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Parity and the risk of breast and ovarian cancer in *BRCA1* and *BRCA2* mutation carriers.

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ABSTRACT

Environmental or lifestyle factors are likely to explain part of the heterogeneity in breast and ovarian cancer risk among *BRCA1* and *BRCA2* mutation carriers. We assessed parity as a risk modifier in 515 and 503 Spanish female carriers of mutations in *BRCA1* and *BRCA2*, respectively. Hazard ratios (HR) and their corresponding 95% confidence intervals (CI) were estimated using weighted Cox proportional hazards regression, adjusted for year of birth and study centre. The results for ever being parous and number of live-births were very similar for carriers of mutations in both genes. For all mutation carriers combined, the estimated HR associated with ever having had a live-birth was 0.74 (95% confidence interval [CI]=0.55-1.01, p=0.06), and that associated with each live-birth was 0.87 (95%CI=0.77-0.98, p=0.02). The latter association was observed only in women aged 40 and above (HR=0.81, 95%CI=0.70-0.94, p=0.004 versus HR=0.99, 95%CI=0.83-1.18, p=0.9 for women under age 40), and this trend was highly consistently observed for carriers of mutations in each gene. There was no evidence of an association between breast cancer risk and age at first birth for parous *BRCA1* or *BRCA2* mutation carriers (p-trend$\geq$0.3). The power to detect associations with ovarian cancer risk was much lower, especially for *BRCA2* mutation carriers. Nevertheless, having a live-birth was associated with protection for *BRCA1* mutation carriers (HR=0.41, 95%CI=0.18-0.94, p=0.03), and a strong and consistent protective effect of age at first birth was observed for parous carriers of mutations in both genes (HR=0.65, 95%CI=0.52-0.83, p<0.001). This is the third independent study to find that, as in the general population, parity appears to be associated with protection from breast cancer in women with mutations in *BRCA1* and *BRCA2*. Parity appears to be protective for ovarian cancer in

BRCA1 mutation carriers, but its role in *BRCA2* mutation carriers remains unclear. Whether later age at first birth is also protective for ovarian cancer in mutation carriers requires further confirmation.

INTRODUCTION

The incomplete penetrance of mutations in the breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* suggests that there are other genetic and/or environmental factors that modify the risk of these cancers in female mutation carriers. Additional evidence of risk modifiers includes the general observation that estimates of penetrance tend to be higher in studies of multiple-case families than in studies of families of cases unselected for family history [1], as well as the more recent finding that the proportion of breast cancer fenocopies (cases of cancer in non-carrier members of a mutation-carrying family) is greater than that expected according to the disease incidence in the general population [2-4]. Both results suggest that other genetic and/or non-genetic factors may accumulate in some families and influence the risk of cancer in carriers and non-carriers alike. More specific evidence of the existence of non-genetic modifiers, of breast cancer risk in particular, comes from the consistent observation that the penetrance of *BRCA1* and *BRCA2* mutations has increased over the last century [1, 5-8]. Environmental or lifestyle factors, rather than genetic factors, are most likely to explain this trend.

The identification of these risk-modifying factors for mutation carriers is important for several reasons. Firstly, providing these women with information about what they can do with respect to environmental and lifestyle factors to reduce their risk of cancer may be an important complement to screening programs, and a possible alternative to invasive prophylactic surgical interventions. Secondly, the incorporation of these factors into penetrance estimation will lead to more accurate risk modelling and therefore better informed genetic counselling.

It is not clear whether established risk factors for breast and/or ovarian cancer in the general population, such as parity [9, 10], act as risk modifiers in carriers of mutations in *BRCA1* and *BRCA2*. Various studies have investigated such factors as modifiers, but all are subject to potential biases due to the way in which mutation carriers are recruited, and few definitive conclusions have been reached [11]. While prospective studies of cohorts of unaffected carriers are considered best placed to clarify this issue, these will take time to accumulate a sufficient number of incident cancer cases for analysis. It is therefore important that, at least until results from prospective studies become available, the largely retrospective data at hand are taken advantage of to make appropriate inference. Results that are consistently observed across multiple studies are likely to be most reliable.

It has been established in the general population that an increasing number of children is associated with protection from both breast and ovarian cancer [9, 12, 13]. Later age at first birth is associated with increase risk of breast cancer [9], but possibly a reduced risk of ovarian cancer [13]. Parity has been evaluated as a breast cancer risk modifier in a number of studies of mutation carriers [14-20], with largely contradictory results. There are fewer published studies of modifiers of ovarian cancer risk [15, 21, 22]. We aimed to assess parity (ever parous, number of full-term pregnancies and age at first full-term pregnancy) as a modifier of breast cancer risk and ovarian cancer risk in carriers of mutations in *BRCA1* and *BRCA2* recruited by 13 genetic counselling centres in Spain.

