# Genetics and tumor genomics in familial colorectal cancer

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# Increased frequency of 20q gain and copy-neutral LOH in mismatch repair proficient familial colorectal carcinomas

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# Chapter 6

# Increased frequency of 20q gain and copy-neutral LOH in mismatch repair proficient familial colorectal carcinomas

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**Abstract**

Many hereditary non-polyposis colorectal cancers (CRCs) cannot be explained by Lynch syndrome. Other high penetrance genetic risk factors are likely to play a role in these mismatch repair (MMR)-proficient CRC families. Because genomic profiles of CRC tend to vary with CRC susceptibility syndromes, our aim was to analyze the genomic profile of MMR-proficient familial CRC to obtain insight into the biological basis of MMR-proficient familial CRC.

We studied 30 MMR-proficient familial colorectal carcinomas, from 15 families, for genomic aberrations, including gains, physical losses, and copy-neutral LOH (cnLOH) using SNP array comparative genomic hybridization. In addition, we performed somatic mutation analysis for *KRAS*, *BRAF*, *PIK3CA*, and *GNAS*.

The frequency of 20q gain (77%) is remarkably increased when compared to sporadic CRC, suggesting that 20q gain is involved in tumor progression of familial CRC. There is also a significant increase in the frequency of cnLOH and, as a consequence, a reduced frequency of physical loss compared to sporadic CRC. The most frequent aberrations observed included gains of 7p, 7q, 8q, 13q, 20p, and 20q, and physical losses of 17p, 18p, and 18q. Most of these changes are also observed in sporadic CRC. Mutations in *KRAS* were identified in 26% of the MMR-proficient CRCs and mutations in *BRAF* were identified in 12%. No mutations were identified in *PIK3CA* or the chromosome 20 candidate gene *GNAS*.

In conclusion, while the global patterns of MMR-proficient familial CRC resemble sporadic CRC, the chromosomal instability patterns exhibit a distinct pattern of aberrations with increased levels of cnLOH and 20q gain.

## Introduction

Clinical criteria are used for the identification of Lynch syndrome patients. These so-called Amsterdam Criteria and Bethesda criteria include type of cancer, family history, and age at onset of disease.[1,2] Analysis of families that fulfill the strict Amsterdam Criteria I (AC-I) has proved to be successful in identifying germ-line mutations in mismatch repair (MMR) genes to be responsible for the increased CRC susceptibility in Lynch syndrome families. Mutations in MMR genes lead to deficient mismatch repair, which is reflected by microsatellite instability in tumors from Lynch syndrome patients. However, no mutations are identified in the MMR genes *MLH1* and *MSH2* in over half of the patients that meet the AC-I, suggesting that Lynch syndrome cannot explain all AC-I positive families.[3] Some of these AC-I positive families might have mutations in *MSH6*, *PMS2*, or undetected mutations in *MLH1* or *MSH2*; however, this will not explain all AC-1 positive families. Similarly, approximately 40% of families that fulfill the less stringent Amsterdam Criteria II do not display microsatellite instability, a characteristic of MMR deficiency, in their tumors.[4] Moreover, it has been estimated that approximately 32% of the excess CRC risk that is associated with a positive family history of CRC remains unexplained by known genes.[5] In these families, it is likely that other high penetrance genetic risk factors play a role. Analysis of the incidence of cancer in these families showed that they have an increased risk of CRC compared to the general population, albeit to a lesser extent than Lynch syndrome families.[5-7] Lindor and colleagues observed that MMR-proficient AC-I positive families have an increased risk for CRC but not for other cancers. Furthermore, members of MMR-proficient AC-I positive families tend to develop CRC at an older age than individuals in MMR-deficient AC-I positive families.[6] Aaltonen et al. estimated that first-degree relatives (FDRs) of probands with microsatellite stable cancer had a 1.3-fold increase in CRC risk.[5] In addition, a recent study reported that FDRs of CRC patients with microsatellite stable tumors had an increased risk for CRC and observed a strong correlation between the risk of CRC and the number of affected FDRs.[7] Linkage analysis in CRC pedigrees and affected siblings has been performed to identify novel high penetrance risk factors. Several chromosomal regions have been linked to colorectal cancer susceptibility, including 3q21-q24, 7q31, 9q22.2-31.2, 11q23.2, 11q13.4, 14q24.2, and 22q12.1.[8-15] However, none of these studies have led to the identification of a novel CRC susceptibility gene yet.

