

Supplementary Materials

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BRCA1 promoter methylation and clinical outcomes in ovarian cancer: an individual patient data meta-analysis

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Supplementary Table 1. Summary of eligible studies not included in the meta-analysis

| Dataset | Total participants | Study type | Methylation assay* | %BRCA1 methylation | Population included | Reported clinicopathological associations† | Reported progression-free survival association† | Reported overall survival association† |
|------------------------------|--------------------|----------------------|---|--|---|--|---|---|
| Buller 2002 ¹ | 250 | Case control | BC/MSP Region: +52 +141 CpG sites: 7 | 7.6% (n=19) | All histological subtypes and stages High grade only | Better surgical cytoreduction | NR | No association |
| Wang 2004 ² | 64 | Retrospective cohort | BC/MSP Region: +52 +141 CpG sites: 7 | 31.0% (n=20) | All grades, stages and histological subtypes | Serous histology | NR | No association |
| Chiang 2006 ³ | 63 | Retrospective cohort | MRED/SB Region: -86 to + 213 CpG sites: 7 BC/MSP Region: +58 +123 CpG sites: 7 | 17.4% (n=11) <i>(100% correlation between MRED/SB and BC/MSP)</i> | All grades, stages and histological subtypes | No associations | Trend towards worse; median 9.8 months (<i>BRCA1-methylated</i>) vs 25.5 months (<i>non-BRCA1-methylated</i>) p=0.21 | Trend towards worse; median 35.6 months (<i>BRCA1-methylated</i>) vs 61.7 months (<i>non-BRCA1-methylated</i>) p=0.07 |
| Yang 2006 ⁴ | 49 | Retrospective cohort | BC/MSP Region: +52 +141 CpG sites: 7 | 16.3% (n=8) | All grades, stages and histological subtypes | High grade Serous histology | NR | No association |
| Stefansson 2012 ⁵ | 30 | Retrospective cohort | Pyrosequencing Region: Not detailed | 13.3% (n=4) | All stages Serous only | Stage I/II disease | Improved (p<0.005) | Improved (p=0.008) |
| Wang 2012 ⁶ | 44 | Retrospective cohort | BC/MSP Region: +55 +140 CpG sites: 6 | 20.5% (n=9) | High grade serous only; all stages | NR | NR | NR |
| Bai 2014 ⁷ | 142 | Retrospective cohort | BC/MSP Region: +52 +141 CpG sites: 7 | 30.2% (n=50) | All grades, stages and histological subtypes | Bilateral ovarian involvement High CA125 Stage III/IV (trend towards an association) | Improved amongst stage III/IV EOC (p=0.005) | Improved amongst stage III/IV EOC (p=0.007) |
| Yates 2017 ⁸ | 299 | Retrospective cohort | MRED Region: -57 +308 CpG sites: unknown | NR | All grades, stages and histological subtypes | NR | NR | NR |
| Bernards 2018 ⁹ | 332 | Retrospective cohort | BC/MSP Region: +52 +141 CpG sites: 7 | 6.6% (n=22) | All grades, stages and histological subtypes | Younger age, high grade serous histology, no association with platinum sensitivity (p=0.803) | No survival association; HR 0.80, 95% CI [0.51 – 1.27], p=0.36 <i>(for PFS for BRCA1/RAD51 methylated vs non-BRCA1/RAD51 methylated/mutated)</i> | No survival association; HR 0.76, 95% CI [0.51 – 1.26], p=0.3 <i>(for OS for BRCA1/RAD51 methylated vs non-BRCA1/RAD51 methylated/mutated)</i> |

*Genomic position is the location of the 5' nucleotide of the sense primer in relation to the BRCA1 transcriptional start site (positioned at 0) at 41,277,500 on RefSeq NM_007294.1 (hg19 assembly)

†as reported in publication;

BC = bisulfide conversion; MSP = methylation specific PCR; MRED = methylation-sensitive restriction endonuclease digestion; SB = southern blotting; NR = not reported, HR = hazard ratio, CI = confidence interval

Supplementary Table 2. Summary of eligible studies included in meta-analysis – *BRCA1/2* aberrations

(All percentages reflect percentage of total non-missing data)