METHODS

Subjects

All female carriers of deleterious mutations in *BRCA1* and *BRCA2* recruited at 13 genetic counselling centres in Spain (see Table 1) were considered eligible. These included: (i) 799 mutation carriers recruited by 12 centres between 1995 and 2006 from the 319 families included in our previous penetrance study [5]; (ii) 235 mutation carriers from 235 families in which, as at 31st December, 2006, they were the only individual that had tested positive (which meant that they were excluded from the penentrance study, [5]); (iii) 89 mutation carriers from 42 families recruited by the *Hospital Vall d´Hebrón* in Barcelona between 2005 and 2008; and (iv) 107 obligate carriers (untested women with at least one decendent and one other non-decendent blood relative who had tested positive for the same mutation) from families recruited at all 13 centres.

Family selection, mutation testing and other data collection methods have been described previously [5]. Briefly, the youngest member affected with breast and/or ovarian cancer from families with multiple cases of these cancers was generally the first tested for mutations in *BRCA1* and/or *BRCA2*. When a mutation was detected, that specific mutation was tested for in additional family members. Mutations were defined as deleterious if they were classified as clinically important by the Breast Information Core (BIC, http://research.nhgri.nih.gov/bic/) or they met other widely accepted criteria [5]. Information on year of birth, breast and ovarian cancer status, age at diagnosis of breast and/or ovarian cancer (if applicable), current age, age at death (if deceased), age at prophylactic bilateral mastectomy (if applicable), and age at prophylactic oophorectomy (if

applicable), was collected on each family member as part of genetic counselling. We excluded eligible mutation carriers with missing data for any of these items, or for which the year of birth of at least one of their children was unknown.

Statistical methods

We compared the distribution of subjects across centres (CNIO, Sant Pau, HCSC, ICO, Vall d´Hebron, FPGMX, all others combined - for *BRCA1* mutation carriers; and CNIO, Sant Pau, HCSC, ICO, Valencia, all others combined - for *BRCA2* mutation carriers) between affected and unaffected mutation carriers using Pearson´s chi-squared test. The distributions of age at censoring (see below) and year of birth were compared by affection status using logistic regression, fitting each of these as continuous variables.

Associations with the risk of breast and ovarian cancer were assessed separately for each of the parity variables considered, by estimating hazard ratios (HR) and their corresponding 95% confidence intervals (CI) using weighted multivariable Cox proportional hazards regression with robust estimates of variance [23]. For each mutation carrier, we modeled the time to diagnosis of breast or ovarian cancer from birth, censoring at the first of the following events: bilateral prophylactic mastectomy, bilateral prophylactic oophorectomy, breast cancer diagnosis, ovarian cancer diagnosis, death and date last know to be alive. For the analysis of breast cancer, subjects were considered affected if their age at censoring corresponded to their age at diagnosis of breast cancer and unaffected otherwise. For the analysis of ovarian cancer, subjects were considered affected if their age at censoring corresponded to their age at diagnosis of ovarian cancer

and unaffected otherwise. Weights were assigned separately for the breast and ovarian cancer analyses, by affection status, age and gene mutated, so that the weighted observed incidence rate agreed with established estimates [1], summarized as "external rates" in Antoniou *et al*. [23]. The age categories considered were <25, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69 and ≥70, with the first three categories combined for the ovarian cancer analysis due to the small number of affecteds observed. These weights have been shown to correct for the bias inherent in the oversampling of affected women due to the ascertainment criteria applied [23].

We evaluated associations with parity (nuliparous, parous), number of live births (0, 1, 2, 3, ≥4) and age at first live birth (15-19, 20-24, 25-29, ≥30), with trends assessed for the latter two based on the corresponding continuous variables. For the analysis of categories of age at first live birth, 20-24 was used as the reference group because it was the most common. All these measures were modeled as time-varying variables. Additional independent variables included in all analyses were year of birth (<1930, 1930-1939, 1940-1949, 1950-1959, 1960-1969, ≥1970) and centre (in defined above and presented in Table 2). Heterogeneity in HRs by age was assessed based on the Wald-statistic p-value corresponding to the interaction term for the variable in question, by age (dichotomized into (<40, ≥40). Mutation carriers from the present study included in previous studies of parity as a modifier of cancer risk by the International BRCA1/2 Carrier Cohort Study (IBCCS) [17, 22] were excluded and weights recalculated in sensitivity analysis. The influence of survival bias was evaluated by repeating all analyses (based on re-calculated

weights) after excluding affecteds who were genetically tested more than three years after their breast cancer diagnosis, or more than one year after their ovarian cancer diagnosis.

All statistical analyses were carried out using Stata: Release 10 [24]. Robust estimates of variance were calculated using the *cluster* subcommand, applied to an identifier variable unique to each family. All p-values were two-sided and those less than 0.05 were considered statistically significant.

RESULTS

Of the 626 eligible *BRCA1* mutation carriers and 604 eligible *BRCA2* mutation carriers, 515 (82%) and 503 (83%), respectively, were included in the analyses of parity as a risk modifier. Details are given in Table 1. These were members of 253 and 246 famlies, respectively. The distrubition of the number of carriers per family were very similar for *BRCA1* and *BRCA2* mutations, with, overall, 51% of families with just one member, 23% with two, 13% with three, 6% with four, 3% with five, 2% with six, 1% with seven and less than 1% of families with eight or more members represented in the dataset. Table 2 summarises the characteristics of included mutation carriers according to affection status, and gene mutated. For ovarian cancer, but not breast cancer, affecteds tended to be older than unaffecteds, regardless of the gene mutated (both $p < 0.001$). For carriers of mutations in both genes and for both cancers, affected women tended to be born before unaffected women (all $p \leq 0.001$).