Target genes for somatic mutations tend to vary by cancer type. In CRC, p.V600E (c.1199T>A) mutations in *BRAF* are predominantly seen in tumors that have sporadic promoter hypermethylation of *MLH1*.[16] *BRAF* mutations are, on the other hand, rare in the tumors of Lynch syndrome patients. Similarly, in *MUTYH*-associated polyposis G>T transversions are observed, with GAA>TAA mutations in *APC* and specific GGT>TGT mutations in codon 12 of *KRAS* (c.34 G>T, p.Gly12Cys). Tumors in Lynch syndrome patients, in contrast, often carry mutations in codon 13 of *KRAS* (c.38 G>A, p.Gly13Asp).[17,18] In addition to somatic mutations, distinct patterns of genetic instability are associated with specific CRC syndromes. Lynch syndrome carcinomas are characterized by microsatellite instability and copy-neutral

loss of heterozygosity (cnLOH) at the locus of the mutated mismatch repair gene, and rarely have large chromosomal aberrations.[19,20] In contrast, at the genomic level, carcinomas of *MUTYH*-associated polyposis patients are characterized by a high level of cnLOH throughout the genome. In addition, the cancer cells are generally near-diploid or near-triploid.[21] Similarly, characteristic genomic aberrations are observed in breast cancers from patients with a germ-line mutation in *BRCA1* or, to a lesser extent, *BRCA2*.[22-24] Therefore, genomic profiling of familial CRCs may provide insight into the biological basis of CRC in these families.
We studied the pattern of genomic aberrations in carcinomas from MMR-proficient familial CRC patients. Our goal was to generate a profile of genomic aberrations in MMR-proficient familial CRC that might provide insight into the biological basis of the increased CRC susceptibility of these families.

## Material and Methods

### Tumor samples

Thirty MMR-proficient familial colorectal carcinomas and corresponding histologically normal tissues were selected (Table 1). The formalin-fixed paraffin-embedded (FFPE) tumors originate from fifteen families. All families have a positive history of CRC, with either one or two affected generations. Two families fulfill the AC-I and in the other families, at least two family members are affected with CRC. Two tumors from each of the fifteen families were analyzed. Tumor samples were enriched for tumor tissue by taking 0.6 mm tissue punches from the tumor field using a tissue microarrayer (Beecher Instruments, Sun Prairie, WI, USA), guided by a haematoxylin and eosin (H&E)-stained slide. DNA was isolated using a method, described previously; subsequently, DNA was cleaned using the Genomic Wizard kit (Promega, Leiden, the Netherlands).[4] DNA concentrations were measured using the picogreen method (Invitrogen-Molecular Probes, Breda, the Netherlands).
Tumors were tested for microsatellite instability using the marker set recommended by the National Cancer Institute Workshop on Microsatellite Instability, supplemented with three additional mononucleotide repeat markers (BAT40, MSH3, and MSH6), as described previously.[4,25] All of the tumors were microsatellite-stable (MSS).
The study was approved by the local Medical Ethical Committee (protocol P01.019); samples were handled according to the medical ethical guidelines described in the Code Proper Secondary Use of Human Tissue established by the Dutch Federation of Medical Sciences.[1]

### SNP array profiling

We analyzed thirty carcinomas from fifteen CRC families for genomic aberrations, including gains, physical losses and cnLOH. We used Illumina Beadarrays in combination with the linkage mapping panel IV_B4b and human linkage V panel (Illumina, San Diego, CA, USA). The

[1] http://www.federa.org

| Family | Colorectal cancers[a] | Other cancers[b] | Affected with CRC | Generations[c] | AC-I[d] | Relationship[e] |
|---|---|---|---|---|---|---|
| **F1** | C46, C55, C56, C69 | / | 4 of 10 sibs | 1 | - | FDR (sibs) |
| **F2** | C34, C50, C60 | / | 3 of 14 | 2 | - | TDR |
| **F3** | C48, C53 | Thyroid55 | 2 of 4 sibs | 1 | - | FDR (sibs) |
| **F4** | C51, C60, C76, C77, C83 | / | 5 of 8 | 2 | - | FDR |
| **F5** | C40, C56 + unknown | Unknown | unknown | unknown | unknown | unknown |
| **F6** | C56, C58, C63, C72, C73, C76[f] | Breast, Ovary67 | 5 of 11 sibs | 1 | - | FDR (sibs) |
| **F7** | C48, C52, C54, C55, C55, C60, C60, C66, C68 | Lung65, Breast52, Breast55, Breast49 | 9 of 24 | 2 | + | Fourth-degree relatives |
| **F8** | C52, C61, C73 | / | 3 of 10 | 2 | - | FDR (sibs) |
| **F9** | C51, C53 | / | 2 of 7 sibs | 1 | - | FDR (sibs) |
| **F10** | C53, C54, C63, C65 | / | 4 of 5 sibs | 1 | - | FDR (sibs) |
| **F11** | C49, C51, C76 | / | 3 of 4 | 2 | + | FDR (sibs) |
| **F12** | C50, C71 | Pancreas65 | 2 of 6 sibs | 1 | - | FDR (sibs) |
| **F13** | C42, C42, C60, C64 | / | 4 of 15 | 2 | - | FDR (sibs) |
| **F14** | C28, C36, C50, C56 | Thyroid58 | 4 of 14 | 2 | - | TDR |
| **F15** | C52, C65, C66, C78 | Leukemia54, Eusop/ Gastric68, Gastric45 | 4 of 12 | 2 | - | TDR |

