Cesarean Section Is Associated with an Increased Risk of Acute Lymphoblastic Leukemia and Hepatoblastoma in Children from Minnesota

Lindsay A. Williams[1,2,3], Michaela Richardson[1], Logan G. Spector[1,3], and Erin L. Marcotte[1,2,3]

ABSTRACT

Background: In recent decades, Cesarean section (C-section) rates have increased. C-section is hypothesized to negatively impact the developing immune system by altering activation of the hypothalamic–pituitary–adrenal axis and the infant microbiome, among other mechanisms, thereby potentially modulating childhood cancer risk.

Methods: Using linked birth and cancer registry data from Minnesota (1976–2014), we included individuals ages 0–14 at diagnosis with one of 19 cancers. Cases and controls were frequency matched by birth year. We used logistic regression to estimate ORs and 95% confidence intervals (95% CI) as the measure of association between C-section and cancer. We assessed sex–C-section interactions for each cancer and conducted stratified analyses in acute lymphoblastic leukemia (ALL) for birth year, age at diagnosis, and maternal race.

Results: There were 3,166 cases and 20,589 controls. One third ($n = 1{,}174$) of controls born during 2004–2014 were delivered via C-section compared with 42.2% of cases ($n = 285$). C-section was associated with ALL ($n = 819$; OR: 1.20; 95% CI: 1.01–1.43) and hepatoblastoma ($n = 50$; OR: 1.89; 95% CI: 1.03–3.48), particularly among females (ALL OR: 1.34; 95% CI: 1.04–1.72; hepatoblastoma OR: 3.87; 95% CI: 1.30–11.57). The risk of ALL was highest during 2005–2014 (OR: 1.62; 95% CI: 1.11–2.34) and among children ages 1–5 years (OR: 1.28; 95% CI: 1.02–1.61).

Conclusions: C-section was associated with an increased risk of ALL and hepatoblastoma.

Impact: These associations require investigation to determine causality and rule out confounding by indication or reverse causality. The mechanisms underlying these associations may depend on neonatal immune system processes altered during C-section deliveries.

Introduction

Caesarean section (C-section) has been examined as a risk factor for some childhood cancers as it has been shown that C-section can modify immune system function in the offspring (1). This is of particular concern as the rates of C-section have increased over recent decades to now include nearly one-third of live births in the United States, annually (2). Data from both the United States and international sites have shown a decrease in the vaginal birth after cesarean rate (3), an increase in prelabor C-sections (4), and an increase in C-sections following a previous C-section delivery over the past 30 years (2, 5–7). Mechanisms of action for C-section in childhood cancer development may be due to the neonate not experiencing the necessary stress of vaginal delivery that is thought to properly activate the hypothalamic–pituitary–adrenal axis and prime the immune system for future function (8). The absence of this priming event in children born via C-section, particularly prelabor C-section, is hypothesized to (i) contribute to poor innate and adaptive immune system formation demonstrated by higher rates of asthma and other conditions in these children (1, 9); (ii) lead to variation in gene regulation modified by epigenetic changes conferred via C-section (8); and (iii) result in failure to seed the neonatal gut microbiome with necessary bacterial species from the maternal vaginal canal (8, 10–12). These C-section–driven changes to the neonatal immune system may create a permissive environment for malignancies to develop.

We and others have reported an approximate 20% increased risk of B-cell acute lymphoblastic leukemia (ALL) among children born via C-section (13–16), particularly prelabor C-section (5, 13). Other studies have reported null associations between C-section and ALL (5, 17); however, older studies where C-section, particularly that without trial of labor, was less common may be driving the reported lack of association. In addition, C-section has been found to be associated with some embryonal tumors (16), including neuroblastoma (18, 19), though results have been inconsistent and are often based on small sample sizes and older studies where C-section was not as common as recent decades.

The incidence of C-section has increased substantially in the United States and around the world in recent decades (4, 20). Simultaneously, the incidence of some childhood cancers, including leukemias (21), hepatoblastoma (22), and malignant brain tumors, is also increasing (23). Therefore, using linked birth and cancer registry data spanning nearly four decades of cancer diagnoses in the state of Minnesota, we examined the association between C-section and childhood cancer risk overall and for 19 different cancer types. We performed sex-stratified analyses to elucidate the role of C-section in the observed sex differences in childhood cancer risk (24). C-section is a potentially modifiable risk factor; therefore, it is important to understand the contribution of C-section to childhood cancer development as a potential means to lessen the burden of pediatric malignancies.

[1]Division of Epidemiology and Clinical Research, Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota. [2]Brain Tumor Program, University of Minnesota, Minneapolis, Minnesota. [3]Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Erin L. Marcotte, 420 Delaware Street SE, MMC 715, Minneapolis, MN 55455; Phone: 612-626-3281; E-mail: marcotte@umn.edu

Cancer Epidemiol Biomarkers Prev 2021;30:736–42

doi: 10.1158/1055-9965.EPI-20-1406

Materials and Methods

Study population

This analysis includes incident primary cancers diagnosed in children ages 0–14 years identified from the population-based cancer registry in Minnesota from 1976 to 2014. These data are the result of a linkage between the Department of Health, Minnesota Cancer Surveillance System and the Minnesota birth registry for all cancers diagnosed, as described previously (25). Cases and controls were frequency matched approximately 1:5 on birth year (1989–2010). Approval for the study was obtained from the Minnesota Department of Health and from the University of Minnesota Institutional Review Board. Informed consent was waived.

Cancers of interest

Cancer type was classified using the International Classification of Childhood Cancer, Third Edition (26). Cancers included were ALL, acute myeloid leukemia (AML), Hodgkin lymphoma, non-Hodgkin lymphoma, Burkitt lymphoma, ependymomas, astrocytomas, intracranial/intraspinal embryonal tumors, neuroblastoma, retinoblastoma, Wilms tumor, hepatoblastoma, osteosarcoma, Ewing sarcoma, rhabdomyosarcoma, fibrosarcoma, intracranial/intraspinal germ cell tumors (GCT), extracranial/extragonadal GCTs, and thyroid carcinoma. Tumor types with fewer than five cases born via C-section or vaginal delivery were excluded from the analyses. Children with down syndrome listed on their birth certificate ($n = 25$ cases and $n = 7$ controls) were also excluded.

