LETTER TO JMG

# Screening for genomic rearrangements of the *MMR* genes must be included in the routine diagnosis of HNPCC

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In hereditary non-polyposis colorectal cancer (HNPCC), the most common form of inherited colorectal cancer, detection of the causal alteration of the mismatch repair (*MMR*) gene involved is essential for proper management of the families. This will allow the identification of relatives with high risk for colorectal or endometrial cancer, who require the appropriate screening and, conversely, will avert useless surveillance in non-carrier relatives. Mutational studies,[1] based on conventional screening methods, have indicated that point mutations of *MSH2*, *MLH1*, or *MSH6* can be detected in approximately 55% of the families, fulfilling the Amsterdam (AMS) criteria. These stipulate:

- at least three relatives with colorectal cancer, or 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 the age of 50 years.[2]

In a recent study, we showed that genomic rearrangements of *MSH2* are involved in approximately 20% of the AMS+ HNPCC families without detectable point mutations within *MSH2* or *MLH1*.[3] This study was performed using quantitative multiplex PCR of short fluorescent fragments (QMPSF), which can easily detect heterozygous genomic deletions and duplications.[3–7] This method is based on the simultaneous amplification of short genomic sequences under quantitative conditions, using dye labelled primers, and the superimposition of the electropherograms of patients and controls.

**Table 1** Frequency of *MSH2* and *MLHI* exonic rearrangements detected by QMPSF in HNPCC families according to their status

| Status | *MSH2* | *MLH1* |
|---|---|---|
| **AMS+ families** | 120* | 86† |
| Without IHC‡ information | 101 | 75 |
| -with a rearrangement | 16 (16%) | 2 (3%) |
| With selective extinction of the MMR protein | 19 | 11 |
| -with a rearrangement | 11 (58%) | 4 (36%) |
| -AMS+ families with a rearrangement | 27 (22%) | 6 (7%) |
| **AMS− families** | 212* | 106† |
| Without IHC‡ information | 200 | 85 |
| -with a rearrangement | 9 (4%) | 2 (2%) |
| With selective extinction of the MMR protein§ | 12 | 21 |
| -with a rearrangement | 7 (58%) | 1 (5%) |
| -AMS families with a rearrangement | 16 (8%) | 3 (3%) |

*Without *MSH2* or *MLH1* point mutation.
†Without *MSH2* or *MLH1* point mutation, or *MSH2* exonic rearrangement.
‡IHC staining of the tumour.
§In the tumour.

## Key points

- In hereditary non-polyposis colorectal cancer (HNPCC), point mutations of *MSH2*, *MLH1*, or *MSH6* are detected in approximately half of the families involved, which therefore fulfil the Amsterdam criteria (AMS).
- We analysed *MSH2* in 120 AMS+ and 212 AMS− HNPCC families without *MSH2* or *MLH1* point mutations, using quantitative multiplex PCR of short fluorescent fragments(QMPSF). We identified in 22% of the AMS+ and in 8% of the AMS− families 19 distinct exonic deletions and two cases of duplication of *MSH2*. We detected seven distinct 5′ breakpoints in the deletions removing exon 1. Specific QMPSF analysis of the *MSH2* promoter in 65 AMS+ families, without *MSH2/MLH1* point mutations or *MSH2* exonic deletion, revealed only one case of promoter deletion. Among 86 AMS+ and 106 AMS− families, we detected seven distinct *MLH1* exonic deletions in 7% of the AMS+ families and in 3% of the AMS− families. We found that the selective extinction of *MMR* protein in the tumours was highly predictive of an *MMR* rearrangement.
- We conclude that *MSH2* rearrangements are involved in at least 10% of the AMS+ families, which justifies screening for these in the routine diagnosis of HNPCC. The presence of *MLH1* rearrangements should be considered in AMS+ HNPCC patients, when there is a selective loss of *MLH1* expression in the tumours.

