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STAT3 polymorphisms may predict an unfavorable response to first-line platinum-based therapy for women with advanced serous epithelial ovarian cancer

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Abstract

Cancer stem cells (CSC) contribute to epithelial ovarian cancer (EOC) progression and therapeutic response. We hypothesized that germline single nucleotide polymorphisms (SNPs) in CSC-related genes may predict an initial therapeutic response for women newly diagnosed with EOC. A nested case–control design was used to study 361 women with advanced-stage serous EOC treated with surgery followed by first-line platinum-based combination therapy at Moffitt Cancer Center or as part of The Cancer Genome Atlas Study. “Cases” included 102 incomplete responders (IRs) and “controls” included 259 complete clinical responders (CRs) to therapy. Using Illumina genotyping arrays and imputation, DNA samples were evaluated for 5,509 SNPs in 24 ovarian CSC-related genes. We also evaluated the overall significance of each CSC gene using the admixture maximum likelihood (AML) test, and correlated genotype with EOC tumor tissue expression. The strongest SNP-level associations with an IR to therapy were identified for correlated ($r^2 > 0.80$) SNPs within signal transducer and activator of transcription 3 (*STAT3*) [odds ratio (OR), 2.24; 95% confidence interval (CI), 1.32–3.78; $p = 0.0027$], after adjustment for age, population stratification, grade and residual disease. At the gene level, STAT3 was significantly associated with an IR to therapy (p_{AML} 5 0.006). rs1053004, a *STAT3* SNP in a putative miRNA-binding site, was associated with *STAT3* expression ($p = 0.057$). This is the first study to identify germline *STAT3* variants as independent predictors of an unfavorable therapeutic response for EOC patients. Findings suggest

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Additional Supporting Information may be found in the online version of this article.

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that *STAT3* genotype may identify high-risk women likely to respond more favorably to novel therapeutic combinations that include *STAT3* inhibitors.

Keywords

polymorphisms; cancer stem cells; ovarian cancer; STAT3

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer death among women in the United States.[1] Nearly 80% of cases present with advanced-stage (III/IV) high-grade serous disease when the 5-year survival rate is 30%.[1] Standard treatment for the initial management of advanced EOC is cytoreductive surgery followed by first-line platinum-based combination chemotherapy (cisplatin or carboplatin) +/− taxane (paclitaxel).[2] Approximately 70% of patients have a complete clinical response (CR) to this therapy as defined by clinical examination, radiologic imaging or serum CA-125 measurement[3] and are initially "chemosensitive" (but eventually recur), whereas 30% have an initial incomplete response (IR) characterized by persistent or progressive "chemoresistant" disease and endure toxicities with little clinical benefit.[2,4] Although chemoresistance in EOC is influenced by age, tumor stage, grade, histology and extent of surgical debulking,[2,4,5] the field of pharmacogenomics suggests that certain drugs will be most effective and safe for a particular individual based on their unique genomic sequence or expression profile.[6] Germline variants affecting the pharmacokinetics and pharmodynamics of platinum therapy have been shown to predict chemoresponse (and associated toxicities) in EOC,[6,7] and one intriguing yet underexplored area involves the evaluation of single nucleotide polymorphisms (SNPs) in cancer stem cell (CSC)-related genes as predictors of chemoresponse in EOC.

CSCs are a rare population of aggressive tumor cells characterized by their unique capacity to self-renew and give rise to cells that have high proliferative and invasive potential.[8] Evidence demonstrates that CSCs may be a root cause of EOC progression and act as promising targets for new combinations of therapeutic drugs.[8,9] The ovarian CSC phenotype has been characterized at the molecular level by cell surface markers and transcription factors shown to be upregulated in clinical samples and cell lines.[10] Moreover, high expression levels of several of these markers have been associated with recurrent disease, chemoresistance and poor survival.[11–15]

Germline variants in the CSC genes *CD44*, *CD133* and *STAT3* have been linked to clinical outcomes in patients with localized gastric adenocarcinoma[16] and colon cancer,[17] metastatic colorectal cancer[18] and metastatic renal cell carcinoma[19,20] and acute myeloid leukemia,[21] respectively. Variants in CSC genes may also alter gene expression and/or activity in EOC tissues and ultimately contribute to clinical outcomes, yet no published investigations of this topic area exist. The primary goal of this nested case–control study was to comprehensively evaluate associations between germline variants in ovarian CSC-related genes and response to first-line platinum-based therapy among women with advanced-stage serous EOC. We also aimed to evaluate the overall significance of each CSC gene by combining SNP-level

evidence, and then correlated genotype with tumor tissue expression of the most promising CSC gene(s).

