Breast Cancer Research

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Breast Cancer Research 2010, **12**:R109 doi:10.1186/bcr2796

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ISSN 1465-5411

Article type Research article

Submission date 26 July 2010

Acceptance date 23 December 2010

Publication date 23 December 2010

Article URL http://breast-cancer-research.com/content/12/6/R109

Breast Cancer Research

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A PALB2 mutation associated with high risk of breast cancer

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Abstract

Introduction: As a group, women who carry germline mutations in PALB2 are at increased risk of breast cancer. Little is known about by how much, or whether risk differs by mutation or family history, due to the paucity of studies of cases unselected for family history.

Methods: We screened 1,403 case-probands participating in a population-based, age-at-onset-stratified study of Australian women with invasive breast cancer for PALB2 mutations. The age-specific risk of breast cancer was estimated from the cancer histories of first- and second-degree relatives of mutation carrying probands using a modified segregation analysis that included a polygenic modifier and conditioned on the carrier case proband. Further screening for PALB2 c.3113G>A (W1038X) was conducted for 779 multiple-case breast cancer families ascertained through family cancer clinics in Australia and New Zealand and 764 population-based controls.

Results: We found five independent case probands in the population-based sample with the protein-truncating mutation PALB2 c.3113G>A (W1038X); two of 695 diagnosed before age 40 years and three of 708 diagnosed when aged 40-59 years. Both the two early-onset carrier case probands had very strong family histories of breast cancer. Further testing found that the mutation segregated with breast cancer in these families. No c.3113G>A (W1038X) carriers were found in 764 population-based unaffected controls. The hazard ratio was estimated to be 30.1 (95% CI 7.5 to 120; $P < 0.0001$) and the corresponding cumulative risk estimates were 49% (95% CI 15 to 93) to age 50 and 91% (95% CI 44 to 100) to age 70. We found a further eight families carrying this mutation in 779 multiple-case breast cancer families ascertained through family cancer clinics.

Conclusions: The PALB2 c.3113G>A mutation, appears to be associated with substantial risks of breast cancer that are of clinical relevance.

Introduction

A increasing number of so-called “moderate-risk” [1] breast cancer susceptibility genes have been identified. Protein-truncating mutations in *ATM*, *BRIP1*, and *PALB2,* and *CHEK2*, have been observed to be nearly 10-times more likely in cases with strong breast cancer family histories than in unaffected controls [2-4]. The implications of these observations are not straightforward, it is difficult to use the cancer histories of these families to make informative estimates of risk (penetrance) when the reason for studying them in the first place has been their family cancer history [2-5]. Inference is more informative when based on testing families unselected for family history, but there is a paucity of such data for these genes.

Predicated on the assumption that the increased risk associated with a mutation multiplies a woman’s underlying familial/genetic risk, for these genes it has been estimated that rare protein-truncating mutations are associated with, on average, increases in risk of 2- to 3-fold [1-4]. Therefore, this increased risk must be interpreted by taking into account a carrier’s family history. Consequently, for a given gene, the absolute risk is not the same for all carriers of such mutations [5].

It is also possible that the increased risk is not the same for all mutations. Some mutations in moderate risk genes are high-risk. For example, population-based studies have shown that the 7217T>G variant in *ATM* is associated with a substantially increased risk comparable to that for mutations in *BRCA1* and *BRCA2* [6], as has a subset of rare substitutions in *ATM* [7]. Another example is the Finnish founder *PALB2* mutation; from studying cases unselected for family history it has been estimated to be

associated with a 6-fold increased risk and a cumulative breast cancer risk of 40% by age 70 years [8].

We tested Australian women, affected and unaffected with breast cancer, and selected and unselected for family history for *PALB2* mutations in order to estimate prevalence and penetrance of these mutations in the Australian population.

Materials and methods

Subjects

The Australian Breast Cancer Family Registry (ABCFR) is a population-based, case-control-family study of breast cancer, with an emphasis on early-onset breast cancer, carried out in Melbourne and Sydney, Australia [9-11] that is part of the Breast Cancer Family Registry [12]. All adult women living in the metropolitan areas of Melbourne and Sydney who were diagnosed with a histologically confirmed first primary cancer of the breast were invited to participate in the ABCFR. From January 1, 1992, through September 30, 1999, in Melbourne and from January 1, 1993, through December 31, 1998, in Sydney, women aged younger than 40 years at diagnosis were selected; after January 1, 1996, random samples of women aged 40–49 years and 50–59 years at diagnosis were selected. All recruitment was done irrespective of family history.

Cases were identified by use of the Victorian and New South Wales cancer registries, to which notification of cancer diagnoses is a legislative requirement. Overall, we approached 2303 eligible cases (ages at diagnosis: 1208 = <40 years, 551 = 40–49 years, 544 = 50–59 years) which resulted in 1610 cases and a blood sample was

collected from 94%. Cancers in relatives were verified by cancer registry reports, medical records or death certificates."