Associations with breast cancer risk

Results from the multivariable analysis of the three parity variables and breast cancer risk are summarized in Table 3. The results for ever being parous and number of live-births were very similar for carriers of mutations in *BRCA1* and *BRCA2*, with HR estimates below 1, although none were statistically significant (all $p \geq 0.08$). After combining mutation carriers in both genes, the estimated HR associated with ever having had a live-birth was 0.74 (95% confidence interval [CI]=0.55-1.01, p=0.06), and that associated with each live-birth was 0.87 (95%CI=0.77-0.98, p=0.02). Analyses stratified by age suggested that this association with number of live-births was only apparent in women

aged 40 and above (HR=0.81, 95%CI=0.70-0.94, p=0.004 versus HR=0.99, 95%CI=0.83-1.18, p=0.9 for women under age 40). While the difference in HR by age was not statistically significant (p=0.1), this result was consistently observed for *BRCA1* mutation carriers (HR=0.82, 95%CI=0.69-0.98, p=0.03 for women aged 40 and above and HR=1.02, 95%CI=0.81-1.29, p=0.9 for younger women) and *BRCA2* mutation carriers (HR=0.81, 95%CI=0.63-1.04, p=0.09 and HR=0.97, 95%CI=0.74-1.28, p=0.8, respectively). We observed no evidence of an association between breast cancer risk and age at first birth for parous *BRCA1* or *BRCA2* mutation carriers (both p-trend $\geq$ 0.3).

There were 67 mutation carriers that were included in a previous study of parity and breast cancer risk by the IBCCS [17], 38 with mutations in *BRCA1* and 29 with mutations in *BRCA2*. Excluding these made no substantial difference to the results obtained. The estimated HR per live-birth was 0.86 for *BRCA1* mutation carriers, 0.90 for *BRCA2* mutation carriers and 0.87 (p=0.03) for all carriers combined. The corresponding HR estimates for women aged less than 40 were 0.96, 1.03 and 0.97, respectively, while those for women aged 40 and above were 0.82, 0.83 and 0.81 (p=0.008), respectively. There were 299 affected mutation carriers who were diagnosed with breast cancer more than three years prior to their mutation testing, 154 with mutations in *BRCA1* and 145 with mutations in *BRCA2*. Excluding these similarly made no substantial difference to the results obtained. The estimated HR per live-birth was 0.77 for BRCA1 mutation carriers, 0.88 for BRCA2 mutation carriers and 0.79 (p=0.002) for all mutation carriers combined.

Associations with ovarian cancer risk

Results from the multivariable analyses of the three parity variables and ovarian cancer risk are also summarized in Table 3. For *BRCA1* mutation carriers, ever having had a live-birth was associated with reduced risk of ovarian cancer (HR=0.41, 95%CI=0.18-0.94, p=0.03). There was some evidence of a dose-response effect, with *BRCA1* mutation carriers with four or more children at even lower estimated risk relative than those with no children (HR=0.15, 95%CI=0.04-0.56, p=0.005), but the trend per birth was not statistically significant (HR=0.80, 95%CI=0.61-1.05, p=0.1). There was no evidence of association with number of live-births for *BRCA2* mutation carriers (all $p \geq 0.3$). Age at first birth appeared to be inversely associated with ovarian cancer risk, with very similar HR estimates for *BRCA1* and *BRCA2* mutation carriers (p-trend=0.001 and 0.1 for *BRCA1* and *BRCA2* mutation carriers, respectively). The estimated HRs for carries of mutations in both genes combined, per five years of age, was 0.65 (95%CI=0.52-0.83, p<0.001).

There were 116 mutation carriers that were included in a previous study of parity and ovarian cancer risk by the IBCCS [22], 59 with mutations in *BRCA1* and 57 with mutations in *BRCA2*. Excluding these gave slightly stronger evidence of the associations reported above. For *BRCA1* mutation carriers the estimated HRs were 0.32 (p=0.01) for ever having had a live-birth and 0.74 (p=0.04) per live-birth. The estimated HR associated with increments of 5 years in age at first birth was 0.64 (p=0.004) for *BRCA1* mutation carriers, 0.46 (p=0.02) for *BRCA2* mutation carriers and 0.60 (p=0.001) for all carriers combined. There were 55 affected mutation carriers who were diagnosed with ovarian cancer more than one year prior to their mutation testing, 36 with mutations in

BRCA1 and 19 with mutations in *BRCA2*. Results were consistent after excluding these women. For *BRCA1* mutation carriers the estimated HRs were 0.29 (p=0.007) for ever having had a live-birth and 0.72 (p=0.08) per live-birth. The estimated HR associated with increments of 5 years in age at first birth was 0.58 (p=0.03) for *BRCA1* mutation carriers, 0.78 (p=0.5) for *BRCA2* mutation carriers and 0.65 (p=0.04) for all carriers combined. It should be noted that there was likely to be over-fitting of these latter models due to the small number of affecteds in this reduced sample set (37 and 16 for *BRCA1* and *BRCA2* mutation carriers, respectively).

