Fine-Scale Mapping at 9p22.2 Identifies Candidate Causal Variants That Modify Ovarian Cancer Risk in BRCA1 and BRCA2 Mutation Carriers


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Abstract

Population-based genome wide association studies have identified a locus at 9p22.2 associated with ovarian cancer risk, which also modifies ovarian cancer risk in BRCA1 and BRCA2 mutation carriers. We conducted fine-scale mapping at 9p22.2 to identify potential causal variants in BRCA1 and BRCA2 mutation carriers. Genotype data were available for 15,252 (2,462 ovarian cancer cases) BRCA1 and 8,211 (631 ovarian cancer cases) BRCA2 mutation carriers. Following genotype imputation, ovarian cancer associations were assessed for 4,873 and 5,020 SNPs in BRCA1 and BRCA2 mutation carriers respectively, within a retrospective cohort analytical framework. In BRCA1 mutation carriers one set of eight correlated candidate causal variants for ovarian cancer risk modification was identified (top SNP rs10124837, HR: 0.73, 95%CI: 0.68 to 0.79, p-value 2× 10^{-16}). These variants were located up to 20 kb upstream of BNC2. In BRCA2 mutation carriers one region, up to 45 kb upstream of BNC2, and containing 100 correlated SNPs was identified as causal variant (top SNP rs62543585, HR: 0.69, 95%CI: 0.59 to 0.80, p-value 1.0 × 10^{-6}). The candidate causal in BRCA1 mutation carriers did not include the strongest associated variant at this locus in the general population. In sum, we identified a set of candidate causal variants in a region that encompasses the BNC2 transcription start site. The ovarian cancer association at 9p22.2 may be mediated by different variants in BRCA1 mutation carriers and in the general population. Thus, potentially different mechanisms may underlie ovarian cancer risk for mutation carriers and the general population.

Introduction

Once age is taken into account, family history is the strongest risk factor for ovarian cancer. Women with a first-degree relative with ovarian cancer are at a 3-fold increased risk of
developing the disease, indicating the importance of genetic factors in ovarian cancer predisposition. The most important genes in the context of genetic counseling for ovarian cancer susceptibility are BRCA1 and BRCA2, which account for approximately 24% of the familial risk among first-degree relatives [1]. In contrast to the general population, in which the lifetime risk of developing ovarian carcinoma is 1.6% (average age at diagnosis 63 years), women carrying a BRCA1 mutation have a lifetime risk of 35–60% with an average age of diagnosis of 50 years [2]. The ovarian cancer penetrance is lower for BRCA2, with a lifetime risk of 12–25% and an average age of diagnosis of 60 years [2]. The majority of BRCA1/2 associated ovarian cancers present as high-grade serous histology in advanced stage [3].

Genome wide association studies have identified several common germline variants associated with ovarian cancer risk. The 9p22 locus was first found to be associated with ovarian cancer risk in the general population, and subsequently to be an ovarian cancer risk modifier in BRCA1 and BRCA2 mutation carriers [4,5]. The SNP showing the strongest association in the general population was rs3814113, which was associated with a decrease in the risk of ovarian cancer in carriers of the minor allele (OR per allele = 0.82, 95%CI: 0.79 to 0.86, p-value = 5.1 × 10−19) [5] and had a similar association with ovarian cancer risk for BRCA1 and BRCA2 mutation carriers [4]. rs3814113 lies in a 150-kb linkage disequilibrium (LD) block. The closest genes to rs3814113 are Basonuclin 2 (BNC2) and Centlein (CNTLN). BNC2 is a zinc-finger protein spanning nucleotides 16409503 to 16870706. It is expressed in ovary, testis and the male germ line where it regulates cell cycle progression [6]. CNTLN spans nucleotides 17134982 to 17503923, it is ubiquitously expressed and localises at centrosomes to ensure centrosome function during cell division [7,8]. However, no fine-scale mapping of this locus has been reported yet in either the general population or in mutation carriers. Therefore, it is unclear which are the likely causal variants in the region.

