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The role of *KRAS* rs61764370 in invasive epithelial ovarian cancer: implications for clinical testing

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Abstract

Purpose—An assay for the single nucleotide polymorphism (SNP) rs61764370 has recently been commercially marketed as a clinical test to aid ovarian cancer risk evaluation in women with family histories of the disease. rs67164370 is in a 3′UTR miRNA binding site of the *KRAS* oncogene, and is a candidate for epithelial ovarian cancer (EOC) susceptibility. However, only one published paper, analyzing fewer than 1,000 subjects in total, has examined this association.

Experimental Design—Risk association was evaluated in 8,669 cases of invasive EOC and 10,012 controls from nineteen studies participating in the Ovarian Cancer Association Consortium, and in 683 cases and 2,044 controls carrying *BRCA1* mutations from studies in the Consortium of Investigators of Modifiers of *BRCA1/2*. Prognosis association was also examined in a subset of five studies with progression-free survival data and eighteen studies with all-cause mortality data.

Results—No evidence of association was observed between genotype and risk of unselected EOC (odds ratio (OR)=1.02, 95% confidence interval (CI)=0.95-1.10), serous EOC (OR=1.08, 95%CI=0.98-1.18), familial EOC (OR=1.09, 95%CI=0.78-1.54), or among women carrying deleterious mutations in *BRCA1* (OR=1.09, 95%CI=0.88-1.36). There was little evidence for association with survival time among unselected cases (hazard ratio (HR)=1.10, 95%CI=0.99-1.22), among serous cases (HR=1.12, 95%CI=0.99-1.28), or with progression-free survival in 540 cases treated with carboplatin and paclitaxel (HR=1.18, 95%CI=0.93-1.52).

Conclusions—These data exclude the possibility of an association between rs61764370 and a clinically significant risk of ovarian cancer or of familial ovarian cancer. Use of this SNP for ovarian cancer clinical risk prediction therefore appears unwarranted.

INTRODUCTION

Epithelial ovarian cancer (EOC) is the fifth-most common cancer in women. It generally presents as advanced disease with poor prognosis. Family and twin studies have suggested that inherited genetic variation plays an appreciable part in determining individual risk. However, until recently, knowledge of genetic susceptibility was limited to rare, highly-penetrant alleles in a handful of genes including *BRCA1, BRCA2* and the mismatch repair genes (1). In the past two years, genome-wide association studies have identified common susceptibility alleles at four loci at highly stringent levels of statistical significance ($P<10^{-8}$), but these alleles have small effects on disease risk (per-allele OR<1.3) and explain a small fraction of the genetic component of disease risk (2-4). Many candidate gene studies have identified possible common ovarian cancer susceptibility alleles, but most are likely to represent false-positive associations as none have been reported at the levels of statistical significance required when testing hypotheses with low prior probabilities of association (1).

In July 2010, a single nucleotide polymorphism (SNP), rs61764370, located in the 3′UTR of the *KRAS* oncogene, was reported to be associated with risk of unselected epithelial ovarian cancer (5). The variant was also reported to be associated with a stronger risk in women carrying *BRCA1* mutations, in women not carrying *BRCA1* or *BRCA2* mutations but with a family history of the disease, as well as associated with poorer progression-free survival (5). This SNP was thought to be a strong candidate for cancer risk as it lies in a miRNA binding site, and associations between miRNA mutations or mis-expression and risk of some human cancers have been seen. These observations suggested that miRNAs can function as tumor suppressors or oncogenes (6). An assay to determine genotype at rs61764370 has subsequently been marketed as a commercial test to determine risk in women with a family history of ovarian cancer (http://www.miradx.com). However, as with other candidate gene studies, the reported association was not at a level of statistical significance that is regarded as definitive for common susceptibility alleles (7), nor was the magnitude of risk sufficient for this SNP to be acceptable as a useful clinical marker of ovarian cancer risk. The present work therefore sought to: i) replicate the association in a robust manner in multiple study populations genotyped to a high standard with stringent quality assurance procedures; ii) assess the association between genotype at this locus and ovarian cancer risk in women with family histories or who carry deleterious mutations in *BRCA1*; and iii) examine the hypothesis that the SNP is associated with differences in post-diagnosis progression-free survival or all-cause mortality.

