Whole-Genome Sequencing Identifies PPARGC1A as a Putative Modifier of Cancer Risk in BRCA1/2 Mutation Carriers

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Simple Summary: In search of genetic factors that affect cancer risks in BRCA carriers, we carried out the first whole-genome sequencing study in a unique registry of familial ovarian cancer, selected to enrich with BRCA1/2 carriers. We are the first to survey rare variants, particularly the non-coding variants for BRCA modifier genes and identified PPARGC1A, a master regulator of mitochondrial biogenesis, as a novel putative BRCA modifier. This finding can help improve cancer risk prediction and provide personalized preventive care for BRCA carriers.

Abstract: While BRCA1 and BRCA2 mutations are known to confer the largest risk of breast cancer and ovarian cancer, the incomplete penetrance of the mutations and the substantial variability in age at cancer onset among carriers suggest additional factors modifying the risk of cancer in BRCA1/2 mutation carriers. To identify genetic modifiers of BRCA1/2, we carried out a whole-genome sequencing study of 66 ovarian cancer patients that were enriched with BRCA carriers, followed by validation using data from the Pan-Cancer Analysis of Whole Genomes Consortium. We found PPARGC1A, a master regulator of mitochondrial biogenesis and function, to be highly mutated in BRCA carriers, and patients with both PPARGC1A and BRCA1/2 mutations were diagnosed with breast or ovarian cancer at significantly younger ages, while the mutation status of each gene alone

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did not significantly associate with age of onset. Our study suggests PPARGC1A as a possible BRCA modifier gene. Upon further validation, this finding can help improve cancer risk prediction and provide personalized preventive care for BRCA carriers.

**Keywords:** BRCA modifier; cancer susceptibility gene; whole-genome sequencing; ovarian cancer; breast cancer

### 1. Introduction

BRCA1 and BRCA2 are the two most well-known cancer predisposition genes. Inheritance of a BRCA1 or BRCA2 mutation greatly increases lifetime risk of breast cancer and ovarian cancer [1,2]. While lifetime risk of developing ovarian cancer for women in the general population is about 1.2% [3], the risk was estimated to be 39–59% and 11–17% for women who carry BRCA1 and BRCA2 mutations, respectively, by age 70−80 [4–7]. Preventive strategies have therefore been implemented to reduce cancer risk in BRCA1/2 carriers [8–10]. On the other hand, the penetrance of BRCA1/2 mutations is not complete, and there is substantial variability in age of cancer onset among carriers. These observations support the hypothesis that cancer risks in BRCA1/2 mutation carriers are modified by other factors. Over the past two decades, significant efforts have been invested in search of these modifying factors, among the most influential of which is the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) [11]. These efforts resulted in the discovery of a number of risk modifiers for BRCA1/2 mutations, including environmental, reproductive, and genetic factors [12–15].

To date, studies to identify genetic modifiers of BRCA1/2 were mainly carried out in three ways: candidate gene studies, investigation of specific variants discovered by genome-wide association studies (GWAS) to associate with cancer risks in the general population, and GWAS carried out specifically in BRCA1 or BRCA2 carriers [13,16–19]. The potential BRCA1/2 genetic modifiers identified in these studies are all common variants with relatively small effect sizes. On the other hand, the contribution of rare variants, which are more likely to have large effect sizes and/or direct functional consequences, in modifying cancer risks of BRCA1/2 carriers has not been systematically explored. Therefore, we employed whole-genome sequencing (WGS) technology to identify genetic modifiers of BRCA1/2 that are driven by rare variants.

To maximize the likelihood of discovering genetic modifiers of BRCA1/2, we performed WGS on a total of 66 ovarian cancer (OC) patients that were enriched with BRCA carriers. A total of 49 of these patients were selected from the Familial Ovarian Cancer Registry (FOCR) [20,21] to have a strong family history of OC. The discovered candidates were evaluated using independent WGS data of 247 ovarian and breast cancer patients from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium [22]. To our knowledge, this is the first and largest WGS study of BRCA1/2 modifiers to date, and we report PPARGC1A as a novel putative genetic modifier of ovarian and breast cancer risk for BRCA1/2 carriers.