Methods

Study population, data and biospecimens

This study included data and specimens from women newly diagnosed with pathologically confirmed invasive advanced-stage (III/IV) serous EOC. All women were treated with cytoreductive surgery and subsequent first-line platinum-based combination chemotherapy and were followed to assess their response to therapy; patients who had neoadjuvant therapy were excluded. For the present analysis, we focused on self-reported white women since the burden of EOC is highest in this racial group.[22] We included two cohorts: 45 women diagnosed and treated at our institution (Moffitt Cancer Center) between 2001 and 2012, and 316 women diagnosed and treated between 1993 and 2009 who had samples analyzed as part of The Cancer Genome Atlas (TCGA) project.[23] Eligible subjects contributed germline DNA for prior genotyping[23,24] or had genomic DNA available for genotyping through this study, and most had matched primary EOC tumor tissue that had undergone gene expression profiling.

Individuals at Moffitt had provided written consent for data and biospecimens to be donated for research through several protocols approved by the Institutional Review Board (IRB) of the University of South Florida, including Total Cancer Care (http://moffitt.org/patient-services/total-cancercare/research).[25] Demographic, clinical and pathologic data (such as age at pathological diagnosis, degree of debulking/cytoreduction, histology, stage, grade, chemotherapy regimens and response and follow-up data regarding recurrence and survival) from Moffitt cases were obtained from the Moffitt Cancer Registry and other source systems, and were supplemented with data abstracted from the medical record and clinical databases maintained by the Women's Oncology program. Pertinent data elements and biospecimens were collected by the TCGA project as described elsewhere.[23] The publicly available TCGA ovarian cancer gene expression dataset was accessed at http://cancergenome.nih.gov, and prior approval was granted to publish on individual-level TCGA genotype data.

Measurement of the outcome

Treatment response was retrospectively evaluated for each cohort member using standard criteria for patients with measurable disease WHO guidelines.[26] CA-125 level was used to classify responses in the absence of a measurable lesion based on established guidelines.[27] CRs had a complete disappearance of all measurable disease for 4 weeks or, in the absence of measurable lesions, a normalization of CA-125 level for at least 4 weeks and were therefore "chemosensitive." Patients who demonstrated a partial response (defined as a 50% or greater reduction in tumor burden obtained from measurement of each bidimensional lesion for at least 4 weeks, or a drop in CA-125 by >50% for at least 4 weeks), no response (had stable disease) or progressed during adjuvant therapy were classified as IRs, and were "chemoresistant."

Genotyping, quality control, imputation and data harmonization

Whole blood served as the primary source of germline DNA and was collected in the course of clinical care. DNA was isolated using Autopure reagents (Qiagen Sciences Inc, Germantown, MD) and stored at 4°C. For the minority of cases, the source of DNA was adjacent normal tissue, and the DNeasy mini kit was used for isolation (Qiagen Sciences Inc, Germantown, MD). Genotypes were generated for each cohort using ~250 ng DNA and different versions of Illumina Infinium Arrays; Moffitt samples were genotyped on the 610-quad Array (at Mayo Clinic, Rochester, MN) or the Human OmniExpress 12v1-1B Array (at Moffitt, Tampa, FL), and TCGA samples were genotyped using the Human 1M-Duo Array.

Sample and SNP quality control (QC) procedures have been described previously.[23,24] Briefly, samples were excluded if they had a call rate <95%, >1% discordance, <80% European ancestry or ambiguous gender. SNPs were excluded if they had call rates <95% or they were monomorphic. To integrate different genotyping platforms and improve genomic coverage and power,[28] we imputed genotypes for all subjects based on data from the 1000 Genomes Project (1KGP) using IMPUTE2 version 2 after prephasing with SHAPEIT.[29] We used all 14 populations in the 1KGP as the reference. Before imputation, we excluded poor performing SNPs according to the genotyping success rates, deviation from Hardy–Weinberg equilibrium (HWE) ($p < 1 \times 10^{-7}$), replicate errors and minor allele frequency (MAF). To ensure the quality of the imputed genotypes, maximum likelihood genotype imputation was carried out and an estimate of the squared correlation between the imputed and true genotypes was calculated. Imputed SNPs with an $r^2 < 0.3$ were excluded.