METHODS

Study populations

Nineteen ovarian cancer case-control sets and one case series participating in the Ovarian Cancer Association Consortium (OCAC), and one additional case series, contributed data to

the analyses (Table 1). Three of the case-control sets were each comprised of a case series matched to controls from the same geographical region: PVM, UK2 and UK-GWAS. Survival time analysis was based on data from eighteen case series including the additional publicly available data for 359 ovarian cancer cases from The Cancer Genome Atlas (http://cancergenome.nih.gov/) that had information on all-cause mortality. The analysis of progression-free survival was based on data from five case series. Finally, data from 683 cases and 2,044 controls enrolled in a stage I project of the Consortium of Investigators of Modifiers of *BRCA1/2* (see reference (8) for details of studies participating in CIMBA) were used to examine risk among women carrying deleterious *BRCA1* mutations. Each study was approved by a governing research ethics committee and all study subjects provided written informed consent. Clinical and questionnaire data included tumor behavior, histology, stage and grade, age at diagnosis (or at comparable date for controls), family history of ovarian cancer, and ethnicity/race.

Survival-time data were available for cases from eighteen studies (BEL, DOV, UCR, GER, HOP, LAX, MAY, NCO, PVD, RMH, SEA, SOC, SRO, TBO, TCGA, UCI, UKO, USC) and clinical information on chemotherapy, residual disease after surgery and time to progression was collected in five studies (BEL, LAX, MAY, SRO, TCGA). All of the women included in the analysis of progression-free survival (PFS) had at least four cycles of carboplatin and paclitaxel as part of primary treatment. PFS was defined as the time interval between the date of histologic diagnosis and the first confirmed sign of disease recurrence or progression (9).

Genotyping

Genotyping of thirteen case-control sets was performed in a single laboratory using a 5′ nuclease Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA) as part of a 96-SNP Fluidigm multiplex (10 studies) or--with the same batch of Taqman reagents--using the 7900HT Sequence Detection Software (Applied Biosystems) (three studies) (Table 1). Details of OCAC's quality control (QC) criteria have been described previously (10); they include genotyping of a common set of 95 DNAs (90 CEPH trios and five duplicate samples) and comparison to the genotypes for the same samples as reported by HapMap. However, rs61764370 was not genotyped in the HapMap project. Based on sequence data for 57 individuals of European origin from the 1000Genomes project (http://www.1000genomes.org), a HapMap SNP, rs17388148, was found to be strongly correlated (r^2=0.97) with rs61764370. The concordance between the CEPH trio genotype data for rs61764370 and the HapMap genotypes for rs17388148 was 100%. Therefore, data on rs17388148 were obtained from three genome-wide association studies (GWAS), where cases and controls had been genotyped using Illumina genome-wide SNP arrays (1, 2, 4, 8) (Table 1). Neither rs61764370 nor rs17388148 were included on the Illumina arrays used in these GWAS studies, but imputed genotypes were available for rs17388148. These genotypes were provided as the estimated number of rare alleles carried (0 to 2 on a continuous scale). The accuracy of the imputation as calculated by the program MACH of Li and Abecasis (11) for the North American studies (BWH, MAY, NCO, TBO and TOR) was r^2=0.977. This high accuracy of the imputation was evidently due to the presence of a nearby SNP (rs12305513, 17kb away) in high LD (r^2=1 in HapMap) with rs17388148.

