

# Prenatal X-ray Exposure and Rhabdomyosarcoma in Children: A Report from the Children's Oncology Group

Seymour Grufferman,<sup>1</sup> Frederick Ruyman,<sup>2</sup> Simona Ognjanovic,<sup>3</sup>  
Erik B. Erhardt,<sup>1</sup> and Harold M. Maurer<sup>4</sup>

<sup>1</sup>Division of Epidemiology and Biostatistics, Department of Internal Medicine, University of New Mexico, Albuquerque, New Mexico; <sup>2</sup>Division of Hematology/Oncology/BMT, Nationwide Children's Hospital, Columbus, Ohio; <sup>3</sup>Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota; and <sup>4</sup>University of Nebraska Medical Center, Omaha, Nebraska

## Abstract

The association between antenatal diagnostic X-ray exposure and risk of rhabdomyosarcoma in children was assessed in a national case-control study of 319 rhabdomyosarcoma cases and 319 matched controls. Data were collected by telephone interviews of subjects' parents. Overall, an odds ratio (OR) of 1.9 [95% confidence interval (CI), 1.1-3.4] was found for any X-ray examination of the mother during pregnancy. Risk was greatest for X-ray exposure during the first trimester (OR, 5.7; 95% CI, 1.2-27.8) and was also increased for the third trimester (OR, 2.0; 95% CI, 0.9-4.6), whereas second trimester exposure was not associated with increased risk. A nonsignificant increase in risk was found for any X-rays of the abdomen, pelvis, chest, or back. Increased

risk was significantly associated with "other" X-ray exposures (relative risk, 2.9; 95% CI, 1.1-7.7), primarily composed of dental X-rays. The association was strongest between embryonal rhabdomyosarcoma and first trimester exposure (relative risk, 10.5; 95% CI, 1.5-458.4). This observation regarding embryonal rhabdomyosarcoma, and our previous report of an increased frequency of major malformations in rhabdomyosarcoma are compatible with findings from animal studies in which Ptc heterozygous knockout mice exhibited an increased risk of radiation-induced developmental defects and of spontaneously occurring embryonal rhabdomyosarcoma. (Cancer Epidemiol Biomarkers Prev 2009;18(4):1271-6)

## Introduction

Prenatal X-ray exposure is one of the few well-established environmental risk factors for childhood cancer. Following the initial report by Giles et al. in 1956 (1), there have been numerous studies linking diagnostic radiation exposure *in utero* with an increased risk of childhood cancer (2-17). Most report an increased risk of childhood leukemia following *in utero* radiation exposure and the odds ratios (OR) have been on the order of 1.5. Many studies were limited by considering only children dying of cancer and only mothers' abdominal or pelvic X-ray examinations. Less attention has been paid to the association between prenatal X-ray exposure and risk of cancers other than leukemia. In general, the increased risk of "solid" tumors (usually considered as all other nonleukemia childhood cancers) is significantly greater than unity, but lower than that for leukemia (11, 18, 19). There has been no study which specifically assessed the risk of rhabdomyosarcoma following prenatal X-ray exposure.

The conduct of a large national case-control study of childhood rhabdomyosarcoma in the United States allowed us to evaluate *in utero* radiation exposure as a risk factor for the disease (20). This study has several advantages over many previous studies that assessed associations between prenatal X-ray exposure and childhood cancer. Extensive questionnaire data were collected directly from subjects' parents regarding the type and time of mothers' diagnostic X-ray examinations during pregnancy. Data were collected for both surviving and dying patients. Detailed obstetric histories permitted the consideration of possible indications for diagnostic radiographic examinations as potential confounders. Lastly, the rhabdomyosarcoma cases ranged in age at diagnosis from 0 to 20 years, allowing for assessment of late childhood effects of prenatal radiation exposures.

