# Variants in Genes Encoding Small GTPases and Association with Epithelial Ovarian Cancer Susceptibility

Madalene Earp[#1], Jonathan P Tyrer[#2], Stacey J Winham[1], Hui-Yi Lin[3,4], Ganna Chornokur[5], Joe Dennis[2], Katja KH Aben[6,7], Hoda Anton-Culver[8], Natalia Antonenkova[9], Elisa V Bandera[10], Yukie T Bean[11,12], Matthias W Beckmann[13], Line Bjorge[14,15], Natalia Bogdanova[16], Louise A Brinton[17], Angela Brooks-Wilson[18,19], Fiona Bruinsma[20], Clareann H Bunker[21], Ralf Butzow[22,23], Ian G Campbell[24-26], Karen Carty[27,28], Jenny Chang-Claude[29,30], Linda S Cook[31], Daniel W Cramer[32], Julie M Cunningham[33], Cezary Cybulski[34], Agnieszka Dansonka-Mieszkowska[35], Evelyn Despierre[36], Jennifer A Doherty[37,38], Thilo Dörk[16], Andreas du Bois[39,40], Matthias Dürst[41], Douglas F Easton[42,43], Diana M Eccles[44], Robert P Edwards[45], Arif B Ekici[46], Peter A Fasching[13,47], Brooke L Fridley[48], Aleksandra Gentry-Maharaj[49], Graham G Giles[20,50], Rosalind Glasspool[27], Marc T Goodman[51], Jacek Gronwald[34], Philipp Harter[39,40], Alexander Hein[13], Florian Heitz[39,40], Michelle AT Hildebrandt[52], Peter Hillemanns[16], Claus K Hogdall[53], Estrid Høgdall[54,55], Satoyo Hosono[56], Edwin S Iversen[57], Anna Jakubowska[34], Allan Jensen[54], Bu-Tian Ji[17], Audrey Y Jung[29], Beth Y Karlan[58], Melissa Kellar[11,12], Lambertus A Kiemeney[7], Boon Kiong Lim[59], Susanne K Kjaer[54,60], Camilla Krakstad[14], Jolanta Kupryjanczyk[35], Diether Lambrechts[61,62], Sandrina Lambrechts[63], Nhu D Le[64], Shashi Lele[65], Jenny Lester[58], Douglas A Levine[66], Zheng Li[1,67], Dong Liang[68], Jolanta Lissowska[69], Karen Lu[70], Jan Lubinski[34], Lene Lundvall[53] , Leon FAG Massuger[71], Keitaro Matsuo[56], Valerie McGuire[72], John R McLaughlin[73], Ian McNeish[73], Usha Menon[49], Roger L Milne[20,50], Francesmary Modugno[21,45,74], Kirsten B Moysich[65], Roberta B Ness[75], Heli Nevanlinna[23], Kunle Odunsi[76], Sara H Olson[77], Irene Orlow[77], Sandra Orsulic[58], James Paul[27], Tanja Pejovic[11,12], Liisa M Pelttari[23], Jenny B Permuth[5], Malcolm C Pike[77], Elizabeth M Poole[78,79], Barry Rosen[80], Mary Anne Rossing[37], Joseph H Rothstein[81], Ingo B Runnebaum[41], Iwona K Rzepecka[35], Eva Schernhammer[78,79], Ira Schwaab[82], Xiao-Ou Shu[83], Yurii B Shvetsov[84], Nadeem Siddiqui[84], Weiva Sieh[81], Honglin Song[4], Melissa C Southey[26], Beata Spiewankiewicz[85], Lara Sucheston-Campbell[65], Ingvild L

Tangen[14], Soo-Hwang Teo[86,87], Kathryn L Terry[79,88], Pamela J Thompson[51], Lotte Thomsen[89], Shelley S Tworoger[5,78,79] , Anne M van Altena[90], Ignace Vergote[63], Liv Cecilie Vestrheim Thomsen[14], Robert A Vierkant[1], Christine S Walsh[58], Shan Wang-Gohrke[91], Nicolas Wentzensen[17], Alice S Whittemore[92], Kristine G Wicklund[37], Lynne R Wilkens[84], Yin-Ling Woo[59,93], Anna H Wu[94], Xifeng Wu[52], Yong-Bing Xiang[95], Hannah Yang[17], Wei Zheng[96], Argyrios Ziogas[8], Alice W Lee[97], Celeste L Pearce[88], Andrew Berchuck[98], Joellen M Schildkraut[99], Susan J Ramus[100,101], Alvaro NA Monteiro[5], Steven A Narod[102], Thomas A Sellers[5], Simon A Gayther[103], Linda E Kelemen[104], Georgia Chenevix-Trench[105], Harvey A Risch[106], Paul DP Pharoah[2,107], Ellen L Goode*[1], and Catherine M Phelan[@5]

1. Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA.
2. Department of Oncology, University of Cambridge, Strangeways Research Laboratory, Cambridge, UK.
3. Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, FL, USA.
4. School of Public Health, Louisiana State University Health Sciences Center, New Orleans, LA, USA.
5. Division of Population Sciences, Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, FL, USA.
6. Netherlands Comprehensive Cancer Organization, Utrecht, The Netherlands.
7. Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, The Netherlands.
8. Genetic Epidemiology Research Institute, UCI Center for Cancer Genetics Research and Prevention, School of Medicine, Department of Epidemiology, University of California Irvine, Irvine, CA, USA.

9. Byelorussian Institute for Oncology and Medical Radiology Aleksandrov N.N., Minsk, Belarus.
10. Cancer Prevention and Control, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA.
11. Department of Obstetrics and Gynecology, Oregon Health & Science University, Portland, OR, USA.
12. Knight Cancer Institute, Oregon Health & Science University, Portland, OR, USA.
13. University Breast Center Franconia, Department of Gynecology and Obstetrics, University Hospital Erlangen, Erlangen, Germany.
14. Department of Gynecology and Obstetrics, Haukeland University Hospital, Bergen, Norway.
15. Centre for Cancer Biomarkers, Department of Clinical Medicine, University of Bergen, Bergen, Norway.
16. Gynaecology Research Unit, Hannover Medical School, Hannover, Germany.
17. Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA.
18. Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada.
19. Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada.
20. Cancer Epidemiology & Intelligence Division, The Cancer Council Victoria, Melbourne, Australia.
21. Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.
22. Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland.
23. Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland.
24. Cancer Genetics Laboratory, Research Division, Peter MacCallum Cancer Centre, Melbourne, Australia.
25. Sir Peter MacCallum Department of Oncology, University of Melbourne, Australia.