| Dataset | Study type | Total n= | BRCA1 methylation assay* | BRCA1 methylation assay interpretation | BRCA1-methylated n= (%) | BRCA1-methylated in HGSC | BRCA1 mutations [†] n= (%) | BRCA2 mutations [†] n= (%) | Dual BRCA1/2 aberrations (excluded) |
|-------------------------------|----------------------|----------|--|---|-------------------------|--------------------------|--------------------------------------|-------------------------------------|---|
| Wiley 2006 ¹⁰ | Retrospective cohort | 201 | BC/MSP Region: +52 +141 CpG sites: 7 | MSP products visualised on gel | 43 (21.4%) | 22 (30.6%) | Not assessed | | |
| No data; n= | | | | | 0 | 0 | 201 | 201 | |
| Swisher 2009 ¹¹ | Retrospective cohort | 129 | BC/MSP Region: +52 +141 CpG sites: 7 | MSP products visualised on gel | 8 (6.2%) | 6 (6.4%) | 15 (11.6%) | 5 (3.9%) | None |
| No data; n= | | | | | 0 | 0 | 0 | 0 | |
| Srinivasan 2009 ¹² | Prospective cohort | 35 | BC/MSP Region: -58 +123 CpG sites: 7 | MSP products visualised on gel | 15 (42.9%) | | Not assessed | | |
| No data; n= | | | | | 0 | 35 | 35 | 35 | |
| MDACC 2010 | Retrospective cohort | 184 | MRED Region: -57 +308 CpG sites: unknown | Quantitative; threshold>10% | 16 (8.6%) | 12 (8.3%) | 24 (13.0%) | 8 (4.3%) | 1 BRCA1mut + BRCA2mut |
| No data; n= | | | | | 0 | 0 | 0 | 0 | |
| Radosa 2011 ¹³ | Retrospective cohort | 27 | BC/MSP Region: +47 +120 Sites: 8 | MSP products visualised on gel | 3 (11.1%) | 2 (16.7%) | Not assessed | | |
| No data; n= | | | | | 0 | 0 | 27 | 27 | |
| TCGA 2011 ¹⁴ | Retrospective cohort | 482 | Genome wide methylation array (Illumina Infinium HumanMethylation 27k Beadchip) CpG sites: 4/9 | Samples with min 2 CpG probes correlating with low BRCA1 mRNA | 56 (11.6%) | 56 (11.6%) | 35 (11.2%) | 31 (10.3%) | 2 BRCA1mut + BRCA2mut; 1 BRCA1meth + BRCA2mut |
| No data; n= | | | | | 0 | 0 | 170 | 170 | |
| McAlpine 2012 ¹⁵ | Prospective cohort | 131 | BC/MSP Region: +52 +141 CpG sites: 7 | Quantitative; PMR > 4% | 21 (16.0%) | 19 (20%) | 18 (13.7%) | 8 (6.1%) | 2 BRCA1mut + BRCA1meth |
| No data; n= | | | | | 0 | 0 | 0 | 0 | |
| Montavon 2012 ¹⁶ | Retrospective cohort | 80 | BC/MSP Region: Unknown CpG sites: Unknown | MSP products visualised on gel electrophoresis | 13 (16.3%) | 11 (14.3%) | Not assessed | | |
| No data; n= | | | | | 0 | 0 | 80 | 80 | |
| Rzepecka 2012 ¹⁷ | Retrospective cohort | 147 | BC/MSP Region: +18 +122 CpG sites: 8 | Quantitative; PMR > 4% | 23 (15.6%) | 16 (14.7%) | Only select BRCA1 mutations assessed | | 1 BRCA1mut + BRCA1meth |
| No data; n= | | | | | 0 | 0 | 147 | 147 | |
| Cunningham 2014 ¹⁸ | Retrospective cohort | 481 | Genome wide methylation array (Illumina Infinium HumanMethylation 450k Beadchip) CpG sites: 21/46 | Quantitative (correlated with low BRCA1 mRNA); threshold>15% | 44 (9.1%) | 39 (10.8%) | 17 (5.6%) | 13 (4.3%) | 1 BRCA2mut + BRCA1meth |
| No data; n= | | | | | 0 | 0 | 178 | 178 | |
| Ignatov 2014 ¹⁹ | Retrospective cohort | 217 | BC/MSP Region: +18 +122 CpG sites: 8 | MSP products visualised on gel electrophoresis | 73 (33.6%) | 47 (30.3%) | Not assessed | | |
| No data; n= | | | | | 0 | 0 | 217 | 217 | |

| | | | | | | | | | |
|----------------------------|----------------------|------|---|---|-------------|-------------|---------------------------------------|------------|----------------------------|
| Ruscito 2014 ²⁰ | Retrospective cohort | 257 | BC/MSP Region: +43 +136 CpG sites: 4 | Quantitative; threshold>4% | 38 (14.8%) | 38 (14.8%) | Only exon 11 BRCA1 mutations assessed | | |
| No data; n= | | | | | 0 | 0 | 257 | 257 | |
| Patch 2015 ²¹ | Retrospective cohort | 80 | Genome wide methylation array (Illumina Infinium HumanMethylation 450k Beadchip) CpG sites: 8/46 | Samples with min 7 of 8 methylated CpG probes (β value for methylation cut-off correlating with low BRCA1 mRNA) | 12 (15.0%) | 12 (15.4%) | 18 (22.5%) | 3 (3.8%) | None |
| No data; n= | | | | | 0 | 0 | 0 | 0 | |
| Irish 2017 | Retrospective cohort | 109 | MRED Region: -57 +308 CpG sites: unknown | Quantitative; threshold>10% | 9 (8.2%) | 9 (14.3%) | 5 (4.6%) | 13 (11.9%) | 1 BRCA2mut/ + BRCA1meth |
| No data; n= | | | | | 0 | 0 | 0 | 0 | |
| Prieske 2017 ²² | Retrospective cohort | 76 | BC/MSP Region: +52 +141 CpG sites: 7 | MSP products visualised on gel electrophoresis | 56 (73.7%) | 48 (71%) | Not assessed | | |
| No data; n= | | | | | 0 | 0 | 170 | 170 | |
| TOTAL | | 2636 | | | 430 (16.3%) | 337 (13.0%) | 132 (10.6%) | 81 (6.5%) | |