Here, we report the fine-scale mapping of the 9p22.2 locus using data from 15252 BRCA1 and 8211 BRCA2 mutation carriers of European ancestry. We comprehensively characterized the associations of genetic variants in the region with ovarian cancer risk for BRCA1 and BRCA2 mutation carriers.

Materials and Methods

Study Population

Epidemiological and genotype data were obtained from BRCA1 and BRCA2 mutation carriers participating in the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA, [9]). Eligibility to CIMBA was restricted to women older than age 18 years who carried pathogenic mutations in the BRCA1 or BRCA2 genes. For each mutation carrier, date of birth, age at cancer diagnosis, age at bilateral prophylactic mastectomy and/or oophorectomy, age at interview or last follow-up, exact BRCA1 and BRCA2 mutation description and self-reported ethnicity were recorded, together with tumor pathology, survival, treatment and other established lifestyle/hormonal risk factors for breast or ovarian cancer. Participants were recruited from 25 countries under ethically approved protocols and provided written informed consent.

Genotyping and Imputation

Genotyping was performed using the iCOGS Illumina array [10]. The quality control (QC) of the genotyping data has been described in detail previously [11,12]. The iCOGS array included SNPs for fine mapping of the 9p22.2 region. The fine mapping region was defined as Chromosome 9 positions: 16407967 to 17407967 (NCBI build 37). To select the SNPs for inclusion on iCOGS, we considered all variants with minor allele frequencies of >0.02 from the 1000 Genomes Project (March 2010 version) and selected SNPs that were correlated at r²>0.1 with
the SNP that had been identified through the GWAS (rs3814113), and the set of SNPs that tagged all remaining SNPs in the region with $r^2 > 0.9$. A total of 407 and 401 SNPs that were included in iCOGS in the 9p22.2 region passed QC and were available for the analyses for BRCA1 and BRCA2 mutation carriers, respectively. Imputation of genotypes was based on the phase 3 release of the 1000 Genome Project spanning nucleotides 16407967 to 17407967 (build 37) at chromosome 9 with a buffer region of 500bp, using IMPUTE2 v2 [13]. SNPs with an “info” metric lower than 0.3 were considered poorly imputed and excluded from downstream analyses. In addition, SNPs with a minor allele frequency (MAF) lower than 0.005 were excluded from the association analyses.

Statistical Analysis and Computational Methods

The primary analysis evaluated the association between each variant and ovarian cancer risk. To account for the non-random sampling of mutation carriers with respect to disease status, the analysis was conducted within a retrospective cohort framework by modeling the likelihood of the observed genotypes conditional on the disease phenotypes as previously described [14]. Each mutation carrier was followed until the first of: ovarian cancer diagnosis, risk-reducing salpingo-oophorectomy or age at last observation. Only those diagnosed with ovarian cancer were considered as cases. The effect of each SNP was modeled as a per-allele Hazard Ratio (HR). To account for related individuals in the sample, a kinship-adjusted version of the score test for association was used which accounts for the correlation between the genotypes of the relatives [15]. Analyses were carried out separately for BRCA1 and BRCA2 mutation carriers and all analyses were stratified by country of residence and year of birth. The USA and Canada strata were further subdivided by reported Ashkenazi Jewish ancestry.

Ovarian cancer associations were combined in a meta-analysis between BRCA1 and BRCA2 mutation carriers. A fixed effect meta-analysis weighted by the inverse variance was conducted for imputed and genotyped SNPs when risk estimates were available in both datasets. For BRCA1 and BRCA2 mutation carriers, logarithms of per-allele HR estimates were used. The Cochran Q test was carried out to assess heterogeneity.

