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SHORT REPORT

Partial duplications of the *MSH2* and *MLH1* genes in hereditary nonpolyposis colorectal cancer

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Numerous reports have highlighted the contribution of *MSH2* and *MLH1* genomic deletions to hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch's syndrome, but genomic duplications of these genes have been rarely reported. Using quantitative multiplex PCR of short fluorescent fragments (QMPSF), 962 and 611 index cases were, respectively, screened for *MSH2* and *MLH1* genomic rearrangements. This allowed us to detect, in 11 families, seven *MSH2* duplications affecting exons 1–2–3, exons 4–5–6, exon 7, exons 7–8, exons 9–10, exon 11, and exon 15, and three *MLH1* duplications affecting exons 2–3, exon 4 and exons 6–7–8. All duplications were confirmed by an independent method. The contribution of genomic duplications of *MSH2* and *MLH1* to HNPCC can therefore be estimated approximately to 1% of the HNPCC cases. Although this frequency is much lower than that of genomic deletions, the presence of *MSH2* or *MLH1* genomic duplications should be considered in HNPCC families without detectable point mutations.
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Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch's syndrome, which represents the most common form of inherited colorectal cancer, results from germline alterations of the DNA mismatch repair genes *MSH2*, *MLH1*, *MSH6* and *PMS2* (for review see Gruber[1]). Identification of the causal alteration is essential for an appropriate clinical management of the families as it allows to offer to carriers an efficient tumour detection, based on colonoscopy and hysteroscopy every 2 years from 20 years of age and to avoid an inappropriate follow-up in noncarriers. Point mutations within *MSH2*, *MLH1*, and *MSH6* account approximately for 25, 25 and less than 5% of the HNPCC families, respectively. Numerous reports, initially based on Southern blot or RT-PCR, then on QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments), MLPA (Multiplex Ligation-dependent Probe Amplification) or conversion analysis have now underlined the significant contribution of *MSH2* and *MLH1* genomic deletions to HNPCC.[2–24] At present, 43 *MSH2* and 33 *MLH1*, distinct exonic deletions, removing either a single or multiple exons, have been reported.[2–24] We previously estimated that genomic deletions of *MSH2* and *MLH1* respectively, account for approximately 10 and 4% of HNPCC families fulfilling Amsterdam criteria.[6,13] All the *MSH2* genomic deletions detected so far have been shown to result from unequal *Alu*-mediated recombinations[6,8,11,12,18,20] and we found that the density of *Alu S* repeats within the *MSH2* 5′ region was remarkably high, compared with other human genes.[18] In contrast, *MLH1* genomic deletions have been found to involve not only *Alu* repeats, but also nonhomologous sequences.[2,3,9,20,21] As expected, exonic *MSH2* and *MLH1* duplications, the reciprocal genomic alterations of deletions resulting from unequal crossingover, have also

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been detected in HNPCC families, but at a much lower frequency. We reported, using QMPSF, the first case of a *MSH2* duplication involving exons 9–10 that was confirmed by long-range PCR[5] and we subsequently detected another *MSH2* duplication involving exons 7–8.[13] Only eight other *MMR* duplications have been reported in studies based either on MLPA or conversion analysis: for *MSH2*, duplication of exons 5–6,[19] exon 7,[15] exons 11–16;[17] for *MLH1*, duplication of exon 1,[16] exon 3,[16] exon 19,[16] exons 6–12,[19] and exons 7–12.[25] We now report the detection of 10 *MSH2* and *MLH1* exonic duplications, 7 of which are novel. The aim of this report was to highlight the contribution to HNPCC of *MMR* genomic duplications and to estimate the frequency of these alterations.

Materials and methods

Patients

Patients analysed in this study, either fulfilled Amsterdam (AMS) criteria (at least three relatives with colorectal cancer, cancer of the endometrium, small bowel, ureter or renal pelvis, one of whom is a first-degree relative of the other two; at least two successive generations affected; and at least one cancer diagnosed before age 50[26]) or were selected because of a suggestive familial history associated to a microsatellite instability (MSI) phenotype in the tumour. A total of 962 and 611 index cases were, respectively, screened for *MSH2* and *MLH1* genomic rearrangements.

QMPSF analysis

The QMPSF protocol used to screen for genomic rearrangements of *MSH2* and *MLH1* has been described previously.[5,6] Briefly, short fragments corresponding to the 16 *MSH2* and 19 *MLH1* exons and to control genes, were simultaneously PCR-amplified using dye-labeled primers. Primer sequences are available upon request. PCR products were separated by electrophoresis on an ABI-377 or 3100 automated sequencer (Applied Biosystems, Foster, USA). Data were analysed using the Gene Scanner Model 672 Fluorescent Fragment Analyzer (Applied Biosystems, Foster, USA). For each index case, QMPSF profile was superposed to that generated from a control-DNA by adjusting to the same level the peaks obtained for the control amplicon. Based on an extensive number of QMPSF analyses (including >50 genes and >1000 tests) performed in our laboratory, we have estimated the variability of gene copy number measurements to be 0.1 among control samples. Thus, copy number ratios between 0.9 and 1.1 indicate two gene copies, and copy number ratios between 1.4 and 1.6 indicate three copies.[5,27–29] Each positive result was confirmed by another QMPSF analysis performed on an independent sample.