## Materials and Methods

### Study Population

**Cases.** Cases were patients with rhabdomyosarcoma in the previous Intergroup Rhabdomyosarcoma Study, which in the year 2000, became part of the Children's Oncology Group. A few of our case-control study results have been reported previously (20, 21). The Intergroup Rhabdomyosarcoma Study was a clinical trial group that coordinated treatment protocols for 80% to 85% of all childhood rhabdomyosarcoma cases in the United States

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**Requests for reprints:** Seymour Grufferman, MSC 10 5550, University of New Mexico, Albuquerque, NM 87131. Phone: 626-241-1513; Fax: 626-445-4334. E-mail: sgrufferman@salud.unm.edu

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**Table 1. Characteristics of subjects and their families**

Characteristics	Cases, N (%) <sup>*</sup>	Controls, N (%) <sup>*</sup>
Sex of child		
Male	214 (67.1%)	214 (67.1%)
Female	105 (32.9%)	105 (32.9%)
Race of child		
White	284 (89.0%)	288 (90.3%)
Black	20 (6.3%)	21 (6.6%)
Other	15 (4.7%)	10 (3.1%)
Mother's educational level		
Less than high school	45 (14.1%)	38 (12.0%)
High school	132 (41.4%)	126 (39.7%)
More than high school	142 (44.5%)	153 (48.3%)
Father's educational level		
Less than high school	54 (17.1%)	36 (11.6%)
High school	112 (35.3%)	111 (35.8%)
More than high school	151 (47.6%)	163 (52.6%)
Total annual family income		
\$0-19,999	103 (32.8%)	75 (23.9%)
\$20,000-39,999	129 (41.1%)	154 (49.0%)
\$40,000+	82 (26.1%)	85 (27.1%)
Age at diagnosis of index child <sup>†</sup>		
Child	7.6 (5.3)	7.5 (5.5)
Maternal	33.8 (6.8)	33.8 (7.0)
Paternal	36.6 (7.5)	36.6 (8.0)

<sup>\*</sup>Percentage of respondents to each question (excluding unknowns).

<sup>†</sup>Mean (SD).

(22, 23). Cases were aged 0 to 20 y at diagnosis and were consecutively entered into the Intergroup Rhabdomyosarcoma Study-III trial from April 1982 to July 1988. The diagnoses of all cases were confirmed by central expert pathology review.

There were 511 patients aged 0 to 20 y in the Intergroup Rhabdomyosarcoma Study-III during the study period, of whom 440 cases were eligible for this study and 351 had completed interviews. Ineligible cases ( $n = 71$ ) had no home telephone ( $n = 29$ ), were not U.S. residents ( $n = 9$ ), their families did not speak English or Spanish ( $n = 15$ ), or their institutions' review board did not approve the study ( $n = 18$ ). In addition, 89 eligible cases did not participate due to parental ( $n = 41$ ) or physicians' ( $n = 30$ ) refusals, whereas 18 families could not be located (24). In summary, 73% (322) of eligible cases were interviewed and matched with controls.

**Controls.** Controls were selected by random-digit telephone dialing (22, 25). Cases' telephone area code and first five digits of their number were used with two randomly selected terminal digits to search for controls by telephone calling. Controls were matched to cases on race, sex, and age (within 1 y for cases aged 0-5 y at diagnosis and within 3 y for cases aged 5-20 y). Of homes with a matching child, 22% refused to participate. Controls could not be obtained for 8% of cases.

**Data Collection.** Data were collected by telephone interview of parents using a structured questionnaire. The mean duration of the interviews was 70 min for case and 68 min for control families. Interviews were conducted in English or Spanish (six case and two control families). The interviews collected data on a variety of childhood environmental exposures, family history, past medical history, parents' occupational history, and mothers' obstetric history. On average, parents were asked to recall perinatal exposures that occurred 8 to 9 y prior to the interview.

An obstetric history, which was focused on the subjects' gestation and delivery, was obtained from the subjects' mothers. Mothers were specifically asked "Did you have any X-rays during the pregnancy?", "What parts of your body were X-rayed?", and "During which trimesters?". Mothers were also asked about a variety of problems and illnesses that they might have had during the index pregnancy.

**Data Analysis.** Conditional logistic-regression models were used to obtain OR estimates of the relative risk of rhabdomyosarcoma associated with factors such as mothers' history of X-ray studies during pregnancy (SAS version 6; ref. 26). Potentially confounding variables were considered in the multivariate models used to obtain adjusted OR. These are identified in the table footnotes.