Table 1. Characteristics of the cohort of MMR-proficient CRC families.
[a] Colorectal cancer cases in the families, including the corresponding age at diagnosis for each CRC; [b] Other cancers diagnosed in the families, including the corresponding age at diagnosis for each tumor; [c] The number of generations affected with CRC in the family; [d] Fulfillment of the Amsterdam Criteria I; [e] The relationship of the two family members from which the tumors were analyzed in this study. FDR, first-degree relative; SDR, second-degree relative; and TDR, third-degree relative; [f] One family member had two colorectal cancers (C56 and C76).

GoldenGate assay was performed at the Leiden Genome Technology Center[2] following the manufacturer's instructions, with minor adjustments: 0.5 µg input DNA was used for multi-use activation and resuspended in 60 µl RS1.[26] Genotypes were extracted using BeadStudio (V3.2, Illumina, San Diego, CA, USA). Genomic profiles were generated from the tumors and their corresponding normal tissue using the "Beadarray SNP" package, as described previously.[19,21,27]
The nature of the tissue, archival FFPE tissue, did not allow us to study germ line copy num-

[2] http://www.lgtc.nl

ber changes. Such small inherited germ line deletions and amplifications might be the underlying cause of the inherited risk for CRC in these patients. SNP arrays with a higher resolution should be used to study these types of copy number changes; however, these arrays are not yet suitable for DNA isolated from FFPE tissue.

**Homozygosity mapping**

To study the possibility of a recessively inherited risk locus in MMR-proficient familial CRC patients, we analyzed the normal tissue DNA for regions of homozygosity. For each SNP the chance of homozygosity was calculated as 1 minus the frequency of the heterozygote genotype. These calculations used the frequency of the heterozygote genotype found in the Hapmap project for the CEU population.[28] A measure for the extent of homozygosity was derived by multiplying the chances for homozygosity for each SNP in a consecutive stretch of homozygous SNPs. The extent of homozygosity along the chromosomes was visualized as a weighted grey value (darker grey corresponds with an increasing extent of homozygosity), with a cut-off of 10-3, representing stretches of at least ten homozygous SNPs with minor allele frequencies of approximately 0.5.

**Somatic mutation analysis**

Tumor samples were screened for mutations in *KRAS* exons 1 and 2, *BRAF* exon 15, and *PIK3CA* exons 9 and 20 by Sanger sequence analysis. PCR was performed on DNA extracted from FFPE material using iQ supermix (Bio-Rad Laboratories, Veenendaal, the Netherlands) using standard conditions.[29] Sequence analysis was performed at the Leiden Genome Technology Center.

Tumor samples were analyzed for the hotspot mutation in *GNAS* (c.601C>T, p.Arg201Cys) using the Taqman SNP Genotyping assay, following the manufacturer's protocol (Applied Biosystems), in a LightCycler 480 (Roche Applied Science, Almere, The Netherlands).

Some DNA fragments isolated from the FFPE tissues failed to amplify because of the limited fragment size that can be amplified from FFPE tissue. Details of the reaction conditions are available upon request. Primer sequences are provided in supplementary table 1.

Mutation frequencies were compared with the mutation data obtained from the Sanger Institute COSMIC (Catalogue of Somatic Mutations in Cancer) database[3].[30]

## Results and discussion

We analyzed 30 colorectal carcinomas from 15 families with a history of CRC, using Illumina 6K Beadarrays, to study the genomic profile of MMR-proficient familial CRC. Characteristics of the studied cohort are detailed in Table 1. The studied families were predominantly affected with CRC; however, other malignancies were observed in family members, including leuke-

[3] http://www.sanger.ac.uk/cosmic

mia, gastric cancer, thyroid, breast, ovarian, lung, and pancreatic cancers.