We have now integrated QMPSF into the routine diagnosis of HNPCC. We first analysed, as previously described,[3] the 16 exons of *MSH2* in 332 families, without point mutations within *MSH2* and *MLH1* (table 1). These families corresponded to 120 families fulfilling AMS criteria and 212 AMS- families. Immunohistochemical (IHC) staining of the tumours was performed in 19 AMS+ and 12 AMS− patients, and revealed a selective extinction of the *MSH2* protein. Among the AMS+ patients without IHC information, an *MSH2* genomic rearrangement was detected in 16% of the cases; the detection rate reached 58% in AMS+ patients, showing a selective loss of expression of *MSH2* in their tumours. Among the AMS− patients negative for *MSH2* and *MLH1* mutations, we found an *MSH2* genomic rearrangement in 4% of the cases, when IHC analysis had not been performed, and in 58% of the cases with IHC *MSH2* extinction. QMPSF analysis of the 19 exons of *MLH1* was then carried out in 192 families, corresponding to 86 AMS+

**Table 2** Summary of *MSH2* and *MLH1* exonic rearrangements detected by QMPSF in HNPCC families

| *MSH2* | Families | *MLH1* | Families |
|---|---|---|---|
| del* exon 1 | R1†, R2†, P14 | del exons 1–19 | U4,U5 |
| del exons 1–2 | P15, R12, R13, R20 | del exon 4–6 | Lu4 |
| del exons 1–4 | L7† | del exon 6 | S10 |
| del exons 1–6 | Li8†, R14, P16, U1, U2, S1, R18 | del exons 7–9 | R19 |
| del exons 1–7 | R9†, Lu1, Lu2, S2, R21 | del exons 9–10 | P20 |
| del exons 1–8 | R10†, R11†, S3, S4 | del exon 11 | S11, U6 |
| del exons 1–11 | U3 | del exon 14 | S12 |
| del exons 1–15 | P12† | | |
| del exon 2 | S5 | | |
| del exon 3 | R3†, P17 | | |
| del exons 4–6 | Lu3 | | |
| del exon 5 | P5† | | |
| del exon 5–6 | P13† | | |
| del exon 7 | L6† | | |
| del exon 7–10 | S6 | | |
| del exon 8 | P18, R15, S7, R16 | | |
| del exons 9–10 | P19 | | |
| del exons 12–13 | R17 | | |
| del exons 13–15 | S8 | | |
| Dup‡ exons 7–8 | S9 | | |
| Dup exons 9–10 | L14† | | |

*del, deletion.
†Previously published in Charbonnier *et al*.[3]
‡Dup, duplication.
We have not included in this table two *MLH1* rearrangements removing exon 2 and exons 12–13 that we had initially detected by RT-PCR and documented in Charbonnier, *et al*.[4]

families (including 11 families in which IHC staining of the tumours had been undertaken and revealed a selective extinction of *MLH1*) and 106 AMS− families. Among the AMS+ patients, we found an *MLH1* genomic deletion in 3% of the cases when IHC had not been performed, and in 36% of the cases when IHC had revealed a selective *MLH1* extinction. Although the patients for whom IHC staining of the tumours revealed a selective loss of expression were low in number, our results indicate that the selective extinction of an *MMR* protein within a tumour is predictive, in HNPCC families, of the genetic alteration.

We detected a total of 21 distinct *MSH2* exonic rearrangements, including 19 deletions and two duplications, in 43 families; and seven exonic deletions of *MLH1* in nine families (table 2). In families with an *MSH2* rearrangement removing exon 1, QMPSF scanning of 50 Kb of genomic sequences upstream of the *MSH2* transcription initiation site (table 3) revealed at least seven distinct 5′ breakpoints. Furthermore, this analysis showed that the recurrent exonic deletions that we detected (deletions of exon 1, exons 1–2, 1–6, 1–7) had been independently generated (tables 2 and 3), thus excluding a founder effect. The numerous breakpoints within the 5′ *MSH2* region led us to screen for rearrangements affecting the promoter selectively, which would have escaped the initial QMPSF analysis of *MSH2* restricted to the 16 exons. We therefore performed a specific QMPSF assay for the 4.4 kb promoter region, using the promoter amplicons indicated in table 3, and reanalysed 65 AMS+ families without *MSH2*/*MLH1* point mutations or *MSH2* exonic deletion. We identified in a single family a 1.7 kb partial deletion of the promoter removing the -1770–60 region.

This study confirms the following.

- The frequency of *MSH2* exonic rearrangements in AMS+ HNPCC families without detectable point mutations of *MSH2* or *MLH1* can be estimated to approximately 20% (table 1).
- We identified, on the basis of exonic and promoter rearrangements, 30 distinct genomic alterations that demonstrate the remarkable heterogeneity of *MSH2* rearrangements (tables 2 and 3).
- The rearrangements that affect the *MSH2* promoter selectively occur in less than 2% of AMS+ families.
- *MLH1* rearrangements are involved in 7% of AMS+ families without point mutations.

