The role of *TTN* and *BAG3* in therapy-related cardiomyopathy among long-term survivors of childhood cancer: A report from the St. Jude Lifetime Cohort (SJLIFE) and the Childhood Cancer Survivor Study (CCSS)

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Background: Cancer therapy-related cardiomyopathy (CCM) is a well-established late effect among long-term survivors of childhood cancer. In the general population, some rare variants in *TTN* and *BAG3* increase the risk of familial cardiomyopathy, while their common missense variants (rs3829746 and rs2234962) are associated with a decreased risk of sporadic cardiomyopathy. The role of variants in *TTN* and *BAG3* in the risk of CCM among survivors of childhood cancer is unknown.

Methods: Five-year survivors of childhood cancer of European ancestry exposed to cardiotoxic therapies (anthracyclines and/or radiation (RT) exposing the heart) participating in the St. Jude Lifetime Cohort (SJLIFE; n=1,645) and Childhood Cancer Survivor Study (CCSS; n=4,604) were included. Association of common (MAF>5%) missense variants within *TTN* (n=40) and *BAG3* (n=6) with risk of CCM (CTCAE grade ≥2) was evaluated using logistic regression adjusting for age at primary cancer diagnosis, sex, cumulative anthracycline dose, mean heart RT dose, age at last contact and the top 10 PCs. Linear regression models were fit to assess the correlation between the nominally replicated SNPs and the ventricular and atrial functions determined by echocardiography in SJLIFE survivors. Carrier status of rare (MAF<1%) pathogenic/likely pathogenic (P/LP) variants in *TTN* (n=15) and *BAG3* (n=1) was examined with CCM risk using Fisher's Exact test. RNAseq data on human induced pluripotent stem cells derived cardiomyocytes (hiPSC-CMs) from patients with (n=3) and without (n=3) CCM was evaluated for changes in gene expression when treated with doxorubicin.

Results: In a combined analysis of SJLIFE and CCSS (median age at last contact = 38.3 years; range = 32.1-45.5 years), 15 of 46 common missense variants were associated with CCM risk ($p<0.05$). Of these, minor alleles of 13 missense variants including those of rs3829746 and rs2234962 were associated with a decreased risk of CCM (ORs, 0.78-0.83; $p<0.05$), while the minor alleles of the

remaining two were associated with an increased risk (ORs, 1.36 and 1.52). In SJLIFE, the minor allele of rs2234962 (*BAG3*) was also associated with increased ejection fraction (β=0.12; p=0.042) and decreased global longitudinal strain (β=-0.13; p=0.029), end diastolic (β=-0.12; p=0.042) and end systolic (β=-0.15; p=0.019) volumes. Rare P/LP variants in *TTN* and *BAG3* were not associated with CCM risk (p>0.52). Expression of *BAG3* was downregulated (fold change=0.79; p=0.052) in hiPSC-CMs from CCM patients compared to those without CCM.

Conclusions: Leveraging data from two large cohorts of long-term survivors of childhood cancer of European ancestry, we identified association of common missense variants in *TTN* and *BAG3* with decreased risk of CCM but no increased risk among survivors with rare variants in these genes.