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Mismatch repair gene polymorphisms and survival in invasive ovarian cancer patients

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Abstract

Aims—Inherited genetic factors may help partially explain variability of survival length amongst ovarian cancer patients. Of particular interest are genes involved in DNA repair, specifically those involved in mismatch repair (MMR). The aim of this study was to investigate the possible association between the common variants in MMR genes and invasive ovarian cancer overall survival.

Method/results—We examined associations between 44 variants that tag the known common variants (minor allele frequency ≥0.05) in seven MMR genes (*MLH1*, *MLH3*, *MSH2*, *MSH3*, *MSH6*, *PMS1* and *PMS2*) and survival of invasive ovarian cancer patients in three case–control studies from United Kingdom (UK), Denmark and California of United States of America (USA). DNA from up to 1495 women were genotyped. The genotypes of each polymorphism were tested for association with survival using Cox regression analysis stratified by study. A nominally significant association ($P = 0.04$) between genotype and ovarian cancer survival was observed for

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rs2228006 in *PMS2*. The per-rare allele hazard ratio (HR 95%CI) was 0.84 (0.71–0.99), however, it was not significant after adjusting for multiple covariants (P = 0.47). When the analyses were restricted to serous type ovarian cancer, two SNPs showed marginal significant associations; the per-rare allele HR was 1.3 (1.05–1.6) (P = 0.02) for rs1799977 in MLH1 and 1.4 (1.03–1.9) (P = 0.04) for rs6151662 in *MSH3*. Neither was significant after adjusting for multiple covariants.

Conclusion—It is unlikely that common variants in the MMR pathways examined have moderate effects on survival after diagnosis with ovarian cancer. Much larger studies would be needed to exclude common variants with small effects.

Keywords

Mismatch repair; SNPs; Ovarian cancer; Survival

1. Introduction

Ovarian cancer is the sixth most common female cancer and also the seventh most common cause of cancer death in women worldwide. Over 204,000 new ovarian cases are diagnosed and caused 125,000 deaths globally per year.[1] It is often advanced at presentation and is associated with a poor prognosis. Despite advances in the treatment of ovarian cancer over the past 20 years, the overall 5-year survival is still about one-third.

The survival time of ovarian cancer patients varies amongst individuals, and inherited genetic factors may explain some of this variability. Several studies investigating common genetic variation and prognosis in ovarian cancer have been published,[2–15] some of which have reported significant associations at a nominal $P < 0.05$.[6–13] However, most of these studies were small (<220 cases), and none reached the level of statistical significance that has been suggested as appropriate for genetic association studies in candidate genes where the prior probability of an association is low ($P < 10^{-4}$).[16] If germ-line genetic markers of prognosis can be reliably identified, they might be used to predict the outcome for ovarian cancer as well as offer insights into the biological mechanism of response to treatment and prognosis.

Mismatch repair (MMR) is one of the most important DNA repair processes for maintaining genetic fidelity.[17,18] It corrects nucleotide mismatches during replication, thus preventing proliferation of mutations in genes. Mutations in MMR genes can result in microsatellite instability (MSI). This occurs when a germ-line microsatellite allele has gained or lost repeat units, thus undergoing somatic change in length.[19] A disrupted MMR system has been identified in several cancers, including prostate, pancreatic, gastric and hereditary nonpolyposis colorectal cancer (HNPCC).[20–23] Individuals with HNPCC are at increased risk of endometrial, gastric and ovarian cancer.[24] Although highly penetrant mutations in MMR genes are rare, common polymorphic variation in these genes may influence cancer risk and tumour biology and possibly affect outcome after diagnosis. Recently, a polymorphism in the *MSH2* gene was reported to be associated with poor survival for non-small cell lung cancer.[25]

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We have previously investigated the association of 44 tag SNPs (tSNP) that capture the common variation in seven genes involved in mismatch repair pathway (MMR) (*MLH1*, *MLH3*, *MSH2*, *MSH3*, *MSH6*, *PMS1* and *PMS2*) using three case–control studies.[26] We found one borderline significant association with ovarian cancer-susceptibility: *PMS2* rs7797466 (*P* trend = 0.013). The purpose of the analyses reported here was to evaluate the association of germ-line genetic variation in MMR genes and survival after the diagnosis of ovarian cancer. In order to do this, we have linked the genetic data from the analysis of the seven MMR genes described above to the outcome data from regional cancer registries in approximately 1500 invasive ovarian cancer cases from the United Kingdom (UK), Denmark and United States of America (USA).

