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# Detection of large scale 3′ deletions in the *PMS2* gene amongst Colon-CFR participants – have we been missing anything?

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## Abstract

Current screening practices have been able to identify *PMS2* mutations in 78% of cases of colorectal cancer from the Colorectal Cancer Family Registry (Colon CFR) which showed solitary loss of the PMS2 protein. However the detection of large-scale deletions in the 3′ end of the *PMS2* gene has not been possible due to technical difficulties associated with pseudogene sequences. Here, we utilised a recently described MLPA/long-range PCR-based approach to screen the remaining 22% (n = 16) of CRC-affected probands for mutations in the 3′ end of the *PMS2* gene. No deletions encompassing any or all of exons 12 through 15 were identified; therefore, our results suggest that 3′ deletions in *PMS2* are not a frequent occurrence in such families.

### Keywords

Lynch syndrome; *PMS2*; germline testing; large deletions; pseudogenes; colorectal cancer

In a recent publication, Vaughn *et al.* [1] described a combinatorial approach of long-range PCR and MLPA (multiplex ligation-dependant PCR amplification) to detect deletions specific to *PMS2* at the 3′ end of the gene. The accurate screening for mutations in *PMS2*

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has, until fairly recently, been plagued by problems associated with the large number of highly homologous sequences within pseudogenes that flank the functional gene.[2,3] Previous studies have led to long-range PCR becoming the method of choice for the detection of point mutations as it circumvents these pseudogene-associated problems.[4–6] The detection of large-scale deletions has, however, not been so reliable, particularly at the 3′ end of the gene due to technical issues caused by the high degree of homology between the real *PMS2* gene and its pseudogenes. The technique most commonly used for large scale deletion detection is a multiplex ligation-dependant PCR amplification method more commonly referred to as MLPA.[7] For *PMS2*, this technique has taken advantage of small differences between the *PMS2* gene and the pseudogenes for the positioning of its analytical probes. This has proved successful for the majority of exons within *PMS2* but towards the 3′ end of the gene purported differences between the real gene and the pseudogenes (paralogous sequence variants (PSVs)) have turned out to be shared polymorphism sites (SPSs),[8] i.e. they are polymorphic in both sequences, which has resulted in un-interpretable findings at these locations.[9]

MRC Holland recently addressed this issue by modifying their *PMS2* kit to include non-specific probes as well as probes designed to hybridise to both alleles of these SPSs. When this kit is used in conjunction with gene and pseudogene specific sequencing, Vaughn *et al.* found that it can be used to accurately assess the deletion status at the 3′ end of the gene.[1] In their methodological study, sample selection was biased towards cases likely to harbour a 3′ deletion, therefore the true extent of such deletions still remains unclear in a clinical setting.

In this study, we utilise this new method (Supplementary Document 1) to screen for 3′ deletions in a cohort of CRC-affected cases from the Colon Cancer Family Registry[10] who were suspected of carrying deleterious mutations in the *PMS2* gene, based on the loss of expression of the PMS2 protein by immunohistochemistry (IHC), but for whom no mutation could be identified using the standard long-range PCR approach and the previous MLPA method for large deletion detection of exons 1–11.

Because of the complexity of the method, we first utilised it to screen a small reference set of cases with colorectal cancer from other sources who were predicted to have a 3′ deletion. As in Vaughn *et al*, they had been selected because they had a deletion of at least exon 11, or the previous MLPA kit suggested a deletion in conjunction with suggestive sequence data (homozygosity) for any of the terminal 3 exons. Among this reference set, we identified an exon 14 deletion in *PMS2*, a deletion of exons 14 and 15 from the pseudogene and confirmed the deletion of the complete *PMS2* gene in two subjects (Figure 1). Further investigation of the exon 14 deletion enabled us to determine the location of the breakpoint (c.2276-113_c.2245+1596del) and allowed for the design of a breakpoint specific PCR which simplifies mutation detection (Supplementary Document 2). This mutation has previously been identified amongst a Dutch cohort through an RNA based screening approach.[9]

The test set consisted of 76 CRC-affected cases that were identified as candidates for a mutation in *PMS2* based on the solitary loss of expression of PMS2 following IHC analysis, and microsatellite instability (MSI-H phenotype) as determined by the analysis of a 10 microsatellite marker panel.[11] These 76 CRC-affected cases were identified from a total of 4402 CRC-affected cases whose tumours had undergone IHC analysis for all four mismatch repair proteins. Standard mutation screening via long-range PCR and large deletion detection for the 5′ end of the *PMS2* gene identified pathogenic mutations in 59 cases (59/76; 78%). Of the remaining 17 cases whose tumours showed solitary loss of PMS2 expression but *did not* have a pathogenic mutation detected in *PMS2*, 16 had sufficient DNA available to test for large deletions in the 3′ end of the *PMS2* gene using the method

described by Vaughn *et al.*[1] No deletions in exons 12–15 were detected from these 16 CRC-affected cases and so the causative mutation still remains to be identified.

In this study, we have confirmed that an integration of MLPA and long-range PCR can be used to detect aberrations in the 3′ end of the gene; however, we did not identify any deletions of exons 12–15 in our test set of 16 CRC-affected cases. Our data therefore suggest that, whilst plausible, large scale deletions at the 3′ end of the *PMS2* gene are not a substantive cause of disease in cases for which a mutation cannot be identified by previous methods. In contrast to our findings, Vaughn *et al.* have recently followed up on their original methods paper with a study similar to the one outlined here, wherein they identified 7 cases with a 3′ deletion.[12] In this study, they screened a total of 58 samples with unexplained loss of PMS2 emanating from an initial cohort of 117 samples. When compared to our own, the findings of Vaughn *et al.* highlight a considerable disparity between the type of mutations as well as the total percentage of mutations identified. Even in the absence of any 3′ deletions, our own study identifies mutations in 78% (59/76) of cases, where as Vaughn *et al.* find a mutation in 55% (64/117) of cases, with 11% (7/64) of these being due to 3′ terminal deletions. Both of these cohorts have been screened using very similar methods and so we would expect that the ability to detect *PMS2* mutations is the same in each study, which leaves a significant proportion (particularly in the Utah cohort) of *PMS2* suspected cases which have not had a causative mutation identified in the coding regions of the gene.

