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Genetically inferred birthweight, height, and puberty timing and risk of osteosarcoma

D. Matthew Gianferante [a], Amy Moore [a], Logan G. Spector [b], William Wheeler [c], Tianzhong Yang [d], Aubrey Hubbard [a], Richard Gorlick [e], Ana Patiño-Garcia [f], Fernando Lecanda [g], Adrienne M. Flanagan [h,i], Fernanda Amary [i], Irene L. Andrulis [j], Jay S. Wunder [j], David M. Thomas [k,l], Mandy L. Ballinger [k,l], Massimo Serra [m,n], Claudia Hattinger [m,n], Ellen Demerath [o], Will Johnson [p], Brenda M. Birmann [q], Immaculata De Vivo [q,r], Graham Giles [s,t,u], Lauren R. Teras [v], Alan Arslan [w,x], Roel Vermeulen [y,z], Jeannette Sample [b], Neal D. Freedman [a], Wen-Yi Huang [a], Stephen J. Chanock [a], Sharon A. Savage [a], Sonja I. Berndt [a,1], Lisa Mirabello [a,*,1]

[a] Division of Cancer Epidemiology and Genetics, NCI, NIH, Rockville, MD, USA
[b] Department of Pediatrics, University of Minnesota, Minneapolis, MN 55455, USA
[c] Information Management Services, Rockville, MD, USA
[d] Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN, USA
[e] University of Texas MD Anderson Cancer Center, Houston, TX, USA
[f] Department of Pediatrics and Solid Tumor Division CIMA, IdiSNA, Clínica Universidad de Navarra, Pamplona, Spain
[g] Center for Applied Medical Research (CIMA)-University of Navarra, IdiSNA, and CIBERONC, Pamplona, Spain
[h] UCL Cancer Institute, Huntley Street, London WC1E 6BT, UK
[i] Royal National Orthopaedic Hospital NHS Trust, Stanmore, Middlesex HA7 4LP, UK
[j] Litwin Centre for Cancer Genetics, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada
[k] Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
[l] St Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia
[m] Laboratory of Experimental Oncology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy
[n] IRCCS Istituto Ortopedico Rizzoli, Osteoncology, Bone and Soft Tissue Sarcomas and Innovative Therapies, Pharmacogenomics and Pharmacogenetics Research Unit, Bologna, Italy
[o] Division of Epidemiology and Clinical Research, School of Public Health, UMN, USA
[p] School of Sport, Exercise, and Health Sciences, University of Loughborough, UK
[q] Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA
[r] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[s] Cancer Epidemiology Division, Cancer Council Victoria, Melbourne, Victoria, Australia
[t] Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Victoria, Australia
[u] Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, Victoria, Australia
[v] Department of Population Science, American Cancer Society, Atlanta, GA, USA
[w] Department of Obstetrics and Gynecology, New York School of Medicine, New York, NY, USA
[x] Department of Population Health, New York University School of Medicine, New York, NY, USA
[y] Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands
[z] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands

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ABSTRACT

Introduction: Several studies have linked increased risk of osteosarcoma with tall stature, high birthweight, and early puberty, although evidence is inconsistent. We used genetic risk scores (GRS) based on established genetic loci for these traits and evaluated associations between genetically inferred birthweight, height, and puberty timing with osteosarcoma.

* Correspondence to: Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, USA.
E-mail address: mirabellol@nih.gov (L. Mirabello).
[1] These authors contributed equally to this work

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Birthweight
Genetic risk score

Methods: Using genotype data from two genome-wide association studies, totaling 1039 cases and 2923 controls of European ancestry, association analyses were conducted using logistic regression for each study and meta-analyzed to estimate pooled odds ratios (ORs) and 95% confidence intervals (CIs). Subgroup analyses were conducted by case diagnosis age, metastasis status, tumor location, tumor histology, and presence of a known pathogenic variant in a cancer susceptibility gene.
Results: Genetically inferred higher birthweight was associated with an increased risk of osteosarcoma (OR =1.59, 95% CI 1.07–2.38, P = 0.02). This association was strongest in cases without metastatic disease (OR =2.46, 95% CI 1.44–4.19, $P = 9.5 \times 10^{-04}$). Although there was no overall association between osteosarcoma and genetically inferred taller stature (OR=1.06, 95% CI 0.96–1.17, P = 0.28), the GRS for taller stature was associated with an increased risk of osteosarcoma in 154 cases with a known pathogenic cancer susceptibility gene variant (OR=1.29, 95% CI 1.03–1.63, P = 0.03). There were no significant associations between the GRS for puberty timing and osteosarcoma.
Conclusion: A genetic propensity to higher birthweight was associated with increased osteosarcoma risk, suggesting that shared genetic factors or biological pathways that affect birthweight may contribute to osteosarcoma pathogenesis.

1. Introduction

Osteosarcoma is the most common primary bone tumor in children and occurs more commonly in males than in females, with an overall male-to-female incidence rate ratio of 1.43–1 [1–4]. The peak incidence occurs during adolescence [1] and corresponds with the onset of puberty and the adolescent growth spurt with the peak incidence for girls occurring before boys [1,2]. Osteosarcoma survival rates vary by age, presence of metastatic disease, tumor histologic subtype and tumor location, with particularly poor prognosis for older aged cases, metastasis at diagnosis, and axial tumor locations [1,2]. Established risk factors for developing osteosarcoma include radiation, chemotherapy, a previous retinoblastoma diagnosis, and numerous cancer predisposition syndromes, including Li-Fraumeni, Diamond-Blackfan anemia, Rothmund-Thomson, Werner, and Bloom syndromes [2]. Rare variants in cancer predisposing genes are important in osteosarcoma etiology. It has been reported that up to one quarter of osteosarcoma cases have a germline pathogenic variant in an established cancer susceptibility gene with *TP53* mutations being the most prevalent [5].

