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Alpha-1-Antitrypsin Deficiency and Smoking as Risk Factors for Mismatch Repair Deficient Colorectal Cancer: a Study from the Colon Cancer Family Registry

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Abstract

In a previous study, alpha-1-antitrypsin (A1AT) deficiency alleles were found to be over represented among individuals with microsatellite unstable (MSI-high) colorectal cancers, and this was most significant in former or current smokers. We evaluated this association in a larger case-control study, stratified by microsatellite instability phenotypes. Concordant with prior observations, gender (female) and smoking history were positively associated with colorectal cancers having an MSI-high phenotype. No difference in frequency of A1AT deficiency alleles was found between cases and controls, irrespective of the MSI subtype.

Keywords

protease inhibitor; microsatellite instability; DNA mismatch repair; smoking

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1. Background

Microsatellite instability (MSI) refers to altered, short, repetitive DNA sequences and occurs in most tumors from patients with hereditary non-polyposis colon carcinoma (HNPCC-Lynch Syndrome) as well as a subset of nonhereditary cancers, including colorectal, gastric and endometrial [1]. In nonhereditary colorectal cancers (CRC), three MSI phenotypes have been described: the MSI-high phenotype (MSI-H; ≥ 30 of loci examined show instability in short repetitive DNA sequences); MSI-low phenotype (MSI-L; instability in less than 30% of loci examined) [1–3]; and tumors lacking any evidence of MSI are defined as microsatellite stable (MSS). Current data suggests that the MSI-H phenotype in nonhereditary CRC results from defective DNA mismatch repair: over 95% are attributable to somatic loss of *MLH1* gene function [2–4].

In a prior clinic-based study, we unexpectedly found a nominally significant association between heterozygosity for α_1 -antitrypsin deficiency (α_1 ATD-ht) and risk of CRC with an MSI-H phenotype: the relative risk of having MSI-H CRC for current and former smokers (regardless of α_1 ATD-ht status) was 6.6 and 2.7, respectively [5].

Given that 5–10% of those of European descent are carriers of a α_1 ATD allele, the contribution of α_1 ATD-ht status to the overall colorectal cancer burden could be significant. Our hypothesis for this study was that α_1 ATD-ht inactivating alleles (i.e., S and Z) [5,6] may represent a hereditary risk factor for the development of CRC, specifically for MMR deficient CRC, an effect that may be accentuated by cigarette smoking.

2. Methods

2.1 Study Population

CRC cases came from the Colon Cooperative Family Registry (C-CFR.) (<http://epi.grants.cancer.gov/CFR/>). C-CFR registries used to identify eligible cases were at Fred Hutchinson Cancer Research Center, University of Hawaii Cancer Research Center, Cancer Care Ontario, Australasia CFR, and Mayo Clinic. Each registry provided one unrelated control matched on gender, age at interview (± 2 years), and ethnicity. A total of 940 cases were identified with available mismatch repair data, smoking data, and available DNA. Fifty three percent were females; of the 940 pairs, 79% were Caucasian. They were classified into four different groups:

- **Group 1 (n=96)** included cases diagnosed under age 50 years in which CRC showed evidence of DNA mismatch repair (MMR) deficiency, defined as an MSI-H phenotype or loss of immunohistochemical expression (IHC) of one or more of the DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*). Results of IHC are shown for Groups 1 and 2 in Table 2.
- **Group 2 (n=301)** were cases diagnosed over age 49 years, also with tumor MMR deficiency.
- **Group 3 (n=173)** included cases with MSI-low tumors, diagnosed at any age.
- **Group 4 (n=370)** included cases with MSS tumors, diagnosed at any age.

This was a one-to-one matched case-control study. All cases and controls had provided written informed consent for use of their blood, tumor, and risk factor questionnaires; all were approved by the respective institutional research review boards.

2.2 Smoking exposure history

All subjects completed a risk factor questionnaire around the time of C-CFR enrollment. Cigarette smoking status was ascertained in the year prior to diagnosis for cases and a comparable period for control and was described as never smoked, former smoker, or current smoker.

2.3 Immunohistochemistry

For each CRC case, paraffin-embedded tissue sections were stained with antibodies to hMLH1, hMSH2 and hMSH6 as described [4]. hPMS2 protein expression was assessed using a purified mouse anti-PMS2 monoclonal antibody (clone A16-4, BD PharMingen). Tumor cells showing absence of nuclear staining in the presence of normal nuclear staining of normal colonic mucosa or lymphocytes were interpreted as having absence of expression [4].

2.4 Tumor Microsatellite Instability

DNA from paraffin-embedded normal and tumor tissue was microdissected and extracted as described. Only areas containing >70% tumor cells were used. MSI was assessed with 10 microsatellite markers, four mononucleotide (BAT26, BAT25, BAT40, BAT34c) and 6 dinucleotide (D5S346, D17S250, D18S55, D10S197, mycL and ACTC). A minimum of five informative markers was required to score for MSI. MSI phenotypes were defined as MSS, MSI-L or MSI-H if 0, 1–30, or ≥ 30 of the informative markers exhibited MSI, respectively [1].

2.5 Genotyping

α_1 AT genotypes were determined by a Roche LC480 light cyclers assay, as described [7].

