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LETTER

Stability of *BAT26* in Lynch syndrome colorectal tumours

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In a recent report published in the *European Journal of Human Genetics*, Pastrello *et al*[1] elegantly demonstrated that in individuals with Lynch syndrome, large germline deletions of *MSH2*, which span exon 5 are likely to somatically delete the commonly used microsatellite instability (MSI) marker *BAT26* from the remaining wild-type allele. As a consequence, *BAT26* appears to be stable when assessed in tumour tissue. This work has highlighted a limitation of using *BAT26* alone for the detection of MMR deficiency, a limitation of considerable magnitude given that large deletions of *MSH2,* of which up to one-half span exon 5,[2,3] comprise 15% of all known deleterious MMR mutations.

Several panels of MSI markers have been proposed, and have demonstrated excellent clinical utility.[4–6] Tumours showing instability in 30–40% or more of these microsatellite markers are designated MSI-H (high-level MSI) and this phenotype is tightly associated with Lynch syndrome in early onset cancers. The microsatellite marker, *BAT26*, has shown particularly high sensitivity and specificity in the detection of an MSI phenotype in colorectal tumours, and it has been suggested that this marker alone could be used to screen for Lynch syndrome.[4,7–9] *BAT26* is located immediately downstream of exon 5 in the *MSH2* gene, and consists of a poly-A repeat of an invariant number of nucleotides, which is particularly susceptible to deletion-type replication errors in states of MMR deficiency. Its apparent, though not total, lack of polymorphism[10] also makes it an especially useful MSI marker as normal reference DNA need not be tested alongside that from an individual tumour. Stability at *BAT26,* however, has been previously reported in Lynch syndrome tumours, occurring in three tumours with absent immunostaining for MSH2.[11] As large intragenic deletions of *MSH2* can account for up to one-third of its mutations,[12] this finding may in part be owing to deletions that remove *BAT26*.

We investigated the stability of *BAT26* in a series of 108 confirmed germline MMR mutation carriers from the Australasian Colorectal Cancer Family Study of which 6 (5%) were derived from a population-based subset, and the remainder from high-risk colorectal cancer family clinics. Of these 108 cases, 55 (51%) carried mutations in *MSH2* and 53 (49%) in *MLH1*. All cases had undergone tumour MSI analysis and immunohistochemistry for MMR genes as described previously.[13] Fifteen of 55 (27%) *MSH2* mutation carriers were found to have large exonic deletions, seven of which spanned exon 5. Of these seven, four showed stability at *BAT26* in their colorectal tumours. In the remaining 48 cases, where exon 5 was not deleted, only six were stable at *BAT26* (Figure 1). Therefore, the rate of *BAT26* stability in cases with large deletions spanning exon 5 (4/7 or 57%) was significantly higher than in all other types of *MSH2* mutation (6/48 or 12.5%) ($P=0.016$, Fisher's exact test). These findings are consistent with the data of Pastrello *et al*,[1] and suggest that a finding of *BAT26* stability in a tumour that demonstrates no MSH2 protein is suggestive of a large genomic deletion encompassing exon 5. Assessment of the prevalence of *BAT26* stability in *MSH2*-mutation carriers other than those who carried exon 5 deletions (6/48 or 12.5%) showed only borderline statistical significance ($P=0.051$, Fisher's exact test) when tested against that of *MLH1*-mutation carriers (1/53 or 1%). In our current data set, the average number of unstable markers per tumour (81.5%) in MLH1-deficient tumours from Lynch syndrome cases was significantly higher than that seen in MSH2-deficient tumours (69.9%) ($P=0.0030$; two-tailed *t*-test). Although this observation remains significant when cases with exon 5 deletions are removed ($P=0.018$), this does not remain so if all cases of *BAT26* stability are removed ($P=0.11$), indicating that *BAT26* stability in MSH2-deficient tumours is the likely reason for the difference in overall stability.