### 2. Materials and Methods

#### 2.1. Study Population

A total of 50 hereditary OC patients from 48 families were selected from FOCR (formerly known as the Gilda Familial Ovarian Cancer Registry) for WGS based on DNA availability, prior genetic test results of BRCA1/2, and strong family history. The FOCR housed at Roswell Park Comprehensive Cancer Center (RPCCC) recruits families with two or more cases of OC, families with three or more cases of cancer on the same side of family with at least one being OC, families with at least one female having two or more primary cancers and one of the primaries being OC, and families with two or more cases of cancer with at least one being OC diagnosed at an early age of onset (45 years old or
younger) [21]. Families provide written informed consent under an institutional protocol CIC95-27. Cases are verified by medical record and/or death certificate when required, and a registry pathologist verifies stage and histology. The registry comprises 50,401 individuals including 5614 ovarian cancers from 2636 unique families. A total of 27 of the 50 FOCR patients were known carriers of BRCA from prior genetic testing performed by Myriad or inhouse [21]. An additional 18 RPCCC patients with sporadic OC were also included for WGS.

2.2. Whole-Genome Sequencing, Variant Calling, and Variant Filtering

Sequencing library preparation and whole-genome sequencing was performed at The American Genome Center at the Uniformed Services University, Bethesda, MD (Supplementary Methods). The GATK data pre-processing workflow was used to generate analysis-ready alignments (Supplementary Methods). DeepVariant (v0.5) was used to call single nucleotide variants (SNVs) and small insertions and deletions (indels) in standard VCF format for each sample with a convolutional neural network model [23]. Structural variants (SV) were detected using a structural variant calling workflow developed by bcbio, which used an integrative caller MetaSV [24] that combines the results from four separate methods, CNVkit [25], Manta [26], LUMPY [27], and Wham [28]. We only considered SVs longer than 50 bp and required SVs to be detected by at least two of the four methods with ≥3 supporting reads (split read or disconcordant read) in each method. Using the genotypes of SNVs, we performed sample level quality assessment using the Bioconductor package SeqSQC [29]. One FOCR patient and one sporadic OC patient were identified as population outliers and hence were excluded from further analyses.

A series of filters were applied to keep only rare and functional variants in our analysis (Supplementary Methods). Variants in the eight genes with significantly higher mutation rate in BRCA carriers of our WGS discovery cohort (Table 1) were manually inspected to ensure reliable variant calls.

2.3. Statistical Analysis

One-sided Fisher’s exact test was used to test whether the gene mutation frequency is higher in BRCA carriers than non-carriers.

2.4. Network Propagation and Pathway Enrichment Analysis

Using HotNet2 [30] and the Reactome functional interaction networks [31], we applied a network propagation analysis on the −log10 scores of the one-sided Fisher’s exact test p-values calculated by comparing gene mutation frequencies between BRCA carriers and non-carriers within the 49 hereditary OC patients. Only genes with Fisher’s exact test p-values ≤ 0.6 were included in the analysis. The statistical significance of the identified sub-networks was based on the number and size of the identified sub-networks compared to those found using a permutation test. We used 100 permutations and a minimum network size of 2 for statistical testing.

To examine the biological functions of each significant gene sub-network, pathway enrichment analysis of genes in each sub-network was performed using hypergeometric testing based on the Reactome pathway database [31]. Multiple testing was corrected using the Benjamini–Hochberg method. For each sub-network, pathways with adjusted p-values < 0.05 were considered significantly enriched.

2.5. Validation Using PCAWG Breast and Ovarian Cancer Cohorts

We obtained germline genetic variants called by PCAWG and kept only breast and ovarian cancer patients that were of European ancestry. Sample level quality assessment was performed using the Bioconductor package SeqSQC [29], which resulted in the removal of nine problematic samples. Germline variants of the remaining PCAWG samples were filtered and annotated in the same way as described above for the germline variants in our
WGS data. We kept only the variants whose target genes were $BRCA1$, $BRCA2$, $PPARGC1A$, and $PBX1$ in our analysis.

2.6. Study Approval

The study was approved by Institutional Review Boards. All participants provided written informed consent.

3. Results

3.1. Study Population in the Discovery Stage

Our discovery WGS cohort consisted of 49 OC patients from the FOCR (formerly known as the Gilda Familial Ovarian Cancer Registry) with a strong family history of OC [20,21] and 17 sporadic OC patients (Figure 1, Tables S1–S3); all were of European descent. Among the 49 hereditary OC patients, 27 were known $BRCA$ carriers from prior genetic testing and were purposely included to enrich for $BRCA$ carriers in our discovery cohort (Materials and Methods).