26. Department of Pathology, University of Melbourne, Melbourne, Victoria, Australia.
27. CRUK Clinical Trials Unit, The Beatson West of Scotland Cancer Centre, Glasgow, UK.
28. Department of Gynaecological Oncology, Glasgow Royal Infirmary, Glasgow, UK.
29. Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany.
30. University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
31. Division of Epidemiology and Biostatistics, University of New Mexico, Albuquerque, NM, USA.
32. Obstetrics and Gynecology Center, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.
33. Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA.
34. International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland.
35. Department of Pathology, The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland.
36. Division of Gynecologic Oncology, Department of Obstetrics and Gynecology and Leuven Cancer Institute, University Hospitals Leuven, Belgium.
37. Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.
38. Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA.
39. Department of Gynaecology and Gynaecologic Oncology, Dr. Horst Schmidt Kliniken Wiesbaden, Wiesbaden, Germany.
40. Department of Gynaecology and Gynaecologic Oncology, Kliniken Essen-Mitte/ Evang. Huyssens-Stiftung/ Knappschaft GmbH, Essen, Germany.
41. Department of Gynecology, Friedrich Schiller University, Jena, Germany.

42. Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, UK.
43. Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK.
44. Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, UK.
45. Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
46. Institute of Human Genetics, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany.
47. David Geffen School of Medicine, Department of Medicine Division of Hematology and Oncology, University of California at Los Angeles, CA, USA.
48. Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA.
49. Gynaecological Cancer Research Centre, Department of Women's Cancer, Institute for Women's Health, University College London, London, UK.
50. Centre for Epidemiology and Biostatistics, School of Population and Global Health, University of Melbourne, Melbourne, VIC, Australia.
51. Samuel Oschin Comprehensive Cancer Institute, Cedars Sinai Medical Center, Los Angeles, CA, USA.
52. Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
53. Department of Gynaecology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.
54. Virus, Lifestyle and Genes, Danish Cancer Society Research Center, Copenhagen, Denmark.

55. Molecular Unit, Department of Pathology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark.
56. Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Aichi, Japan.
57. Department of Statistics, Duke University, Durham, NC, USA.
58. Women's Cancer Program, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA.
59. Department of Obstetrics and Gynaecology, University Malaya Medical Centre, University Malaya, Kuala Lumpur, Malaysia.
60. Department of Gynecology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.
61. Laboratory for Translational Genetics, Department of Oncology, University of Leuven, Leuven, Belgium.
62. Vesalius Research Center, VIB, University of Leuven, Leuven, Belgium.
63. Division of Gynecologic Oncology; Leuven Cancer Institute, University Hospitals Leuven, KU Leuven, Leuven, Belgium.
64. Cancer Control Research, BC Cancer Agency, Vancouver, BC, Canada.
65. Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY, USA.
66. Gynecology Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.
67. Department of Gynecologic Oncology, The Third Affiliated Hospital of Kunming Medical University (Yunnan Tumor Hospital), Kunming, China
68. College of Pharmacy and Health Sciences, Texas Southern University, Houston, TX, USA.
69. Department of Cancer Epidemiology and Prevention, M. Sklodowska-Curie Memorial Cancer Center & Institute of Oncology, Warsaw, Poland.

70. Department of Gynecologic Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
71. Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands.
72. Department of Health Research and Policy, Stanford University School of Medicine, Stanford, CA, USA.
73. Public Health Ontario, Toronto, ON, Canada.
74. Womens Cancer Research Program, Magee-Womens Research Institute and University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.
75. The University of Texas School of Public Health, Houston, TX, USA.
76. Department of Gynecologic Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA.
77. Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.
78. Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.
79. Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA.
80. Department of Gynecology-Oncology, Princess Margaret Hospital, and Department of Obstetrics and Gynecology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada.
81. Department of Population Health Science and Policy, Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
82. Institut für Humangenetik Wiesbaden, Wiesbaden, Germany.
83. Epidemiology Center and Vanderbilt, Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN, USA.
84. Cancer Epidemiology Program, University of Hawaii Cancer Center, HI, USA.

85. Department of Gynecologic Oncology, Institute of Oncology, Warsaw, Poland.
86. Division of Cancer Etiology and Genetics, National Cancer Institute, Bethesda, MD, USA.
87. University Malaya Medical Centre, University Malaya, Kuala Lumpur, Malaysia.
88. Department of Epidemiology, University of Michigan, Ann Arbor, MI, USA.
89. Department of Pathology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.
90. Department of Obstetrics and Gynecology, Radboud University Medical Center, Nijmegan, The Netherlands.
91. German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany.
92. Department of Health Research and Policy, Department of Biomedical Data Science, Stanford University School of Medicine, Stanford, CA, USA.
93. Cancer Research Malaysia.
94. Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.
95. Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China.
96. Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, TN, USA.
97. Department of Health Science, California State University, Fullerton, Fullerton, CA, USA.
98. Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA.
99. Department of Public Health Sciences, The University of Virginia, Charlottesville, VA, USA.
100. School of Women's and Children's Health, University of New South Wales, Sydney, Australia.
101. The Garvan Institute, Sydney, New South Wales, Australia.
102. Women's College Research Institute, University of Toronto, Toronto, Ontario, Canada.
103. Center for Cancer Prevention and Translational Genomics, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA.

104. Department of Public Health Sciences, Medical University of South Carolina and Hollings Cancer Center, Charleston, SC, USA.

105. Department of Genetics, QIMR Berghofer Medical Research Institute, Brisbane, Australia.

106. Department of Chronic Disease Epidemiology, Yale School of Public Health, New Haven, CT, USA.

107. Department of Public Health and Primary Care, University of Cambridge, Strangeways Research Laboratory, Worts Causeway, Cambridge, UK.

