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The contribution of deleterious germline mutations in *BRCA1, BRCA2* and the mismatch repair genes to ovarian cancer in the population

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ABSTRACT

Background: The aim of this study was to estimate the contribution of deleterious mutations in *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6* and *PMS2* to invasive epithelial ovarian cancer in the population.

Methods: The coding sequence and splice site boundaries of all six genes were amplified in germline DNA from 2,240 invasive EOC cases and 1,535 controls. Barcoded fragment libraries were sequenced using the Illumina GAII or HiSeq and sequence data for each subject de-multiplexed prior to interpretation. GATK and Annovar were used for variant detection and annotation. After quality control 2,222 cases (99.2 per cent) and 1,528 controls (99.5 per cent) were included in the final analysis.

Results: We identified 193 EOC cases (8.7 per cent) carrying a deleterious mutation in at least one gene compared to 10 controls (0.65 per cent). Mutations were most frequent in *BRCA1* and *BRCA2*, with 84 EOC cases (3.8 per cent) carrying a *BRCA1* mutation and 94 EOC cases (4.2 per cent) carrying a *BRCA2* mutation. The combined *BRCA1* and *BRCA2* mutation prevalence was 11 per cent in high-grade serous disease. Seventeen EOC cases carried a mutation in a mismatch repair gene, including 10 *MSH6* mutation carriers (0.45 per cent) and 4 *MSH2* mutation carriers (0.18 per cent).

Conclusions: At least one in ten women with high-grade serous EOC has a *BRCA1* or *BRCA2* mutation. The development of next generation sequencing technologies enables rapid mutation screening for multiple susceptibility genes at once, suggesting that routine clinical testing of all incidence cases should be considered.

Introduction

Epithelial ovarian cancer (EOC) is the most fatal gynaecological malignancy, resulting in approximately 140,000 deaths worldwide per year (1). Despite some recent advances in treatment, there have been only slight improvements in survival in patients diagnosed with EOC in over four decades. Approximately 70 per cent of EOC cases are diagnosed with advanced stage disease, in whom five-year survival is less than 30 per cent. By contrast, survival is over 90 per cent for patients with Stage I disease (2). Prophylactic salpingo-oophorectomy reduces the risk of ovarian/fallopian tube cancer in carriers of high-penetrance alleles of *BRCA1* or *BRCA2* by 75 - 96 per cent (3,4).

Individual susceptibility to EOC has a substantial inherited genetic component - women with a single-first degree relative with ovarian cancer have a 3-fold greater risk of developing the disease than women without a family history (5). The known ovarian cancer susceptibility genes, which include *BRCA1* and *BRCA2*, are estimated to explain less than 40 per cent of the excess familial risk of ovarian cancer. Genes other than *BRCA1* and *BRCA2* that confer >20 fold lifetime risk of EOC are unlikely to exist, given that *BRCA1* and *BRCA2* explain most multi-generation, multi-case EOC families (6,7).

Common, low penetrance susceptibility alleles also contribute to familial EOC risk, and genome wide association studies (GWAS) performed in large-scale, case-control studies with sample sizes in excess of 40,000 subjects have so far identified eleven confirmed ovarian cancer susceptibility alleles, each conferring relative risks of less than 1.5 (8-14). However, in combination, these alleles account for just 4 per cent of

the excess familial ovarian cancer risk (13) suggesting other susceptibility alleles are likely to exist. A wide variety of genetic models have been suggested to account for the "missing heritability" including the possibility that there are several rare alleles that confer relative risks greater than 3. Evidence for this has emerged through the recent identification of deleterious alleles in *RAD51C*, *RAD51D* and *BRIP1* that are associated with an increased risk of EOC (15-17). Truncating mutations in the DNA mismatch repair genes are also associated with modest risks of EOC (18,19). In particular mutations of *MLH1*, *MSH2*, *MSH6* and *PMS2* are reported to be associated with ovarian cancer as part of the Lynch Syndrome (18,20,21).

Many studies have investigated the contribution of *BRCA1* and/or *BRCA2* to EOC incidence in the population but most of these have involved fewer than 500 subjects (22). Only two studies have reported *BRCA1* and *BRCA2* mutation prevalence based on more than 1000 samples (23,24). Mutation prevalence reported in population-based studies that screened the full coding sequence varies widely from 3.4 per cent to 9.8 per cent for *BRCA1* and from 0.6 per cent to 5.7 per cent for *BRCA2* (22). A handful of studies have reported the prevalence of deleterious mutations in the MMR genes to be 0.5-3 per cent in EOC (21,25-28). Furthermore, no large studies have evaluated the frequency of deleterious mutations in these genes in non-cancer controls. The aim of this study was to establish the contribution of predicted deleterious mutations in the *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6* and *PMS2* to ovarian cancer risk in the population using targeted next generation sequencing in two large case-control studies.

Results

Sequence data for *BRCA1 BRCA2*, *MLH1*, *MSH2*, *MSH6* and *PMS2* were available in 2,222 cases and 1,528 controls after quality control. The clinical-pathological characteristics of cases in this study are presented in Table 1. Controls were individuals with no known diagnosis of ovarian or any other cancer.

We detected a total of 813 different variants of which 85 (10 per cent) were frameshift indels, 10 (1.2 per cent) were variants predicted by MaxEntScan (29) to affect gene splicing, 37 (4.6 per cent) were nonsense substitutions and 506 (62 per cent) were missense substitutions and 175 (22 percent) were synonymous substitutions. Of the missense substitutions, 24 had an alternate allele frequency greater than 2 per cent and were not considered deleterious. We classified six rare missense variants in *BRCA1* as deleterious, two in *BRCA2*, one in *MLH1* and two in *MSH6*. Three hundred and forty missense variants were predicted by SIFT/polyphen-2 to be neutral and 131 were predicted to have some functional effect. These were all considered unclassified. One nonsense substitution – *BRCA2* K3326X – had a carrier frequency of 2.0 per cent in controls and 2.5 per cent in the cases (P=0.31), and so was not considered deleterious.

Two hundred and three subjects carried at least one of the 143 different deleterious variants (henceforth mutations) identified. The mutation prevalence was 8.7 per cent in cases (193 out of 2,222) and 0.65 per cent in controls (10 out of 1,528). Details of each protein truncating mutation and potential deleterious missense/synonymous mutation we identified are given in Supplementary Table S1 and Supplementary Table S2, respectively. In EOC cases, we identified 84 *BRCA1* mutation carriers (3.8

per cent), 94 *BRCA2* mutation carriers (4.2 per cent), 10 *MSH6* mutation carriers (0.45 per cent), 4 *MSH2* mutation carriers (0.2 per cent), and 2 *MLH1* mutation carriers (0.1 per cent) and 1 *PMS2* mutation carrier (0.05 per cent) (Table 2). One case with a mucinous subtype ovarian cancer had a deleterious mutation in both *MSH6* and *BRCA1;* another case with a clear cell subtype ovarian cancer had a deleterious mutation in both *BRCA1* and *MLH1*. Of the deleterious mutations in controls, we identified one deleterious mutation in *BRCA1*, 4 in *BRCA2*, 2 in *MLH1* and 3 in *MSH6* (Table 2).

