Response to Yang et al.

We thank Yang et al. for their interest in our recent paper[1] and for sharing their findings of a suspected Lynch syndrome (LS) family meeting the Amsterdam I criteria and having a biallelic frameshift variant (p.Asp206Thrfs*18) in *MLH3*. We fully agree that more research is needed to establish a firm basis for *MLH3* variant interpretation and that strict criteria need to be applied to any novel disease-causing variants. We would like to address some points raised by Yang et al.[2] as to why *MLH3* variants might be considered disease-causing.

MLH3 is one of three alternative partners known to complex with MLH1 in postreplicative mismatch repair (MMR), the other two being PMS1 and PMS2.[3] The MLH1/PMS2 (MutLα) complex is by far the most abundant. Cell line studies suggest that MLH1/MLH3 (MutLγ) can partially compensate for the lack of MutLα. Reduced penetrance is a common feature of cancer-associated variants of *PMS2*. Pathogenic variants of *PMS2* give rise to LS with low penetrance when heterozygous and display full penetrance with the clinical phenotype of constitutional mismatch repair deficiency (CMMRD) syndrome when homozygous.[4] Many CMMRD patients with pathogenic MMR gene variants develop gastrointestinal adenomas ranging from a few polyps to profuse polyposis, resembling attenuated adenomatous polyposis.[5]

Consistent with observations from CMMRD, adenomatous polyposis was the leading phenotypic manifestation in homozygous carriers of our *MLH3* p.Ser1188Ter variant. Combined with the lack of any microsatellite instability in tumor tissue, a clear distinction relative to LS was made.[1] All biallelic carriers had polyposis (up to 100 polyps) and/or cancer; the delayed onset (around 50 years) suggested reduced penetrance. Some proven and obligatory heterozygous carriers, too, were affected with mild polyposis or cancer. Similarly, in the recessive polyposis syndromes associated with pathogenic variants in *MUTYH*[6] or *NTHL1*,[7] a proportion of heterozygous carriers are affected with cancers considered to be part of the respective tumor spectra. Varying penetrance and expressivity may complicate transmission patterns especially if combined with inbreeding. In the family described by Yang et al.,[2] the mode of inheritance could, in our opinion, be interpreted as autosomal dominant with reduced penetrance, or autosomal recessive with dosage effect and increased cancer risk in heterozygous carriers. In agreement with our families, Yang et al.[2] report the absence of microsatellite instability in their family. We agree that the family is unlikely to represent LS. We would be very interested to know if the variant carriers exhibited any polyps, which is currently not mentioned.

Our five families all shared a core haplotype of 0.8 Mb around the *MLH3* p.Ser1188Ter variant,[1] suggesting a common ancestral origin even though the pedigree information available to us did not indicate any apparent connection between the families. The shared region encompasses 15 other genes, with none linked to any known cancer syndrome. We detected no variants of possibly pathogenic significance in any of these other genes by exome sequencing, leaving the *MLH3* p.Ser1188Ter alteration the only variant of interest. Nevertheless, we agree with Yang et al.[2] that genome sequencing might be useful to rule out possible regulatory alterations in noncoding regions.

According to Yang et al.,[2] their *MLH3* p.Asp206Thrfs*18 variant is predicted to be present in 4 of 7 *MLH3* transcripts, which would imply a chance of functional compensation by wild-type isoforms. However, a recent investigation[8] identifies only two major isoforms, whereas the remaining five are predicted in silico. Both the *MLH3* p.Ser1188Ter variant and the p.Asp206Thrfs*18 variant would equally affect the two major isoforms; thus, no unaffected wild-type isoforms would remain in either case.

Yang et al.[2] note that contrary to the typical proximal location of LS-associated colorectal tumors, the tumors in their family resided in the rectum. Interestingly, in one of the original papers proposing a connection between heterozygous *MLH3* variants and hereditary colon cancer predisposition,[9] a strong predominance of left-sided colon cancer was reported. Furthermore, in CMMRD caused by pathogenic biallelic *PMS2* variants, left colon is the typical site of colon cancer.[4]

Even in the case of the established LS predisposition genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*, genotype–phenotype relationships are poorly understood, including mechanisms responsible for the essential clinical differences between monoallelic and biallelic variant carriers. An exceptionally high mutational load ("ultrahypermutation" phenotype) following replication repair failure was recently detected in CMMRD-associated brain tumors, which might explain why brain tumors are common in CMMRD and develop at early age; however, gastrointestinal polyps from CMMRD patients showed no increased mutational load compared with adult polyps, thus offering no insights to polyp predisposition in CMMRD.[10] In the case of *MLH3*, failing MMR may not be a particularly promising candidate for a phenotypic determinant in the first place, considering the observed redundancy between MutLγ and MutLα and the resulting minor role for *MLH3* in MMR.[3] As pointed out in Olkinuora et al.,[1] *MLH3* is known to have many other cancer-relevant functions, whose roles in tumors developing in *MLH3* variant carriers remain to be addressed by future studies.

In summary, we acknowledge the limitations of our research, including the restriction of genetic screening to

coding regions and lack of functional verification. However, we do believe that the genetic and clinical evidence we provide[1] define a novel *MLH3*-associated polyposis syndrome. Likewise, it seems likely that the family described by Yang et al.[2] represents a condition distinct from LS. Additional multidisciplinary and multicenter research is clearly warranted to better understand the role of *MLH3* and its variants in cancer predisposition.

DISCLOSURE

The authors declare no conflicts of interest.

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A. Olkinuora, MSc [1], *T. T. Nieminen, PhD*[1,2],
M. Nordling, PhD[3,4] *and P. Peltomäki, MD, PhD*[1]

[1]Department of Medical and Clinical Genetics, Medicum, University of Helsinki, Helsinki, Finland; [2]Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA; [3]Department of Pathology and Genetics, Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden; [4]Department of Clinical Pathology and Genetics, Sahlgrenska University Hospital, Gothenburg, Sweden. Correspondence: A. Olkinuora (alisa.olkinuora@helsinki.fi)

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