Prognostic Value and Implication for Chemotherapy Treatment of ABCB1 in Epithelial Ovarian Cancer: A Meta-Analysis

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Abstract

Background
Chemotherapy resistance is reported to correlate with up-regulation of anti-tumor agent transporter ABCB1 (p-gp) in epithelial ovarian cancer (EOC), but the results remain controversial. To reconcile the results, a systematic review followed by meta-analysis was performed to assess the association between high ABCB1 status or ABCB1 gene variants and overall survival (OS), progression free survival (PFS), and total response rate (TR) in patients with EOC.

Materials and Methods
Electronic searches were performed using Pubmed, EMBASE, Web of Science and Chinese Wanfang databases from January 1990 to February 2016. Summary hazard ratio (HR), risk ratio (RR) and 95% confidence intervals (CIs) were combined using fixed or random-effects models as appropriate.

Results
Thirty-eight retrospective studies of 8607 cases qualified for meta-analysis were identified. Our results suggested that ABCB1 over-expression was significantly associated with unfavorable OS (HR = 1.54; 95% CI, 1.25–1.90), PFS (HR = 1.49; 95% CI, 1.22–1.82) and TR (RR = 0.63; 95% CI, 0.54–0.75). After adjustment for age, clinical stage, residual disease, histological type and tumor grade, high ABCB1 status remained to be a significant risk factor for adverse OS and PFS. Patients with recurrent ABCB1 positivity suffered from poorer OS than those with primary ABCB1 positivity. However, stratified by chemotherapy regimen, inverse correlation between high ABCB1 status and poor OS, PFS and TR were only found in patients underwent platinum-based chemotherapy but not in patients received standard platinum/paclitaxel-based chemotherapy. No evidence was found for any association between ABCB1 gene polymorphisms and OS, PFS or TR.
Conclusion
High ABCB1 status is significantly associated with chemo-resistance and poor prognosis in patients with EOC. Large-scale, prospective studies are needed to assess the clinical value of ABCB1 expression in EOC more accurately.

Introduction
Ovarian cancer is the most lethal gynecological malignancy worldwide [1]. Epithelial ovarian cancer (EOC) accounts for over 90% of all ovarian cancers [2]. As its progression is usually asymptomatic and unpredictable, most (approximately 85%) patients were diagnosed at late stage, in whom cytoreductive surgery combined with platinum-based chemotherapy represents the current standard procedure [3]. Six cycles of taxane plus platinum chemotherapy represents the most widely used regimen for EOC; other platinum-based regimens included PAC (platinum + doxorubicin + cyclophosphamide) and PC (platinum + cyclophosphamide). Although this treatment procedure can achieve an overall response rate higher than 70% in patients with ovarian cancer, a great proportion (approximately 75%) of them suffer a recurrence within 2 to 3 years after initial treatment, which leads to poor outcomes [4]. The high recurrence rate is associated with the development of acquired chemo-resistance. Repeated chemotherapeutic stimulation induces biological changes in tumor cells allowing them to survive under chemotherapy. Identification of prognostic biomarkers predicting chemotherapy response was essentially necessary for individual therapy.

Insufficient accumulation of anti-cancer agents in tumor cells resulting from increased efflux and/or decreased influx is a critical mechanism of chemo-resistance [5,6]. ABCB1 (ATP binding cassette B1, also known as multidrug resistance protein 1, MDR1 or p-glycoprotein, p-gp) is a cellular surface protein, which transports a variety of chemotherapy drugs such as paclitaxel, docetaxel, doxorubicin and topotecan and plays a major role in cellular detoxification [7–10]. Functional experiments revealed that up-regulated ABCB1 expression impaired the sensitivity to cisplatin, paclitaxel, docetaxel and doxorubicin in ovarian cancer cell lines [11]. A substantial literature interrogated the relationship between ABCB1 status (expression or polymorphisms) and clinical outcomes such as overall survival (OS), progression free survival (PFS) and total response (including complete response and partial response according to Response Evaluation Criteria In Solid Tumors) to chemotherapy (TR) in EOC patients [12–55]. Although a great number of studies evaluated the effect of ABCB1 on EOC patients, the association between ABCB1 and EOC prognosis was still under debate. While certain amount of studies reported that high ABCB1 could predict unfavorable prognosis [23,28,30,31,34,35], quite a few did not find any significant association between ABCB1 and survival in patients with EOC [18,27,32]. The possible reasons for these inconsistent results might include (i) heterogeneities of the basic characteristics of the studies such as sample sizes, EOC subtypes, clinical stage, and populations, (ii) different intervention such as residual disease after surgery, time point of ABCB1 measurement and (iii) different methodology such as detection methods and procedures. Therefore, we performed a systematic and comprehensive meta-analysis to assess more precisely the correlation between ABCB1 status and EOC prognosis based on individual studies.

Methods
This meta-analysis was performed according to the PRISMA-statement for systematic review and meta-analysis of genetic association studies (S1 and S2 Checklists).
Search strategy

Thorough electronic search of Pubmed, Embase, Web of Science and Chinese Wanfang databases from January 1990 to Feb 2016 was conducted with the following key words: ‘ovarian cancer’, ‘ovarian carcinoma’ or ‘ovarian neoplasm’ and ‘ABCB1’ or ‘p-gp’. The search strategy was complemented by reviewing the similar articles and by examining the references of the retrieved literature. Additionally we examined the authors of each study to avoid overlap of cases. Only the most recent study was included when overlap occurred between studies.

