

Effects of *BRCA1* and *BRCA2* mutations on female fertility

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Women with *BRCA1/2* mutations have a significantly higher lifetime risk of developing breast or ovarian cancer. We suggest that female mutation carriers may have improved fitness owing to enhanced fertility relative to non-carriers. Here we show that women who are carriers of *BRCA1/2* mutations living in natural fertility conditions have excess fertility as well as excess post-reproductive mortality in relation to controls. Individuals who tested positive for *BRCA1/2* mutations who linked into multi-generational pedigrees within the Utah Population Database were used to identify putative obligate carriers. We find that women born before 1930 who are mutation carriers have significantly more children than controls and have excess post-reproductive mortality risks. They also have shorter birth intervals and end child-bearing later than controls. For contemporary women tested directly for *BRCA1/2* mutations, an era when modern contraceptives are available, differences in fertility and mortality persist but are attenuated. Our findings suggest the need to re-examine the wider role played by *BRCA1/2* mutations. Elevated fertility of female mutation carriers indicates that they are more fecund despite their elevated post-reproductive mortality risks.

Keywords: fertility; mortality; *BRCA1*; *BRCA2*; pleiotropy; telomeres

1. INTRODUCTION

Women with *BRCA1* or *BRCA2* mutations have an estimated 40 to 85 per cent lifetime risk of developing breast cancer and 16 to 64 per cent risk of ovarian cancer [1–3]. These mutations are relatively prevalent, raising the possibility that these mutations may have beneficial effects despite the significant excess cancer risk. Could women with these mutations, whose breast and ovarian cancers arise typically after menopause, bear more children and have a longer reproductive interval? No studies have been conducted that focus exclusively on whether fertility as well as survival differences exist between female *BRCA1/2* mutation carriers and non-carriers *under natural fertility conditions*, and whether these differences persist when modern contraception is available (see the studies of Moslehi *et al.* [4] and Mai *et al.* [5] that share some important features of this analysis).

The purpose of this study is to address two fundamental issues. First, what mechanisms may explain why *BRCA1* and *BRCA2* mutations persist in human populations given their adverse health consequences? Genetic variants have been shown to have pleiotropic functions, with beneficial effects possibly outweighing their adverse effects [6]. Excess mortality (due primarily to breast

and ovarian cancer) risks that occur among mutation carriers could be adequate to result in strong negative selection [7]. We hypothesize that while *BRCA1/2* gene mutations increase cancer incidence and mortality in middle and later adulthood, mutation carriers may have enhanced reproductive fitness because of elevated fertility rates, which may compensate for their excess levels of post-menopausal mortality. Second, we also hypothesize that the fertility effects of being a *BRCA1* or *BRCA2* mutation carrier are different in periods when no modern contraception available compared with periods where individuals may be making family planning decisions based on predictive genetic risk information heretofore unavailable. We hypothesize that family cancer history and the advent of genetic testing, along with the availability of effective family planning methods, lead to lower levels of fertility for female *BRCA1/2* mutation carriers in relation to non-carriers. These hypotheses are tested using a unique resource, the Utah Population Database (UPDB), which identifies putative obligate carriers of *BRCA1* or *BRCA2* mutations whose descendants were tested directly for these mutations.

2. STUDY DESIGN

BRCA1/2 mutation carriers were identified from two longitudinal studies. The first was a large, prospective study that analysed the fertility behaviours and attitudes of *BRCA1* mutation carriers. Participants of this study

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2011.1697> or via <http://rspb.royalsocietypublishing.org>.