![Figure 1](image-url). Schema of the analyses to identify genetic modifiers of ovarian and breast cancer risks in $BRCA$ carriers.

3.2. Discovery of Candidate Genes That Modify Cancer Risks of $BRCA1/2$

The germline genetic variants were detected from the discovery WGS data using a deep learning variant calling algorithm [23], which has been shown to achieve higher sensitivity and specificity in pathogenic variant detection than standard methods [32]. A series of stringent variant filtering steps were carried out to retain only rare (MAF < 0.5%...
in European population) and functional variants for subsequent analyses (Materials and Methods). The 27 known BRCA carriers were confirmed to carry BRCA1/2 mutations from our WGS analysis, and 12 more patients in the discovery cohort were found to be BRCA carriers, including 9 hereditary OC patients and 3 sporadic OC patients.

To identify BRCA modifiers that increase cancer risks in BRCA carriers, we utilized three different approaches. First, we compared mutation frequency of each gene between BRCA carriers and non-carriers in our discovery cohort using Fisher’s exact test and focused on genes that are more frequently mutated in BRCA carriers (Tables 1 and S4), requiring uncorrected Fisher’s exact test p-value \( \leq 0.05 \) in consideration of our relatively small sample size. In addition, we also required the genes to have significantly higher mutation frequency in BRCA carriers in the analysis of the 49 hereditary OC patients, assuming the effect of BRCA modifier is most enriched in cancer patients with family history. Eight genes satisfied both criteria, including BRCA1/2 and a known cancer gene PBX1.

Table 1. Comparison of gene mutation frequency between BRCA carriers and non-carriers in the discovery cohort.

| Gene     | Hereditary OC (36 BRCA Carriers vs. 13 Non-Carriers) | Hereditary OC + Sporadic OC (39 BRCA Carriers vs. 27 Non-Carriers) |
|----------|-----------------------------------------------------|------------------------------------------------------------------|
|          | # and Fraction of Mutated BRCA Carriers | # and Fraction of Mutated Non-Carriers | p-Value * | # and Fraction of Mutated BRCA Carriers | # and Fraction of Mutated Non-Carriers | p-Value * |
| BRCA1    | 29 0.81 | 0 0.00 | 2.95 \( \times 10^{-7} \) | 30 0.77 | 0 0.00 | 3.84 \( \times 10^{-11} \) |
| BRCA2    | 10 0.28 | 0 0.00 | 3.09 \( \times 10^{-2} \) | 12 0.31 | 0 0.00 | 7.94 \( \times 10^{-4} \) |
| PPARGC1A | 11 0.31 | 0 0.00 | 2.06 \( \times 10^{-2} \) | 11 0.28 | 1 0.04 | 9.99 \( \times 10^{-3} \) |
| PBX1     | 14 0.39 | 1 0.08 | 3.49 \( \times 10^{-2} \) | 14 0.36 | 3 0.11 | 2.15 \( \times 10^{-2} \) |
| LMNTD1   | 9 0.25  | 0 0.00 | 4.58 \( \times 10^{-2} \) | 9 0.23  | 0 0.00 | 5.73 \( \times 10^{-3} \) |
| AHDC1    | 9 0.25  | 0 0.00 | 4.58 \( \times 10^{-2} \) | 9 0.23  | 1 0.04 | 3.01 \( \times 10^{-2} \) |
| MADD     | 9 0.25  | 0 0.00 | 4.58 \( \times 10^{-2} \) | 9 0.23  | 1 0.04 | 3.01 \( \times 10^{-2} \) |
| TRERF1   | 9 0.25  | 0 0.00 | 4.58 \( \times 10^{-2} \) | 10 0.26 | 1 0.04 | 1.75 \( \times 10^{-2} \) |

* Raw p-value from one-sided Fisher’s exact test (H₀: gene mutation frequency in BRCA carriers ≥ the frequency in non-carriers).

