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Frequent inactivating germline mutations in DNA repair genes in patients with Ewing sarcoma Germline mutations in Ewing sarcoma

Andrew S Brohl, M.D.[1,*], **Rajesh Patidar, M.S.**[2], **Clesson E Turner, M.D.**[3], **Xinyu Wen, M.S.**[2], **Young K Song, Ph.D.**[2], **Jun S Wei, Ph.D.**[2], **Kathleen A Calzone, Ph.D.**[2], and **Javed Khan, M.D.**[2,**]

[1]Sarcoma Department, H. Lee Moffitt Cancer Center, Tampa, FL, 33612, USA

[2]Genetics Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, 20892, USA

[3]Cancer Genetics Services, Walter Reed National Military Medical Center, Bethesda, MD, 20889, USA

Abstract

Purpose—Ewing sarcoma is a highly malignant small round blue cell tumor that predominantly affects the adolescent and young adult population. It has long been suspected that a genetic predisposition exists for this cancer, but the germline genetic underpinnings of this disease have not been well established.

Methods—We performed germline variant analysis of whole genome or whole exome sequencing of samples from 175 patients affected by Ewing sarcoma.

Results—We discovered pathogenic or likely pathogenic germline mutations in 13.1% of our cohort. Pathogenic mutations were highly enriched for genes involved with DNA damage repair and for genes associated with cancer predisposition syndromes.

Conclusion—Our findings reported here have important clinical implications for patients and families affected by Ewing sarcoma. Genetic counseling should be considered for patients and families affected by this disease to take advantage of existing risk management strategies. Our study also highlights the importance of germline sequencing for patients enrolled on precision medicine protocols.

Keywords

Ewing sarcoma; genetics; germline; DNA repair; next-generation sequencing

*Corresponding Authors: Andrew S. Brohl, M.D., 12902 Magnolia Drive, Tampa, FL 33612-9416, (813)745-3242 (p), (813)745-8337 (f), andrew.brohl@moffitt.org. **Corresponding Authors: Javed Khan, M.D., 37 Convent Drive, Building 37, Room 2016B, Bethesda, MD 20892, khanjav@mail.nih.gov.

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Introduction

Ewing sarcoma is a highly malignant small round blue cell tumor that predominantly affects the adolescent and young adult population. It has long been suspected that a genetic predisposition exists for this cancer due to the young age of patients involved and wide demographic variations in its incidence[1]. Furthermore, it has recently been recognized that there are an excess of cancers amongst relatives of Ewing sarcoma patients, including a subset of patients that have a pedigree suggestive of a familial cancer syndrome[2].Ewing sarcoma has also been significantly associated with hereditary retinoblastoma based on a meta-analysis that included 10 such cases reported in the literature [3]. Germline mutations in cancer predisposition genes have recently been described in 8.5-12% of pediatric cancer cases across a range of cancer types[4-6]. An excess of pathogenic germline variants has also recently been reported in a large cohort of sarcoma patients encompassing a variety of histologic subtypes [7].

Here we report a germline next-generation sequencing analysis of 175 patients with Ewing sarcoma, which is the largest and most comprehensive germline genomics analysis to date in this rare tumor type. Our data is derived from three cohorts that have been previously analyzed for somatic mutational spectrum: the National Cancer Institute (NCI, 56 subjects), the International Cancer Genome Consortium (ICGC, 100 subjects), and the Pediatric Cancer Genome Project (PCGP, 19 subjects)[8,9].

Materials and Methods

Raw sequencing data from the ICGC and PCGP cohorts was accessed from the European Genome-phenome archive, accession numbers EGAS00001000855 (ICGC) and EGA00001000839 (PCGP), respectively. Details of patient selection, informed consent, and clinical characteristics have been previously reported[8,9]. Additional data description is provided in Supplementary Methods.

WGS/WES were processed and mapped and variants called using methods employed previously by our group with very high validation rates[5,8,10]. To limit the number of variants for manual review, we implemented a bioinformatics pipeline that included filters for quality, rarity in population databases, and curated knowledge databases such as ClinVar (supplementary methods, Figure S1). After application of the above bioinformatics pipeline, the resultant “Tier 1” variants were manually evaluated and classified by a medical oncologist and medical geneticist according to American College of Medical Genetics and Genomics (ACMG) guidelines[11]. The ACMG classification was the final result used for reporting.