were members of a large Utah multi-generational pedigree or kindred (K2082) with an identified mutation at the *BRCA1* locus. To increase our sample size and include carriers of deleterious mutations in both *BRCA1* and *BRCA2*, participants from the High-Risk Breast Cancer Clinic (HRBCC) at the University of Utah's Huntsman Cancer Institute (HCI) were also selected. The HRBCC is a research resource for individuals with a family history of breast and ovarian cancer. A detailed description of methods, eligibility criteria and protocols for both studies has been described elsewhere [1,8]. There were 133 female mutation carriers from 49 kindreds (each with a unique founder) selected for this study based on the availability of their genetic test results from these two sources. Genetic testing for individuals in K2082 and the HRBCC were performed by Clinical Laboratory Improvement Act-approved laboratories. Tests for a few initial enrollees were done by the University of Utah's DNA Diagnostic Laboratory, but nearly all the remainder were conducted by Myriad Genetics [1,9,10] after these individuals were provided with extensive genetic counselling and gave their informed consent. Subjects were then classified as a *BRCA1* or *BRCA2* mutation carrier. Both studies were approved by the University of Utah's Institutional Review Board and the Resource for Genetic and Epidemiologic Research.

Information about each carrier's relatives was obtained from the UPDB. UPDB is a population-based resource used for biomedical research. UPDB holds data on approximately seven million individuals derived from genealogical, demographic and medical records spanning the past two centuries [11,12]. Linked genealogical and birth certificate records are contained within the UPDB and permit the identification of multi-generational pedigrees that range from 2 to 11 generations. Data from the Utah Cancer Registry, Idaho Cancer Registry and Utah death certificates are linked to the family history (genealogical) records to construct an extensive biodemographic research database.

Pedigree information of carriers was used to identify ancestors who are putative mutation carriers. Mutation status of ancestors in the UPDB was derived based on pedigree position and relationship to multiple tested carriers. Families with multiple tested mutation carriers were reviewed to identify the transmission of the mutation through the pedigree. This was done by first identifying two or more individuals who were directly tested and found to be carriers of the same *BRCA1/2* mutation. The closest common ancestor (i.e. founder) between the two (or more) tested individuals was then identified based on the UPDB. All individuals that connect the founder and the tested individuals were identified as putative obligate mutation carriers.

Several selection criteria were used to identify the sample from the UPDB. Each individual was required to have complete information on birth dates, fertility history and age at last birth. The UPDB contains comprehensive birth history information, but no data exist for pregnancies and stillbirths. The few individuals who were members of a polygamous relationship were excluded. To focus the assessment of fertility on monogamous unions, only individuals who were married once are included. Individuals who never married or had incomplete spouse information were excluded. All offspring were required to have complete birthdates and to

have been born in Utah or Idaho. The birth years before 1930 were used to approximate an era when women would have limited access to modern birth control (e.g. oral contraceptives) until their mid-30s at the earliest. Tested carriers born after 1975 were also not selected as a result of their severely censored fertility experience.

The first step in selecting controls from the UPDB was to identify founders for the 49 HRBCC/K2082 kindreds with a known mutation. Any descendants of these 49 founders were excluded from consideration as a control. Controls were selected by matching birth year of the mutation carriers. We restricted the analysis to parous individuals. This specification was used because the study design requires that all putative obligate carriers had at least one child survive to reproductive age. In other words, since there needs to be an unbroken chain of descendants connecting the founders to tested individuals among the obligate carriers, it was necessary to impose the same pedigree structure on the controls. Controls, like the carriers, were also required to have one spouse and complete fertility information.

The final sample of 181 carriers comprised carriers born before 1930 (obligate $n = 48$, directly tested $n = 11$) and tested female carriers ($n = 122$) born between 1930 and 1975, and 15 : 1 matched controls ($n = 885$ controls for the pre-1930 carriers and $n = 1830$ controls for the post-1930 women). Drawing on the UPDB, the potential pool of population-based controls (as well as mutation carriers) were those who were born before 1975, had no ancestors or descendants who were carriers, and, like the carriers, were once married with complete spouse information, had at least one child where the mother's age at first birth was known and gave birth to all children in Utah or Idaho. A control is unlikely to be a carrier given that none of their tested descendants are carriers and they themselves were not descendants of obligate carriers. If a carrier was incorrectly counted as a control, this misclassification would create a conservative bias. This protocol resulted in a pool of 101 023 potential controls that excluded 5536 descendants of the *BRCA1* or *BRCA2* founders. Women were restricted to survive to at least age 45 in order to observe completed (or nearly completed) fertility. We also report post-reproductive mortality risks associated with the *BRCA1* and *BRCA2* mutations. This survival restriction excludes 12 per cent of carriers and 5 per cent of controls, a difference that might again yield conservative results.