2. Materials and methods

2.1. Study population

This study examines the case data originally collected for three case–control studies previously described.[26]

SEARCH (the studies of epidemiology and risk factors in cancer heredity) ovarian cancer study: this is an ongoing, population-based ovarian cancer case–control study covering the regions served by the East Anglia and West Midlands cancer registries in the UK. All patients diagnosed in East Anglia with invasive epithelial ovarian cancer under the age of 70 years since 1991 and still alive in 1998 when recruitment started are invited to take part (prevalent cases). Incident cases are those diagnosed <70 years since 1998 in East Anglia and since 2003 in the West Midlands. To date, we have invited 1750 women to participate of whom 1157 have provided a blood sample – the first 722 cases were available for this analysis. The study is approved by the Eastern Multi-centre Research Ethics Committee. DNA was extracted from blood samples by Whatman International Ltd. (Ely, UK).

MALOVA (the Danish malignant ovarian tumour) study: this is a population-based, Danish case–control study of ovarian cancer. Eligible cases were women aged 30–80 years, who were diagnosed with an ovarian tumour from December 1994 to May 1999. The study included 18 different hospitals from the municipalities of Copenhagen and Frederiksberg as well as the counties of Copenhagen, Frederiksborg, Roskilde, Western Sealand, Storstrøm, Funen, Southern Jutland and Northern Jutland. By the end of the study period, 861 were invited to take part of whom 652 (76%) provided a blood sample. Samples were collected at the time of diagnosis. Samples from 446 invasive cases were available for this study. DNA was extracted from blood samples by Whatman International Ltd. (Ely, UK). This study has been approved by the scientific ethical committee in the study area (KF01-384/95) and all subjects provided a written consent.

GEOCS (the genetic epidemiology of ovarian cancer study): this is a population-based case–control study in six counties in the San Francisco bay area which began 1st March, 1997 and was completed on 31st July, 2002. Patients with epithelial ovarian cancer were identified *via* rapid case ascertainment through the Greater Bay Area Cancer Registry operated by the Northern California Cancer Centre as part of the Surveillance, Epidemiology and End Results (SEER) Programme of the National Cancer Institute. Eligible patients were those

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diagnosed with invasive or low malignant potential epithelial ovarian cancer aged 20–64 years who resided in six Bay Area counties (Alameda, Contra Costa, Marin, San Francisco, San Mateo or Santa Clara). By the end of the study period, 682 patients were interviewed. Of these, 579 (85%) genomic DNA were isolated from leucocytes of peripheral blood using the Puregene Kit (Gentra Systems, Minneapolis, MN). Genomic DNA was also isolated from exfoliated cells in buccal mouthwash rinses as previously described.[27] Research was conducted with protocols approved by the Institutional Review Boards of Stanford University School of Medicine and Roswell Park Cancer Institute. This analysis is restricted to the 327 white cases with invasive epithelial ovarian cancer, for whom DNA was available.

2.2. Follow-up

The SEARCH study: follow-up was carried out by the Eastern and West Midlands cancer registries at 3 and 5 years after diagnosis, and then at 5-year interval. This was done by searching hospital information systems for recent visits. When it was observed that a patient had no recent visit, the general practitioner of the patient was contacted to ascertain the patient's vital status. Active follow-up of subjects by contacting general practitioners was carried out until the end of 2005. Subsequently patients' vital status was ascertained by using the National Health Service (NHS) Strategic Tracing Service. In addition, the registries obtain notification of deaths through death certificate flagging with the Office for National Statistics. There is a lag time with this process of a few weeks for cancer deaths and 2 months to a year for non-cancer deaths. At the time of analysis 188 patients had died within 10 years of diagnosis (the latest update was 30th September 2006).