*Genomic position is the location of the 5' nucleotide of the sense primer in relation to the BRCA1 transcriptional start site (positioned at 0) at 41,277,500 on RefSeq NM_007294.1 (hg19 assembly);

† Include germline and/or somatic BRCA1/2 mutations depending on cohort

BC = bisulfide conversion; MSP = methylation specific PCR; MRED = methylation-sensitive restriction endonuclease digestion; PMR = percentage methylated reference; HGSC = high grade serous cancer; BRCA1mut = BRCA1 mutation; BRCA2mut = BRCA2 mutation; BRCA1meth=BRCA1 methylation; min = minimum

Supplementary Table 3. Summary of eligible studies included in meta-analysis – Participants and disease characteristics

(All percentages reflect percentage of total non-missing data)

| Dataset | Total participants; n= | Median age | Total FT; n= | Total PP; n= | Total serous; n= (%) | Total stage 3/4; n= (%) | Total high grade; n= (%) | Total stage 3/4 HGSC; n= (%) | Total < 1cm cytoreduction; n= (%) | Total first line platinum; n= (%) | Total neoadjuvant; n= (%) |
|-------------------------------|------------------------|------------|--------------|--------------|----------------------|-------------------------|--------------------------|------------------------------|-----------------------------------|-----------------------------------|---------------------------|
| Wiley 2006 ¹⁰ | 201 | 58 | 0 | 0 | 82 (40.8%) | 140 (69.7%) | 143 (71.1%) | 66 (32.8%) | 88 (43.8%) | 175 (87.1%) | 0 |
| No data; n= | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Swisher 2009 ¹¹ | 129 | 59 | 3 | 10 | 95 (73.6%) | 111 (86.7%) | 123 (95.3%) | 88 (68.2%) | 82 (64.6%) | 122 (97.6%) | 16 (12.4%) |
| No data; n= | | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 4 | 0 |
| Srinivasan 2009 ¹² | 35 | 48 | 0 | 0 | 33 (94.2%) | 35 (100%) | | | 8 (22.9%) | 35 (100%) | 35 (100%) |
| No data; n= | | 0 | 0 | 0 | 0 | 0 | 35 | 35 | 0 | 0 | 0 |
| MDACC 2010 | 184 | 60 | 0 | 0 | 157 (85.3%) | 161 (88.5%) | 165 (91.2%) | 127 (68.6%) | 118 (69.4%) | 163 (96.4%) | 22 (12.1%) |
| No data; n= | | 6 | 2 | 2 | 0 | 2 | 3 | 5 | 14 | 15 | 4 |
| Radosa 2011 ¹³ | 27 | 58 | 1 | 0 | 17 (63%) | 27 (100%) | 18 (66.7%) | 12 (44.4%) | 27 (100%) | 27 (100%) | 0 |
| No data; n= | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TCGA 2011 ¹⁴ | 482 | 59 | 0 | 0 | 482 (100%) | 458 (95.4%) | 474 (100%) | 456 (95%) | 312 (72.1%) | 440 (100%) | 1 (0.002%) |
| No data; n= | | 0 | 0 | 0 | 0 | 3 | 9 | 12 | 49 | 42 | 0 |
| McAlpine 2012 ¹⁵ | 131 | 56 | | | 100 (76.3%) | 94 (71.8%) | 103 (78.6%) | 83 (64.8%) | 81 (61.8%) | 131 (100%) | 16 (12.2%) |
| No data; n= | | 0 | 131 | 131 | 0 | 0 | 3 | 3 | 0 | 0 | 0 |
| Montavon 2012 ¹⁶ | 80 | 58 | 0 | 1 | 78 (97.5%) | 68 (85%) | 78 (98.7%) | 66 (82.5%) | 51 (63.8%) | 80 (100%) | 0 |
| No data; n= | | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| Rzepecka 2012 ¹⁷ | 147 | 54 | 0 | 0 | 112 (76.2%) | 124 (84.4%) | 134 (91.2%) | 101 (68.7%) | 42 (28.6%) | 143 (97.3%) | NA |
| No data; n= | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 147 |
| Cunningham 2014 ¹⁸ | 481 | 62 | | | 363 (75.5%) | 393 (81.7%) | 430 (89.4%) | 336 (69.7%) | 341 (88.6%) | 391 (92.9%) | 0 |
| No data; n= | | | 481 | 481 | 0 | 0 | 0 | 0 | 96 | 60 | 0 |
| Ignatov 2014 ¹⁹ | 217 | 64 | 0 | 0 | 169 (77.9%) | 169 (77.9%) | 178 (82%) | 129 (59.4%) | 192 (88.5%) | 212 (97.7%) | 0 |
| No data; n= | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ruscito 2014 ²⁰ | 257 | 58 | 0 | 0 | 257 (100%) | 242 (94.2%) | 257 (100%) | 242 (94.2%) | 235 (31.4%) | 234 (91.1%) | 0 |
| No data; n= | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Patch 2015 ²¹ | 80 | 59 | 2 | 15 | 78 (97.5%) | 80 (100%) | 80 (100%) | 78 (97.5%) | 51 (63.8%) | 80 (100%) | 5 (6.3%) |
| No data; n= | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Irish 2017 | 109 | 59 | 1 | 0 | 70 (64.2%) | 69 (63.3%) | 83 (78.3%) | 58 (53.2%) | 66 (82.5%) | 91 (84.3%) | 6 (5.5%) |
| No data; n= | | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 29 | 1 | 0 |
| Prieske 2017 ²² | 76 | 62 | 0 | 0 | 62 (81.6%) | 76 (100%) | 76 (100%) | 62 (81.6%) | 53 (71.5%) | 74 (98.7%) | 13 (17.3%) |
| No data; n= | | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| TOTAL | 2636 | 59 | 7 | 26 | 2155 (81.8%) | 2247 (85.4%) | 2342 (90.7%) | 1904 (73.9%) | 1747 (71.5%) | 2396 (95.4%) | 45 (2.1%) |

