Original Article
Case-parent analysis of variation in pubertal hormone genes and pediatric osteosarcoma: a Children's Oncology Group (COG) study

Jessica RB Musselman[1], Tracy L Bergemann[2], Julie A Ross[1,3], Charles Sklar[4], Kevin AT Silverstein[5], Erica K Langer[1,3], Sharon A Savage[6], Rajaram Nagarajan[7], Mark Krailo[8], David Malkin[9], Logan G Spector[1,3]

[1]Division of Pediatric Epidemiology and Clinical Research, University of Minnesota, Minneapolis MN 55455; [2]Medtronic, Mounds View, MN 55112; [3]University of Minnesota Masonic Cancer Center, Minneapolis MN 55455; [4]Memorial Sloan-Kettering Cancer Center, New York City, New York 10065; [5]Supercomputing Institute for Advanced Computational Research University of Minnesota, 55455; [6]Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD 20852; [7]Division of Oncology, Cincinnati Children's Hospital Medical Center, Cincinnati OH 45229; [8]Department of Preventive Medicine, University of Southern California, Los Angeles CA 90089; [9]Division of Hematology/Oncology, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto CA M5G 1X8

Abstract: Osteosarcoma (OS) is a rare malignant bone tumor with an overall incidence rate of 4.6 cases per million children aged 0-19 years in the United States. While the etiology of OS is largely unknown, its distinctive age-incidence pattern suggests that growth and development is crucial in genesis. Prior studies have suggested that variants in genes in the estrogen metabolism (ESTR) and insulin-like growth factor/growth hormone (IGF/GH) pathways are associated with OS. We examined 798 single nucleotide polymorphisms (SNPs) in 42 genes from these pathways in a case-parent study (229 complete triads and 56 dyads) using buccal cell samples. Relative risks (RR) and 95% confidence intervals (CI) associated with transmitting one or two copies of the variant were estimated using log-linear models. After Bonferroni correction, 1 SNP within the ESTR pathway (rs1415270: RR = 0.50 and 8.37 for 1 and 2 vs. 0 copies, respectively; p = 0.010), and two SNPs in the IGF/GH pathway (rs1003737: RR = 0.91 and 0.0001 for 1 and 2 vs. 0 copies, respectively; p <0.0001 and rs2575352: RR = 2.62 and 0.22 for 1 and 2 vs. 0 copies; p < 0.0001) were significantly associated with OS incidence. These results confirm previous findings that variation in the estrogen metabolism and bone growth pathways influence OS risk and further support a biologically and epidemiologically plausible role in OS development.

Keywords: Osteosarcoma, case-parent study, growth and development, insulin-like growth factor pathway, estrogen metabolism pathway

Introduction

Osteosarcoma (OS) is the most commonly occurring bone cancer in children <20 years of age, with about 440 new cases diagnosed annually in the United States and Canada [1, 2]. Several lines of evidence link OS etiology to factors related to pubertal development and bone growth. First, incidence of OS is distributed bimodally, with the primary peak occurring during adolescence, corresponding to the pubertal growth spurt and its accompanying increase in endogenous sex and growth hormone levels [3-5]. OS incidence peaks earlier in females, which corresponds to the earlier onset of puberty in females [6]; however, overall incidence is higher in males, who are generally taller than females. The association of OS incidence with growth and puberty corroborates findings of associations among greater height [5, 7-12], rate of growth [12], and birth weight [13, 14] and increased risk of OS in both humans and canines, although not all studies have found such associations [15, 16]. Furthermore, the

most common sites of OS are areas of rapid bone growth during puberty [6, 17, 18].

There are few known causes of OS, and these account for only a small proportion of cases. The only widely accepted exogenous risk factors are high-dose ionizing radiation [19, 20] and prior chemotherapy with alkylating agents [19, 20]. In addition, there are several associated syndromes [21-23] including Li-Fraumeni Syndrome [24-26], hereditary retinoblastoma [27-29], Werner [30], Bloom [31], and Rothmund-Thomson Syndromes [32, 33]. Because growth and puberty are heritable traits, it is possible that other forms of genetic variation may explain sporadic OS incidence.

