Cytochrome P450 17A1 and Catechol O-Methyltransferase Polymorphisms and Age at Lynch Syndrome Colon Cancer Onset in Newfoundland

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Abstract

Purpose: Lynch syndrome is a cancer predisposition syndrome which includes colon cancer. It is caused by inherited defects in DNA mismatch repair genes. Sporadic colon cancers are influenced by exogenous hormones (e.g., postmenopausal hormones); we hypothesized that polymorphisms which influence endogenous hormones would therefore modify age at colon cancer onset among Lynch syndrome mutation carriers.

Experimental Design: We genotyped 146 Caucasian Lynch syndrome mutation carriers for a 5′-untranslated region polymorphism in cytochrome P450 17A1 (CYP17; c.-34T→C) and an exon 4 polymorphism in catechol O-methyltransferase (COMT; c.472G→A). 50 mutation carriers had developed colon or rectal cancer at last contact. We used χ² tests to assess differences in counts. Kaplan-Meier survival curves and Cox proportional hazard models assessed age at onset of colorectal cancer stratified by CYP17 and COMT genotypes.

Results: Homozygous carriers of the CYP17 C allele were diagnosed with colorectal cancer 18 years earlier than homozygous carriers of the T allele. Hazard ratios identified that, relative to homozygous carriers of the T allele (T/T), carriers of one copy (T/C) and two copies (C/C) of the rare allele were, respectively, at 1.9-fold and 2.9-fold increased risk of colon cancer at any age. The COMT rare allele suggested a nonstatistically significant trend of decreased colon cancer risk.

Conclusions: This study showed that a polymorphism in CYP17 (c.-34T→C) modifies age at onset of Lynch syndrome. Because of the high risk of colorectal cancer among this group, knowledge of the CYP17 genotype is warranted for genetic counseling and risk assessment. Future work should assess polymorphisms associated with steroid hormones in Lynch syndrome mutation carriers.

The incidence and mortality rates for colorectal cancer in the Canadian province of Newfoundland and Labrador rank among the highest in the world (1), partly explained by a high prevalence of familial cancer (2). Lynch syndrome is an autosomal dominant genetic syndrome characterized by early age at colon cancer diagnosis and the common occurrence of tumors in the gastrointestinal, upper urologic, and female reproductive tracts (3). Lynch syndrome is caused by germ line mutations in genes involved with postreplicative nucleotide mismatch repair; ~90% of the causative mutations occur in MLH1 (located on chromosome 3p21.3) or MSH2 (located on chromosome 2p22-21; ref. 4). Loss of DNA repair ability leads to microsatellite instability in tumor tissue, a hallmark of Lynch syndrome (5).

Penetrance of MSH2 germ line mutations is high but variable. The consistent finding of lower penetrance for colon cancer in women than in men with the same mismatch repair defects (6–10) suggests that steroidal hormones, such as estrogen or testosterone, might modify risk. Exogenous estrogens (postmenopausal hormones and oral contraceptives) likely reduce the risk of sporadic colon cancer (11, 12). More specific to Lynch syndrome, oral contraceptives and postmenopausal hormones are linked with decreased microsatellite instability colorectal cancer risk (13). Recent work by our group suggests that hormonal contraceptives and postmenopausal hormones are associated with decreased colorectal cancer risk among women from families that meet the Amsterdam or revised Bethesda criteria for Lynch syndrome.5

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Common genetic polymorphisms have been reported to influence endogenous hormone levels (14, 15). Polymorphisms in the sex steroid synthesis and metabolism genes cytochrome P450 17A1 (CYP17) and catechol-O-methyltransferase (COMT) have been studied extensively in relation to risk of breast cancer (16–19), and to a lesser extent, cancers of the ovary (20), endometrium (21), and prostate (22, 23).

CYP17 encodes a protein involved in the production of precursors to testosterone, estrone, and estradiol. A common single base pair substitution (c.-34T→C) in the 5′ untranslated region of CYP17 creates an additional promoter site and increases its transcription (24). Relative to the common allele (T), the rare allele (C) correlates with higher serum levels of various sex steroids (e.g., testosterone, progesterone, estrone, and estradiol; refs. 25–28).