FT = fallopian tube cancer; PP = primary peritoneal cancer; HGSC = high grade serous cancer

Supplementary Table 4. Summary of eligible studies included in meta-analysis – survival outcomes

(All percentages reflect percentage of total non-missing data; PFI = platinum free interval; PFS = progression-free survival; OS = overall survival; HR = hazard ratio; CI = confidence interval)

| Dataset | Total participants; n= | Total PFI >12 mths; n= | Median PFS B1-meth (months) | Median PFS non-B1-meth (months) | Univariate HR for B1-meth PFS [95% CI] | Total censored for PFS | Median OS B1-meth (months) | Median OS non-B1-meth (months) | Univariate HR for B1-meth OS [95% CI] | Total censored for OS |
|-------------------------------|------------------------|------------------------|-----------------------------|---------------------------------|--|------------------------|----------------------------|--------------------------------|---------------------------------------|-----------------------|
| Wiley 2006 ¹⁰ | 201 | 87 (64.0%) | 18.0 | 26.4 | 1.41 [0.94 – 2.13] | 82 (40.8%) | 50.4 | 49.6 | 1.08 [0.67 – 1.73] | 109 (54.2%) |
| No data, n= | | 29 | 0 | | | 0 | 0 | 0 | | 0 |
| Swisher 2009 ¹¹ | 129 | 58 (61.7%) | 17 | 26 | 0.77 [0.31 – 1.91] | 18 (14.0%) | 39 | 45 | 0.65 [0.26 – 1.61] | 32 (24.8%) |
| No data, n= | | 31 | 29 | | | 29 | 0 | 0 | | 0 |
| Srinivasan 2009 ¹² | 35 | NA | 27.3 | 19.3 | 0.35 [0.14 – 0.98] | 15 (42.8%) | NA | NA | NA | NA |
| No data, n= | | 35 | 0 | | | 0 | 15 | 20 | | 35 |
| MDACC 2010 | 184 | 60 (38.4%) | 16.9 | 14 | 0.90 [0.46 – 1.77] | 26 (14.1%) | 44.4 | 46.3 | 1.10 [0.55 – 2.18] | 83 (45.1%) |
| No data, n= | | 20 | 25 | | | 25 | 0 | 0 | | 0 |
| Radosa 2011 ¹³ | 27 | 10 (37%) | 14 | 15.4 | 0.69 [0.16 – 2.96] | 0 | 27.8 | 37.4 | 0.98 [0.29 – 3.33] | 0 |
| No data, n= | | 0 | 0 | | | 0 | 0 | 0 | | 0 |
| TCGA 2011 ¹⁴ | 482 | 144 (44.5%) | 14.8 | 16.9 | 0.90 [0.46 – 1.77] | 110 (22.8%) | 39 | 44.5 | 1.43 [0.98 – 2.09] | 210 (43.6%) |
| No data, n= | | 78 | 67 | | | 67 | 0 | 0 | | 0 |
| McAlpine 2012 ¹⁵ | 131 | 73 (56.6%) | 15.6 | 22.5 | 1.64 [0.99 – 2.72] | 39 (29.8%) | 101.6 | 74.4 | 1.28 [0.63 – 2.59] | 84 (64.1%) |
| No data, n= | | 1 | 0 | | | 0 | 0 | 0 | | 0 |
| Montavon 2012 ¹⁶ | 80 | 32 (64%) | 18 | 16 | 0.66 [0.30 – 1.42] | 0 (0.0%) | 38 | 62 | 0.69 [0.34 – 1.40] | 16 (20%) |
| No data, n= | | 30 | 30 | | | 30 | 0 | 0 | | 0 |
| Rzepecka 2012 ¹⁷ | 147 | 65 (51.6%) | 15.4 | 19.7 | 1.50 [0.90 – 2.49] | 20 (13.6%) | 28.7 | 44.1 | 1.71 [1.03 – 2.82] | 50 (34.0%) |
| No data, n= | | 19 | 21 | | | 21 | 0 | 0 | | 0 |
| Cunningham 2014 ¹⁸ | 481 | NA | 20 | 22 | 1.27 [0.91 – 1.77] | 95 (19.8%) | 59 | 53 | 0.94 [0.65 – 1.35] | 134 (27.9%) |
| No data, n= | | 481 | 0 | | | 0 | 0 | 0 | | 0 |
| Ignatov 2014 ¹⁹ | 217 | 126 (62.3%) | 41 | 18 | 0.51 [0.35 – 0.73] | 73 (33.6%) | 54 | 47 | 0.49 [0.28 – 0.86] | 65 (70%) |
| No data, n= | | 0 | 0 | | | 0 | 0 | 0 | | 0 |