In particular, evidence suggests that genes underlying pubertal growth might be key in OS etiology. Linear bone growth is under the influence of several genes, including those from the sex steroid hormone control pathway (e.g., estrogen metabolism (ESTR)) and insulin-like growth factor/growth hormone (IGF/GH) pathway, which regulate rates of bone growth [34-39] through release of estrogen, IGF1, and growth hormones. The timing and extent of adolescent bone growth is also under substantial genetic control, with heritability estimates ranging from 50 to 80% for the time of onset of puberty and peak bone mineral density [40, 41]. Hence, variation in genes in the IGF/GH and ESTR pathways are reasonable candidates for exploration in OS etiology. A candidate gene study by Mirabello et al. identified 12 SNPs in the DNA repair gene and growth/hormone pathways as being associated with OS in a study of ~5000 tag SNPs in 255 candidate genes [3]. In the same population, Savage et al. identified two polymorphisms of the IGF2 receptor gene that were significantly more frequent in OS cases [42, 43]. That study included adult and pediatric cases and a similar approach has not been taken in a study comprised solely of pediatric OS cases.

In order to assess the association of OS with genes putatively related to adolescent bone growth, we conducted a case-parent study consisting of nearly 300 cases of pediatric OS recruited through the Children's Oncology Group and one or both of their biological parents. Using these triads/dyads, we employed log-linear regression to assess 798 SNPs across 42 candidate genes in biologically plausible pathways for an association with OS.

Materials and methods

Study population

OS cases were identified through the Childhood Cancer Research Network (CCRN)--the registry component of the Children's Oncology Group (COG) [44]. As part of registering all pediatric patients with the COG data center, the CCRN consent process allows for informed consent for the release of personal identifiers on patients and parents along with permission to contact in the future. Eligible cases were patients with a primary diagnosis of OS (ICCC 9180-9200; OMIM #259500) at age <20 years made at a North American COG institution between December 24, 2007 and March 31, 2010, who had at least one living biological parent willing to participate and understand English or Spanish. Initial contact was through an introductory letter followed by a telephone call during which study staff summarized the goals of the study and provided respondents with an opportunity to ask questions and assess eligibility. If consent over the telephone was given, assent forms (if appropriate), buccal cell collection kits (mouthwash or cytobrushes), a paper questionnaire, and return mailers were sent. The study sample consisted of the 290 cases who returned a buccal cell kit for themselves and at least one biological parent (mean age at diagnosis 12.9 years). Thus, this study population represents one of the largest and highly powered studies for assessing genetic variants associated with OS in a pediatric population.

SNP selection

Tag SNPs were selected as follows: first, transcripts and gene boundaries for gene symbols were obtained from Ensembl release 50. SNPs were then found by uploading the list of gene names to Ensembl's BioMart, and then additional SNPs were obtained directly at hapmap. org [45] that were 20,000 bp up and downstream of gene boundaries using the data from the International HapMap Project Data Rel 23a/phase II Mar08 data file where tag SNP data was downloaded from the CEU population using the "tagger pairwise method" with an R-square cutoff of 0.8 and a minor allele frequency (MAF) cutoff of 0.0. The final set of SNPs were then chosen if they were annotated as verified in the dbSNP or SNP500 databases [46] or were coding SNPs predicted to be dele-

terious [47, 48] or SNPs selected from the Phase I HapMap data using the Tagger algorithm with $r^2 > 0.8$.

Genotyping assays

Buccal cell samples were stored at 4°C and batched for DNA extraction. Samples were centrifuged, cells pelleted and washed, and DNA was extracted using the Puregene kit (Gentra Systems, Minneapolis, MN). DNA was quantified, aliquoted and stored in hydration solution at -20°C. The Sequenom platform iPLEX Gold method was used for genotyping. Multiplexed PCR was performed in 5µl reactions on a 384-well plate. Reactions contain 0.5 U HotStar Taq polymerase (Qiagen), 100 nM primers, 1.25X HotStar Taq buffer, 1.625 mM MgC1$_2$2, and 500 µM dNTPs. Enzyme activation was performed at 94°C for 15 minutes, followed by DNA amplification with 45 cycles at 94°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minute, followed by a three minute extension at 72°C. Unincorporated dNTPs were removed using shrimp alkaline phosphatase (0.3 U, Sequenom, San Diego). SBE primers at concentrations from 0.625 µM to 1.25 µM and iPLEX enzyme and buffers in 9 µl reactions were used to carry out single base extension. Reactions were desalted and SBE products were measured using the MassARRAY system. TYPER software (Sequenom) was used to analyze mass spectra in order to generate genotype calls and allele frequencies.