## Results

**Characteristics of Study Subjects.** Cases and controls were quite similar with regard to a number of characteristics such as mean age at time of diagnosis of the case child (7.6 versus 7.5 years). The mean ages of subjects' mothers and fathers were identical at the time of cases' diagnoses (33.8 years for mothers and 36.6 for fathers). Case families had significantly lower annual income levels ( $P = 0.03$ ) than did control families (ref. 24; Table 1). No other differences in characteristics of cases and controls reached statistical significance (data not shown).

Table 2 compares cases and controls with regard to several obstetric factors. Cases and controls were quite comparable in birth order, type of delivery, numbers and outcomes of their mothers' prior pregnancies, mean number of prenatal medical visits, mean parents' ages at birth of the index child, and mean birth weight. Cases were more likely to be premature at birth and controls

**Table 2. Pregnancy characteristics of cases and controls**

Characteristics	Cases, N (%) <sup>*</sup>	Controls, N (%) <sup>*</sup>
Birth rank of index child		
First	138 (43.4%)	139 (43.9%)
Second	102 (32.1%)	99 (31.2%)
Third+	78 (24.5%)	79 (24.9%)
Type of delivery		
Vaginal	259 (81.2%)	272 (86.3%)
Cesarean	60 (18.8%)	43 (13.7%)
Length of pregnancy		
Normal	257 (81.3%)	255 (81.2%)
Premature	25 (7.9%)	9 (2.9%)
Overdue	34 (10.8%)	50 (15.9%)
Spotting/cramping/abnormal vaginal bleeding during pregnancy	67 (21.1%)	37 (11.9%)
Number of pregnancies <sup>†</sup>		
Previous pregnancies	3.1 (1.6)	3.2 (1.6)
Stillbirths/miscarriages/abortions	0.4 (0.9)	0.4 (0.8)
Living infants	2.6 (1.3)	2.7 (1.3)
Number of prenatal visits <sup>†</sup>	13.3 (4.1)	13.5 (4.8)
Index child birth weight (lbs) <sup>†</sup>	7.4 (1.4)	7.5 (1.1)
Age at index child birth (y) <sup>†</sup>		
Paternal	29.0 (5.9)	29.0 (6.4)
Maternal	26.2 (5.2)	26.2 (5.2)

<sup>\*</sup>Percentage of respondents to each question (excluding unknowns).

<sup>†</sup>Mean (SD).

**Table 3. Reported mothers' diagnostic X-ray examinations during pregnancy by exposure site**

Site category	Cases, N (%) <sup>*</sup>	Controls, N (%) <sup>*</sup>
Pelvis	15 (4.8%)	11 (3.5%)
Abdomen	9 (2.9%)	4 (1.3%)
Back	1 (0.3%)	0 (0.0%)
Chest	4 (1.3%)	2 (0.6%)
Dental	12 (3.8%)	3 (1.0%)
Sinus	1 (0.3%)	0 (0.0%)
Neck	1 (0.3%)	0 (0.0%)
Lower extremity	4 (1.3%)	3 (1.0%)

<sup>\*</sup>Percentage of respondents to each question (excluding unknown).

were more likely to be overdue at delivery. Case mothers were also more likely to have had spotting/cramping/abnormal vaginal bleeding during pregnancy.

**Possible Indications for Pregnancy X-rays.** Possible indications for obtaining X-ray studies during pregnancy were assessed. Prematurity occurred significantly more frequently in cases than in controls [OR, 4.4; 95% confidence interval (CI), 1.7-11.6] as did cramping, spotting, or abnormal bleeding (OR, 1.9; 95% CI, 1.2-2.9). There were no other significant case-control differences in intrapartum illnesses that might possibly lead to X-ray examinations of the mothers. Consideration of prematurity and cramping/bleeding in multivariate analyses did not change the results very much from univariate analyses. We report the percentage of reported X-ray exams by cases and controls in Table 3 and the unadjusted and adjusted results in Table 4.