To perform burden testing for genes with pathogenic/likely pathogenic variants, we compared the rate of these classes of variants in these genes in our Ewing sarcoma cohort to that in the ExAC population database, minus samples contributed from TCGA. Variants downloaded from the ExAC database were subject to the same classification methods as the study group. Two-sided Fischer exact test was used for statistical comparison.

Pathway analysis of pathogenic/likely pathogenic variants was performed using Ingenuity Pathway Analysis software (http://www.ingenuity.com/products/ipa).

Results

In the cohort of 175 Ewing sarcoma patients analyzed, we identified 52 Tier 1 variants for further manual classification. Of these, we classified 23 as pathogenic or likely pathogenic (Table S1), 12 as variants of unknown significance (VUS) (Table S2), 4 as likely benign and 4 were heterozygous pathogenic variants in *MUTYH* (Table S3). The nine remaining variants we placed in a separate category as truncating mutations in a tumor suppressor gene that is not reported to be a germline cancer predisposition gene (Table S4). To help ensure that no potential pathogenic variants were missed due to strict initial filtering, we manually reviewed all lower tier mutations in the 22 genes in which a pathogenic or likely pathogenic mutation was found. No additional pathogenic/likely pathogenic mutations were identified from this expanded review.

The pathogenic/likely pathogenic variants were all mutually exclusive by patient, and were therefore found in 13.1% of the population studied. These variants were found in similar percentages amongst the different cohorts studied (NCI 14.3% vs. ICGC 12.0% vs. PCGP 15.8%, p=0.85), between WGS and WES samples (12.0% vs. 16.0%, p = 0.62) and between matched sequencing and tumor-only sequencing (12.3% vs. 16.2%, p=0.58). Variants of unknown significance were similarly well distributed between groups (Table S2). Truncating mutations in non-syndromic tumor suppressor genes, however, were much more commonly observed in tumor-only sequencing samples (19.4% vs. 2.2%, p=0.003), suggesting that some of these mutations are likely somatic variants that have not been previously reported simply because they are not highly recurrent in this tumor type (Table S4).

The 23 variants deemed to be pathogenic/likely pathogenic included 22 different genes, with only *BLM* having multiple pathogenic mutations (Table 1). There were, however, several functional or disease-related clusters of genes affected. Pathway analysis revealed a striking enrichment for hereditary breast cancer signaling, DNA repair pathways, and notably DNA double-strand break repair (Table 2). Enrichment in DNA damage response elements, such as ATM signaling and GADD45 signaling, embryonic stem cell pluripotency and molecular mechanisms of cancer were also noted.

To evaluate the possibility that the pathogenic/likely pathogenic variants were detected at a rate that is similar to that of the general population, we evaluated germline variants from these same 22 genes in the ExAC database (minus TCGA), which included data from 53,105 subjects. We identified 1367 pathogenic/likely pathogenic variants in this population, or 2.57%, which is significantly lower than the 13.1% affected in the Ewing sarcoma cohort (p=2e-10). We performed similar analysis for heterozygous mutations in *MUTYH*. We considered this gene separately as heterozygous pathogenic mutations in this gene are not uncommon in the general population. The rate of *MUTYH* pathogenic/likely pathogenic mutation was not significantly different between groups (2.29% vs. 1.08%, p=0.12).

