RESEARCH ARTICLE

Variants in genes encoding small GTPases and association with epithelial ovarian cancer susceptibility

Madalene Earp1a, Jonathan P. Tyrer2a, Stacey J. Winham1, Hui-Yi Lin3,4, Ganna Chornokur5, Joe Dennis6, Katja K. H. Aben1,2, Hoda Anton-Culver7, Natalia Antonenkov9, Elsisa V. Bandera10, Yukei T. Bean11,12, Matthias W. Beckmann13, Line Bjorge14,15, Natalia Bogdanova16, Louise A. Brinton17, Angela Brooks-Wilson18,19, Fiona Bruinsma20, Clareann H. Bunker21, Ralf Butzow22,23, Ian G. Campbell24,25,26, Karen Carty27,28, Jenny Chang-Claude29,30, Linda S. Cook31, Daniel W. Cramer32, Julie M. Cunningham33, Cezary Cybulski34, Agnieszka Dansonka-Mieszkowska35, Evelyn Despierre36, Jennifer A. Doherty37,38, Thilo Dörk16, Andreas du Bois39,40, Matthies Dürst41, Douglas F. Easton12,43, Diana M. Eccles44, Robert P. Edwards45, Arif B. Ekici46, Peter A. Fasching13,47, Brooke L. Fridley48, Aleksandra Gentry-Maharaj49, Graham G. Giles50,51, Rosalind Glasspool52,77, Marc T. Goodman53, Jacek Gronwald54, Philipp Harter55,56, Alexander Hein57, Florian Heitz58,90, Michelle A. T. Hildebrandt59, Peter Hillemanns60, Klaus K. Hodgall61,62, Estrid Hodgall61,62, Satoyo Hosono63, Edwin S. Iversen64, Anna Jakubowska65, Allan Jensen66, Bu-Tian Ji17, Audrey Y. Jung67, Beth Y. Karian68, Melissa Keliar69,70, Lambertus A. Kiemeneij69, Boon Kiong Lim17,71, Susanne K. Kjaer66,67, Camilla Krakstad15, Jolanta Kupryjanczyk26, Diether Lambrechts21,62, Sandra Lambrechts72, Nhu D. Lê73, Shashi Lele68, Jenny Lester74,75, Douglas A. Levine76, Zheng Li1,67, Dong Liang69,70, Joel Lissowska69, Karen Lu63, Jan Lubinski64, Lene Lundvall65, Leon F. A. G. Massuger61,71, Keitaro Matsuo70,76, Valerie McGuire72, John R. McLaughlin73, Iain McNeish74, Usha Menon75, Roger L. Milne20,79, Francescmary Modugno21,46,74, Kirsten B. Moysich76, Roberta B. Ness75, Heli Nevanlinna80, Kunle Odusami81, Sara H. Olson77, Irene Orlow77, Sandra Orsulic58, James Paul62, Tanja Pejovic1,12, Liisa M. Pelttari23, Jenny B. Permutt8, Malcolm C. Pike77, Elizabeth M. Poole78,79, Barry Rosen80, Mary Anne Rossing81, Joseph H. Rothstein81, Ingo B. Runnebaum81, Iwona K. Rzepecka35, Eva Schernhammer79,80, Ira Schwaab62, Xiao-Ou Shu83, Yuri B. Shvetsov84, Nadeem Siddiqui84, Weina Sieh85, Honglin Song78,79, Melissa C. Southey26, Beata Spiewankiewicz86, Lara Sucheston-Campbell65, Ingivild L. Tangen14, Soo-Hwae Teo86,87, Kathryn L. Terry79,88, Pamela J. Thompson51, Lotte Thomsen89, Shelley S. Tvoroger24,1,78,79, Anne M. van Altena90, Ignace Vergote63, Liv Cecilia Vestreheim31,80, Robert A. Vierkant24,25,26, Roberta B. Ness75, Nicole Wentzensen17, Alice S. Whitemore82, Kristine G. Wicklund57, Lynne R. Wilkens94, Yin-Ling Woo59,93, Anna H. Wu64, Xinfeng Wu62, Yong-Bing Xiang95, Hannah Yang17, Wei Zheng96, Argyrios Ziozas8, Alice W. Lee97, Celeste L. Pearce88, Andrew Berchuck98, Joellen M. Schildkraut99, Susan J. Ramus100,101, Alvaro N. A. Monteiro102, Steven A. Narod102, Thomas A. Sellers5, Simon A. Gayther103, Linda E. Kelemen104, Georgia Chenevix-Trench105, Harvey A. Risch106, Paul D. P. Pharoah3,107, Ellen L. Goode1 *, Catherine M. Phelan51