Publication Selection and Quality Assessment

Two authors reviewed the studies independently to decide which were eligible for inclusion. The included studies had to meet the following criteria: 1) focused on epithelial ovarian carcinoma and evaluated correlation between ABCB1 status and prognosis of the patients; 2) published as full text in English or in Chinese; 3) analyzed ABCB1 status with practical and comparable method; 4) abided by the Hardy Weinberg Equilibrium (HWE) when performing gene associated analysis. The primary outcome was OS and the secondary outcome included PFS and TR. The methodology of each study was assessed independently by two authors according to the Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analysis. Each study was scored according to three aspects: selection, comparability and exposure. The maximum score of each aspect was 4, 2 and 3 respectively, adding up to a total score of 9. Higher score stood for better quality (S1 File).

Data Extraction and Statistical Methods

The data extracted from the included studies were authors, year of publication, countries, sizes of population, histology, clinical stage, follow-up time, treatment, tissue type, point of time when transporters are measured, assay method and corresponding scoring system, the results of survival analysis, the number of cases and controls, and response to chemotherapy. Two authors reviewed the literatures and extracted the data independently. If disagreement occurred, a third author was conferred with thus the issue be discussed altogether.

For time to event survival analysis, we assessed the effect of ABCB1 status on prognosis by hazard ratio (HR). For each study, the HR and its 95% confidence interval (CI) were retrieved. If these parameters were not available in studies, we used the software GetDATA Graph Digitizer 2.25 to extract the specific survival rates according to the Kaplan-Meier curves to calculate the HR by the methods described by Tierney et al [56]. For dichotomous outcomes such as TR, we measured the impact of ABCB1 status on the prognosis by risk ratio (RR). For each study, we extracted the number of patients who experienced the events of interest and the number of patients who were free from the events at the end point to calculate the RR and its 95% CI.

Meta-analysis was performed for OS, PFS and treatment response. For each endpoint, the analyses were stratified by ABCB1 detection methods, histology, clinical stage, geographical region of studies and chemotherapy regimens. Generally the individual HR and RR were pooled according to the method reported by DerSimonian, R et al. and Mantel, N et al. Fixed inverse variance model and random model were used to calculate pooled HRs and RRs of studies with class I-II heterogeneity (I² <50%) and those with class III-IV heterogeneity (I² ≥50%), respectively [57,58]. The heterogeneity between studies was assessed by I² and p-value according to the method described by Higgins et al [59]. The effect of ABCB1 on prognosis was considered significant only when the 95% CI for HR and RR did not overlap with 1. Visual inspection of symmetry of funnel plots and Egger's test were used to assess publication bias.
[60]. A two-sided $P$ value < 0.05 was considered of statistical significance. All analyses were carried out using STATA14 (MP-Parallel Edition, College Station, Texas 77845 USA).

**Results**

**Study characteristics**

The procedure of study selection was presented in a flowchart (Fig. 1). Initially, 653 articles related to the keywords were identified after removal of duplicates. Among these articles, 595 were excluded by review of title and abstract as they did not report on ABCB1, published as reviews or focused on ABCB1 cytological mechanisms and function. Full texts were retrieved of the remaining 58 studies and 20 of them were excluded after review of full text (S1 Table). Finally, 38 eligible studies involving 8607 cases were identified, including 23 studies for ABCB1 protein (p-gp) expression (Table 1), 9 for ABCB1 mRNA expression, and 7 for ABCB1 SNPs (Table 2).

The number of studies addressing OS, PFS, and TR was 19, 19, and 21 respectively. In addition to the basic characteristics, 6 and 4 studies assessed the impact of ABCB1 on OS [20,23,29,37,45,48] and PFS [20,29,37,45], respectively, adjusted for age, clinical stage, residual disease, histological type and tumor grade by multivariate analysis (Tables 1 and 2). For p-gp intensity, IHC results were appraised by measuring the intensity of reaction and proportion of positive cells. For measurement of ABCB1 gene expression by PCR, most of the studies (8/9) used the median as the cut-point according to the distribution of each cohort. The distributions of ABCB1 genotype frequencies in all 7 studies did not depart from Hardy-Weinberg equilibrium. Most of the studies revealed no correlation between common ABCB1 variants and poor prognosis (6/7).

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**Fig 1. Flow chart of literature search and selection.**

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Table 1. Characteristics of studies that identify ABCB1 protein (p-gp) expression in epithelial ovarian cancer.