Previous mutation screening performed on the germline DNA of these women included screening of *BRCA1*, *BRCA2*, *TP53* and *ATM* using a variety of mutation detection techniques [13, 14-20]. These identified *BRCA1* (n=47), *BRCA2* (n=48), *ATM* (n=1) and *TP53* (n=5) mutation carriers that were not excluded from *PALB2* mutations screening performed in this study. Six hundred and ninety five women diagnosed with breast cancer under the age of 40 years were available from the ABCFR for HRM curve analysis of *PALB2* and 708 women diagnosed with breast cancer at the age of 40 years and over were screened for *PALB2* c.3113G>A by taqman assay. The study was approved by the ethics committees of The University of Melbourne and The Cancer Council Victoria and all participants provided written informed consent for participation in the study.

The Kathleen Cuningham Foundation Consortium for Research in Familial Breast Cancer (kConFab) collected the same epidemiological, family history, lifestyle data, as well as biospecimens as the ABCFR from more than 1,500 Australasian multiple-case breast cancer families encompassing 10,000 subjects ascertained through family cancer clinics in Australia and New Zealand [21]. We obtained 778 DNAs from the youngest affected member, who had provided a blood sample, of families who had been tested and found not to carry *BRCA1* or *BRCA2* mutations. All participants provided written informed consent for participation in the study.

High Resolution Melt curve analysis and sequencing analysis of *PALB2*

The *PALB2* genomic sequence was obtained from NCBI (Reference sequence number NG_007406.1). Primers (Geneworks, South Australia, Australia) were designed by using Primer3 software (Whitehead Institute and Howard Hughes Medical Institute, Cambridge, USA, http://frodo.wi.mit.edu/primer3/). For optimal performance of the HRM, primers were designed to amplify DNA products between 100 and 310 base pairs. A total of 35 fragments were designed to cover the coding and flanking intronic regions of *PALB2*. The primer sequences and annealing temperatures are listed in additional file 1. Initially, 96 DNAs were Sanger-sequenced as described in Tischkowitz *et al.*, (2008) [22]. This data and the corresponding DNAs were then used to establish the optimal conditions for HRM analysis. DNA extracted from peripheral blood samples provided by 1,473 case probands (695 from women diagnosed with breast cancer under the age of 40 years participating in the ABCFR and 778 from kConFab) were screened for germline *PALB2* mutations using HRM analysis [23-24]. DNAs were then systematically screened using this established method. HRM reactions were carried out in 15µl volumes and included 1.5 µl of 10X PCR Buffer (Applied Biosystems, Victoria, Australia), 3mM final concentration of $MgCl_2$ (Applied Biosystems), 100µM final concentration of dNTPs (Bioline, New South Wales, Australia), 200nM final concentration of each primer (Geneworks), 2.3µM final concentration of Syto9 (Invitrogen, Victoria, Australia), 0.25 units of AmpliTaq Gold (Applied Biosystems) and 3µl of Q solution (Qiagen, Victoria, Australia). Each reaction underwent a hold of 10 minutes at 95˚C and 40 cycles of amplification of 30s at 95˚C and 1 minute at annealing temperature followed by melting to dissociate double-stranded DNA. The temperature

range for melting was set at ±10˚C of the T_m of each amplicon with a rise in temperature of 0.05˚C/s. HRM analysis was performed using Rotor-Gene 6000 Series Software 1.7 (Qiagen). Fragments displaying aberrant melt curves were sequenced to determine potential underlying genetic variations. Sequencing reactions utilised larger amplicons than those generated during HRM analysis (additional file 2). Sequencing was carried out in 10µl reactions which included 1µl of 10X PCR Buffer (Applied Biosystems), 3mM final concentration of $MgCl_2$ (Applied Biosystems), 100µM of final concentration of dNTP (Bioline), 200nM of each primer (Geneworks), 0.25 units of AmpliTaq Gold (Applied Biosystems) and 3µl of Q solution (Qiagen). PCR products were purified and analysed on a 3130xl Genetic Analyser and the results were viewed using Chromas 1.45 (Technelusium, Queensland, Australia).

Taqman assays

Using the methods of Orlando *et al.*, (1998) [25] and Ratnasinghe *et al.*, (2004) [26], DNA extracted from the peripheral blood of 708 ABCFR probands diagnosed with breast cancer over the age of 40 years, 403 probands selected from the kConFab resource and 764 unaffected population controls from the ABCFR, were analyzed for *PALB2*, c.3113G>A by Taqman assay. Each 10µl reaction contained 5µl of 2X Taqman mastermix (Applied Biosystems) and 0.125µl of 40X assay mix (Applied Biosystems) and were performed using a LightCycler 480 SW1.5 (Roche, Penzberg, Germany).

Statistical methods for penetrance analyses

The age-specific hazard ratio (HR) for breast cancer (the ratio of the age-specific breast cancer incidence rates for carriers of the mutation to that for non-carriers) was estimated using modified segregation analysis [27]. Models were fitted by the method of maximum likelihood using the statistical package MENDEL version 3.2 [28]. To adjust for ascertainment, the likelihood for each pedigree was conditioned on the proband's phenotype (breast cancer status and age of onset) and genotype. Each pedigree involved data for the carrier proband and her first- and second-degree relatives.