3. Statistical Analysis

Separate analyses were performed for the four case groups versus controls in this one-to-one case control matching study. Before model based analysis, we looked at the frequency of each variable. Univariate and multivariable conditional logistic regression models were then fit to evaluate the relationship between colon cancer and carrier of deficiency alleles and smoking, both between CRC groups and between cases and controls. SAS9.2 was used to generate the odds ratios (OR) and 95% confidence intervals from these models.

4. Results

Table 1 shows the most notable analyses. There was no statistical difference between the proportion of cases with α_1 ATD-ht alleles compared to age/gender/ethnically matched controls across any of the CRC DNA mismatch repair subsets. Among the 301 cases with MSI-H tumors, 63.5% smoked (n=191), compared to 56.9% of age/gender/ethnically matched controls (P=0.01). Of the MSI-H cases versus controls that had α_1 ATD-ht alleles, 60.5% of both cases and controls smoked, i.e., smoking exposure was not associated with increased rates of α_1 ATD-ht alleles in cases compared to controls. Female gender was positively associated with MSI-H colorectal cancers and smoking was also confirmed as an independent risk factor for MSI-H colorectal cancers. MSI-L cancers showed a similar trend toward smoking like MSI-H cancers but opposite trend with respect to gender, more like MSS tumors. An earlier study indicated carriers of deficient allele S had elevated risk of proximal colon cancer among female smokers, using a protein-based testing (isoelectric focusing) (5); this was not confirmed in this DNA-based study.

5. Conclusion

This study did not confirm the prior observation of an association between α_1 ATD-ht and MSI-high colon cancers. Smoking does appear to be an independent risk factor for MSI-H CRCs over age 49 years, possibly for MSI-L CRCs, but not for MSS CRCs or in young onset MSI-H cases. This is consistent with other recent reports [8]. The over representation of females in the MSI-H >49 years of age subgroup has also been reported before [9].

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Table 1

Odds ratios between each case and control group reported by using logistic regression models. The models are fitted for each covariate separately (univariate) as well as jointly (multivariable). Interactions between allele and sex, allele and smoke status were also tested. For simplicity, only main multivariable effects are presented.

	1. MSI-H or loss of MMR by IHC <age 50 (n=96)	2. MSI-H or loss of MMR by IHC >age 49 (n=301)	3. MSI-L Any age (n=173)	4. MSS Any age (n=370)
	Odds ratio (CI)	Odds ratio (CI)	Odds ratio (CI)	Odds ratio (CI)
	<i>P</i> value N (% of group)	<i>P</i> value N (% of group)	<i>P</i> value N (% of group)	<i>P</i> value N (% of group)
S_allele (at least one copy)	0.65 (0.25, 1.67) <i>0.37</i> N=5(5.3%)	1.01 (0.61, 1.67) <i>0.97</i> N=25 (8.6%)	0.74 (0.38, 1.45) <i>0.38</i> N=11(6.4%)	0.91 (0.56, 1.46) <i>0.69</i> N=28(7.6%)
Z_allele (at least one copy)	0.97 (0.28, 3.31) <i>0.96</i> N=3(3.1%)	1.19 (0.58, 2.45) <i>0.64</i> N=12(4.0%)	1.55 (0.70, 3.43) <i>0.28</i> N=9(5.2%)	0.80 (0.38, 1.70) <i>0.56</i> N=10(2.7%)
Smoke (ever)	0.94 (0.60, 1.47) <i>0.71</i> N=53 (55.2%)	1.46 (1.10, 1.95) 0.01 N=191 (63.5%)	1.38 (0.97, 1.95) <i>0.07</i> N=114(65.9%)	0.99 (0.77, 1.29) <i>0.96</i> N=212 (57.3%)
Males vs Females	1.52 (0.97, 2.38) <i>0.07</i> N=42 (43.7%)	0.53 (0.40, 0.71) <0.0001 N=202 (67.1%)	1.23 (0.88, 1.72) <i>0.23</i> N=83 (48%)	1.30 (1.01, 1.69) <i>0.04</i> N=175 (47.3%)

Table 2

Available immunohistochemistry groups in the MSI-H cases: Group 1 (MSI-H and age < age 50 years) and Group 2 (MSH-H, age > 49 years). These results suggest the age cut-offs did create groups that were more (Group 1) and less (Group 2) likely to have germline mutations underlying the MSI phenotype. Germline testing, tumor *BRAF* status, and tumor *MLH1* methylation results were not sufficiently consistently available at the time of this study to further refine these groups while maintaining adequate sample sizes.

	Group 1	Group 2
MLH1		
# samples with interpretable results	89	273
# samples with loss of expression	41	213
% interpretable samples with loss of expression	46%	78%
MSH2		
# samples with interpretable results	87	281
# samples with loss of expression	35	30
% interpretable samples with loss of expression	40%	10.7%
MSH6		
# samples with interpretable results	72	247
# samples with loss of expression	2	9
% interpretable samples with loss of expression	3.6%	3%
PMS2		
# samples with interpretable results	59	171
# samples with loss of expression	3	8
% interpretable samples with loss of expression	5%	4.7%