Two supporting complementary analyses were also conducted. The first, and the most historical, examined female fertility of the *BRCA1/2* mutation founders ($n = 49$) with 15 : 1 matched (by birth year) controls ($n = 733$). This analysis (hereafter the 'founder' analysis) was conducted to assess fertility at the earliest possible point where natural fertility conditions prevailed. The second, and the most contemporary, considered women who enrolled at the HRBCC and completed questionnaires that asked about their reproductive history. They are from high-risk breast cancer families who enrolled at the HRBCC between 1994 and 2006, all of whom were tested for *BRCA1/2* mutations. Given that contemporary women have substantially lower fertility, it is more informative to assess whether these women differed in their ability to conceive according to their mutation status, information obtained from the HRBCC

questionnaire and unavailable for the larger sample of carriers in the UPDB.

(a) Statistical methods

We examined several measures of fertility. The first indicator is the number of children ever born (CEB). To examine the relationship between CEB and mutation status we used ordinary least squares (OLS) as well as Poisson regression (results were very similar; accordingly, we show here the OLS regressions results; Poisson results are given in the electronic supplementary material). This was done for both the full sample and the founder analysis. The availability of effective birth control methods was considered by performing separate regressions for carriers born before 1930 and born during 1930–1974. The year 1930 was selected because women born after that date would be fecund at the time modern family planning methods, notably exogenous hormones, became available. Mutation status was represented by a dummy variable, carrier (=1) versus control (=0). The other covariates used in all fertility models were birth year, age at first birth, and, when appropriate, age at marriage and the number of offspring who died as children. A dummy variable that describes whether a woman was an active member of the Church of Jesus Christ of Latter-day Saints (LDS, or Mormon) status was used in the pre-1930 models. A second fertility model was estimated to assess whether there was effect modification due to historical time. This was done by introducing a two-way interaction between mutation status and birth year (or whether born before or after 1930).

The effect of the subjects' parental fertility patterns on CEB was also considered, since a subject's parents' fertility might be associated with both the subject's mutation status and fertility. The number of siblings was therefore included in the regressions. The addition of parental fertility patterns did not alter the effect of mutation status and was therefore excluded from the models shown.

We investigated whether carriers were more likely to exceed the average number of children for their cohort. The association between fertility and mutation status was estimated by multiple logistic regression. The mean number of children for the sample was 3.73, so subjects with four or more children were classified as having 'more' children. The dependent variable was coded 1 if the subjects had four or more children; all other subjects were coded 0.

Additional measures of reproductive behaviours are examined that reflect underlying fecundity (particularly for those born during the pre-1930 era), as well as efforts to limit fertility through family planning (primarily for those born in 1930 or later). Moreover, we sought to avoid any artefacts that might produce an association between fertility and mutation status. In particular, it is possible that contemporary individuals chose to be tested genetically (with some testing positive) because they come from large families where the consequences of the test results could be particularly significant. If seeking genetic testing is linked to family size, then pedigrees containing mutation-positive persons may also contain putative obligate carriers with greater fertility. Accordingly, we have expanded the measures of fertility that are less sensitive to this potential limitation: age at first birth, first birth interval, average birth interval (mean

length of time between all consecutive births with adjustments for twinning) and age at last birth. While all of these measures are related to CEB, they do provide additional assessments of fecundity, particularly for those bearing children before the advent of modern contraception. An examination of these characteristics also provides an opportunity to isolate the specific demographic components of fertility through which *BRCA1/2* mutations may be operating. These additional outcomes are all continuous variables and are analysed using OLS regression.

