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Response

Reply to Evans and Woodward

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We thank Drs Evans and Woodward for their correspondence (1) in which the following points related to our publication entitled "Heterozygous *BRCA1* and *BRCA2* and mismatch repair gene pathogenic variants in children and adolescents with cancer" are raised.

Point 1: The frequency of pathogenic and/or likely pathogenic variants (PVs) in the German control group is lower than the frequency observed in published series. We agree with this point, and for this reason, we included an analysis of a cancer-free Genome Aggregation Database (gnomAD) control group. The enrichment of PVs in *BRCA1* and 2 and mismatch repair (MMR) genes was reproduced—with borderline statistical significance for PVs in *BRCA1* and 2 combined—when we used the gnomAD control group. Similar results were reproduced in a supplementary analysis including 17 studies. In addition, analysis of a validation patient cohort confirmed an enrichment of PVs in *BRCA2* and *MSH2*—but not in *PMS2*—compared with a gnomAD control group. Notably, there are other independent recent publications (2-5) including one study focusing on the somatic mutation landscape (2) supporting the association between Lynch syndrome and childhood cancer. Other recent papers have demonstrated an enrichment of PVs in *BRCA2* in children with cancer (6,7). Together, these studies suggest that these syndromes have a low penetrant pediatric spectrum.

Point 2: Variants may have been missed (eg, in *PMS2*), and copy number variants were not included. Different sequencing pipelines and differences in pathogenicity assessment can confound burden testing. Therefore, we restricted our meta-analysis that included studies employing different pipelines to ClinVar PVs. This led to the intentional exclusion of copy number variants not included in ClinVar; however, this factor affects both cases and controls. We agree that difficulties in detecting variants are likely to influence the reported PV frequencies of our study, however, this factor affects both cases and controls. Future studies should analyze cases and controls employing the same pipeline and pathogenicity assessment strategy and should include copy number variants.

Point 3: Biallelic gene variants were not ruled out. This point cannot be addressed because of the retrospective nature of our study. Based on our results, we now recommend functional assays (eg, chromosomal breakage test) to rule out Fanconi anemia in children with cancer who are found to have a heterozygous PV in *BRCA1*, *BRCA2*, or *PALB2*. It is possible that a second germline PV on the other allele is missed when sequencing is employed alone. We recommend a similar procedure in children with cancer who are found to harbor a heterozygous germline PV in a MMR gene to rule out constitutional MMR deficiency because of biallelic PVs in one of the MMR genes.

Despite these limitations, our data provide evidence supporting the hypothesis that heterozygous PVs in *BRCA* and MMR genes (with the strongest signal observed for *MSH2*) are associated with pediatric cancer with a low penetrance not necessitating changes to current predictive testing recommendations. Prospective studies are needed to independently confirm these findings and to define the pediatric cancer spectra, tumor risks, and somatic mutation landscapes associated with PVs in these genes.

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Data availability

No new data were generated or analyzed for this response.

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