The mean age at diagnosis of CRC was 56.4 years (range 28-77). All carcinomas had a microsatellite stable (MSS) phenotype. The majority of CRCs were Dukes B (14/28) or C (8/28) and nearly all were left-sided (25/29). The tumor location varies in different CRC syndromes. Seventy percent of the tumors of Lynch syndrome patients are located in the right side of the colon, whereas almost all familial adenomatous polyposis (FAP) cancers develop in the left side of the colon.[31-33] In addition, carcinomas from patients with *MUTYH*-associated polyposis are predominantly localized in the proximal (right) colon.[34] Familial MSS tumors have been reported to be predominantly located on the left side of the colon, which is consistent with the distribution we observed in our study.[35]

We analyzed the 30 MMR-proficient familial colorectal carcinomas for genome-wide chromosomal gains, physical losses, and cnLOH.[21] To study possible family specific tumor phenotypes, two carcinomas from each family were analyzed. We observed between 0 and 23 genomic aberrations in the MMR-proficient familial CRCs, with a mean of 11.4 aberrations per carcinoma. In addition, we observed a gradual increase in the number of aberrations per tumor from Dukes stage A to stage D. However, we did not detect a significant correlation between the number of aberrations and the Dukes stage of the tumor. This might be explained by the low number of Dukes stages A and D carcinomas in our series.

In a previous study, Rahman et al. reported an average of 5.9 copy number aberrations per MMR-proficient familial CRC, using comparative genomic hybridization. In addition, half of the MMR-proficient familial CRCs displayed less than 5 aberrations.[36] In order to compare our results with this earlier study, we counted the number of copy number aberrations (excluding cnLOH) in our series. In our series of MMR-proficient familial CRCs, there was on average 7.5 copy number aberrations per carcinoma, which is increased compared to the study of Rahman et al. We observed fewer than 5 copy number aberrations in 30% (9/30) of the CRCs. The most frequent aberrations, which we observed in at least 30% of the MMR-proficient familial CRCs, included gains of chromosome 7p (40%), 7q (33%), 8q (30%), 13q (57%), 20p (37%) and 20q (77%); and physical losses of 17p (37%), 18p (37%), and 18q (53%) (Figure 1). The observed aberrations that were most frequent in the MMR-proficient familial CRCs are well-known colorectal cancer aberrations, which are typically observed in sporadic colorectal cancers.[37,38]

We compared the profile of aberrations in our MMR-proficient familial CRCs series to that of sporadic CRC, MAP carcinomas, and Lynch carcinomas series that we analyzed previously, using the same methodology.[19,21,38] The frequencies of gains at 8q and 13q, physical losses at 17p and chromosome 18, and cnLOH at 5q are similar in MMR-proficient familial CRC and sporadic CRC. Gains of chromosomes 7 and 20 were observed more frequently in MMR-proficient familial CRC than in sporadic CRC. On the contrary, physical loss of 8p is observed less frequently in the familial carcinomas. cnLOH of 8p and 17p is observed more frequently in familial CRC than in sporadic CRC. We observed many aberrations that occur in only 10% to 20% of the familial tumors, suggesting heterogeneity among MMR-proficient

familial CRC (Figure 1).

The observed frequency of 20q gain (77%) is remarkably high in the series of MMR-proficient familial CRC compared to sporadic CRC. Based on the literature and our work, a frequency of 30-50% has been reported for 20q gain in sporadic CRC.[37,38] When compared with the 20q gain frequency in a series of sporadic CRCs (36%), which was previously analyzed using the same methodology, we found that the frequency of 20q gain is significantly increased in MMR-proficient familial CRC (p=3.4 x 10-3).[38] In addition, we extended the comparison to a previously reported large series of sporadic CRCs, in which both tumor and paired normal tissue were analyzed by SNP array CGH. [39] While the frequency of 20q gain in this large Japanese series of sporadic CRC is higher (56%), there is still a significant increase of 20q gain in familial CRC (p=4.7 x 10-2).[39] Furthermore, our results are in line with a study of Finnish colorectal cancers, which reported an 85% frequency of 20q gain in MMR-proficient familial CRCs using CGH.[40] In addition, a 70% frequency of 20q gain was reported in a group of early-onset MSS CRC patients.[41] This high frequency of 20q gain suggests that 20q is involved in tumor progression, particularly in MMR-proficient familial CRC. Several genes on 20q, including *AURKA*, *TH1L*, *ADRM1*, and *TCFL1*, have already been described to be differentially expressed in tumors with 20q gain when compared to tumors without 20q gain. [42] Analysis of the coding sequence of eleven CRCs by Sjöblom et al. identified three activating missense mutations in *GNAS*, which is located on 20q13.32.[43] Therefore, we analyzed our cohort of MMR-proficient familial CRC for the most frequent mutation (p.Arg201Cys, c.601C>T) in *GNAS*; however, we did not identify any mutation.