DNA was quantified and normalized to a housekeeping gene. A total of 866 SNPs were selected and genotyped following our SNP selection procedure. SNPs were excluded if they had less than a 90% genotyping rate in our sample, were non-variable, or had a sample MAF of < 1%. Individuals were excluded if >10% genotypes were missing. If the individual excluded was a case, the entire triad/dyad was excluded. In addition, an algorithm was created to assess for discrepancy between the genotypes of the parents and their associated case child. Individuals were excluded whose genotypes demonstrated >5% inconsistency between the parents and the associated case child (e.g. paternal genotype contains two copies of the variant and the child's genotype contains no copies). A total of 798 tag SNPs, 186 complete triads and 72 dyads met the above quality con-

trol criteria and were included for analysis. All original candidate genes had at least one SNP included in the final analysis except for IGFBP6.

Statistical analysis

This study uses the case-parent design, which involved enrolling and genotyping OS cases and their parents [49, 50] and testing whether the distribution of candidate susceptibility alleles in cases significantly deviates from what would be expected by Mendelian inheritance. We used a log-linear regression model to assess for significant over- or undertransmission of a variant allele among OS cases and their biological parents [51, 52]. Briefly, the log-linear model estimates relative risks (RR) of possessing 0, 1, or 2 copies of the variant by assessing the joint likelihood of the case, maternal, and paternal genotypes where the statistical model is conditioned on the presence of disease in the offspring. A likelihood ratio test (LRT) determines the statistical significance for each SNP variant. The least common allele among cases was selected to serve as the variant for each SNP. Families were informative if at least one biological parent was available for genotyping. Estimation and inference were performed in the presence of missing parental data using a likelihood-based algorithm [51]. Bonferroni corrections were performed within each of two pathways--the ESTR pathway (29 genes, 519 SNPs after quality control) and the IGF/GH pathway (13 genes, 279 SNPs after quality control)--to correct for multiple statistical comparisons. Statistical analyses were performed using custom programs in R (http://www.r-project.org).

Results

A summary of case identification, enrollment, and DNA collection is provided in **Figure 1**. During the eligibility period, a total of 660 OS cases were identified at COG institutions. Of those, families of 602 (91%) cases consented to participation in the CCRN and potential future contact for study participation. Twenty of those who consented to CCRN were considered ineligible due to lack of an available biological parent (n=17) or lack of a parent who spoke either English or Spanish (n=3). Of those who were contacted and eligible (n=582), 445 families (76% contacted) gave consent over the
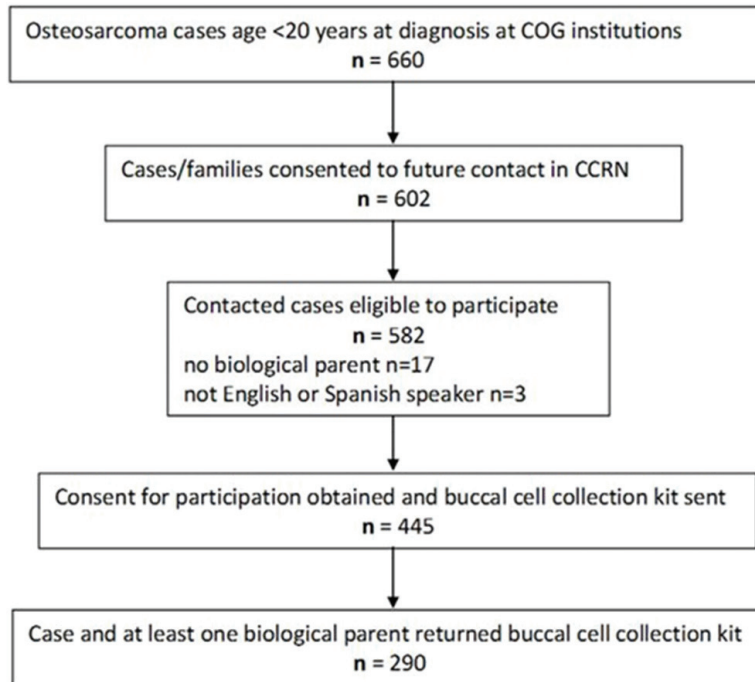
Figure 1. Flow chart describing the identification and enrollment of the cases that make up the study population from the initial population of childhood osteosarcoma cases treated at Children's Oncology Group institutions during the eligibility period. Patient Identification, Contact, Consent, Enrollment, and Participation in a case-parent study of childhood osteosarcoma; April 1, 2007-March 31, 2010.

