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# Allele-specific loss of heterozygosity in multiple colorectal adenomas: toward an integrated molecular cytogenetic map II

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**Abstract** Colorectal cancer (CRC) remains a significant public health challenge despite our increased understanding of the genetic defects underlying the pathogenesis of this common disease. It has been thought that multiple mechanisms lead to the malignant phenotype, with familial predisposition syndromes accounting for only a small proportion of all CRC cases. To identify additional loci likely involved in CRC and to test the hypothesis of allele-specific loss of heterozygosity (LOH) for the localization of CRC susceptibility genes, we initially conducted a genome-wide allelotyping analysis of 48 adenomas from a patient with familial adenomatous polyposis coli (FAP) and 63 adenomas from 7 patients with sporadic CRC using 79 fluorescently tagged oligonucleotide primers amplifying microsatellite loci covering the human genome. Frequent allelic losses were identified at *D17S802* (41%), *D7S518* (40%), *D18S53* (38%), *D10S249* (32%), *D2S391* (29%), *D16S419* (27%), *D15S1005* and *D15S120* (24%), *D9S274* and *D11S1318* (23%), *D14S65* (20%), *D14S274* and *D17S953* (19%), *D19S424* (18%), *D5S346* and *D1S397* (15%), and *D6S468* (13%) in multiple FAP adenomas. Common LOH was also detected at *D4S1584* (42%), *D11S968* (31%), *D17S953* (28%), *D5S394*, *D9S286* and *D10S249* (24%), *D8S511* (23%), *D13S158* (21%), *D7S669* (20%), *D18S58* (19%), *D2S162* and *D16S432* (16%), *D2S206* (15%), *D7S496* and *D17S946* (14%), *D6S292* (13%), *D4S1586* and *D8S283* (11%), and *D1S2766* (10%) in multiple CRC adenomas. In addition, allele-specific LOH at *D5S346*, *D15S1005*, and *D15S120* was observed in multiple FAP adenomas ($P < 0.01$) and at *D2S206* and *D16S423* in multiple CRC ($P < 0.05$). To compare our data to previous reports, we determined the band-specific frequency of chromosomal imbalances in CRC karyotypes reported in the Mitelman database, and from the CGH results of cases accessible through the PROGENETIX website. Furthermore, published genome-wide allelotyping analysis of CRC and other allele-specific LOH studies were compiled and collated with our LOH data. The combined results not only provide a comprehensive view of genetic losses in CRC, indicating the comparability of these different techniques, but they also reveal different novel loci in multiple adenomas from FAP and sporadic CRC patients, suggesting that they represent a distinct subtype of CRC in terms of allelic losses. Allele-specific LOH is an alternative approach for cancer gene mapping. 

## 1. Introduction

Colorectal cancer (CRC) represents a group of heterogeneous epithelial malignancies, of which familial adenomatous polyposis coli (FAP) and hereditary nonpolyposis colon cancer (HNPCC) are two major CRC predisposition syndromes [1–3]. FAP usually presents in the second decade and is characterized by large numbers of adenomatous polyps (usually more than 100), carpeting the large bowel. Malignant change usually takes place in one or more polyps by the age of 50 years [4–7]. Almost all FAP cases result from truncating mutations in the *APC* gene [5–10]. In contrast, HNPCC patients have a normal or only slightly elevated tendency to develop adenomas, but the probability and rate of progression to carcinoma is increased [11–13] and an increased risk of other carcinomas is also

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a recognizable feature of this syndrome [14]. Germline defects in one or more of a group of DNA mismatch repair genes, including *MLH1*, *MSH2*, *MLH3*, *PMS1*, *PMS2*, *MSH6*, and *GTBP*, are associated with HNPCC [15–21]. Approximately 15% of CRC are caused by dominantly inherited predisposition to the disease [22,23]. Only 2–6% of cases have been attributed to FAP or HNPCC [8,9,12], however, suggesting the presence of additional predisposition genes [24]. A proportion of this residual risk may be due to primary predisposition to colorectal adenomas, which subsequently progress to carcinoma. Previous epidemiological studies have shown that the relatives of CRC patients have a two- to threefold risk of developing adenomas [25–27], and relatives of probands with adenomas are at a twofold risk of developing CRC [28]. Furthermore, it has been suggested that predisposition to colorectal adenoma is common in the general population and that colorectal adenomas and carcinomas may occur predominantly in susceptible individuals [22].

