

The Association of the COMT V158M Polymorphism with Endometrial/Ovarian Cancer in HNPCC Families Adhering to the Amsterdam Criteria

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Abstract

Catechol-O-methyltransferase (COMT) is vital for the conjugation of catechol estrogens that are produced during oestrogen metabolism. The efficiency of this process varies due to a polymorphism in COMT, which changes valine to methionine (V158M). The Met genotypes slow the metabolism of catechol oestrogens, which are agents that are capable of causing DNA damage through the formation of DNA adducts and reactive oxygen species (ROS) production. The slower metabolism of catechol oestrogens results in there being a higher circulating concentration of these oestrogens and consequently greater probability of DNA damage. To determine whether metabolic inefficiencies of oestrogen metabolism are associated with the development of malignancy in hereditary non-polyposis colorectal cancer (HNPCC), we studied the V158M polymorphism in COMT in a large cohort of 498 HNPCC patients from Australia and Poland that were either mutation positive (n=331) or negative (n=167) for mismatch repair (MMR) gene mutations (hMLH1 or hMSH2). HNPCC is a familial predisposition to colorectal cancer (CRC) and extracolonic cancers that include endometrial cancer.

Using Real Time PCR, the COMT V158M polymorphism was examined and its association with disease expression, age of diagnosis of cancer, mutation status and mutation type was assessed in the HNPCC MMR mutation positive and negative groups. This study showed that the V158M polymorphism had no association with disease risk in the HNPCC MMR mutation positive population. However, the polymorphism was significantly associated with endometrial/ovarian cancer risk in HNPCC MMR mutation negative patients ($p=0.002$). The heterozygous (Val/Met) genotype was associated with an increased risk of developing endometrial/ovarian cancer whereas the homozygous mutant (Met/Met) showed a decreased risk. The results suggest heterosis, where there is an apparent greater effect of the heterozygous state in this dichotomous trait. In conclusion, this study shows that the COMT V158M polymorphism alters the risk of developing endometrial/ovarian cancer in patients that adhere to the Amsterdam HNPCC criteria but do not have a DNA mismatch repair gene mutation.

Introduction

Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant inherited disorder associated with a familial predisposition to colorectal cancer (CRC) and endometrial cancer (EC). It is characterised by early age of disease (CRC) onset, neoplastic lesions, microsatellite instability (MSI) and an increased incidence of extracolonic cancers [1]. Familial colorectal cancer syndromes, HNPCC and familial adenomatous polyposis (FAP), account for around 3% of all colorectal cancer cases; however, approximately 20% show familial inheritance of which there is no known genetic cause [2]. Mutations of the genes involved in the mismatch repair pathway, hMLH1 and hMSH2, account for a large proportion of patients that fit the HNPCC clinical criteria [1, 3]. Approximately 80% of men and 40% of women that have germline mutations in MMR genes develop CRC [1, 4] and 25-50% and 8-12% of women develop endometrial cancer and ovarian cancer, respectively [5]. While environmental factors are thought to play an important role in HNPCC disease aetiology, other as yet unknown genetic factors are also likely to contribute to HNPCC disease susceptibility, and it has been suggested that single nucleotide polymorphisms (SNPs) contribute to disease. Polymorphisms in genes involved in many biological pathways (DNA repair, xenobiotic clearance, immune response and a number of other pathways) have been examined, but the role of polymorphisms in oestrogen metabolism genes has not been characterised in the HNPCC population.

Catechol-O-methyltransferase (COMT) is a phase II enzyme involved in oestrogen metabolism. It catalyses the addition of a methyl group to catechol oestrogens and converts them into methoxy derivatives [6]. Catechol oestrogens are believed to contribute to oestrogen-induced cancer through their ability to initiate DNA damage by the formation of DNA adducts and reactive oxygen species (ROS) [7, 8]. 2-Methoxyestrone has a protective role in the development of cancer since it is antioestrogenic, inhibits tumour growth, stimulates apoptosis and inhibits angiogenesis [6-9]. Therefore, the conversion of catechol oestrogen into 2-methoxyestrone is important in the elimination of toxic agents by conjugation [10]. The highest COMT activity occurs within the brain, liver, kidney, endometrium and breast [11].