MLPA

MLPA was performed using the SALSA P003 (*MLH1*/*MSH2*) MLPA kit (MRC Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol. Fragments were separated and analyzed with a 377XL genetic analyzer and Genescan 3.1 software as well as with a 3100-Avant capillary genetic analyzer and GeneMapper 3.5 software (Applied Biosystems). Peak areas and heights were then exported to a Microsoft Excel spreadsheet (http://leedsdna.info/science/dosage/Conventional_MLPA/Conventional_MLPA.htm) and calculations were carried out according to the method described by Taylor *et al*.[10]

Results

Using QMPSF, we detected 10 distinct genomic duplications of *MSH2* or *MLH1* genes in 11 families: seven duplications involved *MSH2* exons 1–2–3, exons 4–5–6, exon 7, exons 7–8, exons 9–10, exon 11, and exon 15, and three duplications involved *MLH1* exons 2–3, exon 4 and exons 6–7–8 (Table 1). As illustrated by Figure 1, exonic duplications could easily be detected by QMPSF by a 1.5 increase of the corresponding peaks. Only the duplication of *MSH2* exons 9–10 detected in family L14 had previously been confirmed by long-range PCR.[5] MLPA was therefore used in the present study to confirm the nine other duplications. In each case, MLPA confirms the presence of the exonic duplication(s) detected by QMPSF. These duplications generate in MLPA an increase of the corresponding peak area(s) above 1.3. In one family (G1), we observed a discrepancy between QMPSF and MLPA concerning the extent of duplication. Indeed, QMPSF revealed the presence of a *MSH2* duplication involving exons 5–6, whereas MLPA suggested that this duplication also involved exon 4. In order to clarify the situation, we designed new QMPSF assays exploring specifically intron 3, exon 4 and intron 4 of *MSH2*. These complementary QMPSF analyses revealed that, within the G1 family, the duplication included exon 4, but did not extend to nucleotide c.646–121 corresponding to the sense primer used in the first QMPSF assay, and therefore did not involve the first 1920 nucleotides of intron 3.

Discussion

One hypothesis that might explain the difference in the numbers of *MMR* genes genomic deletions and duplications reported so far is the technical difficulty to detect duplications *versus* deletions. Indeed, the largest series of HNPCC patients screened for *MMR* genomic rearrangements have been analysed using QMPSF[13] or MLPA.[19] In both methods, heterozygous deletions result in a 0.5 reduction of the corresponding peaks, whereas duplications generate a 1.5 increase. This could explain why duplications are more difficult to detect than deletions, and this might represent a technical bias in the estimation of *MMR* duplications frequency. QMPSF analysis allowed us in this large series of suspected HNPCC patients to detect

Table 1 Duplications of *MSH2* or *MLH1* in HNPCC families

Family	*Duplicated gene*	*Duplicated exon(s)*	*Amsterdam criteria*	*Earliest age of tumour onset (in years)*
R1	*MSH2*	Exons 1–2–3	+	52
G1		Exons 4–5–6	+	42
B1		Exon 7[a]	+	42
S1[b]		Exons 7–8	–	45
L14[b]		Exons 9–10	+	37
B2		Exons 9–10	+	49
R2		Exon 11	–	30
M2		Exon 15	+	42
M1	*MLH1*	Exons 2–3	+	28
SC1		Exon 4	–	38
G2		Exons 6–7–8	+	45

[a]This duplication has already been detected by Casey *et al*.[15]
[b]We already reported this family.[5,13]