¶ Authors contributed equally to this manuscript.

*To whom correspondence should be addressed: Ellen L. Goode, Ph.D., M.P.H., Department of Health Sciences, Research, Mayo Clinic, Email egoode@mayo.edu.

@Catherine M Phelan became deceased in September 2017.

# Abstract

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer mortality in American women. Normal ovarian physiology is intricately connected to small GTP binding proteins of the Ras superfamily (Ras, Rho, Rab, Arf, and Ran) which govern processes such as signal transduction, cell proliferation, cell motility, and vesicle transport. We hypothesized that common germline variation in genes encoding small GTPases is associated with EOC risk. We investigated 322 variants in 88 small GTPase genes in germline DNA of 18,736 EOC patients and 26,138 controls of European ancestry using a custom genotype array and logistic regression fitting log-additive models. Functional annotation was used to identify biofeatures and expression quantitative trait loci that intersect with risk variants. One variant, *ARHGEF10L* (Rho guanine nucleotide exchange factor 10 like) rs2256787, was associated with increased endometrioid EOC risk (OR=1.33, $p=4.46 \times 10^{-6}$). Other variants of interest included another in *ARHGEF10L*, rs10788679, which was associated with invasive serous EOC risk (OR=1.07, p=0.00026) and two variants in *AKAP6* (A-kinase anchoring protein 6) which were associated with risk of invasive EOC (rs1955513, OR=0.90, p=0.00033; rs927062, OR =0.94, p=0.00059). Functional annotation revealed that the two *ARHGEF10L* variants were located in super-enhancer regions and that *AKAP6* rs927062 was associated with expression of GTPase gene *ARHGAP5* (Rho GTPase activating protein 5). Inherited variants in *ARHGEF10L* and *AKAP6*, with potential transcriptional regulatory function and association with EOC risk, warrant investigation in independent EOC study populations.

# Introduction

In 2017, in the United States, more than 21,000 women are expected to be diagnosed with epithelial ovarian cancer (EOC), and more than 14,000 women are predicted to die from the disease.[1] EOC is heterogeneous and therefore classified into major histological subtypes of invasive disease - serous, endometrioid, clear cell, and mucinous – and two histological subtypes of borderline disease – serous and mucinous. These histological subtypes have differences in genetic and epidemiologic risk factors, molecular events during oncogenesis, response to chemotherapy, and prognosis.[2]

Approximately 20% of the familial component of EOC risk is attributable to high-to-intermediate risk gene mutations.[3] In European populations, genome-wide association studies (GWAS) have identified more than 30 EOC susceptibility alleles, as reviewed previously.[4] Known common genetic variants explain 3.9% of the inherited component of EOC risk, and additional susceptibility loci are likely to exist, particularly for the less common, non-serous histological subtypes.

Normal ovarian physiology is intricately connected to tightly regulated small GTP binding proteins of the Ras superfamily (Ras, Rho, Rab, Ral, Arf, and Ran) which regulate key cellular processes such as signal transduction, cell proliferation, cell motility, and vesicle transport.[5] These proteins function in a highly coordinated manner through signaling networks and feedback loops within and among the small GTPase subfamilies.[6] The Rab and Ral GTPases are thought to function in membrane trafficking in exocyst assembly and vesicle-tethering processes;[7, 8] Rho-related proteins function to integrate extracellular signals with specific targets regulating cell morphology, cell aggregation, tissue polarity, cell motility and cytokinesis.[5] Ras family genes cycle between their inactive GDP forms in the cytoplasm and the active GTP-bound forms on the plasma membrane and are associated with signaling pathways contributing to

normal and aberrant cell growth.[9]

As regulation of the RAS signal transduction pathway involves a highly complex, highly polymorphic machinery of genes, we conducted a large-scale candidate pathway association study, hypothesizing that variation in small GTPase genes is associated with EOC risk.

# Materials and Methods

## *Variant Selection*

RAS pathway genes were selected based on the Cancer Genome Anatomy Project and review of the published literature (www.pubmed.gov). Within 115 candidate genes, 6103 single nucleotide polymorphism (SNPs) were interrogated in early GWAS analysis of 7931 EOC patients and 9206 controls[10]; 339 SNPs in 88 of these genes showed nominal evidence of association with risk of EOC or of serous EOC ($p<0.05$ using all participants or North American participants only)[10] and were targeted in the present analysis (SI Table 1).

## *Study Participants and Genotyping*

We studied 18,736 EOC patients (10,316 of serous histology) and 26,138 controls who participated in Ovarian Cancer Association Consortium studies; all participants were of European ancestry.[11] This included participants from the GWAS which was used for variant selection (described above)[10] and an additional 10,243 patients and 16,932 controls. Genotyping used a custom Illumina Infinium array.[11] SNPs were excluded according to the following criteria: no genotype call; monomorphism; call rate less than 95% and minor allele frequency > 0.05 or call rate less than 99% with minor allele frequency < 0.05; evidence of deviation of genotype frequencies from Hardy-Weinberg equilibrium ($p < 10^{-7}$); greater than

2% discordance in duplicate pairs. Overall, 322 small GTPase gene SNPs were genotyped and passed QC; numbers of participants with data for each SNP vary, as some DNA samples failed QC for particular SNPs. This study was reviewed and approved by the Mayo Clinic Institutional Review Board as protocol 1367-05.

## *Genetic Association*

We followed STREGA guidelines for genetic association studies.[12] Unconditional logistic regression treating the number of minor alleles carried as an ordinal variable (log-additive model) was used to evaluate the association between each SNP and EOC risk adjusted for age, study site, and principal components to account for residual differences in European ancestry. Six series of analyses were conducted considering the following groups: all invasive EOC combined, each of the four main invasive histological subtypes (serous, endometrioid, clear cell and mucinous), and all borderline tumors combined. No corrections were made for multiple testing.