Next, we adopted a network-based approach to identify the pathways that are altered in BRCA carriers based on the hypothesis that malfunction of certain biological processes increases cancer risk in BRCA carriers, and within those processes, multiple genes instead of a unique gene can be targeted by germline genetic mutations. Specifically, we mapped genes that showed elevated mutation rates in BRCA carriers within the hereditary OC cohort onto Reactome functional interaction networks [31] and used the network propagation method HotNet2 [30] to detect sub-networks that contain multiple contributing neighboring genes (Materials and Methods). A total of 15 significant sub-networks were identified (HotNet2 permutation p-value = 0.01). The largest sub-network contained BRCA1/2 and 45 other genes (Figures 2 and S1). The biological pathways that were significantly enriched in this sub-network included transcriptional regulation of white adipocyte differentiation, transcriptional regulation by Notch3 and Notch1, circadian clock, transcriptional activation of mitochondrial biogenesis, and SUMOylation (Table S5). Assuming the BRCA modifiers alter the same biological processes as BRCA1/2, we focused on the 47 genes within the same sub-network as BRCA1/2 in further analysis.
Finally, under our prior hypothesis that any BRCA modifier gene would be more highly mutated in BRCA carriers, we would expect the expression of any such modifier gene to differ between BRCA carriers and non-carriers, assuming the genetic mutation affects its gene expression. Therefore, we utilized both RNAseq and whole-exome sequencing (WES) data from The Cancer Genome Atlas (TCGA) Pan-Cancer analysis project to detect differentially expressed genes (DEGs) between BRCA carriers and non-carriers, respectively, (Figure 1). We then sought to validate these two genes using independent WGS cohorts of breast and ovarian cancer from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium [22], consisting of both the TCGA and the International Cancer Genome Consortium (ICGC). Among the 156 and 91 breast cancer and OC patients from PCAWG, only 17% and 19% were BRCA carriers, respectively, which was similar to the value in the sporadic OC cases of our discovery cohort but much lower than in the hereditary OC cases of our discovery cohort, where 73% were BRCA carriers. It was significantly up-regulated in BRCA carriers than non-carriers (Figure 3a, Table S6). This trend was also found in PCAWG’s ovarian cancer cases from TCGA, but not in those from ICGC (Table S6).

3.3. Independent Validation of BRCA Modifier Candidate Genes

Using the three different approaches described above, we found two potential BRCA modifier candidates, PPARGC1A and PBX1, which were identified by three and two approaches, respectively, (Figure 1). We then sought to validate these two genes using independent WGS cohorts of breast and ovarian cancer from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium [22], consisting of both the TCGA and the International Cancer Genome Consortium (ICGC). Among the 156 and 91 breast cancer and OC patients from PCAWG, only 17% and 19% were BRCA carriers, respectively, which was similar to the value in the sporadic OC cases of our discovery cohort but much lower than in the hereditary OC cases of our discovery cohort, where 73% were BRCA carriers.

In PCAWG’s breast cancer cohort, we observed a trend of higher PPARGC1A mutation frequency in BRCA carriers than non-carriers (Figure 3a, Table S6). This trend was also found in PCAWG’s ovarian cancer cases from TCGA, but not in those from ICGC (Table S6).
When combining the discovery and validation cohorts, the PPARGC1A gene in BRCA carriers possessed more deleterious mutations than in non-carriers with borderline significance ($p = 0.055$, Figure 3b). The other candidate gene, PBX1, did not show a higher mutation rate in BRCA carriers in either PCAWG breast or ovarian cancer cohort (Table S7).

Figure 3. Fraction of BRCA carriers and non-carriers that contained PPARGC1A mutations: (a) analysis within the PCAWG validation cohorts; (b) analysis within our discovery cohort, the PCAWG validation cohorts, and all cohorts combined. The numbers inside the bars are the numbers of BRCA carriers and non-carriers.

3.4. PPARGC1A as a Novel BRCA Modifier Candidate Gene

We then investigated whether PPARGC1A mutations affect age of onset of breast and ovarian cancer in the PCAWG patients. We found that the interaction between PPARGC1A mutation status and BRCA status significantly associated with an earlier age of cancer onset ($p = 0.03$), while the main effect of each gene alone was not significant ($p = 0.33$ and 0.96, respectively, for PPARGC1A status and BRCA status). Patients who carried both BRCA1/2 mutations and PPARGC1A mutations were diagnosed with breast or ovarian cancer at a significantly younger age (Figure 4). The median age of onset was 48, 55.5, 60.5, and 58 respectively for patients carrying mutations in both PPARGC1A and BRCA1/2 genes, in BRCA1/2 genes only, in PPARGC1A only, or in none of the three genes. The interaction term remained significant when restricting on breast cancer patients, but not in a regression model on the smaller cohort of ovarian cancer patients, where only one patient carried both BRCA and PPARGC1A mutations (Table S8). Consistent patterns were observed when BRCA1 and BRCA2 status were included in the regression model (Table S9).