COMT encodes an enzyme responsible for metabolizing catechol estrogens into their less active derivatives (29). A G→A transition in exon 4 of COMT creates a valine-to-methionine substitution; the substitution was reported to decrease protein activity and stability by 2- to 3-fold in vitro (30, 31). The rare allele (MET) was associated with higher concentrations of several sex hormones in both premenopausal (32) and postmenopausal women (28).

To our knowledge, only one study has examined the influence of one of these polymorphisms (COMT) with risk of sporadic colorectal cancer (33), and no study has been conducted among confirmed Lynch syndrome mutation carriers. Given the putative evidence for a link between steroidal hormone exposures and colorectal cancer, the current interest was to identify whether polymorphisms in CYP17 and COMT modified Lynch syndrome age at colon cancer onset among confirmed Lynch syndrome mutation carriers. In addition to the reported influences of these polymorphisms on endogenous steroid levels, they were also selected for study because both rare alleles were reported to be sufficiently common in Caucasian populations (15, 34–37).

Materials and Methods

Over the last 25 years, members of more than 100 nuclear families with extensive colorectal cancers were referred to the Provincial Medical Genetics Program at Memorial University of Newfoundland in St. John’s, Newfoundland and Labrador, Canada. Members of more than 50 of these families received MSH2 germ line mutation screening for Lynch syndrome (6). Twelve families harbored a founder mutation in MSH2 (c.942+3A→T). One of these families was used in the original linkage study of the 2p locus (38). A second mutation in MSH2 was recently identified, c.846-T>2802+C (a genomic deletion of exons 4-16), in a very large kindred (39). A third mutation in MSH2, c.1277-1_1386+2del (a genomic deletion of exon 8), was identified in five families (39).

Genomic DNA was extracted from leukocytes using a common salting out procedure (40). Techniques for MSH2 mutation detection were reported previously (6, 39). For the current study, we selected all archived mutation-positive DNA samples for which we had data on age and site of cancer diagnoses, colon screening history, polyp detection, and vital status (n = 161). From those 161 samples, 15 were excluded because of low DNA yield. Written informed consent was obtained from all subjects for use of their DNA samples for research purposes consistent with this study. The ethics review board of the Memorial University of Newfoundland approved this study.

CYP17 and COMT genotypes were determined by PCR and RFLP techniques as previously described (20), with slight adaptation. A 459 bp fragment of CYP17 was amplified using the following primers: forward 5′-TCTTCCACAGGCAAGAGA-3′; reverse 5′-TTGGCCCAA-AACAAATG-3′. The 25 μl PCR reaction for CYP17 included 100 ng of genomic DNA, 1× reaction buffer, 3.0 mmol/L of MgCl2, 0.2 mmol/L of each deoxynucleotide triphosphate, 0.04 units/μl of Taq polymerase, and 0.6 μmol/L of each forward and reverse primers. The region containing the COMT exon 4 polymorphism was amplified using the primers: forward 5′-TCATGTGGCTACTCTGTGC-3′; and reverse 5′-GTGAACGTGCTGTAACACCC-3′. The 20 μl PCR reaction for COMT included 200 ng of genomic DNA, 1× reaction buffer, 0.2 mmol/L of each deoxynucleotide triphosphate, 0.05 units/μl of Taq polymerase, and 0.2 μmol/L of each of forward and reverse primers.