Thirty-one deleterious mutations were detected in more than one individual, with the most common, 4065_4068delTTGA in *BRCA1*, being found in seven individuals. The *BRCA1* mutation 5266dupC (known as 5382insC), which is relatively common in individuals of Eastern European origin, was identified in 5 individuals. Of the total 195 deleterious mutations in cases, 127 (65 per cent) were insertion or deletion frameshifts, 41 (21 per cent) were nonsense substitutions, 12 (6.2 per cent) were variants situated near exon/intron boundary that were predicted to affect gene splicing, and 15 (7.7 per cent) were previously reported pathogenic/likely pathogenic missense substitutions (30-33) (Supplementary Table S2). In controls, 4 mutations were frameshift indels, 4 were nonsense mutations and 2 variants that were predicted to affect splicing.

Previous studies have indicated that mutations located in specific regions of *BRCA1* (cDNA 2282-4066) (7,34) and *BRCA2* (cDNA 2807-6401, termed the Ovarian Cancer Cluster Region or OCCR) (7,35) are associated with a relatively higher risk of ovarian cancer compared to breast cancer. In our study, the proportion of *BRCA1* mutations located in this region was similar to that would be expected by chance if mutations

occurred evenly cross the coding region (33 per cent vs 32 per cent). The proportion of mutations in the OCCR region of *BRCA2* was significantly higher than that expected by chance (54 per cent vs 35 per cent, P=0.0017).

We compared the frequency of deleterious mutation carriers in cases and controls using an odds ratio. The odds ratio was 60 (95% confidence interval 10 - 2100) for *BRCA1*, 17 (6.3 - 63) for *BRCA2* and 2.3 (0.83 – 8.2) for MMR gene mutation carriers. The average cumulative risks of ovarian cancer by age 80 years are estimated to be 61 per cent (15 – 99 per cent) for *BRCA1*, 24 per cent (10 – 62 per cent) for *BRCA2* and 3.7 per cent (1.4 – 13 per cent) for MMR genes.

Associations with clinical and histopathological characteristics of ovarian cancer:

BRCA1 mutation carriers were diagnosed at a median age of 52 years (range 33-82 years); this compared to 57 years (range 33 – 84 years) for *BRCA2* carriers, 54 years (range 43-73 years) for MMR gene mutation carriers and 59 years (range 19 -91) for non-mutation carriers (Table 3). The proportion of cases of the serous subtype was higher in the Mayo Clinic case-series (74 per cent) than the SEARCH case-series (46 per cent) (Table 1). Of these, 34 per cent of SEARCH cases (N=451) and 72 per cent of Mayo Clinic cases (N=654) were classified as high-grade serous subtype. There are two primary reasons for this: first, the SEARCH cases had 16 per cent unspecified subtype derived from routine pathology report, whereas the Mayo Clinic cases all had central pathology review and only 5 per cent were unspecified; second, SEARCH included prevalent cases and so the proportion of good prognosis tumours (non-serous) will be higher. In total, 57 per cent of the cases were diagnosed with serous tumours; the remainder were a mix of endometrioid, clear cell, mucinous or

other/unknown subtypes (Table 1). The prevalence of deleterious mutations varied by histopathological subtype and by study (Table 2). In the combined results, 122 of the 178 *BRCA1* and *BRCA2* mutation carriers (69 per cent) were identified in patients diagnosed high-grade serous EOC, and *BRCA1*/*BRCA2* mutation prevalence in high-grade serous disease was 11 per cent compared to 5 per cent for other subtypes. A total of 17 MMR gene mutations were identified. Five EOC cases with MMR gene mutations were clear cell tumours, three were undifferentiated tumours, four were high-grade endometrioid tumours, two were high-grade serous tumours and one each of low-grade serous, low-grade-endometrioid and high-grade-mucinous tumour.

Information on breast and/or ovarian cancer history in first-degree relatives was available for 1,862 of 2,222 cases. A family history of breast cancer only was reported by 302 cases, 88 reported a family history of ovarian cancer only and 18 reported a family history of both breast and ovarian cancer. Of the 408 cases reporting a family history, 78 (19 per cent) carried a deleterious variant in either *BRCA1* or *BRCA2* compared to 70 mutation carriers out of 1,454 cases (4.8 per cent) with no family history (Table 4). Information on family history was available for 11 of the 17 MMR gene mutation carriers; five of these women reported a first- or second-degree relative with endometrial cancer and three reported a first- or second-degree relative with colorectal cancer.

Association between rare missense variants and ovarian cancer risk

In addition to the probable deleterious variants, we identified 471 unclassified missense variants across all six genes of which 131 are predicted to have a functional effect based on SIFT and polyphen-2. We compared the relative burden of the

possibly functional variants in cases and controls for each gene using the rare admixture maximum likelihood test (RAML) (36). We found little evidence for association of rare missense variation in any of these genes and ovarian cancer risk (all $p > 0.05$).

Discussion

This is the largest population-based ovarian cancer study to estimate the prevalence of mutations in *BRCA1*, *BRCA2* and the MMR genes reported to date. Overall, 8.7 per cent of epithelial ovarian cancer cases had a mutation in one of the six genes compared with 0.65 per cent in controls. We have almost certainly underestimated the true mutation prevalence in this series of genes, as some deleterious mutations will have been missed using our next generation sequencing approach. For example, we did not sequence the entire coding sequence in all individuals; the mean coverage for the targeted gene regions was 84 per cent ranging from 71 per cent- 92 per cent for the six genes at read depth at least 15 (Supplementary Table 3). In addition, some missense substitutions of uncertain significance, which were excluded from the prevalence estimates, may be true deleterious mutations. Finally, our PCR based method would not have been able to detect large genomic rearrangements, which we have previously shown account for ~10 per cent of mutations in *BRCA1*/*BRCA2* (7) and 5 to 20 per cent of all *MMR* gene mutations in Lynch syndrome families (37,38). By extrapolation, we calculate that this study may have underestimated the true prevalence of deleterious *BRCA1*, *BRCA2* and *MSH6* mutations by about 17, 24 and 30 per cent respectively which would increase the deleterious mutation prevalence estimates in EOC to 4.6 per cent for *BRCA1*, 5.6 per cent for *BRCA2* and 0.6 per cent for *MSH6*.

