Perinatal factors associated with clinical presentation of osteosarcoma in children and adolescents

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ABBREVIATIONS:

CCR	California Cancer Registry
CDPH	California Department of Public Health
ICCC-3	International Classification of Childhood Cancer, Third Edition
ICD-O-3	International Classification of Disease for Oncology, Third Edition
SES	Socioeconomic Status
SEER	Surveillance, Epidemiology, and End Results Program
POBW	Proportion of Optimal Birthweight

ABSTRACT:

Background: Osteosarcoma typically develops during puberty with tumors arising at sites of rapid bone growth, suggesting a role for growth-regulating pathways in tumor etiology. Birthweight is one measure of perinatal growth that has been investigated as an osteosarcoma risk factor. Whether birthweight affects clinical features of osteosarcoma remains unexplored.

Methods: 670 patients with osteosarcoma, ages 0-19 years, were identified through the California Cancer Registry. We analyzed birth certificate data from the California Department of Public Health vital statistics unit for these patients and 2,860 controls, matched by sex, birth-year, and race/ethnicity. We examined the impact of birthweight on the risk, timing, and clinical presentation of pediatric osteosarcoma, including: tumor location, size, extension, differentiation, presence of metastasis, and age-at-onset. Regression models were adjusted for race, sex, gestational age, socio-economic status, and tumor site.

Results: Higher birthweight was associated with more advanced tumor stage (P=0.017), a trend toward greater tumor extension into surrounding tissues (P=0.083), and with occurrence of tumors in sites other than the long bones of the arms/legs ($P=9.7x10^{-3}$). Higher birthweight was also associated with an increased likelihood of metastases present at diagnosis (P=0.047), with each 200g increase in birthweight associated with a 1.11-fold increase in the odds of having metastatic disease (95% CI: 1.01-1.22).

Conclusions: The association between higher birthweight and more aggressive osteosarcoma, frequently occurring at sites other than the long bones, suggests that growth pathways active during gestation may play an important role in future osteosarcoma progression, especially at anatomic sites with diminished rates of osteoblastic proliferation.

INTRODUCTION:

Osteosarcoma is the most prevalent pediatric primary bone malignancy, with roughly seven new cases per million diagnosed each year in the US[1]. This aggressive tumor occurs most frequently in children and adolescents around the time of puberty, after which the risk declines markedly[2]. Osteosarcoma arises most often at sites of rapid bone growth, such as the metaphysis of long bones. This suggests that rapid proliferation of osteoblastic cells near bone growth plates is associated with carcinogenesis, presumably through increased propensity of multiplying osteoblasts to acquire mutations leading to malignant transformation. Additionally, the incidence of osteosarcoma is higher in males than females, possibly related to increased duration and rate of bone growth during male puberty[1,3,4]. In females, osteosarcoma diagnosis tends to occur at a younger age, corresponding with an earlier pubertal growth spurt relative to males[5,6]. Because peak osteosarcoma incidence parallels pubertal growth acceleration in both males and females, when growth hormone levels are highest, it seems biologically plausible that pathways activated during bone growth also contribute to development and severity of osteosarcoma.

A subset of pediatric osteosarcoma is attributable to radiation therapy and heritable cancer predisposition syndromes (*e.g.* Li-Fraumeni syndrome, Diamond-Blackfan anemia, Rothmund-Thompson syndrome, and hereditary retinoblastoma)[7-10]. However, most cases appear sporadic. Research exploring additional risk factors for pediatric osteosarcoma has been limited[11]; however, male sex and above-average stature are two factors consistently linked to increased osteosarcoma risk[1,2]. Taller stature is a confirmed osteosarcoma risk factor in both males and females, likely related to prolonged growth of long bones in taller individuals[2,12-15].

Birthweight is an additional factor undergoing investigation in epidemiologic studies of osteosarcoma[2,6,16]. Higher birthweight is indicative of increased fetal growth *in utero*, and potentially, greater exposure to insulin-like growth factors (IGFs). High birthweight has previously been linked to increased risk of a variety of childhood cancers, including: acute lymphoblastic leukemia, Wilm's tumor, neuroblastoma, non-Hodgkin's lymphoma, soft tissue sarcomas, and germ cell tumors[17-22]. However, relationships between birthweight and osteosarcoma risk remain poorly understood.

Because osteosarcoma onset closely mirrors the timing of puberty in males and females, clinical presentation, such as age at diagnosis, may be influenced by measures of childhood growth. Few studies have examined potential relationships between birthweight and the timing of osteosarcoma development. Patient age at diagnosis is an important prognostic indicator for osteosarcoma, as increasing patient age is associated with poorer survival and increased risk of metastatic disease at initial diagnosis[23,24]. Whether perinatal factors, including birthweight, influence osteosarcoma timing and clinical presentation remains unexplored but could provide further insight into the pathogenesis, progression, and prognosis of this disease.