Although the data are inconsistent, studies have linked the development of osteosarcoma with growth and puberty [6,7], tall stature [8,9], and higher birthweight [8,10]. Epidemiologic studies of measured height have either reported no association between height and osteosarcoma risk [11,12] or a positive association between taller than average height and osteosarcoma risk [13,14] with two large meta-analyses supporting the association between tall stature and osteosarcoma [8,9]. Two large meta-analyses of birthweight reported that individuals with high birthweight had an increased risk of osteosarcoma [8,10], although not all studies have been consistent [12,13]. A recent case-control study identified an association of higher birthweight associated with more advanced osteosarcoma at diagnosis [15].

The heterogeneity in these study-specific association results for birthweight, height, and osteosarcoma risk could be due to differences in how these characteristics were ascertained in cases and controls, modeled, and/or the covariates used in the models as well as biases in case-control ascertainment and selection. Recall bias and unmeasured confounding in epidemiologic studies of environmental and lifestyle factors can lead to biases in the results and incorrect inferences. For example, early-life factors/exposures, including nutrient availability, therapeutic interventions (i.e., chemotherapy and radiation), and socioeconomic circumstances that affect height [16] are often not captured and accounted for in epidemiologic studies evaluating the association with osteosarcoma risk, which could lead to bias in risk estimates. In the era of genome-wide association studies (GWAS), investigators have turned to using genetic risk scores (GRS) constructed from established loci as a proxy for estimating relative birthweight, attained height, and puberty timing. As GRS are constructed from germline variants present at birth, they can provide an unbiased measure of the association between anthropometric traits and disease risk, reducing or eliminating some of the biases of observational studies, including recall bias and unmeasured confounding.

GWAS have identified multiple loci for height, [17,18] birthweight, [19] and puberty timing [20,21]. Together, these genetic variants explain approximately 19.7% of the variance in height, 2% of the variance in birthweight, and 7.4% of the variance in age at menarche. One study evaluated a polygenic score for adult height in 864 osteosarcoma cases and reported an increased risk of osteosarcoma for participants with higher scores for genetically inferred height [22]. Here, we used previously established genetic loci associated with birthweight, height, and puberty timing to construct genetic risk scores (GRS) and evaluate the association between those scores and risk of osteosarcoma.

2. Materials and methods

2.1. Study populations

A total of 1039 unique osteosarcoma cases of European ancestry from two separate studies were included in the analysis. Case set 1 included 968 cases that were ascertained from 10 centers as part of a previously published genome-wide association study (Table S1) [23–25]. Patients were diagnosed in the individual hospitals, and study centers provided data on patient and clinical variables that were harmonized across studies, including age at diagnosis, sex, presence of metastatic disease, histologic subtype, and tumor location. Cancer-free controls in case-control set 1 (N = 2923; Table S1) were drawn from the following previously genotyped and published studies: the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), Non-Hodgkin Lymphoma (NHL) case-control study, the Nurses' Health Study (NHS), and from the Instituto de Oncologia Pediátrica GRAACC/UNIFESP and Universidade Federal de Sao Paulo, as previously described [5,24,26–28], and matched to the cases on genetic ancestry and genotyping array at a 3:1 ratio. Case set 2 consisted of 71 osteosarcoma cases (non-overlapping with case set 1) with DNA extracted from blood spots archived at the Michigan Neonatal Biobank between 1992 and 2008 [29]. Other than sex, no clinical variables were available for these cases. Set 2 controls (N = 212) were non-overlapping cancer-free adults from PLCO, matched to set 2 cases on genetic ancestry and genotyping array at a ratio of 3:1. All tumors were histologically confirmed at each institution. All cases and controls were restricted to European ancestry (described below). All participants provided written consent and were recruited through an IRB-approved protocol, additional study-specific acknowledgments, funding, and grant information are listed in the acknowledgment section.

2.2. Genotyping

Germline genomic DNA was extracted from either blood or buccal cells (case set 1) [5,23] or archived blood spots (case set 2) [30], as

previously described. Genotyping was performed using either the Illumina OmniExpress or the Omni2.5 single nucleotide polymorphism (SNP) microarray for case set 1[23] and the Infinium Global Screening Array (GSAv2-MD; Illumina Inc., San Diego, USA) for set 2 cases and controls at the Cancer Genomics Research Laboratory, DCEG, NCI. Standard quality control and filtering was performed as previously described [23]. In brief, SNPs were required to have a minor allele frequency (MAF) of $\geq$ 1%, a $\geq$ 95% completion rate and no evidence of deviation from Hardy-Weinberg proportion ($P > 1 \times 10^{-7}$). Samples were excluded if they had abnormal heterozygosity values of $<$ 20% or $>$ 31%, abnormal X-chromosome heterozygosity or sex-discordance, evidence of contamination or a low call rate (>2% missing).