## *Functional Annotation*

For SNPs of interest, dbSUPER [13] and Haploreg v4.1[14] were used to evaluate publicly available data for variant overlap with human super-enhancers,[15] known expression quantitative trait loci (eQTL), GWAS hits, and other regulatory marks. In addition, we assessed correlations between germline genotype with tumor expression levels (eQTL analysis) using 312 Mayo Clinic patients (226 serous, 54 endometrioid, 22 clear cell, 5 mucinous, and 5 of other histological subtypes). Expression data were obtained using fresh frozen tumor RNA and Agilent whole human genome 4×44 expression arrays and were analyzed in the form of log ratios of signals from individual tumors compared to signals from a reference mix of 106 tumor samples[16, 17]versus signals from a reference mix of 106 tumor samples[16, 17]. Expression levels for

minor allele carriers versus non-carriers were compared using the Wilcoxon rank sum statistic.

# Results and discussion

Demographic and clinical characteristics of the study sample (18,736 EOC patients and 26,138 controls) have been described previously.[11] In brief, compared to controls, patients were older, attained menarche at older ages, and had higher body mass index. As expected, most tumors (57.6%) were of serous histology with 14.2% endometrioid, 7.1% clear cell, 6.5% mucinous, and 14.6% other/unknown.

From among 322 SNPs in 88 RAS pathway small *GTPase* genes, we observed that 99 SNPs in 43 genes were nominally associated with EOC risk ($p<0.05$) (SI Table 2). These associations were from six separate analyses that evaluated all patients with invasive disease, patients with one of the four main invasive histological subtypes, serous [n=8,372], endometrioid [n=2,068], clear cell [n=1,025] and mucinous [n=943], as well as patients with borderline tumors.

In *ARHGEF10L*, which encodes the Rho guanine nucleotide exchange factor 10-like protein, SNP rs2256787 was associated with invasive endometrioid EOC risk (OR= 1.33, 95% CI: 1.18-1.50, $p= 4.5x10^{-6}$) (Table). The Figure shows the ORs and 95% CIs associated with the G allele at this SNP overall and by contributing study.

Figure: Association of rs2256787 in the ARHGEF10L gene with invasive endometrioid EOC risk by study site and combined.

Table. Association of variants in small GTPase genes with epithelial ovarian cancer risk (p-value<$10^{-4}$) and functional annotation.

| | | | | | Genetic Association | | | Functional Annotation | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gene** | **SNP** | **Chr:Position** | **Alleles** | **MAF** | **Histology** | **OR (95% CI)** | **P-value** | **Conserved site** | **eQTL** | **Tissues with enhancer histone mark** | **Tissues with DNAse site** | **In super-enhancer** |
| *ARHGEF10L* | rs2256787 | 1:17,765,403 | A/C | 0.07 | Endometrioid | 1.33 (1.18-1.50) | $4.5 \times 10^{-6}$ | No | No | ESC, ESDR, IPSC, FAT, STRM, BRST, BRN, SKIN, VAS, LIV, GI, HRT, MUS, LNG, OVRY, PANC | None | Yes |
| | rs10788679 | 1:17,789,549 | A/G | 0.42 | Serous | 1.07 (1.03-1.11) | $2.6 \times 10^{-4}$ | No | No | None | None | Yes |
| *AKAP6* | rs1955513 | 14:32,245,693 | C/A | 0.07 | All invasive | 0.90 (0.85-0.95) | $3.3 \times 10^{-4}$ | Yes | No | FAT, SKIN, VAS, BRN, MUS, GI, BLD | SKIN,MUS,MUS,T HYM,BLD | No |
| | rs927062 | 14:32,164,800 | G/A | 0.21 | All invasive | 0.94 (0.90-0.97) | $5.9 \times 10^{-4}$ | No | Yes, ARHGAP5 | None | GI | No |

SNP, single nucleotide polymorphism; alleles show minor/major; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; eQTL, expression quantitative locus with p<0.05 in EOC tumors; histone marks and DNAse I hypersensitive sites from HaploReg v 4.1 indicating tissue types as defined therein; super enhancer information based on the human super-enhancer database available at http://bioinfo.au.tsinghua.edu.cn/dbsuper/index.php; none of these SNPs had previous GWAS associations with any phenotype based on the EBI GWAS catalog or resided within promoter histone marks; all SNPs are intronic to the gene indicated.

Three other variants were associated at p-value<$10^{-4}$ (Table and SI Figures 1-3). rs10788679 in an intron of *ARHGEF10L* was associated with risk of invasive serous EOC (OR= 1.07, 95% CI: 1.03-1.11, p= 2.6x$10^{-4;}$); *ARHGEF10L* SNPs rs2256787 and rs10788679 are independent ($r^2$ = 0.02, 1000 Genomes Project EUR). In addition, rs1955513 was most strongly associated with all invasive EOC risk (OR= 0.90, 95% CI: 0.85-0.95, p= 3.3x$10^{-4}$). This variant lies in an intron of A-kinase (PRKA) anchor protein 6 (*AKAP6*). Another variant in *AKAP6*, intronic SNP rs927062, was also associated with all invasive EOC risk (p= 5.9x$10^{-4}$); *AKAP6*SNPs rs1955513 and rs927062 are in modest linkage disequilibrium ($r^2$ = 0.15, 1000 Genomes Project EUR).

We investigated whether the four variants of interest, rs2256787, rs10788679, rs1955513, rs927062, which are all intronic, alter expression of their proximal GTPases, or coincide with regulatory marks that may affect expression (Table). In publicly available databases,[13, 14] the *ARHGEF10L* SNPs rs2256787and rs10788679 coincide with a human ovary super-enhancer, a region of the genome with unusually strong enrichment for the binding of transcriptional coactivators in this tissue. As *ARHGEF10L* rs2256787 associated with endometrioid EOC risk, we were particularly interested in eQTLs in the 54 endometrioid patients; however, there was no evidence of association between rs2256787 genotype and *ARHGEF10L* expression in endometrioid EOC tumors or other tumor subtypes. In 312 invasive EOC tumors, the G allele of *AKAP6* rs927062 correlated with reduced expression of Rho GTPase activating protein 5 (ARHGAP5), a GTPase ~150kb upstream of *AKAP6* (β= -0.22, 95% CI: -0.41 to -0.03, p = 6.6x$10^{-3}$). Other unstudied variants may also be associated with expression of *ARHGAP5* (or may be more strongly associated than rs927062), thus future genome-wide or pathway-based analysis of GTPase SNP-expression relationships are of great interest. In other histology-specific eQTL analyses, none of the four variants tested were associated with EOC tumor mRNA expression.