Statistical Analyses

Analyses were restricted to white non-Hispanic women based on self-reported ethnic origin for all of the studies, with the exception of the TCGA, MAY, NCO, TOR, TBO and UK-GWAS controls. For these studies, genome-wide genotype data were used to estimate intercontinental ancestry and women of less than 90 percent European ancestry were excluded (see methods in reference (3) for details). Cases with borderline (low malignant

potential) epithelial ovarian cancer were also excluded, as were 22 cases from TCGA that had been provided to TCGA as part of the MAY case-control study. Departure of genotype frequencies from those expected under Hardy-Weinberg equilibrium was assessed using a chi-square test for each study that was directly genotyped. The association between SNP and disease risk was evaluated using unconditional logistic regression in which number of copies of the minor (infrequent) allele was treated as a continuous variable. This provides an estimate of the per-allele odds ratio (OR) and 95% confidence interval (95% CI). Models adjusted for age categories (<40, 40-49, 50-59, 60-69 and ≥70 years) were also considered. Each case-control set was analyzed individually and the pooled result was obtained by combining the log odds-ratios using standard inverse variance-weighted meta-analytic methods. Analysis of the *BRCA1* mutation carrier cohort was carried out using a time-to-event analysis framework that models the association between genotype and ovarian cancer risk as a hazard ratio. Because mutation carriers were not sampled randomly with respect to their disease status, standard methods of survival analysis may lead to biased estimates of associations. Therefore, analyses were carried out by modeling the retrospective likelihood of observed genotype conditional on disease phenotype (see references (8, 12) for details).

Associations between genotype and progression-free survival and all-cause mortality were evaluated using proportional hazards regression. Because the EOC cases were recruited at variable times after diagnosis, regression analysis of all-cause mortality allowed for left truncation, with time at risk starting on date of diagnosis and time under observation beginning at the time of study entry. This method generates an unbiased estimate of the hazard ratio provided that the proportional hazards assumption is reasonably correct (13). Cause-specific mortality was not available for most studies, so the analysis of all-cause mortality was right-censored at five years after diagnosis in order to minimize the proportion of deaths from causes other than ovarian cancer. The analysis of progression-free survival was adjusted for stage and residual disease, and survival time ended at time of progression or was censored at time of last follow-up.

RESULTS

Details of the nineteen case-control sets used in our analyses are given in Table 1. Genotype data from these sets were available for 8,669 cases and 10,012 controls (Table 2). All studies passed the OCAC criteria for genotyping quality. Genotype frequencies were close to those expected under Hardy Weinberg equilibrium in both cases and controls for the thirteen directly genotyped studies. No evidence was found for association between rs61764370 and invasive EOC in univariate analysis (OR = 1.02, 95% CI 0.95-1.10, P = 0.44), with minimal heterogeneity of risk between studies (P = 0.28). Study specific odds ratios are shown in Figure 1a. When studies with directly genotyped data and with imputed data were analyzed separately, the overall OR in the genotyped studies was 0.96 (95% CI 0.87-1.06, P = 0.42) compared to 1.08 (95% CI 0.97-1.20, P = 0.15) in the imputed data studies. Adjusting for age at diagnosis/interview made little difference to the results (data not shown). No differences in risk were observed when analyses were restricted to cases who provided blood samples within 18 months of diagnosis (n = 6,550, OR = 1.02, 95% CI 0.93-1.10, P = 0.72), cases with serous tumors (Figure 1b, n = 4,706, OR = 1.08, 95% CI 0.98-1.18, P = 0.11), or cases reporting a family history of ovarian cancer in a first-degree relative (from 6 studies) (Figure 1c, n = 249, OR = 1.09, 95% CI 0.78-1.54, P = 0.62). Tests for heterogeneity of risk between studies in all analyses were not statistically significant. A similar result was seen for risk of EOC by genotype among 683 cases and 2,044 controls who were carriers of *BRCA1* mutations (HR = 1.09, 95% CI 0.88-1.36, P = 0.40).

Survival-time data were available for 6,002 cases of the 6,826 total from eighteen case series, including 13,696 person-years at risk and 2,044 deaths. Little evidence of association

was observed between rs61764370 genotype and all-cause mortality within five years of diagnosis (HR = 1.10, 95% CI 0.99-1.22, P = 0.08), with no evidence of heterogeneity of the hazard ratio between studies (P = 0.89). Results of analyses restricted to serous subtype (HR = 1.12, 95% CI 0.99-1.28, P = 0.08) or adjusted for tumor stage and grade (HR = 1.06, 95% CI 0.96-1.18, P = 0.27) were similar. There was also little evidence for association between genotype and progression-free survival in 540 high-grade serous cases known to have been treated with carboplatin and paclitaxel (HR = 1.18, 95% CI 0.93-1.52, P = 0.16).