A mixed model was employed which incorporates an unmeasured polygenic factor to model the effect on breast cancer risk of a large number of unmeasured genes, in addition to the measured major gene [29]. The polygenic part of this model was implemented via a hypergeometric polygenic model with four loci [30] and postulates a normally-distributed random variable G for each person so that these variables are correlated within families (see section 8.9 of Lange et al 1998 [31]). A woman's age at breast cancer diagnosis was modeled as a random variable whose hazard was, for non-carriers, exp(G) times the Australian breast cancer incidence rate for 1992-2002 (Australian Institute of Health and Welfare & National Breast Cancer Centre (2006) [32]) or, for carriers, the product of this hazard by the age-specific HR. As in Antoniou et al., 2002 [33] the variance of G was chosen to be 1.67 and the mean was chosen so that the average hazard for non-carriers equaled the population incidence. When testing for an age dependence of the HR, the model with a constant HR was compared to one where the HR was a continuous, piece-wise linear function of age which was constant

before age 40 years, linear between ages 40 and 60 years and constant after age 60 years.

All models assumed Hardy-Weinberg equilibrium at the *PALB2* locus, a dominant action of *PALB2* c.3113 G>A on breast cancer risk, conditional independence of all phenotypes given genotypes and an allele frequency of 0.001 for the variant in the Australian population. Two-sided P-values for the modified segregation analyses were based on the likelihood ratio test.

Age-specific cumulative risk estimates were calculated from these hazards as one minus the exponential of the cumulative hazard and the corresponding confidence intervals were calculated using a parametric bootstrap with 5000 replications. More specifically, 5000 draws were taken from the normal distribution that the parameter estimates would be expected to follow under asymptotic likelihood theory. For each age, corresponding values of the cumulative risk were calculated and the 95% CI was taken to be the 2.5^{th} and 97.5^{th} percentile of this sample. To test the accuracy of this estimate, we compared an appropriate weighted average of the corresponding survival functions (which are one minus the cumulative risk) with the Kaplan-Meier survival curve [33].

Results

We tested for *PALB2* mutations using high resolution melt (HRM) analysis to scan DNA extracted from peripheral blood samples taken from a sample, unselected for family history, of women diagnosed with breast cancer before the age of 40 years (probands) from a population-based case-control-family study (Australian Breast Cancer

Family Registry; ABCFR). Two of the 695 probands diagnosed before the age of 40 years were found to carry the *PALB2* c.3113G>A (W1038X) mutation and both had strong family histories of breast cancer. Figure 1 shows that only 8% of all tested probands had two or more first- or second-degree relatives with breast or ovarian cancer [13], yet both these carriers were in this extreme group.

Proband 1 was diagnosed at the age of 37 years with a Grade 3 infiltrating ductal carcinoma. Figure 2 (a) shows that she had three sisters who had all been diagnosed with breast cancer (one had two primary diagnoses) at the ages of 36, 40, 45 and 51 years. Her mother had been diagnosed with breast cancer at the age of 68 years and a maternal cousin had a breast cancer diagnosis at the age of 51 years. The proband's father had been diagnosed with bladder cancer at the age of 65 years and prostate cancer at the age of 68 years. Two paternal aunts had breast cancer diagnoses at ages 67 and 54 years and a paternal cousin (daughter of an affected paternal aunt) had been diagnosed with breast cancer at the age of 47 years. Predictive testing was performed for this PALB2 mutation using blood samples collected from twenty two family members. Four affected female relatives were found to carry this mutation (one an obligate carrier) that involved the paternal lineage. The proband's mother, maternal uncle, and a maternal cousin with breast cancer did not carry the mutation.

Proband 2 was diagnosed with a Grade 3 infiltrating ductal carcinoma at the age of 27 years. Figure 2(b) shows that she had two paternal aunts diagnosed with breast cancer at ages 34 and 35 years and a paternal grandmother diagnosed at the age of 50 years (all deceased). Predictive testing was performed for this *PALB2* mutation using blood samples collected from seven family members, and paraffin-embedded tissue for

three of the affected relatives (one paternal aunt and two paternal grandparents). The proband's father, and two affected female relatives related to the father, carried the mutation. One unaffected sister of the proband, aged 42 years at last contact, carried *PALB2* c.3113G>A. The proband's mother, a maternal aunt and two sisters, all unaffected, did not carry the mutation.

We then used a Taqman assay to screen in women diagnosed with breast cancer between the ages of 40 and 59 years and control probands participating in the ABCFR for the *PALB2* c.3113G>A mutation. One of the 360 probands diagnosed when aged 40-49 years, and two of the 348 diagnosed when aged 50-59 years, were found to carry the mutation. Figures 2(c)-(e) show that all these carriers had a family cancer history and one included verified reports of breast cancer in relatives. None of 764 tested population-based controls carried this mutation.

Using the five population-based mutation carrier families we estimated the breast cancer hazard ratio (HR) using the cancer histories of first- and second-degree relatives and found it to be 30.1 (95% CI 7.5 – 120; $p<0.0001$), and independent of age ($p=0.8$; though due to low power a modest age-dependence cannot be ruled out). This corresponded to an estimated age-specific cumulative risk (penetrance) of 49% (95% CI 15 - 93) to age 50 years and 91% (95% CI 44 - 100) to age 70 years; see Figure 3. Our confidence in these estimates was enhanced by their high degree of agreement with the Kaplan-Meier survival curves for female first- and second-degree relatives of the probands which accounted for the fact that these relatives are a mix of carriers and non-carriers (see Figure 4). The HR corresponding to the *PALB2* W1038X variant could not be distinguished from the average of the age-specific HRs for *BRCA2* mutations

reported in Antoniou et al. (2003) [34] of 13.6 (p=0.3). It was higher than a HR of 2.3 which is roughly equivalent to the OR of 2.3 for *PALB2* variants reported by Rahman et al., (2007) [4] (p=0.0003).