Gene and SNP selection

Twenty-four genes were chosen for this study based on published literature, which directly showed or suggested a role for the respective gene in the biology or regulation of ovarian CSCs. The genes included: *ABGC2, ALDH1A1, BMI1, CD44, COMT, CXCR4, ENG, EPCAM, ESRRB, HES1, KIT/CD117, KLF4, LIN28A, LIN28B, MYC, NANOG, NES, NOTCH1, OCT4/POU5F1, PROM1/CD133, RONIN/THAP11, SOX2, STAT3* and *THY1/CD90*. A total of 5,509 SNPs from the 24 genes of interest were extracted from the imputed dataset and were available for statistical analysis (Supporting Information Table S1).

Microarray gene expression profiling analysis of EOC tissue

Before the current investigation, under an IRB-approved protocol[25] matched frozen tumor tissue from 23 ovarian serous adenocarcinoma patients treated at Moffitt was previously arrayed on Affymetrix HuRSTA-2a520709 GeneChips (Santa Clara, CA) which contained ~60,000 probe sets representing ~25,037 unique genes (Affymetrix HuRSTA-2a520709, GEO: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc5GPL10379). Matched gene expression profiling data (level 3) for 316 high-grade serous EOC samples were also obtained from the TCGA Data Portal. The profiling of the TCGA samples was performed on the Human Agilent 244K Gene Chip (Agilent, Santa Clara, CA).

Statistical analysis

Descriptive statistics were generated using means and standard deviations for continuous variables and frequencies and percents for categorical variables. To minimize the potential

for population stratification, European ancestry was confirmed using principal component analysis[30] with HapMap CEU populations. We then performed SNP-level association tests for the 5,509 identified SNPs. For each variant, unconditional logistic regression under a dominant model (carriers *versus* noncarriers) was used to evaluate individual SNP associations with response status (IR *vs.* CR). Models were adjusted for five principal components (PCs) representing ancestry and age at pathological diagnosis. Models were also adjusted for five PCs, age at pathological diagnosis, high-grade disease (yes *versus* no) and residual disease at debulking (suboptimal *versus* optimal). At the SNP level, we focused on results significant at $p < 0.005$ after adjustment for prognostic variables.

For genes having multiple SNPs associated with response status, we calculated pairwise linkage disequilibrium (LD) and reported SNPs with $r^2 < 0.80$. Gene-level tests were also conducted to combine association evidence from SNPs within each evaluated gene. We used the admixture maximum likelihood (AML) test,[31] an approach that simultaneously tests the global null hypothesis (of no SNP–outcome associations) and estimates the proportion of underlying false hypotheses. The AML uses univariate SNP-level results to calculate the AML Cochran–Armitage trend test. Compared to other methods, AML has been shown to have similar or higher statistical power to detect associations except under the unlikely scenario that >20% of all variants are associated with the outcome.[31] For significant genes ($p < 0.05$) that contained regions of high LD ($r^2 > 0.80$), we tested for associations between haplotypes and response status using the Haplo.stats program and R software according to the methods proposed by Schaid *et al.*[32] Rarer haplotypes (frequencies < 10%) were combined into a single category to minimize sparse cell counts. Haplotype effects were considered significant using a threshold of $p < 0.01$. This exploratory gene-level and haplotype-based approach was undertaken to complement SNP-level findings, and aimed to reduce the degrees of freedom, avoid model fitting issues due to multicollinearity from LD and to improve statistical power. As such, SNP-level results were not adjusted for multiple testing.

We performed a *cis*-expression quantitative trait locus (eQTL) analysis to examine the association between genotype and corresponding gene expression in matched EOC tissue samples. The expression levels between carriers *versus* noncarriers of the response-associated genotype were compared using the Wilcoxon rank sum test. The *in silico* tools ANNOVAR[33] (http://www.openbioinformatics.org) and SNPInfo[34] (http://www.niehs.nih.gov/snpinfo) were used to evaluate the putative function of candidate SNPs.

Results

Subject characteristics

Select clinical and pathologic characteristics of the 361 study participants (102 IRs and 259 CRs) are summarized in Table 1. The mean age at diagnosis (59 years) was similar in the IRs and CRs, all participants had stage III or IV serous ovarian carcinomas and the stage distribution was similar in the IR and CR groups. Most (88%) women had high-grade, aggressive disease. Suboptimal surgical debulking was more common among the IRs compared to the CRs ($p < 0.0001$). Most women received a combination of paclitaxel and

carboplatin for first-line therapy. The mean survival time was 25 months among IRs and 44 months among CRs.