To assess the number of variants independently associated with ovarian cancer risk in BRCA1 and BRCA2 mutation carriers, each SNP was included in a Cox-regression model conditioned on the most strongly associated variant for each dataset and further adjusting by year of birth, and stratifying by country of residence. This approach has been shown to yield valid tests of association [16]. All SNPs with a MAF > 0.005, and imputation accuracy higher than 0.3, were included. For single SNP associations, associations were considered significant if $p < 5 \times 10^{-8}$. The most parsimonious model in the conditional analyses was identified using a threshold of $p < 10^{-4}$ for retaining SNPs in the model.

The set of potential causal SNPs was defined by those SNPs for which their likelihood ratio relative to the most significant variant was equal or less than 100 and having a pair-wise correlation ($r^2$) with the top SNP higher than 0.1 [17].

BEDTools was used to intersect positions of ovarian cancer risk-associated variants with functional genomic features generated by Coetzee et al [18] including FAIRE-seq identified regulatory elements and enhancers identified by histone modification ChIP-seq. Variants implicated by overlap were then queried with HaploReg v3 (http://www.broadinstitute.org/mammals/haploreg/haploreg_v3.php).

Ethics statement

Each of the host institutions recruited under ethically approved protocols. A list of the local Institutional Review Boards that provided ethical approval for this study is given in S1 Table.
Results

Association of the 9p22.2 Locus with Ovarian Cancer Risk in BRCA1 Mutation Carriers

Data were available for 15,252 BRCA1 mutation carriers of whom 2,462 were censored at ovarian cancer diagnosis (S2 Table). After quality control, data for 407 SNPs genotyped through the iCOGS array spanning chromosome 9 from positions 16424985 to 174 04464 (Genome built 37) were available. A further 36,769 SNPs were imputed using the 1000 Genome Project as reference panel. Of those, 4,873 had a MAF higher than 0.005 and were considered reliably imputed (IMPUTE2 "info" score > 0.3), and were included in the association analysis.

The strongest associated variant was the imputed SNP rs10124837 (per allele HR = 0.73; 95%CI = 0.68–0.79; p = 2.0×10−16, Fig 1A, Table 1 and S1 Table) located 12 kb upstream of BNC2. SNP rs3814113 that was originally identified through the GWAS demonstrated a weaker association (p = 5.2×10−13). The correlation between the top SNP and the rs3814113 was 0.56 (Table 2). In total, 292 SNPs showed evidence of association with ovarian cancer risk (p: 10−4 to 10−16, Fig 1A). The correlation between the top SNP and the SNPs in this set varied from 0.1 to 0.9 (Fig 1). Results for all SNPs are presented in S3 Table.

Association of the 9p22.2 Locus with Ovarian Cancer Risk in BRCA2 Mutation Carriers

A total of 8,211 BRCA2 mutation carriers were included in the analysis, of whom 631 were censored at ovarian cancer diagnosis (S2 Table). The association analysis included 5,020 SNPs (401 genotyped) with MAF>0.005 that were reliably imputed (IMPUTE2 "info" score greater than 0.3). The strongest associated SNP with ovarian cancer risk was rs62543585, with a MAF of 0.20 and a per-allele HR = 0.69 (95%CI = 0.59–0.80; p = 1.0 × 10−6, Table 1). SNP rs3814113 demonstrated a slightly weaker association (p = 6.7×10−6 Table 1, r² with SNP rs62543583 = 0.48, Table 2). Although for BRCA2 mutation carriers the p-values did not reach GWAS statistical significance (5x10−8), given the strong prior evidence of association between SNPs in the region and risk for BRCA1 carriers and in the general population we selected the most significant SNPs as associated with ovarian cancer risk. Results for all SNPs with p<0.01 are presented in S3 Table.