We evaluated the potential association between germline pathogenic mutation and the known recurrent somatic mutations in *STAG2*, *TP53* and *CDKN2A* in Ewing sarcoma. There were no significant differences between patients with a pathogenic/likely pathogenic germline mutation and those without with regards to rates of somatic mutations in *STAG2* (18.1% vs. 17.8%, p=1.0), *CDKN2A* (13.6% vs. 12.3%, p=1.0), or *TP53* (9.1% vs. 8.9%, p=1.0). Interestingly, we noted that the two patients with a somatic mutation in *TP53* in the pathogenic/likely pathogenic group include the two patients with germline mutations in either *TP53* itself or the *TP53*-associated gene *WRAP53,* suggesting a germline/somatic oncogenic synergy in these two cases. For matched samples, we evaluated for additional examples of second hits in tumors from patients affected by a pathogenic/likely pathogenic germline mutation, either by loss of heterozygosity or by a truncating somatic mutation, but observed no additional cases. We additionally evaluated for potential associations between pathogenic/likely pathogenic mutations and demographic or outcomes characteristics in the subset of patients for which this data was available[8,9]. There were no observed differences between groups in gender. There was a trend towards younger age amongst patients with pathogenic/likely pathogenic germline mutation than those without (57.1% vs. 42.3% under age 12, 42.9% vs. 50.8% age 12-24, 0 vs. 6.9% over age 24), but this result did not reach statistical significance (p=0.31). There was no significant difference in rates of death due to disease. All of these comparisons were limited by small numbers of patients with available data (Table S5).

Discussion

We present the largest and most comprehensive germline genomics analysis to date in patients with Ewing sarcoma, utilizing whole genome or whole exome sequencing data from 175 patients. We discovered a high rate of pathogenic or likely pathogenic mutations, accounting for 13.1% of the population. This rate is similar but slightly higher than what has recently been reported in pediatric malignancies more generally, including in smaller subsets of Ewing sarcoma patients [4-6]. Differences in methodology likely account the difference in incidence between our study and previous, in particular how broad of a gene set was utilized for reporting. For example, we considered heterozygous deleterious mutation in any member of the Fanconi anemia gene family to likely predispose to solid malignancies and thus considered these as likely pathogenic in our population. Next generation sequencing has increasingly identified heterozygous deleterious mutation of members of this gene family outside of the more well established *BRCA1* and *BRCA2* as predisposing to solid tumors [12,13], supporting our choice. Furthermore, a strong association has recently been reported between heterozygous germline variants in Fanconi anemia genes and translocation-associated sarcomas [7]. We additionally performed comparison to a large population database and found that pathogenic/likely pathogenic mutations in the genes reported are much more common in our cohort as compared to controls.

We found that pathogenic germline mutations in Ewing sarcoma are not highly recurrent in a single gene, but rather spread across a number of genes with potentially similar functional clustering. Mutations affecting DNA double strand repair, in particular, were highly enriched. We found that second somatic hits in the same genes were uncommon, which is consistent with one previous report in which 0 of 5 Ewing sarcoma patients with pathogenic/

likely pathogenic germline mutation had a second somatic hit [4]. Given that gain of a somatic EWS-ETS fusion is believed to be the seminal event in Ewing sarcoma development [8,9,14], we speculate that the pathogenic germline mutations observed may be permissive to the development of DNA breaks and subsequent translocation. Previous work has also noted a similar association between deleterious germline mutation in DNA repair genes and other translocation-associated sarcomas [7]. We do caution that similar gene classes of pathogenic germline mutations have been described in the previously aforementioned pediatric malignancies studies that were not Ewing sarcoma-specific. Further study is therefore warranted to clarify whether a functional association between this class of mutation and the development of fusion-driven cancer truly exists.

One potential limitation of our study is including a portion of patients with tumor-only sequencing. In this smaller subset of patients, we were therefore unable to confirm our findings as germline versus somatic. However, the rate of pathogenic/likely pathogenic mutations discovered is very similar in this subgroup to the overall cohort, and removing these patients from analysis results in a virtually identical rate of pathogenic/likely pathogenic mutation overall (13.1% vs. 12.3%). Additionally, three large sequencing efforts in Ewing sarcoma have not reported recurrent somatic mutation any of the genes identified here [8,9,14].

It should be noted that our study was designed to capture only the most clinically relevant germline mutations (i.e. those that would be considered pathogenic or likely pathogenic). The vast majority of VUS mutations, some of which may in time be proven to be clinically important, would have been filtered by design and are outside the scope of this evaluation. Additionally, we did not pursue copy number evaluation as part of our analysis, as we feel that copy number detection from whole genome or whole exome sequencing is more prone to false positive detection than small variant detection. We also did not include variants that did not affect either a coding region or splice site. Finally, as most of our data was derived from online repositories or samples from outside collaborators, we did not have access to family history or familial samples, so were unable to assess whether variants were *de novo* or inherited.