The first study examining the function of COMT revealed that its activity is low, intermediate or high [12]. The three levels of activity correspond to a trimodal distribution. In 1995, Lotta et al. [13] identified a G to

A polymorphism in COMT, which results in a valine to methionine amino acid change at position 158 of the gene (known as V158M). COMT is polymorphic within the general population since approximately 50% have the intermediate and 25% have the low activity forms of the polymorphism [14]. The A allele is thought to be associated with a 4-5 times less efficient metabolism of oestradiol than the G allele [12], which subsequently allows the accumulation of higher circulating levels of oestradiol. Heterozygous individuals have intermediate COMT activity [14].

The V158M polymorphism has been studied in a variety of hormonally influenced cancers such as prostate [15], breast [16 and references within], ovarian [17, 18] and endometrial cancer [19, 20]. Some of these studies have found positive associations between the low activity allele and cancer risk but other studies have not found any association and in some cases the opposite association has been reported. For that reason, the role of V158M polymorphism in COMT and cancer risk remains unresolved.

To our knowledge there have been no studies examining the COMT V158M polymorphism and colorectal and endometrial cancer risk in HNPCC patients. So far, there have been only two studies that have looked at the V158M polymorphism and endometrial cancer and three studies involving colorectal cancer. A study by Doherty et al. [19] showed a modest decreased risk of developing endometrial cancer with the Met allele, which was not expected. Another study by McGrath et al. [20] found no association between endometrial cancer and the polymorphism. In addition, a study by Sasaki et al. [21] showed that promoter region of membrane bound COMT (MB-COMT) was methylated in 47/60 endometrial cancer tumours. Methylation of the promoter region silences the gene and they concluded that this may contribute to endometrial carcinogenesis. All of the studies that examined the polymorphism and colorectal cancer susceptibility showed no associations [22-24]. Also, Garner et al. [17] and Sellers et al. [18] studied ovarian cancer susceptibility and the COMT V158M polymorphism. Garner et al. [17] concluded that Val/Met variant of COMT decreases the risk for mucinous tumours, but both studies reported no other associations. In conclusion, the role of the V158M polymorphism in COMT and its relation to cancer have previously shown inconsistent findings.

Since endometrial cancer is the most common cancer in women that have HNPCC and the genetics of endometrial and ovarian cancer within the context

of HNPCC are poorly understood, it is important to identify other genes involved in susceptibility to disease. COMT is a good candidate as another gene involved in disease since COMT is highly expressed within the endometrium and previous studies have shown associations between the COMT polymorphism and oestrogen-influenced cancers. Additionally, the functioning of COMT is important for the degradation of catechol oestrogen and the conversion to 2-methoxyestrone to prevent the formation of DNA adducts and ROS. For this reason, the role of the V158M polymorphism in COMT is important to elucidate in the HNPCC population.

Materials and methods

Subjects

498 patients were included in this study. The patients were selected from across the state of New South Wales and from Poland because they fulfilled the clinical criteria of hereditary non-polyposis colorectal cancer (HNPCC). Approval for this study was obtained from the Hunter Area Research Ethics Committee (Australia), the University of Newcastle Human Research Ethics Committee (Australia) and the Ethics Committee of the Pomeranian Academy of Medicine (Poland). All patients enrolled in this study had given informed consent for their anonymous DNA to be used for research into genetic predispositions to colorectal cancer. Two HNPCC groups were examined: those with mutations in MMR genes, hMLH1 or hMSH2 (mutation positive – 331 patients) and those without mutations in these genes (mutation negative – 167 patients).