| Detection method | First Author | Year | Country | No. of Cases | Stage | Histology | Anti-body | Score | Cut-off point | Tissue Type | Time point | Chemotherapy | End-point |
|------------------|--------------|------|---------|--------------|-------|-----------|-----------|-------|---------------|-------------|------------|--------------|-----------|
| IHC              | Matsuo       | 2014 | USA     | 112          | I-IV  | s         | JSB1      | standard | 5%            | FFPE        | 1          | 2           | OS, PFS    |
|                  | Lu D         | 2011 | China   | 80           | I-IV  | s,m,e     | ZSGB      | standard | 10%           | FFPE        | 1          | 1,2         | OS         |
|                  | O'Tool       | 2007 | Ireland | 77           | I-IV  | s,m,c     | JSB1      | standard | 10%           | FFPE        | 1          | 1,2         | OS, PFS    |
|                  | Oda          | 2007 | Japan   | 53           | I-IV  | s,e       | JSB1      | standard | 10%           | FFPE        | 1          | 2           | OS, PFS    |
|                  | Wei          | 2007 | China   | 42           | III   | all       | C219      | standard | 20%           | FFPE        | 1          | 2           | TR         |
|                  | Yakirevich   | 2006 | USA     | 60           | I-IV  | s         | JSB1      | standard | 10%           | FFPE        | 1          | 2           | OS, TR     |
|                  | Surowiak     | 2006 | Poland  | 43           | III-IV| all       | C219      | standard | 20%           | FFPE        | 1,2        | 1           | OS, PFS, TR |
|                  | Obata        | 2006 | Japan   | 60           | III-IV| s,e       | C-19      | standard | 5%            | FFPE        | 1          | 1,2         | TR         |
|                  | Goto         | 2006 | Japan   | 21           | IIIC-IV| s        | JSB1      | standard | 20%           | FFPE        | 1          | 2           | TR         |
|                  | Raspollini   | 2005 | Italy   | 52           | III   | s         | MDR8      | standard | 30%           | FFPE        | 1          | 1           | TR         |
|                  | Penson       | 2004 | USA     | 32           | II-IV | s,e,c     | C219,c494 | standard | 20%           | FFPE        | 1,2        | 2           | OS, TR     |
|                  | Ikeda        | 2003 | Japan   | 93           | II-IV | all       | C-19      | standard | 10%           | FFPE        | 1          | 1,2         | OS         |
|                  | Raspollini   | 2003 | Italy   | 83           | III   | s         | MDR8      | standard | 30%           | FFPE        | 1          | 1           | OS, PFS, TR |
|                  | Raspollini   | 2002 | Italy   | 22           | III   | s,e       | MDR8      | standard | 30%           | FFPE        | 1          | 1           | PFS, TR    |
|                  | Itamochi     | 2002 | Japan   | 131          | III-IV| s,c       | C494,C-20 | standard | 10%           | FFPE        | 1          | 1           | TR         |
|                  | Zhang        | 2001 | China   | 27           | I-IV  | all       | C219      | standard | 10%           | FFPE        | 1          | 2           | TR         |
|                  | Baekelandt   | 2000 | Norway  | 73           | III   | all       | JSB1      | standard | 10%           | FFPE        | 1          | 1           | OS, PFS, TR |
|                  | Yokoyama     | 1999 | Japan   | 22           | I-IV  | all       | LSAB      | standard | 30%           | FFPE        | 1          | 1           | PFS, TR    |
|                  | Arts         | 1999 | Netherlands | 112      | I-IV  | all       | JSB1      | standard | 10%           | FFPE        | 1          | 1,2         | PFS, TR    |
|                  | Khalifa      | 1997 | Canada  | 75           | I-IV  | all       | JSB1      | standard | 10%           | FFPE        | 1          | 2           | OS         |
|                  | Van          | 1995 | Netherlands | 89        | I-IV  | all       | JSB1      | standard | 5%            | FFPE        | 1          | 1           | PFS, TR    |
|                  | Izquierdo    | 1995 | Netherlands | 57        | III-IV| s,m,e     | JSB1      | standard | 10%           | FFPE        | 1          | 1           | TR         |
|                  | WB Joncourt  | 1998 | Switzerland | 39        | I-IV  | all       | Customized* | -  | positive Frozen | 1          | 1          | TR         |

IHC: Immunohistochemistry; WB: Western blot
First Author: bolded when the result of ABCB1 association was significant
Histology: all = serous, mucinous, endometrial and clear cell carcinoma with or without mixed histology, s = serous ovarian cancer, m = mucinous ovarian cancer, e = endometrial ovarian cancer, c = clear cell ovarian cancer
Score: standard = score procedures were performed double-blindly by at least one pathologist according to standard scoring systems
Tissue Type: FFPE = formalin-fixed and paraffin-embedded
Time point: the time point of specimen obtained, 1 = specimens obtained during primary surgery, 2 = specimens obtained from recurrent tumor
Treatment: 1 = platinum-based chemotherapy, 2 taxane-containing chemotherapy
*The antibody used was a polyclonal rabbit antiserum raised against amino acids 1205–1224 of the human mdr protein. This peptide (ALDTEKV-VQEAELDKAREG) was made by Multiple Peptide Systems, Inc. (San Diego, CA).

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Nineteen studies with 6635 patients were included in the meta-analysis of OS. Fifteen studies with 1140 patients reported the association between high ABCB1 expression and OS. Overall, high ABCB1 expression was correlated with a poor OS (HR, 1.54; 95% CI: 1.25–1.90) in EOC. The effect of ABCB1 expression on OS remained significant in the IHC (HR, 1.63; 95% CI: 1.22–2.19), the PCR (HR, 1.42; 95% CI: 1.08–1.88), the FIGO III-IV (HR, 1.92; 95% CI: 1.20–3.07), the ABCB1 expression in recurrent tumor (HR, 3.09; 95% CI: 1.79–5.34), the platinum-based non-taxane-containing chemotherapy (HR, 2.55; 95% CI: 1.60–4.05), the America (HR, 1.68; 95% CI: 1.16–2.44) and the Europe (HR, 2.55; 95% CI: 1.57–4.13) subgroups. Similarly, when adjusted for residual disease (N = 6), age (N = 4), FIGO stage (N = 4), histological type (N = 2) and tumor grade (N = 1), high ABCB1 expression remained significantly associated with OS (HR, 1.94; 95% CI: 1.52–2.46). However, an inverse correlation was not found in the serous tumor, in the Asia, in the Oceania, in the patients with primary tumors, nor in the taxane-containing chemotherapy subgroups. We found that in the serous subgroup, the