# Conclusion

We investigated 322 SNPs in 88 genes encoding small GTP binding proteins of the Ras superfamily (Ras, Rho, Rab, Ral, Arf, and Ran) in germline DNA of over 17,000 EOC patients and 26,000 controls. The 88 genes were derived from G protein (guanine nucleotide-binding proteins) signaling, Ras-GTPases, regulation of Rho GTPase protein signal transduction and activation of Rac GTPase activity. [18] Ras-GTPases are activated at the plasma membrane by guanine nucleotide exchange factors (GEF) such as: son of sevenless homologs 1 and 2 (Drosophila) (SOS-1 and SOS-2); Ras protein-specific guanine nucleotide-releasing factor 1 (GRF1); Rap guanine nucleotide exchange factor 1 (GRF2); and RasGEF domain family, members 1A, 1B and 1C (RasGRF). They are inactivated by GTPase activating proteins (GAP) which include RAS p21 protein activator (GTPase activating protein) 1 (p120RasGAP). GEF factors are recruited to the plasma membrane by scaffold and adaptor complexes such as SHC/Grb2 that associate with activated tyrosine kinase receptors (TKR).[19] These factors exchange GTP for GDP on the Ras protein. The resulting GTP-Ras protein activates various downstream effectors such as MAP-kinase Raf-1 which activates the MEK/ERK gene regulation cascade, a primary cell growth and anti-apoptosis pathway.[6]Ras-GTPases family members regulate the action of other GTPase pathways involving Rap, Ral, Rac and Rho Ras-GTPase. Ras-GTPases also regulate phosphoinositide 3-kinase (PI3K) and phospholipase C (PLC) activities.[5] Several of these genes are mutated in ovarian tumors.[20]

Overall, analysis at only one SNP yielded a p-value < $10^{-5}$: rs2256787 in *ARHGEF10L* which was associated with 33% increased endometrioid EOC risk. Of note, the experiment-wide error rate for this SNP, accounting for the initial overall set of 6103 candidate SNPs equals 0.027 (Bonferroni-corrected p-value $4.5 \times 10^{-6} \times 6103$); additionally accounting for six case groups analyzed, this value increases to 0.16 (Bonferroni-corrected p-value $4.5 \times 10^{-6} \times 6103 \times 6$). However, as SNPs, as well as case groups, are not

independent, simulation studies are necessary to derive an empirical p-value. Another *ARHGEF10L* SNP, rs10788679, in showed the smallest p-value in analysis of serous EOC and was the second-most strongly associated SNP in all analyses. ARHGEF10L is a member of the RhoGEF family GEFs that activate Rho GTPases.[21] The Rho branch of the Ras super family encompasses 20 genes in humans, of which Rho, Rac and Cdc42 are the best characterized. Rho GTPases regulate the actin cytoskeleton and control changes in cell morphology and cell motility triggered by extracellular stimuli. Rho GTPases are regulated by GDP/GTP exchange factors and GAPs. Members of this subfamily are activated by specific GEFs and are involved in signal transduction. SNPs in this gene are also associated with obesity[22] and cutaneous basal cell carcinoma.[23]

The SNP most associated with risk of invasive EOC was rs1955513 in the *AKAP6* gene. This gene is involved in overall G protein signaling. SNPs in this gene are also associated with neurologic functioning[24] and anorexia.[25] Functionally, rs927062 in *AKAP6* was associated with expression of the Rho GTPase activating protein 5, *ARHGAP5*, also known as p190 RhoGAP, which negatively regulates RHO GTPases. The p190 RhoGAP gene contains a carboxy-terminal domain that functions as a GAP for the Rho family GTPases. In addition to its RhoGAP domain, p190 contains an amino-terminal domain that contains sequence motifs found in all known GTPases.

In conclusion, our study identified potentially functional genetic variants in small GTPase genes that may have roles in EOC susceptibility. To interpret these associations, we suggest consideration of effect sizes and directionality in the context of the sets of histotype-specific analyses conducted; whether a more conservative or liberal statistical significance threshold is applied, the small set of variants highlighted for detailed functional follow-up remain the same. A limitation of this work is that nearby imputed variants were not examined and thus other ungenotyped variants may be driving the reported

associations. Nonetheless, four variants in two genes show promising associations that have not been reported previously but point to known pathways that are mutated in ovarian tumors. The results of our investigation suggest that further assessment of this important pathway is warranted in additional collections of densely genotyped EOC patients and controls.

# Acknowledgements

We thank all the individuals who took part in this study and all the researchers, clinicians and technical and administrative staff who have made possible the many studies contributing to this work. In particular, we thank: D. Bowtell, A. deFazio, D. Gertig, A. Green, P. Parsons, N. Hayward, P. Webb and D. Whiteman (AUS); G. Peuteman, T. Van Brussel and D. Smeets (BEL); the staff of the genotyping unit, S LaBoissiere and F Robidoux (Genome Quebec); U. Eilber (GER); L. Gacucova (HMO); P. Schurmann, F. Kramer, W. Zheng, T. W. Park, Simon, K. Beer- Grondke and D. Schmidt (HJO); S. Windebank, C. Hilker and J. Vollenweider (MAY); the state cancer registries of AL, AZ, AR, CA, CO, CT, DE, FL, GA, HI, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, and WYL. The authors assume full responsibility for analyses and interpretation of these data (NHS); L. Paddock, M. King, L. Rodriguez-Rodriguez, A. Samoila, and Y. Bensman (NJO); M. Sherman, A. Hutchinson,N. Szeszenia--- Dabrowska, B. Peplonska, W. Zatonski, A. Soni, P. Chao and M. Stagner (POL); C. Luccarini,P. Harrington the SEARCH team and ECRIC (SEA); I. Jacobs, M. Widschwendter, E. Wozniak, N. Balogun, A. Ryan and J. Ford (UKO); Carole Pye (UKR); A. Amin Al Olama, K. Michilaidou, K. Kuchenbaker (COGS). The Australian Ovarian Cancer Study acknowledges the cooperation of the participating institutions in Australia and acknowledges the contribution of the study nurses, research assistants and all clinical and scientific collaborators to the study. The complete Australian Ovarian Cancer Study Management Group can be found at *www.aocstudy.org (*Georgia.Trench@qimrberghofer.edu.au). We would like to thank all of the women who participated in these research programs.