1 Department of Health Sciences Research, Mayo Clinic, Rochester, MN, United States of America, 2 Department of Oncology, University of Cambridge, Strangeways Research Laboratory, Cambridge, United Kingdom, 3 Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, FL, United States of America, 4 School of Public Health, Louisiana State University Health Sciences Center, New Orleans, LA, United States of America, 5 Division of Population Sciences, Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, FL, United States of America, 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, United States of America, 9 Byelorussian Institute for Oncology and Medical Radiology, Minsk, Belarus, 10 Cancer Prevention and Control, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, United States of America, 11 Department of Obstetrics and Gynecology, Oregon...
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 the Human Epigenome project. These authors contributed equally to this work.

**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

**Competing interests:** The authors have declared that no competing interests exist.

The authors have declared that no competing interests exist.

*egoode@mayo.edu.*

These authors contributed equally to this work.

† Deceased.

*egoode@mayo.edu.*
logistic regression fitting log-additive models. Functional annotation was used to identify bio-
features 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 x 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 var-
ients in ARHGEF10L and AKAP6, with potential transcriptional regulatory function and asso-
ciation with EOC risk, warrant investigation in independent EOC study populations.

Introduction

In 2017, in the United States, more than 21,000 women were expected to be diagnosed with
epithelial ovarian cancer (EOC), and more than 14,000 women were 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 sub-
types 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-inter-
mediate 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 feed-
back loops within and among the small GTPase subfamilies.[6] The Rab and Ral GTPIases are
thought to function in membrane trafficking in exocyst assembly and vesicle-tethering pro-
cesses;[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 contribut-
ing 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 (S1 Table).

**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 4x44 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 \( (p<0.05) \) (S2 Table). 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 \( (OR = 1.33, 95\% CI: 1.18–1.50, p = 4.5 \times 10^{-6}) \) (Table 1). (Fig 1) shows the ORs and 95% CIs associated with the G allele at this SNP overall and by contributing study.

Three other variants were associated at \( p \text{-value}<10^{-4} \) (Table 1, S1, S2 and S3 Figs). rs10788679 in an intron of ARHGEF10L was associated with risk of invasive serous EOC \( (OR = 1.07, 95\% CI: 1.03–1.11, p = 2.6 \times 10^{-4}) \); ARHGEF10L SNPs rs2256787 and rs10788679 are independent \( (r^2 = 0.02, 1000 \text{ Genomes Project EUR}) \). In addition, rs1955513 was most strongly associated with all invasive EOC risk \( (OR = 0.90, 95\% CI: 0.85–0.95, p = 3.3 \times 10^{-5}) \). 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 \( (p = 5.9 \times 10^{-4}) \); AKAP6 SNPs rs1955513 and rs927062 are in modest linkage disequilibrium \( (r^2 = 0.15, 1000 \text{ Genomes Project EUR}) \).