| First Author | Year | Country | No. of Cases | Stage | Histology | Detection method | Tissue Type | Time Point | Chemotherapy | End-point |
|--------------|------|---------|--------------|-------|-----------|-----------------|-------------|------------|--------------|----------|
| ABCB1 mRNA expression | | | | | | | | | | |
| Johnatty | 2013 | Australia | 143 | I-IV | s | PCR | frozen | 1 | 2 | OS, PFS |
| Lu L | 2007 | USA | 206 | I-IV | all | PCR | frozen | 1 | 1,2 | OS, PFS |
| Materna | 2004 | Germany | 61 | I-IV | all | PCR | frozen | 1 | 1,2 | OS, PFS |
| Nakayama | 2002 | Japan | 82 | I-IV | all | PCR | frozen | 1 | 1,2 | OS |
| Galani | 2002 | Greece | 22 | III-IV | all | PCR | frozen | 1 | 1,2 | TR |
| Kamazawa | 2002 | Japan | 27 | III-IV | s,e,c | PCR | frozen | 1 | 2 | TR |
| Wang | 1998 | China | 27 | I-IV | all | PCR | frozen | 1 | 1 | TR |
| Zhu L | 1997 | China | 32 | III | s,e,c | PCR | frozen | 1 | 1 | TR |
| Kavallaris | 1996 | Australia | 53 | I-IV | all | PCR | frozen | 1 | 1 | PFS |
| ABCB1 gene variants | | | | | | | | | | |
| Tecza K | 2015 | Poland | 126 | I-IV | all | ASA-PCR, multiplex-PCR, RFLP-PCR | blood | 1 | 2 | PFS |
| Johnatty | 2013 | Australia | 4450 | I-IV | all | Illumina Infinium iSelect BeadChip | blood | 1 | 2 | OS, PFS |
| Tian | 2012 | USA | 511 | III-IV | all | Sequenom iPLEXTMGOLD Assay and MALDI-TOF | blood | 1 | 2 | OS, PFS |
| Prema | 2011 | USA | 445 | I-IV | all | Illumina Veracode Assay | blood | 1, 2 | 1 | PFS |
| Bergmann | 2011 | Denmark | 119 | II-IV | all | Pyrosequencing and TaqMAN® predesigned SNP genotyping assays | FFPE | 1 | 1 | OS |
| Grimm | 2010 | Austria | 106 | I-IV | all | Pyrosequencing | blood | 1 | 2 | OS, PFS |
| Johnatty | 2008 | Australia | 309 | I-IV | all | MALDI-TOF | blood | 1 | 2 | OS, PFS |

First Author: bolded when the result of ABCB1 association was significant
Histology: all = serous, mucinous, endometrial and clear cell carcinoma with or without mixed histology, s = serous ovarian cancer, m = mucinous ovarian cancer, e = endometrial ovarian cancer, c = clear cell ovarian cancer
Tissue Type: FFPE = formalin-fixed and paraffin-embedded
Time point: the time point of specimen obtained, 1 = specimens obtained during primary surgery, 2 = specimens obtained from recurrent tumor
Treatment: 1 = platinum-based chemotherapy, 2 taxane-containing chemotherapy

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Meta-analysis of relationship between ABCB1 status and OS in EOC

Nineteen studies with 6635 patients were included in the meta-analysis of OS. Fifteen studies with 1140 patients reported the association between high ABCB1 expression and OS. Overall, high ABCB1 expression was correlated with a poor OS (HR, 1.54; 95% CI: 1.25–1.90) in EOC. The effect of ABCB1 expression on OS remained significant in the IHC (HR, 1.63; 95% CI: 1.22–2.19), the PCR (HR, 1.42; 95% CI: 1.08–1.88), the FIGO III-IV (HR, 1.92; 95% CI: 1.20–3.07), the ABCB1 expression in recurrent tumor (HR, 3.09; 95% CI: 1.79–5.34), the platinum-based non-taxane-containing chemotherapy (HR, 2.55; 95% CI: 1.60–4.05), the America (HR, 1.68; 95% CI: 1.16–2.44) and the Europe (HR, 2.55; 95% CI: 1.57–4.13) subgroups. Similarly, when adjusted for residual disease (N = 6), age (N = 4), FIGO stage (N = 4), histological type (N = 2) and tumor grade (N = 1), high ABCB1 expression remained significantly associated with OS (HR, 1.94; 95% CI: 1.52–2.46). However, an inverse correlation was not found in the serous tumor, in the Asia, in the Oceania, in the patients with primary tumors, nor in the taxane-containing chemotherapy subgroups. We found that in the serous subgroup, the
correlation between ABCB1 expression and OS became significant after excluding study specifically identifying high-grade serous ovarian cancer (HGSOC) (HR, 1.31; 95% CI: 1.07–1.59). For ABCB1 gene polymorphisms, although rs2235023, rs13237132, rs12334183, rs10264990, and rs4148732 were previously reported to be associated with ovarian cancer outcome, evidences from a study of more than 10000 cases suggested these candidate polymorphisms were not correlated with EOC prognosis [19]. Therefore we only evaluated the impact of rs2032582, rs1128503 and rs1045642 on prognosis. Five studies with 5495 patients reported association between these polymorphisms and OS in EOC [20,21,24,25,54]. No evidence of correlation between ABCB1 gene SNPs and OS was found (HR, 0.99; 95% CI: 0.95–1.02). The heterogeneity between studies in these analyses was acceptable (Figs 2–4 and Table 3).