Lastly, HRM screening for *PALB2* mutations for 779 multiple-case breast cancer families recruited through Australian and New Zealand clinics by the kConFab consortium identified a further eight (1.0%) families that carried the *PALB2 c.*3113G>A mutation. We did not attempt to estimate penetrance using these family history since they were non-systematically selected by their family histories of cancer.

Discussion

Studies conducted in the UK, Finland, Italy, Spain and Canada have shown that, while rare, mutations in *PALB2* are far more common in breast cancer cases with a strong family history than in unaffected population-based controls. In the UK study, 10 of 923 (1.1%) strongly familial cases, compared with 0 of 1084 unaffected controls, were mutation carriers (*p*=0.004) [4]. The Finnish recurrent mutation *PALB2* (c.1592delT) was carried by three of 113 familial breast cancer cases (2.7%) compared with six of 2,501 (0.2%) controls, an 11-fold difference (95% CI: 3-44) [35]. A French-Canadian founder mutation, c.2323C>T, resulting in Q775X, was found in two of 356 (0.6%) unselected early-onset cases compared with one of 50 (2%) familial cases and none of 6442 (0%) newborns [36]. Papi *et al*. (2009) screened 132 Italian breast cancer families without BRCA1 or BRCA2 mutations and identified one protein truncating mutation (c.2257C>T, R753X) that was not observed in 300 control DNAs [37]. Garcia *et al.* (2009) reported one protein-truncating mutation (c.1056-1057delGA, K3531X) in a

screen of 95 multiple-case breast cancer families without BRCA1 or BRCA2 mutations [38]. These observations, while showing that *PALB2* mutations are associated with increased risk of breast cancer, cannot be used to obtain direct or precise estimates of the magnitude of increased risk [4-5].

Estimation of the average increased risk associated with *PALB2* mutations has been performed previously using an indirect method by presuming a polygenic modifier model. This method estimated that on average *PALB2* protein-truncating mutations conferred a 2.3-fold increased risk of breast cancer (95% CI 1.4–3.9, P = 0.0025) [4]. For one specific Finnish founder mutation, a direct estimate has been made from a sample of population-based cases, unselected for family history, screened for the *PALB2* 1592delT mutation [8]. Eighteen of the 1,918 (0.9%) cases carried the mutation. It was estimated from their family histories that carriers in this setting are at about 6-times population risk (95% CI: 2-17; p=0.01), equivalent to a breast cancer risk by age 70 years of 40% (95% CI: 17-77) comparable to the 45% estimate found for *BRCA2* mutation carriers from a large study of unselected carrier families [34].

In Australia, we have now found a *PALB2* mutation, c.3113G>A, carried by five of 1,403 (0.4%) unselected population-based cases diagnosed before age 60 years, eight of 779 (1%) cases from multiple-case families, and none of 764 unaffected population-based controls. This mutation was found twice by a UK study that screened 923 cases from multiple-case breast cancer families [4]. The Australian carrier families reported here are predominantly of Australian, English and Scottish heritage. These are the only reports of this mutation we know of so it is possible that this mutation originated in the UK.

We estimated from analyses of case carrier families that carriers of the *PALB2* *c.*3113G>A mutation who are relatives of unselected case carriers have a high risk of developing breast cancer; about 50% to age 50 years and 90% to age 70 years. The lower bound of the 95% confidence interval of the latter estimate is about the same as the average risk for *BRCA2* mutation carriers estimated using the same design and statistical methodology [39]. Therefore this mutation is associated with as high a risk as other mutations being tested for by cancer family genetics services across the world.

For multiple-case families currently attending cancer genetics services in Australia, at most 20% of those screened for *BRCA1* and *BRCA2* are found to carry mutations [21], similar to the experience in the UK [40] and USA [41]. Although *PALB2* c.3113G>A is rare, testing for it is inexpensive and has clinical utility; by virtue of being based on case families, our risk estimate applies to women with at least some family history so is appropriate for counselling carriers identified in multiple-case families attending cancer family genetics services.

Conclusions

Given carriers of this *PALB2* mutation appear to be at least at the conventional level of “high-risk”, thought to apply to BRCA1 and BRCA2 mutation carriers, testing would seem justified in a clinical genetics setting. For the women and their family members who carry these mutations, it is potentially important that they be identified so that they can be offered appropriate prevention, screening and clinical management. There may be other *PALB2* mutations that are also as highly penetrant, and this paradigm might apply to other genes, such at *ATM, BRIP1* and *CHEK2*, for which

mutations appear to be - on average - associated with 'moderately' increased risks but for which some mutations might be associated with "high risk". Targeted clinical testing of such high-risk mutations in these genes could be justified.