Associations between CSC germline variation and treatment response

Table 2 includes noncorrelated SNPs ($r^2 < 0.80$) from eight genes (*ALDH1A1*, *COMT*, *ENG*, *ESRRB, LIN28, NOTCH1, PROM1* and *STAT3*) that were associated with treatment response in EOC. After adjustment for age at pathological diagnosis and population substructure, SNPs in *STAT3* such as rs62075772 [OR (95% CI): 2.42 (1.47–3.98), $p = 0.0005$] demonstrated the most significant associations with an incomplete/unfavorable response to platinum-based therapy followed by SNPs in *ALDH1A1* such as rs1364455 [OR (95% CI): 2.14 (1.29–3.55), $p = 0.0031$]. Also shown in Table 2, *ESRRB* and *LIN28* were the only two candidate genes that contained SNPs that were significantly associated with a complete/favorable response to first-line therapy. After adjustment for additional prognostic factors (including high-grade disease and residual disease at debulking), magnitudes of association were slightly attenuated for SNPs in *STAT3* (ORs ranging from 2.18 to 2.24), and results remained statistically significant ($p < 0.005$). For top-ranked *STAT3* SNP, rs62075772, stratified analyses revealed that minor allele G carriers with high-grade disease were less likely to be CRs to therapy when compared to noncarriers with high-grade disease ($p = 0.08$) (Supporting Information Fig. 1). Aside from rare SNP rs74561951 in *PROM1* (MAF < 1%), most response-associated SNPs were common (MAF > 0.05) (Table 2).

Gene-level analysis revealed that two of the 24 evaluated CSC-related genes, *STAT3* and *NOTCH1*, were globally associated with an IR to therapy ($p < 0.05$) (Table 3). Overall, *STAT3* was most significantly associated with an IR to therapy (AML $p_{trend} = 0.006$), consistent with SNP and haplotype-level analyses, followed by *NOTCH1* (AML $p_{trend} = 0.03$). Several regions of strong LD ($r^2 > 0.80$) were identified throughout *STAT3*, namely in blocks 3, 4 and 5 (Figs. 1a and 1b). Haplotype analysis revealed that block 4 in STAT3 (spanning ch17:40499804–40505202; Fig. 1c) that includes rs62075772 was most strongly associated with an incomplete response to therapy ($p = 0.0054$), complementing SNP-level analyses. Regions of strong LD were also identified in *NOTCH1*, but haplotype analysis did not yield statistically significant findings ($p > 0.01$). To be comprehensive, we examined haplotype associations for the other six genes (*ALDH1A1, COMT, ENG, ESRRB, LIN28* and *PROM1*) containing SNPs associated with response status and did not observe any statistically significant associations.

Correlations between germline SNPs in STAT3 and gene expression in tumor tissue

Analysis of *STAT3* gene expression in EOC tissue from 316 cases revealed slightly higher mean *STAT3* tissue expression in 93 IRs compared to 223 CRs, though this finding was not statistically significant ($p = 0.15$). To determine the potential functional consequences of germline variants in *STAT3*, we investigated whether *STAT3* expression in the 316 serous EOC tumors varied by allele. Most identified *STAT3* variants were intronic, and no differences were observed in tumor *STAT3* expression by *STAT3* genotype. However, for rs1053004 (G>A), a variant that lies in a predicted miRNA-binding site for miR-423-5p within the 3′ untranslated region (UTR) of *STAT3*, slightly lower *STAT3* tumor expression was observed in a dose-response manner in those with AA *vs.* GA *vs.* GG genotype ($p =$

0.058) and in A allele carriers compared to noncarriers ($p = 0.043$) (Figs. 2a and 2b, respectively). SNP rs1053004 and top-ranked STAT3 SNP rs62075772 (Table 2) are in moderate LD within our cohort population ($r^2 = 0.67$) as well as within European populations of 1000 genome project (phase 3, CEU + TSI + FIN + GBR + IBS: $r^2 = 0.62$).

Using dbSNP and ANNOVAR,[33] we identified two coding *STAT3* missense variants (rs1064122 and rs11547455) within the same haplotype block as rs1053004 (spanning 17:40466092-40485239), which are predicted to be probably damaging. Neither of these SNPs was directly genotyped or imputed in this study, precluding accurate estimation of LD. Nonetheless, it is possible that these and other unidentified SNPs may have a functional role in regulating *STAT3* expression and contributing to treatment response in EOC.