HNPCC MMR Mutation Positive Population

The selection criteria for the HNPCC MMR mutation positive group were based on the molecular diagnosis of HNPCC; 331 patients harboured a confirmed causative mutation in either hMLH1 or hMSH2, of which there were 285 nonsense, insertion, deletion or splice mutations (leading to a truncated protein) and 46 missense mutations described as pathogenic in the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) mutation database. There were two subpopulations of Caucasians in this study – Australian and Polish. In the Australian population, there were 197 samples collected in the state of New South Wales from 1998 to 2004, and in the Polish population there were 134 samples collected from 1997 to 2002. Of the 331 individuals, 149 had been diagnosed with colorectal cancer: 94 in the Australian population

and 55 in the Polish population. Of the 197 Australian and 134 Polish patients, 107 (54%) and 78 (58%) were relatives of probands, respectively.

Population subgroups

To determine any association between the disease characteristics of the mutation positive group and the V158M polymorphism, the samples were subdivided into different subgroups according to: (i) gene mutation status (hMLH1 or hMSH2); (ii) mutation type: truncation/deletion (including insertion, deletion, nonsense and splice site changes) or pathogenic missense mutations and; (iii) disease expression (affected/unaffected with CRC or affected/unaffected with endometrial/ovarian cancer). The age of onset of CRC was defined as the patient's age at diagnosis, while the age of the unaffected patients was determined by subtracting their date of birth from their age at the time of testing. A subgroup of patients unaffected with CRC over the age of 45 years was prepared to compare with the patients affected with CRC. This was performed since patients under the age of 45 years whom are not affected with disease could possibly still develop disease later in life. The age of diagnosis of CRC was unknown for 7 Australian and 5 Polish patients, and the disease expression status was unknown for 4 Australian patients.

Combined HNPCC mutation positive populations (Australian and Polish)

The Australian and Polish populations were combined to determine any association between the disease characteristics of the mutation positive group and the V158M polymorphism. The analysis was performed in the same way as mentioned above (population subgroups).

HNPCC Mutation Negative Population

To determine if the V158M polymorphism is associated with disease expression in HNPCC mutation negative patients, the samples were divided into those affected with CRC and affected or unaffected with endometrial/ovarian cancer. The mutation negative population was previously tested to determine whether they harboured a germline mutation in the hMLH1 or hMSH2 genes by denaturing high performance liquid chromatography (dHPLC) analysis followed by direct sequencing, multiplex ligation probe amplification (MLPA) assay and denaturing gradient gel electrophoresis (DGGE). From all analyses performed, no

mutation was found in the samples. This group of patients was collected from 1997 to 2004. Within this group there were 167 Australian samples, all of which were affected with CRC or affected/unaffected with endometrial/ovarian cancer (155 affected with CRC, 21 affected with endometrial/ovarian cancer, 9 affected with both CRC and endometrial/ovarian cancer). Of the 167 patients, 6 (3.5%) were relatives or probands. The age of diagnosis of CRC was unknown for 2 patients.

DNA isolation

Genomic DNA was isolated from Na₂EDTA blood according to the method previously described by Miller et al. [25].

Real-time PCR SNP genotyping

DNA samples were genotyped to determine the allele frequency of the COMT V158M polymorphism. Allelic discrimination was performed on an ABI PRISM 7900HT sequencing detection system (PE Applied Biosystems, Foster City). Assay-by-DesignSM, a service offered by Applied Biosystems (PE Applied Biosystems), was used to design primers and probes. The primers and probes used were 5'-CCCAGCGGATGGTGGAT-3" (forward primer), 5'-CAGGCATGCACACCTTGTC-3" (reverse primer), 5'-VIC-TTCGCTGGCATGAAG-3" (wildtype probe) and 5'-FAM-TCGCTGGCGTGAAG-3" (mutant probe). The assay functions under universal conditions with each reaction containing: 50 ng DNA, 0.125 μ l 40 \times Assay Mix and 2.5 μ l TaqMan Universal PCR master mix made up to 5 μ l with sterile water. The thermal cycling conditions were 50°C for 2 min, 95°C for 10 min, and 70 cycles of 92°C for 15 sec and 60°C for 1 min. Post PCR, the plate was scanned to allow discrimination between the different genotypes.