BRCA1 or *BRCA2* mutations were more common in the high-grade serous ovarian cancer subtype. This is consistent with the previous findings that DNA double strand break repair is associated with high-grade serous EOC (39). The estimated true

prevalence of *BRCA1* and *BRCA2* in high-grade serous ovarian cancer is 14.1 per cent: this is consistent with The Cancer Genome Atlas (TCGA) project, which identified germline *BRCA1/BRCA2* in 16 per cent of high-grade serous EOC cases (40).

The frequency of mutations in *MSH6* for EOC cases (10/2222 or 0.45 per cent) is similar to that reported by Walsh et al (2/360 or 0.55 per cent) (28). Similar to this study, and as previously reported for epithelial ovarian cancer cases in Lynch syndrome families (20), mutations in MMR genes were mostly of the non-serous subtype.

The frequency of mutations in *BRCA1* and *BRCA2* is somewhat lower than that reported in the two previously largest published studies (8 per cent in SEARCH, 8 per cent in Mayo Clinic cases, 13 per cent in Zhang et al (23) and 14 per cent in Alsop et al.(24)). However, the published data include data for large genomic rearrangements. In addition the ancestry of the populations is different - the three variants common in the Ashkenazi Jewish population accounted for 11 to 14 per cent of all deleterious variants identified in the two large published case series compared to 3 per cent in our study (Supplementary Table 4). The deleterious variant prevalence in the high grade serous subtype was substantially higher in the Australian cases series (23 per cent) than in SEARCH (14 per cent), Mayo Clinic cases (9 per cent) or the Canadian case series (18 per cent). The clinical characteristics of the four case series are broadly similar apart from a smaller proportion of serous cases in SEARCH and a higher proportion of cases diagnosed at age 60 or older in Mayo Clinic cases (Supplementary Table 5). Thus, reasons for this difference are not clear.

In summary, we estimate that *BRCA1* and *BRCA2* together are major contributors to at least one in every 12 invasive EOC cases, and one in every 10 high-grade serous EOC cases. Mutations in MMR genes are responsible for at least one in every 131 EOC cases. The recently published NICE Guideline for the management of women with a family history of breast cancer has recommended that individuals with a 10 per cent or greater probability of carrying a deleterious mutation in *BRCA1* or *BRCA2* should be offered genetic testing (NICE clinical guidelines Issued: June 2013, CG164). Thus, all women with high-grade serous ovarian would be eligible for *BRCA1* and *BRCA2* testing under the NICE Guideline. Given that PARP inhibitors have been shown to improve progression-free survival among patient with platinum-sensitive, relapsed, high-grade serous ovarian cancer (41), such testing may have important implications for the patient. The potential implications for the family of *BRCA1* or *BRCA2* mutation carriers are clearer, as cascade testing of unaffected female relatives will identify unaffected carriers for whom the benefits of prophylactic salpingo-oophorectomy in reducing risk of incident ovarian, fallopian tube and peritoneal cancer are well established. Furthermore, it will be feasible to use a targeted sequencing approach for clinical testing of multiple genes in a single assay to replace the conventional clinical genetic testing which was done gene by gene with relatively high cost. The widespread application of panel sequencing in unselected women in the UK population and the provision of prophylactic salpingo-oophorectomy to mutation carriers has the potential to reduce the incidence of epithelial ovarian cancer by 8 per cent.

Materials and Methods

Study subjects

Confirmed invasive EOC cases and unaffected controls were from two case-controls studies: the population based SEARCH study (1321 cases/1389 controls) from the United Kingdom, and the hospital-based Mayo clinic study (919 cases/146 controls) from the USA. The studies have been previously described (42). Briefly, the SEARCH ovarian cancer study comprises 1321 invasive epithelial ovarian cancer cases <75 years from East Anglian West Midlands & Trent regions of England. Prevalent cases diagnosed between 1991-1998; incident cases diagnosed 1998 onwards. All eligible cases were identified through Cancer Registry. Pathology information was abstracted from routine pathology reports. Cancer free controls are from a population based controls series from East Anglia (UK) and frequency matched to cases on age. The Mayo Clinic ovarian cancer study comprises 919 cases diagnosed from 2000 onwards at Mayo Clinic, for whom a diagnosis of histologically-confirmed primary epithelial ovarian cancer was ascertained within one year of consent. The pathology of all eligible cases were centrally reviewed by a group of gynecologic pathologists. Controls were frequency matched to cases on race and age from the same study area. Based on data from single nucleotide polymorphism genotyping, 2,214 cases and 1,518 controls were of European ancestry (13). For quality control purposes we also included 43 duplicate samples and 34 positive controls known to be *BRCA1* or *BRCA2* mutation carriers. Both studies have ethics committee approval, and all study subjects provided written, informed consent.

Sequencing library preparation and sequencing

We used the 48.48 Fluidigm Access Arrays™ for target sequence enrichment. This method uses a 4-primer chemistry that allows the addition of barcode and adapter sequences to small target regions (<210bp) during up to 10-plex PCR amplification. A total of 338 primer-pairs were designed to cover the exons and splice sites of *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6* and *PMS2* (Supplementary Table S3). The combined sequencing target for these six genes was about 28kb with some of the sequence being covered by multiple amplicons. The primer design achieved more than 94 per cent coverage of the coding exons and potential splice site (i.e. 20 bp in the intron for 3' acceptor sites and 6 bp in the intron for donor 5' sites) sequence for each gene. Sequencing library preparation used 1.25µl germline DNA at 75ng/µl according to the manufacturer's protocol (Fluidigm, San Francisco, CA, US). Sequencing libraries were quantified using a KAPA library quantification kits with specific probes for the ends of the adapters according to the manufacturer's protocol (Kapabiosystems, Boston, MA, US). The sequence library was sequenced using either the single end sequencing on the Illumina GAII (1,443 samples) or paired end sequencing on the Illumina HiScan (2,332 samples) according to the manufacturer's protocol (Illumina Inc, San Diego, CA, US). Sample barcoding enabled 384 individual's sample to be sequenced on each lane of the sequencer.

Data analysis

Sequenced reads were de-multiplexed using standard Illumina software. We used the Burrows-Wheeler Aligner (BWA) (43) for sequencing read alignment against the human genome reference sequence (GRCh37, UCSC hg19). The Genome Analysis Toolkit (GATK) (44) was then used for base quality-score recalibration, local insertion/deletion (indel) realignment, substitution and indel discovery. Variants

were only considered if they satisfied the set of recommended GATK filters, as described in the GATK best practices guide. ANNOVAR (45) was used to annotate the sequence variation detected. We used PolyPhen-2 (46) and SIFT (47) to predict the function of missense variants. We used MaxEntScan (29) to predict the pathogenic potential of putative splicing mutations in sequences from 3 bp in the exon to 20 bp in the intron for 3' acceptor sites and 3 bp in the exon and 6 bp in the intron for the donor 5' sites. MaxEntScan provides a score for the strength of the splice site and enables the scores for the consensus and variant sequences to be compared. It has been suggested that a score for a variant sequence 20 per cent lower than the consensus sequence score as likely to result in abnormal splicing (48). However, after applying this criterion we classified multiple splice site variants as deleterious in both cases and controls. We therefore applied a more stringent criterion of 40 per cent lower, which identified 17 variants, 16 of these occurring in cases.