phone and were sent a DNA kit, of which 290 (65% sent) were returned.

Demographics and clinico-pathologic characteristics of the cases are presented in **Table 1**. A majority (95%) of the families came from the United States. Cases were also primarily white (86%). Tumors were most often located in the long bones (92%). For the log-linear analysis, six families were not included because there was either a diagnosis of or a family history consistent with Li-Fraumeni Syndrome. An additional 30 triads were excluded due to excessive missing genotyping data from the case, and an additional 42 parents were excluded for the same reason. A further four fathers were excluded due to discordance above our 5% cutoff between paternal and progeny genotypes. A total of 68 SNPs were excluded, leaving a total of 798 SNPs for analysis, 519 on the ESTR pathway and 279 on the IGF/GH pathway. After Bonferroni correction (for 519 SNPs within ESTR and 279 within IGF/GH), two SNPs on the IGF/GH pathway remained significant (**Table**

2, **Figure 2**). The first SNP, rs1003737 is located in the intronic region of the IGF2R gene on chromosome 6, and was associated with a RR of 0.91 and 0.0001 for one and two copies versus no copies, respectively, of the minor allele (un-corr $p<0.0001$; corr-p <0.0001). The second SNP, rs2575352, is upstream of the IGFALS gene on chromosome 16 and was associated with a RR of 2.62 and 0.22 for one and two copies versus no copies of the minor allele, respectively (un-corr $p<0.0001$; corr-p <0.0001). An additional SNP from the ESTR pathway also maintained significance after Bonferroni correction: rs1415270 downstream of the androgen receptor (AR) gene of the X chromosome was associated with a RR of 0.53 and 8.26 for one and two copies, respectively, compared to no copies of the minor allele (un-corr $p<0.0001$; corr-p $=0.02$).

Discussion

We employed a candidate gene based approach in a case-parent study to assess the involvement of genes in biologically plausible pathways for a possible association with pediatric OS. The IGF/GH and ESTR pathways are reasonable candidates for analysis due to previously established associations of growth and puberty with OS. The heritability of growth and hormone regulation provides additional rationale for examining genes in these pathways [3-7, 10, 11, 40, 41].

After Bonferroni correction for multiple statistical tests, we identified one SNP in each of three genes of interest as significant. In the IGF/GH pathway, the IGF2R gene (on chromosome 6) plays a role in activating growth factor beta and degrading IGF2, while IGFALS (on chromosome 16) increases the half life of IGF. SNPs in the IGF2R gene were previously reported to be

Table 1. Demographic Characteristics from a Case-Parent Study of Childhood Osteosarcoma, Children's Oncology Group, United States and Canada, 2007-2010 (n=290)

Case Trait	Category	Count	%
Gender	Male	170	58.6
	Female	120	41.4
Age at diagnosis (years)	0-4	2	0.7
	5-9	51	17.6
	10-14	130	44.8
	15-19	107	36.9
Race[a]	White	237	85.6
	Black	21	7.6
	Other	19	6.9
Ethnicity[b]	Non-Hispanic	239	86.0
	Hispanic	39	14.0
Country of Residence	United States	274	94.5
	Canada	16	5.5
Tumor Location[c]	Lower Limb	221	77.5
	Upper Limb	42	14.7
	Chest	6	2.1
	Hip	9	3.2
	Spine	2	0.1
	Head	5	1.8
Histology	Chondroblastic	47	20.2
	Osteoblastic	68	29.2
	Fibroblastic	16	6.9
	Telangiectatic	15	6.4
	High grade	74	31.8
	Low grade	5	2.1
	Periosteal	4	1.7
	Mixed	4	1.7