Unlike sporadic CRCs, the MMR-proficient familial CRCs displayed increased levels of cn-LOH genome-wide and, as a consequence, reduced physical loss. In the MMR-proficient familial CRCs, 37% of the aberrations were gains, 31% were physical losses, and 32% were cnLOH. This distribution is different from sporadic CRC, where 31% of the aberrations are gains, 55% are physical losses, and only 14% are cnLOH.[38] The percentage of cnLOH is significantly increased in MMR-proficient familial CRC over that of sporadic CRC (p=6.1 x 10-3). Additionally, the percentage of physical losses is significantly reduced in familial CRC compared to sporadic CRC (p=0.034).

Interestingly, we have previously shown that cnLOH is associated with tumor types that are deficient in DNA repair. cnLOH is the most frequent type of aberration (71%) in MUTYH-associated polyposis (MAP) colorectal carcinomas, which is caused by base excision repair deficiency.[21] Furthermore, in Lynch syndrome carcinomas, which are caused by DNA mismatch repair deficiency, cnLOH is predominantly observed at the locus of the mutated MMR gene. [19] While the MMR-proficient familial CRCs profile does not resemble the MAP carcinoma nor the Lynch syndrome carcinoma profile, the increased level of cnLOH suggests that an unknown DNA repair defect might be involved in the tumorigenesis of MMR-proficient familial CRC.

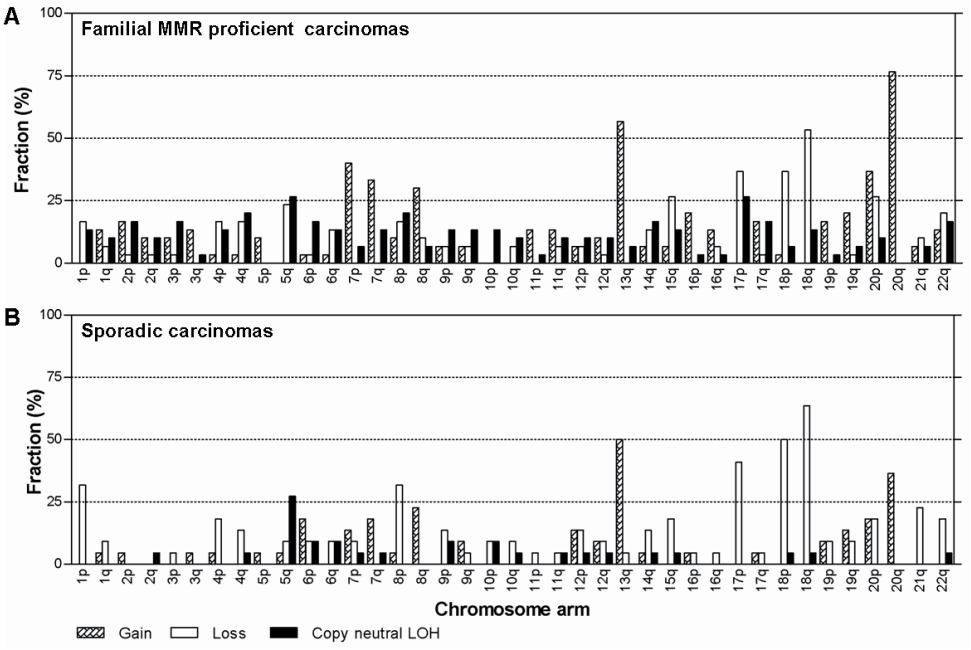


**Figure 1. Chromosomal aberrations in MMR-proficient familial colorectal carcinomas**
(A, B) The bars indicate the percentage of the 30 mismatch repair proficient familial colorectal carcinomas and 22 sporadic carcinomas that exhibit an event of gain, loss or cnLOH of a chromosome. This percentage has been calculated for the respective chromosome arms. White bars, chromosomal gains; checked bars, physical losses of chromosomes; black bars, cnLOH. Panel B shows the results from a previous study of Lips et al.[38]