| Ruscito 2014 ²⁰ | 257 | 119 (56.9%) | 20 | 20 | 1.09 [0.74 – 1.61] | 67 (26.1%) | 39 | 44.5 | 1.43 [0.98 – 2.09] | 174 (67.7%) |
| No data, n= | | 0 | 0 | | | 0 | 0 | 0 | | 0 |
| Patch 2015 ²¹ | 80 | 21 (26.3%) | 10.3 | 12.4 | 0.88 [0.47 – 1.64] | 4 (5%) | 26.9 | 29.2 | 1.23 [0.62 – 2.41] | 9 (11.3%) |
| No data, n= | | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 |
| Irish 2017 | 109 | 54 (58.7%) | 10 | 29 | 2.25 [1.14 – 4.42] | 39 (35.8%) | 31 | 91 | 1.84 [0.87 – 3.89] | 53 (48.6%) |
| No data, n= | | 1 | 0 | 0 | | 0 | 0 | 0 | | 0 |
| Prieske 2017 ²² | 76 | NA | 16.8 | 12.7 | 0.86 [0.51 – 1.47] | 0 (0.0%) | 41.9 | 41.2 | 0.79 [0.45 – 1.39] | 10 (13.2%) |
| No data, n= | | 76 | 0 | 0 | | 0 | 0 | 0 | | 0 |
| TOTAL | 2636 | 849 (46.2%) | 20 | 18.5 | 1.01 [0.87 – 1.16] | 588 (23.9%) | 46.6 | 48 | 1.02 [0.87 – 1.18] | 1029 (39.6%) |

Supplementary Table 5. Quality and risk of bias assessment for internal validity of included studies according to the ROBINS-I* tool²³

| Dataset | Confounding | Selection | | Measurement of intervention | Deviation from intended intervention | Missing data | | | Measurement of outcomes | | | Selection of reported result | Overall risk of bias |
|-----------------|-------------|------------|----------|-----------------------------|--------------------------------------|--------------|------------|---------|-------------------------|----|---------|------------------------------|----------------------|
| | | O1, O2, O3 | O1 | | | O2, O3 | O1, O2, O3 | O1 | O2 | O3 | O1 | | |
| Wiley 2006 | Moderate | Low | Low | Low | Low | Low | | | Low | | | Low | Moderate |
| Swisher 2009 | Moderate | Low | Low | Low | Low | Low | Moderate | Low | Low | ? | Low | Low | Moderate |
| Srinivasan 2009 | Serious | Low | Low | Low | Low | Serious | Low | Serious | ? | | Serious | Low | Serious |
| MDACC 2010 | Moderate | Low | Low | Low | Moderate | Low | Moderate | Low | Low | | | Low | Moderate |
| Radosa 2011 | Moderate | Low | Low | Low | Moderate | Low | | | Low | | | Low | Moderate |
| TCGA 2011 | Moderate | Moderate | Low | Low | Low | Low | Moderate | Low | Low | | | Low | Moderate |
| McAlpine 2012 | Moderate | Low | Low | Low | Low | Low | | | Low | | | Low | Moderate |
| Montavon 2012 | Moderate | Moderate | Low | Low | Low | Low | Moderate | Low | Low | | | Low | Moderate |
| Rzepecka 2012 | Moderate | Moderate | Moderate | Low | Low | Moderate | | | Moderate | | | Moderate | Serious |
| Cunningham 2014 | Moderate | Low | Low | Low | Moderate | Low | | | Low | | | Low | Moderate |
| Ignatov 2014 | Moderate | Low | Low | Low | Moderate | Low | | | Low | | | Low | Moderate |
| Ruscito 2014 | Moderate | Moderate | Low | Low | Low | Low | | | Low | | | Low | Moderate |
| Patch 2015 | Moderate | Moderate | Low | Low | Low | Low | | | Low | | | Low | Moderate |
| Irish 2017 | Moderate | Low | Low | Low | Moderate | Low | | | Low | | | Low | Moderate |
| Prieske 2017 | Moderate | Low | Low | Low | Low | Low | | | ? | | | Low | Moderate |