**Diagnostic X-rays and Rhabdomyosarcoma Risk.** Table 4 presents the results of analyses of mothers'

diagnostic X-rays during the subjects' pregnancies. X-ray examinations were classified with regard to the trimester and site of exposure. The ORs presented have been adjusted for the matching factors (age, sex, and race), the length of pregnancy, type of delivery, and presence of spotting, cramping, or abnormal vaginal bleeding during pregnancy, given that these conditions could be indications for further evaluation by X-ray. Adjustment for socioeconomic status did not change the ORs; therefore, this adjustment was not included in the presented data. Likewise, the adjustment for matching factors did not significantly affect the ORs; both unadjusted and adjusted ORs (95% CIs) are shown in Table 4, although we discuss only adjusted values in the text. Overall, the OR for the association between any diagnostic X-ray exposure during pregnancy and risk of rhabdomyosarcoma was 1.9 (95% CI, 1.1-3.4). It seems that "any" X-ray exposure during the first trimester was associated with the strongest risk (OR, 5.7; 95% CI, 1.2-27.8); however, subgroup analyses should be interpreted with caution due to the small numbers in some subgroups. Although most previously reported findings on *in utero* radiation exposure and childhood cancer risk were focused on pelvic and abdominal X-rays, we found an increased, but not statistically significant OR (1.4; 95% CI, 0.7-2.9) for rhabdomyosarcoma and such exposures. When we grouped all truncal exposures, the OR was 1.5 (95% CI, 0.8-3.1), whereas the OR for all other exposures was 2.9 (95% CI, 1.1-7.7). This category included dental (12 case and 3 control mothers), lower extremity (4 case and 3 control mothers), and head and neck X-rays (2 case and 0 control mothers).

Although the questionnaire did not ask about the use of lead shielding during X-ray procedures, four case

**Table 4. Risk of rhabdomyosarcoma and mothers' diagnostic X-ray examinations during pregnancy by trimester and site of exposure**

Trimester of X-ray	Site of X-ray examination <sup>*</sup>				
	Cases (%) / controls (%) <sup>†</sup>				
	Unadjusted OR (95% CI) <sup>‡</sup>				
	Adjusted OR (95% CI) <sup>§</sup>				
	Abdomen / pelvis <sup>  </sup>	Other truncal <sup>¶</sup>	All truncal	Other <sup>**</sup>	Any
First	4 (1.3) / 2 (0.6)	2 (0.6) / 0 (0.0)	5 (1.6) / 2 (0.6)	8 (2.6) / 0 (0.0)	12 (3.9) / 2 (0.6)
	2.0 (0.4-11.0)	n.a.	2.5 (0.5-13.0)	n.a.	6.2 (1.4-27.8)
	1.4 (0.2-8.7)	n.a.	1.9 (0.3-10.7)	n.a.	5.7 (1.2-27.8)
Second	4 (1.3) / 4 (1.3)	1 (0.3) / 1 (0.3)	5 (1.6) / 4 (1.3)	6 (1.9) / 5 (1.6)	9 (2.9) / 8 (2.6)
	1.0 (0.2-4.0)	1.0 (0.1-16.0)	1.2 (0.3-4.7)	1.2 (0.4-4.0)	1.1 (0.4-2.9)
	0.8 (0.2-3.5)	n.a.	0.8 (0.2-3.5)	0.8 (0.2-3.1)	0.9 (0.3-2.4)
Third	16 (5.1) / 9 (2.9)	1 (0.3) / 1 (0.3)	17 (5.5) / 10 (3.2)	4 (1.3) / 1 (0.3)	21 (6.8) / 11 (3.5)
	1.8 (0.8-4.1)	1.0 (0.1-16.0)	1.7 (0.8-3.8)	4.0 (0.4-36.1)	2.0 (0.9-4.1)
	1.8 (0.7-4.6)	1.2 (0.1-20.4)	1.7 (0.7-4.2)	4.8 (0.5-45.6)	2.0 (0.9-4.6)
Any	24 (7.7) / 15 (4.8)	5 (1.6) / 2 (0.6) <sup>††</sup>	28 (9.0) / 16 (5.2)	18 (5.8) / 6 (1.9)	42 (13.5) / 21 (6.8)
	1.6 (0.8-3.2)	2.5 (0.5-13.0)	1.8 (0.96-3.4)	3.1 (1.2-7.9)	2.1 (1.2-3.7)
	1.4 (0.7-2.9)	2.2 (0.4-12.4)	1.5 (0.8-3.1)	2.9 (1.1-7.7)	1.9 (1.1-3.4)

<sup>\*</sup>Includes multiple X-ray exposure.