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 1). In publicly available databases,\([13, 14]\) the ARHGEF10L SNPs rs2256787 and rs10788679 coincide with a human ovary super-enhancer, a region of the genome with unusually strong enrichment for the binding of

Table 1. Association of variants in small GTPase genes with epithelial ovarian cancer risk (p-value<10^{-4}) and functional annotation.

| Gene      | SNP          | Chr:Position | Alleles | MAF   | Histology | OR (95% CI) | P-value | 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 | None | Yes |
|           | rs1955513    | 14:32,245,693| C/A    | 0.07  | All invasive | 0.90 (0.85–0.95) | 3.3 \times 10^{-3} | Yes  | No | FAT, SKIN, VAS, BRN, MUS, GI, BLD | SKIN, MUS, MUS, THYM, 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.

https://doi.org/10.1371/journal.pone.0197561.t001
| Source | Case/Control | MAF | OR (95% CI)       | PVal |
|--------|--------------|-----|-------------------|------|
| AUS    | 109/951      | 0.07| 1.37 (0.81, 2.32) | 0.2454 |
| BAV    | 13/142       | 0.08| 1.78 (0.47, 6.66) | 0.3933 |
| BEL    | 21/1256      | 0.07| 2.38 (0.94, 6.05) | 0.0677 |
| DAN    | 68/810       | 0.06| 1.50 (0.83, 2.71) | 0.18  |
| DOV    | 150/1346     | 0.07| 0.97 (0.60, 1.57) | 0.916 |
| GER    | 37/411       | 0.07| 0.73 (0.25, 2.13) | 0.5656 |
| HAW    | 12/156       | 0.04| 2.92 (0.51, 16.58)| 0.2277 |
| HJO    | 24/269       | 0.06| 2.24 (0.74, 6.75) | 0.1514 |
| HMO    | 12/131       | 0.06| 0.73 (0.09, 6.07) | 0.7681 |
| HPE    | 102/1465     | 0.07| 1.46 (0.90, 2.39) | 0.1289 |
| LA2    | 87/984       | 0.07| 1.94 (1.21, 3.11) | 0.006 |
| MAY    | 96/743       | 0.06| 2.10 (1.25, 3.52) | 0.0047 |
| MCC    | 6/58         | 0.07| 7.54 (0.64, 88.82)| 0.1085 |
| MDA    | 28/383       | 0.08| 0.51 (0.12, 2.15) | 0.3566 |
| MSK    | 20/555       | 0.08| 1.59 (0.61, 4.17) | 0.3443 |
| NCO    | 108/781      | 0.07| 1.20 (0.68, 2.09) | 0.531 |
| NEC    | 126/997      | 0.06| 1.57 (0.98, 2.54) | 0.0626 |
| NHS    | 13/383       | 0.07| 0.50 (0.06, 3.95) | 0.5098 |
| NJO    | 27/179       | 0.07| 1.16 (0.36, 3.72) | 0.8016 |
| NOR    | 27/370       | 0.06| 2.11 (0.80, 5.58) | 0.1316 |
| NTH    | 64/323       | 0.06| 1.18 (0.55, 2.57) | 0.6693 |
| OVA    | 101/741      | 0.07| 1.26 (0.75, 2.10) | 0.3864 |
| POC    | 39/416       | 0.07| 0.76 (0.27, 2.17) | 0.6115 |
| POL    | 33/211       | 0.06| 1.62 (0.62, 4.21) | 0.3247 |
| SEA    | 215/5839     | 0.06| 1.05 (0.70, 1.56) | 0.8157 |
| STA    | 30/334       | 0.06| 1.01 (0.34, 3.01) | 0.9793 |
| TOR    | 132/440      | 0.06| 1.52 (0.90, 2.56) | 0.1152 |
| UCI    | 48/366       | 0.06| 1.50 (0.65, 3.46) | 0.3385 |
| UK2    | 188/1009     | 0.07| 1.32 (0.89, 1.95) | 0.173 |
| WOC    | 20/204       | 0.06| 1.65 (0.47, 5.79) | 0.4365 |
| Combined | 1984/22700 | 0.07| 1.33 (1.18, 1.50) | 4.5x10^-6 |

Odds Ratio
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 ($\beta = -0.22$, 95% CI: -0.41 to -0.03, $p = 6.6 \times 10^{-3}$). 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 $4.5 \times 10^{-6}$); additionally accounting for six case groups analyzed, this value increases to 0.16 (Bonferroni-corrected p-value $4.5 \times 10^{-6}$ x 6103 x 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.