Our findings reported here have important clinical implications for patients and families affected by Ewing sarcoma. Given the high rates of pathogenic germline mutations in this population, we believe referral to a genetic specialist should be considered for all patients and families affected by this disease. Though screening for Ewing sarcoma itself is unlikely to be undertaken given the rarity of the disease even in those with a predisposing mutation, patients that survive their cancer and/or potentially family members may benefit from existing risk management strategies for those with deleterious mutations in genes such as *APC* or *BRCA1* that are associated with cancer syndromes that have a screening or surgical risk reduction management option. For the patients themselves, many of these germline variants may also influence cancer treatment or at least suggest the use of novel therapies, ideally in the setting of clinical trial. As examples, carriers of germline *BRCA1* or *BRCA2* mutations, even outside of breast or ovarian cancer, may benefit from treatment with PARP inhibitors[15,16], while Hedgehog pathway inhibitors have activity in tumors associated with germline mutations in *PTCH1* or *PTCH2*[17,18]. The high frequency of potentially actionable

germline alterations in Ewing sarcoma should also be considered as personalized medicine approaches are designed and contemplated for patients affected by this disease. This study adds to a growing list that highlights the importance of germline sequencing for patients enrolled on precision therapy protocols[5,19].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1
Pathogenic/likely pathogenic variants Identified in Ewing sarcoma patients

Gene	Variant	dbSNP	Notes
APC	NM_001127511:c.806_807insAACAGCC:p.E269fs		
BLM	NM_000057:c.3384_3385insTATTTGTATACTT:p.S1128fs		*1*
BLM	NM_000057:c.C1933T:p.Q645X	rs373525781	*3*
BRCA1	NM_007300:c.3481_3491del:p.E1161fs	rs80357877	
BRIP1	NM_032043:c.C2392T:p.R798X	rs137852986	*2*
ERCC3	NM_000122:c.1421dupA:p.D474fs		*1*
EXT2	NM_000401:c.69+2insAGGG (splice site)		
FANCC	NM_000136:c.C553T:p.R185X	rs121917783	*2*
FANCD2	NM_033084:c.2715+1G>A (splice site)	rs201811817	*1*
FANCM	NM_020937:c.2191_2192del:p.L731fs		
FLCN	NM_144606:c.G918A:p.W306X	rs142934950	*1*
MITF	NM_000248:c.G952A:p.E318K	rs149617956	
PMS2	NM_000535:c.G137T:p.S46I	rs121434629	
POLE	NM_006231:c.4090dupC:p.R1364fs		
PTCH2	NM_001166292:c.3311_3312insA:p.L1104fs		
PTPN11	NM_002834:c.A1529G:p.Q510R	rs121918470	
RAD51	NM_001164269:c.G452A:p.R151Q	rs121917739	*1*
RAD51D	NM_001142571:c.293delA:p.D98fs		*1*
RET	NM_020630:c.G2370C:p.L790F	rs75030001	*2*
SLX4	NM_032444:c.C5242T:p.Q1748X		
TINF2	NM_001099274:c.C936A:p.Y312X	rs201677741	
TP53	NM_001126115:c.C451T:p.R151C	rs149633775	
WRAP53	NM_018081:c.1558dupG:p.C519fs		

1=tumor only sequencing

2=previously reported Zheng et al. [4]

3=previously reported Chang et al. [5]

Table 2

Top enriched pathways affected by pathogenic/likely pathogenic germline variants in Ewing sarcoma patients.

Ingenuity Canonical Pathway	p value
Hereditary Breast Cancer Signaling	3.16E-13
Role of BRCA1 in DNA Damage Response	7.94E-13
ATM Signaling	3.02E-09
Ovarian Cancer Signaling	1.70E-07
Molecular Mechanisms of Cancer	1.20E-06
Basal Cell Carcinoma Signaling	5.01E-05
DNA Double-Strand Break Repair by Homologous Recombination	8.71E-05
Mouse Embryonic Stem Cell Pluripotency	1.15E-04
GADD45 Signaling	1.62E-04
DNA damage-induced 14-3-3σ Signaling	1.62E-04