Abbreviations

ABCFS: Australian Breast Cancer Family Study; HR: Hazard Ratio; HRM: High resolution melt; kConFab: The Kathleen Cuningham Foundation Consortium for Research in Familial Breast Cancer; NCBI: National Centre for Biotechnology Information; UK: United Kingdom; USA: United States of America

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MCS, led this study including the conception, design, acquisition of data, analysis, interpretation on data and preparation of the manuscript; ZLT, conducted molecular analyses and contributed to the preparation of the manuscript; JGD led aspects of the analyses and contributed to the preparation of the manuscript; FAO, conducted molecular analyses and contributed to the preparation of the manuscript; DJP, provided critical input to the molecular analysis and manuscript preparation; MT, conducted molecular analyses and contributed to the preparation of the manuscript; NS, conducted molecular analyses and contributed to the preparation of the manuscript; GBB, contributed to aspects of the analyses and contributed to the preparation of the

manuscript; CA, contributed data from the Australian Breast Cancer Family Study resource and contributed to the preparation of the manuscript; IW, provided critical input to the molecular analysis and manuscript preparation; LB led aspects of the analyses and contributed to the preparation of the manuscript; GGG, is co-founder of the ABCFS resource, contributed data from the ABCFS and contributed to the preparation of the manuscript; DEG provided critical input to the molecular analysis, statistical analyses and manuscript preparation; WDF conducted molecular analyses and contributed to the preparation of the manuscript; JLH is co-founder of the ABCFS resource, contributed data from the ABCFS, contributed to analyses and the preparation of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The Australian Breast Cancer Family Registry was supported by the National Health and Medical Research Council of Australia, the New South Wales Cancer Council, the Victorian Health Promotion Foundation (Australia), and the National Cancer Institute, National Institutes of Health under RFA-CA-06-503 and through cooperative agreements with members of the Breast Cancer Family Registry (Breast CFR) and P.I.s. The University of Melbourne (U01 CA69638) contributed data to this study. The content of this manuscript does not necessarily reflect the views or the policies of the National Cancer Institute or an of the collaborating centres in the Breast CFR, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government or the Breast CFR. We extend our thanks to the many women and

their families that generously participated in the Australian Breast Cancer Family Study and consented to us accessing their pathology material.

We wish to thank Heather Thorne, Eveline Niedermayr, all the kConFab research nurses and staff, the heads and staff of the Family Cancer Clinics, and the Clinical Follow Up Study (funded by NHMRC grants 145684, 288704 and 454508) for their contributions to this resource, and the many families who contribute to kConFab. kConFab is supported by grants from the National Breast Cancer Foundation, the National Health and Medical Research Council (NHMRC) and by the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia, and the Cancer Foundation of Western Australia.

We wish to thank Margaret McCredie for key role in the establishment and leadership of the ABCFS in Sydney, Australia. MCS is a National Health and Medical Research Council Senior Research Fellow and Victorian Breast cancer Research Consortium (VBCRC) Group Leader. JLH is an Australia Fellow of the National Health and Medical Research Council and a VBCRC Group Leader. MT is supported by the Susan G. Komen Foundation for the Cure, the Jewish General Hospital Weekend to End Breast Cancer and Fonds de la Recherche en Santé du Québec (FRSQ). WDF is a Chercheur National of the FRSQ.

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Figure legends

Figure 1. A schematic representation of women diagnosed with breast cancer under age 40 years participating in the Australian Breast Cancer Family Registry.

The majority of breast cancer cases diagnosed before the age of 40 years have no affected family members (0 affected, the “sporadic” breast cancer group), some probands have one affected family member (1 affected), and ~8% have a stronger family history[#] (the “familial” breast cancer group). #; strong family history defined as the case proband having two or more first- or second-degree relatives affected with breast or ovarian cancer. x; represents the 22 *BRCA2* mutation carriers identified by previous

testing in the ABCFR [13, 19]. The filled black stars represent the two probands found to carry *PALB2* c.3113G>A who both have a very strong family history of breast cancer. Adapted from Hopper, 2001 [42].

Figure 2.

Pedigrees of the *PALB2* c.3113G>A mutation carrying families.

Pedigrees of the *PALB2* c.3113G>A mutation carrying families with probands (indicated by arrows) (a) and (b) families with probands diagnosed under the age of 40 years identified in the population-based study (c) family with proband diagnosed under the age of 50 years and (d) families diagnosed under the age of 60 years.

Breast cancer indicated by black filled symbols and others cancers by grey filled symbols. All primary cancer diagnoses are indicated for each individual. Numbers within symbols represent multiple individuals. Breast, breast cancer; V, cancer verified; +, *PALB2* c.3113G>A positive; -, *PALB2* c.3113G>A negative; (+), obligate carrier; / , deceased

Figure 3.

Age-specific cumulative risks for *PALB2* c.3113G>A mutation carriers.

Age-specific cumulative risks of breast cancer for women carrying the PALB2 c.3113G>A mutation (unbroken lines) and for women in the general Australian population (dotted line).

Figure 4.

The Kaplan-Meier survival curves for *PALB2* c.3113G>A mutation carriers.

The Kaplan-Meier survival curve, with breast cancer as the outcome, for female first- and second-degree relatives of the probands with the fitted survival curve overlaid. The fitted survival curve was calculated as the average of the survival curves for carriers and non-carriers, each of these being equal to the exponential of minus the appropriate cumulative incidences. The averaging of survival curves used age-specific weights equal to the expected proportion of relatives at risk of breast cancer at a given age who carry (for the carrier survival curve) or do not carry (for the non-carrier survival curve) the *PALB2* mutation. Carrier probabilities for the relatives were calculated from known genotypes and family relationships, but not affected statuses or other phenotypes, using a modified version of Mendel 3.2 [28].