The GEOCS: follow-up of subjects was carried out until 2002. Updates of the vital status from the Greater Bay Area Cancer Registry, which is operated by the Northern California Cancer Centre as part of the SEER Programme, were carried out for patients once or twice during the study and most recently in 2004. In the state of California, cancer is a state mandated reportable condition. Cancer registry staff periodically review computerised hospital tumour registry data or medical records for updated information. They also receive updates on the person's vital status from the state's death index (lag time of around 18 months). At the time of analysis 147 patients had died within 10 years of diagnosis.

The MALOVA study: follow-up was carried out until 2003. In Denmark all inhabitants have a unique personal identification number, used universally in Danish society. These identification numbers are registered in the computerised Danish National Central Population Register. Cases in this study were traced in the register for date of death or emigration. Hospital files were collected from all patients in the study. Women who died during follow-up were linked to a Danish Hospital Reference System and information about the cause of death was assessed by matching against clinical records. At the time of analysis a total of 301 patients had died within 10 years of diagnosis, amongst them 286 (95%) patients had died from ovarian cancer and 15 (5%) patients had died from other disease than ovarian cancer.

2.3. Tag SNP selection

Details of the approach used for selection of tag SNPs have been reported previously.[26] Briefly, we used a comprehensive SNP tagging approach in which tag SNPs were chosen to capture all the known common genetic variation in each gene with a minimum correlation coefficient (r^2) of 0·8. In total, 44 SNPs were chosen to tag 259 common variants in seven MMR genes.

2.4. Genotyping

All samples were genotyped using the Taqman™ 7900HT Sequence Detection System according to manufacturer's instructions. Assays were carried out in 384-well plates and included 12 duplicate samples in each plate for quality control. Genotypes were determined using Allelic Discrimination Sequence Detection Software (Applied Biosystems, Warrington, UK). Genotyping of SEARCH and GEOCS samples were carried out in Department of Oncology, University of Cambridge and MALOVA samples were genotyped at the Translational Research Laboratory, University of College London. For MALOVA, 31 of the SNPs were genotyped in a reduced sample set (n = 278) and the other 13 SNPs were genotyped in the complete sample set (n = 446). Call rates ranged from 90% to 99% for all the individual studies for all the SNPs except rs1799977 and rs3136317 which failed for MALOVA and rs1233255 where the call rate in MALOVA was 84%. Overall concordance between duplicate samples was over 98%. Individual samples with failed calls were not repeated. Hence, there are variations in the number of samples successfully genotyped for each polymorphism.

2.5. Statistics

The effect of each SNP on survival was assessed using Cox regression analysis stratified by study. Because there is a variable time between diagnosis and patient recruitment, analyses were conducted allowing for left-truncated data. Time at risk began on date of diagnosis, but time under observation began at the date of blood draw and ended at the date of death from any cause, or, if death did not occur, after 10 years follow-up or on the date of last follow-up, whichever was first. This generates an unbiased estimate of the hazard ratio provided the proportional hazard assumption is correct.[28] The primary end-point was all cause mortality (data on cancer specific death were not available for SEARCH and GEOCS). The primary tests were the likelihood ratio test for trend (1 degree of freedom) based on the number of rare alleles carried and the hazard ratio (HR) per-rare allele carried was estimated from the Cox regression. The assumption of proportional hazards was assessed graphically by log–log survival curves.

Data on histopathology subtype, tumour stage, grade and age at diagnosis were available for 100%, 79%, 76% and 100% of the cases, respectively (Table 1). This enabled us to evaluate the significance of each polymorphism after adjusting for known prognostic factors. Factors were grouped as follows: histological subtype (serous, clear cell, endometrioid, mucinous and other); tumour stage (localised defined as FIGO stage I/II and advanced defined as FIGO stage III/IV); grade (well differentiated, moderately differentiated, and poorly/undifferentiated) and age at diagnosis (<40, 40–49, 50–59, >60 years). These factors were tested for association with outcome in univariate analyses and included in multivariate

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models using cox regression. The SNPs significantly associated with survival at the 5% level were re-tested in multivariate analysis models to adjust these prognostic factors. For analyses including covariates, individuals with missing data were not included. All analyses were performed in STATA version 8.0.