We further investigated the association between *BAT26* stability and MSH2 immunodeficiency in a series of colorectal cancer cases referred to the Mayo Clinic Molecular Genetics Laboratory over a 5-year period (2001–2005), for MMR testing. These cases generally represented a moderate to high-risk group for having HNPCC, owing to young age of onset, presence of a family history of colon cancer or HNPCC-related malignancy, or the presence of a tumour's histology suggestive of defective MMR. Both MSI and IHC were used to define the presence of defective DNA MMR in tumour specimens, following established methodology.[13] Of the 1724 colon cancer cases with unequivocal test results, 469 (26%) had a tumour phenotype of MSI-H. Of these, 242 showed a loss of MLH1, 122 MSH2/MSH6, 27 MSH6 alone, 15 PMS2 alone and 14 showed normal expression for all four of these proteins (Table 1). The remaining 49 cases had either missing or equivocal IHC data, or different and less frequent combi-

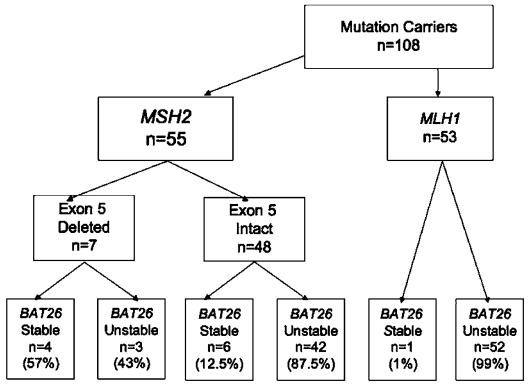

Figure 1 Diagram of breakdown of *MSH2* mutation carriers into those with and without deletions spanning exon 5. The rate of *BAT26* stability in cases with large deletions (4/7 or 57%) was significantly higher than in all other types of *MSH2* mutation (6/48 or 12.5%) ($P=0.016$, Fisher's exact test).

Table 1 Mayo clinic cases with MMR immunohistochemistry and rates of *BAT26* stability

MMR protein absent in tumour tissue	*No. with MSI-H*	*No. with BAT26 stable*
MLH1	242 (58%)	2 (0.8%)
MSH2	122 (29%)	8 (6.6%)
MSH6 only	27 (6.4%)	1 (3.7%)
PMS2 only	15 (3.6%)	0
Normal IHC	14 (3.3%)	4 (28.6%)

nations of protein loss. The frequency of stability with the mononucleotide repeats *BAT26* varied considerably across these five different groups. Stability at this marker was detected in less than 1% of the MLH1-deficient tumours. On the other hand, *BAT26* stability was detected in 6.6% of the MSH2-deficient cases ($P=0.0031$, Fisher's exact test). Although it was not possible to screen this series of tumours for germline mutations in MMR genes owing to the unavailability of corresponding blood samples, the results suggest that *BAT26* stability occurs more commonly in the carriers of germline *MSH2* gene mutations, at least a proportion of which are likely to be large deletions that span the *BAT26* repeat.

These findings suggest that although *BAT26* may be a good marker of MSI in *MLH1* mutation carriers, it is not so for those with *MSH2* mutation. It is also not surprising that *BAT26* has been shown in prior studies to be a good marker for MSI with very few false negative results, as the majority of sporadic cases of colon cancer with defective MMR result from promoter hypermethylation of *MLH1*. When screening higher risk patient groups, however, the abnormal gene distribution is quite different with nearly half representing other MMR gene combinations, and approximately 70% of the non-*MLH1* cases are associated with MSH2 immunodeficiency. As a result, it is clear that *BAT26* cannot be used alone to identify defective MMR (or the MSI-H tumour phenotype) in high-risk patient groups.

