TP53 Germline Pathogenic Variant Frequency in Anaplastic Rhabdomyosarcoma: A Children's Oncology Group Report

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Abstract

Rhabdomyosarcoma (RMS) is a well-described cancer in Li-Fraumeni Syndrome (LFS), resulting from germline TP53 pathogenic variants (PVs). RMS exhibiting anaplasia (anRMS) have been associated with a high rate of germline TP53 PVs. This study provides an updated estimate of the prevalence of TP53 germline PVs from a large cohort of patients (n=239) enrolled in five Children's Oncology Group (COG) clinical trials. Although the prevalence of germline TP53 PVs in anRMS patients in this series is much lower than previously reported, this prevalence remains significantly elevated. Germline genetic evaluation for TP53 PVs should be strongly considered in patients with anRMS.

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Abbreviations key:

RMS	Rhabdomyosarcoma
LFS	Li-Fraumeni Syndrome
PV	Pathogenic variant
anRMS	Rhabdomyosarcoma exhibiting anaplasia
COG	Children's Oncology Group
WT	Wild type

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Abstract

Rhabdomyosarcoma (RMS) is a well-described cancer in Li-Fraumeni Syndrome (LFS), resulting from germline *TP53* pathogenic variants (PVs). RMS exhibiting anaplasia (anRMS) have been associated with

a high rate of germline *TP53* PVs. This study provides an updated estimate of the prevalence of *TP53* germline PVs from a large cohort of patients (n=239) enrolled in five Children's Oncology Group (COG) clinical trials. Although the prevalence of germline *TP53*PVs in anRMS patients in this series is much lower than previously reported, this prevalence remains significantly elevated. Germline genetic evaluation for *TP53* PVs should be strongly considered in patients with anRMS.

INTRODUCTION

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood[1]. An association between RMS and other early-onset cancers was first described in 1969[2], became known as Li-Fraumeni Syndrome (LFS), and was subsequently shown to be caused by germline pathogenic variants (PVs) in the *TP53*tumor suppressor gene[3]. Evidence suggests that RMS patients who harbor germline *TP53* pathogenic variants (PVs) are more likely to exhibit anaplasia (anRMS)[4]. In 2014, Hettmer et al. reported in a retrospective limited series that the overall frequency of germline *TP53* PV in pediatric patients with anRMS was 73% (11 of 15 cases)[5]. These findings formed the basis of recommendations to test for *TP53* germline PV in patients with anRMS in the most recent Chompret Criteria and were adapted for testing criteria by the National Comprehensive Cancer Network (NCCN)[6, 7].

We sought to expand on the prior analysis of anaplasia and germline*TP53* PV and to avoid the potential for selection bias inherent in a limited institution study. We investigated a large RMS study population derived from Children's Oncology Group (COG) clinical trials which included central pathology review for the presence of anaplasia and germline *TP53* PV status from exome sequencing[8]. From this cohort, we estimated the prevalence of germline *TP53* PVs with and without anaplasia, as well as assessed *TP53* PV associations with other RMS tumor and patient characteristics.

METHODS

The study population was derived from patients enrolled on one of the five COG clinical trials for which central pathology review was performed prospectively to determine the presence of anaplasia and for which germline *TP53* data from exome sequencing was recently reported[8]. Two hundred and thirty-nine patients were identified from the five following COG studies: D9602 (n=18), D9802 (n=14), D9803 (n=29), ARST0331 (n=61), ARST0531 (n=117).

RESULTS

TP53 germline status, tumor histology, anaplasia status, sex of patient, age at diagnosis, primary tumor site, tumor size, nodal status, and tumor FOXO1 fusion status were evaluated. Histology, including anaplasia status, was performed through central review by three expert pediatric pathologists. Anaplasia was defined as the presence of enlarged hyperchromatic nuclei with or without multipolar mitotic figures[9, 10]. Focal anaplasia was defined as anaplastic cells loosely scattered among non-anaplastic cells, whereas diffuse anaplasia was defined as anaplastic cells that were aggregated in clusters or that formed continuous sheets[9]. Exome sequencing, variant filtering, and identification of *TP53* PVs, followed methods described in Li and colleagues[8].