Variables of interest

The exposure of interest was mode of delivery defined as vaginal (referent) or C-section as listed on the birth certificate. Covariates considered in adjusted models were selected *a priori* on the basis of established associations with childhood cancer (27–30) and in consideration of the study design of matching by birth year. Covariates include sex (male, female), birth weight category (g; <2,500, 2,500–4,000, >4,000), gestational age (weeks; <38, 38–40, 41–42; gestational ages ≥43 were excluded), maternal race (non-Hispanic white, non-Hispanic black, Hispanic, other), maternal age (years; <25, 25–<35, ≥35), maternal education [<high school (HS) grad, HS grad/GED/some college, college grad], plurality (single, multiples), and birth year (1976–1989, 1990–1995, 1996–2001, 2002–2014).

Statistical analysis

We used logistic regression to estimate ORs and 95% confidence intervals (95% CI) as the measure of association between C-section and childhood cancer overall and by type. Crude models were adjusted for the matching variable, birth year. Fully adjusted models accounted for sex, birth weight, maternal race, maternal age, maternal education, plurality, and birth year. Gestational age was not included in the fully adjusted models as it was missing for 8% of cases and is largely correlated to birth weight, which was included in the models. To account for potential reverse causation related to cancers diagnosed prenatally, we conducted sensitivity analyses restricting to cases 2 months and older at the age of diagnosis. An interaction between C-section and sex was evaluated in logistic regression models for each cancer and a likelihood ratio test $P_{interaction}$ value was estimated. The association between C-section and cancer was estimated for males and females separately using the fully adjusted models and only including cases diagnosed at 2 months of age or older. To further examine the associations between C-section and ALL, we conducted additional analyses stratified by birth year, age at diagnosis, and maternal race. All statistical analyses were conducted in SAS version 9.4 (SAS, Cary, NC).

Results

From 1976 to 2014, there were 3,166 cancers in children <14 years of age at diagnosis in Minnesota that met the inclusion criteria for this study. There were 20,598 controls frequency matched by birth year included in this analysis. A higher percentage of cases were male (cases 56.5%, controls 51.1%), weighed more than 4,000 g at birth (cases 14.5%, controls 13.0%), born to mothers with college education (cases 58.9%, controls 55.8%), and were non-Hispanic white (cases 87.6%, controls 84.9%; **Table 1**). Cases were more frequently delivered by C-section than controls, 21.3% compared with 19.1%, respectively. In 2002–2014, C-section was more common overall and was more frequent among cases (cases 42.2%, controls 30.7%). There was much variation in the percentage of births born via C-section when considering cancer type with osteosarcoma having the lowest at 11.9% and extracranial/extragonadal GCTs having the highest at 42.2%.

When considering the association between C-section and childhood cancer overall, in the fully adjusted model the estimate was elevated but included the null (OR_{adj}: 1.08; 95% CI: 0.98–1.20). There was much variation in the association between C-section and cancer risk when considering cancer type. While many estimates had CIs that spanned the null, elevated estimates were observed for tumors occurring in various anatomic locations (**Table 2**).

We observed a statistically significant association between C-section and ALL (OR_{adj}: 1.20; 95% CI: 1.01–1.42) and hepatoblastoma (OR_{adj}: 1.90; 95% CI: 1.05–3.44) in the crude and fully adjusted models (Supplementary Table S1). These results remained statistically significant in analyses that excluded cases diagnosed at <2 months of age (**Table 2**). Elevated, though nonstatistically significant, associations were observed for C-section and intracranial/intraspinal embryonal tumors (OR_{adj}: 1.30; 95% CI: 0.88–1.91), neuroblastoma (full OR: 1.17; 95% CI: 0.87–1.58), Wilms tumor (OR_{adj}: 1.30; 95% CI: 0.95–1.79), intracranial/intraspinal GCT (OR_{adj}: 2.07; 95% CI: 0.94–4.54), and extracranial/extragonadal GCTs (OR_{adj}: 1.99; 95% CI: 0.99–3.98). Results were similar when excluding children diagnosed at less than 2 months of age for all cancers except extracranial/extragonadal GCTs where the association was attenuated (**Table 2**).

There was evidence of a statistical interaction between C-section and sex for ALL, AML, ependymomas, neuroblastoma, and hepatoblastoma (all $P_{interaction}$ <0.20; **Table 3**). In sex-stratified analyses, we observed a statistically significant association between C-section and ALL (OR_{adj}: 1.34; 95% CI: 1.04–1.72) and hepatoblastoma (OR_{adj}: 3.87; 95% CI: 1.30–11.57) among females. There was an elevated, though nonstatistically significant, association between C-section and neuroblastoma (OR_{adj}: 1.59; 95% CI: 0.99–2.55) and AML among females as well (OR_{adj}: 1.30; 95% CI: 0.71–2.40). In males, there was an elevated association between C-section and ependymomas (OR_{adj}: 1.53; 95% CI: 0.84–2.82).

To better understand the observed association between C-section and ALL, we conducted analyses stratified by birth year, age at diagnosis, and maternal race/ethnicity. When considering year of birth, there was a statistically significant association between C-section and ALL for 2005–2014 (OR_{adj}: 1.62; 95% CI: 1.11–2.34), which had the highest percentages of controls (25.4%) and ALL cases born via C-section at 34.0% (**Table 4**). When we considered age at diagnosis, the ALL cases diagnosed from 1–5 years of age were most strongly associated with C-section (OR_{adj}: 1.28;

Table 1. Characteristics of childhood cancer cases and controls from Minnesota, 1976–2014.

