Maternal hormone levels and risk of cryptorchism among populations at high and low risk of testicular germ cell tumors. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:1732–7.
19. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001;**16**:972–8.
20. Clemmesen J. Is pregnancy smoking causal to testis cancer in sons? A hypothesis. *Acta Oncol* 1997;**36**:59–63.
21. Kaijser M, Akre O, Cnattingius S, Ekbom A. Maternal lung cancer and testicular cancer risk in the offspring. *Cancer Epid Biomarkers Prev* 2003;**12**:643–6.
22. Pettersson A, Kaijser M, Richiardi L, Askling J, Ekbom A, Akre O. Women smoking and testicular cancer: one epidemic causing another? *Int J Cancer* 2004;**109**:941–4.
23. Swerdlow AJ, Huttly SR, Smith PG. Prenatal and familial associations of testicular cancer. *Brit J Cancer* 1987;**55**:571–7.
24. Møller H, Skakkebaek NE. Risks of testicular cancer and cryptorchidism in relation to socio-economic status and related factors: case control studies in Denmark. *Int J Cancer* 1996;**66**:287–93.
25. Coupland CAC, Forman D, Chilvers CED, Davey G, Pike MC, Oliver RTD. Maternal risk factors for testicular cancer: a population-based case–control study (UK). *Cancer Causes Contr* 2004;**15**:277–83.
26. McGlynn KA, Zhang Y, Sakoda LC, Rubertone MV, Erickson RL, Graubard BI. Maternal smoking and testicular germ cell tumors. *Cancer Epidem Biomarkers Prev* 2006;**15**:1820–4.
27. Pettersson A, Akre O, Richiardi L, Ekbom A, Kaijser M. Maternal smoking and the epidemic of testicular cancer – a nested case–control study. *Int J Cancer* 2007;**120**:2044–6.
28. Sonke GS, Chang S, Strom SS, Sweeney AM, Annegers JF, Sigurdson AJ. Prenatal and perinatal risk factors and testicular cancer: a hospital-based case–control study. *Oncol Res* 2007;**16**:383–7.
29. Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry. Experience in Finland. *Acta Oncol* 1994;**33**:365–9.
30. Levi M, Dempsey DA, Benowitz NL, Sheiner LB. Population pharmacokinetics of nicotine and its metabolites. I. Model development. *J Pharmacokin Pharmacodyn* 2007;**34**:5–21.
31. Dempsey D, Jacob III P, Benowitz NL. Accelerated metabolism of nicotine and cotinine in pregnant smokers. *J Pharmacol exp Therap* 2002;**301**:594–8.
32. Riboli E, Haley NJ, De Waard F, Saracci R. Validity of urinary biomarkers of exposure to tobacco smoke following prolonged storage. *Int J Epidemiol* 1995;**24**:354–8.
33. Pukkala E, Andersen A, Berglund G, et al. Nordic biological specimen banks as basis for studies of cancer causes and control – more than 2 million sample donors, 25 million person-years and 100,000 prospective cancers. *Acta Oncol* 2007;**46**:286–307.
34. Farrar HC, Blumer JL. Fetal effects of maternal drug exposure. *Annu Rev Pharmacol Toxicol* 1991;**31**:525–47.
35. Lenz W. A short history of thalidomide embryopathy. *Teratology* 1988;**38**:203–15.
36. Kullander S, Källen BA. A prospective study of smoking and pregnancy. *Acta Obstet Gynecol Scand* 1971;**50**:83–94.
37. Raatikainen K, Huurinainen P, Heinonen S. Smoking in early gestation or through pregnancy: a decision crucial to pregnancy outcome. *Prev Med* 2007;**44**:59–63.
38. Zevin S, Benowits NL. Drug interactions with tobacco smoking. An update. *Clin Pharmacokinet* 1999;**36**:425–38.
39. Windham GC, Mitchell P, Anderson M, Lasley BL. Cigarette smoking and effects on hormone function in premenopausal women. *Environ Health Perspect* 2005;**113**:1285–90.
40. Jauniaux E, Burton GJ. Morphological and biological effects of maternal exposure to tobacco smoke on the feto-placental unit. *Early Hum Dev* 2007;**83**:699–706.
41. IARC. Genetic and related effects. *IARC monographs on the evaluation of carcinogenic risks to humans, tobacco smoke and involuntary smoking* 2004;**83**:1103–17.
42. Wilhelm D, Palmer S, Koopman P. Sex determination and gonadal development in mammals. *Physiol Rev* 2007;**87**: 1–28.
43. Reuter VE. Origins and molecular biology of testicular germ cell tumors. *Mod Pathol* 2005;**18**:S51–60.
44. Walsh TJ, Davies BJ, Croughan MS, Carroll PR, Turek PJ. Racial differences among boys with testicular germ cell tumors in the United States. *J Urol* 2008;**179**:1961–5.
45. Huyghe E, Plante P, Thonneau P. Testicular cancer variations in time and space in Europe. *Eur Urol* 2007;**170**:5–11.
46. Högberg L, Cnattingius S. The influence of maternal smoking habits on the risk of subsequent stillbirth: is there a causal relation? *BJOG* 2007;**114**:504–699.

47. Henderson BE, Benton B, Jing J, Yu MC, Pike MC. Risk factors for cancer of the testis in young men. *Int J Cancer* 1979;**23**:598–602.
48. Brown LM, Pottern LM, Hoover RN. Prenatal and perinatal risk factors for testicular cancer. *Cancer Res* 1986;**46**: 4812–6.
49. Pärna K, Rahu M, Youngman LD, Rahu K, Nygård-Gibur M, Koupil I. Self-reported and serum cotinine-validated smoking in pregnant women in Estonia. *Maternal Child Health J* 2005;**9**:385–92.
50. Lindqvist R, Lendahls L, Tollbom O, Aberg H, Håkansson A. Smoking during pregnancy: comparison of self-reports and cotinine levels in 496 women. *Acta Obstet Gynecol Scand* 2002;**81**:240–4.
51. Wetzels JJ, Kremers SP, Vitória PD, de Vries H. The alcohol-tobacco relationship: a prospective study among adolescents in six European countries. *Addiction* 2003;**98**:1755–63.
52. Damgaard IN, Jensen TK. The Nordic Cryptorchidism Study Group, Petersen JH, Skakkebaek NE, Toppari J, Main KM. *Cryptorchidism and maternal alcohol consumption during pregnancy. Environ Health Persp* 2007;**115**:272–7.
53. Chen Z, Robison L, Giller R, et al. Risk of childhood germ cell tumors in association with parental smoking and drinking. *Cancer* 2005;**103**:1064–71.