Discussion

CSCs have been associated with EOC progression and chemoresponse.[8] Here, we conducted the first comprehensive association study of germline polymorphisms in CSC-related genes and response to first-line platinum-based combination therapy in women with advanced-stage ovarian cancer. At the SNP, haplotype and gene level, the most statistically significant associations with an increased risk for an incomplete response to therapy were observed for signal transducer and activator of transcription 3 (*STAT3*), a transcription factor that has been shown to act as an oncogene in several malignancies.[35,36]

STAT3 is activated in response to the binding of numerous cytokines, hormones and growth factors to their receptors and by activation of intracellular kinases.[36] Phosphorylation of *STAT3* (pSTAT3) causes it to dimerize and translocate from the cytoplasm to the nucleus where it promotes the transcription of genes that affect growth, differentiation and survival.[36] A study in China showed that STAT3 and pSTAT3 protein expression was significantly higher in EOC tissues than in normal ovarian epithelial tissues or benign ovarian tumor tissues,[37] supporting an oncogenic role. Moreover, women who had tumors with positive expression of pSTAT3 had poorer survival compared with women who had tumors with negative pSTAT3 expression ($p < 0.01$).[37] Although we did not evaluate pSTAT3 expression, we did observe increased expression of *STAT3* in IRs (shorter term survivors) compared to CRs (longer term survivors). Thus, *STAT3* signaling appears to contribute to invasion, prognosis and chemoresistance in EOC, and our findings suggest that germline *STAT3* genotype may help mediate these outcomes.

Indeed, *STAT3* genotype has previously been associated with response to standard chemotherapy in other malignancies. A G>C SNP in the 5′ region of *STAT3* (rs4796793) was the most significant predictor of a favorable response to interferon-a therapy in patients with metastatic renal cell carcinoma.[19,20] rs4796793 C allele carriers, which were observed more frequently among IFN-α responders, tended to have lower STAT3 expression in their matched tumor tissue. rs4796793 was not a top-ranked SNP in our dataset. In patients with acute myeloid leukemia,[21] rs9909659 was associated with increased resistance to daunorubicin chemotherapy and was marginally associated ($p = 0.049$) with an incomplete response to platinum-based therapy among women with EOC in our dataset.

Our observational findings combined with recent experimental investigations showing the involvement of *STAT3* signaling in the induction of chemoresistance in EOCs[38–40] suggest that germline *STAT3* polymorphisms may influence sensitivity of EOCs to platinum-based therapy. Thus, for women newly diagnosed with EOC, *STAT3* genotype status could possibly serve as a diagnostic marker of initial platinum resistance. Importantly, based on emerging data regarding the promise of JAK2-STAT3 inhibitors for therapeutic intervention in EOC, *STAT3* genotype may also act as a "trigger" for offering novel rational combination therapies involving such inhibitors. For example, inhibition of JAK2 with diindolymethane induced apoptosis in EOC cells,[41] and the JAK2 inhibitor AZD1480 increased sensitivity of EOC cell lines by inhibiting STAT3 activation.[42] Additionally, curcumin has been shown to suppress STAT3 activation and growth of EOC cells by upregulating the STAT3 inhibitor PIAS-3,[43] corosolic acid enhanced antitumor effects of chemotherapy on EOC cells by inhibiting *STAT3* signaling[38] and HO-3867, a safe *STAT3* inhibitor, has been shown to inhibit EOC migration, invasion and survival.[44] Furthermore, inhibition of the JAK2/STAT3 pathway using CYT387 in combination with paclitaxel suppressed the "stemness" profile in chemotherapy-treated residual cells *in vitro* and *in vivo*, leading to reduced tumor burden.[45,46] Simultaneous or sequential combinations of JAK2/STAT3 inhibitors with platinum compounds therefore hold promise and warrant evaluation as part of future experimental investigations and clinical trials in EOC.