Since FAP and HNPCC patients usually develop multiple independent adenomas, the presence of colorectal adenomas may offer additional support for the localization of susceptibility genes by genetic linkage analysis in families with multiple affected cases. If the underlying susceptibility gene were a tumor suppressor gene, there should be loss of heterozygosity (LOH) occurring in a substantial proportion of tumors within the vicinity of the gene, as previously shown for *APC* [29]. With multiple tumors occurring in the same individual, each tumor should lose the same allele inherited from the non–mutation-carrying parent. This type of allelic losses has been described as allele-specific LOH [30]. Other LOH events not related to a susceptibility gene locus, which might even occur at high frequency, would usually not be allele specific. The utilization of allele-specific LOH in individuals with multiple tumors may obviate the requirement for ascertainment of multiple cases from the same family and, hence, is applicable to susceptibility syndromes with low or variable penetrance. In addition, since allelic losses in tumors often span large chromosomal distances, the marker map used in an allele-specific LOH search for a susceptibility gene could be less dense than the 10–20 cM usually employed in conventional linkage analysis. Allele-specific LOH analysis has previously been used to investigate the clonal origin and progression of several types of tumors [30–47]. To our knowledge, however, it has not been tested for cancer susceptibility gene identification.

As a model for both multistep and multipathway carcinogenesis [48,49] CRC provides paradigms of alterations of tumor suppressor genes (TSG) and oncogenes in malignant transformation [50]. These genetic changes can be detected by different techniques, including conventional cytogenetics, metaphase or array-based comparative genomic hybridization (CGH), and allelotyping. Previous cytogenetic studies have revealed chromosomal abnormalities in 30–80% of CRC, including deletions of 1p, 3p, 5p, 10p, and 17p, as well as loss of 18 [51–54]. CGH studies have shown DNA copy number losses of 5q, 10q, 11q, 17p, and 18q [55]. Molecular studies have demonstrated frequent allelic losses at 1p, 5q, 7q, and 15q [56–58]. However, none of these have put the cytogenetic data [banding, CGH, multiplex fluorescent in situ hybridization (M-FISH)] and molecular data (LOH, genomic and expression microarrys) of a specific tumor type together to create a user-friendly map in a single setting. We have constructed the integrated molecular cytogenetic maps for Sézary syndrome and breast cancer via this approach, facilitating the direct assessment of genetic alterations at chromosomal and molecular levels [59,60]. This provides a basis for the comparison between different techniques to create integrated molecular cytogenetic maps for different tumors.

To identify additional loci likely to be associated with the pathogenesis of CRC through the assessment of allele-specific LOH, and to construct the integrated molecular cytogenetic map for CRC, we initially conducted a genome-wide allelotyping analysis of 48 adenomas from 1 FAP patient and 63 adenomas from 7 patients with sporadic CRC using 79 fluorescently tagged oligonucleotide primers amplifying microsatellite (MS) loci covering the human genome. We then combined our LOH data with published cytogenetic, CGH, and allelotyping data of CRC by using dedicated karyotype parsing softwares and conventional literature searches.

## 2. Materials and methods

### 2.1. Allelotyping

#### 2.1.1. Specimens and DNA extraction

Two sets of samples were collected for genome-wide allelotyping analysis. The first one included 48 adenomas and a normal control tissue (appendix) that were microdissected from formalin-fixed, paraffin-embedded tissue sections from a 45-year-old male patient with FAP who had 5,409 discrete adenomas including tubular, villous, and tubulovillous adenomas. The second one consisted of 63 adenomas and 7 normal control tissues that were dissected from paraffin sections from 7 patients with sporadic CRC (5 male and 2 female, 39–80 years old, average adenoma number $<10$). Dissected tissue samples were incubated in 10 mmol/L Tris hydrogen chloride (pH 7.5), 1 mmol/L ethylenediaminetetraacetic acid, 1% (wt/vol) sodium dodecyl sulfate, and 500 μg/mL proteinase K at 37°C for 72 hours. The mixture was then heated at 100°C for 10 minutes and directly used for polymerase chain reaction (PCR) amplification without further purification.

#### 2.1.2. Primers, PCR, data, and statistic analysis

Our previous study showed that DNA extracted from paraffin sections could not reliably yield products greater than 200 base pairs (bp) upon PCR [61], hence MS markers