Meta-analysis of the correlation between ABCB1 status and PFS in EOC

Nineteen studies with 7133 patients were included for meta-analysis of PFS. Fourteen studies with 1186 patients reported the association between high ABCB1 expression and PFS. Overall, high ABCB1 expression was associated with a poor PFS (HR 1.49; 95% CI: 1.22–1.82) in EOC. The effect of ABCB1 expression on PFS remained significant in the IHC (HR, 1.37; 95% CI: 1.05–1.79), the PCR (HR 1.67; 95% CI: 1.24–2.26), the FIGO III-IV (HR, 1.97; 95% CI: 1.40–2.78), the platinum-based non-taxane-containing chemotherapy (HR, 1.91; 95% CI: 1.46–2.50), the Asia (HR, 2.04; 95% CI: 1.30–3.18) and the Oceania (HR, 1.34; 95% CI: 1.10–1.62) subgroups. Similarly, when adjusted for residual disease (N = 4), age (N = 3), FIGO stage (N = 2), histological type (N = 1) and tumor grade (N = 1), high ABCB1 expression remained significantly associated with PFS (HR, 2.13; 95% CI: 1.60–2.83). However, such correlations were not found in the serous, the taxane-containing chemotherapy, the America and the Europe subgroups. Six studies with 5947 patients reported the association between rs2032582, rs1128503 or rs1045642 and PFS. No evidence of correlation between ABCB1 gene SNPs and PFS was found (HR, 1.02; 95% CI: 0.99–1.05). Significant heterogeneities were found in overall meta-analysis of high ABCB1 expression (I² = 73.6%, P = 0.000), the IHC (I² = 81.3%, P = 0.000), the PCR (I² = 52.1%, P = 0.099), the rs2032582 (I² = 73.6%, P = 0.004), the FIGO stage (I² = 88.0%, P = 0.000), the platinum-based (I² = 88.6%, P = 0.000), the all chemo (I² = 64.7%, P = 0.027), the Europe (I² = 87.6%, P = 0.000) subgroups and when adjusted for residual disease (I² = 85.4%, P = 0.000) (Figs 5–7 and Table 3).

Meta-analysis of the correlation between ABCB1 status and TR in EOC

Twenty-one studies involving 966 patients were included for meta-analysis of TR. Overall, high ABCB1 expression was associated with a poor TR (RR, 0.63; 95% CI: 0.54–0.75) in EOC. The effect of ABCB1 expression on OS remained significant in the IHC (RR, 0.63; 95% CI: 0.52–0.75), the PCR (RR, 0.52; 95% CI: 0.33–0.80), the FIGO III-IV (RR, 0.57; 95% CI: 0.47–0.68), the platinum-based non-taxane-containing chemotherapy (RR, 0.59; 95% CI: 0.48–0.72), the Asia (RR, 0.62; 95% CI: 0.46–0.85) and the Europe (RR, 0.62; 95% CI: 0.51–0.76) subgroups. No evidence for correlations between ABCB1 expression and TR were found in the serous, the taxane-containing and the all chemo subgroups. We found that in the serous subgroup, the correlation between ABCB1 expression and TR became significant in studies specifically identified low-grade serous ovarian cancer (LGSOC) and remained non-significant in studies identified HGSOC. The heterogeneities of overall meta-analysis (I² = 66.6%, P = 0.000), the IHC (I² = 72.4%, P = 0.000), the FIGO stage (I² = 69.9%, P = 0.000), the serous (I² = 68.1%, P = 0.024), the platinum-based (I² = 73.2%, P = 0.000), the America (I² = 80.3%, P = 0.024) and the Europe (I² = 77.2%, P = 0.000) subgroups were significant (Figs 8–10 and Table 3).
Fig 2. Forest plots presenting HRs of EOC OS for high ABCB1 expression, adjusted results and ABCB1 gene variants. HR = hazard ratio; EOC = epithelial ovarian cancer; OS = overall survival.

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### Fig 3. Forest plots of subgroup meta-analysis presenting HRs of EOC OS for high ABCB1 expression.

HR = hazard ratio; EOC = epithelial ovarian cancer; OS = overall survival.

