

BRIEF COMMUNICATION

Germline TP53 Variants and Susceptibility to Osteosarcoma

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Abstract

The etiologic contribution of germline genetic variation to sporadic osteosarcoma is not well understood. Osteosarcoma is a sentinel cancer of Li-Fraumeni syndrome (LFS), in which approximately 70% of families meeting the classic criteria have germline TP53 mutations. We sequenced TP53 exons in 765 osteosarcoma cases. Data were analyzed with χ^2 tests, logistic regression, and Cox proportional hazards regression models. We observed a high frequency of young osteosarcoma cases (age <30 years) carrying a known LFS- or likely LFS-associated mutation (3.8%) or rare exonic variant (5.7%) with an overall frequency of 9.5%, compared with none in case patients age 30 years and older ($P < .001$). This high TP53 mutation prevalence in young osteosarcoma cases is statistically significantly greater than the previously reported prevalence of 3% ($P = .0024$). We identified a novel association between a TP53 rare variant and metastasis at diagnosis of osteosarcoma (rs1800372, odds ratio = 4.27, 95% confidence interval = 1.2 to 15.5, $P = .026$). Genetic susceptibility to young onset osteosarcoma is distinct from older adult onset osteosarcoma, with a high frequency of LFS-associated and rare exonic TP53 variants.

Osteosarcoma, the most common primary bone malignancy, has a bimodal age incidence distribution, with a primary peak in adolescence and a smaller peak in the elderly (1). There are no substantial differences in incidence by ancestry; worldwide incidence patterns in adolescents and young adults are similar (1,2). Osteosarcoma risk factors include tall stature, high birth weight, and previous therapeutic radiation (3,4).

Candidate gene studies and a recent genome-wide association (GWAS) study have found several common single nucleotide polymorphisms (SNPs) associated with osteosarcoma (5–7). Osteosarcoma occurs at higher-than-expected frequencies in individuals with certain rare cancer predisposition syndromes (eg, Li-Fraumeni Syndrome [LFS]) (8). However, collectively these rare syndromes and common SNPs account for

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a small portion of the genetic contribution to osteosarcoma etiology (6).

LFS is a highly penetrant, autosomal dominant (AD) cancer predisposition syndrome associated with a wide range of cancer types occurring at younger-than-expected ages (9–12). Osteosarcoma is diagnosed in approximately 12% of individuals with LFS and may be the first cancer suggestive of LFS in a family (13–15). AD germline *TP53* mutations are identified in approximately 70% of classic LFS families and approximately 20% of families that meet the Chompret criteria (16,17).

The presence of germline *TP53* mutations in sporadic osteosarcoma has been reported to be 3% to 7% (18,19). Twelve *TP53* SNPs were evaluated in a small case-control study which found associations between two SNPs (rs1642785 and rs1042522 [p.P72R]) and osteosarcoma (20), but other coding variants in *TP53* were not evaluated. A subsequent study did not confirm the risk association between osteosarcoma and rs1042522, although they found that patients with this variant had a statistically significantly increased mortality risk (21). *TP53* is somatically mutated in many sporadic osteosarcoma tumors (22); somatic *TP53* mutations have been suggested to be associated with reduced survival in osteosarcoma patients (23).

We determined the frequency of *TP53* exonic germline variants in a set of 765 unselected osteosarcoma case patients (Supplementary Table 1, available online) using targeted next-generation sequencing. Participating subjects provided informed consent under the auspices of local institutional review boards. Information on patient family histories and tumor samples was not available. Germline DNA was extracted from blood or buccal cells and sequenced using custom Ion Torrent Ampliseq panels for all *TP53* exons and intronic flanking regions (Supplementary Table 2, available online). Variants were validated with Sanger sequencing. The IARC germline *TP53* database Version R17 (November 2013) (<http://www-p53.iarc.fr/Germline.html>) was used to identify *TP53* mutations that have been reported in families with LFS (termed “LFS-associated mutations”) (24). Variants were considered “likely LFS-associated mutations” if absent from publically available databases and predicted nonfunctional or deleterious/disease-causing using multiple algorithms (24–29) (Supplementary Table 3, available online). Variants were considered “rare exonic variants” if their minor allele frequency (MAF) was less than 2% in the National Heart, Lung, and Blood Institute Exome Sequencing Project (ESP) (<http://evs.gs.washington.edu/EVS/>) and/or the 1000 Genomes Project (<http://www.1000genomes.org/>) (30) and they had uncertain clinical significance (Supplementary Table 3, available online). European ancestry was determined based on eigenvectors reported in our previously published GWAS (5).

Statistical significance for the difference between case patients with an LFS-associated or likely LFS mutation or rare exonic variant and case patients without these variants was assessed using chi-squared tests. The mutation frequency confidence intervals (CI) were estimated based on the standard normal approximation. Overall survival was assessed with Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals adjusted for study population, age, and sex. The assumption of proportional hazards was assessed and verified based on the Schoenfeld residuals (31). Overall survival time was estimated as the time from the date of diagnosis until the date of death or the last known alive date; patients were censored at the last date to be alive or if lost to follow-up. Logistic regression models were used to calculate the odds ratio (OR) and 95% confidence interval per copy of the minor allele assuming a multiplicative (log-additive) genetic model adjusted

for study population, age, and sex. Analyses were performed with SPSS version 21.0 and R version 3.1.2; all statistical tests were two-sided.