Meta-analysis of BRCA1 and BRCA2 Mutation Carriers

Since the majority of both BRCA1 and BRCA2 ovarian cancer associated cancer tumors are high-grade serous ([19] and S2 Table) to increase the power of the association analyses, a meta-analysis combining HRs for the association of variants with ovarian cancer risk in BRCA1 and BRCA2 was conducted. Variants available in only one of the datasets were excluded from the analysis (40 removed from BRCA1 and 187 from BRCA2). In the meta-analysis, the strongest associated variant was the genotyped SNP rs7046326 with a MAF of 0.25 and 0.24 in BRCA1 and BRCA2 mutation carriers, respectively. It displayed an HR = 0.74 (95% CI = 0.69–0.79; p = 6.2 × 10−21, Table 1 and Fig 2). The correlation with the top SNP in BRCA1 mutation carriers was 0.88 and with the top SNP in BRCA2 mutation carriers 0.69 (Table 2). In addition, 148 SNPs reached genome wide significance (p < 5×10−8) for the association with ovarian cancer risk, including the original GWAS hit rs3814113 (Fig 2). No evidence for heterogeneity in the associations for BRCA1 and BRCA2 mutation carriers was observed (Q-test, p-values >0.5, data not shown).
Identifying Independent Signals for the Association of 9p22 and Ovarian Cancer in BRCA1 and BRCA2 Mutation Carriers

In BRCA1 mutation carriers, no variant displayed evidence of an association at a \( p < 10^{-4} \) after analyses conditioning on rs10124837 (S1A Fig). The association with rs3814113, the original
Table 1. Associations between selected SNPs from 9p22.2 and ovarian cancer in BRCA1, BRCA2 and combined analysis of BRCA1/2 mutation carriers.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>R</th>
<th>E</th>
<th>T</th>
<th>Info</th>
<th>MAF</th>
<th>HR</th>
<th>95%CI</th>
<th>p-value</th>
<th>Info</th>
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<td>0.24</td>
<td>0.73</td>
<td>(0.79,0.68)</td>
<td>2.0E-16</td>
<td>0.98</td>
<td>0.23</td>
<td>0.74</td>
<td>(0.85,0.64)</td>
<td>2.4E-05</td>
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<td>0.73</td>
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<td>0.99</td>
<td>0.25</td>
<td>0.74</td>
<td>(0.69,0.79)</td>
<td>2.9E-16</td>
<td>1</td>
<td>0.24</td>
<td>0.74</td>
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<td>1.3E-05</td>
<td>0.90</td>
<td>0.74</td>
<td>(0.69,0.79)</td>
</tr>
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<td>1.3E-05</td>
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<td>0.25</td>
<td>0.74</td>
<td>(0.79,0.69)</td>
<td>4.4E-16</td>
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<td>0.24</td>
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<td>(0.79,0.68)</td>
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<td>0.9</td>
<td>0.27</td>
<td>0.77</td>
<td>(0.88,0.67)</td>
<td>2.4E-04</td>
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<td>0.74</td>
<td>(0.69,0.79)</td>
<td>1.0E-15</td>
<td>0.98</td>
<td>0.23</td>
<td>0.74</td>
<td>(0.64,0.85)</td>
<td>1.8E-05</td>
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<td>0.74</td>
<td>(0.69,0.79)</td>
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<td>(0.69,0.81)</td>
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<td>1</td>
<td>0.19</td>
<td>0.69</td>
<td>(0.59,0.80)</td>
<td>1.0E-06</td>
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<td>6.7E-06</td>
<td>0.37</td>
<td>0.76</td>
<td>(0.73,0.83)</td>
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</table>

Selected SNPs correspond to the 8 strongest associated in BRCA1 mutation carriers plus the strongest associated SNP in BRCA2 mutation carriers and the initial GWAS hit rs3814113. SNPs indicated in bold indicate the strongest associated in BRCA1 mutation carriers, the strongest associated in the BRCA1/2 meta-analysis, in BRCA2 mutation carriers and rs3814113. “R” and “E” correspond to reference and effector allele, respectively. “T” corresponds to genotyped, eSNP(p) displays the p-value for expressed Single Nucleotide Polymorphism association for the BNC2 gene based on whole blood tissue extracted from GTEx Portal (http://www.gtexportal.org/home/). “Info” quantifies the accuracy of the imputation. “MAF”, “HR” and “CI” correspond to minor allele frequency, hazard ration and confidence interval, respectively. P-Het corresponds to the p-value for testing heterogeneity between BRCA1 and BRCA2 coefficients of association.