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| Study ID                  | HR (95% CI) | % Weight |
|---------------------------|-------------|----------|
| Patients of FIGO III-IV   |             |          |
| Surowiak a (2006)         | 2.09 (0.89, 4.90) | 39.81    |
| Surowiak b (2006)         | 1.59 (0.77, 3.27) | 39.81    |
| Raspolini (2002)          | 2.35 (1.15, 4.82) | 20.37    |
| Overall (I-squared = 0.0%, p = 0.739) | 1.92 (1.20, 3.07) | 100.00   |
| Patients with serous ovarian cancer HGSOC |             |          |
| Matsuo (2014)             | 1.18 (0.66, 2.10) | 35.56    |
| Subtotal (I-squared = %, p = .) | 1.18 (0.66, 2.10) | 35.56    |
| HGSOC and LGSOC           |             |          |
| Johnatty (2013)           | 1.20 (0.92, 1.55) | 45.40    |
| Yakirevich (2006)         | 1.60 (1.24, 2.06) | 19.05    |
| Subtotal (I-squared = 65.0%, p = 0.091) | 1.31 (1.07, 1.59) | 64.44    |
| Overall (I-squared = 44.2%, p = 0.166) | 1.26 (0.99, 1.61) | 100.00   |
| Specimen of the first surgery |             |          |
| Matsuo (2014)             | 1.18 (0.66, 2.10) | 14.38    |
| Lu D (2011)               | 1.41 (1.14, 1.76) | 10.27    |
| O'Tool (2007)             | 1.90 (0.44, 8.27) | 10.01    |
| Lu L (2007)               | 1.21 (0.73, 2.02) | 26.44    |
| Oda (2007)                | 0.62 (0.08, 4.57) | 6.80     |
| Yakirevich (2006)         | 1.60 (1.24, 2.06) | 7.70     |
| Surowiak a (2006)         | 2.09 (0.85, 4.90) | 5.52     |
| Pensio a (2004)           | 2.33 (0.55, 9.89) | 4.11     |
| Ikeda (2003)              | 1.10 (0.50, 2.60) | 11.94    |
| Raspolini (2002)          | 2.35 (1.15, 4.82) | 2.82     |
| Overall (I-squared = 0.0%, p = 0.580) | 1.33 (1.00, 1.78) | 100.00   |
| Specimen of the second surgery |             |          |
| Surowiak b (2006)         | 1.59 (0.77, 3.27) | 28.87    |
| Pensio b (2004)           | 4.57 (1.59, 13.13) | 21.33    |
| Khalifa (1997)            | 3.83 (1.55, 9.45) | 50.00    |
| Overall (I-squared = 49.9%, p = 0.136) | 3.09 (1.79, 5.34) | 100.00   |
| Platinum-based chemotherapy |             |          |
| Taxane-containing         |             |          |
| Matsuo (2014)             | 1.18 (0.66, 2.10) | 9.22     |
| Johnatty (2013)           | 1.20 (0.92, 1.55) | 11.77    |
| Oda (2007)                | 0.62 (0.08, 4.57) | 4.36     |
| Yakirevich (2006)         | 1.60 (1.24, 2.06) | 4.94     |
| Pensio a (2004)           | 2.33 (0.55, 9.89) | 2.63     |
| Pensio b (2004)           | 4.57 (1.59, 13.13) | 2.63     |
| Subtotal (I-squared = 45.5, p = 0.102) | 1.33 (0.95, 1.85) | 35.56    |
| Non-taxane-containing     |             |          |
| Surowiak a (2006)         | 2.09 (0.89, 4.90) | 3.54     |
| Surowiak b (2006)         | 1.59 (0.77, 3.27) | 3.54     |
| Raspolini (2002)          | 2.35 (1.15, 4.82) | 1.81     |
| Khalifa (1997)            | 3.83 (1.55, 9.45) | 6.17     |
| Subtotal (I-squared = 0.0%, p = 0.445) | 2.55 (1.60, 4.05) | 15.06    |
| All chemo                 |             |          |
| Lu D (2011)               | 1.41 (1.14, 1.76) | 6.58     |
| O’Tool (2007)             | 1.90 (0.44, 8.27) | 6.42     |
| Lu L (2007)               | 1.21 (0.73, 2.02) | 16.95    |
| Materna (2004)            | 3.70 (1.09, 6.63) | 5.02     |
| Ikeda (2003)              | 1.10 (0.50, 2.60) | 7.65     |
| Nakayama (2002)           | 1.41 (0.79, 2.52) | 6.75     |
| Subtotal (I-squared = 0.0%, p = 0.491) | 1.46 (1.07, 2.03) | 49.38    |
| Overall (I-squared = 26.7%, p = 0.155) | 1.54 (1.25, 1.90) | 100.00   |
Publication bias was first assessed by visual inspection of funnel plots and then estimated with bias \( p \)-value from Egger’s test. While asymmetry of funnel plots was observed in overall meta-analysis of OS, PFS and TR, the results of egger’s test indicated that no publication bias existed in studies included for overall meta-analysis of OS, PFS and most subgroup meta-analysis. Publication bias was only found in studies for overall meta-analysis of TR (\( P = 0.019 \)), analysis of PFS when adjusted for residual disease (\( P = 0.003 \)), the FIGO stage (\( P = 0.010 \)), the platinum-based (\( P = 0.032 \)) and the taxane-containing (\( P = 0.032 \)) subgroups of TR. After excluding certain studies to eliminate publication bias, the results of these analyses remained consistent to the original results (Fig 11 and Table 3).

Discussion

Most EOC patients develop resistance to chemotherapy and eventually demise from the recurrent disease due to drug resistance. The development of chemo-resistance significantly impairs the survival in patients with EOC. The identification of predictive factors for chemo-resistance and poor survival might improve the individualized therapy for EOC. In the present study, we systematically assessed the impact of ABCB1, a transporter participating in efflux of a series of drugs, on chemotherapy response and survival in patients with EOC. We found that a high ABCB1 expression was significantly associated with decreased OS, PFS, and TR in EOC.
The table below shows the pooled hazard ratios (HR) or relative risks (RR) with heterogeneities and publication bias of meta-analyses and subgroup analyses for ABCB1 expression, FIGO stage, tumor type, chemotherapy regimen, and geographical region.