Cases and controls were considered European based on $>$ 80% infered genetic European ancestry using SNPWEIGHTS version 2.1 [31] and available SNP GWAS microarray data, with outliers removed. Cases and controls were matched using the R package CGEN (v3.8.0). Case-control set 1 and case-control set 2 were imputed separately using the 1000 Genomes Project Reference Panel.

2.3. Genetic Risk Score Construction

We constructed weighted GRS for birthweight, height, and puberty timing. The GRS for each participant was computed as the sum of their allelic dosages for each SNP multiplied by the reported effect or weight of the association for that SNP with birthweight, height, or puberty timing (i.e., female age at menarche and male age at voice breaking) respectively. The equation is shown below where w_j is the weight or coefficient for the *j*th SNP derived from the literature and x_{ij} is the allelic dosage of the *j*th SNP for the *i*th individual.

$$GRS_i = \sum_{j=1}^{k} w_j x_{ij}$$

The SNPs identified in previous studies in European ancestry individuals to be significantly associated with birthweight (k = 206), height (k = 3296), age of menarche for girls (k = 375), and age at voice breaking for boys (k = 75) were used to generate each GRS (Table S2) [20,21,32,33]. The effect estimates reported in these studies were used as the weights (e.g., standardized birthweight and height, age of puberty onset). Poorly imputed SNPs (info score $<$ 0.3) were excluded.

2.4. Logistic regression

We performed unconditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association of each GRS with risk of osteosarcoma [5]. Each GRS was modeled as a continuous variable and also categorized into quartiles with cut points inferred from the distribution among controls. Since the two case-control sets were genotyped on different microarrays, they were analyzed separately and the results combined via a fixed-effects meta--analysis. All models were adjusted for significant principal components; sex-combined models were also adjusted for sex. For case-control set 1, we were able to conduct stratified analyses by clinical characteristics, including age at diagnosis, presence of metastatic disease at diagnosis, tumor location, and histology. Age at diagnosis was additionally grouped based on typical puberty times for girls (10–16 years of age) and boys (12–18 years of age) versus timing before or after those age ranges. Tumor location was grouped as either appendicular or axial locations. Appendicular tumor locations included tumors of the extremities, shoulder and pelvic girdle, and axial locations included skull, mandible, vertebral column, and thorax. 'Conventional' histology included osteoblastic, chondroblast, and fibroblastic. 'Other' was made up of all other histologic subtypes. In addition, polytomous regression models were used to test for heterogeneity of the effect between the genetically inferred trait and osteosarcoma across comparison groups (e.g., no metastatic disease vs. metastatic disease).

Germline pathogenic variants in cancer susceptibility genes, particularly in *TP53*, have been shown to be important in osteosarcoma etiology.[2,34] To explore GRS associations in cases carrying a known pathogenic or likely pathogenic cancer susceptibility gene variant, we conducted analyses within a subset of cases with available germline exome sequencing data (N = 789 cases from set 1). The variant pathogenicity classifications for these 789 cases were previously described [34]. In brief, pathogenicity was based on the American College of Medical Genetics and Genomics (ACMG) pathogenicity guidelines [35] and focused on rare variants (MAF<0.5%) in candidate cancer susceptibility genes. A hierarchical system of ClinVar,[36] The Human Gene Mutation Database [37], and InterVar [38] was then used to assign pathogenicity. Of the 789 cases with exome sequencing data, 154 cases had a known pathogenic or likely pathogenic variant in a candidate cancer susceptibility gene, and 30 had a known pathogenic or likely pathogenic *TP53* variant. Due to the limited number of cases with a pathogenic variant, the GRS analyses for these cases were performed using the GRS as a continuous variable, and only limited stratified analyses by sex, diagnosis age within the puberty peak, and appendicular vs axial tumor locations, were performed.

2.5. Mendelian Randomization and Polytomous Regression Analyses

To evaluate the extent to which our findings may be causally associated with risk, we also conducted a two-sample Mendelian randomization analysis using the inverse-variance weighted (IVW) method [39] and summary statistics from our GWAS and the published GWAS of birthweight [33], height,[17,18] and puberty timing [20,21]. To validate the results from the IVW method and identify possible pleiotropy, Egger regression (MR-Egger) [40] was also performed.

2.6. Correlations with Measured Exposures and Transmission Disequilibrium Tests

A subset of 143 cases (all from set 1), with a similar male/female distribution to the full set (59% were male, N = 85), from the Children's Oncology Group (COG) had measured height during childhood from pediatrician records and birthweight from birth records. Relative and absolute puberty timing were assessed by self-report using detailed questionnaire data. Height based on sex and growth curves for each age were modeled using previously published methods [41,42]. We estimated the correlation between genetically inferred birthweight and actual birthweight within individuals by sex and between genetically inferred height and actual height within individuals by age and sex. Genetically inferred puberty timing was correlated with patient-reported characteristics. Those characteristics included questionnaire-derived data on puberty timing, including degree of breast development and having started menarche for girls, and degree of voice deepening and facial hair development for boys [43–45].

For 86 of the COG cases mentioned above, genotyping was also available for both their parents. Using 64 complete trios of European ancestry, we performed a polygenic transmission disequilibrium test (pTDT) analysis [46] for each GRS. pTDT assumes that the average offspring GRS for each trait would be greater than the average mid-parent GRS due to the ascertainment for the cases. We additionally performed a sensitivity analysis using all 86 trios, including 64 European and 22 non-European ancestry trios, with available genotype data, assuming the polygenic effects are homogenous across different ancestries.