Strengths of our study include the novelty of the topic area, the multitiered genomic evaluation and the larger sample size compared to other studies that reported associations between CSC-related SNPs and response to therapy.[16,17,19–21] Another strength is the relatively homogeneous population of women treated for their diagnosis of advanced serous EOC, though it has been recognized that there is vast diversity in the combinations of platinum- and taxane-based adjuvant treatments that these patients have endured.[47] We recognize the possibility of false-positive results and the importance of attempting to replicate these findings, and plan to do so once denser genotype and imputed data and detailed clinical follow-up data become available from a large, independent cohort of EOC cases genotyped by the international Ovarian Cancer Association Consortium (OCAC).[48] Mechanistic studies to assess how the most promising STAT3 polymorphisms affect oncogenic phenotypes will also be important. For example, interplay between miRNAs and the STAT3 signaling pathway has recently been reported.[49] Given our detection of an association between *STAT3* tissue expression and rs1053004, a SNP in a putative miRNA-binding site for miR-423-5p, a regulator of cell proliferation, invasion and progression,[50,51] investigations of *STAT3* genotype, miRNA expression and *STAT3* expression seem warranted and are the focus of future research efforts.

In summary, this study provides the first evidence that germline polymorphisms in CSC-related genes, and *STAT3* in particular, may influence response to first-line platinum-based combination therapy in EOC. This information may someday help to select a subgroup of women who may benefit from newly developed CSC-targeting drugs. Future biomarker-embedded translational trials are needed to extend upon these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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What's new?

This study identified genomic biomarkers that may predict a woman's response to ovarian cancer treatment. Self-renewing cancer stem cells are key to ovarian cancer's development and spread, and the authors tested more than 5,500 SNPs in 24 ovarian cancer stem cell-related genes and compared the relationship between genotype and the patient's response to first-line ovarian cancer treatment. They identified variants in the STAT3 gene as predicting an unfavorable response to treatment, suggesting that testing this gene could help identify patients who may respond more favorably to combination therapy, possibly including STAT3 inhibitors.

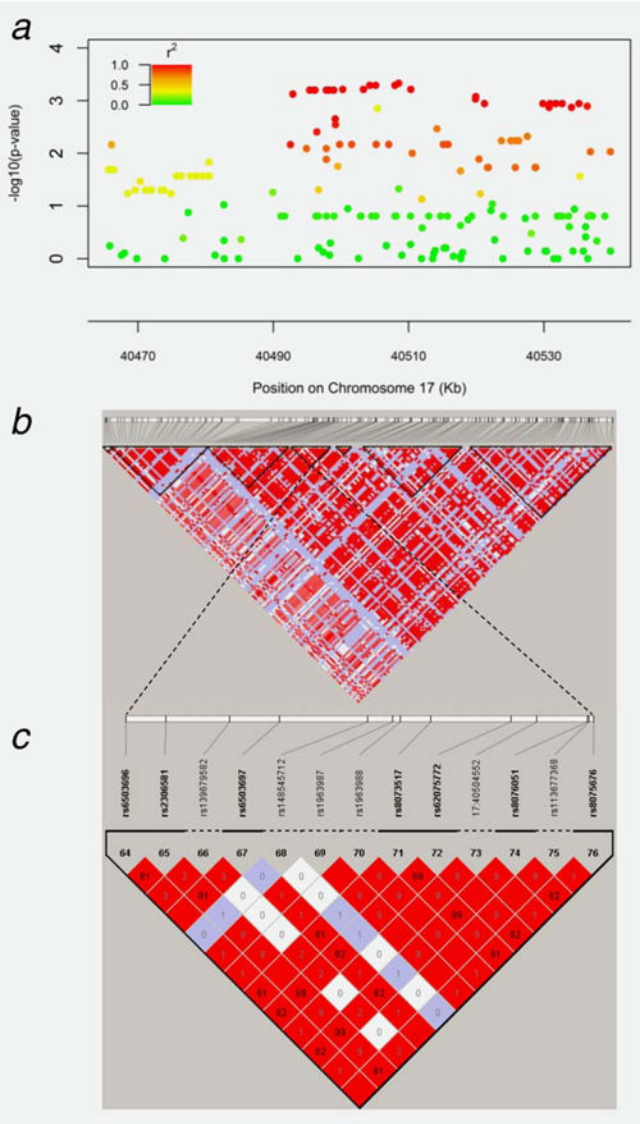

Figure 1.

Linkage disequilibrium pattern of the *STAT3* gene. (*a*) Regional association plot demonstrating that several SNPs within *STAT3* are significantly associated with response to platinum-based chemotherapy in EOC. (*b*) LD map of *STAT3* gene based on *D′* (coefficient of linkage disequilibrium). (*c*) Polymorphisms within haplotype block 4, including rs62075772, are strongly correlated, and are associated with an unfavorable response to platinum-based chemotherapy.