To assess HRBCC women with respect to the effects of *BRCA1/2* on difficulties becoming pregnant, we estimated logistic regressions for the dependent variable (1 = yes, 0 = no) based on the following question: 'Did you ever try for one straight year or more to become pregnant and, during that time, not become pregnant?' We analysed all HRBCC participants between ages 18 and 64 ($n = 419$) who answered this question. Covariates included in the model are age at interview, birth control usage, total live births at time of interview and marital status.

Mortality differences between *BRCA1/2* carriers and controls for survival past age 45 are presented as shown in figure 1*d* (see electronic supplementary material, table S1), based on Cox proportional hazard regression analyses for persons born before 1930. They show significant excess mortality risks associated with being a carrier of a *BRCA1* or *BRCA2* mutation.

3. RESULTS

Descriptive statistics are displayed in table 1 and show that mutation carriers had approximately two more children (CEB) than controls. For example, controls born before 1930 had a mean CEB of 4.19 while carriers from the same era had 6.22.

Figure 1*a* and electronic supplementary material, table S2 report the results of OLS regressions showing the effects of mutation status on CEB. There is a significant association between mutation status and CEB for pre-1930 carriers who have an average of 1.91 more children than controls. Excess and significant levels of CEB are also detected for post-1930 carriers, though the increase is attenuated to 0.61 CEB. The decline in the fertility effect of carrier status before and after 1930 is statistically significant ($p < 0.001$), a result that is largely attributable to the effects of *BRCA1* carriers specifically. The significant fertility-enhancing effects of *BRCA1/2* mutations are also detected based on the founder sample (electronic supplementary material, table S3): carriers have 1.17 more children than controls ($p < 0.01$) and are 2.67 times more likely to have four or more children (95% CI: 1.26–5.68).

To address the possibility that these fertility influences are attributable to unique mutations found among specific founders [10], we identified 23 distinct mutations for those born prior to 1930 and 37 for the more recent cohort (the two birth cohorts share 20 mutations; see electronic supplementary material, table S4). The most frequent mutation, c.3937C>T [13,14], is associated with K2082, with the remaining mutations comprising very small percentages; the next most frequent mutations comprised approximately 10 per cent of their respective cohorts (c.213-11T>G for the pre-1930 cohort,