[a]13 unknown; [b]12 unknown; [c]5 missing.

associated with increased risk of OS. However, the SNP found in our study, rs1003737, was in low LD with the previously reported SNPs (r-squared 0.10) [42]. The third SNP is located on the X chromosome about 20 kb downstream of the AR gene in a region that plays a role in the N-terminal, the DNA-binding, and the androgen-binding domains [42, 43, 45]. To our knowledge, neither the particular function of these specific SNPs nor their involvement in other malignancies has been reported.

Previous studies used a case-control approach, which is limited by the need to identify an appropriate control group and the possibility of population stratification in genetic analyses. In contrast, for the case-parent approach, no controls are needed and population stratification is inherently corrected for. The statistical model employed in a case-parent design also allows for the estimation of RRs rather than odds ratios-- the standard measure of association in case-control studies. Further, our focus on pediatric malignancies is a strength given the availability and willingness of biological parents to participate in research, particularly when compared to the underwhelming participation rates for population based controls. Including solely pediatric OS cases could also improve our understanding of etiology should it differ from that of older patients; this is especially the case in light of our large sample size, given the rarity of the tumor. We appear to have taken the first comprehensive look at genetic variation in the ESTR pathway as an OS risk factor. Our identification of a significant SNP associated with the AR gene suggest that this pathway might also be of biological relevance. It should also be noted that while Bonferroni corrections were used in both our analyses and that of previous studies, we elected to correct at the pathway level and thus our criteria for statistical significance was considerably more stringent. This could partially explain both the reduction

Table 2. Significant SNPs from the IGF/GH and ESTR pathways and associated relative risks after correction for multiple testing from a study of childhood osteosarcoma

Pathway	Gene	SNP id	Chromosome	Region	MAF[a]	RR1[b]	RR2[c]	Un-corr-p	Corr-p[d]
IGF/GH	IGF2R	rs1003737	6	intronic	0.30	0.91	0.0001	<0.0001	<0.0001
	IGFALS	rs2575352	16	upstream	0.35	2.62	0.22	<0.0001	<0.0001
ESTR	AR	rs1415270	X	downstream	0.22	0.53	8.26	<0.0001	0.02

[a]: minor allele frequency; [b]: relative risk for one vs. no copies; [c]: relative risk for two vs. no copies; [d]: Bonferroni corrected within pathway (519 SNPs for ESTR and 279 SNPs for IGF/GH).
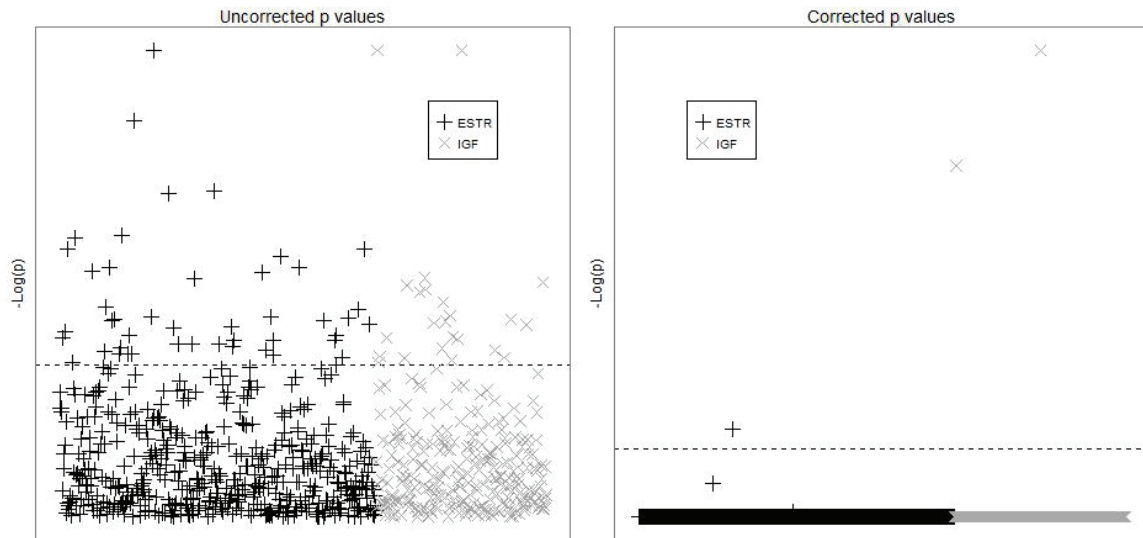