In the current analysis, we explore how birthweight and other perinatal factors influence osteosarcoma risk and clinical presentation. Although several studies have examined whether birthweight is associated with osteosarcoma incidence in case-control comparisons, such studies have not explored how birthweight influences patients' clinical presentation in case-only analyses. In addition to case-control comparisons, we investigate whether birthweight is associated with tumor location, size, extension, differentiation, presence of metastasis, and age-at-onset using a large, population-based cohort of Californian children (N=670 osteosarcoma cases, 2,860 controls). While past osteosarcoma research has primarily focused on non-Hispanic

white populations, this multi-ethnic cohort of Californian children enables examination of birth characteristics in populations frequently omitted from clinical research.

METHODS:

Study Population

A population-based case-control analysis of pediatric osteosarcoma was conducted using data from the California Cancer Registry (CCR) linked with birth certificate data from the California Department of Public Health's (CDPH) vital statistics unit. The investigated cohort included 670 unique patients with diagnosis of osteosarcoma and 2,860 matched controls, born in California between 1978 and 2005. Cases were identified through the CCR via International Classification of Childhood Cancer, 3rd edition (ICCC-3) ICD-O-3 codes 9180-9183, 9185-9187, and 9192-9195 and diagnosed with osteosarcoma between ages 0-19 years. Each patient was matched to four controls based on sex, birth-year, and self-report of race/ethnicity obtained through record-linkage between the CCR and vital statistics unit of CDPH. Controls were cancer-free at age 19 or the year 2011, whichever came first. This study was approved by Institutional Review Boards at the University of California, Berkeley and the California Department of Public Health.

Data Collection

California Cancer Registry data included age-at-diagnosis, tumor characteristics (*e.g.* tumor site, histological subtype, lateralization, stage, differentiation, size, tumor extension, lymph node involvement, metastasis) and sociodemographic data, including an index of socioeconomic status (SES) based on a principal components analysis[25]. Data obtained from

147 the CDPH for both patients and controls provided information on sex, birth-year, child
148 race/ethnicity, parental race/ethnicity, parental ages and years of education at birth, birthweight,
149 gestational age, plurality, mode of delivery, number of older siblings, prenatal care start date,
150 pregnancy complications, labor complications, congenital anomalies, and expected methods of
151 payment for prenatal care and delivery.

152 Codes for CCR data were obtained through the California Cancer Registry Data
153 Dictionary website and the National Cancer Institute Surveillance, Epidemiology, and End
154 Results Program Coding and Staging Manual, 2015. Histological subtypes of osteosarcoma were
155 classified via ICD-O-3 codes as osteosarcoma, not otherwise specified (9180), chondroblastic
156 osteosarcoma (9181), fibroblastic osteosarcoma (9182), telangiectatic osteosarcoma (9183),
157 small cell osteosarcoma (9185), central osteosarcoma (9186), intraosseous, well-differentiated
158 osteosarcoma (9187), parosteal osteosarcoma (9192), periosteal osteosarcoma (9193), and high-
159 grade surface osteosarcoma (9194). Cancer site was grouped into four categories including long
160 bone upper limb (C400), long bone lower limb (C402), craniofacial bones including skull and
161 mandible (C410-C411), and other (C401, C403, C409, C412-C414, C418-C419). Tumor stage
162 was defined using the SEER Summary Stage at Diagnosis variable, a derived combination of the
163 various SEER stage variables used between 1988 and 2012. The presence of metastasis was
164 defined using the combined TMN and AJCC general and site-specific metastasis variables from
165 the CCR.

166 For all patients with osteosarcoma, data on race and ethnicity was combined into a single
167 category derived from the California Cancer Registry variable ‘RACE08’, which describes the
168 race/ethnicity of the patient, including Hispanic origin. Classifications include: non-Hispanic
169 white, non-Hispanic black, Hispanic, Asian/Pacific Islander (includes Native Hawaiian), and

Other/Unknown (includes American Indian/Alaska Native and Middle Eastern origins). For controls, birth certificate data was used to re-code and combine race/ethnicity into these same five classifications.