Characteristic	Controls *N* (%)	Cases *N* (%)
Child	20,598	3,166
Sex		
Male	10,183 (51.1)	1,776 (56.5)
Female	9,762 (48.9)	1,365 (43.5)
Missing	653	125
Birth weight (g)		
<2,500	1,166 (5.7)	177 (5.4)
2,500–4,000	16,711 (81.3)	2,606 (80.1)
>4,000	2,668 (13.0)	471 (14.5)
Missing	53	12
Plurality (%)		
Singleton	20,004 (97.1)	3,194 (97.8)
Multiple	593 (2.9)	71 (2.2)
Missing	1	1
Age at diagnosis (years)		
Mean (SD)	—	5.9 (4.4)
Gestational age (weeks)		
<38	2,857 (14.9)	470 (15.7)
38–40	13,129 (68.5)	2,072 (69.0)
41–42	3,191 (16.6)	460 (15.3)
Missing	1,421	264
Maternal		
Age (years)		
<24	5,086 (24.8)	749 (23.1)
24–<28	4,886 (23.8)	826 (25.4)
28–<32	5,286 (25.7)	817 (25.2)
32–<35	2,838 (13.8)	441 (13.6)
≥35	2,449 (11.9)	416 (12.8)
Missing	53	17
Educational attainment		
<HS graduate	2,160 (10.9)	284 (9.0)
HS grad/GED	6,596 (33.3)	1,008 (32.1)
Some college	5,200 (26.2)	856 (27.2)
College grad	5,881 (29.6)	998 (31.7)
Missing	761	120
Maternal race		
Non-Hispanic, white	17,352 (84.9)	2,841 (87.6)
Non-Hispanic, black	1,083 (5.3)	110 (3.4)
Hispanic	751 (3.7)	124 (3.8)
American Indian/Alaska Native	391 (1.9)	45 (1.4)
Asian/Pacific Islander	848 (4.2)	121 (3.7)
Other	21 (0.1)	2 (0.1)
Missing	152	23
Mode of delivery		
Vaginal	16,198 (80.9)	2,491 (78.7)
C-section	3,830 (19.1)	675 (21.3)
1976–1989	778 (20.3)	151 (22.4)
1990–1995	940 (24.5)	118 (17.5)
1996–2001	938 (24.5)	121 (17.9)
2002–2014	1,174 (30.7)	285 (42.2)
Missing	570	100

95% CI: 1.02–1.61). Again, sample size was too small to estimate an association between C-section and ALL for infants less than 1 year of age at diagnosis. We observed an association between C-section and ALL among children born to mothers classified as non-Hispanic white (OR_{adj}: 1.24; 95% CI: 1.03–1.48), but this is the only racial/ethnic group for which there was adequate sample size to detect an association.

Discussion

In our analysis of 3,166 children diagnosed with childhood cancers in Minnesota from 1976–2014, we observed a statistically significant, positive association between C-section and ALL and hepatoblastoma after adjusting for suspected confounders. In crude models, we observed significant associations between C-section and Wilms tumor, neuroblastoma, and extracranial/extragonadal GCTs, but these associations were no longer statistically significant when excluding cases diagnosed prior to 2 months of age (neuroblastoma and extracranial/extragonadal GCTs) and upon adjustment for suspected confounders (Wilms tumor). For neuroblastoma and extracranial/extragonadal GCTs, the findings align with these tumors forming and oftentimes diagnosed *in utero* requiring a C-section for safe delivery so cancer treatment can begin (31, 32). We did not observe an association between C-section and AML, and this finding is consistent with a previous report (13). Similarly, we also did not observe an association between C-section and lymphomas, which is consistent with our previous report (33) but contrary to the increased risk for non-Hodgkin lymphoma among children born via C-section that was reported in another small study (34). We also did not observe an association between C-section and sarcomas, which are generally diagnosed during adolescence and likely depend on the growth changes accompanying puberty, particularly bone sarcomas (24, 35–37). There were suggested statistical interactions between sex and C-section for ALL, AML, ependymomas, neuroblastoma, and hepatoblastoma with females having a statistically significant increased risk of ALL and hepatoblastoma when born via C-section. We also observed an increased, though nonstatistically significant, risk of neuroblastoma for females born via C-section while a suggested increased risk of ependymoma among males was observed. Collectively, the findings of our study suggest that C-section may be associated with increased risk for some childhood cancers, namely, ALL and hepatoblastoma, and that this increase may be stronger among females, among children born in the early 2000s for ALL, and among young children diagnosed with ALL.

Our findings of an increased risk of ALL among children born via C-section agrees with many prior reports on this association (13, 14, 38–43). We also observed that this increased risk was strongest in females, which has not been reported, and among children diagnosed from ages 1–5 years (14, 39). The finding of a higher risk of ALL among females born via C-section is counter to the increased risk of ALL among males we have reported previously (24, 44); however, Black and colleagues (2015) have reported that female neonates had higher proportion of prelabor C-sections (1), which are more strongly associated with ALL risk (13). Therefore, a female excess in planned C-sections may underlie our observation, but as this is registry data we cannot investigate this hypothesis. C-section rates have increased in recent decades, as shown in our controls, and we observed a nearly 1.6-fold increase in ALL risk among children born via C-section during 2005–2014. These findings suggest that ALL incidence may continue to increase, particularly in young children, if C-section rates remain constant or continue to rise.

Previous reports have also described an elevated, though often nonstatistically significant, risk of embryonal tumors, including neuroblastoma and some central nervous system (CNS) tumors, among children born via C-section (16, 18, 19). In our study, we did not observe a statistically significant association between C-section and neuroblastoma and the size of our effect estimate was smaller than others have reported, 1.17 in our study and 1.40 elsewhere; however, we were able to account for suspected confounders including

Table 2. OR and 95% CI as the measure of association between C-section delivery in childhood cancers diagnosed in children ≥2 months of age in Minnesota, 1976–2014.