The majority of the PPARGC1A mutations we identified in both the discovery and the validation cohorts were non-coding variants (Figure 5). To investigate how these non-coding variants affect PPARGC1A expression, we compared PPARGC1A expression between the carriers of non-coding variants and the non-carriers using PCAWG RNA-seq data [33] and observed a trend of lower expression in the carriers ($p = 0.09$ and 0.44 for ovarian cancer and breast cancer, respectively, Figure S2).
**Figure 4.** The distribution of cancer age onset by BRCA and PPARGC1A mutation status in PCAWG breast and ovarian cancer patients. The number in parenthesis is the sample size of each group.

**Figure 5.** The PPARGC1A variants identified in the discovery stage (a); and validation stage (b); respectively.

### 4. Discussion

In this first WGS study to discover putative BRCA genetic modifiers, we performed WGS on 66 OC patients that were enriched with BRCA carriers and identified two genes, PPARGC1A and PBX1, to be highly mutated in BRCA carriers and within the same gene subnetwork with BRCA1/2. In addition, PPARGC1A was found to be differentially expressed between BRCA carriers and non-carriers within the TCGA breast cancer cohort.
Our independent validation in PCAWG observed a similar trend of a higher PPARGC1A mutation rate in BRCA carriers than non-carriers for patients with breast cancer. Importantly, we found that patients with both PPARGC1A and BRCA1/2 mutations were diagnosed with breast or ovarian cancer at a significantly younger age, while the effect of each gene alone was not significant. Therefore, our results suggest PPARGC1A to be a potential new BRCA modifier. While previous candidate gene studies of common genetic variants linked PPARGC1A with ovarian cancer and familial breast cancer risk [34,35], our WGS study, focusing on rare and functional variants, was the first to reveal the effect of PPARGC1A in the context of BRCA1/2 mutation. The PPARGC1A mutations we identified through WGS were dominantly non-coding variants, which highlights the importance of going beyond just the gene coding regions in search of BRCA modifiers and cancer predisposition genes. It is worth noting that the pathways involving PPARGC1A, such as transcriptional regulation of white adipocyte differentiation and transcriptional activation of mitochondrial biogenesis, were found to be disturbed in BRCA carriers (Table S3). Future studies targeting these pathways may allow identification of additional BRCA modifiers.

PPARGC1A, also known as Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), is a coactivator of PPARγ, which is a crucial gene regulating BRCA1 gene expression [36]. In addition, PPARGC1A is a master regulator of mitochondrial biogenesis and function. It is essential for cancer cells to rapidly adapt to energy-demanding situations. Both increased and decreased PPARGC1A expression have been reported in a range of cancer types and associated with a worse prognosis [37–39]. These contradicting observations are now thought to result from cancer cells exploiting PPARGC1A to provide them metabolic plasticity to support their evolving needs along the course of cancer development [37,38,40,41]. During early tumorigenesis, PPARGC1A may be downregulated to facilitate the increased consumption of glucose and glutamine in cancer cells [37,40]. This is consistent with our findings of significantly earlier cancer development in carriers with mutations in both PPARGC1A and BRCA1/2, as well as lower PPARGC1A expression in the PPARGC1A mutation carriers. On the other hand, a recent study demonstrated inhibition of PPARGC1A in tumor infiltrating T cells leads to T cell exhaustion and tumor escape [42]. BRCA1 and BRCA2 are key homologous recombination genes. Defects in BRCA1/2 result in tumors with extensive genomic instability that stimulates inflammatory signaling [43–49]. Therefore, cancer cells that are genomically unstable must evolve to escape immune surveillance in order to avoid being cleared by the immune system [43]. Because tumor-infiltrating T cells in PPARGC1A mutation carriers experience PPARGC1A inhibition and T cell exhaustion due to metabolic insufficiency [42], BRCA1/2-mutant cancer cells in PPARGC1A mutation carriers have an advantage in escaping immune surveillance and thus can develop into tumors earlier than in individuals without PPARGC1A mutations. In summary, loss of PPARGC1A due to germline PPARGC1A variants might stimulate tumor development by giving tumor cells a metabolic advantage or weakening immune surveillance, or both.