DISCUSSION

Parity and breast cancer risk

We have evaluated the effect of parity on the risk of breast cancer in 515 *BRCA1* mutation carriers and 503 *BRCA2* mutation carriers in Spain. After adjusting for study centre and year of birth, we observed evidence parity is associated with protection from breast cancer in *BRCA1* and *BRCA2* mutation carriers. Each live-birth was associated with an estimated 13% risk reduction. We observed no evidence of an association with age at first birth.

The results from previous studies of the possible effect of parity on breast cancer risk in *BRCA1* and *BRCA2* mutation carriers have been mixed. They are summarized in Table 4. Jernstrom *et al.*, [16] pooled data from carriers of mutations in both genes (although 80% had mutations in *BRCA1*) and found that parous carriers were at an estimate 71% increased risk of breast cancer compared to nuliparous carriers. They also observed a trend effect, with an estimated 24% increased risk per full-term pregnancy. This result was not replicated in a subsequent study by the same group, based on a much larger set of mutation carriers from 55 international collaborating centres [14], most (73%) in North America. They observed that for women with a BRCA1 mutation, having 4 or more children was associated with reduced breast cancer risk compared to being nulliparous. In contrast, among BRCA2 carriers, increasing parity was associated with an increased risk of breast cancer (15% per live-birth). A third study by some of the same authors [15], reported that for Polish *BRCA1* mutation carriers, each live-birth was associated with an estimated 20% increased risk of breast cancer. All three studies matched unaffected

carriers to affected carriers on year of birth, country and gene mutated and estimated odds ratios (OR) using condition logistic regression.

The IBCCS, a predominantly European consortium, has more recently published their analysis of parity as a potential modifier of breast cancer risk in mutation carriers [17]. They obtained similar results for carriers of mutations in *BRCA1* and *BRCA2*. In a pooled analysis, they observed no effect associated with being parous, but among parous women, estimated that each live-birth was associated with a statistically significant 14% decrease in risk. This effect was only observed in women over age 40 years. This group also evaluated the effect of age at first live-birth and found marginally statistically significant evidence that it differed between *BRCA1* and *BRCA2* mutation carriers [17]. While for *BRCA1* mutation carriers, having a child later in life appeared to be associated with protection, the opposite seemed to be the case for *BRCA2* mutation carriers. Antoniou *et al.* [18] subsequently carried out a very similar analysis of a smaller set of mutation carriers from the United Kingdom and found that ever being parous was associated with an estimated 56% reduced risk for all mutation carriers combined, but again, only for women over age 40. For women of all ages, there was marginal evidence of a trend of decreasing risk with increasing parity. They also observed evidence that in parous *BRCA2* mutation carriers, risk is higher for those who have their first child later. Both these studies estimated HR using weighted Cox regression, adjusting for year of birth and other covariates.

Two other studies have examined the effect of age at first birth on breast cancer risk in mutation carriers. Rebbeck *et al.* [20] studied mostly (83%) *BRCA1* mutation carriers and observed that those who had their first birth earlier were at reduced risk of breast cancer. Most recently, members of the aforementioned intenational consortium applied their matched case-control design to the largest set of mutation carriers studied to date [19]. They found no evidence of an association for all carriers combined and reported that this result was consistent in stratified analyses by gene mutated.

Our results are consistent with those of the two other European studies that applied the same analytic approach [17, 23]. This approach adopted allows all mutation carriers with complete data to be included, in contrast to the majority of the other studies in which up to 40% of carriers were excluded because no matched-pair was found [14, 19]. The consistent results from these three independent studies suggest that, as for women in the general population, parity is associated with protection from breast cancer for women at high risk of the disease due to mutations in *BRCA1* and *BRCA2.* This finding may be particularly relavent to unaffected mutation carriers who are concerned about the impact pregnancy may have on their own breast cancer risk.

While our results are also consistent with there being no association between age at first birth and breast cancer risk in mutation carriers, the power of our study in this regard was limited (as discussed further below), and the estimated HRs for trend are in the same (opposing) directions as those reported by the two European studies [17, 18]. It is therefore difficult to reach any definitive conclusions in this regard.

Parity and ovarian cancer risk

Regarding ovarian cancer risk, after adjusting for study centre and year of birth, we observed marginal evidence that for *BRCA1* mutation carriers, ever having had a live-birth is associated with protection. We also observed that for parous *BRCA1*, and possibly *BRCA2*, mutation carriers, later age at first birth is associated with protection.