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GWAS hit, became non-significant (p = 0.2) when rs10124837 was included as covariate in the model (S1A Fig and Table 3). Similarly, in BRCA2 mutation carriers no evidence of an association was observed for any variant after conditioning on rs62543585 (p > 10\(^{-4}\) S1B Fig). Neither rs3814113 nor rs10124837 were significant at p < 0.05 when rs62543585 was included as covariate in the model while the latter still displayed an association with p = 5\times 10^{-3} (S1B Fig and Table 3).

Taken together, these results indicate that in both BRCA1 and BRCA2 mutation carriers there is only one peak of association with ovarian cancer risk at 9p22.

**Association of 9p22 and Ovarian Cancer in BRCA1 and BRCA2 Mutation Carriers.**

SNPs with a likelihood ratio relative to the most significant variant greater than 100 and having an \(r^2 < 0.1\) with the index SNP were excluded from being potentially causative. In BRCA1 mutation carriers, this identified eight highly correlated SNPs (\(r^2 > 0.8\)), referred hereafter as the "BRCA1 peak". These variants clustered in a 20kb region around the transcription start site of BNC2 (positions: 16,847,520–16,891,647). The SNPs in this set displayed MAFs of 0.24–0.28 and imputation accuracy higher than 0.95 and two out of the eight were genotyped (Fig 1A and Table 1 and S4 Table).

In BRCA2 mutation carriers, 100 variants could not be rejected from being potentially causal. The MAFs for these SNPs varied from 0.15 to 0.34 and had pairwise correlations with the index SNP of greater than 0.4 (Fig 1B, S4 Table). The quality of imputation was \(>0.95\) for all except two variants (info = 0.68 and 0.46, S4 Table).

All except one (imputed SNP rs139555631) of the likely causal variants in BRCA1 mutation carriers were included in the set marking the potentially causal variants defined in BRCA2 mutation carriers. However, none of them were ranked within the top 60 associated variants in BRCA2 carriers. The index SNP (rs10124837 in BRCA1 mutation carriers was in linkage disequilibrium with the index SNP (rs62543583) in BRCA2 mutation carriers \(r^2 = 0.76\), Fig 1, Table 2).

The original GWAS hit, rs3814113, was within the set of the strongest associated SNPs in BRCA2 mutation carriers, but was rejected from being potentially causal in BRCA1 mutation carriers.

In the BRCA1/2 meta-analysis, eleven SNPs were the set of potentially causal variants, which included the eight identified in BRCA1 plus three only present in the BRCA2 set. These eleven variants were highly correlated with the lead SNP of the meta-analysis rs7046326 \(r^2 > 0.8\). Of note, the set excluded the original GWAS hit rs3814113 (Fig 2, S5 Table).

Intersection of variants exhibiting the strongest associations with genomic features derived from cultured ovarian and fallopian tube cells revealed several SNPs that may be functionally relevant in influencing risk. Fig 3 shows the location of the sets of SNPs associated with ovarian cancer risk in BRCA1 and BRCA2 mutation carriers relative to the BNC2 gene. Several potentially functional variants are predicted, including SNPs that lie in regulatory regions identified by FAIRE- and ChIP-seq.