Gestational age and labor/pregnancy complication data were obtained from the California Birth Registry for both cases and controls. Gestational age was computed from the interval between the date of mother's last menstrual period and the delivery date. Pregnancy complications were grouped into the following categories: Prenatal Complications, Pre-eclampsia/Eclampsia/Seizures, Diabetes, Blood Disorders, Infections, Non-infectious Comorbidities (cardiac, vascular, renal, or pulmonary disease), Substance Abuse, Invasive Procedures, Previous Infant with Low/High Birthweight (<2500g or >4000g), Previous Premature Birth (<37 weeks), Other (previous caesarean section, electronic fetal monitoring, tocolysis), and Unknown. Labor complications were categorized as Maternal Distress, Fetal Distress, Breech/Abnormal Positioning, Abnormal Delivery, Placental/Vascular Anomalies, Infections, Invasive Procedures, Other (electronic fetal monitoring, tocolysis, ultrasound) and Unknown. Congenital anomalies were coded as present or absent and included: Anencephalus/Spina Bifida, Congenital Hydrocephalus, Other CNS Anomalies, Eyes, Cleft lip/Palate, Other Ear/Face/Neck, Cardiovascular, Respiratory, Digestive, Genito-Urinary, Musculoskeletal, Skin/Hair/Nails, Down Syndrome, and Other.

Statistical Analyses

Statistical analyses included both univariate and multivariate modeling conducted in SAS v9.1.3 (Cary, NC). Whenever possible, continuous or ordinal variables were modeled without categorization into discrete groups. For associations between perinatal factors and clinical variables (*e.g.* metastatic disease, age-at-diagnosis), regression models were adjusted for race,

sex, birth-year, SES, and tumor site (long bones versus other). Maternal age, birth order, plurality, and start of prenatal care were assessed as potential confounders, but did not change effect estimates more than 10%. When child's birthweight was modeled as the exposure of interest, regression models were additionally adjusted for gestational age. We estimate that we have 80% power to detect a difference in birthweight of 68g between osteosarcoma cases and controls, at an alpha level of 0.05. In case-only analyses, we have 80% power to detect a difference in birthweight of 198g between patients with metastatic disease and patients without, at an alpha level of 0.05.

In case-control analyses, multivariable logistic regression was performed. Maternal age, birth order, plurality, and start of prenatal care were assessed as potential confounders but did not change risk estimates more than 10%. No adjustment was made for ethnicity, sex, or birth-year (matching variables), but regression models were adjusted for gestational age when child's birthweight was the exposure of interest.

RESULTS:

The birth characteristics of 670 patients with osteosarcoma and 2,860 matched controls appear in **Supplemental Table S1**. A total of 666 patients had histologically-confirmed osteosarcoma, two had cytologically-confirmed osteosarcoma, and two had radiographically-diagnosed osteosarcoma. Five patients had multiple diagnoses of a primary osteosarcoma, while the remaining 665 patients had a single recorded primary tumor. Cases were 41% non-Hispanic white, 39% Hispanic, 11% non-Hispanic black, and 9% Asian/Pacific Islander. In line with previous reports, patients with osteosarcoma were more likely to be male than female (56% vs.

44%). Associations between case-control status and sex, birth-year, and maternal race/ethnicity were not calculated because these were matching variables used for control selection.

Table 1 summarizes clinical features of cases, including: age at diagnosis, tumor location, and measures of disease severity, as well as the relationship between these variables and child's birthweight. All measures of effect are calculated for a 200 gram increase in birthweight. In general, patients with higher birthweight tended to have more aggressive disease. After adjustment for child's sex, race, gestational age, SES, and tumor location, higher birthweight was statistically significantly associated with more advanced tumor stage (P=0.017). For each 200g increase in birthweight, there was a 1.14-fold increase in odds of having distant/remote tumor extension, versus presenting with localized disease (OR=1.14; 95%CI=1.02, 1.27) (**Table 1**). Higher birthweight was also associated with an increased risk of metastatic disease (P=0.047). Each 200g increase in birthweight was associated with a 1.11-fold increase in the odds of a patient with osteosarcoma presenting with metastatic disease, after adjustment for race, sex, gestational age, tumor site, and SES (95%CI=1.01-1.22).

Tumor location was also associated with birthweight. Tumor location was classified as long bone (including upper and lower extremity), craniofacial bones (including skull, face and mandible), and other (including short bones of the upper and lower extremity, vertebral column, ribs, sternum, clavicle, pelvis, sacrum, and coccyx). Patients with higher birthweight were more likely to have tumors in craniofacial bones and "other" sites than in long bones (P_{ANOVA}=0.035). When comparing osteosarcoma of the long bones to all other sites, a 200g increase in birthweight was associated with a statistically significant reduction in risk of having a tumor in a long bone (OR=0.86; 95%CI=0.77-0.97; P=$9.7x10^{-3}$). Patients with osteosarcoma of the long bones were also significantly younger than patients with tumors in other sites (12.46 years versus 13.90

years, P=0.0036 after adjusting for sex). These observations suggest that factors associated with increased *in utero* growth may have a greater impact on osteosarcoma development in sites with diminished rates of osteoblastic proliferation and during later ages (**Table 1**).