| Type                  | No. of studies | HR (95%CI) or RR (95%CI) | HR and RRP value | Heterogeneity P value | I² value (%) | Bias P value |
|-----------------------|----------------|---------------------------|------------------|-----------------------|--------------|--------------|
| **OS**                |                |                           |                  |                       |              |              |
| High ABCB1 expression | 19             | 1.54 (1.25–1.90)          | 0.000            | 0.267                 | 18.0         | 0.078        |
| ABCB1 detected by IHC | 15             | 1.54 (1.25–1.90)          | 0.000            | 0.151                 | 43.4         | 0.206        |
| FIGO III-IV           | 2              | 1.92 (1.20–3.07)          | 0.006            | 0.739                 | 0.0          | -            |
| Serous tumor          | 3              | 1.26 (0.99–1.61)          | 0.061            | 0.166                 | 44.2         | 0.766        |
| ABCB1 expression in primary tumor | 10 | 1.33 (1.00–1.78) | 0.049 | 0.580 | 0.0 | 0.888 |
| ABCB1 expression in recurrent tumor | 3 | 3.09 (1.79–5.34) | 0.000 | 0.136 | 49.9 | 0.182 |
| Platinum-based chemotherapy | 14 | 1.54 (1.25–1.90) | 0.000 | 0.155 | 26.7 | 0.078 |
| Taxane-containing chemotherapy | 5 | 1.33 (0.95–1.85) | 0.093 | 0.102 | 45.5 | 0.613 |
| Non-taxane-containing chemotherapy | 3 | 2.55 (1.60–4.05) | 0.000 | 0.445 | 0.0 | 0.311 |
| All chemotherapy | 6 | 1.48 (1.07–2.03) | 0.017 | 0.491 | 0.0 | 0.505 |
| **Geographical region** |               |                           |                  |                       |              |              |
| America               | 3              | 1.68 (1.16–2.44)          | 0.006            | 0.156                 | 42.6         | 0.957        |
| Asia                  | 5              | 1.16 (0.83–1.64)          | 0.382            | 0.434                 | 0.0          | 0.104        |
| Europe                | 5              | 2.55 (1.57–4.13)          | 0.000            | 0.650                 | 0.0          | 0.327        |
| Oceania               | 1              | 1.20 (0.92–1.56)          | 0.171            | -                     | -            | -            |
| High ABCB1 expression adjusted for residual disease | 4 | 1.94 (1.52–2.46) | 0.000 | 0.000 | 86.4 | 0.080 |
| **PFS**               |                |                           |                  |                       |              |              |
| High ABCB1 expression | 12             | 1.49 (1.22–1.82)          | 0.000            | 0.000                 | 73.6         | 0.146        |
| ABCB1 detected by IHC | 8              | 1.37 (1.05–1.79)          | 0.042            | 0.000                 | 81.3         | 0.122        |
| FIGO III-IV           | 3              | 1.97 (1.40–2.78)          | 0.000            | 0.000                 | 88.0         | 0.068        |
| Serous tumor          | 2              | 1.14 (0.82–1.59)          | 0.431            | 0.323                 | 35.8         | -            |
| Platinum-based chemotherapy | 12 | 1.49 (1.22–1.82) | 0.000 | 0.000 | 73.6 | 0.146 |
| Taxane-containing chemotherapy | 3 | 1.30 (0.91–1.85) | 0.148 | 0.431 | 0.0 | 0.902 |
| Non-taxane-containing chemotherapy | 5 | 1.91 (1.46–2.50) | 0.000 | 0.000 | 88.6 | 0.110 |
| All chemotherapy | 4 | 1.39 (0.97–1.99) | 0.074 | 0.027 | 64.7 | 0.663 |
| **Geographical region** |               |                           |                  |                       |              |              |
| America               | 1              | 0.95 (0.48–1.88)          | 0.883            | -                     | -            | -            |
| Asia                  | 3              | 2.04 (1.30–3.18)          | 0.002            | 0.907                 | 0.0          | 0.119        |
| Europe                | 6              | 1.27 (0.92–1.75)          | 0.141            | 0.000                 | 87.6         | -            |
| Oceania               | 2              | 1.34 (1.10–1.62)          | 0.003            | 0.732                 | 0.0          | 0.199        |
| High ABCB1 expression adjusted for residual disease | 4 | 2.13 (1.60–2.83) | 0.000 | 0.000 | 85.4 | 0.003 |
| ABCB1 gene variants | 6 | 0.95 (0.97–1.99) | 0.074 | 0.027 | 64.7 | 0.663 |
| **TR**                |                |                           |                  |                       |              |              |
| High ABCB1 expression | 21             | 0.63 (0.54–0.75)          | 0.000            | 0.000                 | 66.6         | 0.019        |
| ABCB1 detected by IHC | 16             | 0.63 (0.52–0.75)          | 0.000            | 0.000                 | 72.4         | 0.051        |
| FIGO III-IV           | 13             | 0.57 (0.47–0.68)          | 0.000            | 0.000                 | 69.9         | 0.010        |
| Serous tumor          | 4              | 0.72 (0.57–0.91)          | 0.006            | 0.024                 | 68.1         | 0.823        |
| Platinum-based chemotherapy | 21 | 0.63 (0.54–0.75) | 0.000 | 0.000 | 63.7 | 0.019 |
| Taxane-containing chemotherapy | 5 | 0.76 (0.49–1.16) | 0.199 | 0.164 | 38.7 | 0.032 |
| Non-taxane-containing chemotherapy | 13 | 0.59 (0.48–0.72) | 0.000 | 0.000 | 73.2 | 0.032 |
| All chemotherapy | 3 | 0.82 (0.53–1.28) | 0.389 | 0.180 | 41.6 | 0.171 |
| **Geographical region** |               |                           |                  |                       |              |              |
| America               | 2              | 0.85 (0.46–1.54)          | 0.587            | 0.024                 | 80.3         | -            |
| Asia                  | 9              | 0.62 (0.46–0.85)          | 0.003            | 0.271                 | 19.4         | 0.133        |
| Europe                | 10             | 0.62 (0.51–0.76)          | 0.000            | 0.000                 | 77.2         | 0.547        |

a: HR and its 95%CI were used to assess OS and PFS, RR and its 95%CI were used to assess TR
b: PT (platinum + taxane) regimen
c: PAC (platinum + doxorubicin + cyclophosphamide), PA (platinum + doxorubicin) or PC (platinum + cyclophosphamide) regimen
d: Studies identified patients received PT, PAC, PA or PC regimen.

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Fig 5. Forest plots presenting HRs of EOC PFS for high ABCB1 expression, adjusted results and ABCB1 gene variants. HR = hazard ratio; EOC = epithelial ovarian cancer; PFS = progression free survival.

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patients. These results are convincing because (i) the outcome definition was clear, (ii) the included studies were sub-grouped according to measuring methods to ensure the value of comparability, (iii) multivariate results further validated the association between ABCB1 and EOC prognosis and (iv) the number of studies reporting positive results or non-significant positive results were of greater quantity than the corresponding studies reporting negative results. Although heterogeneities were found in overall analyses for PFS and TR, we conducted sub-group analysis and determined histology and chemotherapy regimen as two main sources of heterogeneity between studies.