**Table 3** QMPSF scanning of the 5′ *MSH2* region in HNPCC families with exonic deletions removing exon 1

| Family | Exonic rearrangement | QMPSF amplicons* −39258 −39107 | −34123 −33918 | −23323 −23186 | −9381 −9243 | −5112 −4942 | −4244† −4084 | −3591† −3387 | −1714† −1494 | +200‡ 327 |
|---|---|---|---|---|---|---|---|---|---|---|
| R2 | del exon 1 | | | | | | | +§ | del¶ | del |
| L7 | del exons 1–4 | | | | | | | + | del | del |
| R18 | del exons 1–6 | | | | | | | + | del | del |
| U1 | del exons 1–6 | | | | | | | + | del | del |
| R14 | del exons 1–6 | | | | | | + | del | del | del |
| U2 | del exons 1–6 | | | | | + | del | del | / | del |
| R1 | del exon 1 | | | + | del | del | del | del | del | del |
| P15 | del exons 1–2 | | | + | del | del | / | del | del | del |
| R9 | del exons 1–7 | | | + | del | del | del | del | del | del |
| R11 | del exons 1–8 | | | + | del | del | del | del | del | del |
| U3 | del exons 1–11 | | | + | del | del | / | del | / | del |
| R12 | del exons 1–2 | | + | del | del | del | / | del | del | del |
| R13 | del exons 1–2 | | + | del | / | del | / | del | / | del |
| S2 | del exons 1–7 | + | del | del | / | del | / | del | / | del |
| R10 | del exons 1–8 | + | del | del | del | del | del | del | del | del |
| Li8 | del exons 1–6 | del | del | del | del | del | del | del | del | del |
| S1 | del exons 1–6 | del | / | del | / | del | / | del | / | del |
| Lu1 | del exons 1–7 | del | / | del | / | del | / | del | / | del |
| Lu2 | del exons 1–7 | del | / | del | / | del | / | del | / | del |
| S3 | del exons 1–8 | del | / | del | / | del | / | del | / | del |
| S4 | del exons 1–8 | del | / | del | / | del | / | del | / | del |
| P12 | del exons 1–15 | del | del | del | del | del | del | del | del | del |

*Numbered from the *MSH2* transcription initiation site (- 68 bp from the ATG) according to the chromosome 2.
†These amplicons correspond to the *MSH2* promoter defined by Iwahashi, *et al* (1998).
‡Amplicon corresponding to exon 1.
§Non-deleted.
¶Deleted.
Working draft sequence (contig NT_034483). Primers and QMPSF conditions are available upon request.

Two recent papers,[8–9] have reported higher detection rates of *MSH2* and *MLH1* rearrangements, respectively, but these remarkable percentages are probably due to the differences between the populations analysed. The first study,[8] performed on 24 AMS+ families without point mutations, using Southern Blot analysis, documented the detection of an *MSH2* genomic deletion in 50% of the cases. This remarkable detection rate is probably explained by the fact that the exons 1–6 deletion, detected in seven families, were shown to be associated to a founder effect.[8] The second study,[9] based on the QMPSF analysis of 52 AMS+ families without point mutations, reported the detection of genomic deletions of *MSH2* and *MLH1* in 12% of the families, for each gene. In this work, the existence of a common haplotype, in four families harbouring a deletion of *MLH1* exons 1–10, suggested a founder effect and may also have led to an overestimation of the relative contribution of *MLH1* deletions in HNPCC.

In conclusion, we recommend that investigation for *MSH2* rearrangements be included systematically in the routine diagnosis of HNPCC. The contribution of these alterations to HNPCC is higher than that of *MSH6* mutations.[10–12] Considering the lower frequency of *MLH1* rearrangements, except in certain populations where they are associated with a founder effect,[8–9] it is probably more efficient to search in HNPCC families only when IHC staining of the tumours has revealed a selective loss of *MLH1* expression.

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# CORRECTION

The authors of the paper by Howell *et al* in the September issue (HRPT2 mutations are associated with malignancy in sporadic parathyroid tumours. *J Med Genet* 2003;**40**:657–63) have notified us of an error. In figure 1, third row from the bottom, for Family ID (Family F1)4†, the histology of the tumour should be Adenoma and not Carcinoma. The authors apologise for the error.