Each 200g increase in birthweight was associated with a 2.88mm increase in tumor size and a 0.74 month older age-at-diagnosis, although neither of these associations reached statistical significance (P=0.16 and P=0.29, respectively). Higher birthweight demonstrated a suggestive association with greater tumor extension after adjustment for sex, race, tumor site, gestational age, and SES (P=0.083). Gestational age was not statistically significantly associated with any clinical features of cases (P>0.05), although there was a suggestive association between older gestational age and reduced tumor stage (P=0.11; **Supplemental Table S2**).

No birth characteristics were associated with osteosarcoma case-control status at P<0.05 in our data (**Supplemental Table S1**). In general, mothers of children with osteosarcoma were slightly older than control mothers (P=0.65), and cases had somewhat fewer birth and pregnancy complications than controls (P=0.16 and 0.053, respectively). Higher birthweight was not significantly associated with osteosarcoma risk in case-control comparisons. For each 200g increase in birthweight, there was a 1.01-fold increase in odds of osteosarcoma, after adjustment for gestational age (P=0.99) (**Supplemental Table S3**). Associations between osteosarcoma risk and gestational age were similarly null (P=0.67) (**Supplemental Table S3**).

DISCUSSION:

Our analyses shed new light on the relationship between infant birthweight and disease severity in patients with osteosarcoma. Higher birthweight was associated with more advanced tumor stage and greater risk for metastatic disease at diagnosis, even after adjustment for tumor

site, gestational age, sex, race and SES. We also observed a suggestive association between higher birthweight and increased tumor extension, indicative of more advanced disease. Furthermore, patients with high birthweight were likelier to have tumors in short bones of upper and lower extremities (*e.g.* carpals, metacarpals, metatarsals, phalanges), vertebral column, ribs, sternum, clavicle, and pelvis than in the long bones of the arms or legs (*i.e.* humerus, radius, ulna, femur, fibula, tibia). This suggests that factors associated with accelerated *in utero* growth, including levels of circulating growth factors, may disproportionately influence osteosarcoma development at anatomic sites with lower rates of osteoblastic proliferation. Furthermore, because patients with osteosarcoma of the long bones develop tumors at significantly younger ages than patients with tumors at other anatomic sites, birthweight may also impact the timing of tumor formation.

Infant birthweight is determined by a variety of factors, including: maternal weight gain, height, parity, nutritional status, comorbid illnesses, and overall health. Variation in fetal growth according to these factors poses a challenge when comparing infant birthweights. Although we adjusted for infant race, sex, SES and gestational age in our case-only analyses, our dataset did not permit inclusion of other important maternal health parameters. A measure that accounts for the influence of gestational duration, fetal sex, maternal height, age and parity, such as proportion of optimal birthweight (POBW), may improve future analyses[26]. Due to limitations of data collected from California birth records, specifically lack of maternal and paternal height and weight, POBW could not be incorporated into current analyses.

Recent studies suggest the involvement of insulin-like growth factors (IGFs) in osteosarcoma development[2,4,6]. Greater exposure to IGFs during critical growth periods *in utero* may contribute to increased birthweight and also to more aggressive potential of

284 osteosarcoma tumors. Indeed, circulating levels of IGF1 and IGF2 are highest in the cord blood
285 of infants with higher birthweights [27,28] and play key roles in promoting osteoblast
286 differentiation and proliferation[29]. Osteosarcoma tumors have been shown to express high
287 levels of IGF1 and IGF2, suggesting potential involvement of IGF signaling in osteosarcoma
288 progression[30]. Furthermore, IGF1 can act as an osteoblast chemotactic factor *in vivo* [29] and
289 as a mitogen for osteosarcoma growth *in vitro* [31]. Dependence on IGF signaling for tumor
290 growth may underlie the findings that both high birthweight and greater *in utero* IGF exposure
291 are associated with more aggressive osteosarcoma. Further analyses of IGF levels which account
292 for birthweight are necessary to elucidate this potential relationship.

293 We did not find an association between birthweight and osteosarcoma risk, consistent
294 with several previous analyses[15,22,32,33]. The most recent of these studies evaluated 251
295 patients and 53,716 controls in the US and 390 patients and 593 controls in the UK and found no
296 significant increase in osteosarcoma risk with each 0.5 kilogram increase in birthweight (OR:
297 1.02 and 1.04, respectively)[22]. The lack of convincing evidence surrounding birthweight and
298 osteosarcoma risk may reflect the complexity of fetal and childhood growth trajectories. A study
299 from the Netherlands (N=5,431) observed that weight and height measurements within the first
300 year of life had the largest impact on total bone mass in later life, and that infants with decreased
301 fetal growth and low birthweight underwent compensatory accelerated growth in the first two
302 years of life[34]. This accelerated growth during infancy may play a significant role in
303 osteosarcoma risk, further complicating analyses of growth trajectories and pediatric cancer risk.