<sup>†</sup>Percentage of respondents to each question (excluding unknowns).

<sup>‡</sup>ORs (95% confidence limits) unadjusted.

<sup>§</sup>ORs (95% confidence limits) adjusted for matching factors (age, sex and race) and the length of the pregnancy, the type of delivery and the presence of spotting, cramping or abnormal vaginal bleeding during pregnancy.

<sup>||</sup>Includes sites: pelvis, pelvimetry, uterus, uterus NOS, abdomen, stomach, stomach NOS, upper gastrointestinal, IVP.

<sup>¶</sup>Includes sites: back, chest.

<sup>\*\*</sup>Includes sites: dental, sinus, neck, lower extremity.

<sup>††</sup>Includes one case for whom the site of X-ray was chest, trimester unknown.

mothers and no control mothers reported that shielding had been used during their examinations. Three of the four mothers reported the use of shielding during dental X-rays (one in the first and two in the second trimester of pregnancy) and one during a first trimester X-ray examination of her foot. If these cases are excluded from the analyses, the overall OR for any X-ray exposure becomes 1.8 (95% CI, 1.0-3.3) and for any first trimester X-rays, the OR becomes 5.1 (95% CI, 1.0-34.1). Because no questions regarding lead shielding were asked and all of the subjects volunteering such information were case mothers, there is a reasonable likelihood of selective recall and reporting by case mothers. It is quite possible that some of the control mothers were also shielded but were not as apt as case mothers to recall and report it. Thus, the results using all reports of X-rays by subjects' mothers are probably less biased estimates than results which exclude volunteered reports of shielding.

**Other Factors.** Some earlier studies considered birth order of cases in their analyses because first-born children might have greater exposure to *in utero* X-rays than might later-born children (1, 2, 6, 13). Our results show an OR of 1.8 (95% CI, 0.8-3.9) for first-born children based on 18 cases and 11 controls being exposed. For second birth order children, the OR was 8.0 (95% CI, 1.8-34.8) based on 17 cases and 3 controls exposed; for third birth rank or higher, the OR was 0.9 (95% CI, 0.3-2.4) based on 7 cases and 8 controls exposed.

The association between radiation exposure during pregnancy and rhabdomyosarcoma risk was also stratified by age of cases at diagnosis. Cases were divided into four 5-year age groups. The ORs for each of the four age groups is greater than unity although only the OR for the 0- to 4-year group is statistically significantly higher (OR, 3.2; 95% CI, 1.2-8.7).

Stratification by rhabdomyosarcoma subtype (Table 5) showed the highest risk for development of embryonal rhabdomyosarcoma after *in utero* X-ray exposure (OR, 2.3; 95% CI, 1.2-4.4), whereas exposure during the first trimester of pregnancy led to the highest risk (OR, 10.5; 95% CI, 1.5-458.4).

## Discussion

*In utero* exposure to X-rays has been identified as a risk factor for childhood leukemia (18). Our results suggest that childhood rhabdomyosarcoma could be added to the list of cancers associated with *in utero* diagnostic X-irradiation. The magnitude of risk of rhabdomyosarcoma associated with X-rays during pregnancy seems to

be somewhat higher than that reported for leukemia and other solid tumors; however, more studies are needed to confirm this.

In our study, all X-ray examinations of subjects' mothers during pregnancy were considered. This led to the observation that X-ray exposure other than those of the abdomen and pelvis might be associated with an increased risk of rhabdomyosarcoma. For example, 11 case mothers reported having had dental X-rays (3 of which were done with lead shielding), but only 3 control mothers reported this. Six of the 11 case mothers reporting dental X-rays had the examinations done during their second or third trimester of pregnancy; all three of the exposed control mothers had their X-ray examinations done in the last two trimesters of pregnancy. This is somewhat surprising because most dental X-rays are not done on an emergency basis, and thus, could be deferred until after pregnancy. Although dental X-ray beams are focused on the teeth and supporting bony structures, there is considerable opportunity for scatter of the rays, particularly when downward-angled beams are used or when old equipment is used or films are taken by inadequately trained personnel. The same opportunity for scatter of X-rays might exist also for the three other nontruncal X-rays reported by case mothers (two lower extremity and one sinus film) and the two reported by control mothers (two lower extremity films). In the absence of systematic data on shielding during these examinations, we can only conjecture about these exposures.