Additional files

Additional file 1.

Title: PCR primer sequences and annealing temperatures.

Description: The primer sequences and annealing temperatures designed to amplify 35 fragments (including the coding and flanking intronic regions) of *PALB2*.

Additional file 2.

Title: Sequencing primer sequences.

Description: Primers designed for DNA sequencing, including DNA fragment size (bp) and annealing temperatures.

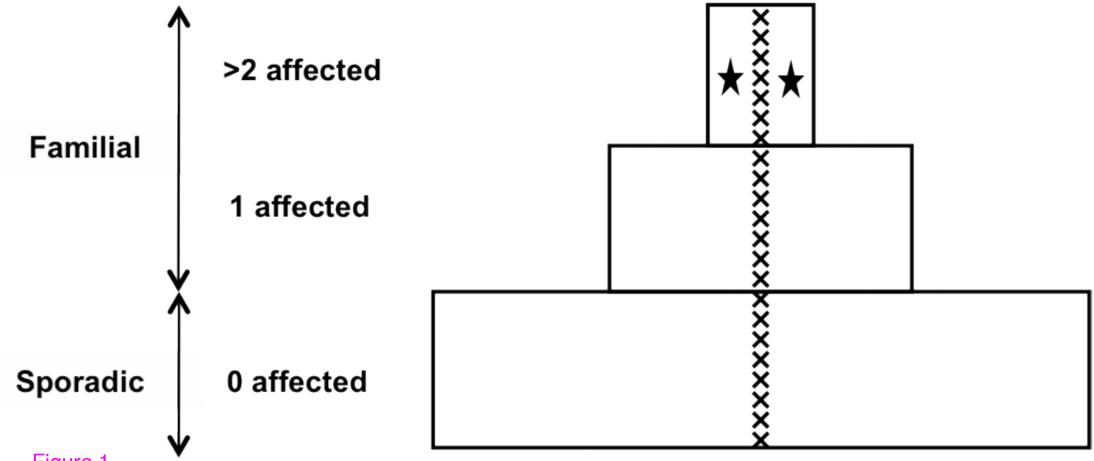

Figure 1

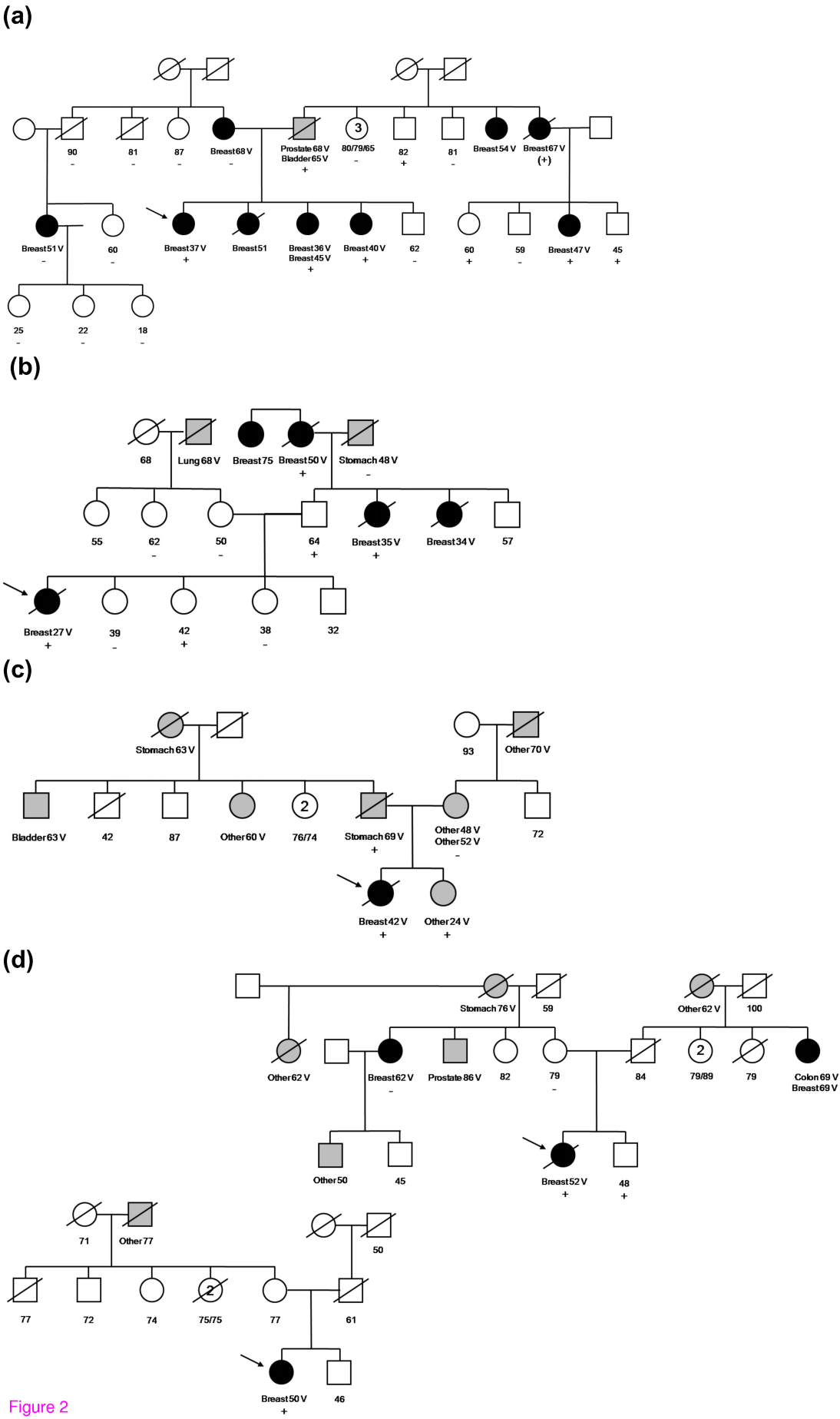
(a)
90 -
81 -
87 -
Breast 68 V -
Prostate 68 V
Bladder 65 V
+
3
80/79/65 -
82 +
81 -
Breast 54 V
Breast 67 V
(+)
Breast 51 V -
60 -
Breast 37 V +
Breast 51
Breast 36 V
Breast 45 V
+
Breast 40 V +
62 -
60 +
59 -
Breast 47 V +
45 +
25 -
22 -
18 -
(b)
68
Lung 68 V
Breast 75
Breast 50 V +
Stomach 48 V -
55
62 -
50 -
64 +
Breast 35 V +
Breast 34 V
57
Breast 27 V +
39 -
42 +
38 -
32
(c)
Stomach 63 V
93
Other 70 V
Bladder 63 V
42
87
Other 60 V
2
76/74
Stomach 69 V +
Other 48 V
Other 52 V -
72
Breast 42 V +
Other 24 V +
(d)
Stomach 76 V
59
Other 62 V
100
Other 62 V
Breast 62 V -
Prostate 86 V
82
79 -
84
2
79/89
79
Colon 69 V
Breast 69 V
Other 50
45
Breast 52 V +
48 +
71
Other 77
50
77
72
74
2
75/75
77
61
Breast 50 V +
46