*Risk Of Bias In Non-randomized Studies of Interventions

**O1 = outcome 1: clinic-pathological features associated with BRCA1-methylated OC; O2 = outcome 2: progression-free survival; O3 = outcome 3: overall survival

Classification of bias as per ROBINS-I criteria:

Low: the study is comparable to a well-performed randomized trial

Moderate: the study provides sound evidence for a non-randomized study but cannot be considered comparable to a well-performed randomized trial

Serious: the study has some important problems

Critical: the study is too problematic to provide any useful evidence and should not be included in any synthesis

?: No information on which to base a judgement about risk of bias

Overall risk of bias: equivalent to the highest risk of bias in any domain

Supplementary Table 6. Participant and disease characteristics

(All percentages represent fraction of non-missing data)

| Parameter | Number of participants (%) |
|--------------------------|----------------------------|
| Age at diagnosis | |
| Mean (SD) | 59 (11.8) |
| Median (range) | 59 (20 – 93) |
| Interquartile range | 51– 68 |
| Unknown | 17 |
| Histology | |
| Serous | |
| Low grade | 52 (2.0) |
| High grade | 2065 (78.3) |
| Grade unknown | 36 (1.4) |
| Endometrioid | |
| Grade 1 | 40 (1.5) |
| Grade 2 | 66 (2.5) |
| Grade 3 | 86 (3.3) |
| Clear cell | 107 (4.1) |
| Mucinous | 50 (1.9) |
| Undifferentiated | 59 (2.2) |
| Mixed mullerian | 18 (0.7) |
| Mixed | 24 (0.9) |
| Other | 33 (1.2) |
| Unknown | 0 |
| FIGO stage | |
| I | 209 (8.0) |
| II | 178 (6.8) |
| III | 1876 (71.3) |
| IV | 367 (13.9) |
| Unknown | 6 |
| Cytoreduction | |
| < 1 cm | 1757 (71.9) |
| ≥ 1 cm | 687 (28.1) |
| Unknown | 192 |
| Platinum sensitivity | |
| Resistant* | 529 (26.7) |
| Sensitive† | 1452 (73.3) |
| No platinum chemotherapy | 123 |
| Unknown/censored | 532 |

*resistant = platinum free interval (PFI) less than 6 months; †sensitive = PFI of 6 months or greater

Supplementary Table 7. Association between *BRCA1* methylation status and clinico-pathological factors in the cohort with known *BRCA1/2* mutation status (All percentages reflect percentage of total non-missing data)

| Parameter | BRCA1/2 intact (n=907) | BRCA1 meth (n=128) | BRCA1 mut (n=132) | BRCA2 mut (n=81) | Adjusted P* | | | |
|------------------------------|---------------------------|-----------------------|----------------------|---------------------|------------------------------------|-----------------------------------|-----------------------------------|-------------------------------|
| | | | | | BRCA1 meth vs BRCA1/2 intact | BRCA1 mut vs BRCA1/2 intact | BRCA2 mut vs BRCA1/2 intact | BRCA1 meth vs BRCA1 mut |
| Age, No. (%) | | | | | | | | |
| <60 | 402 (44.6) | 78 (60.9) | 87 (65.9) | 53 (66.3) | 0.007 | <0.001 | 0.001 | 0.91 |
| ≥60 | 500 (55.4) | 50 (39.1) | 45 (34.1) | 27 (33.8) | | | | |
| Missing | 5 | 0 | 0 | 1 | | | | |
| FIGO stage, No (%) | | | | | | | | |
| I-II | 156 (17.2) | 10 (7.9) | 9 (6.8) | 9 (11.4) | 0.01 | 0.02 | 0.20 | 0.92 |
| III-IV | 750 (82.8) | 117 (92.1) | 123 (93.2) | 70 (88.6) | | | | |
| Missing | 1 | 1 | 0 | 2 | | | | |
| Histology, No (%) | | | | | | | | |
| Serous | 725 (79.9) | 117 (91.4) | 124 (93.9) | 67 (96.3) | 0.009 | 0.005 | 0.001 | 0.91 |
| Non-serous | 182 (20.1) | 11 (8.6) | 8 (6.1) | 3 (3.7) | | | | |
| Missing | 0 | 0 | 0 | 0 | | | | |
| Grade, No (%) | | | | | | | | |
| High | 815 (90.6) | 127 (100.0) | 132 (100.0) | 80 (100.0) | 0.005 | 0.006 | 0.009 | - |
| Low | 85 (9.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) | | | | |
| Missing | 5 | 1 | 0 | 1 | | | | |
| Cytoreduction, No (%) | | | | | | | | |
| Macro <1cm | 586 (74.0) | 87 (73.7) | 88 (73.9) | 56 (78.9) | 0.99 | 0.73 | 0.46 | 0.92 |
| Macro ≥1cm | 206 (26.0) | 31 (26.3) | 31 (26.1) | 21 (21.1) | | | | |
| Missing | 115 | 10 | 13 | 10 | | | | |
| Platinum sensitivity, No (%) | | | | | | | | |
| PFI<6 months | 232 (32.5) | 30 (27.8) | 28 (26.4) | 9 (12.9) | 0.40 | 0.008 | <0.001 | 0.91 |
| PFI≥6 months | 481 (67.5) | 78 (72.2) | 78 (73.6) | 61 (87.1) | | | | |
| No chemo/missing | 194 | 20 | 26 | 11 | | | | |