In addition to comparing the MMR-proficient familial CRC profile with the sporadic CRC profile, we compared the genomic profiles of tumors within one family to identify possible family-specific genomic profiles. Aberrations that were most frequently shared by tumors from family members included the common aberrations at 13q, 17p, 18q, and 20q. There was a high incidence of 20q gain, which was shared by both tumors in over half of the families. Less frequently shared affected regions include 5q, 8p, 8q, 14q, and 15q. No distinctive profile of aberrations was observed in any of the families. The tumor phenotypes of all families roughly fit the overall genomic profile that was observed for MMR-proficient familial CRCs.
In two families, cnLOH of 8p was shared (F3 and F7) and in one family physical loss of part of 8p was shared (F14) by the tumors. In three families (F3, F7, and F13) aberrations at 8q were shared, however, gains, physical losses, as well as cnLOH were observed in these tumors. Three families (F1, F7, and F12) shared physical loss or cnLOH at the region on 5q that encompasses the APC gene. In addition, both tumors that were analyzed from three families (F6, F14, and F15) exhibited physical loss or cnLOH of 14q. Finally, chromosome 15q

displayed aberrations in both tumors from three families (F6, F11, and F15); however, gains, physical losses, and cnLOH were observed.

**Homozygosity analysis**

We searched normal tissue for regions of homozygosity that are shared within a family or between multiple individuals across families. These homozygous regions might suggest the presence of a recessively inherited gene, similar to *MUTYH*. In six of our fifteen families only one generation was affected with CRC, which suggests a recessive mode of inheritance. Moreover, in a previous study, we observed a homozygous region in MAP patients that had a shared haplotype that encompassed the *MUTYH* gene (results not shown).[21] In the current study, we did not identify any shared homozygous regions in the MMR-proficient familial CRCs, with the exception of a small region on chromosome 21q22.13. However, we found that this region was often homozygous in tumors from MAP and Lynch syndrome patients, suggesting that this region of homozygosity is not specific for MMR-proficient familial CRC.[19]

**Somatic mutation analysis**

We also studied the MMR-proficient familial CRCs for somatic mutations in *KRAS*, *BRAF*, and *PIK3CA*. Mutations in *KRAS* were identified in 5 of 19 tumors (26%). Three mutations were identified in codon 12 (one c.34G>T, p.Gly12Cys; and two c.35G>A, p.Gly12Asp), one in codon 13 (c.38G>A, p.Gly13Asp), and one in codon 63 (c.187G>A, p.Glu63Lys) of the *KRAS* gene. In *BRAF*, we detected mutations in codon 600 in 3 of the 26 tumors (12%) analyzed. Two of these mutations were V600E transversions (c.1199T>A, p.Val600Glu) and the third was a V600A transition (c.1199T>C, p.Val600Ala). None of the tumors carried mutations in both *KRAS* and *BRAF*, which has been observed in previous studies also.[16,36,44] The frequency of mutations in *KRAS* is lower and in *BRAF* is slightly increased compared to a previous study of MMR-proficient familial CRC (40% and 4%, respectively).[44] The frequency for both *KRAS* and *BRAF* mutations is similar to the reported mutation frequency in sporadic CRC (COSMIC, 32% and 12%, respectively).[30] We also screened exons 9 and 20 of *PIK3CA* for mutations; however, we did not detect a mutation in any of the 16 tumors that could be analyzed. These results are consistent with a previous report, in which *PIK3CA* mutations were found in only 4% of MSS familial CRCs.[45] The reported *PIK3CA* mutation frequency in sporadic CRC was higher (between 11% (COSMIC) and 32%).[30,46]

In conclusion, we show that the chromosomal instability patterns of MMR-proficient familial CRC are distinct from sporadic CRC, with significantly increased levels of 20q gain and genome-wide cnLOH. However, the overall aberration pattern resembles sporadic CRC.

The increased level of cnLOH in familial MMR-proficient CRC suggests a weak DNA repair defect. The high frequency of 20q gain suggests that there is an important role for this chromosomal region in tumorigenesis, similar to what was found by Laiho and colleagues. Howe-

ver, no gene on 20q has been reported to be mutated in familial CRC.[40] Further evaluation of 20q will be valuable and could include mutation analysis of candidate genes or next-generation sequencing of the entire region.