Three studies have evaluated parity as a modifier of ovarian cancer risk in mutation carriers. A Polish study of 300 *BRCA1* mutation carriers found no evidence of association with number of live-births [15]. The previously mentioned international consortium studied 3,223 mutation carriers, and observed that while women with *BRCA1* mutations (84% of their sample) appeared to be protected from ovarian cancer both by ever having had full-term pregnancy (OR=0.67, 95%CI=0.46-0.96, p=0.03) and with increasing parity (OR=0.87 per birth, 95%CI=0.79-0.95, p=0.003), parous *BRCA2* mutation carriers were at increased risk (OR=2.74, 95%CI=1.18-6.41, p=0.02) [21]. The authors did not assess age at first birth as a risk modifier. Finally, the IBCCS has recently reported on their study of larger sample of 2,281 *BRCA1* and 1,038 *BRCA2* mutation carriers [22]. It also observed evidence that among parous *BRCA1* mutation carriers, ovarian cancer risk decreased with each live-birth after the first (p=0.002), but that risk was also reduced for those who were nulliparous, relative to those who had had just one live-birth (p=0.02). No definitive conclusions were reached regarding the effect of parity for *BRCA2* mutation carriers. No evidence of an association with ovarian cancer risk was seen for age at first birth.

Our finding that later age at first birth is associated with reduced risk of ovarian cancer in mutation carriers is consistent with what has been observed in the general population, based on two large [25, 26] (and a combined analysis of smaller [13]) population-based case-control studies, although inconsistent findings have been reported from much smaller, hospital-based studies [13, 27, 28]. Further investigation is warranted to clarify this issue.

Study biases and limitations

Our study, like those of the IBCCS and the UK group [17, 18, 22], sought to account for the potential biases inherent in these studies of a highly selected and related sample of mutation carriers by modeling time from birth to diagnosis of breast or ovarian cancer in carriers using weighted Cox regression. Weights were calculated to correct for the over-representation of affected individuals at all ages, assuming that the age-specific incidence rates for breast and ovarian cancer in carriers of mutations in both genes estimated by *Antoniou et al*. (2003) are applicable [23]. Our recent study of the penetrance of mutations in *BRCA1* and *BRCA2* in Spanish multiple-case families indicated that this assumption is valid [5]. That our results were maintained after exluding prevalent cases suggests that survival bias was not present.

While we attempted to include obligate carriers wherever possible, in general, a mutation carrier had to be genetically tested in order to be included in the analysis. A further potential bias in this study would therefore be present if affected and unaffected women

were influenced by parity in different ways in terms of their decision to undergo genetic testing. It may be, for example, that women who have already been diagnosed with breast or ovarian cancer are more influenced in this decision by whether or not they have children (at potential genetic risk of the disease), than are unaffected women and this may result in bias in HR estimation. However, it could be hypothesized that this would tend to bias HR estimates in the direction of increased risk associated with being parous, rather than towards the observed protection.

One of the limitations of our study was that we measured time to cancer diagnosis and age at first birth in years, rather than months or days. This would have reduced the power to detect associations, but is unlikely to have introduced bias in HR estimation. Another potential limitation was that we were not able to adjust for potential confounding factors such as education level and other hormonal risk factors because we did not systematically collect this information on all mutation carriers. However, other studies were able to adjust for most of these factors and found that this had little impact on parity-associated HR estimates for breast and ovarian cancer [17, 21]. Finally, the number of mutation carriers with ovarian cancer was relatively low, particularly with regard to *BRCA2*, and so the corresponding results should be interpreted with greater caution.

Conclusions

This is the third independent study to find that, as in the general population, parity appears to be associated with protection from breast cancer in women with mutations in *BRCA1* and *BRCA2*. Nevertheless, results have not been consistent across all studies and

their retrospective designs imply a number of potential biases. Prospective studies of mutation carrier cohorts are therefore likely to be highly informative in this regard. Parity also appears to confer protection from ovarian cancer, at least for *BRCA1* mutation carriers. Whether this is the case for *BRCA2* mutation carriers remains to be confirmed. It may be that later age at first birth is associated with protection from ovarian cancer in both BRCA1 and *BRCA2* mutation carriers, as has been observed in the general population, but again, this finding requires confirmation in independent studies.

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Table 1: Number of eligible and included carriers of mutations in *BRCA1* and *BRCA2*, by centre.

Centre[a]	Carriers from families included in the penetrance study [5]				Sole mutation carriers[b]		All mutation carriers		Proportion of eligible mutation carriers included	
	Tested		Obligate							
	Included/Eligible[c]		Included/Eligible[c]		Included/Eligible[c]		Included/Eligible[c]			
	BRCA1	**BRCA2**	**BRCA1**	**BRCA2**	**BRCA1**	**BRCA2**	**BRCA1**	**BRCA2**	**BRCA1**	**BRCA2**
CNIO	69/84	4/121	1/7	3/6	35/37	20/26	105/128	117/153	82%	76%
Sant Pau	63/64	69/72	12/16	7/8	11/11	7/7	86/91	83/87	95%	95%
HCSC	63/72	52/57	1/16	0/11	14/15	20/20	78/103	72/88	76%	82%
ICO	41/43	57/58	7/9	4/4	4/4	3/3	52/56	64/65	93%	98%
Vall d´Hebron[d]							60/68	25/27	88%	93%
FPGMX	49/58	6/8	0/3	0/0	10/12	0/1	59/73	6/9	81%	67%
Valencia	6/6	43/43	0/0	4/4	3/3	3/5	9/9	50/52	100%	96%
Valladolid	4/17	1/8	2/4	0/2	16/27	31/50	22/48	32/60	46%	53%
Castellón	12/12	12/12	3/3	1/1	0/0	0/0	15/15	13/13	100%	100%
Barakaldo	5/5	18/18	0/0	1/2	0/0	0/0	5/5	19/20	100%	95%
Zaragoza	9/10	5/6	0/0	0/0	5/5	4/7	14/15	9/13	93%	69%
Elche	7/7	7/7	0/2	2/2	1/1	1/1	8/10	10/10	80%	100%
Salamanca	2/5	3/6	0/0	0/1	0/0	0/0	2/5	3/7	40%	43%
Total	**330/383**	**367/416**	**26/60**	**22/41**	**99/115**	**89/120**	**515/626**	**503/604**	**82%**	**83%**