With this in mind, it seems likely that mutation-screening strategies will have to be broadened in order to pinpoint the causative mutation in such cases. One possible source for these elusive mutations might be intronic sequences that are not routinely examined; an example of which we have recently identified in the context of the *MSH2* gene.[13] For many genes the screening of these large genomic regions could eventually become cost-effective with the decreasing cost of next generation sequencing (NGS). It should be noted, however, that the pseudogene sequences which have caused many of the exonic screening problems to date will also impact on the reliability of NGS of the *PMS2* loci.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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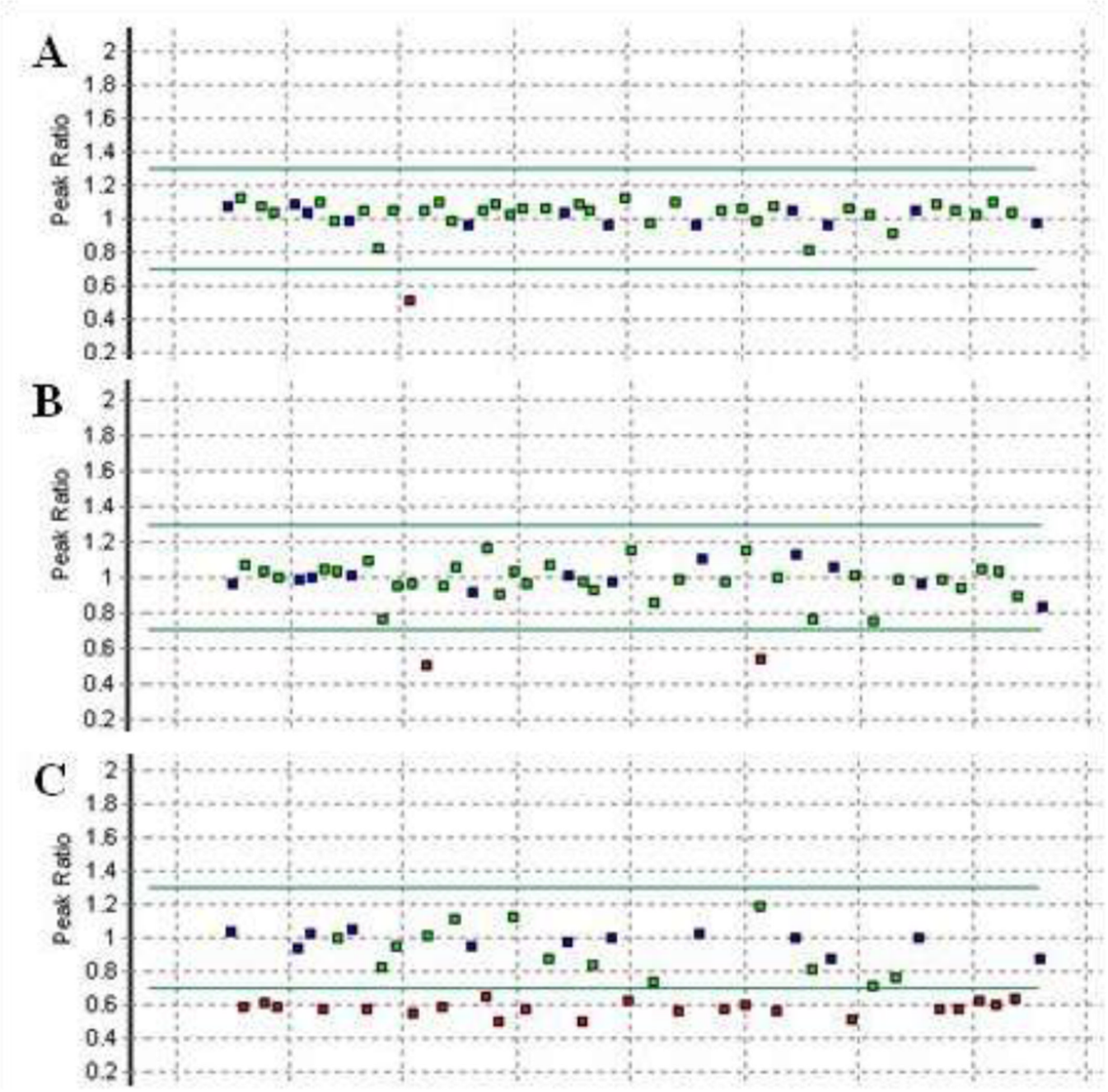


**Figure 1.**

MLPA findings for 3 reference DNAs which showed abnormal probe levels. A) Individual with a deletion of *PMS2* exon 14. B) Individual with an apparent deletion of exons 14 and 15 from *PMS2-CL*. C) Confirmation that the entire *PMS2* coding region is deleted in an individual previously classified as having an exon 1 – 12 deletion with the older version of the MLPA kit. Amounts of specific probes, relative to a set of control samples which have two copies of each probe, are represented by squares. A red square denotes a probe whose copy number is suggestive of a gain or a deletion. Sequencing across the probe binding sites in both *PMS2* and *PMS2-CL* allows for deletions and duplications to be assigned to specific loci. A detailed description of the analyses can be found in Vaughn *et al.* (2011).[1]