Statistical analysis

Statistical analysis was undertaken to assess whether or not the polymorphism segregates with specific types of disease expression, mutation type, mutation status or age of diagnosis of CRC in HNPCC patients. The Hardy-Weinberg equilibrium (HWE) was assessed for the subject groups. All statistical tests were performed on the statistical software package Intercooled Stata 8.0 (Stata Corporation, Texas) and Statistical Package for the Social Sciences (SPSS) 12.0 (SPSS, Chicago). The significance levels for all tests were set at $p < 0.05$. The genotype distribution between the different groups was analysed using Pearson's

chi-squared test and if the number of samples in a given group was less than 5, Fisher's exact test was used. Kaplan-Meier survival analysis was used to compare genotype and age of diagnosis of CRC. To assess the homogeneity of the survival curves the Wilcoxon, log rank and Tarone-Ware tests were used. The log rank p value was only reported when there were no significant results.

Results

HNPCC Mutation Positive Group

Disease expression in Australian HNPCC mutation positive patients compared to Polish patients

There was no significant difference in the frequency of affected and unaffected CRC patients observed between the two populations. The proportion of hMLH1 and hMSH2 carriers was similar in both populations, and the proportion of colorectal cancer patients was similar for hMLH1 and hMSH2 mutation carriers. The subgroup endometrial/ovarian cancer had a similar frequency of individuals affected and unaffected with CRC (11 affected and 12 unaffected in Australia and 5 affected and 9 unaffected in Poland). In addition there was no significant difference between the two populations in the frequency of truncation/deletion and missense mutations.

Allele frequency distribution of COMT V158M in Australian and Polish HNPCC mutation positive patients

The distribution of the V158M polymorphism in this study was in Hardy-Weinberg equilibrium (HWE) in both populations. The three genotypes in the V158M polymorphism were Val/Val (homozygous wildtype/GG), Val/Met (heterozygous/GA) and Met/Met (homozygous mutant/AA). There was a statistically significant difference in the allele frequency distribution of the polymorphism between the two populations ($p = 0.02$). The Australian population had a higher frequency of the heterozygous genotype and a lower frequency of the homozygous mutant genotype compared to the Polish population. When the subject group was subdivided according to their gene mutation status there was a significant difference observed between the Australian and Polish hMLH1 mutation carriers ($p = 0.03$), where the Australian patients had a much higher proportion of the Val/Met (GA) genotype and a much lower proportion of the Met/Met (AA) genotype. There was no statistical difference seen between the Australian and Polish hMSH2 mutation carriers. There was a statistically significant result

Table 1. Allele frequency distribution of the COMT V158M polymorphism in the Australian and Polish HNPCC MMR mutation positive patients

Group	Population	Val/Val (%)	Val/Met (%)	Met/Met (%)	n	Pearson's Chi-squared
subject group	Australia	53 (26.9)	108 (54.8)	36 (18.3)	197	p=0.02
	Poland	33 (24.6)	59 (44.0)	42 (31.3)	134	
hMLH1 mutation carriers	Australia	31 (28.7)	59 (54.6)	18 (16.7)	108	p=0.03
	Poland	17 (23.0)	32 (43.2)	25 (33.8)	74	
hMSH2 mutation carriers	Australia	22 (24.7)	49 (55.1)	18 (20.2)	89	p=0.41
	Poland	16 (26.7)	27 (45.0)	17 (28.3)	60	
mutation type: truncation/deletion	Australia	50 (28.2)	95 (53.7)	32 (18.1)	177	p=0.05
	Poland	26 (24.1)	49 (45.4)	33 (30.6)	108	
mutation type: missense	Australia	3 (15.0)	13 (65.0)	4 (20.0)	20	p=0.20
	Poland	7 (26.9)	10 (38.5)	9 (30.6)	26	
affected with CRC	Australia	24 (25.5)	51 (54.3)	19 (20.2)	94	p=0.88
	Poland	14 (25.5)	28 (50.9)	13 (23.6)	55	
unaffected with CRC	Australia	28 (28.3)	53 (53.5)	18 (18.2)	99	p=0.02
	Poland	19 (24.1)	31 (39.2)	29 (36.7)	79	
unaffected with CRC (>45 years)	Australia	10 (23.3)	24 (55.8)	9 (20.9)	43	P=0.84
	Poland	5 (22.7)	11 (50)	6 (27.2)	22	
endometrial/ovarian cancer	Australia	6 (26.1)	14 (60.9)	3 (13.0)	23	p=0.12
	Poland	5 (35.7)	4 (28.6)	5 (35.7)	14	
affected with CRC and unaffected with endometrial/ovarian cancer	Australia	22 (22.7)	57 (58.8)	18 (18.6)	97	p=0.09
	Poland*	28 (23.3)	55 (45.8)	37 (30.8)	120	