304 A key shortcoming of our analyses is the limitation to birth certificate data provided by
305 the California Department of Public Health's (CDPH) vital statistics unit. Validity of these data
306 is known to vary, and the information recorded differs from year to year. Additionally, the

appropriateness of birthweight as a measure of fetal growth remains unclear. Although our sample size is relatively small compared with analyses of adult solid tumors or pediatric hematological malignancies, it is quite large relative to other studies of sarcomas in children. These analyses provide an enhanced understanding of the factors underlying both pediatric osteosarcoma risk and clinical presentation.

This study provides new insight into the effect of fetal growth on osteosarcoma pathogenesis, suggesting that patients with osteosarcoma who were large at birth may be at risk for more aggressive disease and more refractory subtypes. The strengths of our analysis include a large multi-ethnic population-based cohort, near census-level ascertainment of osteosarcoma patients, and matching of cases and controls based on child's birth-year, sex, and maternal race/ethnicity. Studies in ethnically diverse populations are crucial for revealing the breadth of genetic and environmental factors that may impact osteosarcoma risk and severity. This knowledge can improve cancer risk stratification and enhance targeted therapy, thereby benefitting the overall health and prognosis of patients with osteosarcoma. Additional analyses integrating genomic markers and ancestry data can complement these analyses of birth characteristics and shed further light on the interaction between germline genetics, childhood growth trajectories, and the etiology of osteosarcoma in children and adolescents.

CONFLICT OF INTEREST DISCLOSURE:

The authors declare no conflicts of interest.

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SUPPLEMENTAL TABLE LEGENDS:

Supplemental Table S1: Risk of osteosarcoma associated with birth characteristics among 670 cases and 2680 controls in California, born 1978-2005

Supplemental Table S2: Associations between gestational age and clinical presentation of childhood osteosarcoma in Californian children born 1978-2005

Supplemental Table S3: Risk of osteosarcoma associated with gestational age and birth weight for 670 cases and 2680 controls in California, born 1978-2005

TABLE 1:

Associations between birthweight and clinical presentation of childhood osteosarcoma in Californian children, born 1978-2005.

Clinical Variable	Definition	Mean Birthweight in kg (N)	Effect estimate (95% CI)[1] per 200g increase in birthweight	P-value
Age at Diagnosis[2]	0-4 years	3.21 (8)		
	5-9 years	3.36 (133)		
	10-14 years	3.35 (299)		
	15-19 years	3.50 (230)		
			0.74 months (-0.65, 2.1)	0.29
Tumor Site[3]	Long bone upper limb	3.34 (83)		
	Long bone lower limb	3.39 (516)		
	Craniofacial bones	3.60 (24)		
	Other	3.57 (47)		
	Long bone vs any other site		OR=0.86 (0.77, 0.97)	9.7×10^{-3}
Tumor Histology[4]	Osteosarcoma, NOS	3.41 (467)		
	Chondroblastic osteosarcoma	3.36 (89)		
	Fibroblastic osteosarcoma	3.55 (33)		
	Telangiectatic osteosarcoma	3.39 (33)		
	Smal cell osteosarcoma	3.10 (8)		
	Central osteosarcoma	3.38 (9)		
	Intraosseous well-differentiated osteosarcoma	3.31 (2)		
	Parosteal osteosarcoma	3.34 (23)		
	Periosteal osteosarcoma	3.69 (5)		
	High grade surface osteosarcoma	4.52 (1)		
				0.45
Tumor Lateralization[5]	Right	3.37 (309)	OR=1.0 (Ref)	
	Left	3.41 (328)	OR=1.02 (0.95, 1.10)	
	Not a paired site	3.60 (33)	-	
				0.53
Tumor Stage[6]	Localized	3.38 (197)	OR=1.0 (Ref)	
	Regional Extension	3.37 (337)	OR=1.04 (0.96, 1.13)	
	Distant/Remote	3.54 (122)	OR=1.14 (1.02, 1.27)	
	Unknown/Missing	3.46 (14)	-	
	P(trend)			0.017

Table 1, continued.