Variant alternate allele frequency was defined as the fraction of alternative allele reads compared to the total number of reads at the variant locus. We used the variants called in the sequence data from the 43 duplicate samples and additionally the *BRCA1* and *BRCA2* positive controls to define thresholds, based on coverage and alternate allele frequency, for definitive variant calling in the full data set. Variants with depth < 15 were not called. Alternate allele heterozygotes were called if (1) depth ≥500 and alternative allele frequency ≥10 per cent; (2) 250 ≤ depth <500 and alternative allele frequency ≥15 per cent; (3) 30 ≤ depth <250 and alternative allele frequency ≥20 per cent; (4) 15 ≤ depth <30 and alternative allele frequency ≥30 per cent. Samples with fewer than 80 per cent of amplicons covered at a read depth of ≥ 15 were excluded from subsequent analyses (a total of 7 controls and 18 cases failed in both sequencing

runs). We defined deleterious variants as those that are predicted to result in protein truncation (frame shift indels, consensus splice site substitutions and nonsense substitutions) or those missense mutations that have been previously reported or classified (e.g http://www.insight-group.org/ for MMR missense variants classification) or predicated by MaxEntScan as deleterious.

The median read depth for our targeted sequencing was 102 (IQR 91– 115) and 548 (IQR 492-626) for Illumina GAII and HiSeq, respectively. Eighty-two per cent of targeted sequence base had read depth ≥30 and 85 per cent had read depth ≥15. The average sequencing coverage for these six genes is summarized in Supplementary Table S3. The concordance rate for the 43 duplicates was 100 per cent. Thirty-one of 34 (91 per cent) known *BRCA1* or *BRCA2* positive controls were called correctly. Three mutations were missed because the read depth of the relevant amplicon was less than 15.

Mutation validation

Two hundred and five potentially deleterious variants were identified. We inspected the sequence alignments for all the deleterious variants using the Integrative Genomic viewer (IGV) (49). Ninety-four per cent (193/205) of these had an alternative allele read frequency ≥30 per cent. We validated 24 deleterious variants, including all variants with an alternative allele read frequency of less than 30 per cent (n=12) by PCR amplification and Sanger sequencing.

Unclassified variant analysis

We also identified multiple unclassified, but putative functional missense variants

with allele frequencies of less than two per cent in the samples. Because the statistical power to detect single rare alleles by association, even with large sample sizes is modest, we performed burden tests, which combines the information across multiple variants to increase statistical power. We classified variants with frequency ≤2 per cent into three groups: (1) deleterious variants as defined previously; (2) variants predicted to have a damaging effect on protein function - SIFT score <0.05 and polyphen-2 classified as possibly damaging/probably damaging; (3) variants with probable benign effects. We used the RAML (36) to test for the association of uncommon missense variants (MAF≤ 2 per cent) with ovarian cancer risks on a gene-by-gene basis. RAML takes account of variants that increase or decrease risk. Only subjects with a call rate greater than 80 per cent and variants with a call rate greater than 80 per cent with genotype frequencies consistent with Hardy-Weinberg equilibrium ($P>10^{-5}$) were included in these analyses.

Funding

This work was funded by Cancer Research UK (C490/A10119 and C490/A10124) (SEARCH) and National Institute of Health (R01 CA122443), P50-CA136393, Fred C. and Katherine B. Andersen Foundation (Mayo Clinic study), Cancer Research UK grant C1005/A12677 and National Institute of Health grant R01 CA178535 (USC), and the UK National Institute for Health Research Biomedical Research Centre at the University of Cambridge,

Acknowledgements

We thank all the study participants who contributed to this study and all the researchers, clinicians and technical and administrative staff who have made possible this work. In particular, we thank: Marie Mack, Craig Luccarini, Caroline Baynes, the SEARCH team and Eastern Cancer Registration and Information Centre (SEARCH).

Conflict of Interest Statement

No potential conflicts of interest are declared.

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Table 1. Characteristics of the ovarian cancer subjects (N = 2,222)

	SEARCH (N = 1310)	Mayo Clinic (N= 912)	Total (N)
Mean age at diagnosis, years (range)	55.9 (19-74)	62.5 (23-91)	58.6 (19-91)
Morphology			
Serous	600 (46%)	677 (74%)	1277 (57%)
Endometrioid	212 (16%)	110 (12%)	322 (14%)
Clear Cell	137 (10%)	55 (6.0%)	192 (8.6%)
Mucinous	132 (10%)	25 (3.0%)	157 (7.1%)
Other/Unknown	229 (16%)	45 (5.0%)	274 (12%)
Stage			
I	431 (33%)	141 (15%)	572 (26%)
II	121 (9%)	51 (5.6%)	172 (7.7%)
III	368 (28%)	538 (59%)	1143 (51%)
IV	66 (5.0%)	171 (19%)	237 (11%)
Unknown	324 (25%)	11 (1.2%)	335 (15%)
Grade			
Low grade	169 (13%)	128 (14%)	297 (13%)
High grade	714 (55%)	754 (83%)	1468 (66%)
Unknown	427 (33%)	30 (3%)	457 (21%)

Table 2. Number of mutations identified in 6 genes by histology subtype

	Control (n=1528)		SEARCH cases (n=1310)				Mayo Clinic cases (n=912)				Cases combined (n=2222)			
	n	%	High-grade serous	%	Other	%	High-grade serous	%	Other	%	High-grade serous	%	Other	%
Non-carrier	1518	99.4	388	86	803	93.5	593	91	245	95	981	89	1,048	94
Mutation carrier														
Any	10	0.6	63	14	56	6.5	61	9.3	13	5	124	11	69	6
BRCA1	1	0.07	25	5.5	19	2.2	33	5.1	7	2.7	58	5.3	26	2.3
BRCA2	4	0.3	38	8.4	26	3.0	26	4.0	4	1.6	64	5.8	30	2.7
*MMR**	5	0.3	0	0	13	1.5	2	0.3	2	0.78	2	0.18	15	1.3
Total	1,528	100	451	100	859	100	654	100	258	100	1,105	100	1,117	100

*one case had both *BRCA1* and *MLH1* mutation and another case had both *BRCA1* and *MSH6* mutation.