The thermocycler conditions used were 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 57°C, and extension for 1 min at 72°C. Initial denaturation (95°C for 5 min) and final extension (72°C for 10 min) steps were also used. After amplification, 3.3 μL of digestion buffer, 0.66 μL of restriction enzyme MspAI (New England BioLabs), and 0.35 μL of bovine serum albumin were added to each 25 μL sample of the CYP17 PCR product. Digestion of the total mixture occurred for ~12 h at 37°C. Added to each 20 μL sample of the COMT PCR product were 3 μL of digestion buffer and 1.5 μL of restriction enzyme NalIII (New England BioLabs). Digestion of the total mixture occurred for 1 h at 37°C. The digested products were separated using agarose gel electrophoresis and visualized using ethidium bromide staining. Restriction fragments were detected as previously reported (16, 24, 36). The presence of a single 459 bp fragment for CYP17 indicated the homozygous common genotype (T/T); fragments of 459, 335, and 124 bp indicated heterozygosity (T/C); and fragments of 335 and 124 bp indicated the homozygous rare genotype (C/C). For COMT, the presence of the MET allele (rare) was shown by a 96 bp fragment; the VAL allele (common) was indicated by a 114 bp fragment. Genotypes were determined blinded to the subjects' cancer status. All genotyping was repeated by automated sequencing to confirm the original results. Discrepancies between the two techniques occurred in three samples. The sequencing results were taken as the gold standard.

Hardy-Weinberg equilibrium was tested among both genotypes, stratified by outcome, and in the total sample. Age at first colon cancer was the outcome of interest in this study. Kaplan-Meier survival curves were used to assess age at first colon cancer diagnosis, stratified by genotype. Homogeneity between survival curves was assessed with the log-rank test. Censored observations occurred where a subject had no colon or rectal cancer diagnosis at last follow-up, or had been diagnosed with colon polyps upon screening, or had been diagnosed with extraluminal cancer. Censoring was implemented in the latter two situations because the natural history of Lynch syndrome was likely altered. The median age at diagnosis represents the age at which 50% of the sample was colon cancer–free. Cox proportional hazard models were used to estimate hazard ratios (HR) and 95% confidence intervals (95% CI); age at colon cancer onset was the modeled outcome, whereas genotype, sex, and MSH2 mutation type were included as covariates. We selected 92 DNA samples from population-based controls in Newfoundland to compare allele frequencies in the current study with that of the general population. Controls were identified through random-digit dialing and frequency-matched on age and sex to the case population and were unaffected by any cancer. These analyses were conducted to assess potential population stratification in this study. All analyses were conducted with the SAS software package, version 9.1 (SAS Institute).

Results

As shown in Table 1, 50 of the 146 MSH2 mutation carriers developed colon cancer at last follow-up. The majority of the total sample was female (59%). The majority of the colon cancer sample was male (58%). All participants were
Caucasian. Mean age at colon cancer diagnosis was slightly younger among males (38 years) than females (43 years), and the difference was statistically significant according to a t test \((P = 0.043)\). Mean age at colon cancer diagnosis and age at censoring were similar between the two outcome groups, as shown in Table 1 \((P = 0.89)\).

**Discussion**

Our data suggested that a common polymorphism in CYP17 modified age at colon cancer onset among MSH2 germ line mutation carriers. Compared with homozygote carriers of the common allele (T/T), risk of colon cancer was increased by nearly 2-fold among heterozygote carriers (T/C) and risk was further increased by nearly 3-fold among homozygote carriers of the rare allele (C/C). These results suggest a gene-dosage (or additive) effect for the C allele \((P \text{ for linear trend} = 0.02)\). In our data, a common COMT polymorphism was not associated with age at colon cancer onset; however, homozygous carriers of the rare allele (MET/MET) relative to homozygous carriers of the common allele (VAL/VAL) experienced a nonsignificant 27% reduction in risk of colorectal cancer at any given age.

These findings for CYP17 were consistent with previous reports of the effects of polymorphisms in other genes among Lynch syndrome mutation carriers. For example, clinic-based data from the United States showed that carriers of a variant allele in cyclin D1 developed Lynch syndrome an average of 11 years earlier than noncarriers \((41)\). Similar work from the same group showed that Lynch syndrome age at onset was