Clinical Variable	Definition	Mean Birthweight in kg (N)	Effect estimate (95% CI)[1] per 200g increase in birthweight	P-value
Tumor Differentiation[7]	Grade I - Well Differentiated	3.50 (14)	OR=0.89 (0.68, 1.18)	
	Grade II - Moderately Differentiated	3.37 (33)	OR=0.95 (0.79, 1.13)	
	Grade III - Poorly Differentiated	3.41 (95)	OR=0.94 (0.85, 1.05)	
	Grade IV - Undifferentiated	3.43 (370)	OR=1.0 (Ref)	
	Grade/Differentiation Not Determined	3.35 (158)	-	
	P(trend)			0.31
Tumor Size[2]	<5cm	3.49 (54)		
	5-10cm	3.30 (199)		
	10-15cm	3.46 (140)		
	≥15cm	3.48 (69)		
	Unknown/Missing	3.42 (208)		
			2.38mm (-0.92, 5.68)	0.16
Tumor Extension[8]	Localized	3.37 (204)	OR=1.0 (Ref)	
	Extension beyond periosteum to surrounding tissues	3.39 (332)	OR=1.06 (0.98, 1.14)	
	Adjacent bone/cartilage	3.54 (21)	OR=1.21 (0.98, 1.50)	
	Further contiguous extension	3.42 (8)	OR=1.15 (0.74, 1.78)	
	Discontinuous tumors in primary site	3.52 (69)	OR=1.10 (0.97, 1.24)	
	Unknown/Missing	3.42 (36)	-	
	P(trend)			0.083
Metastasis[9]	No metastases present	3.38 (363)	OR=1.0 (Ref)	
	Distant metastasis present	3.54 (79)	OR=1.11 (1.01, 1.22)	
	Unknown/Missing	3.39 (228)	-	
				0.047
Regional Lymph Nodes[9]	No regional lymph node involvement	3.39 (502)	OR=1.0 (Ref)	
	Regional lymph nodes involved	3.56 (11)	OR=1.05 (0.80, 1.37)	
	Unknown/Missing	3.43 (157)	-	
				0.73
Number of Primary Tumors[9]	Single primary tumor	3.40 (665)	OR=1.0 (Ref)	
	Multiple primary tumors	3.70 (5)	OR=1.23 (0.84, 1.80)	
				0.28

[1]All effect estimates are calculated for a 200g increase in birthweight. Statistical model applied is indicated in footnotes.
[2]Multivariable linear regression, adjusted for: race, sex, gestational age, tumor site, SES
[3]Multivariable logistic regression comparing "long bone" to "all other tumor sites", adjusted for: race, sex, gestational age, SES
[4]ANOVA

[5]Multivariable logistic regression comparing "left side" to "right side", adjusted for: race, sex, gestational age, tumor site, SES
[6]Multivariable linear regression adjusted for: race, sex, gestational age, tumor site, SES. Odds ratios correspond to the increase in tumor stage at diagnosis associated with a 200g increase in birthweight.
[7]Multivariable linear regression adjusted for: race, sex, gestational age, tumor site, SES. Odds ratios correspond to the increase in tumor grade at diagnosis associated with a 200g increase in birthweight.
[8]Multivariable linear regression adjusted for: race, sex, gestational age, tumor site, SES. Odds ratios correspond to the increase in tumor extension at diagnosis associated with a 200g increase in birthweight.
[9]Multivariable logistic regression, adjusted for: race, sex, gestational age, tumor site, SES

Supplemental Table S1

Risk of osteosarcoma associated with birth characteristics among 670 cases and 2680 controls in California, born 1978-2005