[a] The 12 participating centres were the *Centro Nacional de Investigaciones* Oncológicas, Madrid (CNIO); the *Hospital de la Santa Creu i Sant Pau*, Barcelona (Sant Pau); the *Hospital Clínico San Carlos*, Madrid (HCSC); the *Institut Català d'Oncologia*, Barcelona (ICO); the *Hospital Vall d´Hebron*, Barcelona (Vall d´Hebron); the *Fundación Pública Galega de Medicina Xenómica*, Santiago de Compostela (FPGMX); the *Hospital Clínico Universitario de Valencia*, Valencia (Valencia); the *Instituto de Biología y Genética Molecular*, Valladolid (Valladolid); the *Hospital Provincial de Castellón*, Castellón (Castellón); the *Hospital de Cruces*, Barakaldo-Bizkaia (Barakaldo); the *Hospital Universitario Miguel Servet*, Zaragoza (Zaragoza); the *Hospital General Universitario de Elche*, Elche (Elche); and the *Centro de Investigación del Cáncer*, Salamanca (Salamanca).

[b] Sole mutation carriers in their respective families (not included in the penetrance study; [5])

[c] All identified female mutation carriers were considered eligible, but only those with complete data were included in the analyses

[d] Carriers from Vall d´Hebron were not included in the penetrance study [5]. These included 4 of 5 eligible obligate *BRCA1* mutation carriers and 1 eligible obligate *BRCA2* mutation carrier.

Table 2: Distribution of mutation carriers according to study centre (CENTRE), censoring age (AGE) and year of birth (YOB), by type of cancer, affection status and gene mutated

	***BRCA1* mutation carriers, n (%)**				***BRCA2* mutation carriers, n (%)**			
	BREAST CANCER		**OVARIAN CANCER**		**BREAST CANCER**		**OVARIAN CANCER**	
	Affected	**Unaffected**	**Affected**	**Unaffected**	**Affected**	**Unaffected**	**Affected**	**Unaffected**
CENTRE[*]								
CNIO	61 (22)	44 (18)	15 (21)	90 (20)	64 (22)	53 (25)	11 (31)	106 (23)
Sant Pau	46 (17)	40 (17)	9 (12)	77 (17)	43 (15)	40 (19)	6 (17)	77 (16)
HCSC	35 (13)	43 (18)	13 (18)	65 (15)	35 (12)	37 (17)	5 (14)	67 (14)
ICO	31 (11)	21 (9)	5 (7)	47 (11)	32 (11)	32 (15)	5 (14)	59 (13)
Vall d´Hebron	29 (11)	31 (13)	5 (7)	55 (12)				
FPGMX	28 (10)	31 (13)	13 (18)	46 (10)				
Valencia					32 (11)	18 (8)	3 (9)	47 (10)
Others[a]	46 (17)	29 (12)	13 (18)	62 (14)	83 (28)	34 (16)	5 (14)	112 (24)
p-valor[b]		**0.3**		**0.3**		**0.01**		**0.8**
AGE								
<25	6 (2)	23 (10)	0 (0)	29 (7)	2 (1)	21 (10)	0 (0)	23 (5)
25-29	23 (8)	24 (10)	0 (0)	47 (11)	17 (6)	27 (13)	0 (0)	44 (10)
30-34	54 (20)	34 (14)	6 (8)	82 (19)	44 (15)	36 (17)	1 (3)	79 (17)
35-39	57 (21)	34 (14)	6 (8)	85 (19)	62 (21)	23 (11)	0 (0)	85 (18)
40-44	61 (22)	35 (15)	12 (16)	84 (19)	58 (20)	25 (12)	3 (9)	80 (17)
45-49	36 (13)	28 (12)	17 (23)	47 (11)	44 (15)	23 (11)	5 (14)	62 (13)
50-54	23 (8)	21 (9)	14 (19)	30 (7)	26 (9)	19 (9)	5 (14)	40 (9)
55-59	8 (3)	17 (7)	10 (14)	15 (3)	15 (5)	11 (5)	3 (9)	23 (5)
60-64	3 (1)	7 (3)	2 (3)	8 (2)	10 (3)	12 (6)	10 (29)	12 (3)
65-69	2 (1)	9 (4)	4 (5)	7 (2)	5 (2)	11 (5)	5 (14)	11 (2)
70-79	3 (1)	7 (3)	2 (3)	8 (2)	6 (2)	6 (3)	3 (9)	9 (2)
p-valor[c]		**0.2**		**<0.001**		**0.2**		**<0.001**
YOB								
<1930	12 (4)	13 (5)	5 (7)	20 (5)	16 (6)	9 (4)	6 (17)	19 (4)
1930-39	20 (7)	14 (6)	13 (18)	21 (5)	32 (11)	20 (9)	13 (37)	39 (8)
1940-49	54 (20)	32 (13)	18 (25)	68 (15)	54 (19)	20 (9)	9 (26)	65 (14)
1950-59	87 (32)	45 (19)	24 (33)	108 (24)	93 (32)	39 (18)	4 (11)	128 (27)
1960-69	68 (25)	53 (22)	9 (12)	112 (25)	71 (25)	40 (19)	3 (9)	108 (23)
≥1970	35 (13)	82 (34)	4 (5)	113 (26)	23 (8)	86 (40)	0 (0)	109 (23)
p-valor[c]		**<0.001**		**<0.001**		**<0.001**		**<0.001**