3. Results

The characteristics of the study population for whom genotyping and vital status data were available are described in Table 1. During the 5994 person-years of follow-up there were 636 deaths.

The results of the univariate Cox regression analyses for those SNP with $P < 0.2$ are summarised in Table 2. The complete data for all SNPs are given in Supplementary Table S1. None of the SNPs in *MLH1*, *MLH3*, *MSH2*, *MSH3*, *MSH6*, *PMS1* was significantly associated with survival ($P > 0.05$). There was no significant difference in hazard ratio estimates between study strata ($P > 0.05$). The trend test was nominally significant for *PMS2* rs2228006, ($P = 0.04$), with the minor allele being associated with a reduced hazard (per-rare allele HR 0.84 95%CI 0.71–0.99) (Table 2). Data on age at diagnosis, tumour stage, grade, histopathology were recorded and collected differently for each study, and so their completeness varies (Table 1). In particular, the proportion of SEARCH patient with missing data on tumour stage and grade was higher, mostly because insufficient information was available in the medical records. As expected, each of these factors was significantly associated with outcome in univariate analyses ($P < 0.05$). A multivariate model including these variables showed that survival was significantly associated with age greater than 50 years old, disease at the advanced stage, tumour grades 2 and 3 and histology subtype stratified by strata (data not shown). The association of *PMS2* rs2228006 was attenuated after adjusting for these factors (HR 0.93 95%CI 0.77–1.1) and no longer statistically significant ($P = 0.47$).

Statistical power to identify subgroup effects in the combined series of ovarian cancer cases is limited, so we restricted subgroup analyses to serous cases only (the most common histopathological type). The results of the univariate Cox regression analyses of serous type ovarian cancers are summarised in Table 3, with the complete data for all SNPs in Supplementary Table S2. There were nominally significant associations for *MLH1* rs1799977 and *MSH3* rs6151662 ($P = 0.02$ and 0.04, respectively) (Table 3). The minor alleles of these SNPs were associated with an increased hazard – per-rare allele HR 95%CI 1.3 (1.05–1.6) for *MLH1* rs1799977 and 1.4 (1.03–1.9) for *MSH3* rs6151662 (Table 3). There was no heterogeneity between studies ($P > 0.05$). After adjusting for age at diagnosis, stage and grade, neither of the SNPs were significantly associated with survival ($P > 0.05$) (data not shown).

4. Discussion

There are no published studies reporting a systematic investigation of common variation in MMR genes and survival after a diagnosis of ovarian cancer. We have evaluated the effects of 44 SNPs in seven MMR genes on ovarian cancer survival amongst white women from the

UK, USA and Denmark. The major strengths of this study are its large sample size, the length and systematic approach of the follow-up, and the systematic approach to tagging the known common genetic variation in the genes of interest.

We have found no evidence that common variations in *MLH1*, *MLH3*, *MSH2*, *MSH3*, *MSH6* and *PMS1* are associated with outcome after a diagnosis of ovarian cancer. We observed an association between ovarian cancer survival and *PMS2* rs2228006, but this was not highly significant and given the low prior probability of association it is most likely to be a false positive association. If real, the SNP may act through established prognostic factors such as tumour characteristics as the association was attenuated after adjusting for multiple prognostic factors. Alternatively, the loss of statistical significance in the multivariate model may reflect the reduced power when the cases with missing covariate information were removed. We also observed marginally significant associations between survival of invasive serous ovarian cancer and *MLH1* rs1799977 and *MSH3* rs6151662 genotypes, although these are also likely to be false positive associations due to chance.