3. Results

We evaluated a total of 1039 cases of osteosarcoma, of which 58% were male (Table 1). In case-control set 1, the mean age at osteosarcoma diagnosis was 16 years (standard deviation [SD]$\pm$ 8.9: range 2–80) with 85% of cases less than 25 years of age at diagnosis and 98% of cases

Table 1
Demographics and clinical characteristics of osteosarcoma cases.

Variable	Category	N	%
Case-Control Set 1			
Sex	Male	566	58%
	Female	402	42%
Age at Diagnosis	< 25 years	811	85%
	≥ 25 years	141	15%
	Missing	16	
Metastasis at Diagnosis	Yes	149	22%
	No	531	78%
	Missing	288	
Tumor Location	Appendicular	918	98%
	Axial	19	2%
	Missing	31	
Histology	Conventional	517	96%
	Other	21	4%
	Missing	430	
Total Cases		968	
Case-Control Set 2			
Sex	Male	38	54%
	Female	33	46%
Total Cases		71	

Appendicular tumor location includes extremities, shoulder, and pelvic girdle; axial includes skull, mandible, vertebral column, and thorax.

having an appendicular primary tumor location. Histologic subtype and presence of metastasis at diagnosis were available for 55% and 70% of cases, respectively. Of the cases with available data, 22% had a metastatic disease at diagnosis and 96% had conventional histology.

3.1. Correlations between genetically inferred and measured birthweight, height, and puberty timing

We first evaluated how well these GRS predicted measured birthweight, height and puberty timing in a subset of 143 cases with both metrics. Measured birthweight was modestly correlated with the GRS for birthweight (r = 0.22, P = 0.013; Fig. 1A). For all ages evaluated (0–12 years), the GRS for height was moderately correlated with the measured height of these cases (r = 0.30–0.40, P < 0.001; Fig. 1B, Table S3). The GRS for age of menarche in females and the GRS for male puberty timing were each evaluated in relation to the applicable questionnaire questions related to puberty timing traits, including age at breast development and menarche for girls, and voice deepening and facial hair development for boys. There was a trend for each puberty timing GRS and a decreased score for each of the evaluated puberty traits suggesting an earlier onset of puberty (Figs. S1 and S2).

3.2. GRS for Birthweight

In the combined analysis, we observed a statistically significant association between higher genetically inferred birthweight and an increased risk of osteosarcoma (per standard error (SE) increase in Z-score for genetically inferred birthweight, OR = 1.59, 95% CI 1.07–2.38, P = 0.02; Table 2, Fig. 2 A). When the birthweight GRS was categorized into quartiles, with the lowest quartile as the comparison group, a dose-response relationship was observed and the highest GRS quartile showed an increased osteosarcoma risk compared to the lowest quartile (OR=1.28, 95% CI: 1.04–1.58; Fig. 2B, Table S4A).

In subgroup analyses (Fig. 2A, Tables S4A and S5), the association between higher genetically proxied birthweight and osteosarcoma had a higher magnitude of association in males ($OR_{per\ SE}$ =1.73, 95% CI 1.03–2.90, P = 0.04) than females ($OR_{per\ SE}$=1.32, 95% CI: 0.69–2.52, P = 0.40), but the difference was not statistically significant ($P_{heterogeneity}$>0.05). When further evaluating associations by other available patient characteristics (case-control set 1), a higher birthweight GRS was associated with an increased risk of osteosarcoma in cases without metastasis at diagnosis ($OR_{per\ SE}$ =2.46, 95% CI 1.44–4.19, P = 9.50 ×10^{-4}), but not in those with metastatic disease ($OR_{per\ SE}$=0.40, 95% CI 0.16–1.00, P = 0.05) ($P_{heterogeneity}$=6.87 ×10^{-4}). No statistically significant differences were observed for other case characteristics or in cases carrying a cancer susceptibility gene pathogenic variant (Table S5 and S6).