In this report, we have confirmed the findings of Pastrello *et al*, who reported an association between *BAT26* stability and *MSH2* exon 5 deletion. We have also demonstrated additional instances where *BAT26* stability occurs in MSI-H colorectal tumours outside the setting of *MSH2* exon 5 deletion. In all but one case where mutation testing had been completed, *BAT26* stability was associated with a germline mutation in *MSH2*. The explanation for these findings is unclear. However, we have previously reported that the average number of markers that show instability is higher in MLH1 deficient tumours of sporadic origin[11] than in those from Lynch syndrome families, when calculated over an entire panel of mononucleotides and higher-order repeats. Our current data set extends this finding to show that MLH1-deficient tumours from Lynch syndrome cases show greater instability than MSH2-deficient tumours, and that this difference is largely owing to the differences in frequency of *BAT26* stability between the two groups. It is possible that with lower average levels of MSI, the likelihood that *BAT26* will escape instability is slightly increased in MSH2-deficient tumours, although the reason for this is unknown. Alternatively, the lower level of instability in MSH2-deficient tumours may reflect differences in the developmental 'age' of the tumour at presentation. In summary, from a fully characterized series of 108 MMR mutation carriers, we demonstrate that 18% (10/55) of *MSH2* mutation carriers showed stability at *BAT26* in their tumours, a significant proportion of which were due to large deletions of *MSH2* involving exon 5. Further, we have shown in an independent tumour series from a moderate- to high-risk patient subset that *BAT26* stability was more likely to be seen in tumours that showed immunodeficiency of MSH2. Taken together, our findings support those of Pastrello *et al* (2006) and suggest that screening tumours for Lynch syndrome with *BAT26* only may be ill advised.

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Reply to Jaskowski *et al*

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Jaskowski *et al*[1] have confirmed our previous paper[2] reporting an association between *BAT26* stability and germline *MSH2* deletion. Among seven highly unstable tumours with *MSH2* deletion spanning exon 5 they found normal *BAT26* sequences in 4. Although their analysis was based on a small number of tumours, they observed this phenomenon in 57% of cases, a percentage very close to the 68% we obtained in the analysis of 19 tumour DNAs.[2] It is reasonable to suppose that the molecular mechanism leading to somatic loss of *BAT26* sequences, as we described previously,[2] is responsible for the observed *BAT26* stability in both subsets of *MSH2* deleted tumours.

In our current dataset including 29 *MLH1*- and 49 *MSH2*-mutated tumours (27 of which retaining exon 5), only two additional cases displayed *BAT26* stability; these were from two patients who were heterozygous for point mutations in *MSH2* and *MLH1*, respectively. On the contrary, in the study by Jaskowski *et al*[1] 6/48 (12.5%) samples from patients with *MSH2* germline mutations with exon 5 retention and only one out of 53 (1.8%) samples from *MLH1* mutation carriers were found to be stable at *BAT26*. A different prevalence of *BAT26* instability was also detected in a series of moderate–high-risk tumours, for which distinction between *MLH1*- and *MSH2*-related cases was based on immunohistochemistry alone. In the hereditary tumours such differential *BAT26* stability was associated with an overall lower degree of instability in *MSH2* compared to *MLH1*-related cancers (69.9 and 81.5% unstable markers, respectively).

On the other hand, extrapolating data from some previous studies on different series of unstable tumours with *MLH1* and *MSH2* defects and considering only the markers belonging to the reference NCI panel, the average percentage of instability was 92 and 79.4%,[3] 92.3 and 86.8%[4], 80 and 72%,[5] for *MLH1* and *MSH2* tumours, respectively. Nonetheless, despite the constant slight instability excess of *MLH1* tumours, statistically significant differences could not be highlighted, even when these data were pooled (89.8 and 80.1%; $P=0.08$, Fisher's exact test).

We also analyzed in more detail our MSI data on a total of 78 unstable tumours with unequivocal test results (among which 61 were colorectal adenocarcinomas); in this series, we could not find a higher instability in the *MLH1* group. In fact, despite the inclusion of 14 samples with germline *MSH2* intragenic deletion and wild-type