There were 32 LFS-associated or rare *TP53* variants in 62 of the 765 osteosarcoma case patients. Fifteen LFS-associated mutations and six likely LFS-associated mutations were present in a total of 24 individuals (Supplementary Table 3, available online). Eleven rare exonic variants, present in 38 individuals, had MAFs of 0.07% to 1.2%. The characteristics and clinical outcomes of the osteosarcoma cases by mutation/variant status are shown in Supplementary Table 4 (available online). Three case patients had both an LFS-associated mutation and a rare exonic variant; two of these case patients had poor survival (9 months and 25 months from diagnosis), and the third had metastasis at diagnosis.

The overall frequency of case patients with an LFS or likely LFS-associated mutation and/or rare exonic variant was 8.1% (95% CI = 6.3% to 10.3%), and 7.6% (95% CI = 5.5% to 10.4%) of case patients of European ancestry (Table 1). Case patients with an LFS- or likely LFS-associated mutation or rare exonic variant were statistically significantly younger at diagnosis compared with case patients without these variants ($P < .001$) (Supplementary Table 4, available online). Notably, all 32 *TP53* variants were present in case patients less than 30 years of age (“young cases”, $n = 505$) compared with none in older cases ($n = 51$) (Table 1). LFS- or likely LFS-associated mutations, which confer substantial cancer risk, were present in 3.8% of young case patients; another 5.7% of the young case patients had a rare exonic variant of uncertain clinical significance. *TP53* variants did not have an even distribution within the first three age decades in young case patients; LFS- or likely LFS-associated mutations had the highest frequency in patients age 0 to 9 years (4.8% of all case patients, and 6.1% of European case patients), and rare exonic variants were most frequent in patients age 10 to 19 years (6.1% of all case patients, and 5.6% of European case patients) (Table 1; Supplementary Figure 1, available online). *TP53* mutation frequencies were similar when restricted to only case patients of European ancestry (Table 1).

A Cox proportional hazards model suggested that young case patients with LFS-associated mutations had worse overall survival compared with young case patients without an LFS mutation (HR = 1.64, 95% CI = 0.76 to 3.54), although not statistically significant ($P = .21$). None of the *TP53* variants were statistically significantly associated with osteosarcoma survival or relapse.

We performed a logistic regression case-case analysis to determine if any of the *TP53* mutations/variants were associated with metastasis at diagnosis. rs1800372 (p.R213R), a rare synonymous variant leading to an exonic splice site change (variant of uncertain clinical significance) (Supplementary Table 3, available online), was statistically significantly associated with metastasis in cases of European ancestry (OR = 4.27, 95% CI = 1.2 to 15.5, $P = .026$) (Table 2).

We compared the frequency of the common exonic variant, rs1042522 (p.P72R), previously associated with risk of osteosarcoma (20), in this study to the frequency observed in ESP for individuals of European ancestry. rs1042522 was statistically significantly associated with osteosarcoma (OR = 1.22, 95% CI = 1.1 to 1.4, $P = .0098$) (Table 2) and with lower survival independent of presence of metastatic disease (HR = 1.35, 95% CI = 1.00 to 1.83, $P = .048$).

Our findings suggest that LFS- or likely LFS-associated *TP53* mutations and rare exonic *TP53* variants are important osteosarcoma risk factors, especially in individuals within its primary incidence peak (<30 years of age). In this study, we found

Table 1. Frequency of germline TP53 exonic variants in unselected osteosarcoma case patients*

TP53 variant	All case patients (n = 765)†					Cases of European ancestry (n = 498)†‡				
	All (n = 765)	Age 0–9 y (n = 63)	Age 10–19 y (n = 391)	Age 20–29 y (n = 51)	Age ≥30 y (n = 51)	All (n = 498)	Age 0–9 y (n = 33)	Age 10–19 y (n = 269)	Age 20–29 y (n = 40)	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Total LFS mutations	24 (3.13)	3 (4.76)	15 (3.84)	1 (1.96)	0	16 (3.21)	2 (6.06)	9 (3.34)	1 (2.50)	
LFS-associated mutation §	18 (2.35)	3 (4.76)	10 (2.56)	1 (1.96)	0	13 (2.61)	2 (6.06)	6 (2.23)	1 (2.50)	
Likely LFS-associated mutation †	6 (0.78)	0	5 (1.28)	0	0	3 (0.60)	0	3 (1.11)	0	
Total rare exonic variants	38 (4.97)	2 (3.18)	24 (6.14)	3 (5.88)	0	22 (4.42)	0	15 (5.58)	3 (7.50)	
Predicted deleterious ¶	24 (3.14)	1 (1.59)	18 (4.60)	3 (5.88)	0	17 (3.41)	0	10 (3.72)	3 (7.50)	
Predicted neutral or a SNP	14 (1.83)	1 (1.59)	6 (1.54)	0	0	5 (1.00)	0	5 (1.86)	0	
Total	62 (8.10)	5 (7.94)	39 (9.98)	4 (7.84)	0	38 (7.63)	2 (6.06)	24 (8.92)	4 (10.00)	
95% Confidence intervals #	6.32 to 10.33	2.96 to 18.37	7.27 to 13.49	2.54 to 19.75		5.52 to 10.41	1.06 to 21.62	5.92 to 13.15	3.25 to 24.60	

* LFS = Li-Fraumeni syndrome; SNP = single nucleotide polymorphism.