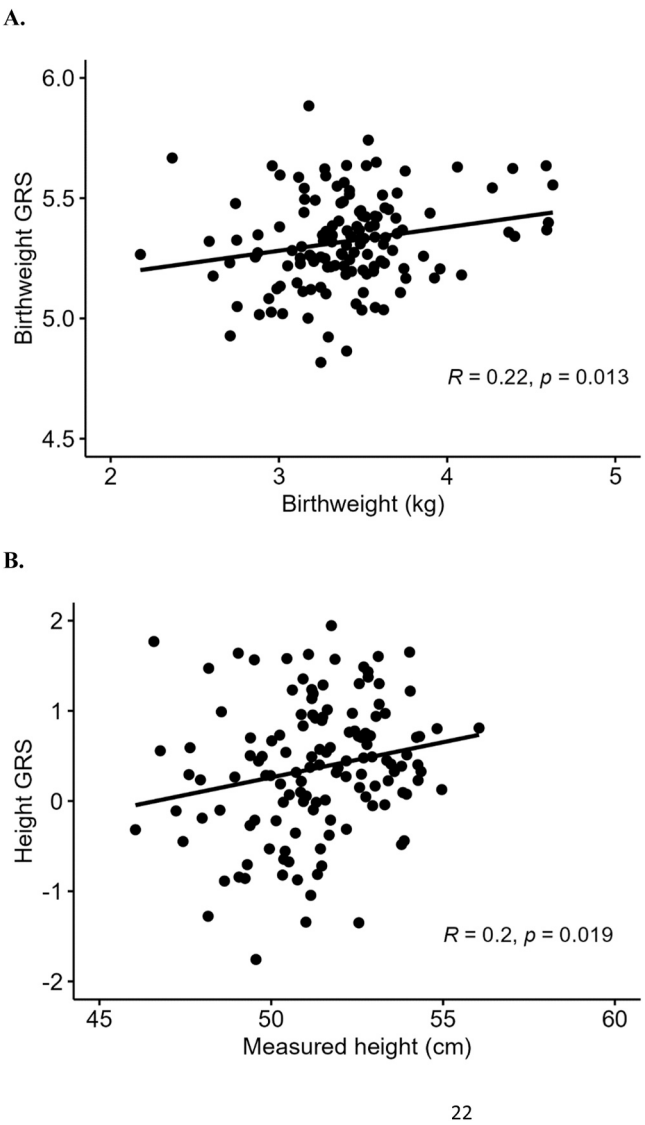

Fig. 1. Scatter plot illustrating the correlation between A) GRS for birthweight and measured birthweight, and B) GRS for height and measured height. A subset of set 1 cases (n = 143) with detailed data on measured birthweight and height were used in the analysis.

For all associations, we additionally used Mendelian randomization to evaluate the extent to which the relationship between genetically proxied birthweight and osteosarcoma risk may be causal (Table S7A). The IVW analysis provided similar risk estimates as the GRS results; the $OR_{per\ SE}$= 1.63 (95% CI 1.03–2.59, P = 0.04) for the association with osteosarcoma overall. The risk estimates for MR-Egger regression and IVW were different, and the MR-Egger intercept approached significance for osteosarcoma overall (P = 0.08) and was significant for non-metastatic disease (P = 0.05), indicating possible directional pleiotropy (Table S7A).

3.3. GRS for Height

There was no significant association between genetically inferred height and osteosarcoma risk in the combined analysis of all cases (OR_{per}

Table 2
Associations between osteosarcoma and genetically predicted birthweight, height, female age of menarche, and male puberty timing.

Variable	Case-Control Set 1				Case-Control Set 2				Combined		
	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI	OR	95% CI	p-value
Birthweight	968	2711	1.61	1.07–2.44	71	212	1.33	0.27–6.44	1.59	1.07–2.38	0.02
Height	968	2711	1.04	0.94–1.16	71	212	1.29	0.89–1.86	1.06	0.96–1.17	0.28
Female age of menarche	402	673	0.94	0.70–1.26	33	133	0.73	0.28–1.89	0.92	0.70–1.22	0.56
Male puberty timing	566	2038	0.66	0.33–1.35	38	79	1.43	0.08–24.34	0.69	0.35–1.38	0.30

Odds ratios (OR) are per standard error comparing cases versus controls with each GRS. Bold indicates p-value < 0.05. Age of menarche and male puberty timing were restricted to female and male sex, respectively, for cases and controls. Abbreviations: OR=odds ratio, CI=confidence interval.