DISCUSSION

This study provides no evidence to support the previously reported associations between rs61764370 and risk of epithelial ovarian cancer. The relative risk given by Ratner and colleagues for their replication data set of unselected cases was 1.70 (95% CI 1.11-2.63, P = 0.016) (5). The confidence intervals in the present study do not overlap with these. The power of the present study to detect an allele conferring a relative risk of 1.3 under either a dominant or log-additive genetic model with a Type 1 error rate of 10^{-4} was greater than 99%, strongly suggesting that the association observed by Ratner and colleagues was a chance finding or possibly due to subtle genotyping errors. This observation is not surprising. Associations with modest P-values that are declared as positive are very likely to be false positives when the prior probability of association is low (14). In genetic association studies, even if evidence exists that a variant has functional effects, the prior probability of association at a relative risk of 1.5 is unlikely to be more than 1:100, and given that only a handful of loci conferring relative risks of more than 1.5 have been found for any cancer, the prior probability is likely to be much less. Based on the methodology of Wacholder and colleagues (14), assuming a prior of 1:100, the probability that the association reported by Ratner and colleagues was a false positive is 86 percent. Under more likely, smaller prior probabilities the false positive probability will approach 100 percent. For familial ovarian cancer, the power of the present study to detect a relative risk of 2.0 was over 95% at a type 1 error rate of 0.05, again suggesting it is unlikely that the present analyses have missed a true association of this magnitude with familial ovarian cancer.

Statistical power to detect an allele with weaker effects is more limited. For example, if the true relative risk were 1.15 under a co-dominant genetic model--similar to the relative risk conferred by an allele with the biggest risk detected in a recent well-powered lung-cancer GWAS (15)--power at a type 1 error rate of 0.001 would be reduced to 70%. A very modest association between the rs61764370 variant and a slightly increased risk of ovarian cancer cannot therefore be excluded. Neither has the present study shown much evidence for association between genotype at this locus and other related phenotypes: EOC risk in carriers of deleterious *BRCA1* mutations or in women with family histories of EOC, all-cause mortality after diagnosis of EOC, and progression-free survival in EOC cases treated with carboplatin and paclitaxel.

In summary, the possibility that the minor (infrequent) allele of rs61764340 is associated with an appreciable risk of epithelial ovarian cancer is excluded. Furthermore, it is debatable whether a single risk allele, even one conferring a relative risk as high as 2.0, has clinical utility, particularly in a disease with lifetime risk as low as it is in invasive epithelial ovarian cancer (1 in 70), and even among women with a family history of the disease (16). The marketing of a commercial assay for rs61764370 for clinical use among unselected cases, or women with family histories of ovarian cancer or who are carriers of *BRCA1* mutations, in the absence of convincing evidence for association, is not warranted.

TRANSLATIONAL RELEVANCE

An assay for a single nucleotide polymorphism (SNP) in a 3′UTR miRNA binding site of the *KRAS* gene has recently been commercially marketed as a clinical test to aid epithelial ovarian cancer (EOC) risk assessment in women with family histories of the disease. The justification for use of this assay was based on one published paper which analyzed fewer than 1,000 subjects in total, including only 67 EOC cases carrying *BRCA1* mutations or with family histories of EOC. The present report found no association between this SNP and ovarian cancer risk among 8,669 cases of unselected invasive EOC and 10,012 controls, or in 683 cases and 2,044 controls carrying *BRCA1* mutations. The results suggest that evaluation of this SNP is not clinically useful for risk prediction in sporadic or familial ovarian cancer.