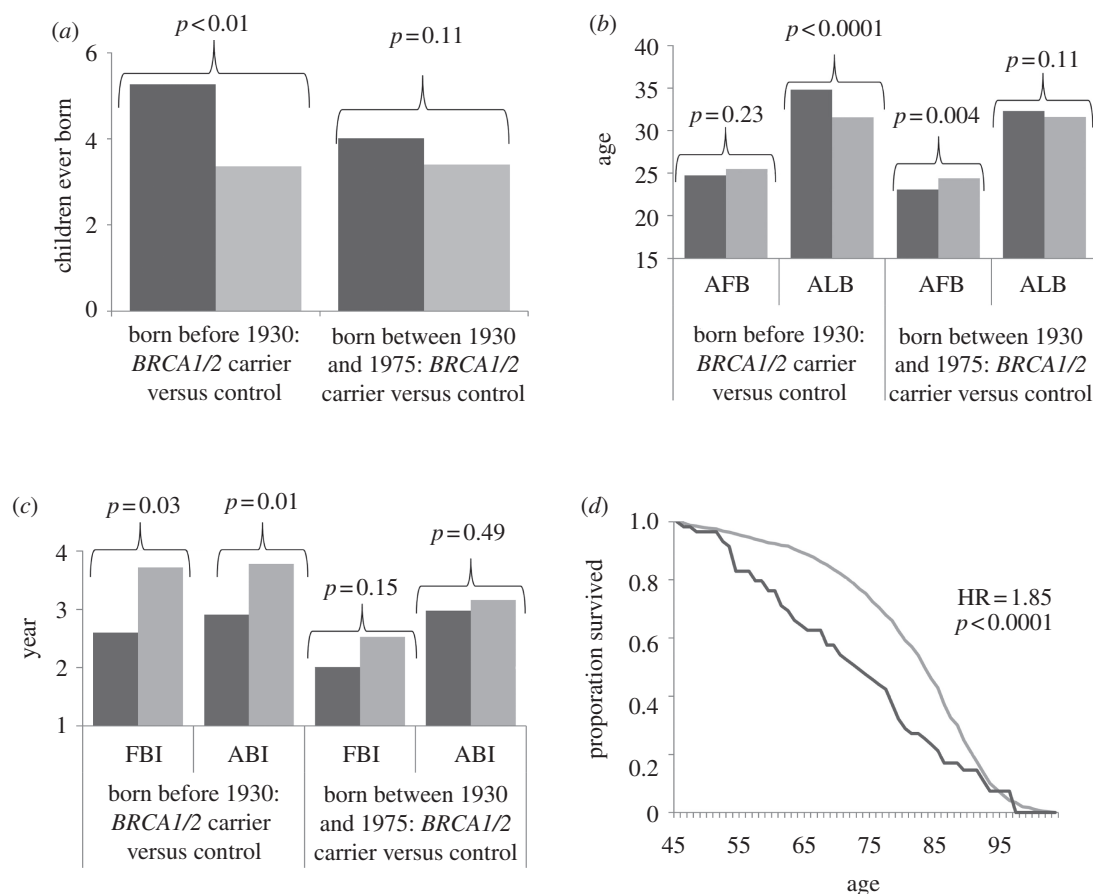


Figure 1. (a) Effects of *BRCA1/2* mutation status on number of children ever born, based on ordinary least squares (OLS) regression controlling for birth year, age at first birth and number of deceased children (who died by age 5). (b) Effects of *BRCA1/2* mutation status on age at first birth (AFB) and age at last birth (ALB), based on OLS regressions controlling for birth year and age at marriage (and AFB for the ALB model). (c) Effects of *BRCA1/2* mutation status on first birth interval (FBI) and average birth interval (ABI), based on OLS regressions controlling for birth year and age at first marriage. (d) Survival probabilities past age 45 by *BRCA1/2* mutation status for women born before 1930, based on Cox proportional hazard regressions controlling for birth year. Models for women born before 1930 also controlled for LDS status. Dark grey bars or lines denote carrier and light grey bars or lines denote control.

c.2035A>T for the later cohort). To address the possibility that members of K2082 may be dominating the results, we excluded their members and re-estimated our models. For pre-1930 carriers (42 carriers and 630 controls), the effects were again significant (1.76 more CEB, $p < 0.001$); a significant (albeit attenuated) effect was also detected for tested women (0.51 more CEB, $p < 0.005$; electronic supplementary material, table S2).

Logistic regressions were also estimated where the dependent variable measures whether an individual had four or more children. In relation to controls, pre-1930 carriers were approximately 3.6 times (95% confidence interval (CI): 1.85–7.15) more likely to have four or more children, whereas tested carriers born after 1930 were approximately 2.04 times more likely (95% CI: 1.37–3.02; see electronic supplementary material, table S5).

The impact of carrier status on the timing and pacing of child-bearing is shown in figure 1*b,c* (electronic supplementary material, table S6). For pre-1930 carriers, their fertility is marked by significantly shorter first and average birth intervals, as well as later ages at last birth. No influence of mutation status was detected for an early age at first birth. Post-1930 carriers show an earlier age at first birth; average and first birth intervals are shorter and age at last birth later; but all are insignificant.

For contemporary high-risk women under age 65 seen at the HRBCC, we compared *BRCA1/2* mutation carriers with non-carriers with respect to their difficulty getting pregnant when intending to do so, according to the HRBCC questionnaire. No differences were detected between these two groups (OR = 0.904, 95% CI: 0.52–1.58), and this null result did not vary by *BRCA1* and *BRCA2* mutation status (*BRCA1*: OR = 1.03, 95% CI: 0.55–1.93; OR = 0.74, 95% CI: 0.35–1.54; electronic supplementary material, table S7). These results suggest that comparisons of contemporary women's fertility by mutation status may be difficult when women's reproductive plans are strongly affected by choice.