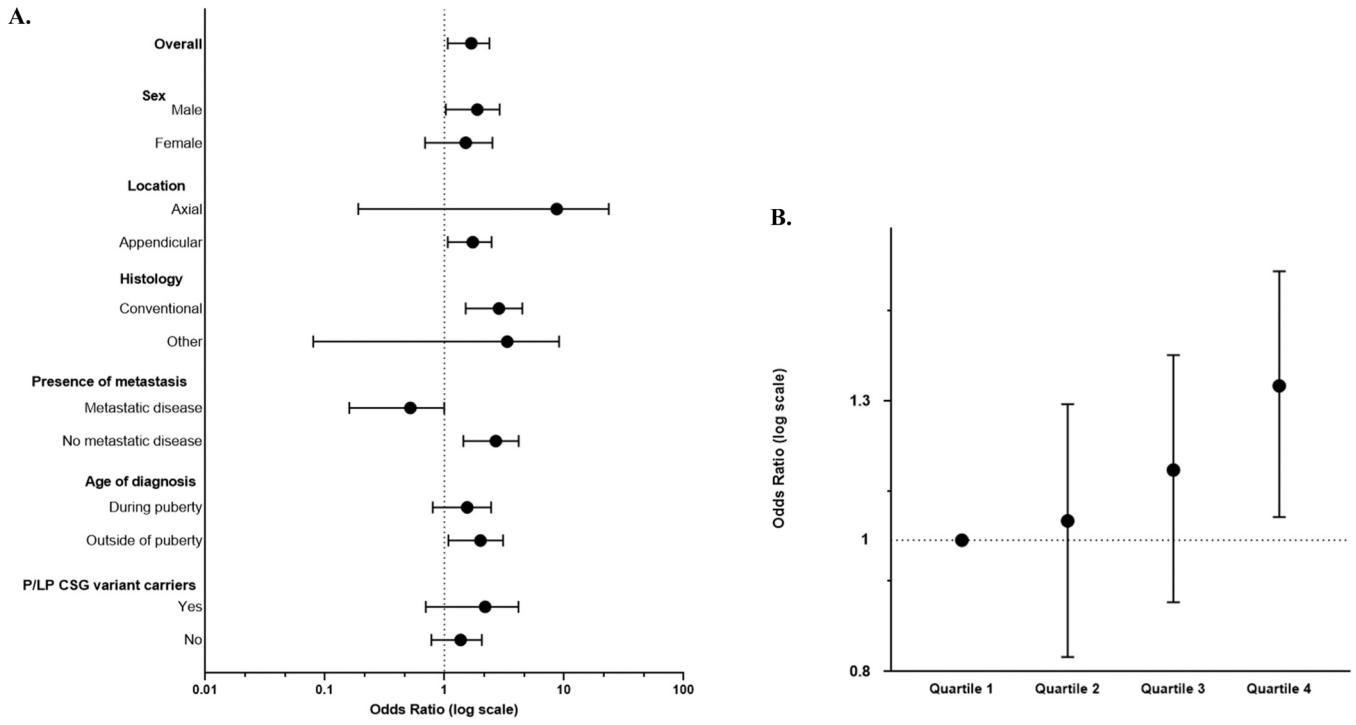

Fig. 2. Risk of osteosarcoma associated with genetically inferred birthweight, A) as a continuous measure, overall cases and by available case clinical characteristics and by presence of pathogenic/likely pathogenic (P/LP) cancer susceptibility gene (CSG) variants in the cases, and B) categorized into quartiles. For Fig. 1A, odds ratios are per standard error increase in the GRS for birthweight comparing cases versus controls. For Fig. 1B, the birthweight GRS was divided into quartiles and analyzed with the lowest quartile as the reference group. For tumor location, appendicular included extremities, shoulder, and pelvic girdle; axial included skull, mandible, vertebral column, and thorax. For age of diagnosis, cases were stratified based whether they were diagnosed during average puberty ages for girls (10–16 years) and boys (12–18 years) versus outside of average puberty ages. 'Conventional' histology included osteoblastic, chondroblast, and fibroblastic. 'Other' was made up of all other histologic subtypes. Overall cases and sex-specific associations were based on a fixed effects meta-analysis of the two case-control sets (n = 1039); other stratified analyses were restricted to cases with available clinical data (case-control set 1, n = 968).

$_{SE}$=1.06, 95% CI 0.96–1.17, P = 0.28; Table 2, Fig. 3A). The Mendelian randomization results were similar to the GRS association results (IVW: OR=1.03, CI 0.95–1.12, P = 0.49), and the MR-Egger intercept was insignificant (Table S7B).

For cases with available germline exome sequencing data[5] (N = 789 cases, all from case-control set 1), genetical predisposition to taller stature was associated with an increased risk of osteosarcoma in 154 cases carrying a germline pathogenic cancer susceptibility gene variant ($OR_{per\ SE}$=1.29, 95% CI 1.02–1.61, P = 0.03), but not associated with osteosarcoma in cases without a pathogenic cancer susceptibility gene variant ($OR=_{per\ SE}$0.98, 95% CI: 0.87–1.11, P = 0.78) ($P_{heterogeneity}$= 0.04) (Tables S5 and S6). After further stratifying cases with a pathogenic cancer susceptibility gene variant based on tumor location, the magnitude of the association was stronger for appendicular tumor location ($OR_{per\ SE}$=1.33, 95% CI 1.05–1.69, P = 0.02) compared to axial location ($OR_{per\ SE}$=1.18, 95% CI: 0.33–4.27, P = 0.80), but there was no significant heterogeneity by tumor location ($P_{heterogeneity}$=0.84). No other osteosarcoma patient characteristics were significantly associated with the height GRS (Fig. 3A, Table S5).

3.4. Puberty timing GRS

No significant associations (P < 0.05) were identified between osteosarcoma and genetically inferred female age of menarche or male puberty timing in the combined analysis (Table 2, Fig. 3B and C), in analyses stratified by any patient characteristics, or in cases carrying a cancer susceptibility gene pathogenic or likely pathogenic variant (Tables S4CD and S6). The Mendelian randomization results were similar to these GRS association results (Table S7CD).

3.5. Polygenic transmission disequilibrium tests (pTDT) for these traits using 86 complete trios

For the 64 European ancestry parent-child trios, no significant deviations from equilibrium, suggesting the genetic variants used in the GRS for each trait were transmitted from parents to their children as