<table>
<thead>
<tr>
<th>Measure</th>
<th>Colon cancer, (n = 50)</th>
<th>No colon cancer, (n = 96)</th>
<th>Total, (n = 146) (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29 (41)</td>
<td>31 (60)</td>
<td>60 (41)</td>
</tr>
<tr>
<td>Female</td>
<td>21 (60)</td>
<td>65 (86)</td>
<td>86 (59)</td>
</tr>
<tr>
<td>Age at colon cancer diagnosis or censoring</td>
<td>Mean (SD)</td>
<td>39.8 (8.9)</td>
<td>39.6 (12.2)</td>
</tr>
<tr>
<td></td>
<td>Minimum and maximum</td>
<td>19-63</td>
<td>19-82</td>
</tr>
<tr>
<td>COMT genotype</td>
<td>Val/Val</td>
<td>10 (20)</td>
<td>26 (27)</td>
</tr>
<tr>
<td></td>
<td>Val/Met</td>
<td>23 (38)</td>
<td>45 (26)</td>
</tr>
<tr>
<td></td>
<td>Met/Met</td>
<td>17 (27)</td>
<td>25 (7)</td>
</tr>
<tr>
<td>CYP17 genotype</td>
<td>T/T</td>
<td>13 (39)</td>
<td>42 (25)</td>
</tr>
<tr>
<td></td>
<td>T/C</td>
<td>28 (39)</td>
<td>47 (7)</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>9 (7)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>hMSH2 germ line mutation type</td>
<td>c.942+3A→T</td>
<td>23 (47)</td>
<td>64 (35)</td>
</tr>
<tr>
<td></td>
<td>c.646-2802+?del (exon 4-16 del)</td>
<td>13 (26)</td>
<td>11 (22)</td>
</tr>
<tr>
<td></td>
<td>c.1277-1386+?del (exon 8 del)</td>
<td>14 (25)</td>
<td>21 (22)</td>
</tr>
</tbody>
</table>

*Parentheses denote percentages, unless otherwise noted.

**Table 2.** Kaplan-Meier survival results for age at onset, stratified by COMT and CYP17 genotypes

<table>
<thead>
<tr>
<th>COMT genotype</th>
<th>Median age at onset* ((n))</th>
<th>Log-rank (\chi^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sample</td>
<td>47 (36)</td>
<td>49 (68)</td>
<td>48 (42)</td>
</tr>
<tr>
<td>Males</td>
<td>47 (13)</td>
<td>41 (29)</td>
<td>43 (18)</td>
</tr>
<tr>
<td>Females</td>
<td>NA (23)</td>
<td>58 (39)</td>
<td>56 (24)</td>
</tr>
<tr>
<td>CYP17 genotype</td>
<td>Total sample</td>
<td>63 (55)</td>
<td>48 (75)</td>
</tr>
<tr>
<td>Males</td>
<td>63 (27)</td>
<td>39 (26)</td>
<td>41 (7)</td>
</tr>
<tr>
<td>Females</td>
<td>NA (28)</td>
<td>58 (49)</td>
<td>45 (9)</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available (<50% of the category was affected by colon cancer).

*Median age at onset was defined as the point at which 50% of the samples were unaffected by a first colon cancer diagnosis.
modified by rare alleles in \( \text{NAT2} \) (42), \( \text{p53} \) (43), \( \text{DNMT3b} \) (44), and \( \text{IGF1} \) (45). Work in Germany reported that polymorphisms in \( \text{RNASEL} \) (46) and \( \text{p53} \) (47) modified age at onset of Lynch syndrome. Work from South Africa recently reported that variants in \( \text{GSTT1} \) and \( \text{GSTM1} \) were associated with earlier age at onset of Lynch syndrome among a homogeneous sample of \( \text{MLH1} \) mutation carriers (48). We found that \( \text{COMT} \) did not significantly modify Lynch syndrome age at onset; similarly, others have reported that age at onset of Lynch syndrome was not modified by polymorphisms in \( \text{ATM} \) (49), \( \text{GSTM1} \) (50), \( \text{CHEK2} \) (51), \( \text{p53} \) (52), and cyclin D1 (53, 54).