# Main funding

The scientific development and funding for this project were funded by the following: NIH R01 CA-1491429 (Phelan PI); the US National Cancer Institute (R01-CA076016); the COGS project is funded through a European Commission's Seventh Framework Programme grant (agreement number 223175 HEALTH F2 2009-223175); the Genetic Associations and Mechanisms in Oncology (GAME-ON): a NCI Cancer Post-GWAS Initiative (U19-CA148112); the Ovarian Cancer Association Consortium is supported by a grant from the Ovarian Cancer Research Fund thanks to donations by the family and friends of Kathryn Sladek Smith (PPD/RPCI.07).

G.C.-T. is supported by the National Health and Medical Research Council; B.K. is funded by the American Cancer Society Early Detection Professorship (SIOP-06-258-01-COUN) and the National Center for Advancing Translational Sciences (NCATS), Grant UL1TR000124; L.E.K. is supported by a Canadian Institute of Health Research New Investigator Award (MSH-87734).

Funding of the constituent studies was provided by the California Cancer Research Program (00-01389V-20170, N01-CN25403, 2II0200); the Canadian Institutes of Health Research (MOP-86727); Cancer Australia; Cancer Council Victoria; Cancer Council Queensland; Cancer Council New South Wales; Cancer Council South Australia; Cancer Council Tasmania; Cancer Foundation of Western Australia; the Cancer Institute of New Jersey; Cancer Research UK (C490/A6187, C490/A10119, C490/A10124); the Danish Cancer Society (94-222-52); the ELAN Program of the University of Erlangen-Nuremberg; the Eve Appeal; the Helsinki University Central Hospital Research Fund; Helse Vest; the Norwegian Cancer Society; the Norwegian Research Council; the Ovarian Cancer Research Fund; Nationaal Kankerplan of Belgium; Grant-in-Aid for the Third Term Comprehensive 10-Year Strategy For Cancer Control from the Ministry of

Health Labour and Welfare of Japan; the L & S Milken Foundation; the Polish Ministry of Science and Higher Education (4 PO5C 028 14, 2 PO5A 068 27); the Roswell Park Cancer Institute Alliance Foundation; the US National Cancer Institute (K07-CA095666, K07-CA143047, K22-CA138563, N01-CN55424, N01-PC67001, N01-PC067010, N01-PC035137, P01-CA017054, P01-CA087696, P01-CA087969, P30-CA072720, P50-CA105009, P50-CA136393, R01-CA014089, R01-CA016056, R01-CA017054, R01-CA049449, R01-CA050385, R01-CA054419, R01- CA058598, R01-CA058860, R01-CA061107, R01-CA061132, R01-CA067262, R01-CA071766, R01-CA074850, R01-CA080742, R01-CA080978, R01-CA083918, R01-CA087538, R01-CA092044, R01-095023, R01-CA122443, R01-CA112523, R01-CA114343, R01-CA126841, R01-CA136924, R03-CA113148, R03-CA115195, U01-CA069417, U01-CA071966, UM1-CA182910, UM1-CA186107 and Intramural research funds); the US Army Medical Research and Material Command (DAMD17-01-1-0729, DAMD17-02-1-0666, DAMD17-02-1-0669, W81XWH-07-0449, W81XWH-10-1-02802); the US Public Health Service (PSA-042205); The National Health and Medical Research Council of Australia (199600 and 400281); the German Federal Ministry of Education and Research of Germany Programme of Clinical Biomedical Research (01GB 9401); the State of Baden-Wurttemberg through Medical Faculty of the University of Ulm (P.685); the Minnesota Ovarian Cancer Alliance; the Mayo Foundation; the Fred C. and Katherine B. Andersen Foundation; the Lon V. Smith Foundation (LVS-39420); the Oak Foundation; the OHSU Foundation; the Mermaid I project; the Rudolf-Bartling Foundation; the UK National Institute for Health Research Biomedical Research Centres at the University of Cambridge, Imperial College London, University College Hospital “Womens Health Theme” and the Royal Marsden Hospital; WorkSafeBC. The Australian Ovarian Cancer Study Group was supported by the U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0729, The Cancer Council Tasmania and The Cancer Foundation of Western Australia and the National Health and Medical Research Council of Australia (NHMRC; ID400413 and ID400281). The Australian Ovarian Cancer

457 Study gratefully acknowledges additional support from S. Boldeman, the Agar family, Ovarian Cancer
458 Action (UK), Ovarian Cancer Australia and the Peter MacCallum Foundation.
459
460
461

# References


1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin* 2017;67(1):7-30. Available from https://www.ncbi.nlm.nih.gov/pubmed/28055103.

2. Soslow RA. Histologic subtypes of ovarian carcinoma: an overview. *Int J Gynecol Pathol* 2008;27(2):161-74. Available from https://www.ncbi.nlm.nih.gov/pubmed/18317227.

3. Pharoah PD, Ponder BA. The genetics of ovarian cancer. *Best Pract Res Clin Obstet Gynaecol* 2002;16(4):449-68. Available from https://www.ncbi.nlm.nih.gov/pubmed/12413928.

4. Kar SP, Berchuck A, Gayther SA, Goode EL, Moysich KB, Pearce CL, et al. Common genetic variation and susceptibility to ovarian cancer: current insights and future directions. *Cancer Epidemiol Biomarkers Prev* 2017 [Epub ahead of print]. Available from https://www.ncbi.nlm.nih.gov/pubmed/28615364.