Figure 2. This figure depicts both the uncorrected *p*-values and the *p*-values after correction for multiple testing for each of the SNPs analyzed in the two pathways. We present the *p*-values (corrected and uncorrected) on the -log scale and provide a horizontal line so that the reader can easily see the threshold necessary for significance in this analysis. *P*-values for each SNP by pathway before and after Bonferroni correction[a] α = 0.05. Inset box indicates pathway symbol, results for 798 SNPs in 42 candidate genes (29 ESTR and 13 IGF/GH). Dashed line represents significance threshold for α.

in number of significant SNPs compared to the findings by Mirabello et al. as well as our failure to confirm significant SNPs from that study.

While we did identify statistically significant SNPs in our proposed pathways, we recognize that interpretation of our results must be considered in the context of the caveats associated with a candidate gene based approach, primarily the reliance on current knowledge regarding the biology of OS and the limitation in the number of SNPs examined compared to a genome-wide approach. Despite these shortcomings, we selected a biologically plausible set of genes based on previous research indicating the primacy of the IGF/GH and ESTR pathways in promoting linear bone growth [34] and on preliminary data implicating the IGF/GH axis in adult and pediatric OS [42], which made candidate genes in the IGF/GH and ESTR path-

ways obvious choices for a study in children. Confirmation of these results in future studies would lend additional support to the association of bone growth pathways in pediatric OS.

Acknowledgements

Address correspondence to: Logan G Spector, Division of Pediatric Epidemiology and Clinical Research, University of Minnesota Cancer Center,

420 Delaware Street SE, MMC 715, Minneapolis, MN 55455. Phone: (612)624-3912; Fax: (612) 624-7147; E-mail: spector@umn.edu

References

[1] McLaughlin. Canadian Cancer Statistics. Toronto: 2005.

[2] Ries L. SEER Cancer Statistic Review, 1975-2002. Bethesda MD: 2004.

[3] Mirabello L, Yu K, Berndt SI, Burdett L, Wang Z, Chowdhury S, Teshome K, Uzoka A, Hutchinson A, Grotmol T, Douglass C, Hayes RB, Hoover RN and Savage SA. A comprehensive candidate gene approach identifies genetic variation associated with osteosarcoma. BMC Cancer 2011; 11: 209.

[4] Mirabello L, Troisi RJ and Savage SA. International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. Int J Cancer 2009; 125: 229-234.

[5] Fraumeni JF Jr. Stature and malignant tumors of bone in childhood and adolescence. Cancer 1967; 20: 967-973.

[6] Staheli LT. Normative data in pediatric orthopedics. J Pediatr Orthop 1996; 16: 561-562.

[7] Gelberg KH, Fitzgerald EF, Hwang S and Dubrow R. Growth and development and other risk factors for osteosarcoma in children and young adults. Int J Epidemiol 1997; 26: 272-278.

[8] Cotterill SJ, Wright CM, Pearce MS and Craft AW. Stature of young people with malignant bone tumors. Pediatr Blood Cancer 2004; 42: 59-63.

[9] Longhi A, Pasini A, Cicognani A, Baronio F, Pellacani A, Baldini N and Bacci G. Height as a risk factor for osteosarcoma. J Pediatr Hematol Oncol 2005; 27: 314-318.

[10] Mirabello L, Pfeiffer R, Murphy G, Daw NC, Patino-Garcia A, Troisi RJ, Hoover RN, Douglass C, Schuz J, Craft AW and Savage SA. Height at diagnosis and birth-weight as risk factors for osteosarcoma. Cancer Causes Control 2011; 22: 899-908.