Figure 2

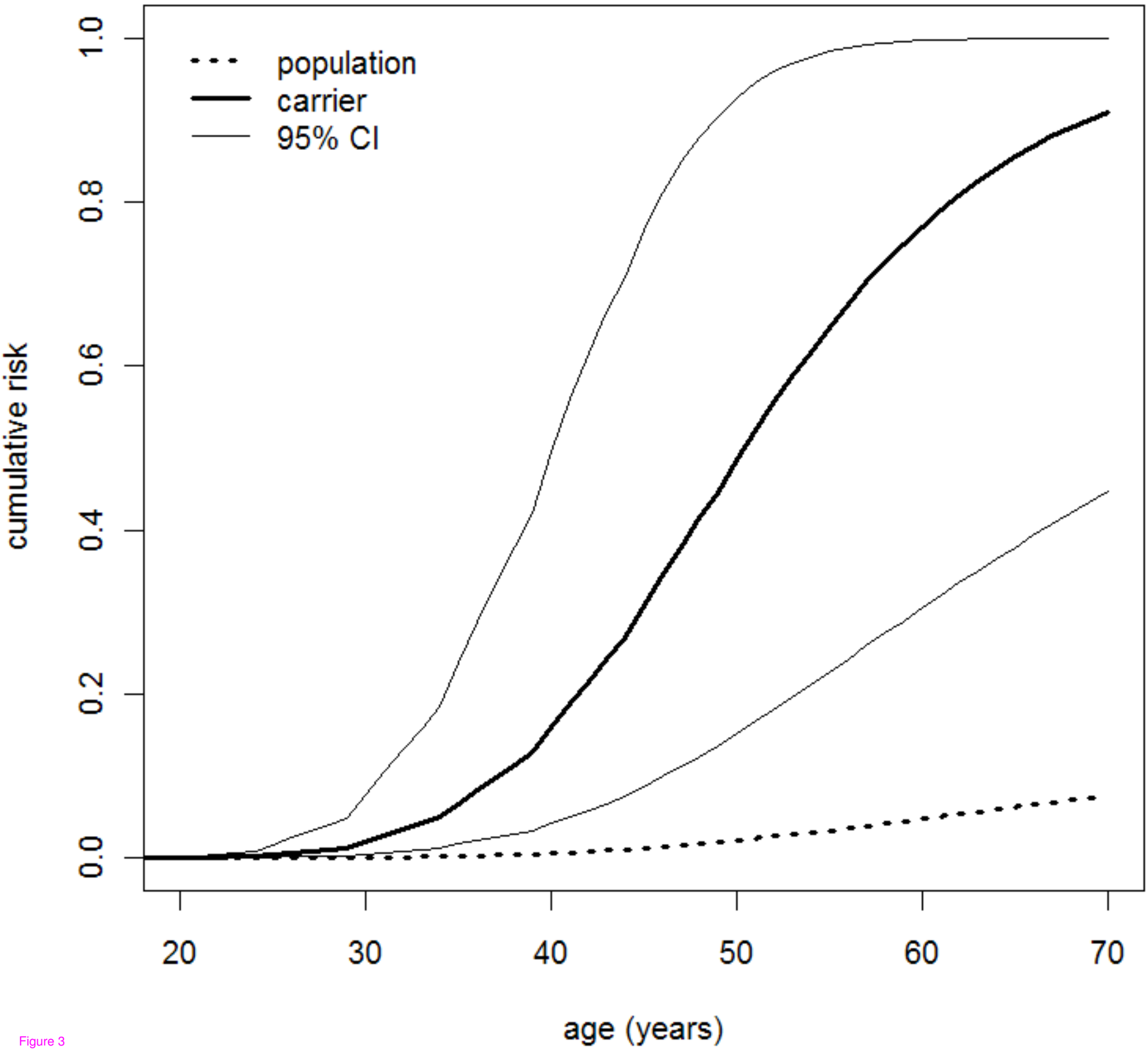
population
carrier
95% CI
cumulative risk
0.0
0.2
0.4
0.6
0.8
1.0
20
30
40
50
60
70
age (years)