5. Lu Y, Ek WE, Whiteman D, Vaughan TL, Spurdle AB, Easton DF, et al. Most common 'sporadic' cancers have a significant germline genetic component. *Hum Mol Genet* 2014;23(22):6112-8. Available from https://www.ncbi.nlm.nih.gov/pubmed/24943595.

6. Pajic M, Herrmann D, Vennin C, Conway JR, Chin VT, Johnsson AK, et al. The dynamics of Rho GTPase signaling and implications for targeting cancer and the tumor microenvironment. *Small GTPases* 2015;6(2):123-33. Available from https://www.ncbi.nlm.nih.gov/pubmed/26103062.

7. Just WW, Peranen J. Small GTPases in peroxisome dynamics. *Biochim Biophys Acta* 2016;1863(5):1006-13. Available from https://www.ncbi.nlm.nih.gov/pubmed/26775587.

8. Li G, Marlin MC. Rab family of GTPases. *Methods Mol Biol* 2015;1298:1-15. Available from https://www.ncbi.nlm.nih.gov/pubmed/25800828.

9. Shirakawa R, Horiuchi H. Ral GTPases: crucial mediators of exocytosis and tumourigenesis. *J Biochem* 2015;157(5):285-99. Available from https://www.ncbi.nlm.nih.gov/pubmed/25796063.

10. Nussinov R, Tsai CJ, Chakrabarti M, Jang H. A new view of Ras Isoforms in cancers. *Cancer Res* 2016;76(1):18-23. Available from https://www.ncbi.nlm.nih.gov/pubmed/26659836.

11. Song H, Ramus SJ, Tyrer J, Bolton KL, Gentry-Maharaj A, Wozniak E, et al. A genome-wide association study identifies a new ovarian cancer susceptibility locus on 9p22.2. *Nat Genet* 2009;41(9):996-1000. Available from https://www.ncbi.nlm.nih.gov/pubmed/19648919.

12. Pharoah PD, Tsai YY, Ramus SJ, Phelan CM, Goode EL, Lawrenson K, et al. GWAS meta-analysis and replication identifies three new susceptibility loci for ovarian cancer. *Nat Genet* 2013;45(4):362-70, 70e1-2. Available from https://www.ncbi.nlm.nih.gov/pubmed/23535730.

13. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, et al. STrengthening the REporting of Genetic Association Studies (STREGA): an extension of the STROBE statement. *PLoS Med* 2009;6(2):e22. Available from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19192942

14. Khan A, Zhang X. dbSUPER: a database of super-enhancers in mouse and human genome. *Nucleic Acids Res* 2016;44(D1):D164-71. Available from https://www.ncbi.nlm.nih.gov/pubmed/26438538.

15. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res* 2016;44(D1):D877-81. Available from https://www.ncbi.nlm.nih.gov/pubmed/26657631.

16. Hnisz D, Schuijers J, Lin CY, Weintraub AS, Abraham BJ, Lee TI, et al. Convergence of developmental and oncogenic signaling pathways at transcriptional super-enhancers. *Mol Cell* 2015;58(2):362-70. Available from https://www.ncbi.nlm.nih.gov/pubmed/25801169.

17. Konecny GE, Wang C, Hamidi H, Winterhoff B, Kalli KR, Dering J, et al. Prognostic and therapeutic relevance of molecular subtypes in high-grade serous ovarian cancer. *J Natl Cancer Inst* 2014;106(10):dju249. Available from https://www.ncbi.nlm.nih.gov/pubmed/25269487.

18. Wang C, Winterhoff BJ, Kalli KR, Block MS, Armasu SM, Larson MC, et al. Expression signature distinguishing two tumour transcriptome classes associated with progression-free survival among rare histological types of epithelial ovarian cancer. *Br J Cancer* 2016;114(12):1412-20. Available from https://www.ncbi.nlm.nih.gov/pubmed/27253175.

19. Hoon JL, Tan MH, Koh CG. The regulation of cellular responses to mechanical cues by Rho GTPases. *Cells* 2016;5(2). Available from https://www.ncbi.nlm.nih.gov/pubmed/27058559.

20. Fritz G, Henninger C. Rho GTPases: novel players in the regulation of the DNA damage response? *Biomolecules* 2015;5(4):2417-34. Available from https://www.ncbi.nlm.nih.gov/pubmed/26437439.

21. Winkler S, Mohl M, Wieland T, Lutz S. GrinchGEF--a novel Rho-specific guanine nucleotide exchange factor. *Biochem Biophys Res Commun* 2005;335(4):1280-6. Available from https://www.ncbi.nlm.nih.gov/pubmed/16112081.

22. Comuzzie AG, Cole SA, Laston SL, Voruganti VS, Haack K, Gibbs RA, et al. Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population. *PLoS One* 2012;7(12):e51954. Available from https://www.ncbi.nlm.nih.gov/pubmed/23251661.

23. Stacey SN, Gudbjartsson DF, Sulem P, Bergthorsson JT, Kumar R, Thorleifsson G, et al. Common variants on 1p36 and 1q42 are associated with cutaneous basal cell carcinoma but not with melanoma or pigmentation traits. *Nat Genet* 2008;40(11):1313-8. Available from https://www.ncbi.nlm.nih.gov/pubmed/18849993.

24. Davies G, Armstrong N, Bis JC, Bressler J, Chouraki V, Giddaluru S, et al. Genetic contributions to variation in general cognitive function: a meta-analysis of genome-wide association studies in

the CHARGE consortium (N=53949). *Mol Psychiatry* 2015;20(2):183-92. Available from https://www.ncbi.nlm.nih.gov/pubmed/25644384.

25. Wang K, Zhang H, Bloss CS, Duvvuri V, Kaye W, Schork NJ, et al. A genome-wide association study on common SNPs and rare CNVs in anorexia nervosa. *Mol Psychiatry* 2011;16(9):949-59. Available from https://www.ncbi.nlm.nih.gov/pubmed/21079607.