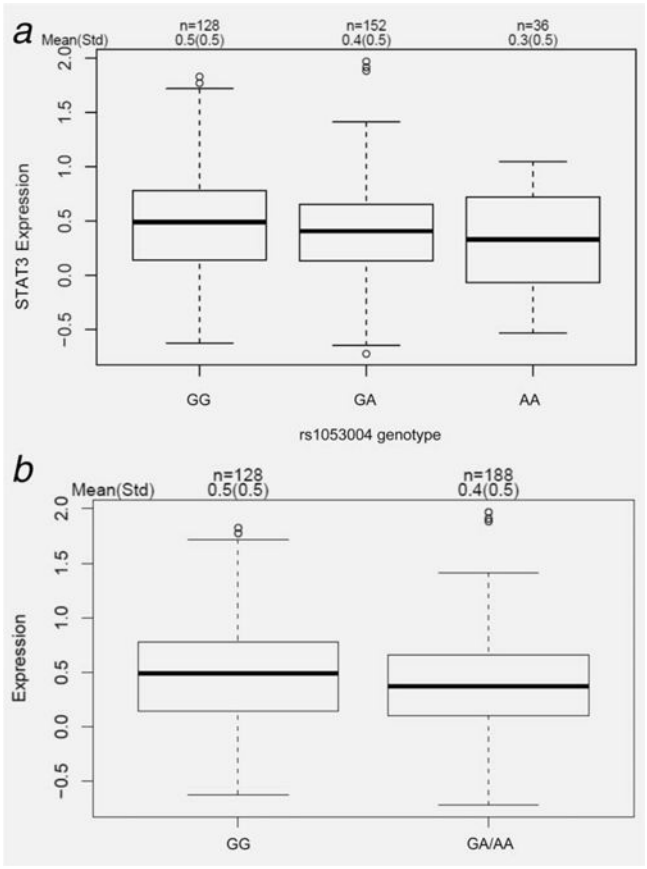

Figure 2.

rs1053004 (G>A) genotype is associated with *STAT3* expression in high-grade serous ovarian tumor tissues. (*a*) *STAT3* expression among GG *vs.*GA *vs.* AA genotype. (*b*) Comparison of *STAT3* expression in GG *vs.* GA/AA carriers.

Table 1

Selected clinical and pathologic characteristics of the study population ($N = 361$)

Characteristic	Incomplete responders (IRs) ($N = 102$)	Complete responders (CRs) ($N = 259$)	p
Mean age (years)	59.8	59.4	0.79[1]
FIGO stage (*N*, %)			0.65[2]
III	83 (81)	216 (83)	
IV	19 (19)	43 (17)	
Grade (*N*, %)			0.20[2]
Low (I/II)	15 (15)	26 (10)	
High (III/IV)	86 (85)	232 (90)	
Surgical debulking (*N*, %)			<0.0001[2]
Optimal (<1 cm)	58 (58)	182 (70)	
Suboptimal ≥cm)	40 (39)	42 (16)	

Data represent counts (percentages) unless otherwise indicated. Counts may not add up to the total due to missing values, and percentages may not equal 100 due to rounding.

[1] *t*-Test.

[2] Fisher's exact test.

Table 2

Cancer stem cell SNPs associated with response to first-line platinum-based therapy in advanced serous ovarian cancer ($p < 0.005$)