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Table 2. Copy number changes and cnLOH identified in MMR-proficient familial CRC

| Family | Tumor | Age at diagnosis | Location | MSI | Dukes | Genomic aberrations | Mutations |
|---|---|---|---|---|---|---|---|
| F1 | 1.1 | 60 | Rectum | S | B1 | *1p31.3-p22.2*, 2pter-p14, *4p14-q22.1*, *5q11.2-q31.1*, *8pter-p12*, *12pter-p12.3*, 12q11-q21.33, 13q, *15q*, 16pter-p12.1, *17p*, *18pq*, 19pq, **21q11.2-q21.3**, 21q22.11-qter, *22q* | |
| F1 | 1.2 | 46 | Rectum | S | A | *1p*, **5q14.3**, 7pq, 12pq, 13q, 20pq, *21q* | *KRAS*, c.35G>A, p.Gly12Asp |
| F2 | 2.1 | 65 | Rectum | S | C1 | *1pter-p34.3*, **2pter-p12**, *6pter-p21.2*, *6q*, 8q21.11-q24.22, 11q13.2-qter, *14q21.1-q32.12*, *15q14-q25.3*, *17p*, *18q*, *20p*, 20q | *KRAS*, c.187G>A, p.Glu63Lys |
| F2 | 2.2 | 78 | Sigmoid | S | B2 | 4pter-p15.1, **4q22.1-q22.3**, **6p22.2-qter**, **9pq**, 13q, **14q**, **15q**, *17p*, 17q, *18pq*, *20p*, 20q | |
| F3 | 3.1 | 53 | Rectum | S | B1 | *1pter-p35.1*, **2pq**, *8pter-q13.2*, *9q34.11-qter*, *14q*, 13q, *15q11.2-q25.1*, *17pter-p12*, *18pq*, *20pter-p11.22*, 20p11.22-qter | |
| F3 | 3.2 | 48 | Colon asc. | S | C1 | 1q42.12-qter, *2q21.2-qter*, **3p14.3-p12.2**, **6q13-qter**, 7p, **8p**, 8q, 9q33.3, 14q, 22q11.21-q12.2, *22q12.2-qter* | |
| F4 | 4.1 | 77 | Sigmoid | S | unknown | **1pq**, 2pq, **4pq**, *5q*, 9p, 13q, *14q*, 19q, 20pq, **21q**, 22q | |
| F4 | 4.2 | 51 | Rectosigmoid | S | unknown | **2pter-p24.2**, 2p24.1-p22.3, **2p22.2-qter**, 8q23.3-qter, *18pq*, 20q | |
| F5 | 5.1 | 40 | Rectum | S | B2 | **4pter-q31.1**, 4q31.21-qter, *8p*, *10q21.1-qter*, *11q12.1-14.1*, *15q*, 16pq, **17pq**, **18pq**, 19pter-q13.2, **19q13.31-qter**, 20pq, *22q* | |
| F5 | 5.2 | 56 | Rectosigmoid | S | B1 | 7pq, **9pq**, 13q, **17pq**, *20p*, 20q | |
| F6 | 6.1 | 72 | Colon asc. | S | B1 | **1q**, 7pq, **9pq**, **10pq**, **11pq**, **14q**, **15q**, *18pq*, **19pter-p13.2**, 20pq, **21q** | BRAF, c.1199T>A, p.Val600Glu |
| F6 | 6.2 | 76 | Colon desc. | S | B2 | *3p*, **5q13.2-qter**, *6q16.1, 8q22.1-qter, 9pter-p13.3, 10q23.31-25.1*, **12pter-12.1**, **12q15-q21.1**, 13q, *14q*, *15q11.2-12*, 15q12-q14, *15q14-qter*, *17p*, *18q*, *19q13.31-qter*, **20p**, 20q, 21q22.11-qter, *22q* | |
| F7 | 7.1 | 48 | Sigmoid | S | B1 | **1p36.13**, **5q13.2**, **7pq**, **8p**, 8q, **13q**, **17p**, **18q**, 20pq, **22q** | |
| F7 | 7.2 | 52 | Rectosigmoid | S | D | *1pter-35.2*, *1q43-qter*, 2p16.1-13.3, 2q24.1-qter, 3q24-qter, *4pter-14*, **5q11.2-q33.3**, *5q34-qter*, **6pter-p21.31**, 7pq, **8p**, 8q, **9pter-21.2**, 11q13.2-22.3, 11q23.1-qter, **12pq**, **14q**, *17p*, 17q, *18q*, *20p*, 20q, *21q* | |
| F8 | 8.1 | 61 | Rectum | S | B1 | **2pq**, **5q**, **6pq**, **7q21.11-qter**, 13q, *16q21-qter*, *17p*, *18pq*, 20pq | *KRAS*, c.34G>T, p.Gly12Cys |