A major strength of our study is the enrichment for BRCA carriers from a familial ovarian cancer registry. However, there are limitations to our study. While we have leveraged the largest publicly available WGS collection of breast and ovarian cancer patients from PCAWG, our validation study is limited by the relatively small sample size. Furthermore, the validation power is reduced due to over-representation of sporadic cancer and the small number of BRCA carriers in these publicly available WGS cohorts. A future sequencing study of the entire PPARGC1A locus in large ovarian and breast cancer cohorts, particularly in cancer patients with family history, is warranted to further validate our finding of PPARGC1A as a BRCA1/2 genetic modifier. Knocking out PPARGC1A in breast or ovarian cancer mouse models with mutated BRCA1/2 [50–52] will also help to investigate the effect of PPARGC1A in increasing cancer risk in the context of BRCA1/2 mutations and to reveal the underlying biological mechanism.
5. Conclusions

We conducted the first WGS study of hereditary OC patients enriched with BRCA carriers and followed with a validation study using the largest WGS collection of OC and BC patients to date to identify PPARGC1A as a possible BRCA modifier gene. Given the impact of PPARGC1A on the age of onset of OC and BC among BRCA mutation carriers, our results could have significant implications for cancer risk prediction and personalized preventive care for BRCA carriers. Future follow-up studies including additional sequencing and functional experiments are warranted to confirm these findings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers14102350/s1, Supplementary Methods; Figure S1: The remaining 14 gene sub-networks significantly altered in BRCA carriers. Genes that were significantly highly mutated in BRCA carriers (Table 1) are highlighted by red underscores.; Figure S2: PPARGC1A expression between carriers of non-coding mutations and non-carriers in PCAWG. Gene expression levels (FPKM-UQ) were estimated using the FPKM metric, based on alignments from the TopHat and STAR algorithms and normalized with the Upper Quartile method. FPKM, fragments per kilobase of transcript per million mapped reads. p-values were calculated using one-sided Wilcoxon rank sum test between 11 carriers and 41 non-carriers in breast cancer and between 7 carriers and 60 non-carriers in ovarian cancer; Table S1: Characteristics of the discovery cohort; Table S2: The sequencing coverage and quality statistics of whole-genome-sequenced FOCR patients; Table S3: The sequencing coverage and quality statistics of whole-genome sequenced sporadic OC patients; Table S4: The PPARGC1A variants included in our analyses; Table S5: The pathways significantly enriched in the genes within the largest gene sub-network significantly altered in BRCA carriers; Table S6: Comparison of PPARGC1A mutation frequency between BRCA carriers and non-carriers in the validation cohorts; Table S7: Comparison of PBX1 mutation frequency between BRCA carriers and non-carriers in the validation cohorts; Table S8: Linear regression between cancer age onset and gene mutation status (BRCA, PPARGC1A) in PCAWG; Table S9: Linear regression between cancer age onset and gene mutation status (BRCA1, BRCA2, PPARGC1A) in PCAWG. (References [53–64] cited in the supplementary materials)

Author Contributions: Q.Z. and K.O. conceived the study and wrote the manuscript; N.W.B., C.L.D., M.D.W., G.S., T.P. and G.L.M. generated the FOCR WGS data; Q.Z., J.W., H.Y., Q.H., M.L., S.R. and E.S. performed data analyses; R.-Y.H., J.K., S.B.L., E.Z., J.V., A.L., K.M. and K.O. coordinated samples and clinical data; Q.Z., J.W., H.Y., M.L., S.R. and K.O. interpreted data. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Roswell Park Comprehensive Cancer Center (protocol I215512).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are restricted due to ethical concerns in keeping with the institute’s policies on germline variation data and the level of patient consent gained. Data are available from the Familial Ovarian Cancer Registry (ovarianregistry@roswellpark.org) for researchers who meet the criteria for access to confidential data. The results published here are in part based upon data generated by The Cancer Genome Atlas (dbGaP Study Accession: phs000178.v10.p8) managed by the NCI and NHGRI. Information about TCGA can be found at http://cancergenome.nih.gov.

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