	Crude model[a]				Fully adjusted model[b]			
	Vaginal *N* (%)	C-section *N* (%)	OR	95% CI	Vaginal *N* (%)	C-section *N* (%)	OR	95% CI
Controls	16,198 (80.9)	3,830 (19.1)	Ref		15,086 (81.0)	3,530 (19.0)	Ref	
ALL CANCERS COMBINED	2,427 (79.3)	633 (20.7)	1.08	0.98–1.19	2,267 (79.7)	579 (20.3)	1.07	0.97–1.18
Acute lymphoid leukemia	670 (77.4)	196 (22.3)	**1.20**	**1.02–1.42**	637 (77.7)	182 (22.2)	**1.20**	**1.01–1.43**
Acute myeloid leukemia	122 (83.0)	25 (17.1)	0.84	0.55–1.30	110 (82.7)	23 (17.3)	0.87	0.55–1.38
Hodgkin lymphoma	105 (85.4)	18 (14.6)	0.79	0.48–1.30	101 (85.6)	17 (14.4)	0.81	0.48–1.36
Non-Hodgkin lymphoma	124 (84.9)	22 (15.1)	0.79	0.50–1.25	118 (84.9)	21 (15.1)	0.83	0.52–1.33
Burkitt lymphoma	58 (82.9)	12 (17.1)	0.90	0.48–1.67	56 (82.4)	12 (17.7)	0.92	0.49–1.74
Ependymoma	83 (80.6)	20 (19.4)	0.99	0.60–1.61	76 (80.9)	18 (19.2)	0.97	0.57–1.64
Astrocytoma	347 (81.3)	80 (18.7)	0.96	0.75–1.22	321 (81.3)	74 (18.7)	1.01	0.78–1.31
Intracranial/Intraspinal embryonal	125 (77.2)	37 (22.8)	1.23	0.85–1.78	117 (77.0)	35 (23.0)	1.31	0.89–1.93
Neuroblastoma	197 (75.5)	64 (24.5)	1.23	0.92–1.64	171 (76.3)	53 (23.7)	1.14	0.83–1.58
Retinoblastoma	71 (83.5)	14 (16.5)	0.76	0.43–1.35	62 (87.3)	9 (12.7)	0.60	0.30–1.23
Wilms tumor	173 (74.3)	60 (25.8)	**1.36**	**1.01–1.83**	160 (74.8)	54 (25.2)	1.28	0.93–1.77
Hepatoblastoma	36 (67.9)	17 (32.1)	**1.84**	**1.03–3.30**	33 (66.0)	17 (34.0)	**1.89**	**1.03–3.48**
Osteosarcoma	59 (89.4)	7 (10.6)	0.56	0.26–1.23	57 (89.1)	7 (10.9)	0.58	0.26–1.28
Ewing sarcoma	46 (80.7)	11 (19.3)	1.07	0.55–2.08	45 (81.8)	10 (18.2)	0.86	0.42–1.75
Rhabdomyosarcoma	87 (82.9)	18 (17.1)	0.89	0.54–1.49	83 (84.7)	15 (15.3)	0.77	0.44–1.35
Fibrosarcoma	27 (75.0)	9 (25.0)	1.59	0.73–3.46	20 (71.4)	8 (28.6)	1.49	0.63–3.53
Intracranial/Intraspinal GCT	23 (69.7)	10 (30.3)	1.69	0.77–3.68	19 (67.9)	9 (32.1)	1.97	0.87–4.48
Extracranial/Extragonadal GCT	19 (79.2)	5 (20.8)	1.13	0.42–3.05	18 (78.3)	5 (21.7)	1.13	0.41–3.13
Thyroid	50 (87.7)	7 (12.3)	0.65	0.29–1.44	50 (87.7)	7 (12.3)	0.65	0.29–1.45

Note: Bold values represent effect estimates and confidence intervals that exclude the null value of 1.
[a]Adjusted for matching variable: birth year (1976–1989, 1990–1995, 1996–2001, 2002–2014).
[b]Adjusted for sex (male, female), birth weight (<2,500 g, 2,500–4,000 g, >4,000 g), maternal race (non-Hispanic white, non-Hispanic black, Hispanic, other) maternal age in years (<25, 25–<35, 35+), maternal education (<HS grad, HS grad/GED/some college, college grad), plurality (single, multiples), and matching variable: birth year (1976–1989, 1990–1995, 1996–2001, 2002–2014).

Table 3. Sex-stratified estimates of the association (OR and 95% CI) between C-section and childhood cancers diagnosed ≥2 months of age in Minnesota, 1976–2014.