[a] Includes Valencia for carriers of mutations in *BRCA1*, and includes Vall d´Hebron and FPGMX for carriers of mutations in *BRCA2*

[b] p-value calculated using Pearson´s Chi-squared test
[c] p-value calculated using logistic regression on the continuous variable

Table 3: Estimated hazard ratios (HR) for breast and ovarian cancer associated with parity variables, for *BRCA1* and *BRCA2* mutation carriers

	Breast Cancer		Ovarian Cancer	
	***BRCA1* mutation carriers HR[a] (95%CI); p-value**	***BRCA2* mutation carriers HR[a] (95%CI); p-value**	***BRCA1* mutation carriers HR[a] (95%CI); p-value**	***BRCA2* mutation carriers HR[a] (95%CI); p-value**
Parity				
Nulliparous	1.00	1.00	1.00	1.00
Parous	0.82 (0.55-1.20); 0.3	0.66 (0.39-1.12); 0.1	0.41 (0.18-0.94); 0.03	0.62 (0.10-3.97); 0.6
Number of live-births				
0	1.00	1.00	1.00	1.00
1	0.93 (0.60-1.46); 0.8	0.69 (0.36-1.34): 0.3	0.33 (0.12-0.95): 0.04	0.87 (0.11-6.79); 0.9
2	0.83 (0.54-1.27); 0.4	0.68 (0.38-1.22); 0.2	0.40 (0.17-0.94); 0.04	0.36 (0.05-2.92); 0.3
3	0.64 (0.36-1.17); 0.1	0.54 (0.26-1.10); 0.09	0.74 (0.28-1.99); 0.6	0.61 (0.08-4.96); 0.6
≥4	0.64 (0.32-1.25); 0.2	0.72 (0.29-1.77); 0.5	0.15 (0.04-0.56); 0.005	1.87 (0.19-18.4); 0.6
Trend (per live-birth)	0.88 (0.76-1.02); 0.08	0.88 (0.71-1.08); 0.2	0.80 (0.61-1.05); 0.1	1.21 (0.59-2.46); 0.6
Age at first live birth				
15-19	0.81 (0.41-1.60); 0.5	0.83 (0.30-2.30); 0.7	0.85 (0.30-2.43); 0.8	0.78 (0.11-5.46); 0.8
20-24	1.00	1.00	1.00	1.00
25-29	0.76 (0.48-1.19); 0.2	1.15 (0.68-1.95); 0.6	1.07 (0.51-2.27); 0.9	0.70 (0.18-2.72); 0.6
≥30	0.65 (0.38-1.11); 0.1	1.16 (0.57-2.38); 0.7	0.40 (0.16-1.02); 0.06	0.26 (0.05-1.35); 0.1
Trend (per 5 years)	0.90 (0.73-1.11); 0.3	1.13 (0.83-1.54); 0.4	0.65 (0.49-0.85); 0.001	0.63 (0.35-1.13); 0.1

CI, confidence interval.
[a] adjusting for year of birth and study centre.

Table 4: Summary of published studies of parity as a modifier of breast cancer risk in *BRCA1* and *BRCA2* mutation carriers