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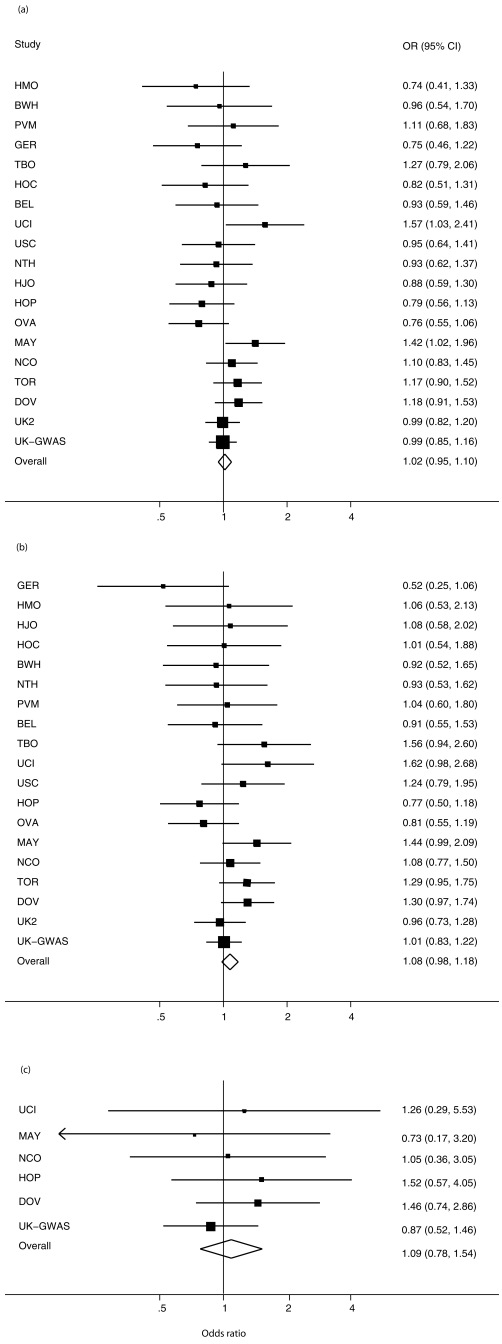

Figure 1.
Funnel plots of study-specific odds ratios for association between rs61764370 and ovarian cancer risk. (a) all cases; (b) serous cases; (c) cases with a family history of ovarian cancer in a first-degree relative.

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Table 1

Description of participating studies

Analysis set	Study name	Study abbrev.	Study population	Study type	Number of subjects		Genotyping method
					Controls	Cases	
Case-control sets							
BEL	Belgium Ovarian Cancer Study	BEL[a]	Belgium	Hospital based	253	173	Fluidigm
BWH	Brigham Women's Hospital Study	BWH	Boston, USA	Population based	142	137	Illumina Hap317
DOV	Diseases of the Ovary and their Evaluation Study	DOV[b]	Washington State, USA	Population based	721	698	Taqman
GER	German Ovarian Cancer Study	GER[b]	Germany	Population based	265	213	Fluidigm
HMO	Hannover-Minsk Ovarian Cancer Study	HMO	Belarus	Hospital based	151	195	Fluidigm
HJO	Hannover-Jena Ovarian Cancer Study	HJO	Germany	Hospital based	426	259	Fluidigm
HOC	Helsinki Ovarian Cancer Study	HOC	Finland	Population based	434	350	Fluidigm
HOP	Hormones and Ovarian Cancer Prediction Study	HOP[b]	Pittsburgh, USA	Population based	368	365	Taqman
MAY	Mayo Clinic Ovarian Cancer Study	MAY[a]	Upper Midwest, USA	Hospital based	520	358	Illumina 610 Quad
NCO	North Carolina Ovarian Cancer Study	NCO[b]	North Carolina, USA	Population based	655	494	Illumina 610 Quad
NTH	Nijmegen Ovarian Cancer Study	NTH	Netherlands	Population based	327	296	Fluidigm
OVA	Ovarian Cancer Study	OVA	Alberta and British Columbia, Canada	Population based	416	494	Fluidigm
PVM	Pelvic Mass Study	PVD[b]	Copenhagen, Denmark	Hospital based		201	Fluidigm
	Malignant Ovarian Cancer Study	MAL	Copenhagen, Denmark	Population based	215		Fluidigm
TBO	Tampa Bay Ovarian Cancer Study	TBO[b]	Tampa, USA	Population based	168	227	Illumina 610 Quad
TOR	Familial Ovarian Tumour Study	TOR	Ontario, Canada	Population based	556	734	Illumina 610 Quad
UCI	UC Irvine Ovarian Cancer Study	UCI[b]	California, USA	Population based	372	192	Fluidigm

