Scratching Below the Ovarian Cancer GWAS Surface

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ABSTRACT

Despite recent notable treatment advancements, ovarian cancer survival rates remain poor, with about half of women surviving five years after diagnosis. Uncovering novel prognostic factors is critical to better understand and reduce mortality from this deadly disease. While genome-wide association studies have identified numerous loci associated with risk of epithelial ovarian cancer, the investigation of genetic factors associated with outcomes among women with ovarian cancer has been limited due to several challenges summarized in the present commentary. Using data from the Ovarian Cancer Association Consortium, Quinn and colleagues conducted a genome-wide association study of patients with ovarian cancer receiving debulking surgery and standard chemotherapy as first-line treatment, revealing a locus at 12q24.33 associated with progression-free survival. Experimental evidence suggests that *ULK1*, a gene coding for a serine/threonine kinase implicated in autophagy, is the target of the association. We discuss the novelty of these findings, unanswered questions, and next steps for the road ahead in translating the work of Quinn and colleagues into clinical practice.

See related article by Quinn et al., p. 1669

Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy in the United States, responsible for an estimated 13,770 deaths in 2021 (1). About half of women with EOC will not survive five years after diagnosis (49% five-year relative survival rate), which is largely attributed to an advanced disease stage at diagnosis for the majority of patients (1). Despite initial response to frontline therapy consisting of a combination of cytoreductive debulking surgery and platinum- and taxane-based chemotherapy, most patients with ovarian cancer with advanced disease will recur and eventually succumb to the disease (2). These poor outcomes highlight the critical unmet need to better understand prognosis and identify factors associated with outcomes in ovarian cancer.

Genome-wide association studies (GWAS) test the association of genomic markers (single nucleotide polymorphisms; SNPs) with a trait (3, 4). During the past decade, international consortia have conducted GWAS with large sample sizes to identify SNPs associated with increased risk of several cancers. In addition, several multi-consortia large scale projects, such as the Collaborative Oncological Gene-Environment study (COG) and the Oncoarray Consortium, revealed hundreds of associations with risk of common cancers (5, 6). These studies have led to the identification of >40 genetic loci associated with increased risk of EOC (7, 8) and contributed to the refinement of risk stratification using polygenic risk scores (9, 10). Notably, these data continue to be leveraged to identify additional risk loci using transcriptome-wide association studies, cross-cancer risk loci analysis and now, with nested studies that can address genetic factors that influence outcomes (11–13). However, preliminary functional dissection has revealed a variety of complex mechanisms underlying risk at each locus, slowing the progress needed to develop mechanism-based preventive and therapeutic interventions (7, 8). In this issue, Quinn and colleagues report a GWAS of progression-free survival (PFS) in 2,352 women with EOC who had undergone cytoreductive surgery and standard carboplatin/paclitaxel chemotherapy and report the identification of a locus near *ULK1* containing seven SNPs reaching genome-wide significance ($P < 5\times10^{-8}$). Importantly, preliminary functional analysis is consistent with *ULK1* being the target of this association. This GWAS of outcomes in patients with ovarian cancer is one of the first to identify loci associated with progression-free survival and with further validation of findings, may provide a more direct approach to interventions to improve outcomes among patients with ovarian cancer.

Why is it so difficult to move GWAS beyond risk?

Several factors contribute to the extreme difficulty of conducting GWAS on clinical outcomes. Multi-site or consortia data are often needed to obtain an adequately powered sample size to investigate genetic associations in cancer, especially in a relatively uncommon malignancy such as EOC. Pooling data from multiple sites can result in several challenges related to inter- and intrastudy variation of treatment and outcomes data. Collection of these data in observational studies is notoriously difficult and can lead to a considerable amount of missing data either at the study- or patient-level. In addition, these multi-site efforts often include institutions and studies across the world with varying standards of care in treating EOC making it challenging to combine data from multiple sites. Moreover, there have been several notable modifications to the management of both newly diagnosed and recurrent ovarian cancer over time. First-line therapy for ovarian cancer traditionally consisted of upfront cytoreductive surgery followed by chemotherapy; however, an alternative approach, neoadjuvant chemotherapy (chemotherapy prior to surgical debulking), has become more common in the last decade (14, 15). Other recent treatment advancements include the utilization of bevacizumab and PARP inhibitors in the recurrent setting (16–18). While many of the existing observational studies contributing genotyping and outcome data to these large data pooling efforts predate these therapeutic advancements, future analyses incorporating more contemporary studies and newly diagnosed cases in prospective cohorts will inevitably face the challenge of increasingly heterogeneous patient groups. Likewise, these new therapeutics will increase the complexity of patient trajectories moving forward, further complicating the