[11] Withrow SJ, Powers BE, Straw RC and Wilkins RM. Comparative aspects of osteosarcoma. Dog versus man. Clin Orthop Relat Res 1991; 159-168.

[12] Tjalma RA. Canine bone sarcoma: estimation of relative risk as a function of body size. J Natl Cancer Inst 1966; 36: 1137-1150.

[13] Gelberg KH, Fitzgerald EF, Hwang SA and Dubrow R. Fluoride exposure and childhood osteosarcoma: a case-control study. Am J Public Health 1995; 85: 1678-1683.

[14] Hartley AL, Birch JM, McKinney PA, Teare MD, Blair V, Carrette J, Mann JR, Draper GJ, Stiller CA, Johnston HE and et al. The Inter-Regional Epidemiological Study of Childhood Cancer (IRESCC): case control study of children with bone and soft tissue sarcomas. Br J Cancer 1988; 58: 838-842.

[15] Operskalski EA, Preston-Martin S, Henderson BE and Visscher BR. A case-control study of osteosarcoma in young persons. Am J Epidemiol 1987; 126: 118-126.

[16] Buckley JD, Pendergrass TW, Buckley CM, Pritchard DJ, Nesbit ME, Provisor AJ and Robison LL. Epidemiology of osteosarcoma and Ewing's sarcoma in childhood: a study of 305 cases by the Children's Cancer Group. Cancer 1998; 83: 1440-1448.

[17] Price CH. Primary bone-forming tumours and their relationship to skeletal growth. J Bone Joint Surg Br 1958; 40-B: 574-593.

[18] Mirabello L, Troisi RJ and Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer 2009; 115: 1531-1543.

[19] Hawkins MM, Wilson LM, Burton HS, Potok MH, Winter DL, Marsden HB and Stovall MA. Radiotherapy, alkylating agents, and risk of bone cancer after childhood cancer. J Natl Cancer Inst 1996; 88: 270-278.

[20] Tucker MA, D'Angio GJ, Boice JD Jr, Strong LC, Li FP, Stovall M, Stone BJ, Green DM, Lombardi F, Newton W and et al. Bone sarcomas linked to radiotherapy and chemotherapy in children. N Engl J Med 1987; 317: 588-593.

[21] Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA and et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990; 250: 1233-1238.

[22] Hickson ID. RecQ helicases: caretakers of the genome. Nat Rev Cancer 2003; 3: 169-178.

[23] Benedict WF, Fung YK and Murphree AL. The gene responsible for the development of retinoblastoma and osteosarcoma. Cancer 1988; 62: 1691-1694.

[24] Li FP, Fraumeni JF Jr, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA and Miller RW. A cancer family syndrome in twenty-four kindreds. Cancer Res 1988; 48: 5358-5362.

[25] Varley JM. Germline TP53 mutations and Li-Fraumeni syndrome. Hum Mutat 2003; 21: 313-320.

[26] Porter DE, Holden ST, Steel CM, Cohen BB, Wallace MR and Reid R. A significant proportion of patients with osteosarcoma may belong to Li-Fraumeni cancer families. J Bone Joint Surg Br 1992; 74: 883-886.

[27] Wong FL, Boice JD Jr, Abramson DH, Tarone RE, Kleinerman RA, Stovall M, Goldman MB, Seddon JM, Tarbell N, Fraumeni JF Jr and Li FP.

Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. JAMA 1997; 278: 1262-1267.

[28] Hansen MF, Koufos A, Gallie BL, Phillips RA, Fodstad O, Brogger A, Gedde-Dahl T and Cavenee WK. Osteosarcoma and retinoblastoma: a shared chromosomal mechanism revealing recessive predisposition. Proc Natl Acad Sci USA 1985; 82: 6216-6220.

[29] Chauveinc L, Mosseri V, Quintana E, Desjardins L, Schlienger P, Doz F and Dutrillaux B. Osteosarcoma following retinoblastoma: age at onset and latency period. Ophthalmic Genet 2001; 22: 77-88.