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Analysis set	Study name	Study abbrev.	Study population	Study type	Number of subjects		Genotyping method
					Controls	Cases	
UK2	SEARCH	SEA[b]	UK	Population based	670		Fluidigm
	Southampton Ovarian Cancer Study	SOC[b]	UK	Population based		389	Fluidigm
	Scottish Randomised Trial in Ovarian Cancer	SRO[a]	UK	Hospital based		866	Fluidigm
	United Kingdom Ovarian Cancer Population Study	UKO[b]	UK	Population based	655		Fluidigm
UK-GWAS	SEARCH	SEA[b]	UK	Population based		1089	Illumina 610 Quad
	United Kingdom Ovarian Cancer Population Study	UKO[b]	UK	Population based		500	Illumina 610 Quad
	Cancer Research UK Familial Ovarian Cancer Register	UCR[b]	UK	Familial cancer register		32	Illumina 610 Quad
	Royal Marsden Hospital study	RMH	UK	Hospital based		147	Illumina 610 Quad
	UK 1958 Birth cohort	58BC	UK	Cohort	1438		Illumina Hap550
	UK Colorectal control	NSCR	UK	Population based	917		Illumina Hap550
USC	Los Angeles County Case-Control Studies of Ovarian Cancer	USC	Los Angeles, USA	Population based	343	260	Taqman
Case-only studies							
LAX	Case series	LAX[a]	Los Angeles, USA	Hospital based		263	Fluidigm
TCGA	The Cancer Genome Atlas	TCGA[a]	USA	Hospital based		359	Illumina 1M Duo

[a]Case data included in analysis of all-cause mortality and progression-free survival

[b]Case data included in analysis of all-cause mortality

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Table 2

Genotype frequency numbers of controls and cases by study

Study abbreviation	Control genotypes[a]					Case genotypes[a]				
	0	1	2	Total	Allele freq.[b]	0	1	2	Total	Allele freq.[b]
BEL	202	49	2	253	0.105	142	28	3	173	0.098
DOV	610	104	7	721	0.082	570	123	5	698	0.095
GER	218	46	1	265	0.091	184	28	1	213	0.070
HJO	354	66	6	426	0.092	217	42	0	259	0.081
HMO	130	19	2	151	0.076	174	20	1	195	0.056
HOC	390	43	1	434	0.052	321	28	1	350	0.043
HOP	297	67	4	368	0.102	308	54	3	365	0.082
LAX						223	39	1	263	0.078
NTH	275	47	5	327	0.087	249	46	1	296	0.081
OVA	332	83	1	416	0.102	417	74	3	494	0.081
PVM	181	33	1	215	0.081	166	34	1	201	0.090
UCI	318	52	2	372	0.075	150	41	1	192	0.112
UK2	1,087	231	7	1,325	0.092	1,039	201	15	1,255	0.092
USC	281	59	3	343	0.095	214	45	1	260	0.090
BWH[c]	118	23	1	142	0.088	115	21	1	137	0.084
MAY[c]	438	78	4	520	0.083	283	71	4	358	0.110
NCO[c]	537	112	6	655	0.095	398	91	5	494	0.102
TBO[c]	141	26	1	168	0.083	181	43	3	227	0.108
TCGA[c]						286	69	4	359	0.107
TOR[c]	458	93	5	556	0.093	587	139	8	734	0.106
UK-GWAS[c]	1,923	410	22	2,355	0.096	1,446	306	16	1,768	0.096
Total	8,282	1,649	81	10,012		7,969	1,241	81	9,291	

[a] Columns labeled 0, 1 and 2 refer to common homozygote, heterozygote and infrequent homozygote, respectively.

[b] Estimated frequency of minor (infrequent) allele: $(f_2+½f_1)/(f_2+f_1+f_0)$.

[c] Estimated genotype frequencies based on imputed data.

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