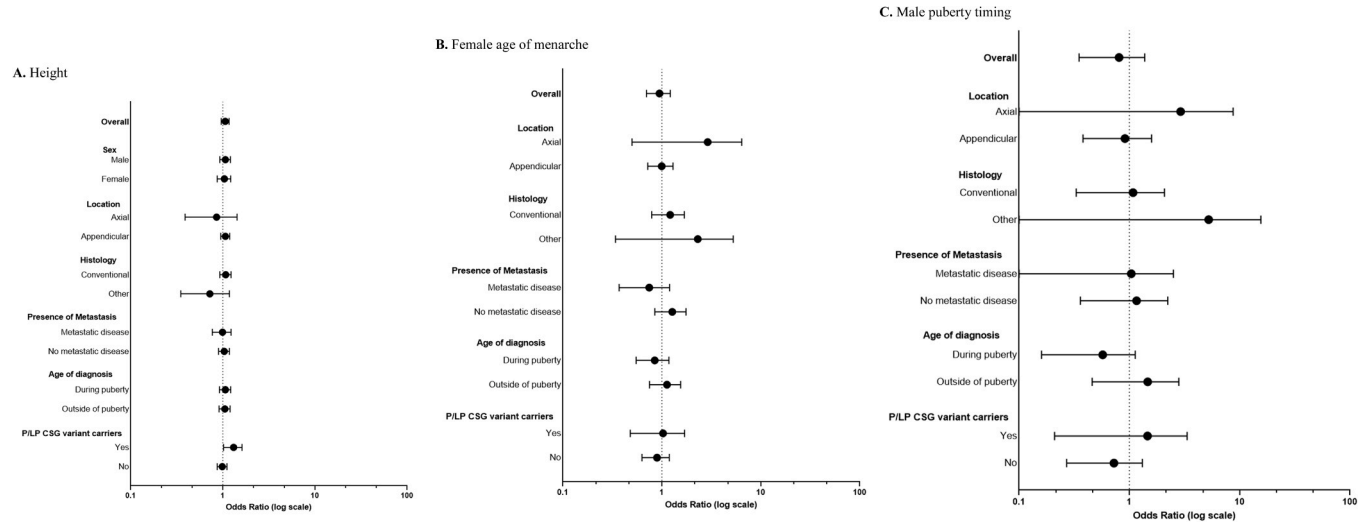

Fig. 3. Risk of osteosarcoma associated with genetically inferred A) height, B) female age of menarche, and C) male puberty timing, as a continuous measure, overall cases and by available case clinical characteristics and presence of pathogenic/likely pathogenic (P/LP) cancer susceptibility gene (CSG) variants in the cases. Odds ratios are per standard error increase in the GRS for corresponding characteristic comparing cases versus controls. For tumor location, appendicular included extremities, shoulder, and pelvic girdle; axial included skull, mandible, vertebral column, and thorax. For age of diagnosis, cases were stratified based whether they were diagnosed during average puberty ages for girls (10–16 years) and boys (12–18 years) versus outside of average puberty ages. 'Conventional' histology included osteoblastic, chondroblast, and fibroblastic. 'Other' was made up of all other histologic subtypes. Overall cases and sex-specific associations were based on a fixed effects meta-analysis of the two case-control sets (n = 1039); other stratified analyses were restricted to cases with available clinical data (case-control set 1, n = 968).

expected (P > 0.1; Table S8A). We conducted a further analysis using all 86 trios with available genotype data, including European and non-European ancestries, and also showed no statistically significant deviations across the different ancestries (P > 0.1; Table S8B).

4. Discussion

Our study is the first investigation of osteosarcoma risk and genetically inferred birthweight and puberty timing and the largest evaluation of osteosarcoma and genetically inferred height, to date. Among 1039 osteosarcoma cases, we identified a statistically significant positive association between risk of osteosarcoma and genetically determined birthweight. This finding suggests that biological pathways and genetic factors that lead to more rapid growth and higher birthweight in *utero*, may also contribute to osteosarcoma pathogenesis. In stratified analyses, the increased risk observed with higher birthweight was only observed for cases with no metastatic disease at presentation. However, this subgroup finding should be interpreted with caution as the sample size was limited for cases with metastatic disease. Although not a strong correlation, genetically constructed birthweight was significantly correlated with measured birthweight, which provides some confidence regarding the validity of our GRS for birthweight.

Measured higher birthweight is a known risk factor for multiple pediatric and adult cancers, including leukemia, non-Hodgkin lymphoma, Wilms's tumor, neuroblastoma, and soft tissue sarcomas [47–52]. Epidemiologic studies have observed a link between increased osteosarcoma risk and higher reported birthweight [8,10,11,15]. Although the biological mechanism for the relationship between higher birthweight and development of osteosarcoma is not clear, it may be related to elevated levels of growth factors. Higher birthweight infants have been observed to have higher circulating insulin-like growth factor (IGF-1 and −2) levels [53], and osteosarcoma tumors have been shown to express high levels of IGF-1 and IGF-2.[54] IGFs also play a role in osteoblast differentiation and proliferation [55].

Smaller case-only studies have observed lower measured birthweights among osteosarcoma cases with primary tumors of the long bones compared to cases with other primary tumor locations [15,56]. Similarly, we observed in a case-case comparison that cases with appendicular tumor locations had lower genetically constructed birthweight than cases with axial tumors (OR=0.64, 95% CI 0.05–7.47), the association was not statistically significant our sample size for axial cases was small, and some cases overlap with one of the previous studies [56]. It has also been previously reported that cases with metastatic disease (based on 79 cases with metastasis) had a higher measured birthweight than cases with non-metastatic disease [15]. We had a larger number of cases with metastatic disease at diagnosis (N = 149) than the previous study, and, in contrast, we found that cases with metastatic disease had a significantly lower GRS for birthweight than those without metastasis (OR= 0.16, 95% CI 0.06–0.45). Although it is possible that one or both findings could be due to chance, this difference could be due to an influence of gestational age or environmental factors on measured birthweight.

Measured birthweight is determined in part by gestational age and environmental factors, such as maternal smoking and nutrition, which is correlated with socioeconomic status and other factors. Failure to account for these confounding factors can lead to incorrect inferences about the contribution of birthweight, as opposed to related factors (e.g., maternal smoking), to risk. Since germline genetic variation is present from birth, genetically predicted birthweight is not correlated or confounded by these environmental factors and thus can be helpful in evaluating causality. The Mendelian randomization analysis that we conducted and found a similar significant association between higher genetically determined birthweight and osteosarcoma risk; however, there was evidence for directional pleiotropy. This suggests that the association between genetically determined birthweight and osteosarcoma may be driven in part by pleiotropy and that there are genetic variants that both lead to increased birthweight and osteosarcoma risk. When we evaluated this further in the secondary analyses excluding HLA variants and SNPs known to be associated with osteosarcoma; however, our results remained the same (data not shown).