	Females[a]				Males[a]				
	Vaginal *N* (%)	C-section *N* (%)	OR	95% CI	Vaginal *N* (%)	C-section *N* (%)	OR	95% CI	$P_{Interaction}$
Controls	7,466 (81.7)	1,674 (18.3)	Ref		7,620 (80.4)	1,856 (19.6)	Ref		
Acute lymphoid leukemia	281 (76.2)	88 (23.9)	**1.34**	**1.04–1.72**	356 (79.1)	94 (20.9)	1.09	0.86–1.38	0.16
Acute myeloid leukemia	46 (76.7)	14 (23.3)	1.30	0.71–2.40	64 (87.7)	9 (12.3)	0.57	0.28–1.17	0.07
Hodgkin lymphoma	35 (85.4)	6 (14.6)	0.90	0.37–2.15	66 (85.7)	11 (14.3)	0.78	0.41–1.49	0.87
Non-Hodgkin lymphoma	44 (89.8)	5 (10.2)	0.60	0.24–1.52	74 (82.2)	16 (17.8)	0.95	0.55–1.65	0.30
Burkitt lymphoma	7 (77.8)	2 (22.2)	—	—	49 (83.1)	10 (16.9)	0.88	0.44–1.75	0.63
Ependymoma	36 (92.3)	3 (7.7)	—	—	40 (72.7)	15 (27.3)	1.53	0.84–2.82	0.03
Astrocytoma	154 (82.8)	32 (17.2)	0.97	0.65–1.43	167 (79.9)	42 (20.1)	1.04	0.74–1.48	0.65
Intracranial/Intraspinal embryonal	55 (80.9)	13 (19.1)	1.06	0.57–1.97	62 (73.8)	22 (26.2)	1.52	0.92–2.51	0.41
Neuroblastoma	64 (71.1)	26 (28.9)	1.59	0.99–2.55	107 (79.9)	27 (20.2)	0.89	0.57–1.38	0.09
Retinoblastoma	35 (85.4)	6 (14.6)	0.67	0.27–1.62	27 (90.0)	3 (10.0)	—	—	0.50
Wilms tumor	75 (75.8)	24 (24.2)	1.27	0.79–2.05	85 (73.9)	30 (26.1)	1.30	0.84–1.99	0.97
Hepatoblastoma	7 (50.0)	7 (50.0)	**3.87**	**1.30–11.57**	26 (72.2)	10 (27.8)	1.35	0.63–2.89	0.13
Osteosarcoma	20 (83.3)	4 (16.7)	—	—	37 (92.5)	3 (7.5)	—	—	0.22
Ewing sarcoma	22 (88.0)	3 (12.0)	—	—	23 (76.7)	7 (23.3)	1.01	0.41–2.48	0.34
Rhabdomyosarcoma	31 (86.1)	5 (13.9)	0.67	0.25–1.78	52 (83.9)	10 (16.1)	0.82	0.41–1.64	0.90
Fibrosarcoma	9 (64.3)	5 (35.7)	2.13	0.67–6.78	11 (78.6)	3 (21.4)	—	—	0.35
Intracranial/intraspinal GCT	8 (72.7)	3 (27.3)	—	—	11 (64.7)	6 (35.3)	2.21	0.79–6.15	0.73
Extracranial/extragonadal GCT	13 (81.3)	3 (18.8)	—	—	5 (71.4)	2 (28.6)	—	—	0.67
Thyroid	37 (88.1)	5 (11.9)	0.65	0.25–1.68	13 (86.7)	2 (13.3)	—	—	0.97

Note: Bold values represent effect estimates and confidence intervals that exclude the null value of 1.
[a]Models adjusted for birth weight (<2,500 g, 2,500–4,000 g, >4,000 g), maternal race (non-Hispanic white, non-Hispanic black, Hispanic, other), maternal age in years (<25, 25–<35, 35+), maternal education (<HS grad, HS grad/GED/some college, college grad), plurality (single, multiples), and matching variable: birth year (1976–1989, 1990–1995, 1996–2001, 2002–2014).

Table 4. Stratified ORs and 95% CIs as the measure of association between acute lymphoid leukemia and C-section delivery in children diagnosed at ≥2 months of age in Minnesota, 1976–2014.

	Controls		Cases			
	Vaginal *N* (%)	C-section *N* (%)	Vaginal *N* (%)	C-section *N* (%)	OR	95% CI
Controls	15,086 (81.0)	3,530 (18.96)	637 (77.8)	182 (22.2)	Ref	
Year of birth[a]						
1976–1984	1,448 (85.5)	245 (14.5)	73 (82.9)	15 (17.1)	1.27	0.71–2.28
1985–1994	5,850 (82.2)	1,263 (17.8)	224 (80.3)	55 (19.7)	1.13	0.83–1.53
1995–2004	6,114 (80.8)	1,453 (19.2)	247 (79.4)	64 (20.6)	1.07	0.80–1.42
2005–2014	1,674 (74.6)	569 (25.4)	93 (65.0)	48 (34.0)	**1.62**	**1.11–2.34**
Age at diagnosis for cases[b]						
<1 year	—	—	19 (86.4)	3 (13.6)	—	—
1–5 years	—	—	328 (75.2)	108 (24.8)	**1.28**	**1.02–1.61**
6–10 years	—	—	190 (79.2)	50 (20.8)	1.16	0.84–1.60
11–14 years	—	—	100 (82.6)	21 (17.4)	1.09	0.68–1.75
Case maternal race[c]						
Non-Hispanic white	12,900 (80.8)	3,061 (19.2)	555 (77.3)	163 (22.7)	**1.24**	**1.03–1.48**
Non-Hispanic black	744 (80.4)	181 (19.6)	12 (66.7)	6 (33.3)	1.59	0.55–4.61
Hispanic	547 (81.0)	128 (19.0)	30 (83.3)	6 (16.7)	0.78	0.31–1.97
Other	895 (84.8)	160 (15.2)	40 (85.1)	7 (14.9)	0.92	0.39–2.19

Note: Bold values represent effect estimates and confidence intervals that exclude the null value of 1.
[a]Model adjusted for sex (male, female), birth weight category (<2,500 g, 2,500–4,000 g, > 4,000 g), maternal race (non-Hispanic white, non-Hispanic black, Hispanic, other), maternal age in years (<25, 25–<35, 35+), maternal education (<HS grad, HS grad/GED/some college, college grad), and plurality (single, multiples).
[b]Model adjusted for sex (male, female), birth weight category (<2,500 g, 2,500–4,000 g, > 4,000 g), maternal race (non-Hispanic white, non-Hispanic black, Hispanic, other), maternal age in years (<25, 25–<35, 35+), maternal education (<HS grad, HS grad/GED/some college, college grad), plurality (single, multiples), and matching variable: birth year categorized (1976–1989, 1990–1995, 1996–2001, 2002–2014).
[c]Model adjusted for sex (male, female), birth weight category (<2,500 g, 2,500–4,000 g, > 4,000 g), maternal age in years (<25, 25–<35, 35+), maternal education (< HS grad, HS grad/GED/some college, college grad), plurality (single, multiples), and matching variable: birth year categorized (1976–1989, 1990–1995, 1996–2001, 2002–2014).

age, maternal education and race, and we restricted to cases 2 months of age and older, all of which were not accounted for in other studies (18, 19). Importantly, we did observe a higher neuroblastoma risk in females born via C-section, which has not been demonstrated to date. In our study, we observed largely null associations for CNS tumors including ependymomas and astrocytomas overall with an elevated, but nonstatistically significant increased effect estimate observed for intracranial/intraspinal embryonal tumors, which are presumably largely medulloblastomas. In the literature, all CNS tumors are generally combined prohibiting us from making direct comparisons between tumor types. In general, there is an elevated, though nonsignificant risk for CNS tumors when the mode delivery is C-section (6, 16, 19).