*Two-sided Cochran-Mantel-Haenszel test, adjusting for study

BRCA1/2 intact = BRCA1/2 wild type non-BRCA1-methylated; BRCA1 meth = BRCA1-methylated; BRCA1 mut = BRCA1-mutated; BRCA2 mut = BRCA2-mutated; Macro = macroscopic residual disease; PFI = platinum-free interval

Supplementary Table 8. Frequency of *BRCA1* locus-specific LOH in *BRCA1*-methylated samples: a pooled analysis

| LOH Methodology | Study | # microsatellites analysed | Methylation methodology | Total patients in study | <i>BRCA1</i> -methylated tumours | | | % <i>BRCA1</i> -methylated tumours without LOH |
|-------------------------|------------------------|----------------------------|-------------------------|-------------------------|----------------------------------|---------------|------------------|--|
| | | | | | Total n (%) | With LOH (n=) | Without LOH (n=) | |
| Microsatellite analysis | Esteller 2000 | 2 | MSP | 31 | 4 (12.9) | 4 | 0 | 0.0 |
| | Baldwin 2000 | 4 | MSP | 98 | 12 (12.2) | 6 | 6 | 50.0 |
| | Geisler 2002 | 3 | MSP | 121 | 16 (13.2) | 12 | 4 | 33.3 |
| | Wang 2004 | 3 | MSP | 64 | 20 (31.3) | 15 | 5 | 25.0 |
| | Press 2008 | 4 | MSP | 49 | 10 (20.4) | 9 | 1 | 10.0 |
| | Rzepecka 2012 | 3 | MSP | 161 | 30 (18.6) | 28 | 2 | 6.7 |
| | Total or weighted mean | | | 524 | 92 (17.6) | 74 | 18 | 19.0 |
| Microarray analysis | Abkevich 2012 | - | MRED | 160 | 15 (9.4) | 15 | 0 | 0.0 |
| | Wang 2012 | - | MSP | 44 | 9 (20.5) | 8 | 1 | 12.5 |
| | Total or weighted mean | | | 204 | 24 (11.8) | 23 | 1 | 2.6 |

MSP = methylation specific PCR; MRED: methylation sensitive restriction endonuclease digestion