The differences between the component studies are a potential weakness, but these differences are unlikely to bias the results, or influence their interpretation. For example, the time between diagnosis and recruitment was longer for SEARCH than for the other studies, and so the cancers tended to have better prognostic features (non-serous subtype, lower grade and earlier stage) and the average annual mortality rate was lower. There was no significant difference in hazard ratio estimates for stage or grade based on the subset of cases recruited within 6 months of diagnosis compared to hazard ratio estimates based on the subset of cases recruited after 6 months ($P = 0.06$ and 0.14, respectively). This shows that the including of prevalent cases has not resulted in a significant bias of the HR estimate. There are also likely to be differences in the way the patients from each study centre were treated. However, we allowed for this in the analysis by stratifying by study centre and allowing for time between diagnosis and recruitment in the analysis. The estimates of the genotype effects will not be biased, provided that the Cox proportional hazards assumption is not violated. There is also a possibility of false negatives for those SNPs/genes where we have not detected an association. The SNPs under study were selected to tag the common variants in each gene, and not because of their predicted effects on structure and function. Tag SNPs were selected using public databases such as HapMap and the Environmental Genome Project and it is thought that most common variants will be efficiently tagged using these data.[29] Nevertheless, it is possible that important, unidentified variants were not efficiently tagged. Furthermore, some known common variants were poorly tagged, because of tSNP assay failure – eight chosen tSNPs failed during assay design: one in *MLH1*, four in *MSH6*, one in *PMS1* and two in *PMS2*.[26] No alternative tSNPs could be genotyped. Power to detect association with these SNPs is limited. It is also possible that rare variants in these genes are important predictors of outcome, but most rare variants will be poorly tagged.

We may also have failed to detect any association with survival because of lack of statistical power to detect modest effects. Despite our large sample size, there were just 636 deaths in our cohort. Assuming a type I error rate of 0·05 we had only 51% power to detect a co-dominant allele of frequency 0·1 that confers a relative hazard of 1.3% and 86% power to detect a similar allele with frequency 0.3. Power to detect recessive alleles with similar

effects is poor. Power may also be reduced by the use of all causes mortality rather than ovarian cancer specific mortality as an end-point because some women will have died from other causes that might not be related to ovarian cancer. However, cause specific mortality data were not available for GEOC or SEARCH. In MALOVA study, 95% of death was caused by ovarian cancer, in the age group of women included in SEARCH and GEOCS studies, the proportion of women dying from cause unrelated to ovarian cancer is likely to be small and any reduction in power will be limited.

In conclusion, common variation in the seven genes of MMR pathways does not appear to be associated with moderate variation in prognosis after a diagnosis of ovarian cancer. Much larger studies would be needed to exclude common variants with small effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1
Characteristics of invasive ovarian cancer cases

	Total	SEARCH	GEOCS	MALOVA
Total number of subjects	1495	722	327	446
Total time at risk (person-years)	5994	2980	1210	1804
Median time from diagnosis to study entry (years)	1.38 (0.00–8.77)[a]	2.68 (0.34–8.77)	0.55 (0.15–4.04)	0.00 (0.00–0.32)
Median time from diagnosis to study exit (years) [b]	6.12 (0.01–10)[a]	7.61 (1.89–10)	4.54 (0.27–8.24)	3.27 (0.01–9.72)
Median time at risk (years)	4.16 (0.00–9.72)[a]	4.69 (0.10–6.58)	3.67 (0.00–7.74)	3.26 (0.01–9.72)
Number of deaths	636	188	147	301
Annual mortality rate	0.11	0.063	0.12	0.17
Median 5-year survival (95% CI)	48% (44–51%)[c]	72% (68–77%)	52% (46–58%)	40% (36–45%)
Median age at diagnosis, years	56 (21–80)[b]	56 (21–74)	51 (23–64)	60 (32–80)
Age at diagnosis				
<40	102 (7%)	50 (7%)	39 (12%)	13 (3%)
40–49	297 (20%)	130 (18%)	96 (29%)	71 (16%)
50–59	529 (35%)	273 (38%)	126 (39%)	130 (29%)
>60	567 (38%)	269 (38%)	66 (20%)	232 (52%)
Total	1495 (100%)	722	327	446
Histopathological type				
Serous	697 (47%)	256 (35%)	166 (51%)	275 (62%)
Endometrioid	234 (16%)	131 (18%)	47 (14%)	56 (13%)
Mucinous	166 (11%)	94 (13%)	29 (9%)	43 (10%)
Clear cell	118 (8%)	62 (9%)	23 (7%)	33 (7%)
Other	280 (19%)	179 (25%)	62 (19%)	39 (9%)
Total	1495	722	327	446
Clinical stage				
Localised tumour	559 (37%)	289 (40%)	122 (37%)	148 (33%)
Advanced disease[d]	629 (42%)	143 (20%)	188 (57%)	298 (67%)
Total known	1188 (79%)	432 (60%)	310 (95%)	446 (100%)
Unknown	309 (21%)	292 (40%)	17 (5%)	0
Grade				
Well differentiated	260 (17%)	111 (15%)	45 (14%)	104 (23%)
Moderately differentiated	376 (25%)	167 (23%)	64 (20%)	145 (33%)
Poorly/undifferentiated	504 (34%)	182 (25%)	154 (47%)	168 (38%)
Total known	1140 (76%)	460 (64%)	263 (80%)	417 (93%)
Unknown	355 (24%)	262 (36%)	64 (20%)	29 (7%)