Our finding of an increased risk of hepatoblastoma for children born via C-section disagrees with a previous report where a null association was observed (45) and requires further investigation. As this is population-based, birth registry data, we were unable to ascertain detailed information on birth defects beyond Trisomy 21; however, based on a recent report by Lupo and colleagues (2019; ref. 46), a number of birth defects including Trisomy 18, congenital heart anomalies, and genitourinary anomalies are all strongly associated with hepatoblastoma risk with HRs ranging from 8 to 80. Therefore, it is likely that some cases of hepatoblastoma may due to the presence of a predisposing birth defect that necessitated a C-section delivery representing confounding by indication in our study (47). However, this should be investigated in other studies of hepatoblastoma cases with information on birth defects and mode of delivery. It is unlikely that our findings of an increased risk of hepatoblastoma are largely due to reverse causation of a prenatal diagnosis as only one case was diagnosed with hepatoblastoma at less than 2 months of age in our study. Should C-section act as a biologic risk factor for hepatoblastoma this will be important information as hepatoblastoma is the fastest rising cancer around the globe and the increased C-section rates may add to this rise in diagnoses in the future (22).

Mechanisms of action for C-section in childhood cancer development may depend on neonatal immune system dysfunction that is the result of not experiencing the necessary stress of a vaginal delivery whereby activation of the hypothalamic–pituitary–adrenal axis occurs leading to normal immune system development, which is a multifaceted process (8). This missed experience in children born via C-section, namely, prelabor C-section, may lead to a poorly formed innate and adaptive immune system, epigenetic changes in gene regulation, and/or underlie variation in the gut microbiota (8, 10, 11). The responsible mechanisms may of course vary by cancer type in children such that hematologic malignancies, namely, ALL, may depend more heavily on the aberrant development of innate and adaptive immune responses, which are known to differ by sex (48). In a study of neonatal inflammatory markers in children that developed B-ALL, there were sex differences in markers including C-reactive protein (49); however, these markers were not associated with mode of delivery, but there were only 16 children born via C-section in this study. Our findings of a higher risk of ALL in females born via C-section taken together with these observations suggests a larger study of inflammatory markers in C-section born children is necessary to better understand the biologic mechanisms underlying the sex differences we have observed. Immune system function has been hypothesized to depend heavily on gut microbiota transferred to the newborn from the mother's vaginal canal, which is absent in C-section deliveries (8, 12). This theory draws on the hygiene hypothesis that the lack of exposure to the vaginal microflora leads to colonization of pathogenic bacterial species from the hospital environment in the neonatal gut that can lead

to dysregulation of the immune system and the development of immune-related diseases, including cancer (20). In animal models, it has been observed that mice with an intact gut microbiome did not develop ALL, further highlighting the importance of a properly seeded microbiome in immune function and leukemogenesis prevention (50). The altered immune system function after a C-section is observed through the higher risk for asthma and other respiratory conditions, type 1 diabetes, celiac, and other gastrointestinal conditions in children born in this manner (1, 9). Furthermore, it has been shown that C-section is often associated with a reduced rate of breastfeeding initiation compared with vaginal delivery and the epidemiologic literature, especially for ALL (51), has shown that breastfeeding reduces risk for developing malignancies in the child. The clear association with C-section and ALL and other cancers, including hepatoblastoma in our study, suggests mode of delivery plays an important role in the development of some childhood cancers and may represent an avenue for prevention intervention (52).

Although we present a large, population-based study of the association between C-section and 19 types of childhood cancer, our study findings should be interpreted with the following limitations in mind. As our data arrived from birth certificates, we are unable to account for type of C-section, prelabor or emergency, which we have found to vary in association with ALL (13). We also do not have information on immunophenotypic or cytogenetic subtypes of ALL, which have varying associations with C-section (13, 53). To that end, our study lacks information on molecular subtypes for other childhood cancers and we do not have data on predisposition syndromes outside of Trisomy 21. Similarly, we are unable to account for all types of birth defects and therefore cannot disentangle the relationship between birth defects, C-section, and hepatoblastoma or other cancers. Because of sample size limitations, we are unable to examine more types of cancer, including additional histologies of CNS tumors, sarcomas, and GCTs. Because of the nature of registry data, we are unable to account for childhood cancer cases among children who were born in Minnesota and left prior to diagnosis and are thus captured in another state's cancer registry. We do not have access to information on other risk factors that may impact C-section rates, such as previous C-section, breastfeeding duration, or maternal preeclampsia rates, which would inform the interpretation of our findings. Our study also lacks information on environmental exposures. Previous reports have found the association between C-section and ALL to be higher among children born to Hispanic mothers; unfortunately, we had a very low proportion of Hispanic mothers in our study resulting in an imprecise estimate of this association.

In conclusion, our population-based, linked birth and cancer registry study in Minnesota confirmed the previously reported statistically significant increased risk of childhood ALL among children born by C-section. We also observed an increased risk of hepatoblastoma for children born via C-section. These associations were strongest among females suggesting an interplay between sex and C-section that may moderate the development of ALL and hepatoblastoma, which warrants further investigations in other studies. We demonstrated that as C-section has increased in recent decades the association between C-section and ALL is higher in magnitude for more recent years. These findings suggest C-section is a strong risk factor for ALL that may impact future incidence rates if C-section rates remain high or increase. If the association between C-section and hepatoblastoma is independent of birth defects, which needs to be verified in other studies, the increase in C-section rates may also contribute to the increased incidence of hepatoblastoma that we are observing globally (22). C-section is a potentially modifiable risk factor for childhood ALL and other cancers that may serve as a future avenue of intervention as we work to reduce the incidence of childhood cancers.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

L.A. Williams: Conceptualization, data curation, formal analysis, writing–original draft, writing–review and editing. **M. Richardson:** Data curation, formal analysis. **L.G. Spector:** Resources, funding acquisition, writing–review and editing. **E.L. Marcotte:** Conceptualization, funding acquisition, investigation, methodology, writing–review and editing.