Variable	Definition	Cases n (%)	Controls n (%)	OR (95% CI)	P-value
CHILD'S BIRTH YEAR	1978-1982	136 (20)	544 (20)	NA[1]	NA[1]
	1983-1987	162 (24)	648 (24)	NA[1]	NA[1]
	1988-1992	194 (29)	776 (29)	NA[1]	NA[1]
	1993-1997	124 (19)	496 (19)	NA[1]	NA[1]
	1998-2005	54 (8)	216 (8)	NA[1]	NA[1]
CHILD'S RACE	Non-Hispanic White	274 (41)	977 (36)	NA[1]	NA[1]
	Non-Hispanic Black	71 (11)	308 (11)	NA[1]	NA[1]
	Hispanic	260 (39)	1104 (41)	NA[1]	NA[1]
	Asian/Pacific Islander	58 (9)	257 (10)	NA[1]	NA[1]
	Other/Unknown	7 (1)	34 (1)	NA[1]	NA[1]
CHILD'S SEX	Male	377 (56)	1508 (56)	NA[1]	NA[1]
	Female	293 (44)	1172 (44)	NA[1]	NA[1]
MOTHER'S AGE AT DELIVERY[2]	<20 years	65 (10)	328 (12)		
	20-29	395 (59)	1552 (58)		
	30-39	198 (30)	753 (28)		
	40+	12 (2)	47 (2)		
				1.03 (0.89, 1.20)	0.65
FATHER'S AGE AT DELIVERY[2]	<20 years	16 (2)	124 (5)		
	20-29	330 (49)	1276 (48)		
	30-39	247 (37)	953 (36)		
	40+	52 (8)	181 (7)		
	Missing	25 (4)	146 (5)		
				0.96 (0.92, 1.02)	0.22
MOTHER'S EDUCATION STATUS AT DELIVERY	High School or less	236 (35)	921 (34)	1.00 (Ref)	
	At least some college	125 (19)	513 (19)	0.97 (0.81, 1.17)	0.79
	Missing	309 (46)	1246 (46)		
FATHER'S EDUCATION STATUS AT DELIVERY	High School or less	201 (30)	825 (31)	1.00 (Ref)	
	At least some college	141 (21)	522 (20)	1.07 (0.90, 1.28)	0.45
	Missing	328 (49)	1333 (50)		
SOURCE OF PAYMENT FOR DELIVERY	Private	187 (28)	663 (25)	1.00 (Ref)	
	Medicaid	139 (21)	604 (23)	0.82 (0.64, 1.04)	0.10
	Self-Pay	11 (2)	45 (2)	0.87 (0.44, 1.71)	0.67
	Other	3 (0)	50 (2)	0.21 (0.07, 0.69)	0.01
	Missing	330 (49)	1318 (49)		
START OF PRENATAL CARE (TRIMESTERS)	No prenatal care	7 (1)	28 (1)	0.98 (0.42, 2.25)	0.96
	First trimester	513 (77)	2005 (75)	1.00 (Ref)	
	Second trimester	122 (18)	501 (19)	0.95 (0.76, 1.19)	0.66
	Third trimester	17 (3)	99 (4)	0.67 (0.40, 1.13)	0.14
	Missing	11 (2)	47 (2)		
NUMBER OF PRENATAL CARE VISITS[3]	No prenatal care	4 (1)	16 (1)	0.96 (0.32, 2.92)	
	Fewer than 5 visits	11 (2)	69 (3)	0.61 (0.31, 1.19)	
	5-10 visits	135 (20)	518 (19)	1.00 (Ref)	
	11-15 visits	170 (25)	676 (25)	0.96 (0.75, 1.24)	
	Over 15 visits	34 (5)	138 (5)	0.95 (0.62, 1.44)	
	Missing	317 (47)	1263 (47)		
					0.69
MODE OF DELIVERY	Vaginal birth	266 (40)	1056 (39)	1.00 (Ref)	
	Cesarean Section	75 (11)	308 (11)	0.97 (0.73, 1.29)	0.82
	Missing	329 (49)	1316 (49)		
PLURALITY	Singleton	656 (98)	2621 (98)	1.00 (Ref)	
	Multiple Birth	14 (2)	59 (2)	0.95 (0.53, 1.71)	0.86
NUMBER OF SIBLINGS[3]	No siblings	271 (40)	1091 (41)		
	1 sibling	224 (33)	843 (31)		
	2 siblings	96 (14)	444 (17)		
	3 siblings	47 (7)	169 (6)		
	4+ siblings	32 (5)	133 (5)		
				0.99 (0.93, 1.06)	0.78
LABOR COMPLICATIONS[3]	No complications	387 (58)	1449 (54)	1.00 (Ref)	
	Single complication	177 (26)	785 (29)	0.84 (0.69, 1.03)	
	Multiple complications	88 (13)	368 (14)	0.90 (0.69, 1.16)	
	Missing	18 (3)	78 (3)		
					0.16
PREGNANCY COMPLICATIONS[3]	No complications	465 (69)	1738 (65)	1.00 (Ref)	
	Single complication	149 (22)	684 (26)	0.81 (0.66, 0.99)	
	Multiple complications	39 (6)	180 (7)	0.81 (0.56, 1.16)	
	Missing	17 (3)	78 (3)		
					0.053
CONGENITAL ANOMALIES	No congenital anomalies	632 (94)	2531 (94)	1.00 (Ref)	
	Congenital anomalies present	19 (3)	73 (3)	1.04 (0.65, 1.74)	
	Missing	19 (3)	76 (3)		

[1]Matching variable for cases and controls.
[2]Odds ratios correspond to a 10 year increase in parental age.
[3]P-value for linear trend.

Supplemental Table S2

Associations between gestational age and clinical presentation of childhood osteosarcoma in Californian children born 1978-2005