Authors (Year)	Methodology	Number[a]	Exposure(s) assessed	RR (95% CI)[b]
Jernstrom *et al.* (1999) [16]	Conditional logistic regression analysis of matched case-control data from pooled female *BRCA1* and *BRCA2* mutation carriers aged ≤40.	189/189 *(BRCA1)* 47/47 *(BRCA2)*	<u>Pooled mutation carriers</u> Parity (ever vs. never) Parity (per birth)	 1.71 (1.13-2.62) 1.24 (1.04-1.47)
Rebbeck *et al.* (2001) [20]	Unconditional logistic regression analysis of affected and unaffected female *BRCA1* and *BRCA2* mutation carriers of all ages pooled.	370/78 (*BRCA1* and *BRCA2* pooled)	<u>Pooled mutation carriers</u> Age at first birth (<30 vs. ≥30 or nulliparous)	 0.33 (0.16-0.66)
Cullinane *et al.* (2005) [14] (Expanded dataset, that includes that of Jernstrom *et al.* (1999) [16]	Conditional logistic regression analysis of matched case-control data from female *BRCA1* and *BRCA2* mutation carriers of all ages, by gene and by age (divided at age 50).	934/934 *(BRCA1)* 326/326 *(BRCA2)*	<u>*BRCA1* mutation carriers</u> Parity (ever vs. never) Parity (≥4 births vs. never) Parity (per birth) <u>*BRCA2* mutation carriers</u> Parity (ever vs. never) Parity (≥2 births vs. never) Parity (per birth) (per birth, age <50) (per birth, age ≥50)	 0.94 (0.75-1.19) 0.62 (0.41-0.94) 0.94 (0.86-1.02) 1.37 (0.93-2.03) 1.53 (1.01-2.32) 1.15 (1.00-1.33) 1.17 (1.01-1.36) 0.97 (0.58-1.53)
Gronwald *et al.* (2006) [15] (data may be included in Cullinane *et al.*, 2005) [14]	Conditional logistic regression analysis of matched case-control data from female *BRCA1* mutation carriers of all ages with mutations in *BRCA1*.	348/348 (*BRCA1)*	<u>*BRCA1* mutation carriers</u> Parity (per birth)	 1.2 (P^c=0.02)
Andrieu *et al.* (2006) [17]	Weighted Cox regression analysis of affected and unaffected female *BRCA1* and *BRCA2* mutation carriers of all ages, by gene and by age (divided at age 40).	602/585 *(BRCA1)* 251/163 (*BRCA2)*	<u>*BRCA1* mutation carriers</u> Parity (ever vs. never) Age at 1st birth (<20 vs. ≥30) <u>*BRCA2* mutation carriers</u> Parity (ever vs. never) Age at 1st birth (<20 vs. ≥20) <u>Pooled mutation carriers</u> Parity (parous women only) (per birth, all ages) (per birth, age ≤40) (per birth, age >40)	 0.86 (0.64-1.15) 1.72 (1.06-2.78) 0.79 (0.46-1.37) 0.5 (not given) 0.86 (0.78-0.94) 1.10 (0.90-1.34) 0.85 (0.77-0.95)

Antoniou *et al.* (2006) [23]	Weighted Cox regression analysis of affected and unaffected female *BRCA1* and *BRCA2* mutation carriers of all ages, by gene and by age (divided at age 40).	248/218 *(BRCA1)* 209/114 (*BRCA2)*	*BRCA1* mutation carriers Parity (ever vs. never) (ever vs. never, age ≤40) (ever vs. never, age >40) Age at 1st birth (<20 vs. ≥30) *BRCA2* mutation carriers Parity (ever vs. never) (ever vs. never, age ≤40) (ever vs. never, age >40) Age at 1st birth (<20 vs. ≥30) Pooled mutation carriers Parity (including nulliparous) (per birth, all ages)	 0.53 (0.34-0.83) 1.17 (0.55-2.52) 0.34 (0.16-0.70) 1.20 (0.61-2.38) 0.58 (0.27-1.24) 0.72 (0.42-1.24) 1.21 (0.37-3.92) 0.21 (0.09-0.48) 0.90 (0.80-1.00)
Kostopoulos *et al.* (2007) [19]	Conditional logistic regression analysis of matched case-control data from female *BRCA1* mutation carriers of all ages with mutations in *BRCA1*.	1405/1405 *(BRCA1)* 411/411 (*BRCA2)*	Pooled mutation carriers Age at 1st birth (trend/year)	 1.01 (0.98-1.03)
Present study	Weighted Cox regression analysis of affected and unaffected female *BRCA1* and *BRCA2* mutation carriers of all ages, by gene	276/239 *(BRCA1)* 289/214 (*BRCA2)*	*BRCA1* mutation carriers Parity (ever vs. never) Parity (per birth) (per birth, age ≤40) (per birth, age >40) Age at 1st birth (trend/year) *BRCA2* mutation carriers Parity (ever vs. never) Parity (per birth) (per birth, age ≤40) (per birth, age >40) Age at 1st birth (trend/year) Pooled mutation carriers Parity (parous women only) (per birth, all ages) (per birth, age ≤40) (per birth, age >40)	 0.82 (0.55-1.20) 0.88 (0.76-1.02) 1.02 (0.81-1.29) 0.82 (0.69-0.98) 0.98 (0.94-1.02) 0.66 (0.39-1.12) 0.88 (0.71-1.08) 0.97 (0.74-1.28) 0.81 (0.63-1.04) 1.03 (0.96-1.09) 0.87 (0.77-0.98) 0.99 (0.83-1.18) 0.81 (0.70-0.94)

[a] Number of affected carriers/number of unaffected carriers (gene in which a mutation is carried in parenthesis)
[b] RR, estimate of relative risk associated with the exposure; CI, confidence interval

[c] P, p-value (95%CI not provided)