Supporting information

S1 Fig. 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.

S2 Fig. 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.

S3 Fig. 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.

S1 Table. Results from prior published EOC GWAS results on the targeted 339 SNPs in 88 RAS pathway genes. More details are available upon request.

S2 Table. 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.

Acknowledgments

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 WY. 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.

Author Contributions

Data curation: Stacey J. Winham, Katja K. H. Aben, Hoda Anton-Culver, Natalia Antonen- kovala, Elisa V. Bandera, Yukie T. Bean, Matthias W. Beckmann, Line Bjorge, Natalia Bogdanova, Louise A. Brinton, Angela Brooks-Wilson, Fiona Bruinsma, Clareann H. Bunker, Ralf Butzow, Karen Carty, Jenny Chang-Claude, Linda S. Cook, Daniel W Cramer, Julie M. Cunningham, Cezary Cybulski, Agnieszka Dansonka-Mieszkowska, Evelyn Despierre, Jennifer A. Doherty, Thilo Dörk, Andreas du Bois, Matthias Dürst, Douglas F. Easton, Diana M. Eccles, Robert P. Edwards, Arif B. Ekici, Peter A. Fasching, Brooke L. Fridley, Aleksandra Gentry-Maharaj, Graham G. Giles, Rosalind Glasspool, Marc T. Goodman, Jacek Gronwald, Philipp Harter, Alexander Hein, Florian Heitz, Michelle A. T. Hildebrandt, Peter Hillemanns, Claus K. Hogdall, Estrid Hogdall, Satoyo Hosono, Edwin S. Iversen, Anna Jakubowska, Allan Jensen, Bu-Tian Ji, Audrey Y. Jung, Beth Y. Karlan, Melissa Kellar, Lambertus A. Kiemeno, Boon Kiong Lim, Susanne K. Kjaer, Camilla Krakstad, Jolanta Kupryjanczyk, Diether Lambrechts, Sandrina Lambrechts, Nhu D. Le, Shashi Lele, Jenny Lester, Douglas A. Levine, Zheng Li, Dong Liang, Jolanta Lissowska, Karen Lu, Jan Lubinski, Lene Lundvall, Leon F. A. G. Massuger, Keitaro Matsuo, Valerie McGuire, John R. McLaughlin, Iain McNeish, Usha Menon, Roger L. Milne, Francesmary Modugno, Kirsten B. Moysich, Roberta B. Ness, Heli Nevanlinna, Kunle Odunsi, Sara H. Olson, Irene Orlow, Sandra Orsulic, James Paul, Tanja Pejovic, Liisa M. Pelttari, Jenny B. Permuth, Malcolm C. Pike, Elizabeth M. Poole, Barry Rosen, Mary Anne Rossing, Joseph H. Rothstein, Ingo B. Runnebaum, Iwona K. Rzepecka, Eva Schernhammer, Ira Schwaab, Xiao-Ou Shu, Yurii B.
Shvetsov, Nadeem Siddiqui, Weiva Sieh, Honglin Song, Melissa C. Southey, Beata Spiewankiewicz, Lara Sucheston-Campbell, Ingvild L. Tangen, Soo-Hwang Teo, Kathryn L. Terry, Pamela J. Thompson, Lotte Thomsen, Shelley S. Tworoger, Anne M. van Altena, Ignace Vergote, Liv Cecilie Vestrheim Thomsen, Robert A. Vierkant, Christine S. Walsh, Shan Wang-Gohrke, Nicolas Wentzensen, Alice S. Whittemore, Kristine G. Wicklund, Lynne R. Wilkens, Yin-Ling Woo, Anna H. Wu, Xifeng Wu, Yong-Bing Xiang, Hannah Yang, Wei Zheng, Argyrios Ziogas, Alice W Lee, Celeste L. Pearce, Andrew Berchuck, Joellen M. Schildkraut.