Table 2 continued. Copy number changes and cnLOH identified in MMR-proficient familial CRC

| Family | Tumor | Age at diagnosis | Location | MSI | Dukes | Genomic aberrations | Mutations |
|---|---|---|---|---|---|---|---|
| F8 | 8.2 | 53 | Sigmoid | S | C2 | 1q, **3pter-p22.3**, **5q**, **6pter-q22.1**, 7p, **7q**, **8pter-p21.3**, **10pter-p13**, 13q, **15q**, **17p**, **18pq**, 19pter-q13.11, **19q13.11-qter**, 20pq, **22q11.23-qter** | |
| F9 | 9.1 | 51 | Colon transv. | S | D | **4pq**, 5p, 6pter-22.1, 6q23.2-23.3, 7pter-q22.1, **8pq**, **10pq**, 11pter-q12.1, **11q22.3-qter**, 12pq, 14q, 15q11.2-25.3, **16pq**, **17pq**, 20q12-qter, **22q11.21-q12.2**, *22q12.3-qter* | |
| F9 | 9.2 | 53 | Sigmoid | S | D | No chromosomal aberrations | |
| F10 | 10.1 | 62 | Rectum | S | B1 | **1pter-p34.1**, *4pq*, *5q13.1-q31.1*, 11p, **13q**, 16pq, **17p**, 17q, *18pq*, 19pq, **20pter-p11.23**, *20p11.22-qter* | |
| F10 | 10.2 | 54 | Sigmoid | S | A | 1q32.1-qter, 2pq, 3pq, 7pq, 8pq, 13q, 16pq, *20p*, 20q | |
| F11 | 11.1 | 50 | Sigmoid | S | B1 | 3pq, 7pq, 8pq, 9pq, 13q, *15q11.2-q21.2*, **15q21.3-qter**, *17p*, *18pq*, 20pq, 22q | *BRAF*, c.1199T>A, p.Val600Glu |
| F11 | 11.2 | 51 | Colon right-side | S | B2 | *1q*, *2p24.3-p21*, **3pq**, *6q14.1-16.3*, **10pq**, **12pq**, 13q, *15q*, *17p*, 17q, *18pq*, *20p*, 20q, *21q*, *22q* | |
| F12 | 12.1 | 50 | Sigmoid | S | C2 | **1pter-35.1**, **1q12-q42.11**, 1q42.12-qter, 3pter-p13, **3p12.3-qter**, *4pq*, *5q14.1-q23.2*, *6q13-qter*, 7pter-14.1, **7p13-qter**, **8p**, 8q, *9pter-p33.1*, **9q33.2-qter**, 13q, **17p**, 17p11.2-q25.1, **17q25.1-qter,** 18p, **18q**, 19pq, **20pter-p12.3**, 22q | |
| F12 | 12.2 | 71 | Rectosigmoid | S | B1 | **5q** | |
| F13 | 13.1 | 42 | Rectum | S | B1 | **4pq**, 5p, **5q**, 7pq, **8q**, 13q, 20pq | |
| F13 | 13.2 | 42 | Rectum | S | C2 | *4pq*, *8q*, *11q*, *12p13.31-qter*, 13q, *15q*, *16q12.1-23.3*, *17pq*, *18pter-q22.3* | *KRAS*, c.35G>A, p.Gly12Asp |
| F14 | 14.1 | 56 | Colon unspec | S | Cx | **4q12-q22.2**, *4q22.3-qter*, *5q22.2-q23.3*, 7pq, *8pter-p11.23*, 8p11.23-qter, 11p, 11q13.4-q23.2, **14q**, 16p, *17p*, *18pq*, **20p**, 20q, **22q** | |
| F14 | 14.2 | 28 | Rectum | S | D | **2p22.3-p11.2**, **2q14.1-qter**, 5p, **5q11.2-q14.2**, 5q14.3-q21.3, **6p**, *8p*, **9q32-q33.1**, 13q, **14q**, *18q12.3-q22.1*, **20p**, 20q, **22q** | |
| F15 | 15.1 | 50 | Rectosigmoid | S | C2 | **3pter-p13**, 11pter-p13, 13q, 16p12.1-q13, **17pq**, *18q21.2-qter*, 20q | *BRAF*, c.1199T>C, p.Val600Ala |
| F15 | 15.2 | 34 | Sigmoid | S | C2 | No chromosomal aberrations | *KRAS*, c.38G>A, p.Gly13Asp |

Chromosomal physical losses are depicted in italics, gains are underlined and cnLOH is depicted in bold face.

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