Although the exact mechanism by which ionizing radiation exposure of the fetus produces childhood cancers is not known, radiation induces genome instability leading to the accumulation of DNA damage and alteration in DNA and histone methylation (27, 28). Rodents have been validated as good models for prediction of risk of perinatal X-ray-induced carcinogenesis in humans (29), despite marked differences in the physiology of gestation (30). A possible mechanism for the induction of rhabdomyosarcoma by radiation exposure is suggested by a report of rhabdomyosarcoma and radiation hypersensitivity in a mouse model of Gorlin (nevoid basal cell carcinoma) syndrome (31, 32). Gorlin syndrome is characterized by a constellation of clinical problems including generalized overgrowth of the body, cysts, skeletal abnormalities, and a predisposition to benign and malignant tumors. Hahn et al. studied the multiple-pass transmembrane receptor patched (Ptc) in a mouse model. Ptc is thought to be a tumor suppressor gene and patients with Gorlin syndrome have germ line mutations in Ptc (33). They found that mice heterozygous for Ptc inactivation develop features consistent with

**Table 5. *In utero* radiation exposure and risk of rhabdomyosarcoma by trimester of exposure and histologic type of rhabdomyosarcoma**

Histologic type	Cases		Trimester of exposure, OR (95% CI)*			
	N	Exposed	First	Second	Third	Any
Embryonal <sup>†</sup>	212	29	10.5 (1.5-458.4) <sup>‡</sup>	0.8 (0.2-3.3) <sup>‡</sup>	2.1 (0.8-5.3)	2.3 (1.2-4.4)
Other <sup>§</sup>	107	13	2.0 (0.1-118.0) <sup>‡</sup>	2.0 (0.3-22.5) <sup>‡</sup>	1.8 (0.4-8.5) <sup>‡</sup>	1.9 (0.7-5.1)

\*Unmatched analyses.

<sup>†</sup>Includes one case for whom the trimester was unknown.

<sup>‡</sup>Exact OR (95% CI).

<sup>§</sup>Includes 66 alveolar cases.

Gorlin syndrome and also have a high incidence of rhabdomyosarcoma of the embryonal type only. It has recently been proposed in *Ptc1*-deficient mice, which develop basal cell carcinoma, that deregulation of the *Shh/Ptc1* pathway represents the initiating step, but that additional genetic damage by radiation in genes serving critical functions in the regulation of cell proliferation, apoptosis, and response to DNA damage, such as *p53*, is required for tumor formation (34). The evidence that the *Ptc* pathway may play an important role in rhabdomyosarcoma development in humans is not limited to Gorlin syndrome patients who possess germ line *Ptc* mutations. In addition, 30% of sporadic rhabdomyosarcoma show genomic loss of *Ptc1* at locus 9q22 (35). *Ptc* is a receptor for sonic hedgehog (*Shh*) and *Shh/Ptc* pathways play a key role in hypaxial skeletal muscle development, and is required for normal differentiation of early developing muscle (36). Deregulation of this pathway is detected in a substantial percentage of sporadic rhabdomyosarcoma (37).

A previous article from our case-control study of rhabdomyosarcoma reported an increased frequency of neurofibromatosis and major malformations in rhabdomyosarcoma cases (20). Several case series of patients with rhabdomyosarcoma have also found an increased frequency of malformations (20, 38). Patients with rhabdomyosarcoma were found to have a particularly increased frequency of central nervous system anomalies (20, 38). The observations of an increased risk of rhabdomyosarcoma in children exposed to ionizing radiation *in utero* and of an increased occurrence of malformations in children with rhabdomyosarcoma raise the question of whether our findings may be related to the *Ptc* receptor and the signaling pathway of muscle development in which it is involved.