**Formal analysis:** Joe Dennis.

**Resources:** Thomas A. Sellers, Simon A. Gayther, Linda E. Kelemen, Georgia Chenevix-Trench, Harvey A. Risch, Paul D. P. Pharoah, Ellen L. Goode, Catherine M. Phelan.

**Writing – original draft:** Madalene Earp, Jonathan P. Tyrer.

**Writing – review & editing:** Stacey J. Winham, Hui-Yi Lin, Ganna Chornokur, Katja K. H. Aben, Hoda Anton-Culver, Natalia Antonenkova, Elisa V. Bandera, Yukie T. Bean, Matthias W. Beckmann, Line Bjoerge, Natalia Bogdanova, Louise A. Brinton, Angela Brooks-Wilson, Fiona Bruinsma, Clareann H. Bunker, Ralf Butzow, Ian G. Campbell, Karen Carty, Jenny Chang-Claude, Linda S. Cook, Daniel W Cramer, Julie M. Cunningham, Cezary Cybulski, Agnieszka Dansonka-Mieszkowska, Evelyn Despierre, Jennifer A. Doherty, Thilo Dörk, Andreas du Bois, Matthias Dürst, Douglas F. Easton, Diana M. Eccles, Robert P. Edwards, Arif B. Ekici, Peter A. Fasching, Brooke L. Fridley, Aleksandra Gentry-Maharaj, Graham G. Giles, Rosalind Glasspool, Marc T. Goodman, Jacek Gronwald, Philipp Harter, Alexander Hein, Florian Heitz, Michelle A. T. Hildebrandt, Peter Hilleman, Klaus K. Hogdall, Estrid Hogdall, Satoyo Hosono, Edwin S. Iversen, Anna Jakubowska, Allan Jensen, Bu-Tian Ji, Audrey Y. Jung, Beth Y. Karlan, Melissa Kellar, Lambertus A. Kiemeney, Boon Kiong Lim, Susanne K. Krakstad, Jolanta Kupryjanczyk, Dietmar Lambrechts, Sandrina Lambrecht, Nhu D. Le, Shashi Lele, Jenny Lester, Douglas A. Levine, Zheng Li, Dong Liang, Jolanta Lissowska, Karen Lu, Jan Lubinski, Lene Lundvall, Leon F. A. G. Massuger, Keitaro Matsuo, Valerie McGuire, John R. McLaughlin, Iain McNeish, Usha Menon, Roger L. Milne, Francesmary Modugno, Kirsten B. Moysich, Roberta B. Ness, Heli Nevanlinna, Kunle Odunsi, Sara H. Olson, Irene Orlow, Sandra Orsulic, James Paul, Tanja Pejovic, Liisa M. Pelttari, Jenny B. Permuth, Malcolm C. Pike, Elizabeth M. Poole, Barry Rosen, Mary Anne Rossing, Joseph H. Rothstein, Ingo B. Runnebaum, Iwona K. Rzepecka, Eva Schernhammer, Ira Schwaab, Xiao-Ou Shu, Yurii B. Shvetsov, Nadeem Siddiqui, Weiva Sieh, Honglin Song, Melissa C. Southey, Beata Spiewankiewicz, Lara Sucheston-Campbell, Ingvild L. Tangen, Soo-Hwang Teo, Kathryn L. Terry, Pamela J. Thompson, Lotte Thomsen, Shelley S. Tworoger, Anne M. van Altena, Ignace Vergote, Liv Cecilie Vestrheim, Thomsen, Robert A. Vierkant, Christine S. Walsh, Shan Wang-Gohrke, Nicolas Wentzensen, Alice S. Whittemore, Kristine G. Wicklund, Lynne R. Wilkens, Yin-Ling Woo, Anna H. Wu, Xifeng Wu, Yong-Bing Xiang, Hannah Yang, Wei Zheng, Argyrios Ziogas, Alice W Lee, Celeste L. Pearce, Andrew Berchuck, Joellen M. Schildkraut, Susan J. Ramus, Alvaro N. A. Monteiro, Steven A. Narod, Thomas A. Sellers, Simon A. Gayther, Linda E. Kelemen, Georgia Chenevix-Trench, Harvey A. Risch, Paul D. P. Pharoah, Ellen L. Goode, Catherine M. Phelan.