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ability to adequately harmonize treatment and outcomes data for analyses. Despite their own challenges, future studies with clinic-based recruitment and enrollment of patients would be ideal to address some of the inherent limitations of observational and population-based studies in moving GWAS beyond risk. In particular, clinic-based studies would allow for better access to medical records to abstract detailed clinical and treatment data, include women with rapidly fatal disease who are often excluded from population-based studies, and encompass all treatment trajectories of patients including the more recent therapeutic advancements.

Functional follow-up and the road ahead

Functional follow-up studies typically include the identification of the functional risk-associated SNPs which are enriched in transcriptional regulatory elements such as promoters and enhancers (19). Because enhancers interact with the promoter of target genes via chromatin looping, putative regulatory elements containing risk-associated SNPs can be used to identify the target gene(s) by physical approaches such as chromosome conformation capture or Chromatin Interaction Analysis by Paired-End Tag Sequencing (20, 21). Alternatively, functional approaches such as eQTL (Expression Quantitative Trait Loci) analysis which tests the association between variation in expression and SNP alleles can also be used to link risk-associated SNPs with putative target genes (22). Using a combination of publicly available data and *in vitro* experiments, Quinn and colleagues hone in on *ULK1* as the likely target gene for the association. High expression of *ULK1* was associated with shorter PFS irrespective of histology and treatment with any chemotherapy. Several SNPs with evidence for association with PFS showed eQTL signals with *ULK1*. Moreover, chromosome looping interaction between a putative regulatory element (PRE) containing risk-associated SNPs and the promoter of *ULK1* was suggested by H3K27Ac (Histone H3 acetylated at Lysine 27) HiChIP data and validated by chromosome conformation capture in ovarian cancer cell lines. The lack of allele-specific activity (no difference between risk and reference haplotypes) in the luciferase experiments assessing the PRE suggests that the in vivo mechanism of regulation may be context dependent. Interestingly, forced reduction of *ULK1* transcriptional levels confers sensitivity to two different classes of chemotherapeutics: DNA damaging platinum compounds and microtubule function disruptors such as taxanes, although the effect was less pronounced in the latter. Taken together, results from Quinn and colleagues indicate that *ULK1* is likely to be, at least in part, the target gene driving the association at the locus. Further studies including a systematic perturbation of other candidate genes at the locus and exposure to a range of concentrations of different chemotherapeutic drugs in a larger panel of EOC cell lines will be needed to determine the robustness and generalizability of these results.

The findings of Quinn and colleagues are specific to patients with ovarian cancer who are receiving upfront debulking surgery and at least four cycles of standard dose platinum- and taxane-based chemotherapy as their first-line treatment regimen. While this patient group represents the majority of patients with ovarian cancer (2), it is unclear whether *ULK1* will confer prognostic significance in patients receiving other therapeutic regimens or nonstandard therapies. The generalizability of these findings to other racial/ethnic groups besides women of European ancestry is also unknown. In particular, investigating genetic loci associated with outcomes among women of African ancestry is a crucial next step as these women experience the worst survival of all racial/ethnic groups and little is known in regards to predictors of outcomes among African-American women with ovarian cancer (1, 23). The study by Quinn and colleagues represents an important step in the road ahead toward improving outcomes in ovarian cancer.

Authors' Disclosures

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