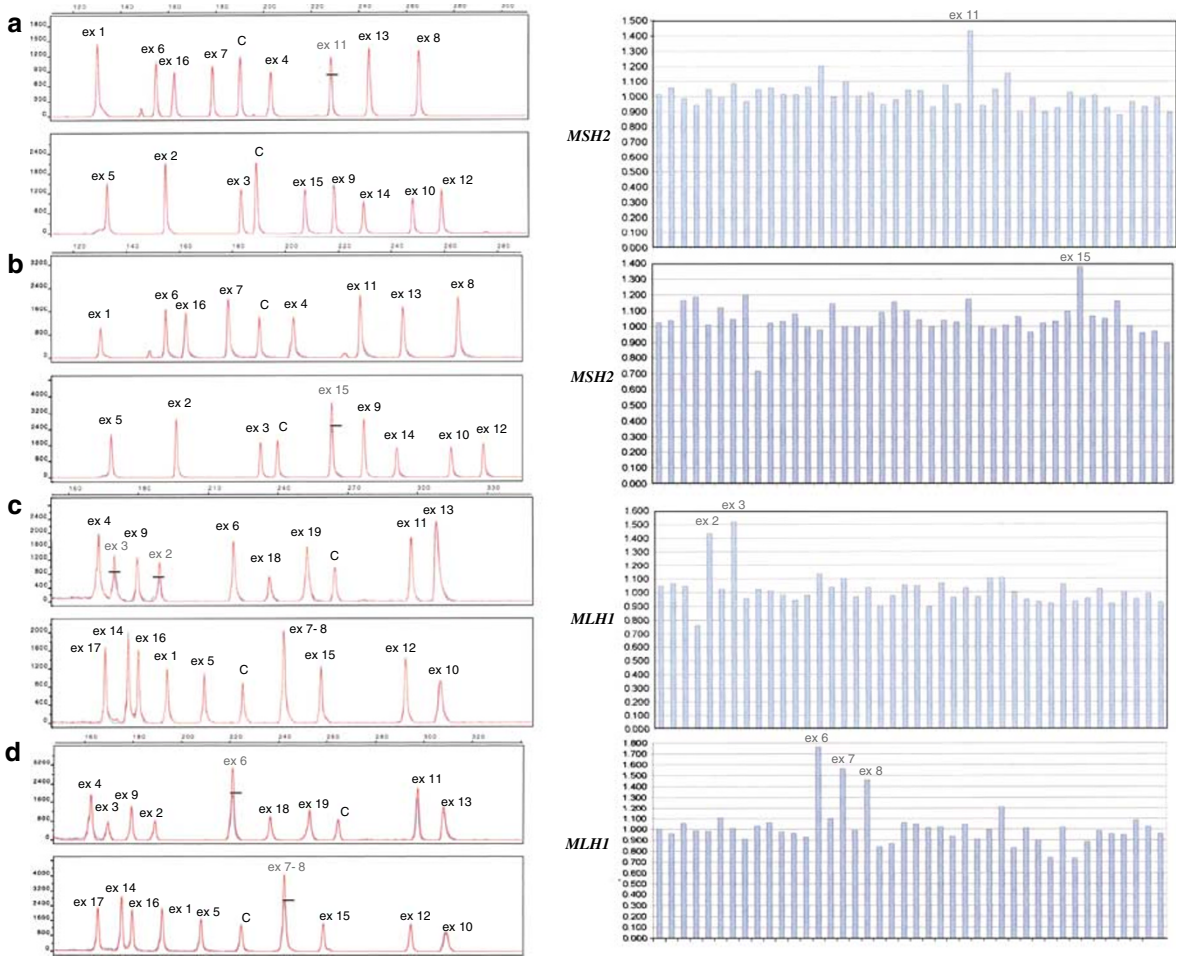

Figure 1 Detection by QMPSF of duplications of the *MSH2* and *MLH1* genes. The left panels show the QMPSF electropherograms in four families and the right panels the results of the corresponding MLPA analyses. In each QMPSF panel, the electropherogram of the patient (in red) was superimposed to that of a normal control (in blue) by adjusting to the same level the peaks obtained for the control (C) amplicon. The *Y*-axis displays fluorescence in arbitrary units, and the X-axis indicates the size in bp. *MSH2* or *MLH1* exons are indicated. Lines indicate the top of the normal blue peak. Duplications are detected by a 1.5 increase of the peaks compared with a normal control. (**a**) Detection of an *MSH2* exon 11 duplication in family R2; (**b**) Detection of an *MSH2* exon 15 duplication in family M2; (**c**) detection of an *MLH1* exons 2 and 3 duplication in family M1; (**d**) detection of an *MLH1* exons 6, 7 and 8 duplication in family G2.

10 distinct *MSH2* or *MLH1* partial genomic duplications, which were all confirmed by another independent method. This shows, as previously illustrated for the *BRCA1*,[27] *BRCA2*,[28] and *APP* genes,[29] that QMPSF is a reliable method for the detection of genomic duplications. Although we cannot provide in this study an accurate estimation of the contribution of *MSH2* and *MLH1* duplications to HNPCC, as the population analysed was heterogeneous including both AMS+ patients and AMS− patients with an MSI phenotype in the tumour, the frequency of *MSH2* and *MLH1* duplications appears low. In our series, 962 and 611 index cases were, respectively, screened for *MSH2* and *MLH1* genomic rearrangements. This allowed us to detect, in 115 families, 30 and 18 distinct exonic deletions of *MSH2* and *MLH1*, respectively (Charbonnier *et al*.[18] and unpublished results). In contrast, in the same series, we detected in 11 families, 7 and 3 exonic duplications of *MSH2* and *MLH1*. Therefore, *MSH2* and *MLH1* duplications account probably for approximately 1% of the HNPCC cases. Although genomic duplications of *MSH2* and *MLH1* are less frequent that genomic deletions, their presence should nevertheless be considered in HNPCC families without detectable point mutations.

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