To our knowledge, no previous study has examined the effect of the \( \text{CYP17} \) or \( \text{COMT} \) polymorphisms on colon cancer risk among Lynch syndrome mutation carriers. Furthermore, we are not aware of any studies which have evaluated the risk of sporadic colon cancer with the \( \text{CYP17} \) genotype. The \( \text{CYP17} \) c.-34T—C polymorphism has been examined extensively in breast cancer with earlier studies, suggesting an increased risk of disease from the C allele (16, 17), whereas more recent studies and meta-analyses do not support this link (18, 19). Evidence for a link between the \( \text{CYP17} \) c.-34T—C polymorphism and cancers of the ovary (20), endometrium (21), and prostate (22, 23) were similarly inconsistent and controversial. Landi et al. (33) reported no association between the current \( \text{COMT} \) polymorphism and risk of sporadic colon cancer in a case-control study. Given the relatively small sample size in our study, and the suggestive trend reported herein, future studies of this allele are warranted.

These polymorphisms are plausible candidates for colon cancer studies because of their effects on altered endogenous sex hormone levels. Given the higher levels of endogenous estrone (55), estradiol (25, 55), and progesterone (25, 55) associated with the \( \text{CYP17} \) C allele relative to the T allele, combined with the consistent evidence that supplementation with these hormones decreases the risk of colorectal cancer in women (11, 12), our finding of a deleterious effect for the C allele was partly unexpected. This unexpected finding might be explained by pleiotropy: whereas C allele–associated increases in estrogens may decrease risk of colorectal cancer in women, the C allele has also been associated with increased levels of testosterone, androstenedione, and SHBG (28, 55–57). Several of these hormones have been implicated in colon cancer.
tumorigenesis (58–63). Supplemental estrogens in males caused insulin resistance and hyperinsulinemia (64); markers of insulin resistance and diabetes are both major risk factors for colorectal cancer (65), supporting the link between the C allele and increased colorectal cancer risk in males. Alternatively, the C allele may be in linkage disequilibrium with another, unknown, polymorphism that confers risk on colon cancer. We found no evidence to support this alternative explanation in the literature or in online repositories of gene sequence data.

The strengths of this study include that it was conducted among participants who largely share common geographical and environmental conditions; this homogeneity should, by design, hold other potentially confounding issues to a minimum and likely decrease the risk of population stratification. Additionally, all subjects in this study shared deleterious defects in the same gene. Although the environmental and genetic homogeneity strengthens the internal validity of this study, the external validity is weakened, further highlighting the need for future studies in other populations. Frequencies of the rare alleles (COMT, 52%; CYP17, 37%) in this study were similar to previous reports among Caucasian populations in the United Kingdom, United States, and Canada (15, 34–37, 66–71), strengthening the external validity in this study. Furthermore, the frequencies of the rare alleles in the current study were similar to a randomly selected sample of 92 population-based controls from Newfoundland (COMT, 48%; CYP17, 37%), supporting the fact that population stratification did not account for these results. Ideally, future studies would also collect data on lifestyle and demographic data; it was not possible for us to collect this information.

One potential bias in this study was from the reported effect of the C allele on postmenopausal hormone use; female carriers with the C/C relative to the T/T genotype were previously reported to be about half as likely to use postmenopausal hormones (72). Although we do not have data on postmenopausal hormone use, we feel this potential for bias was likely minimal. The women in this cohort were relatively young and colon cancers and censoring occurred at relatively young ages (mean age at colon cancer diagnosis among females, 42.8 years; mean age at censoring among females, 40.2 years). All but 3 of the 21 female colon cancers occurred prior to age 50 years. Postmenopausal hormones were largely irrelevant in this sample because their cancers occurred, on average, about one decade prior to menopause. Of the censored women, 10 of 65 were older than 50 years. In the absence of questionnaire or pharmacy record data, we cannot exclude the possibility that some of them used postmenopausal hormones, and we cannot rule out that postmenopausal hormone use might have differed according to CYP17 genotype. Although anecdotal evidence from clinic staff suggests that exogenous hormone use in this population is very low.

Given the dramatic increased lifetime risk of colon and extracolonic cancers among germ line mutation carriers linked with Lynch syndrome, knowledge of genetic and environmental modifiers is needed for risk counseling and targeted prevention strategies. This study found that a common allele in CYP17 modified age at colon cancer onset among MSH2 mutation carriers. Future studies are needed to confirm this association; examination of other polymorphisms involved with steroid production and metabolism is also warranted.

References


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