Although we observed a moderate and significant correlation between genetically determined height and patient measured height, generally supporting the utility of our GRS as a valid instrument for genetically inferred height, we did not observe a significant association between genetically determined height and the risk of osteosarcoma. A previous study of osteosarcoma reported a positive association between

risk of osteosarcoma and a genetic predisposition to taller stature [22]; however, that study had a smaller sample size (N = 670) and used fewer SNPs to calculate the polygenic score for height, 416 vs. the 3296 SNPs utilized in our study [22]. After restricting to the same set of SNPs used in the previous study [22], we did not replicate their findings (P = 0.41; data not shown). We did identify a significant association between taller inferred height and increased osteosarcoma risk in the cases with a previously identified pathogenic cancer susceptibility gene variant. However, this association should be interpreted cautiously due to the limited number of cases with a predicted pathogenic cancer susceptibility gene variant. Future studies with a larger number of cases carrying a pathogenic variant are needed to better understand the association between genetic determinants of height and osteosarcoma risk in the presence of a pathogenic germline cancer susceptibility gene variant.

The strengths of our study include our large osteosarcoma sample size and ability to evaluate different subgroups of cases. The GRS instruments used to in our study explain a substantial portion of the variance in birthweight [19] and height [17,18] and we were able to validate them in a subset of cases. However, there is still more to learn about the genetic determinants of birthweight, height, and puberty timing, and our associations could be attenuated by the limited identified genetic loci for each trait. Puberty timing had a small number of SNPs included in the GRS and limited correlation with the measured trait, particularly for male puberty timing, and perhaps could be related to the lack of associations observed with puberty timing. As more SNPs become identified for these traits, especially across ethnicities, prediction of these traits will improve, and GRS analyses will be able to provide a more precise evaluation of osteosarcoma risk. We restricted our analysis to subjects of European ancestry, which was important for limiting bias due to population stratification, but it also limits generalizability. Additional studies are needed in other populations to confirm and extend these associations.

5. Conclusions

In summary, we performed a large study of genetically determined birthweight, height, and puberty timing and risk of osteosarcoma. We observed that a genetic propensity to higher birthweight was associated with increased osteosarcoma risk, suggesting that genetic variants or biological pathways that affect birthweight may also contribute to osteosarcoma pathogenesis and thus that osteosarcoma may have an *in-utero* origin. The treatment and outcomes of osteosarcoma have remained relatively unchanged for 30 years [2]; a better understanding of the genetic etiology of this disease may provide clues to underlying biological mechanisms that could be exploited to improve risk classification and treatment strategies.

Ethics statement

All participants provided written consent and were recruited through an IRB-approved protocol.

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CRediT authorship contribution statement

D. Matthew Gianferante: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft. **Amy Moore:** Conceptualization, Formal analysis, Writing - Review & Editing. **Logan Spector:** Methodology, Formal Analysis, Writing – Review and Editing. **Tianzhong Yang**: Methodology, Formal Analysis, Writing – Review and Editing. **Aubrey Hubbard**: Formal Analysis, Writing – Review and Editing. **Richard Gorlick**: Resources, Writing – Review and Editing. **Ana Patiño-Garcia**: Resources, Writing – Review and Editing. **Fernando Lecanda**: Resources, Writing – Review and Editing. **Adrienne M. Flanagan**: Resources, Writing – Review and Editing. **Fernanda Amary**: Resources, Writing – Review and Editing. **Irene L. Andrulis**: Resources, Writing – Review and Editing. **Jay S. Wunder**: Resources, Writing – Review and Editing. **David M. Thomas**: Resources, Writing – Review and Editing. **Mandy L. Ballinger**: Resources, Writing – Review and Editing. **Massimo Serra**: Resources, Writing – Review and Editing. **Claudia Hattinger**: Resources, Writing – Review and Editing. **Ellen Demerath:** Resources, Writing – Review and Editing. **Will Johnson**: Formal Analysis, Writing – Review and Editing. **Brenda M. Birmann**: Resources, Writing – Review and Editing. **Immaculata De Vivo**: Resources, Writing – Review and Editing. **Graham Giles**: Resources, Writing – Review and Editing. **Lauren R. Teras**: Resources, Writing – Review and Editing. **Alan Arslan**: Resources, Writing – Review and Editing. **Roel Vermeulen**: Resources, Writing – Review and Editing. **Jeannette Sample:** Formal Analysis, Writing – Review and Editing. **Neal D. Freedman**: Resources, Writing – Review and Editing. **Wen-Yi Huang**: Resources, Writing – Review and Editing. **Stephen J. Chanock**: Conceptualization, Writing – Review and Editing. **Sharon A. Savage**: Conceptualization, Writing – Review and Editing. **Sonja I. Berndt:** Conceptualization, Methodology, Writing - Review & Editing, Supervision. **Lisa Mirabello:** Conceptualization, Methodology, Writing - Review & Editing, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability

GWAS data has been previously published and available in the database of Genotypes and Phenotypes (dbGaP) as per referenced studies.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.canep.2023.102432.

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