Table 3. Mutation status by age of disease onset

Mutation status	Age at diagnosis (n, %)			
	< 40yrs	40-49 yrs	50-59yrs	≥ 60yrs
Non-carrier (n= 2029)	94 (5)	286 (14)	648 (32)	1,001 (49)
Mutation carrier (n= 193)	6 (3)	50 (27)	76 (39)	61 (31)
BRCA1	4 (5)	29 (35)	28 (33)	23 (27)
BRCA2	2 (2)	16 (17)	42(45)	34 (36)
*MLH1/MSH2/MSH6/PMS2**	0 (0)	6 (35)	6 (35)	5 (29)

*one case had both *BRCA1* and *MLH1* mutation and another case had both *BRCA1* and *MSH6* mutation.

Table 4. Mutation status by 1st degree of family history of ovarian/breast cancer in ovarian cancer cases

Gene	Without FH (n= 1454)	%	With FH (n=408)					
			OvFH[1]	%	BrFH[2]	%	OvBrFH[3]	%
Non-carrier	1374	94	63	72	251	83	12	67
Mutation carrier	80	5.5	25	26	51	16	6	33
BRCA1	37	2.5	13	15	19	6.3	5	28
BRCA2	33	2.3	10	11	30	10	1	5.6
*MLH1**	2	0.14	0	0	0	0	0	0
MSH2	2	0.14	0	0	1	0.33	0	0
*MSH6**	7	0.48	2	2.3	1	0.33	0	0
PMS2	1	0.07	0	0	0	0	0	0

[1] OvFH: ovarian cancer family history only; [2] BrFH: breast cancer family history only; [3] OvBrFH: both ovarian and breast cancer family history. *one case had both *BRCA1* and *MLH1* mutation and another case had both *BRCA1* and *MSH6* mutation.

Supplementary Table S1: Catalogue of protein truncating mutations found in the study

Status	Gene	Exon	cDNA	Protein	MutationType	Refage	Morphology
control	*BRCA1*	8	c.520delC	p.Q174fs	fs del	62	NA
control	*BRCA2*	11	c.3158T>G	p.L1053*	nonsense	54	NA
control	*BRCA2*	11	c.2330dupA	p.D777fs	fs ins	55	NA
control	*BRCA2*	8	c.658_659delGT	p.V220fs	fs del	55	NA
control	*BRCA2*	3	c.145G>T	p.E49*	nonsense	52	NA
control	*MLH1*	18	c.2080G>T	p.E694*	nonsense	59	NA
control	*MLH1*	12	c.1333C>T	p.Q445*	nonsense	37	NA
control	*MSH6*	4	c.1629_1630delAA	p.K543fs	fs del	48	NA
case	*BRCA1*	2	c.34C>T	p.Q12*	nonsense	50	clear cell
case	*BRCA1*	I_5	c.213-11T>G	-	splicing	57	serous
case	*BRCA1*	7	c.427G>T	p.E143*	nonsense	50	serous
case	*BRCA1*	7	c.427G>T	p.E143*	nonsense	56	serous
case	*BRCA1*	7	c.427G>T	p.E143*	nonsense	43	serous
case	*BRCA1*	7	c.427G>T	p.E143*	nonsense	33	undifferentiated
case	*BRCA1*	I_7	c.442-3_442-3delT	-	splicing	62	serous
case	*BRCA1*	I_10	c.670+1G>A	-	splicing	63	serous
case	*BRCA1*	11	c.823_824insTA	p.G275fs	fs ins	82	clear cell
case	*BRCA1*	11	c.843_846delCTCA	p.S281fs	fs del	51	serous
case	*BRCA1*	11	c.843_846delCTCA	p.S281fs	fs del	49	serous
case	*BRCA1*	11	c.929delA	p.Q310fs	fs del	35	serous
case	*BRCA1*	11	c.929delA	p.Q310fs	fs del	51	undifferentiated
case	*BRCA1*	11	c.929delA	p.Q310fs	fs del	62	serous
case	*BRCA1*	11	c.1039_1040delCT	p.L347fs	fs del	44	serous
case	*BRCA1*	11	c.1374delC	p.D458fs	fs del	62	clear cell
case	*BRCA1*	11	c.1504_1508delTTAAA	p.L502fs	fs del	44	serous
case	*BRCA1*	11	c.1512dupT	p.K505fs	nonsense	40	serous
case	*BRCA1*	11	c.1556delA	p.K519fs	fs del	52	serous
case	*BRCA1*	11	c.1687C>T	p.Q563*	nonsense	48	serous
case	*BRCA1*	11	c.1823_1826delAGAA	p.K608fs	fs del	57	serous
case	*BRCA1*	11	c.1961dupA	p.K654fs	fs ins	55	serous
case	*BRCA1*	11	c.1961dupA	p.K654fs	fs ins	55	serous
case	*BRCA1*	11	c.2035A>T	p.K679*	nonsense	46	serous
case	*BRCA1*	11	c.2071delA	p.R691fs	fs del	55	serous
case	*BRCA1*	11	c.2071delA	p.R691fs	fs del	53	endometrioid
case	*BRCA1*	11	c.2071delA	p.R691fs	fs del	43	serous
case	*BRCA1*	11	c.2188G>T	p.E730*	nonsense	42	endometrioid
case	*BRCA1*	11	c.2188G>T	p.E730*	nonsense	64	mixed cell
case	*BRCA1*	11	c.2217dupA	p.V740fs	fs ins	41	serous
case	*BRCA1*	11	c.2457delC	p.S819fs	fs del	61	serous
case	*BRCA1*	11	c.2475delC	p.D825fs	fs del	57	serous
case	*BRCA1*	11	c.2678delA	p.K893fs	fs del	43	mucinous
case	*BRCA1*	11	c.2681_2682delAA	p.K894fs	fs del	47	endometrioid
case	*BRCA1*	11	c.2681_2682delAA	p.K894fs	fs del	55	undifferentiated
case	*BRCA1*	11	c.2681_2682delAA	p.K894fs	fs del	65	serous
case	*BRCA1*	11	c.2761C>T	p.Q921*	nonsense	54	serous
case	*BRCA1*	11	c.2857dupT	p.C953fs	fs ins	47	serous
case	*BRCA1*	11	c.2940delA	p.I980fs	fs del	52	serous
case	*BRCA1*	11	c.2989_2990dupAA	p.N997fs	fs ins	45	serous
case	*BRCA1*	11	c.3005delA	p.N1002fs	fs del	59	undifferentiated