4. DISCUSSION

Our findings indicate that female mutation carriers bear more children, have shorter birth intervals and reproduce later in life than matched controls, a difference that is large initially when effective contraception is absent. Mutation carriers also have excess mortality after age 45 in relation to controls. This finding is probably owing to mortality from breast and ovarian cancer in *BRCA1* mutation carriers, and cancers of the breast, ovary, pancreas and melanoma in *BRCA2* mutation carriers. For

Table 1. Descriptive statistics.

| variables | BRCA1/2 mutation carriers | | | controls | | |
|--------------------------------|---------------------------|---------|-------|----------|---------|-------|
| | <i>n</i> | mean | s.d. | <i>n</i> | mean | s.d. |
| <i>born before 1930</i> | | | | | | |
| birth year | 59 | 1906.78 | 17.72 | 885 | 1906.78 | 17.58 |
| death year (if applicable) | 52 | 1974.50 | 24.25 | 716 | 1981.90 | 21.51 |
| year of first marriage | 47 | 1929.70 | 18.36 | 850 | 1928.23 | 17.53 |
| year of first birth | 59 | 1930.22 | 18.67 | 885 | 1930.96 | 18.49 |
| age at first marriage | 47 | 21.77 | 3.34 | 850 | 21.40 | 3.53 |
| age at first birth | 59 | 23.44 | 3.31 | 885 | 24.18 | 4.80 |
| age at last birth | 59 | 36.76 | 5.15 | 885 | 33.94 | 6.27 |
| first birth interval (years) | 47 | 1.68 | 1.49 | 850 | 2.82 | 3.38 |
| average birth interval (years) | 59 | 2.69 | 1.10 | 877 | 3.64 | 2.62 |
| number deceased children | 59 | 0.39 | 0.87 | 885 | 0.35 | 0.78 |
| children ever born | 59 | 6.22 | 2.74 | 885 | 4.19 | 2.68 |
| devout Mormon (dummy) | 59 | 0.76 | 0.43 | 885 | 0.83 | 0.38 |
| <i>born 1930–1974</i> | | | | | | |
| birth year | 122 | 1949.10 | 9.43 | 1830 | 1949.10 | 9.39 |
| death year (if applicable) | 22 | 1997.41 | 6.67 | 102 | 2001.74 | 5.57 |
| year of first marriage | 63 | 1971.03 | 11.67 | 1670 | 1971.06 | 11.71 |
| year of first birth | 122 | 1972.19 | 10.10 | 1830 | 1973.52 | 11.59 |
| age at first marriage | 63 | 21.08 | 4.21 | 1670 | 22.00 | 4.45 |
| age at first birth | 122 | 23.09 | 3.51 | 1830 | 24.42 | 5.16 |
| age at last birth | 122 | 31.70 | 5.37 | 1830 | 31.72 | 5.45 |
| first birth interval (years) | 63 | 2.29 | 3.00 | 1670 | 2.50 | 2.87 |
| average birth interval (years) | 120 | 2.98 | 1.29 | 1824 | 3.17 | 2.15 |
| number deceased children | 122 | 0.03 | 0.22 | 1830 | 0.07 | 0.29 |
| children ever born | 122 | 4.13 | 1.82 | 1830 | 3.40 | 1.75 |

those with death certificates in the pre-1930 cohort, carriers ($n = 33$) had 18 per cent who died from breast cancer and 27 per cent from ovarian cancer. Among controls ($n = 621$), the figures were 3 per cent and 1 per cent, respectively, estimates very close to those for the general population (www.cancer.org). Mai *et al.* [5] suggest that other causes of death are also elevated among mutation carriers.