* This group contained males and females. The sex was unknown for all of the patients.

involving the patients unaffected with CRC between the two populations ($p=0.02$). The Australian population had a higher frequency of the heterozygous genotype and a lower frequency of the homozygous mutant genotype in comparison to the Polish population (see Table 1).

Allele frequency distribution in the Australian and Polish groups analysed separately and combined

When assessing disease expression, mutation status and mutation type between the different subgroups in the two populations separately, there were no significant difference observed in the Australian and Polish populations. In addition there were no significant differences involving disease expression, mutation status and mutation type when the populations were combined (see Table 2).

Median age of diagnosis of CRC in the subject groups

The median age of diagnosis of CRC was similar in both populations, 42.5 years in the Australian group with a range from 17 to 70 years and 44 years in the Polish group with a range from 18 to 78 years. In individuals with hMLH1 and hMSH2 mutations the median age of diagnosis was 42 years for both in the Australia population, with a range from 17 to 64 years in hMLH1 mutation carriers and 22-76 years in hMSH2 mutation carriers. In the Polish population the median age of diagnosis was 45 years for hMLH1 mutation carriers (32-78 years) and 41 years for hMSH2 mutation carriers (18-78 years).

Kaplan-Meier survival analysis

There was no significant difference between genotype and age of diagnosis of CRC in the

Table 2. Allele frequency distribution of the COMT V158M polymorphism in the Australian and Polish HNPCC MMR mutation positive patients combined

Group	Val/Val (%)	Val/Met (%)	Met/Met (%)	n	Pearson's Chi-squared
subject group	86 (26.0)	167 (50.5)	78 (23.6)	331	
hMLH1 mutation carriers	48 (26.4)	91 (50.0)	43 (23.6)	182	p=0.98
hMSH2 mutation carriers	38 (25.5)	76 (51.0)	35 (23.5)	149	
mutation type: truncation/deletion	76 (26.7)	144 (50.5)	65 (22.8)	285	p=0.65
mutation type: missense	10 (21.7)	23 (50.0)	13 (28.3)	46	
affected with CRC	38 (25.5)	79 (53.0)	32 (21.5)	149	p=0.50
unaffected with CRC	47 (26.4)	84 (47.2)	47 (26.4)	178	
unaffected with CRC (>45 years)	15 (23.1)	35 (53.8)	15 (23.1)	65	p=0.92**
endometrial/ovarian cancer	11 (29.7)	18 (48.6)	8 (21.6)	37	p=0.66
affected with CRC and unaffected with endometrial/ovarian cancer*	50 (23.0)	112 (51.6)	55 (25.3)	217	

* This group contained males and females. The sex was unknown for all of the patients.

** This group was compared to patients affected with CRC

Australian and Polish groups (Australian population p=0.19 and Polish population p=0.48). However, there was a trend observed in the Australian population where patients with the Met/Met genotype had a later age of onset of CRC compared to the other genotypes.

HNPCC Mutation Negative Group

Disease expression in Australian HNPCC mutation negative patients

The distribution of the V158M polymorphism was in Hardy-Weinberg equilibrium (HWE) in this population. There was a statistically significant difference in genotype frequency between patients affected with endometrial/ovarian cancer compared to those unaffected (p=0.002) (see Table 3). The endometrial/ovarian cancer group had a higher frequency of the heterozygous (GA) genotype and lower frequency of the other genotypes in comparison to the patients unaffected with endometrial/ovarian cancer (see Fig. 1).