### Table 2. Pairwise correlations \((r^2)\) between selected SNPs.

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs10124837</th>
<th>rs62543583</th>
<th>rs7046326</th>
<th>rs3814113</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10124837</td>
<td>1</td>
<td>0.76</td>
<td>0.88</td>
<td>0.56</td>
</tr>
<tr>
<td>rs62543583</td>
<td>0.76</td>
<td>1</td>
<td>0.69</td>
<td>0.48</td>
</tr>
<tr>
<td>rs7046326</td>
<td>0.88</td>
<td>0.69</td>
<td>1</td>
<td>0.49</td>
</tr>
<tr>
<td>rs3814113</td>
<td>0.56</td>
<td>0.48</td>
<td>0.49</td>
<td>1</td>
</tr>
</tbody>
</table>

DOI:10.1371/journal.pone.0158801.t002
candidate causal variants lies within a ~10 kb region likely to carry regulatory activity encompassing the BNC2 transcription start site. Multiple transcription factor motifs are altered by these variants (S6 Table). Although, no special features were observed for the variants in...
BRCA1 or BRCA1/2 meta-analysis (Fig 3), four of the eight candidate causal SNPs in BRCA1 mutation carriers are expressed single nucleotide polymorphism (eSNP) for the BNC2 gene in whole blood samples (Table 1, data extracted from GTex Portal http://www.gtexportal.org/home/).

Discussion

In this study, we performed fine-scale mapping of the 9p22.2 locus using dense genotype data from the iCOGS array in BRCA1 and BRCA2 mutation carriers of European ancestry. We identified a set of variants that provided stronger evidence of association than the original GWAS hit.

In BRCA1 mutation carriers, one independent set of eight highly correlated ($r^2 > 0.8$) SNPs could not be excluded as being potentially causal for the reported association with ovarian cancer, designated the "BRCA1 peak". The BRCA1 peak covers positions 16847520 to 16891647, which lie within or up to 20 kb upstream BNC2. Of note, the original GWAS hit rs3814113 was excluded from the candidate causal variants in this peak.

For BRCA2 mutation carriers, 100 correlated variants ($r^2 > 0.4$) could not be excluded as potentially causal ("BRCA2 peak"). The BRCA2 peak spanned positions 16847520 to 16915021, which are up to 44 kb upstream of BNC2 and more than 200 kb upstream of CNTLN. The increased number of variants in this case is most likely due to reduced statistical power, as the number of BRCA2 mutation carriers diagnosed with ovarian cancer was only one quarter of the number of affected BRCA1 carriers. The candidate causal SNPs in the BRCA2 peak were mostly contained within the BRCA2 peak but the strongest associated SNP in BRCA2 was excluded from the BRCA1 peak. The current analysis was underpowered to investigate whether the association in BRCA2 mutation carriers is driven by a different set of genetic variants.

Under the model of one shared causal variant explaining the association in both BRCA1 and BRCA2 mutation carriers, the meta-analysis would be expected to increase power for refining the set of potential causal variants. However, the combined analysis of BRCA1 and BRCA2 mutation carriers defined a set of eleven variants as potentially causal, which corresponded to the eleven strongest associated variants in BRCA1. This set included rs3814113 that was reported in the ovarian cancer GWAS [5]. The set of candidate causal variants included three additional SNPs that were confidently discarded on the basis of being less than 100 times likely to be causal relative to the strongest associated SNP in the analysis of BRCA1 carriers only.

Important differences emerged when we compared the patterns of association in the fine-scale mapping of 9p22.2 between BRCA1 mutation carriers and results for the most strongly associated SNPs in samples from the general population.

Table 3. Conditional associations for BRCA1 and BRCA2 top SNPs. The table shows the HR estimate, 95% CI and p-value for the conditional analysis adjusting for the lead SNP in the univariate analysis for BRCA1 (left hand side) or BRCA2 mutation carriers (right hand side). SNPs correspond to: rs10124837, the strongest associated in BRCA1; rs62543583, the strongest associated in BRCA2 mutation carriers; rs7046326, the strongest associated in BRCA1/2 meta-analysis; rs3814113, was the strongest associated variant in the initial GWAS analysis. "HR", hazard ratio; "CI", confidence interval.