What are the mechanisms that connect *BRCA1* mutations to female fertility? Recent studies suggested that *BRCA1/2* mutations limit embryogenesis [15–18], suggesting *impaired* reproductive fitness. Murine models have shown that homozygous deletions of *BRCA1/2* result in embryonic lethality [16], while heterozygous mice developed normally and were fertile. Homozygous *BRCA1/2* human embryos have been shown to spontaneously abort [3]. Pal *et al.* [19] argued that numerous mechanisms alter the process of cell cycle and division and DNA repair, and given that many are affected by BRCA proteins, *BRCA1/2* mutations may limit rather than promote reproduction.

These predictions have not, however, been supported by the human data. Indeed, three recent investigations examining this association show that mutation status has no effect on female fertility. Pal *et al.* [19] asked whether female fertility was affected among carriers of *BRCA1/2* mutations in relation to family controls, all in a contemporary population. They found that parity was the same between carriers and non-carriers. Friedman *et al.* [3] found no differences between carriers and non-carriers in terms of spontaneous abortions. Moslehi *et al.* [4] compared the fertility of mutation carriers and non-carriers with ovarian cancer and controls based on a largely contemporary sample of

Ashkenazi Jewish women (born after 1930). They concluded that there was no evidence that BRCA mutations affected female fertility.

What mechanisms may be involved in a positive association between *BRCA1/2* mutations and fertility? We suggest that an association between telomeres and BRCA mutations are consistent with the findings reported here (see also [19]). This proposed mechanism relies on (at least) two linkages: one between BRCA mutations and telomere length, and another between telomere length and fertility. With respect to the former, French *et al.* [20] reported that the disruption of *BRCA1* may result in telomere lengthening (see also [21]). They also confirmed that the overexpression of *BRCA1* limits telomerase activity and reduces telomere length. Their results suggest a possible role of *BRCA1* mutations in protecting telomeres. Ballal *et al.* [22] also found that *BRCA1* overexpression is associated with telomere shortening (for an exception, see [23]).

While previous studies have shown that longer telomeres are associated with increases in longevity [24] as well as reductions in the risk of major causes of death (except cancer), early evidence suggests that longer telomeres play a role in enhancing reproduction. Keefe *et al.* [25,26] note that telomerase, the enzyme that maintains telomere length, is not active in oocytes (but is during the blastocyst stage) so oocytes have their full telomere length at the earliest point of development. They argue that the lengthy period between foetal life and ovulation in mid-life would expose oocytes to the effects of reactive oxygen that would shorten telomeres. Accordingly, telomeres in oocytes would probably shorten with increasing age owing to the combined effects of late ovulation and

the prolonged interval before ovulation. Keefe *et al.* examined these mechanisms by shortening telomeres in mice, which produced a phenotype comparable with age-related oocyte dysfunction in women. They also examined eggs donated by women aged 25–42 undergoing *in vitro* fertilization (IVF). Telomere lengths were longer in eggs from women who conceived than in those from women who did not after IVF. No women conceived with a mean telomere length from spare eggs shorter than 6.3 kb. They concluded that shorter telomere lengths in eggs predicted conception rates in women undergoing IVF. Earlier, Aydos *et al.* [27] studied telomere lengths of 37 females aged 50, and found a positive association between reproductive life-span and telomere length.

The association between telomere length and reproductive ageing was also analysed by Hanna *et al.* [28], who compared women with recurrent miscarriages (RMs), premature ovarian failure (POF) and two control groups. RM women had significantly shorter age-adjusted average telomeres than controls [28]. Women with POF had longer age-adjusted mean telomeres for one of the two control groups, an inconsistency probably owing to the very small number of POF subjects, as the authors point out. The telomere length differences between RM and control women (who had viable pregnancies later in life) are consistent with the hypothesis that telomere length affects the rate of reproductive ageing in women. Our results on shorter first and average birth intervals are consistent with Hanna *et al.*'s finding.

Previous evidence indicates that fertility may be elevated among mutation carriers. A study investigating the effect of parity on breast cancer risk found that women with a *BRCA2* mutation had elevated CEB (mean CEB: carrier = 3.3, negative = 3.0, control = 3.2), though statistical testing was not done [29]. Jernstrom *et al.* [30] examined the association between parity and cancer risk in mutation carriers and found a significantly higher mean number of births for carriers (1.6) versus controls (1.4).