Median age of diagnosis of CRC

The median age of diagnosis of CRC in the HNPCC MMR mutation negative group was 51 years with a range of 19 to 74 compared to 42.5 years with a range of 17 to 70 years in the mutation positive Australian group. The 8.5 years difference in median age was not statistically significant.

Kaplan-Meier survival analysis

There was no significant difference between genotype and age of diagnosis of CRC in the Australian mutation negative group (p=0.81).

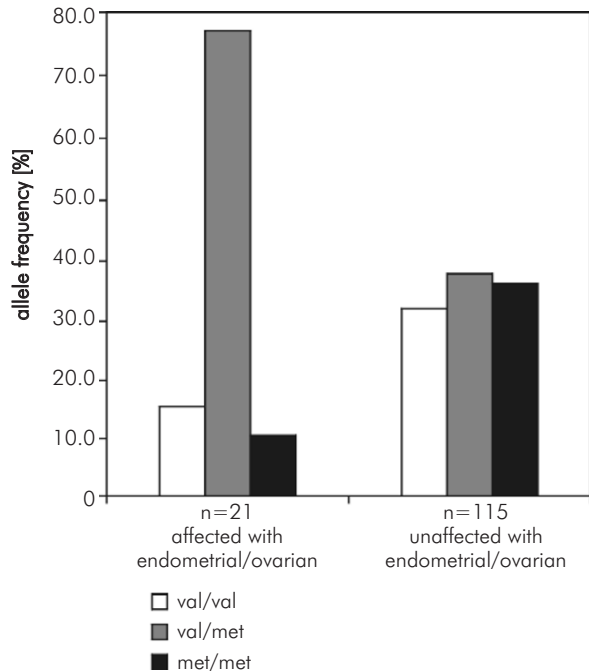


Fig. 1. Allele frequency distribution of the COMT V158M polymorphism in the Australian HNPCC MMR mutation negative patients assessed by endometrial/ovarian cancer disease expression

Table 3. Allele frequency distribution of the COMT V158M polymorphism in the Australian HNPCC MMR mutation negative patients

Group	Val/Val (%)	Val/Met (%)	Met/Met (%)	n	Pearson's Chi-squared
subject group	45 (26.9)	78 (46.7)	44 (26.4)	167	
endometrial/ovarian cancer	3 (14.3)	16 (76.2)	2 (9.5)	21	p=0.002
affected with CRC and unaffected with endometrial/ovarian cancer*	35 (30.4)	41 (35.7)	39 (33.9)	115	

* This group contained females only.

Discussion

There have been numerous candidate SNP studies performed in the past involving colorectal cancer and endometrial cancer, which have focused on a variety of biological pathways. The role of modifier genes in disease is becoming recognised as an important factor in understanding the variation that can be observed in individuals who harbour mutations in the same gene. In addition they could possibly account for a proportion of patients that do not harbour a mutation in a known gene, yet still fit the clinical characteristics of the syndrome.

In this study, we examined the V158M polymorphism in COMT to determine its association with colorectal cancer and endometrial/ovarian cancer in two different groups: an Australian and a Polish population that harboured a mutation in hMLH1 or hMSH2, and an Australian population that did not harbour a mutation in hMLH1 or hMSH2. COMT is involved in oestrogen metabolism and it functions to methylate catechol derivatives to render these carcinogens inactive [6]. Functional studies performed demonstrate that the Met allele hinders the metabolism of oestradiol and allows for greater levels of oestradiol to circulate [12]. One recent study showed that the Met allele has the same level of activity as the Val allele but has greater susceptibility to 4-hydroxyequilenin (4-OHEN) mediated inhibition and thermolability [26].

The frequency of the three genotypes Val/Val, Val/Met and Met/Met in the subject groups were 26.9%, 54.8% and 18.3% Australian MMR mutation positive, 24.6%, 44%, 31.3% Polish MMR mutation positive, and 27.0%, 46.7% and 26.3% in the Australian MMR mutation negative genotype frequencies. The Polish and Australian MMR mutation negative frequencies are in accordance with a number of Caucasian control groups [18, 27-29] and provide evidence that there is no difference in the allele frequencies between HNPCC patients in our study and other Caucasian populations. However, the genotype frequency in the Australian MMR mutation positive group

was statistically significantly different to the Polish population in addition to the other control populations. This is most likely due to the high numbers of relatives involved in the study.