<table>
<thead>
<tr>
<th>SNP</th>
<th>BRCA1 (adj. rs10124837)</th>
<th>BRCA2 (adj. rs62543583)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 95%CI p-value</td>
<td>HR 95%CI p-value</td>
</tr>
<tr>
<td>rs62543583</td>
<td>1.0 (0.76, 1.24) 0.99</td>
<td>0.67 (0.51, 0.88) 4.0x10^-3</td>
</tr>
<tr>
<td>rs10124837</td>
<td>0.8 (0.72, 0.88) 9.0x10^-6</td>
<td>0.99 (0.78, 1.27) 0.96</td>
</tr>
<tr>
<td>rs62543583</td>
<td>0.75 (0.61, 0.92) 5.0x10^-3</td>
<td>0.87 (0.74, 1.03) 0.11</td>
</tr>
<tr>
<td>rs3814113</td>
<td>0.87 (0.74, 1.03) 0.20</td>
<td>0.9 (0.86, 1.03) 0.20</td>
</tr>
<tr>
<td>rs10124837</td>
<td>0.8 (0.72, 0.88) 1.5x10^-5</td>
<td>0.9 (0.86, 1.03) 0.20</td>
</tr>
<tr>
<td>rs3814113</td>
<td>0.9 (0.86, 1.03) 0.20</td>
<td>0.9 (0.86, 1.03) 0.20</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pone.0158801.t003
Fine-mapping results based on iCOGS data from the Ovarian Cancer Association Consortium indicate that SNP rs3814113 remains the most strongly associated SNP at the 9p22.2 region with serous ovarian cancer, the original GWAS hit (personal communication). Based on our results, this SNP can be confidently rejected from the set of possible causal variants in \textit{BRCA1} mutation carriers, suggesting that the associations in \textit{BRCA1} mutation carriers and in the general population may be driven by different causal variants at the 9p22.2 locus. These results may indicate differences in the underlying causal mechanisms explaining the ovarian cancer associations between \textit{BRCA1} mutation carriers and the general population. In support of this possibility, differences in the association patterns with ovarian cancer between \textit{BRCA1} and the general population have been reported before. The 4q32.3 locus is associated with ovarian cancer risk in \textit{BRCA1} but not in \textit{BRCA2} mutation carriers or the general population [11], while the opposite is true for the locus 17q11.2 [21]. However, clearer patterns will hopefully emerge once the fine mapping of the 9p22.2 region in samples from the general population is completed.

As both signals lie in close proximity to the \textit{BNC2} gene, and some candidate causal SNPs are eSNPs for \textit{BNC2} in whole blood, they may modulate the expression of \textit{BNC2} through similar, or different, mechanisms. The possibility that the \textit{BRCA1} association signal may differ from that in the general population adds extra complexity and reinforces the value of fine-scale mapping in different populations. These subtle differences in the patterns of associations depending on the underlying genetic landscape may be difficult to uncover by means other

**Fig 3. Genomic features surrounding the 9p22.2 locus.** Illustration of the genomic region (chr9:16,839,835–16,924,468) encompassing peaks (shaded areas) containing candidate causal variants associated with ovarian cancer risk in \textit{BRCA1} and \textit{BRCA2} mutation carriers. Epigenomic data from Coetzee et al., (2015) [20] representing potential regulatory elements in ovarian cells (iOSE4 and iOSE11) and fallopian tube (FTSEC33) cells derived from formaldehyde assisted identification of regulatory elements sequencing (FAIRE-seq) and histone modification ChIP-seq are shown as black bars. Variants which overlap one of these features are coloured red. Data from the ENCODE project including histone modification ChIP-seq for three modifications (H3K4me1, H3K4me3, and H3K27ac) are shown as coloured histograms, as well as DNase hypersensitive site mapping and transcription factor ChIP-seq. The positions of all common SNPs from dbSNP build 142 are shown in the lowest track.