Figure 3

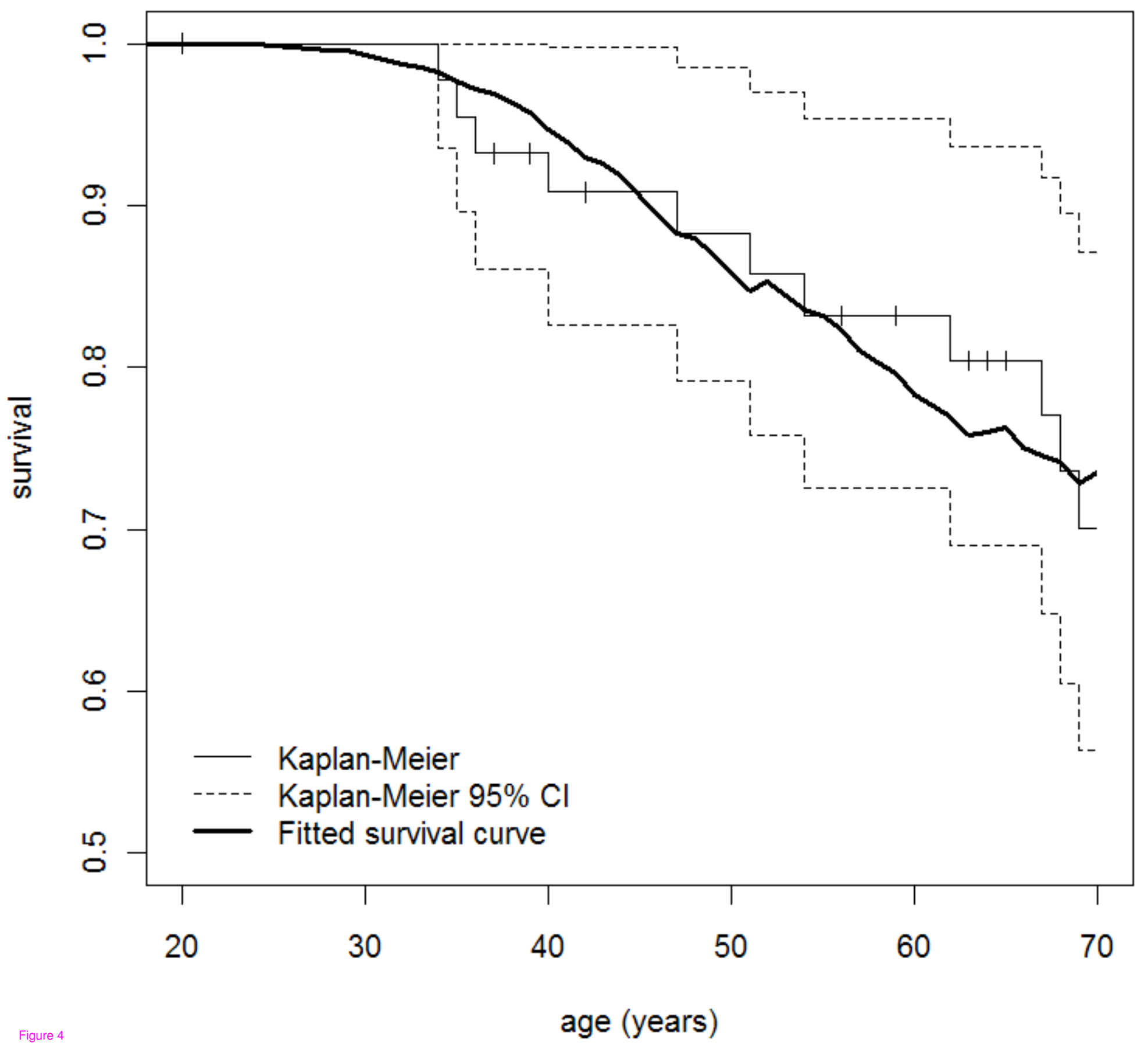
survival
1.0
0.9
0.8
0.7
0.6
0.5
20
30
40
50
60
70
age (years)
Kaplan-Meier
Kaplan-Meier 95% CI
Fitted survival curve

Figure 4

Additional files provided with this submission:

Additional file 1: Additional file 1.pdf, 62K
http://breast-cancer-research.com/imedia/6444151854983168/supp1.pdf
Additional file 2: Additional file 2.pdf, 63K
http://breast-cancer-research.com/imedia/1103663461498317/supp2.pdf