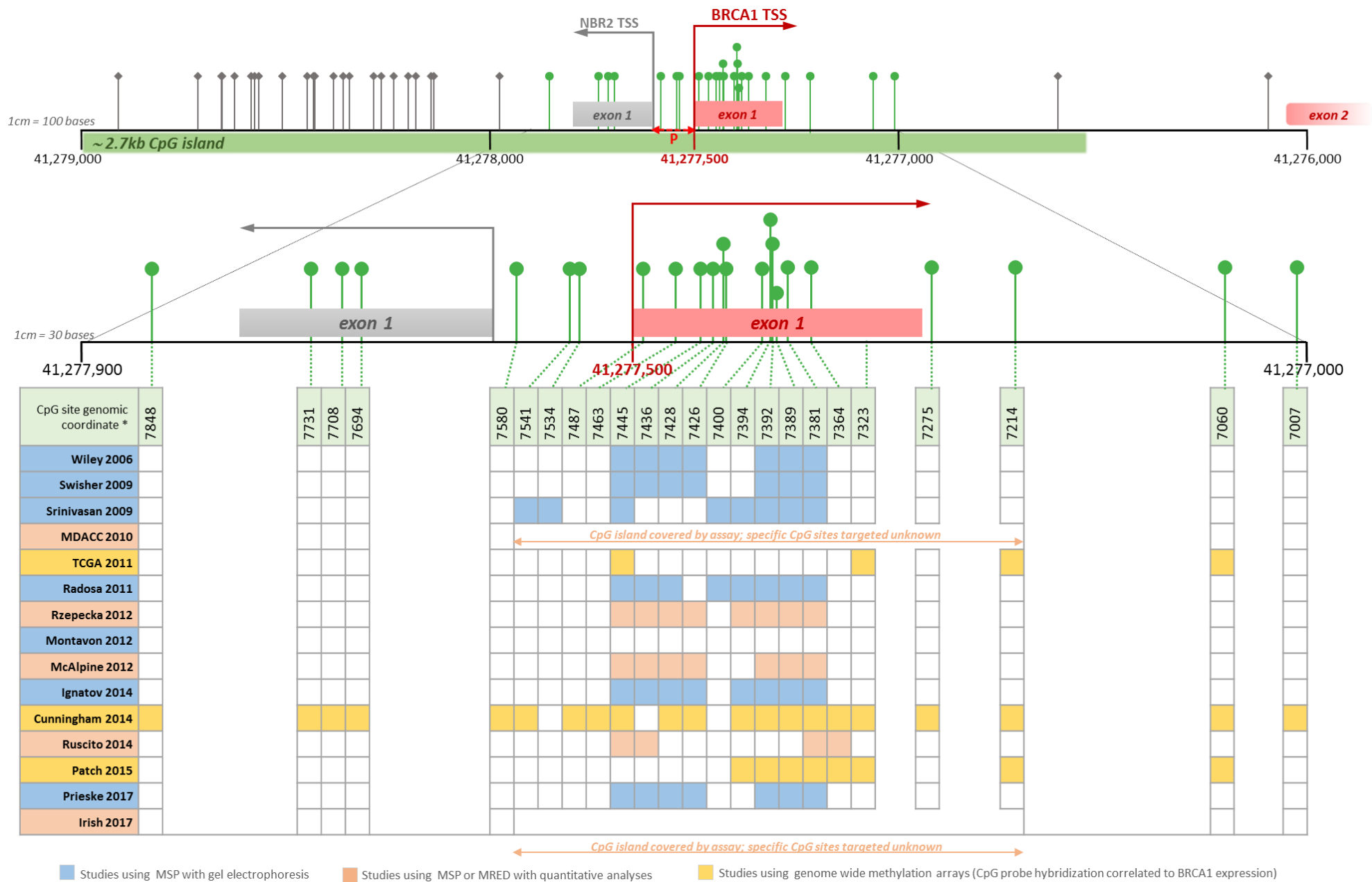
Supplementary Table 9. Univariate analyses of known clinical variables in the entire cohort

| Variable | OS | | PFS | |
|------------------|------------------|--------|------------------|--------|
| | HR [95% CI] | P* | HR [95% CI] | P* |
| Age | 1.36 [1.23-1.51] | <0.001 | 1.23 [1.11-1.35] | <0.001 |
| Stage | 3.77 [3.06-4.64] | 0 | 4.01 [3.36-4.78] | 0 |
| Grade | 3.14 [2.42-4.10] | 0 | 3.80 [3.01-4.81] | 0 |
| Residual Disease | 2.25 [1.98-2.55] | 0 | 2.08 [1.85-2.34] | 0 |
| Histology | | | | |
| Serous | | | | |
| Endometrioid | 0.44 [0.34-0.56] | <0.001 | 0.31 [0.25-0.39] | 0 |
| Clear cell | 0.62 [0.46-0.83] | 0.001 | 0.50 [0.38-0.66] | <0.001 |
| Mucinous | 0.45 [0.28-0.74] | 0.001 | 0.40 [0.26-0.61] | <0.001 |
| Other | 0.95 [0.75-1.22] | 0.69 | 0.82 [0.65-1.02] | 0.08 |

* Two-tailed mixed-effects Cox proportional hazards regression model with p value adjusted for study
 Variables other than histology were dichotomised as follows: Age: 0 for <60 (median age) and 1 for ≥60;
 Stage : 0 for Stage I/II, 1 for Stage III/IV; Grade: 0 for low grade, 1 for high grade; Residual disease: 0 for
 < 1cm, 1 for ≥1cm.

Supplementary Figure 1. Individual CpG sites probed across all studies included in the meta-analysis

Details of CpG sites within the *BRCA1/NBR2* gene locus probed by the studies included in the meta-analysis. Green CpG sites represent those used to determine tumours' methylation status. Grey CpG sites represent those that were probed in studies utilizing GWMA assays, but were not selected for determination of methylation status, as they did not correlate with *BRCA1* expression. Genomic coordinates correspond to the RefSeq NM_007294 (ENSG00000012048), transcript variant 1, using GChr37/hg19 assembly, as obtained from the UCSC genome browser, accessible at <https://genome-euro.ucsc.edu>
 TSS: transcription start site; P: bidirectional promoter; MSP: methylation specific PCR; MRED: methylation-sensitive restriction endonuclease digest; GWMA: genome wide methylation array



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