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than fine-scale mapping, and thus strengthens the value of this approach for generating hypotheses about the functional basis of different sets of variants.

This study cannot exclude the possibility that the actual causal variants were not included in the set of genotyped or well-imputed variants. However, the iCOGs array included variants specifically for fine-scale mapping of the 9p22.2 locus based on data from the 1000 Genomes Project and therefore the region coverage is expected to be high. The relatively low number of ovarian cancer cases with tumor morphology information did not allow performing stratified analyses by ovarian cancer histological subtype. Studies of ovarian tumours in women with BRCA1 or BRCA2 mutations have shown that BRCA1 and BRCA2 carriers predominantly develop serous disease [19,22]. Of the available data in CIMBA, 67% of all ovarian cancer tumours in our analyses were serous ovarian cancers. Our results are therefore more comparable with the associations for serous ovarian cancer in the general population. Larger studies will be required to assess whether the patterns of associations differ by ovarian cancer histological subtyped in BRCA1 and BRCA2 mutation carriers.

Having narrowed down the potential set of causal variants to only eight SNPs in BRCA1 mutation carriers will assist functional studies to identify the gene/s targeted by these variants. BNC2 is an obvious candidate gene, given that the putative causal variants are located in/around its transcription start site. Identifying more strongly associated variants with ovarian cancer in the 9p22.2 region relative to the initial GWAS hit in BRCA1 and BRCA2 mutation carriers will refine the cancer risks associated with this locus further. These novel variants can be included in polygenic risk scores for ovarian cancer and hence inform the identification of patients at greater risk of disease. The results may also help to deepen our understanding of the biology of ovarian cancer development in BRCA1 and BRCA2 mutation carriers, potentially leading to the development of more effective and personalized treatments.

Supporting Information
S1 Fig. Assessment for an independent signal for the association between SNPs in 9p22.2 and ovarian cancer risk in BRCA1 and BRCA2 mutation carriers. The colour code indicates the linkage disequilibrium with respect to the variant used for adjustment.
(TIFF)

S1 Table. List of the local Institutional Review Boards that provided ethical approval for this study.
(XLSX)

S2 Table. Characteristics of study participants.
(PDF)

S3 Table. Association of SNPs with ovarian cancer risk in BRCA1 and BRCA2 mutation carriers (p<0.01).
(XLSX)

S4 Table. SNPs within 100 times likely of being causal for the association with ovarian cancer in BRCA1 and BRCA2 mutation carriers. ‘T’ corresponds to genotyped; ‘Info’ measures the accuracy of the imputation; ‘Ref’ and ‘Eff’ correspond to reference and effector allele, respectively; ‘MAF’ to minor allele frequency, ‘HR’ hazard ratio and ‘CI’ confidence interval. Bold cells correspond to the strongest associated SNP in the indicated dataset. Green and violet text indicates the set of potentially causal variant/s in BRCA1 and BRCA2 mutation carriers, respectively.
(PDF)
S5 Table. SNPs within 100 times likely of being causal for the association with ovarian cancer in the meta-analysis of BRCA1 and BRCA2 mutation carriers. 'T' corresponds to genotyped; 'Ref' and 'Eff' correspond to reference and effector allele, respectively; 'MAF' to minimum allele frequency, 'HR' hazard ratio and 'CI' confidence interval. Bold cells correspond to the strongest associated SNP in the indicated dataset. Green, violet and orange text indicate those SNPs within 100 times likely of being the causal variant/s in BRCA1 and BRCA2 mutation carriers and their meta-analysis, respectively.

(PDF)

S6 Table. Genomic features for selected SNPs associated with ovarian cancer risk in BRCA2 mutation carriers.

(XLSX)

S1 Text. Full list of authors and affiliations.

(ODC)

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References