Acknowledgments

This work was funded by the Children's Cancer Research Fund (to L.A. Williams and E.L. Marcotte).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 25, 2020; revised November 30, 2020; accepted January 29, 2021; published first February 9, 2021.

References

1. Black M, Bhattacharya S, Philip S, Norman JE, McLernon DJ. Planned cesarean delivery at term and adverse outcomes in childhood health. JAMA 2015;314:2271–9.
2. Hehir MP, Ananth CV, Siddiq Z, Flood K, Friedman AM, D'Alton ME. Cesarean delivery in the United States 2005 through 2014: a population-based analysis using the Robson 10-Group Classification System. Am J Obstet Gynecol 2018;219:105.e1–105.e11.
3. Grobman WA, Lai Y, Landon MB, Spong CY, Rouse DJ, Varner MW, et al. The change in the rate of vaginal birth after caesarean section. Paediatr Perinat Epidemiol 2011;25:37–43.
4. Vogel JP, Betrán AP, Vindevoghel N, Souza JP, Torloni MR, Zhang J, et al. Use of the robson classification to assess caesarean section trends in 21 countries: a secondary analysis of two WHO multicountry surveys. Lancet Glob Heal 2015;3:e260–70.
5. Bonaventure A, Simpson J, Ansell P, Roman E. Paediatric acute lymphoblastic leukaemia and caesarean section: a report from the United Kingdom Childhood Cancer Study (UKCCS). Paediatr Perinat Epidemiol 2020;34:344–9.
6. Momen NC, Olsen J, Gissler M, Cnattingius S, Li J. Delivery by caesarean section and childhood cancer: a nationwide follow-up study in three countries. BJOG 2014;121:1343–50.
7. Cnattingius S, Zack MM, Ekbom A, Gunnarskog J, Kreuger A, Linet M, et al. Prenatal and neonatal risk factors for childhood lymphatic leukemia. J Natl Cancer Inst 1995;87:908–14.
8. Cho CE, Norman M. Cesarean section and development of the immune system in the offspring. Am J Obstet Gynecol 2013;208:249–54.
9. Kristensen K, Henriksen L. Cesarean section and disease associated with immune function. J Allergy Clin Immunol 2016;137:587–90.
10. Boutsikou T, Malamitsi-Puchner A. Caesarean section: Impact on mother and child. Acta Paediatr Int J Paediatr 2011;100:1518–22.
11. Wen Y, Jin R, Chen H. Interactions between gut microbiota and acute childhood leukemia. Front Microbiol 2019;10:1300.