Variable	Definition	Mean gestational age in days (N)	Effect estimate (95% CI)[1] per 30-day increase in gestational age	P-value
AGE AT DIAGNOSIS[2]	0-4 years	275 (8)		
	5-9 years	276 (133)		
	10-14 years	277 (299)		
	15-19 years	280 (230)		
			3.9 months (-2.7, 10.5)	0.25
TUMOR SITE[3]	Long bone upper limb	276 (83)		
	Long bone lower limb	278 (516)		
	Craniofacial bones: Skull, face, mandible	273 (24)		
	Other	282 (47)		
	Long bone vs any other site		OR=1.21 (0.66, 2.20)	0.53
TUMOR LATERALIZATION[4]				
	Right	277 (309)	OR=1.0 (Ref)	
	Left	278 (328)	OR=1.05 (0.75, 1.46)	
	Not a paired site	277 (33)	-	
				0.79
TUMOR STAGE[5]	Localized	280 (197)	OR=1.0 (Ref)	
	Regional Extension	276 (337)	OR=0.62 (0.42, 0.93)	
	Distant/Remote	280 (122)	OR=0.78 (0.44, 1.37)	
	Unknown/Missing	277 (14)	-	
	P(trend)			0.11
TUMOR DIFFERENTIATION[6]	Grade I - Well Differentiated	279 (14)	OR=1.37 (0.35, 5.36)	
	Grade II - Moderately Differentiated	278 (33)	OR=1.15 (0.50, 2.65)	
	Grade III - Poorly Differentiated	279 (95)	OR=1.16 (0.70, 1.94)	
	Grade IV - Undifferentiated	278 (370)	OR=1.0 (Ref)	
	Grade/Differentiation Not Determined	276 (158)	-	
	P(trend)			0.55
TUMOR SIZE[7]	<5cm	280 (54)		
	5-10cm	276 (199)		
	11-15cm	279 (140)		
	≥15cm	280 (69)		
	Unknown/Missing	277 (208)		
			7.8mm (-23.7, 8.2)	0.34
TUMOR EXTENSION[7]	Localized	279 (204)	OR=1.0 (Ref)	
	Extension beyond periosteum to surrounding tissues	276 (332)	OR=0.62 (0.42, 0.93)	
	Adjacent bone/cartilage	281 (21)	OR=0.91 (0.25, 3.25)	
	Further contiguous extension	266 (8)	OR=0.17 (0.031, 1.07)	
	Discontinuous tumors in the primary site	281 (69)	OR=0.93 (0.46, 1.90)	
	Unknown/Missing	277 (36)	-	
	P(trend)			0.48
METASTASIS[8]	No metastases present	277 (363)	OR=1.0 (Ref)	
	Distant metastasis present	281 (79)	1.22 (0.70-2.21)	
	Unknown/Missing	278 (228)	-	
				0.45
REGIONAL LYMPH NODES[8]	No regional lymph node involvement	278 (502)	OR=1.0 (Ref)	
	Regional lymph nodes involved	285 (11)	3.95 (0.66-23.4)	
	Unknown/Missing	278 (157)	-	
				0.13
NUMBER OF PRIMARY TUMORS[8]	Single primary tumor	278 (665)	OR=1.0 (Ref)	
	Multiple primary tumors	280 (5)	1.06 (0.13-8.44)	
				0.95

[1]All effect estimates are calculated for a 30-day increase in gestational age. Statistical model applied is indicated in footnotes.
[2]Multivariable linear regression, adjusted for: race, sex, birthweight, tumor site, SES
[4]ANOVA
[4]Multivariable logistic regresion comparing "left side" to "right side", adjusted for: race, sex, birthweight, tumor site, SES
[5]Multivariable linear regresion adjusted for: race, sex, birthweight, tumor site, SES. Odds ratios correspond to the increase in tumor stage at diagnosis associated with a 30-day increase in gestational age.
[6]Multivariable linear regresion adjusted for: race, sex, birthweight, tumor site, SES. Odds ratios correspond to the increase in tumor grade at diagnosis associated with a 30-day increase in gestational age.
[7]Multivariable linear regresion adjusted for: race, sex, birthweight, tumor site, SES. Odds ratios correspond to the increase in tumor extension at diagnosis associated with a 30-day increase in gestational age.
[8]Multivariable logistic regresion, adjusted for: race, sex, birthweight, tumor site, SES

Supplemental Table S3

Risk of osteosarcoma associated with gestational age and birth weight for 670 cases and 2680 controls in California, born 1978-2005

Variable	Definition	Cases n (%)	Controls n (%)	OR[-]	(95% CI)
GESTATIONAL AGE (WEEKS)[1]	Preterm (< 37 weeks)	44 (7)	209 (8)	0.88	(0.64-1.21)
	Normal (37-40 weeks)	363 (54)	1433 (53)	1.00	Ref.
	Post-term (> 40 weeks)	237 (35)	922 (34)	1.01	(0.85-1.21)
	P(trend)			0.67	
BIRTH WEIGHT (GRAMS)[2]	Low (<2500g)	31 (5)	145 (5)	0.89	(0.57-1.39)
	Normal (2500-4000g)	570 (85)	2241 (84)	1.00	Ref.
	High (>4000g)	69 (10)	292 (11)	0.96	(0.72-1.27)
	P(trend)			0.99	

[1]Adjusted for birthweight. Cases and controls were matched on race, sex, and birth-year.
[2]Adjusted for gestational age. Cases and controls were matched on race, sex, and birth-year.