The Fisher's Exact test was used to examine the association between categorical characteristics. The two-sample t-test was performed to compare age at diagnosis between *TP53* PV and wild type (WT) cohorts. Statistical significance was considered at the 0.05 level. The software SAS 9.4 was used for analysis.

The prevalence of germline *TP53* PVs among the entire RMS cohort was 3% (n=7/239). The median age of diagnosis was 2.8 years (range 0.9 to 3.7 years) and 5.8 years (range 0.2 to 28.3 years) (P=0.0003) for the*TP53* PV and the *TP53* WT patients, respectively. Among the entire cohort, histology was classified as alveolar (n=73), embryonal (n=122), botryoid (n=25), spindle cell (n=15), mixed (n=1), not otherwise specified (n=2), and unknown (n=1) (Table 1). Among patients with germline *TP53* PVs, histology included: embryonal (n=3), botryoid (n=2), and spindle cell (n=2); none had alveolar histology. There was

a statistically significant difference in histology type between the germline *TP53* PV patients and those without PVs (P=0.04).

Similar to other clinical reports[10], anaplasia was present in 19% (n=46) of the 239 patients (Table 2); 34 of which had diffuse anaplasia while 12 demonstrated focal anaplasia s (Table 3, supplement). Among the 46 patients with anRMS, 11% (n=5) carried a germline *TP53* PV compared to 1% (n=2) among patients without anaplasia (P=0.003). The proportion of *TP53* PVs in those with diffuse anaplasia and focal anaplasia were 9% (n=3) and 17% (n=2), respectively. Among the seven patients with *TP53* PV patients, 71% (5/7) exhibited anaplasia.

DISCUSSION

Using a population of 239 patients, this report provides the most comprehensive estimate to date of the prevalence of germline *TP53*PVs in pediatric patients with anRMS. We reconfirm the association between RMS and germline *TP53* PVs, and specifically, the association between anRMS and germline *TP53* PVs. However, our findings suggest that the prevalence of *TP53* PVs in anRMS is lower than previously estimated[5]. Specifically, we found in our combined cohort that approximately 11% of patients with anRMS have germline *TP53* PVs compared to the prior description where 75% anRMS patients had *TP53* PVs[5]. It is possible that smaller sample size and selection bias inherent to a limited institution study explains the discordance between our results. Furthermore, we describe the rate of a germline *TP53* PV in RMS without anaplasia to be approximately 1%.

Consistent with other reports[5, 11], we observed a high prevalence of anaplasia (71%) in patients harboring germline*TP53* PVs. We found a statistically significant difference in median age at diagnosis between patients with germline *TP53* PVs and *TP53* WT, in line with previous published reports[5, 11, 12]. A detailed comparison between tumor and clinical features in the germline *TP53* PV versus *TP53*WT cohorts revealed no other significant differences.

Limitations to our current study include the retrospective nature of this investigation and the lack of reporting of heterozygous deletions which may make up 1-5% of *TP53* PVs. Additionally, the number of cases was limited to those in which we had both anaplasia and*TP53* PV information, and therefore could bias the true prevalence of TP53 germline PVs in anRMS.

Although the prevalence of *TP53* PV in anRMS is lower than prior reports and leads to a decrease in the pretest probability in screening for germline *TP53* PVs in anRMS patients, the 11% prevalence of germline *TP53* PVs in anRMS still exceeds the threshold for recommendation for germline genetic evaluation[13]. This study provides the ongoing rationale to evaluate germline *TP53* in the context of RMS so that oncologists can improve the clinical approach to RMS patients and optimize screening practices for LFS in the RMS population.

CONFLICTS OF INTEREST

None

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