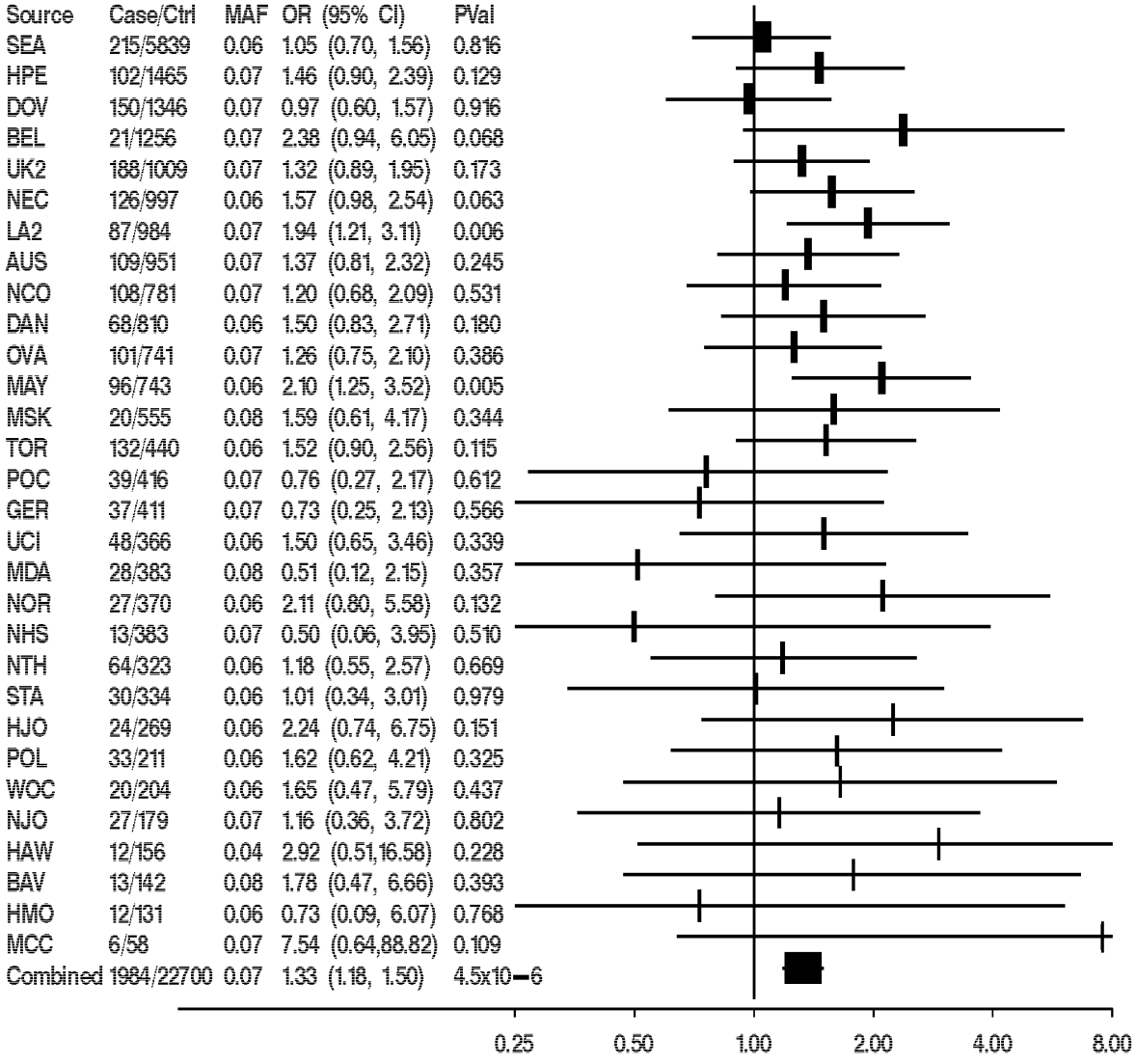
# Figure Legend

**Figure 1: Association of rs2256787 in the *ARHGEF10L* gene with invasive endometrioid EOC risk by study site and combined.**

Squares represent the estimated per-allele odds ratio (OR) and are proportional to sample size for each study; lines indicate its 95% confidence interval (CI); source indicates contributing study;[11] MAF, control minor allele frequency; PVal, per-allele p-value adjusted for age, site, and principal components to account for residual differences in European ancestry.

Figure

Click here to download Figure
Figure_Forestplot_rs2256787_end_20170928.docx



# Supporting Information Legend

**SI Fig 1: Association of rs10788679 in the *ARHGEF10L* gene with invasive serous EOC risk by study site and combined.**

Squares represent the estimated per-allele odds ratio (OR) and are proportional to sample size for each study; lines indicate its 95% confidence interval (CI); Source indicates contributing study [11]; MAF, control minor allele frequency; PVal, per-allele p-value adjusted for age, site, and residual European principal components.

**SI Fig 2: Association of rs1955513 in the *AKAP6* gene with invasive EOC risk by study site and combined.**

Squares represent the estimated per-allele odds ratio (OR) and are proportional to sample size for each study; lines indicate its 95% confidence interval (CI); Source indicates contributing study [11]; MAF, control minor allele frequency; PVal, per-allele p-value adjusted for age, site, and residual European principal components.

**SI Fig 3: Association of rs927062 in the *AKAP6* gene with invasive EOC risk by study site and combined.**

Squares represent the estimated per-allele odds ratio (OR) and are proportional to sample size for each study; lines indicate its 95% confidence interval (CI); Source indicates contributing study [11]; MAF, control minor allele frequency; PVal, per-allele p-value adjusted for age, site, and residual European principal components.

**Supporting Information**

SI_Figure 1_Forestplot_rs10788679_ser.pdf

**Supporting Information**

SI_Figure 2_Forestplot_rs1955513_inv.pdf

**Supporting Information Tables**

**SI Table 1:** Results from prior published EOC GWAS results on the targeted 339 SNPs in 88 RAS pathway genes.

More details are available upon request.

**SI Table 2:** Results from EOC genetic association analysis on 99 SNPs in RAS pathway genes with nominal p-value <0.05 in analysis of all invasive patients, patients with invasive serous, endometrioid, clear cell, or mucinous subtypes, and patients with borderline tumors versus controls.

More details are available upon request.

Click here to access/download

**Supporting Information**

GTPases SIT1 and SIT2 20170613.xls