Chr	Gene	SNP	Location	Alleles[1]	MAF	OR (95% CI)[2]	p^2	OR (95% CI)[3]	p^3
9	ALDH1A1	rs1364455	Intergenic	C/A	0.13	2.28 (1.31–3.98)	0.0036	2.71 (1.44–5.06)	0.0018
9	ALDH1A1	rs4744676	Intergenic	G/A	0.15	2.11 (1.21–3.69)	0.0090	2.71 (1.44–5.11)	0.0020
9	ALDH1A1	rs10869207	Intergenic	G/C	0.14	2.11 (1.21–3.68)	0.0084	2.58 (1.38–4.81)	0.0029
9	ALDH1A1	rs10429484	Intergenic	A/T	0.15	2.14 (1.29–3.55)	0.0031	2.31 (1.32–4.03)	0.0032
9	ALDH1A1	rs7038229	Intergenic	C/T	0.13	2.13 (1.21–3.74)	0.0088	2.55 (1.35–4.81)	0.0038
9	ALDH1A1	rs11143430	Intergenic	T/C	0.08	2.16 (1.23–3.78)	0.0070	2.53 (1.35–4.75)	0.0038
9	ALDH1A1	rs7860295	Intergenic	T/C	0.15	2.07 (1.22–3.52)	0.0072	2.39 (1.32–4.32)	0.0039
22	COMT	rs4818	Exonic	C/G	0.32	1.91 (1.12–3.25)	0.0172	2.37 (1.32–4.24)	0.0036
22	COMT	rs4646312	Intronic	T/C	0.31	1.99 (1.16–3.41)	0.0126	2.34 (1.31–4.19)	0.0041
9	ENG	rs41432051	Intronic	C/T	0.15	2.03 (1.15–3.59)	0.0150	2.58 (1.39–4.77)	0.0026
9	ENG	rs2417056	Intronic	C/A	0.17	1.93 (1.07–3.50)	0.0303	2.56 (1.34–4.88)	0.0045
9	ENG	rs16930129	Exonic	T/A	0.13	1.94 (1.10–3.43)	0.0218	2.45 (1.31–4.55)	0.0048
9	ENG	rs41475247	Intronic	G/A	0.12	1.88 (1.07–3.30)	0.0285	2.29 (1.24–4.24)	0.0083
14	ESRRB	rs2885232	Intronic	C/T	0.41	0.50 (0.31–0.80)	0.0039	0.48 (0.29–0.79)	0.0038
14	ESRRB	rs10149196	Intergenic	G/C	0.40	2.09 (1.27–3.46)	0.0039	2.22 (1.29–3.83)	0.0041
14	ESRRB	rs10083530	Intergenic	G/A	0.27	0.54 (0.34–0.87)	0.0109	0.47 (0.28–0.79)	0.0044
14	ESRRB	rs13379450	Intergenic	G/A	0.40	2.06 (1.25–3.40)	0.0048	2.18 (1.27–3.78)	0.0049
1	LIN28	rs7535902	Intronic	T/A	0.13	0.51 (0.30–0.86)	0.0117	0.44 (0.25–0.77)	0.0040
9	NOTCH1	rs11361183	Intronic	C/T	0.09	2.03 (1.22–3.40)	0.0066	2.22 (1.28–3.85)	0.0044
4	PROM1	rs74561951	Intronic	G/T	0.0009	6.99 (1.71–28.5)	0.0068	23.5 (2.66–207)	0.0045
17	STAT3	rs62075772	Intronic	A/G	0.36	2.42 (1.47–3.98)	0.0005	2.24 (1.32–3.78)	0.0027
17	STAT3	rs3883338	Intronic	G/A	0.25	2.42 (1.48–3.98)	0.0005	2.21 (1.31–3.73)	0.0030
17	STAT3	rs8072391	Intronic	G/A	0.36	2.38 (1.45–3.93)	0.0006	2.20 (1.30–3.73)	0.0033
17	STAT3	rs957971	Intronic	G/C	0.46	2.33 (1.42–3.82)	0.0008	2.18 (1.29–3.67)	0.0035

Sorted alphabetically by gene; SNPs in linkage disequilibrium ($r^2 > 0.80$) with the index SNPs in the table are not featured; dbSNP 141.

[1] Major/minor allele.

[2] Adjusted for age at pathological diagnosis and population substructure principal components.

[3] Adjusted for age at pathological diagnosis, population substructure principal components, high-grade disease (yes *versus* no) and residual disease at debulking (suboptimal *versus* optimal).

Abbreviations: Chr: chromosome; MAF: minor allele frequency; OR: odds ratio; CI: confidence interval.

Table 3

Cancer stem cell genes and their association with an incomplete response to therapy among patients with advanced ovarian cancer

Gene	*N* SNPS	AML (trend)
STAT3	173	**0.006**
NOTCH1	179	**0.03**
ALDH1A1	643	0.094
ENG	130	0.184
LIN28A	60	0.186
PROM1	629	0.189
NES	29	0.24
HES1	3	0.287
COMT	206	0.321
NANOG	59	0.409
LIN28B	277	0.509
ABCG2	667	0.643
KLF4	4	0.683
MYC	20	0.734
BMI1	9	0.752
ESRRB	1072	0.776
KIT	292	0.78
POU5F1B	15	0.781
CXCR4	11	0.781
EPCAM	222	0.947
CD44	486	0.95
THY1	16	0.972
SOX2	1	NA
THAP11	1	NA

Genes with $P < 0.05$ appear in bold.