In searching for additional indirect support for this question in our data, we examined the risks associated with X-ray exposure when stratified by histologic type of rhabdomyosarcoma. This was done to see if embryonal rhabdomyosarcoma, the histologic type observed to occur spontaneously in the mouse model, was more strongly associated with X-ray exposure in humans than were the other histologic types. Table 5 shows the results of this analysis. The highest OR (12.1) was observed for first trimester X-ray exposure in embryonal rhabdomyosarcoma cases. This is based on 10 exposed cases versus 1 exposed control; hence, the wide 95% CI, which nonetheless excludes the null value. Our observation of a very high OR for first trimester exposure is consistent with the suggestion of Zhan and Helman (ref. 39), that perhaps there is a critical window of time in which developing skeletal muscle is sensitive to *Ptc* alterations (39). Nevertheless, observations of an increased frequency of major malformations in rhabdomyosarcoma and of ionizing radiation-induced rhabdomyosarcoma, particularly embryonal rhabdomyosarcoma, suggest that *Ptc* alterations might play a role in the etiology of this malformation. Although the ORs are generally highest for embryonal rhabdomyosarcoma, the ORs seem to be elevated for other types of rhabdomyosarcoma as well. Similar to our findings of the highest risk among the embryonal tumor subtype, a recent study of solid tumor occurrence after *in utero* X-ray exposure revealed that the increased risk of brain tumor development was confined to the embryonal subtype (*PTEN*; ref. 40).

Despite these observations, there are several limitations to our study. Even though this represents one of the largest case-control studies ever conducted on this rare tumor, the infrequency of the exposure precluded detailed analyses. Only 13.5% of cases and 6.8% of controls reported any X-ray exposure during pregnancy. Furthermore, there is concern of recall bias; mothers of case children may tend to over-report exposure compared with mothers of control children. In addition, differential recall bias among cases is possible. For example, case mothers might more accurately recall an X-ray exposure that would be more readily perceived as causal (abdominal/pelvic X-ray) than a more distant exposure (dental X-ray). On the other hand, it is also possible that they would be less likely to report an exposure they may consider causal. It is therefore impossible to address whether differential recall bias played a role in our study. However, it is reassuring that we found an association between X-ray exposure and risk of one (embryonal) but not both rhabdomyosarcoma subtypes. In particular, there is no reason to believe that a mother of a child with embryonal rhabdomyosarcoma would recall the exposure differently than a mother of a child with alveolar rhabdomyosarcoma. We also did not collect information regarding shielding. As we noted, there was an average of 8 years between exposure and interview. Despite these concerns, we found a strong positive association between maternal X-ray exposure and embryonal rhabdomyosarcoma (less so with other rhabdomyosarcomas), which is congruent with the animal data.

A recent large analysis (>1,800 cases) of childhood acute lymphoblastic leukemia found little evidence of an association with prenatal diagnostic X-ray exposure (41). The authors conclude that declining exposure levels through declines in radiation levels in X-rays and a medical practice decrease in X-rays provided to pregnant women may explain these null findings. Mothers in our study were pregnant with study subjects approximately during the 1970s and 1980s, thus covering a period of time when radiation dosages were higher and X-ray practices for women of child-bearing age may have been less adhered to. For example, comparison of two dental surveys from the United Kingdom, one carried out in the late 1980s and the other in the late 1990s, showed that the mean patient entrance dose caused by dental X-rays has decreased by 40% (42).

Although Alice Stewart and colleagues reported on the dangers of *in utero* X-ray exposures 50 years ago, insufficient attention has been paid to the consideration that women of child-bearing age should be assumed to be pregnant when it comes to radiographic examinations. The use of lead apron shielding of uninvolved body areas during X-ray studies has grown widespread but lapses still occur. In addition, concerns remain that a routinely used leaded apron of 0.25 mm lead thickness may not provide sufficient protection (43, 44). Prior to routine shielding of the neck, downward pointing beams from dental X-rays could reach the gravid uterus. Although routine lead shielding of the neck (thyroid) has been added by most dentists, in the current age of digital X-rays, the radiation exposure has been significantly decreased (45), which may reduce adherence to this practice.

In conclusion, our study supports the association between *in utero* X-ray exposure and the risk of rhabdomyosarcoma and supports the view that in addition to brain tumors, other solid tumors may occur after such exposure. This is the largest case-control study of rhabdomyosarcoma to date and the exposure levels were surprisingly high given the public awareness of the possible carcinogenic effects of *in utero* X-ray exposure. Therefore, findings from our study reinforce prevention guidelines to avoid the potential hazards of diagnostic X-ray exposure to unborn children.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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