[30] Goto M, Miller RW, Ishikawa Y and Sugano H. Excess of rare cancers in Werner syndrome (adult progeria). Cancer Epidemiol Biomarkers Prev 1996; 5: 239-246.

[31] German J, Ellis NA and Proytcheva M. Bloom's syndrome XIX. Cytogenetic and population evidence for genetic heterogeneity. Clin Genet 1996; 49: 223-231.

[32] Leonard A, Craft AW, Moss C and Malcolm AJ. Osteogenic sarcoma in the Rothmund-Thomson syndrome. Med Pediatr Oncol 1996; 26: 249-253.

[33] Kansara M and Thomas DM. Molecular pathogenesis of osteosarcoma. DNA Cell Biol 2007; 26: 1-18.

[34] Juul A. The effects of oestrogens on linear bone growth. Hum Reprod Update 2001; 7: 303-313.

[35] Bland R. Steroid hormone receptor expression and action in bone. Clin Sci (Lond) 2000; 98: 217-240.

[36] Le Roith D, Bondy C, Yakar S, Liu JL and Butler A. The somatomedin hypothesis: 2001. Endocr Rev 2001; 22: 53-74.

[37] Gill MS, Tillmann V, Veldhuis JD and Clayton PE. Patterns of GH output and their synchrony with short-term height increments influence stature and growth performance in normal children. J Clin Endocrinol Metab 2001; 86: 5860-5863.

[38] Styne DM. Physiology of Puberty. Adolesc Med 1994; 5: 171-188.

[39] Wang J, Zhou J, Cheng CM, Kopchick JJ and Bondy CA. Evidence supporting dual, IGF-I-independent and IGF-I-dependent, roles for GH in promoting longitudinal bone growth. J Endocrinol 2004; 180: 247-255.

[40] Palmert MR and Boepple PA. Variation in the timing of puberty: clinical spectrum and genetic investigation. J Clin Endocrinol Metab 2001; 86: 2364-2368.

[41] Brown LB, Streeten EA, Shuldiner AR, Almasy LA, Peyser PA and Mitchell BD. Assessment of sex-specific genetic and environmental effects on bone mineral density. Genet Epidemiol 2004; 27: 153-161.

[42] Savage SA, Woodson K, Walk E, Modi W, Liao J, Douglass C, Hoover RN and Chanock SJ. Analysis of genes critical for growth regulation identifies Insulin-like Growth Factor 2 Receptor variations with possible functional significance as risk factors for osteosarcoma. Cancer Epidemiol Biomarkers Prev 2007; 16: 1667-1674.

[43] Savage SA and Mirabello L. Using epidemiology and genomics to understand osteosarcoma etiology. Sarcoma 2011; 2011: 548151.

[44] Steele JR, Wellemeyer AS, Hansen MJ, Reaman GH and Ross JA. Childhood cancer research network: a North American Pediatric Cancer Registry. Cancer Epidemiol Biomarkers Prev 2006; 15: 1241-1242.

[45] The International HapMap Consortium. The International HapMap Project. Nature 2003 Dec 18; 426: 789-96.

[46] Chanock S. Genetic variation and hematology: single-nucleotide polymorphisms, haplotypes, and complex disease. Semin Hematol 2003; 40: 321-328.

[47] Ng PC and Henikoff S. Accounting for human polymorphisms predicted to affect protein function. Genome Res 2002; 12: 436-446.

[48] Ng PC and Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003; 31: 3812-3814.

[49] Ahsan H, Hodge SE, Heiman GA, Begg MD and Susser ES. Relative risk for genetic associations: the case-parent triad as a variant of case-cohort design. Int J Epidemiol 2002; 31: 669-678.

[50] Weinberg CR and Umbach DM. Choosing a retrospective design to assess joint genetic and environmental contributions to risk. Am J Epidemiol 2000; 152: 197-203.

[51] Weinberg CR, Wilcox AJ and Lie RT. A log-linear approach to case-parent-triad data: assessing effects of disease genes that act either directly or through maternal effects and that may be subject to parental imprinting. Am J Hum Genet 1998; 62: 969-978.

[52] Bergemann TL and Huang Z. A new method to account for missing data in case-parent triad studies. Hum Hered 2009; 68: 268-277.