case	*BRCA1*	11	c.3048_3052dupTGAGA	p.N1018fs	fs ins	56	serous
case	*BRCA1*	11	c.3205delC	p.Q1069fs	fs del	46	serous
case	*BRCA1*	11	c.3254_3255dupGA	p.L1086fs	fs ins	67	serous
case	*BRCA1*	11	c.3331_3334delCAAG	p.Q1111fs	fs del	66	serous
case	*BRCA1*	11	c.3627dupA	p.E1210fs	fs ins	46	serous
case	*BRCA1*	11	c.3748G>T	p.E1250*	nonsense	56	serous
case	*BRCA1*	11	c.3756_3759delGTCT	p.L1252fs	fs del	48	serous
case	*BRCA1*	11	c.3756_3759delGTCT	p.L1252fs	fs del	45	mixed cell
case	*BRCA1*	11	c.3817C>T	p.Q1273*	nonsense	69	serous
case	*BRCA1*	11	c.4035delA	p.E1345fs	fs del	75	serous
case	*BRCA1*	11	c.4065_4068delTCAA	p.N1355fs	fs del	36	serous
case	*BRCA1*	11	c.4065_4068delTCAA	p.N1355fs	fs del	45	serous
case	*BRCA1*	11	c.4065_4068delTCAA	p.N1355fs	fs del	58	undifferentiated
case	*BRCA1*	11	c.4065_4068delTCAA	p.N1355fs	fs del	65	serous
case	*BRCA1*	11	c.4065_4068delTCAA	p.N1355fs	fs del	66	serous
case	*BRCA1*	11	c.4065_4068delTCAA	p.N1355fs	fs del	49	serous
case	*BRCA1*	11	c.4065_4068delTCAA	p.N1355fs	fs del	59	undifferentiated
case	*BRCA1*	I_11	c.4096+3A>G	-	splicing	58	serous
case	*BRCA1*	12	c.4165_4166delAG	p.S1389fs	fs del	44	serous
case	*BRCA1*	12	c.4183C>T	p.Q1395*	nonsense	46	serous
case	*BRCA1*	15	c.4574_4575delAA	p.Q1525fs	fs del	39	undifferentiated
case	*BRCA1*	I_18	c.5152+5G>T	-	splicing	64	undifferentiated
case	*BRCA1*	19	c.5153G>A	p.W1718*	nonsense	50	mixed cell
case	*BRCA1*	19	c.5177_5180delGAAA	p.R1726fs	fs del	65	serous
case	*BRCA1*	20	c.5266dupC	p.Q1756fs	fs ins	46	serous
case	*BRCA1*	20	c.5266dupC	p.Q1756fs	fs ins	57	serous
case	*BRCA1*	20	c.5266dupC	p.Q1756fs	fs ins	64	serous
case	*BRCA1*	20	c.5266dupC	p.Q1756fs	fs ins	50	serous
case	*BRCA1*	20	c.5266dupC	p.Q1756fs	fs ins	46	serous
case	*BRCA1*	24	c.5503C>T	p.R1835*	nonsense	44	undifferentiated
case	*BRCA1*	24	c.5503C>T	p.R1835*	nonsense	54	mixed cell
case	*BRCA1*	24	c.5558dupA	p.Y1853fs	nonsense	52	serous
case	*BRCA2*	2	c.22_23delAG	p.R8fs	fs del	47	undifferentiated
case	*BRCA2*	4	c.379delG	p.A127fs	fs del	33	serous
case	*BRCA2*	I_6	c.517-2A>G	-	splicing	58	undifferentiated
case	*BRCA2*	8	c.658_659delGT	p.V220fs	fs del	62	serous
case	*BRCA2*	9	c.754_757delGACA	p.D252fs	fs del	58	clear cell
case	*BRCA2*	10	c.1029delA	p.K343fs	fs del	62	Other
case	*BRCA2*	10	c.1029delA	p.K343fs	fs del	38	serous
case	*BRCA2*	10	c.1265delA	p.N422fs	fs del	56	undifferentiated
case	*BRCA2*	10	c.1875_1876delTT	p.F625fs	fs del	84	serous
case	*BRCA2*	11	c.2330dupA	p.D777fs	fs ins	59	serous
case	*BRCA2*	11	c.2588dupA	p.N863fs	fs ins	63	serous
case	*BRCA2*	11	c.2808_2811delACAA	p.K936fs	fs del	55	serous
case	*BRCA2*	11	c.2808_2811delACAA	p.K936fs	fs del	45	endometrioid
case	*BRCA2*	11	c.2808_2811delACAA	p.K936fs	fs del	66	serous
case	*BRCA2*	11	c.2899_2900delCT	p.L967fs	fs del	52	serous
case	*BRCA2*	11	c.3070_3071delAT	p.I1024fs	fs del	68	serous
case	*BRCA2*	11	c.3405C>A	p.Y1135*	nonsense	50	endometrioid
case	*BRCA2*	11	c.3599_3600delGT	p.C1200fs	fs del	47	serous
case	*BRCA2*	11	c.3599_3600delGT	p.C1200fs	fs del	73	serous
case	*BRCA2*	11	c.3599_3600delGT	p.C1200fs	fs del	51	endometrioid
case	*BRCA2*	11	c.3680_3681delTG	p.L1227fs	fs del	53	endometrioid
case	*BRCA2*	11	c.3689delC	p.S1230fs	fs del	48	endometrioid