The significant differences observed in the mutation positive group when the Australian population was compared to the Polish population (gene mutation status, hMLH1 p=0.03 and disease expression, unaffected with CRC p=0.02) can be accounted for by the fact that this population is not random and contains a large proportion of proband relatives. When the Australian and Polish MMR mutation positive populations were either compared separately or as a combined group there were no significant differences in the frequency of the COMT polymorphism, which suggests that the V158M variant does not influence disease expression, gene mutation status, mutation type or age of diagnosis of CRC.

In the mutation negative group, a statistically significant difference was observed in relation to endometrial/ovarian cancer (p=0.002). Patients affected with endometrial/ovarian cancer had a higher frequency of the Val/Met genotype and a lower frequency of the other genotypes in comparison to those patients unaffected with endometrial/ovarian cancer. The samples used for this part of the study were from the Australian population only; therefore we believe the results to be representative of mutation negative HNPCC families from Australia, and it remains to be seen if these results are similar in other populations.

The significance of these results can be interpreted in two different ways. Firstly, the Val/Met genotype is known to cause intermediate activity of COMT and consequently it is less efficient in the detoxification of the oestrogen metabolites within the endometrium, which ultimately leads to carcinogenesis. Secondly, the low frequency of the Met/Met genotype in the endometrial/ovarian cancer group indicates a protective role of this genotype. The results suggest that endometrial/ovarian cancer susceptibility is more complex than the COMT V158M polymorphism and it is indicative that many other genes or other variants

within COMT will provide further insight into the mechanisms of disease in HNPCC. Li et al. [26] reported that the Ala22Ser polymorphism in COMT showed lower methylation capacity and higher thermolability and thus might be of functional importance in oestrogen-related cancers. These results are of interest since there has previously been no known genetic predisposition in patients that fit the clinical criteria for HNPCC who do not have a MMR gene mutation. COMT V158M therefore could account for some of the endometrial/ovarian cancer cases within the HNPCC population. Alternatively, this may be an example of heterosis [see review 30], where there is an apparent greater effect of the heterozygous state in this dichotomous trait.

Other studies performed involving the COMT V158M polymorphism and CRC, endometrial cancer and ovarian cancer have thus far shown no strong associations with disease. Doherty et al. [19] showed a weak association with the variant alleles displaying a protective role for the development of endometrial cancer. Our results are in accordance with those since the Met/Met genotype in this study appears to be protective for endometrial cancer; however, in this study the heterozygous genotype appears to be causative of disease. Our results regarding CRC risk and V158M are in accordance with the three previous studies performed which showed no association; therefore we conclude that it is highly unlikely that COMT affects CRC risk in HNPCC patients [22-24].

Several limitations in our study warrant caution in the interpretation of the findings presented. Firstly, the size of the subgroups was in some cases quite small and therefore lacked adequate power to detect a small increase in cancer risk. Multiple comparisons were performed to assess disease expression, mutation status and mutation type, which increased the risk of type one errors. Additionally, it is important for future studies to look at the functions of the examined variants and their associated genes to provide a clearer role of susceptibility to disease. The assessment of such interactions will be the focus of future analyses.

Although this study suggests that the heterozygous genotype and homozygous mutant genotype are causative and protective of endometrial/ovarian cancer respectively, these associations should be carefully interpreted and confirmed in a much larger population of women that fit the clinical criteria for HNPCC and have endometrial cancer in addition to a sporadic endometrial cancer population.

In conclusion, the variation observed in MMR gene mutation carriers in regards to disease expression, mutation type, mutation status and age of diagnosis of CRC in HNPCC families is not influenced by the COMT V158M polymorphism. It appears that the polymorphism might account for some of the endometrial/ovarian cancer cases observed in the HNPCC MMR mutation negative population and also in some patients confer a protective role for developing endometrial/ovarian cancer. It is likely that other modifying factors, both genetic and environmental, play a role in the variation in disease expression observed in HNPCC.

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