12. Shao Y, Forster SC, Tsaliki E, Vervier K, Strang A, Simpson N, et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. Nature 2019;574:117–21.
13. Marcotte EL, Thomopoulos TP, Infante-Rivard C, Clavel J, Petridou ETh, Schüz J, et al. Caesarean delivery and risk of childhood leukaemia: a pooled analysis from the Childhood Leukemia International Consortium (CLIC). Lancet Haematol 2016;3:e176–85.
14. Francis SS, Selvin S, Metayer C, Wallace AD, Crouse V, Moore TB, et al. Mode of delivery and risk of childhood leukemia. Cancer Epidemiol Biomarkers Prev 2014;23:876–81.
15. Yen H-Ju, Chen S-H, Chang T-Y, Yang C-P, Lin D-T, Hung I-J, et al. Pediatric acute lymphoblastic leukemia with t(1;19)/TCF3-PBX1 in Taiwan. Pediatr Blood Cancer 2017;64:10.
16. de Paula Silva N, de Souza Reis R, Garcia Cunha R, Pinto Oliveira JF, Santos MO, Pombo-de-Oliveira MS, et al. Maternal and birth characteristics and childhood embryonal solid tumors: a population-based report from Brazil. PLoS One 2016; 11:e0164398.
17. Reynolds P, Von BJ, Elkin EP. Birth characteristics and leukemima in young children. Am J Epidemiol 2002;155:603–13.
18. Hamrick SEG, Olshan AF, Neglia JP, Pollock BH. Association of pregnancy history and birth characteristics with neuroblastoma: a report from the Children's Cancer Group and the Pediatric Oncology Group. Paediatr Perinat Epidemiol 2001;15:328–37.
19. Schüz J, Weihkopf T, Kaatsch P. Medication use during pregnancy and the risk of childhood cancer in the offspring. Eur J Pediatr 2007;166:433–41.
20. Neu J, Rushing J. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. Clin Perinatol 2011;38:321–31.
21. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin 2014;64:83–103.
22. Hubbard AK, Spector LG, Fortuna G, Marcotte EL, Poynter JN. Trends in international incidence of pediatric cancers in children under 5 years of age: 1988–2012. JNCI Cancer Spectr 2019;3:pkz007.
23. Williams LA, Hubbard AK, Scheurer ME, Spector LG, Poynter JN. Trends in pediatric central nervous system tumor incidence by global region from 1988 to 2012. Int J Epidemiol 2020;dyaa176.
24. Williams LA, Richardson M, Marcotte EL, Poynter JN, Spector LG. Sex ratio among childhood cancers by single year of age. Pediatr Blood Cancer 2019;66: e27620.
25. Kehm RD, Spector LG, Poynter JN, Vock DM, Osypuck TL. Socioeconomic status and childhood cancer incidence: a population-based multilevel analysis. Am J Epidemiol 2018;187:982–91.
26. Steliarova-Foucher E, Stiller C, Lacour B, Kaatsch P. International classification of childhood cancer, third edition. Cancer 2005;103:1457–67.
27. O'Neill KA, Murphy MFG, Bunch KJ, Puumala SE, Carozza SE, Chow EJ, et al. Infant birthweight and risk of childhood cancer: international population-based case control studies of 40 000 cases. Int J Epidemiol 2015;44: 153–68.
28. Bjørge T, Sørensen HT, Grotmol T, Engeland A, Stephansson O, Gissler M, et al. Fetal growth and childhood cancer: a population-based study. Pediatrics 2013; 132:e1265–75.
29. Johnson KJ, Carozza SE, Chow EJ, Fox EE, Horel S, McLaughlin CC, et al. Parental age and risk of childhood cancer: a pooled analysis. Epidemiology 2009; 20:475–83.
30. Chow EJ, Puumala SE, Mueller BA, Carozza SE, Fox EE, Horel S, et al. Childhood cancer in relation to parental race and ethnicity: a 5-state pooled analysis. Cancer 2010;116:3045–53.
31. Werner H, Daltro P, Davaus T, Araujo Júnior E. Fetal neuroblastoma: ultrasonography and magnetic resonance imaging findings in the prenatal and postnatal IV-S stage. Obstet Gynecol Sci 2016;59:407–10.
32. Martino F, Avila LF, Encinas JL, Luis AL, Olivares P, Lassaletta L, et al. Teratomas of the neck and mediastinum in children. Pediatr Surg Int 2006; 22:627–34.
33. Marcotte EL, Ritz B, Cockburn M, Clarke CA, Heck JE. Birth characteristics and risk of lymphoma in young children. Cancer Epidemiol 2014;38:48–55.
34. Adami J, Glimelius B, Cnattingius S, Ekbom A, Hoar Zahm S, Linet M, et al. Maternal and perinatal factors associated with Non-Hodgkin's lymphoma among children. Int J Cancer 1996;65:774–7.
35. Granados A, Gebremariam A, Lee JM. Relationship between timing of peak height velocity and pubertal staging in boys and girls. J Clin Res Pediatr Endocrinol 2015;7:235–7.
36. Mirabello L, Pfeiffer R, Murphy G, Daw NC, Patiño-Garcia A, Troisi RJ, et al. Height at diagnosis and birth-weight as risk factors for osteosarcoma. Cancer Causes Control 2011;22:899–908.
37. Gianferante DM, Mirabello L, Savage SA. Germline and somatic genetics of osteosarcoma - Connecting aetiology, biology and therapy. Nat Rev Endocrinol 2017;13:480–91.
38. Cnattingius S, Zack MM, Ekbom A, Gunnarskog J, Linet M, Adami HO, et al. Prenatal and neonatal risk factors for childhood myeloid leukemia. Cancer Epidemiol Biomarkers Prev 1995;4:441–5.
39. Wang R, Wiemels JL, Metayer C, Morimoto L, Francis SS, Kadan-Lottick N, et al. Cesarean section and risk of childhood acute lymphoblastic leukemia in a population-based, record-linkage study in California. Am J Epidemiol 2017; 185:96–105.
40. Greenbaum S, Sheiner E, Wainstock T, Segal I, Ben-Harush M, Sergienko R, et al. Cesarean delivery and childhood malignancies: a single-center, population-based cohort study. J Pediatr 2018;197:292–6.
41. Thomopoulos TP, Skalkidou A, Dessypris N, Chrousos G, Karalexi MA, Karavasilis TG, et al. Prelabor cesarean delivery and early-onset acute childhood leukemia risk. Eur J Cancer Prev 2016;25:155–61.
42. Marcotte EL, Richardson MR, Roesler MA, Spector LG. Cesarean delivery and risk of infant Leukemia: a report from the children's oncology group. Cancer Epidemiol Biomarkers Prev 2018;27:473–8.
43. Sevelsted A, Stokholm J, Bønnelykke K, Bisgaard H. Cesarean section and chronic immune disorders. Pediatrics 2015;135:e92–8.
44. Williams LA, Richardson M, Kehm RD, McLaughlin CC, Mueller BA, Chow EJ, et al. The association between sex and most childhood cancers is not mediated by birthweight. Cancer Epidemiol 2018;57:7–12.
45. Reynolds P, Urayama KY, Von BJ, Feusner J. Birth characteristics and hepatoblastoma risk in young children. Cancer 2004;100:1070–6.
46. Lupo PJ, Schraw JM, Desrosiers TA, Nembhard WN, Langlois PH, Canfield MA, et al. Association between birth defects and cancer risk among children and adolescents in a population-based assessment of 10 million live births. JAMA Oncol 2019;5:1150–8.
47. Walsh CA, MacTiernan A, Farrell S, Mulcahy C, McMahon CJ, Franklin O, et al. Mode of delivery in pregnancies complicated by major fetal congenital heart disease: a retrospective cohort study. J Perinatol 2014;34:901–5.
48. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol 201616:626–38.
49. Søegaard SH, Rostgaard K, Skogstrand K, Wiemels JL, Schmiegelow K, Hjalgrim H. Neonatal inflammatory markers are associated with childhood B-cell precursor acute lymphoblastic leukemia. Cancer Res 2018;78:5458–63.
50. Vicente-Dueñas C, Janssen S, Oldenburg M, Auer F, González-Herrero I, Casado-García A, et al. An intact gut microbiome protects genetically predisposed mice against leukemia. Blood 2020;136:2003–17.
51. Shu XO, Linet MS, Steinbuch M, Wen WQ, Buckley JD, Neglia JP, et al. Breastfeeding and risk of childhood acute leukemia. JNCI J Natl Cancer Inst 1999;91: 1765–72.
52. Prior E, Santhakumaran S, Gale C, Philipps LH, Modi N, Hyde MJ. Breastfeeding after cesarean delivery: a systematic review and meta-analysis of world literature. Am J Clin Nutr 2012;95:1113–35.
53. Williams LA, Yang JJ, Hirsch BA, Marcotte EL, Spector LG. Is there etiologic heterogeneity between subtypes of childhood acute lymphoblastic leukemia? A review of variation in risk by subtype. Cancer Epidemiol Biomarkers Prev 2019; 28:846–56.