case	*BRCA2*	11	c.3785C>G	p.S1262*	nonsense	64	endometrioid
case	*BRCA2*	11	c.3847_3848delGT	p.V1283fs	fs del	81	serous
case	*BRCA2*	11	c.3847_3848delGT	p.V1283fs	fs del	48	serous
case	*BRCA2*	11	c.3865_3868delAAAT	p.K1289fs	fs del	53	serous
case	*BRCA2*	11	c.3873delA	p.Q1291fs	fs del	67	endometrioid
case	*BRCA2*	11	c.4154C>G	p.S1385*	nonsense	60	serous
case	*BRCA2*	11	c.4211_4215delCAAAT	p.S1404fs	fs del	63	serous
case	*BRCA2*	11	c.4284dupT	p.F1428fs	fs ins	58	serous
case	*BRCA2*	11	c.4284dupT	p.F1428fs	fs ins	50	serous
case	*BRCA2*	11	c.4398_4402delACATT	p.L1466fs	fs del	66	serous
case	*BRCA2*	11	c.4472_4475delTGAA	p.L1491fs	fs del	54	serous
case	*BRCA2*	11	c.4477_4480delGAAA	p.E1493fs	fs del	52	serous
case	*BRCA2*	11	c.4477_4480delGAAA	p.E1493fs	fs del	49	serous
case	*BRCA2*	11	c.4477_4480delGAAA	p.E1493fs	fs del	58	undifferentiated
case	*BRCA2*	11	c.4477_4480delGAAA	p.E1493fs	fs del	49	serous
case	*BRCA2*	11	c.4477_4480delGAAA	p.E1493fs	fs del	47	serous
case	*BRCA2*	11	c.4477_4480delGAAA	p.E1493fs	fs del	52	endometrioid
case	*BRCA2*	11	c.4576dupA	p.T1526fs	fs ins	64	serous
case	*BRCA2*	11	c.4631dupA	p.N1544fs	fs ins	51	serous
case	*BRCA2*	11	c.4877_4878delAA	p.N1626fs	fs del	50	serous
case	*BRCA2*	11	c.4965C>G	p.Y1655*	nonsense	62	serous
case	*BRCA2*	11	c.5073dupA	p.K1691fs	fs ins	49	serous
case	*BRCA2*	11	c.5213_5216delCTTA	p.T1738fs	fs del	42	serous
case	*BRCA2*	11	c.5238dupT	p.S1746fs	fs ins	54	serous
case	*BRCA2*	11	c.5290_5291delTC	p.S1764fs	fs del	58	serous
case	*BRCA2*	11	c.5304_5305delTT	p.L1768fs	fs del	54	serous
case	*BRCA2*	11	c.5351_5352delAA	p.N1784fs	fs del	64	serous
case	*BRCA2*	11	c.5351_5352delAA	p.N1784fs	fs del	56	serous
case	*BRCA2*	11	c.5351_5352delAA	p.N1784fs	fs del	58	clear cell
case	*BRCA2*	11	c.5351_5352delAA	p.N1784fs	fs del	57	serous
case	*BRCA2*	11	c.5576_5579delTTAA	p.I1859fs	fs del	65	serous
case	*BRCA2*	11	c.5682C>G	p.Y1894*	nonsense	49	clear cell
case	*BRCA2*	11	c.5864C>A	p.S1955*	nonsense	71	serous
case	*BRCA2*	11	c.5909C>A	p.S1970*	nonsense	56	serous
case	*BRCA2*	11	c.5909C>A	p.S1970*	nonsense	52	serous
case	*BRCA2*	11	c.5909C>A	p.S1970*	nonsense	51	undifferentiated
case	*BRCA2*	11	c.6037A>T	p.K2013*	nonsense	68	serous
case	*BRCA2*	11	c.6052_6053delAG	p.S2018fs	fs del	62	serous
case	*BRCA2*	11	c.6079dupA	p.R2027fs	fs ins	50	serous
case	*BRCA2*	11	c.6276_6277delTT	p.L2092fs	fs del	43	serous
case	*BRCA2*	11	c.6276_6277delTT	p.L2092fs	fs del	54	serous
case	*BRCA2*	11	c.6276_6277delTT	p.L2092fs	fs del	60	serous
case	*BRCA2*	11	c.6276_6277delTT	p.L2092fs	fs del	74	serous
case	*BRCA2*	11	c.6624delT	p.N2208fs	fs del	58	undifferentiated
case	*BRCA2*	11	c.6719delT	p.L2240fs	fs del	49	serous
case	*BRCA2*	11	c.6815_6816delGA	p.R2272fs	fs del	42	mucinous
case	*BRCA2*	14	c.7069_7070delCT	p.L2357fs	fs del	68	undifferentiated
case	*BRCA2*	14	c.7069_7070delCT	p.L2357fs	fs del	69	serous
case	*BRCA2*	14	c.7069_7070delCT	p.L2357fs	fs del	65	other
case	*BRCA2*	14	c.7069_7070delCT	p.L2357fs	fs del	57	serous
case	*BRCA2*	14	c.7069_7070delCT	p.L2357fs	fs del	65	serous
case	*BRCA2*	14	c.7069_7070delCT	p.L2357fs	fs del	60	serous
case	*BRCA2*	14	c.7312delG	p.D2438fs	fs del	48	endometrioid
case	*BRCA2*	14	c.7425delA	p.E2475fs	fs del	64	serous

case	*BRCA2*	15	c.7480C>T	p.R2494*	nonsense	64	serous
case	*BRCA2*	15	c.7566_7567delTC	p.S2522fs	fs del	57	undifferentiated
case	*BRCA2*	16	c.7762delA	p.I2588fs	fs del	59	serous
case	*BRCA2*	16	c.7762delA	p.I2588fs	fs del	50	serous
case	*BRCA2*	17	c.7934delG	p.R2645fs	fs del	57	serous
case	*BRCA2*	I_17	c.7976+5G>C	-	splicing	59	serous
case	*BRCA2*	I_18	c.8331+2T>C	-	splicing	59	serous
case	*BRCA2*	21	c.8633_8634delAA	p.E2878fs	fs del	50	serous
case	*BRCA2*	21	c.8633_8634delAA	p.E2878fs	fs del	54	undifferentiated
case	*BRCA2*	24	c.9196C>T	p.Q3066*	nonsense	48	serous
case	*BRCA2*	25	c.9294C>G	p.Y3098*	nonsense	56	serous
case	*BRCA2*	25	c.9294C>G	p.Y3098*	nonsense	61	serous
case	*BRCA2*	25	c.9382C>T	p.R3128*	nonsense	59	endometrioid
case	*BRCA2*	25	c.9434_9435delTG	p.V3145fs	fs del	51	mixed cell
case	*BRCA2*	27	c.10107dupT	p.T3369fs	fs ins	62	serous
case	*MLH1*	12	c.1333C>T	p.Q445*	nonsense	82	clear cell
case	*MSH2*	I_11	c.1759+1G>A	-	splicing	46	clear cell
case	*MSH2*	I_12	c.2006-5T>A	-	splicing	73	serous
case	*MSH6*	3	c.467C>G	p.S156*	nonsense	70	serous
case	*MSH6*	4	c.2150_2153delTCAG	p.V717fs	fs del	49	undifferentiated
case	*MSH6*	4	c.2194C>T	p.R732*	nonsense	43	clear cell
case	*MSH6*	4	c.2680C>T	p.Q894*	nonsense	43	undifferentiated
case	*MSH6*	4	c.2731C>T	p.R911*	nonsense	47	undifferentiated
case	*MSH6*	4	c.2736G>A	p.W912*	nonsense	43	mucinous
case	*MSH6*	4	c.3103C>T	p.R1035*	nonsense	56	clear cell
case	*MSH6*	4	c.642C>G	p.Y214*	nonsense	54	endometrioid
case	*MSH6*	6	c.3514dupA	p.R1172fs	fs ins	58	clear cell
case	*MSH6*	8	c.3758dupT	p.V1253fs	fs ins	54	endometrioid
case	*PMS2*	11	c.1831dupA	p.I611fs	fs ins	53	endometrioid

[a] fs del: frameshift deletion; fs ins: frameshift insertion. [b] refage: interview age for control and diagnosis age for case. Accession number as follow: *BRCA1*, NM_007294; *BRCA2*, NM_000059; *MLH1*, NM_000249; *MSH2*, NM_000251, *MSH6*, NM_000179; *PMS2*, NM_000535.