In these studies, subjects had access to modern contraception, making it difficult to assess endogenous differences in fecundity between carriers and non-carriers. Knowledge of genetic testing status has been shown previously to affect family planning as our present findings suggest. Carriers, and those who chose not to be tested or did not know their genetic testing status, were less likely to want additional children than non-carriers [31]. Therefore, once effective contraception is accessible, *BRCA* mutation carriers may avail themselves of these family planning methods, allowing them to reduce fertility and in turn reduce the potential risk of burdening their children with cancer. Recent fertility differences may also be the result of women following recommended preventive guidelines for mutation carriers. In a study of behavioural differences 2 years after genetic testing, 46 per cent of carriers had obtained bilateral oophorectomies [32]. A family history of cancer has also been found to influence the decision for prophylactic surgery independent of genetic testing [31]. These findings are consistent with the idea that fertility of tested carriers (i.e. among contemporary women) may be reduced as a result of family history of breast/ovarian cancer.

Finally, Pavard & Metcalf [7] examined evolutionary selection of *BRCA1* mutations and concluded that *BRCA1* alleles may have experienced negative selection throughout human history. Although breast and ovarian cancer typically

arise after menopause, sufficient numbers of incident cases occur before that age. They consider (but do not dismiss) whether antagonistic pleiotropy may be an explanation for the persistent (though rare) allele frequencies for deleterious *BRCA1* mutations, though citing some empirical support for it [7]. They 'show that *BRCA1* mutations are subject to strong negative selection. *BRCA1* is therefore not a good candidate for mutation accumulation'. (p. 7 of [7]). They indicate that for antagonistic pleiotropy to be plausible, positive selection on alleles leading to improved survival or fertility at younger ages must be substantial enough to offset the negative selection on survival at later ages. The specific role of fertility as part of this argument was not directly addressed in their study since their analysis explicitly assumes that *BRCA* mutation carriers and non-carriers do not differ with respect to fertility, and that fertility does not affect the risk of cancer for those with the mutation (p. 4 of [7]). Our results would suggest that the former assumption is not supported. With respect to the latter assumption, there is evidence that increasing fertility may mitigate the cancer risk of *BRCA* mutations [33,34]. This may explain the relatively lower levels of cancer incidence among mutation carriers in the more distant past [22,25]. Accordingly, some susceptibility alleles may have faced lower levels of negative selection in earlier environments and allowed them to have higher allele frequencies in the population. It is worth noting that a number of mutations identified in the sample (e.g. c.4065_4068delTCAA, c.1175_1214del40, c.5266dupC, c.6275_6276delTT, c.6486_6489delACAA, c.3937C>T) have been characterized as having been present 8–170 generations earlier [13,14]. Additional work remains to establish how ancient all the mutations are in this sample.

The higher number of CEB and higher mortality rate of *BRCA1/2* mutation carriers may reflect the antagonistic pleiotropic features of these mutations. This raises a number of clinical issues in that the very individuals who carry these mutations are most likely to transmit them, given their larger family sizes. The fertility that may be elevated among mutation carriers may also offer some protection for mothers from subsequent cancer risk.

This work was supported by National Institutes of Health grant AG022095 (The Utah Study of Fertility, Longevity and Ageing). We wish to thank the Huntsman Cancer Foundation for database support provided to the Pedigree and Population Resource of the HCI, University of Utah. We also thank Dr Richard Cawthon, Dr David Goldgar and Vickie Venne for their comments, and Dr Jeffrey Botkin at the University of Utah School of Medicine and Alison Fraser, Diana Lane Reed, and Deb Ma at the HCI for valuable assistance in managing the data. Partial support for all datasets within the UPDB was provided by the University of Utah HCI and the HCI Cancer Centre Support Grant, P30 CA42014 from National Cancer Institute.

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