Title: Frequency of pathogenic germline variation in pediatric pan-cancer survivors: A report from the Childhood Cancer Survivor Study (CCSS)

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Introduction. Pediatric cancer is the leading cause of death by disease in children despite improved survival rates. Here, we conducted the largest pediatric pan-cancer study to date using exome sequencing (ES) of 5,451 long-term survivors to quantify the frequency of germline pathogenic variation in cancer-susceptibility genes (CSG).

Methods. The CCSS is a multi-center retrospective cohort of children diagnosed <21 years of age who survived at least 5 years from a diagnosis of leukemia, lymphoma, brain tumors, neuroblastoma, Wilms tumor, bone cancers, and soft tissue sarcomas. Germline DNA underwent ES; analyses focused on rare variants in 237 previously published CSGs. Pathogenic/likely pathogenic (P/LP) variants were identified using ClinVar, HGMD with manual review, and InterVar. We compared the frequency of P/LP variants with the same pathogenicity criteria in 5,105 European-ancestry cases (CEU >0.8) vs. 51,377 gnomAD non-Finnish European cancer-free controls (ES data) using the Fisher’s exact test and corrected for multiple testing using a false discovery rate (FDR; q<0.05 considered significant). An unadjusted Kaplan-Meier analysis was used to estimate the differences in overall survival in children with and without P/LP variants.

Results. In 5,105 European-ancestry pediatric cancer survivors, 11% harbored a P/LP variant in a dominant CSG (n=176) vs. 9% in controls (P<0.0001). Eight dominant genes (NF1, WT1, TSC1, REST, KMT2D, EZH2, CDKN2A, MEN1) had significantly more P/LP variants in cases vs. controls (FDR q<0.05). We identified a novel germline cancer risk association in KMT2D (odds ratio [OR] 16.8, 95% CI 4.5-63.5, vs. controls). KMT2D, underlying Kabuki Syndrome, has only previously been associated somatically with cancer. In EZH2 (OR 4.7), CDKN2A (OR 8.6), and MEN1 (OR 20.1), we found multiple novel pediatric cancer associations. Children carrying a
P/LP variant had worse survival compared to children without a P/LP variant (P=0.001). Children with one P/LP variant in *SBDS*, an autosomal recessive CSG, had a significantly increased risk of cancer (FDR q<0.05).

**Conclusion.** To our knowledge, this is the largest pediatric pan-cancer study of pathogenic germline variation to date. In long-term cancer survivors, we found multiple novel gene-cancer associations and worse survival in children with a P/LP variant. These findings could have implications in cancer risk stratification and genetic counseling for patients and families.