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CORRESPONDENCE

Reply to: "Development of an MSI-positive colon tumor with aberrant DNA methylation in a PPAP patient"

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To the Editor:

Yamaguchi et al. [1] identified a germline frameshift variant in the *POLE* gene, c.4191_4192delCT (p.Tyr1398*), located outside the exonuclease domain, in a case with multiple adenomatous polyps and three synchronous colon tumours, directly assigning the truncating variant a causal role in polymerase proofreading-associated polyposis (PPAP) [1].

As its name indicates, PPAP is a polyposis and cancer predisposing syndrome caused by aberrant polymerase proofreading activity, leading, in the associated tumours, to the accumulation of mutations (frequently >100 mutations/Mb), characterized by an overrepresentation of C:G → A:T transversions [2]. To date, the only functionally relevant variants unequivocally linked to PPAP have been missense variants located in the sequence coding for the exonuclease domains of *POLE* and *POLD1*. Loss-of function (LoF) variants and/or genetic changes in these genes outside the exonuclease domain have not been shown to be causal for PPAP [3]. Here, we present evidence showing that LoF *POLE* and *POLD1* variants do not cause PPAP or colorectal cancer predisposition, and in particular, that the tumours from the *POLE* c.4191_4192delCT (p.Tyr1398*) carrier analysed by Yamaguchi et al. do not show the molecular characteristics of cancers driven by pathogenic *POLE* mutations.

We assessed the presence of germline stop-gain, frameshift and start-loss variants in *POLE* and *POLD1* in cancer-free controls (GnomAD v.2.1 non-cancer, non-Finnish Europeans) and familial/early-onset colorectal cancer patients [4] (data access via https://canvar.icr.ac.uk/) [5] (data obtained from publicly available resources). The allele frequency of LoF variants in *POLE* and *POLD1* was 0.12% in controls and 0.16% in familial/early-onset colorectal cancer patients, suggesting no association with predisposition to colorectal cancer (Table 1).

In the case of *POLE* c.4191_4192delCT (p.Tyr1398*), Yamaguchi et al. performed whole-genome sequencing in two of the colon tumours developed by the proband, one of which had microsatellite instability (MSI). The microsatellite stable colon tumour was not ultra/hyper-mutated (2.8 mutations/Mb), clearly arguing against a PPAP-related origin. On the other hand, the other tumour, which exhibited DNA mismatch repair (MMR) deficiency, i.e. MSI, secondary to somatic *MLH1* promoter hypermethylation, was indeed hypermutated (81.5 mutations/Mb). Whether this high mutation burden was caused by the MMR deficiency or a *POLE* proofreading defect, may be easily determined by looking into the analysis of mutational signatures that the authors showed in Supplementary Fig. 3a. According to this, >80% of the signatures' contribution is attributed to COSMIC signatures 6 and 15, unequivocally associated with MMR deficiency, whereas no trace of signatures 10 (*POLE*) or 14 (*POLE* combined with MMR deficiency) [6] is observed [1]. In absence of co-segregation data, the evidence gathered does not link the germline stop-gain variant with the colon tumours analysed by Yamaguchi et al., and it should have been classified, at most, as a variant of uncertain significance for PPAP. In summary, here we show that there is no evidence that loss of function mutations in *POLE* and *POLD1* cause PPAP. Based on this and on the mutational characteristics of the analysed

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Table 1 Allele frequency of LoF variants in controls and familial/early-onset CRC

	Controls[a]	Familial/early-onset CRC[b]
Total allele number	102,460[c]	1864
LoF alleles[d]	122 (0.12%)	3 (0.16%)
p value[e]	0.8571	

CRC colorectal cancer, *LoF* loss of function, *NFE* non-Finnish Europeans

[a]Source: GnomAD v.2.1.1 non-cancer, non-Finnish Europeans (consultation date: July 2019)

[b]Source: https://canvar.icr.ac.uk/

[c]Median of the total allele number considering all variants reported in GnomAD for *POLE* and *POLD1*

[d]Start-loss, stop-gain and frameshift variants were considered. No homozygous individuals were identified in either cases or controls

[e]Chi-square test with Yates correction was used to compare frequencies between cases and controls

tumours, we may now conclude that the patient reported by Yamaguchi et al. should not be considered as PPAP, a syndrome characterized by the presence in the associated tumours of a high mutation rate caused by aberrant proofreading activity of the polymerase. The genetic cause of the polyposis and high colorectal cancer risk in that patient remains to be elucidated.

We feel that special caution should be exercised when interpreting *POLE* and *POLD1* germline variants in routine genetic diagnostics, considering that clinical and scientific communities are used to classifying LoF variants in known cancer-predisposing genes as (likely) pathogenic. As of today, only carefully curated exonuclease domain missense variants should be considered as potentially associated with PPAP.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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