Supplementary Table S2: Catalogue of deleterious missense/synonymous variants found in the study

status	gene	exon	cDNA	protein	refage	Morphology	Mutation type	refere
case	*BRCA1*	2	c.1A>G	p.M1V	67	serous	missense	1
case	*BRCA1*	5	c.181T>G	p.C61G	43	serous	missense	2
case	*BRCA1*	5	c.181T>G	p.C61G	43	serous	missense	2
case	*BRCA1*	5	c.181T>G	p.C61G	44	serous	missense	2
case	*BRCA1*	15	c.4675G>A*	p.E1559K	41	serous	missense	3
case	*BRCA1*	18	c.5095C>T	p.R1699W	50	serous	missense	4
case	*BRCA1*	18	c.5096G>A	p.R1699Q	69	serous	missense	5
case	*BRCA1*	18	c.5096G>A	p.R1699Q	61	serous	missense	5
case	*BRCA1*	18	c.5096G>A	p.R1699Q	66	serous	missense	5
case	*BRCA1*	18	c.5096G>A	p.R1699Q	61	serous	missense	5
case	*BRCA1*	20	c.5213G>A	p.G1738E	61	serous	missense	4
case	*BRCA2*	18	c.8167G>C	p.D2723H	63	serous	missense	6
case	*BRCA2*	24	c.9154C>T	p.R3052W	68	serous	missense	7
case	*BRCA2*	24	c.9154C>T	p.R3052W	67	serous	missense	7
case	*MLH1*	17	c.1961C>T	p.P654L	56	endometrioid	missense	8
case	*MSH2*	7	c.1275A>G*	p.E425E	65	serous	synonymous	8
case	*MSH2*	7	c.1275A>G*	p.E425E	68	endometrioid	synonymous	8
control	*MSH6*	9	c.4001G>A*	p.R1334Q	32	NA	missense	8
control	*MSH6*	4	c.1346T>C	p.L449P	32	NA	missense	8

* variants predicted by MaxEntScan to affect splicing. Accession number as follow: *BRCA1*, NM_007294; *BRCA2*, NM_000059; *MLH1*, NM_000249; *MSH2*, NM_000251, *MSH6*, NM_000179; *PMS2*, NM_000535.

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Supplementary Table S3: Sequencing coverage by gene

Gene	Accession number	No. of coding exons	Total coding length (bp)*	No. of amplicons designed	coding sequence* covered by design (%)	sequence read depth>15 (%)	sequence read depth>30 (%)
BRCA1	NM_007294	22	6138	64	96	92	91
BRCA2	NM_000059	26	10907	118	95	84	83
MLH1	NM_000249	19	2739	35	97	88	87
MSH2	NM_000251	16	3195	38	94	82	80
MSH6	NM_000179	10	4317	50	96	79	79
PMS2	NM_000535	15	2953	33	94	71	69

* The sequence also contains 20 bp in the intron for 3' acceptor sites and 6 bp in the intron for the donor 5' sites.

Supplementary Table 4: Comparison of deleterious *BRCA1/BRCA2* variants detected in SEARCH, MAYO and the two largest published studies[1, 2]

Mutation type	SEARCH	%*	Mayo Clinic	% *	CAN[1]	%*	AUS[2]	%*
Large rearrangement	0	0	0	0	14	1	8	1
BRCA1.185delAG (c.66_67delAG)	0	0	0	0	10	1	4	0
BRCA1.5382insC(c.5266dupC)	2	0	3	0	11	1	8	1
BRCA2.6174delT (c.5946_5946delT)	0	0	0	0	3	0	3	0
Other *BRCA1/2* mutations	106	8	67	7	138	10	118	12
Total *BRCA1/2* mutations	108	8	70	8	176	13	141	14
Total (high grade serous only)	63	14	59	9	135	18	98	23

* Percentage of mutation carriers for all cases in each study.

1 Zhang,S. et al (2011) Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer. Gynecol. Oncol, 121, 353-357.

2 Alsop,K., et al. (2012) BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. J Clin Oncol, 30, 2654-2663.

Supplementary Table 5: Comparison of clinical characteristics of cases in SEARCH, MAYO and the two largest published studies.

	All cases (N and % of all cases)								BRCA1 and BRCA2 carriers (N and % of each subgroup)							
	SEARCH (N=1310)	%	Mayo clinic (N= 912)	%	CAN[1] (N=1342)	%	AUS[2] (N=1001)	%	SEARCH (N = 108)	%	Mayo clinic (N= 70)	%	CAN[1] (N=176)	%	AUS[2] (N=141)	%
Morphology																
Serous	600	46	677	74	751	56	709	71	72	12	60	9	135	18	118	17
Clear Cell	137	10	55	6	91	7	63	6	3	2	3	5	2	2	4	6
Endometrioid	212	16	110	12	287	21	119	12	11	5	2	2	26	9	10	8
Mucinous	132	10	25	3	112	8	0	0	1	1	1	4	0	0	0	0
Other/Unknown	229	17	45	5	101	8	110	11	18	8	4	9	13	13	9	8
Stage																
I	431	33	141	15	NA		122	12	16	4	4	3	NA		8	7
II	121	9	51	6	NA		74	7	9	7	3	6	NA		9	12
III	368	28	538	59	NA		566	57	45	12	43	8	NA		91	16
IV	66	5	171	19	NA		113	11	9	14	20	12	NA		17	15
Unknown	324	25	11	1	NA		126	13	29	9	0	0	NA		16	13
Grade																
Low grade (I)	169	13	128	14	NA		50	5	4	2	2	2	NA		2	4
High grade (II/III)	714	55	754	83	NA		735	73	79	11	67	9	NA		113	15
Unknown	427	33	30	3	NA		216	22	25	6	1	3	NA		26	12

Family history																
Ovarian cancer	67	5	39	41	79	6	NA		20	30	9	23	36	46	NA	
Breast cancer	165	13	155	72	248	18	NA		23	14	32	21	75	30	NA	
Ovarian/breast cancer	224	17	184	07			194	19	42	19	36	20			75	39
No Family history	805	61	649	1	993	74	749	75	40	5	30	5	78	8	62	8
Unknown	281	21	79	9	50	4	58	6	26	9	4	5				
Age at diagnosis, years																
<40	72	5	28	31	82	6	45	4	4	6	2	7	9	11	7	16
40-49	239	18	97	12	283	21	153	15	28	12	17	18	68	24	37	24
50-59	489	37	235	66	392	29	346	35	46	9	24	10	51	13	59	17
>=60	510	39	552	1	574	43	457	46	30	6	27	5	48	8	38	8

1. Zhang,S., et al. (2011) Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer. *Gynecol. Oncol*, **121**, 353-357.

2. Alsop,K., *et al.* (2012) BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. *J Clin Oncol*, **30**, 2654-2663.