[a] Range of variable.

[b] Follow-up censored at 10 years.

[c] 95% confidence interval.

[d] Spread to regional lymph nodes or distant metastases.

Table 2

Genotype frequencies and results of univariate Cox regression analysis of common polymorphisms and invasive ovarian cancer survival with $P < 0.2$

Gene	Rs number[a]	Genotype frequencies				Trend test[c]		Hazard ratio per-rare allele[d]		
		AA[b]	Aa	aa	Total	χ^2	*P*-value	HR	LCL	UCL
MLH1	rs1800734	822	418	66	1306	3.18	0.07	0.87	0.75	1.02
	rs1799977	510	417	97	1024	3.77	0.05	1.2	1.00	1.4
	rs2286939	397	663	265	1325	2.81	0.09	1.1	0.98	1.3
MSH6	rs2348244	936	325	25	1286	1.89	0.17	0.88	0.72	1.06
MSH2	rs3771274	467	637	207	1311	1.64	0.20	1.09	0.96	1.2
MSH3	rs40139	416	667	239	1322	3.71	0.05	0.88	0.78	1.00
	rs26282	697	519	99	1315	1.73	0.19	1.1	0.96	1.3
	rs33008	156	647	451	1254	1.99	0.16	0.90	0.79	1.04
	rs2897298[a]	779	244	19	1042	1.72	0.19	1.1	0.95	1.3
PMS2	rs2228006[a]	1068	372	33	1473	4.29	0.04	0.84	0.71	0.99

[a] SNPs were genotyped in new batch of DNA samples containing 446 cases for MALOVA study, the rest of the SNPs in the table were genotyped in the subset of the MALOVA study (278 cases).

[b] AA, common homozygote; Aa, heterozygote; aa, rare homozygotes.

[c] Stratified by study.

[d] Hazard ratio with 95% confidence interval.

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Table 3

Genotype frequencies and results of univariate Cox regression analysis of common polymorphisms and serous ovarian cancer survival with P < 0.2

Gene	Rs number[a]	Genotype frequencies				Trend test [c]		Hazard ratio per-rare allele[d]		
		AA [b]	Aa	aa	Total	χ^2	P-value	HR	LCL	UCL
MLH1	rs1799977	206	160	48	414	5.71	0.02	1.3	1.05	1.6
	rs2286939	171	286	131	588	2.30	0.13	1.1	0.97	1.3
MSH6	rs2348244	430	134	9	573	1.73	0.19	0.85	0.66	1.09
MSH2	rs2303428	485	97	6	588	2.24	0.13	1.2	0.95	1.6
MSH3	rs6151662	615	73	1	689	4.17	0.04	1.4	1.03	1.9
PMS2	rs2228006	515	163	11	689	3.14	0.08	0.83	0.66	1.03
MLH3	rs7303	153	273	147	573	3.01	0.08	0.87	0.74	1.02
	rs175080	185	274	118	577	1.77	0.18	1.1	0.95	1.3

[a] SNPs were genotyped in new batch of DNA samples containing 446 cases for MALOVA study, the rest of the SNPs in the table were genotyped in the subset of the MALOVA study (278 cases).

[b] AA, common homozygote; Aa, heterozygote; aa, rare homozygotes.

[c] Stratified by study.

[d] Hazard ratio with 95% confidence interval.