**References**

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017; 67(1):7–30. https://doi.org/10.3322/caac.21387 PMID: 28055103
2. Soslow RA. Histologic subtypes of ovarian carcinoma: an overview. Int J Gynecol Pathol. 2008; 27(2):161–74. https://doi.org/10.1097/PGP.0b013e31815ea812 PMID: 18317227

3. Pharoah PD, Ponder BA. The genetics of ovarian cancer. Best Pract Res Clin Obstet Gynaecol. 2002; 16(4):449–68. PMID: 12413928

4. Kar SP, Berchuck A, Gayther SA, Goode EL, Moyaïkb, 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].

5. 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. https://doi.org/10.4161/21541248.2014.973749 PMID: 26103062

6. Just WW, Peranen J. Small GTPases in peryoxisome dynamics. Biochim Biophys Acta. 2016; 1863(5):1006–13. https://doi.org/10.1016/j.bbamcr.2016.01.004 PMID: 26775587

7. Li G, Marlin MC. Rab family of GTPases. Methods Mol Biol. 2015; 1298:1–15. https://doi.org/10.1007/978-1-4939-2569-8_1 PMID: 25800828

8. Shirakawa R, Horiiuchi H. Ral GTPases: crucial mediators of exocytosis and tumourigenesis. J Biochem. 2015; 157(5):181–99. https://doi.org/10.1093/jb/mvv029 PMID: 25796063

9. Nussinov R, Tsai CJ, Chakrabarti M, Jang H. A new view of Ras Isoforms in cancers. Cancer Res. 2016; 76(1):18–23. https://doi.org/10.1158/0008-5472.CAN-15-1536 PMID: 26659836

10. 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. Nat Genet. 2009; 41(9):996–1000. https://doi.org/10.1038/ng.424 PMID: 19648919

11. 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. https://doi.org/10.1038/ng.2564 PMID: 23535730

12. 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. https://doi.org/10.1371/journal.pmed.1000222 PMID: 19192942

13. Khan A, Zhang X. dbSUPER: a database of super-enhancers in mouse and human genome. Nucleic Acids Res. 2016; 44(D1):D164–71. https://doi.org/10.1093/nar/gkv1002 PMID: 26485398

14. 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. https://doi.org/10.1093/nar/gkv1340 PMID: 26657631

15. 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. https://doi.org/10.1016/j.molcel.2015.02.014 PMID: 25801169

16. 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.

17. 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. https://doi.org/10.1038/bjc.2016.124 PMID: 27253175

18. Hoon JL, Tan MH, Koh CG. The regulation of cellular responses to mechanical cues by Rho GTPases. Cells. 2016; 5(2).

19. Fritz G, Henninger C. Rho GTPases: new players in the regulation of the DNA damage response? Biochemicals. 2015; 5(4):2417–34. https://doi.org/10.3390/biom5042417 PMID: 26437439

20. McConkey MK, Ding J, Senz J, Yang W, Melnyk N, Tone AA, et al. Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles. Mod Pathol. 2014; 27(1):128–34. https://doi.org/10.1038/modpathol.2013.107 PMID: 23765252

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. https://doi.org/10.1016/j.bbrc.2005.08.025 PMID: 16112081

22. Cornuzze 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. https://doi.org/10.1371/journal.pone.0051954 PMID: 23251661

23. Stacey SN, Gudbjartsson DF, Sulem P, Berghusson 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. https://doi.org/10.1038/ng.234 PMID: 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. https://doi.org/10.1038/mp.2014.188 PMID: 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. https://doi.org/10.1038/mp.2010.107 PMID: 21079607