EOC consists of several histological subtypes, such as serous, mucinous, clear cell, endometrioid carcinomas. Different subtypes differ in genetic and biological features, clinical progression patterns, response to treatment, and prognosis. Therefore, it is meaningful to analyze the association between ABCB1 and prognosis in different ovarian cancer subtypes separately. However, in our subgroup analysis, we found only 6 out of 38 studies reported exclusively on serous ovarian carcinoma while the remaining reported on all subtypes as a whole. Since none of the studies reported independent data of any other subtypes other than serous ovarian carcinoma, we could not further determine what effects did other subtypes have on the significance association. Even though we find no evidence for correlation between ABCB1 expression and
prognosis in patients with serous carcinoma, which accounts for 70% to 80% of all cases, the result is limited by small number of included studies (N = 6) therefore further validation is essential. Moreover, serous ovarian cancer can be classified into HGSOC and LGSOC according to the origins of tumorigensis, precursor lesions, genetic and molecular characteristics, morphologic features, clinical behaviors and chemotherapy responses [61,62]. HGSOC accounts for most ovarian cancer deaths as nearly 90% of the late stage HGSOC patients develop acquired resistance to platinum-based chemotherapy [63]. The mechanisms underlying acquired chemo-resistance in HGSOC include activation of AKT pathway, the reversion of BRCA1/BRCA2 germline mutations and ABCB1 overexpression [64]. Many argued that although decreased drug accumulation either by decreased influx, increased efflux or enhanced drug detoxification was identified as an important mechanism of drug resistance, most of these mechanisms were not clinically significant in HGSOC [65]. Similarly, we didn't find any evidence for correlation between ABCB1 and prognosis of HGSOC patients. However, we did find unfavorable OS, PFS and TR accompanied by up-regulated ABCB1 in LGSOC patients, which is consistent with the fact that LGSOC is more resistant than HGSOC to initial chemotherapy (N = 2). Nevertheless, more studies were needed to validate the evolvement of ABCB1 in LGSOC chemo-resistance.

The regimen of platinum and taxane combination is the first-line treatment for EOC due to its superiority over other regimens regarding OS and adverse effects [66–68]. Although taxanes
are substrates of ABCB1, our results did not reveal any evidence for a correlation between ABCB1 expression and prognosis in patients who received taxane-containing regimen. Instead, we found an adverse correlation between ABCB1 expression and prognosis in patients who received non-taxane-containing regimen, such as platinum combined with doxorubicin or cyclophosphamide. The biological effects resulted from up-regulated ABCB1 expression in the patients undergoing taxane-containing therapy might be compensated by other mechanisms that need further investigation. On one hand, it seemed that extensive use of taxanes successfully improved the general survival of EOC patients comparing to other drugs [69] and attenuated the sensitivity of ABCB1 as a prognosis indicator. On the other, since both cellular and clinical researches revealed that ABCB1 in ovarian cancer could be up-regulated by chemotherapy [70, 71], taxane induced alteration in ABCB1 through the course of treatment might also be a confounding factor to the measurement of survival analysis because ABCB1 expression was seldom obtained in matched analysis [72–74]. In pre-clinical studies, inhibitors targeting ABCB1 could reverse chemotherapy resistance to some extent [75,76]. However, the efficacies of these ABCB1 inhibitors were somehow discouraging in clinical trials for treatment of EOC patients with initial or acquired chemo-resistance [77]. Unselected patients might be one of the main reasons for that [78]. Our current results indicated that the patients undergoing non-taxane-containing chemotherapy rather than those undergoing taxane-containing chemotherapy might be benefited more from ABCB1 inhibition.

Fig 8. Forest plots presenting RRs of EOC TR for high ABCB1 expression. RR = risk ratio; EOC = epithelial ovarian cancer; TR = total response rate.

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Fig 9. Forest plots of subgroup meta-analysis presenting RRs of EOC TR for high ABCB1 expression.

RR = risk ratio; EOC = epithelial ovarian cancer; TR = total response rate.

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We observed an intimate relationship of ABCB1 expression in recurrent tumor with OS, but not that in primary tumor. Similarly, a whole-genome sequence analysis of a large cohort of sensitive, resistant and refractory ovarian carcinoma tissue specimens performed by Patch et al. revealed that ABCB1 was overexpressed in the recurrent and refractory tumors compared with the sensitive ones, which was caused by recurrent promoter fusion with SLC25A40 and led to acquired resistance to chemotherapy [79]. These findings suggest that the increase in ABCB1 induced by chemotherapy rather than the background ABCB1 expression is response for chemo-resistance and impaired survival and ABCB1 may play an essential role in the development of acquired drug resistance in ovarian cancer. However, the number of included studies for the ABCB1 in recurrent tumor was limited (N = 3) that restricts the reliability of this result.

To sum up, the present meta-analysis showed a significant negative correlation between ABCB1 expression level and prognosis in EOC patients and provided preliminary evidence highlighting this correlation in specific subgroups stratified by chemotherapy regimens, histological subtypes and tissues used for ABCB1 detection. Large-scale, prospective studies are needed to verify the clinical significance of ABCB1 expression in EOC.
Supporting Information

S1 Checklist. PRISMA checklist.
(DOCX)

S2 Checklist. Genetic associated meta-analysis checklist.
(DOCX)

S1 File. Quality assessment of the included studies.
(DOCX)

S1 Table. List of excluded articles and reasons for exclusion.
(DOCX)

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