† Not all case patients had age at diagnosis data available; counts (% of total) are given for the case patients with these data.

‡ Ancestry was determined based on eigenvectors reported in our previously published GWAS (5).

§ Reported in LFS families in the International Agency for Research on Cancer database of TP53 germline mutations.

† Predicted to be deleterious or disease-causing in multiple prediction programs and absent from publically available databases (ESP and the 1000 Genomes Project).

¶ Predicted to be deleterious or disease-causing by at least one prediction program.

95% confidence intervals around the total frequency estimates determined based on the standard normal approximation.

Table 2. TP53 exonic variants associated with osteosarcoma risk or metastasis*

Position	rsID	Variant	Case patients	Control patients		P
			No. (MAF, %)	No. (MAF, %)	OR (95% CI)	
Association with metastasis at diagnosis						
Chr17:g7578210	rs1800372	p.R213R; exonic splice site change	99 (3.03)	269 (0.74)†	4.27 (1.18 to 15.48)	.0269
Association with osteosarcoma risk						
Chr17:g7579472	rs1042522	p.P72R	498 (29.08)	3510 (25.18)‡	1.22 (1.05 to 1.42)	.0098

* Logistic regression models were used to estimate the odds ratios and 95% confidence intervals per copy of the minor allele assuming a log-additive genetic model. CI = confidence interval; MAF = minor allele frequency; OR = odds ratio.

† Case patients of European ancestry with confirmed metastasis at diagnosis (n = 99) compared with case patients of European ancestry without metastasis at diagnosis (n = 269).

‡ Case patients of European ancestry (n = 498) compared with National Heart, Lung, and Blood Institute Exome Sequencing Project individuals of European ancestry (n = 3510).

a 9.5% frequency of LFS-associated or rare exonic TP53 germline variants in young individuals with osteosarcoma (8.8% of young case patients of European ancestry). The two earlier studies by McIntyre et al. (18) and Toguchida et al. (19) estimated that 3% (95% CI = 1.5% to 6.0%) of 235 unselected young osteosarcoma case patients (<20 years of age) and 7.4% (95% CI = 3.6% to 14.4%) of 95 selected (for positive family history or multiple primaries) and unselected case patients of all ages, respectively, had TP53 mutations. In comparison with the study of 235 unselected children with osteosarcoma (18), our TP53 mutation prevalence of 9.69% is statistically significantly higher than their estimate of 3% in a similar age group (<20 years of age, P = .0024). Our data show that osteosarcoma TP53 mutation prevalence is on the high end of the previous estimates of germline TP53 mutations in 2.5% to 10% of early onset sarcomas (<45 years old) (13,19,32–34).

We do not have complete family history information for our retrospectively collected set of patients. Therefore, a limitation of our study is that we cannot comment on the clinical histories

of their families nor whether these TP53 variants were de novo or inherited. A strength of our study is that this case set represents an unselected random set of 765 osteosarcoma case patients. To the best of our knowledge, this is the largest patient population evaluated for TP53 mutations to date, which provides more precise mutation prevalence estimates and has the potential to alter clinical practices. We previously showed that adult onset osteosarcoma has distinct epidemiologic features (incidence rates, including occurrence with Paget's disease and after a prior malignancy, tumor location, survival) (1). Our current data further suggests that young onset osteosarcoma has a different germline genetic etiology. The high rate of pediatric osteosarcoma cases with an LFS- or likely LFS-associated germline TP53 mutation (3.8%) is important because these individuals, and possibly their family members, may be at risk of developing other LFS-associated cancers. The TP53 mutation prevalence in this population is comparable with that observed in early-onset breast cancer patients age 35 years or younger (2% to 5%) (35–37), a population in which clinical TP53 mutation testing

is recommended (National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Breast and Ovarian. <http://www.nccn.org>). Based on our findings, we suggest consideration of genetic counseling and TP53 mutation testing in young patients with osteosarcoma, especially if there is a history of cancer in close relatives. Moreover, the presence of a germline TP53 mutation requires careful follow-up, as there could be a substantial risk for a second malignancy. Recent advances in cancer screening in individuals with LFS, using modalities such as rapid-sequence whole body MRI in combination with other screening tests (38), have the potential to lead to early cancer detection, which in turn could result in improved survival, particularly in what may be a high cancer risk population.

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