Research Article

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Bias Explains Most of the Parent-of-Origin Effect on Breast Cancer Risk in *BRCA1/2* Mutation Carriers

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Abstract

Background: Paternal transmission of a *BRCA* mutation has been reported to increase the risk of breast cancer in offspring more than when the mutation is maternally inherited. As this effect might be caused by referral bias, the aim of this study was to assess the parent-of-origin effect of the *BRCA1/2* mutation on the breast cancer lifetime risk, when adjusted for referral bias.

Methods: A Dutch national cohort including 1,314 proven *BRCA1/2* mutation carriers and covering 54,752 person years. Data were collected by family cancer clinics, via questionnaires and from the national Dutch Cancer Registry. The parent-of-origin effect was assessed using Cox regression analyses, both unadjusted and adjusted for referral bias. Referral bias was operationalized by number of relatives with cancer and by personal cancer history.

Results: The mutation was of paternal origin in 330 (42%, $P < 0.001$) *BRCA1* and 222 (42%, $P < 0.001$) *BRCA2* carriers. Paternal origin increased the risk of prevalent breast cancer for *BRCA1* [HR, 1.54; 95% confidence interval (CI), 1.19–2.00] and *BRCA2* carriers (HR, 1.40; 95% CI, 0.95–2.06). Adjusted for referral bias by several family history factors, these HRs ranged from 1.41 to 1.83 in *BRCA1* carriers and 1.27 to 1.62 in *BRCA2* carriers. Adjusted for referral bias by personal history, these HRs were 0.66 (95% CI, 0.25–1.71) and 1.14 (95% CI, 0.42–3.15), respectively.

Conclusion: A parent-of-origin effect is present after correction for referral bias by family history, but correction for the personal cancer history made the effect disappear.

Impact: There is no conclusive evidence regarding incorporating a *BRCA1/2* parent-of-origin effect in breast cancer risk prediction models. *Cancer Epidemiol Biomarkers Prev; 25(8); 1251–8.* ©2016 AACR.

Introduction

Information about the family history of cancer is an important predictor for estimating both the breast cancer risk and the probability of being a mutation carrier, for example, a carrier of a *BRCA1* or *BRCA2* mutation (1–6). It has been suggested that a paternal origin of the *BRCA* mutation increases the breast cancer risk, whereas it decreases the ovarian cancer risk (7–9). However, it could be that this parent-of-origin effect of the *BRCA* gene may be caused by biases due to the referral criteria, which are mainly based on the number of affected relatives and their ages at diagnosis (8, 10). For these reasons, some studies have taken referral bias into account by including only *BRCA* carriers who were unaffected at baseline and by correcting for breast cancer in first-degree relatives (FDR) in combination with environmental factors (8), or by including all carriers but with correcting for the year of birth and year of referral (7). The paternal *BRCA1* mutation was both with and without bias correction associated with an breast cancer risk increase but for *BRCA2* mutations nonsignificant risk-increasing and risk-decreasing trends have been published (7–9).

It could be that referral bias is only affecting the assessment of the parent-of-origin effect in the index cases and that it does not

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affect the assessment in the majority of the carriers, because once a mutation is detected in the family, genetic testing becomes available to the rest of the family following a cascade protocol irrespective of the family history (11). In the Netherlands, this means that family members who test positive for a (familial) mutation are provided with a letter to help inform their relatives about hereditary cancer. It has, however, been shown that sharing of information about carrier-ship is more likely to be blocked by male relatives in the family (12–16). Therefore, it might be that women with a paternal origin of the mutation are less often 'triggered' to undergo genetic testing until they develop cancer themselves, which causes referral bias (or genetic testing bias).

The aim of this study was to assess the parent-of-origin effect of the *BRCA* mutation on the breast cancer risk in proven *BRCA1/2* mutation carriers and to assess whether this effect still remains when referral biases by family history or personal history of cancer are taken into account. Therefore, we considered it relevant to include only factors related to referral bias and to assess all dimensions of this bias which include the family history of both breast *and* ovarian cancer in first- *and* second-degree relatives (SDR) *and* the personal history of prevalent and/or incident cancer cases.

Materials and Methods

The study cohort

For this study, data from the Dutch national HEBON study (Hereditary Breast and Ovarian cancer study, the Netherlands) were used. The HEBON study was approved by the medical ethical committees of all participating hospitals, and subjects signed an informed consent form upon participation. From 1999 onwards, subjects who underwent genetic testing for the *BRCA1/2* mutation in the course of genetic counselling at any of the Family Cancer Clinics in The Netherlands were invited to participate in the HEBON study. From 2011 onwards, all subjects were invited to fill in a (follow-up) questionnaire on clinical history and risk factors. Thus, the data in this study consist of retrospective data collection with prospective follow-up of carriers' cancer status via linkage with the National Dutch Cancer Registry and questionnaires. The clinics provided data on the date of birth, the cancer status, the mutation status, and the date of the DNA test. Data on the carriers' breast cancer status and ovarian cancer status were also obtained by linkage with the Dutch Cancer Registry with national coverage for the calendar years 1989–2011. Data on cancer status, risk-reducing surgeries, and family history were self-reported by means of the questionnaire.

The current study included females with a pathogenic *BRCA1/2* mutation proven by genetic testing who completed the questionnaire. Exclusion criteria were: being the index carrier in the family, that is, the first member who tested positive for the mutation and who was affected at the time of DNA testing ($n = 655$); no available information on the parental origin of the *BRCA* mutation (data either missing or reported as unknown; $n = 124$); carrying a mutation from both parents or carrying both a *BRCA1* and *BRCA2* mutation ($n = 48$); or when a risk-reducing mastectomy (RRM) was performed, but the age at RRM was unknown in women without breast cancer ($n = 7$).

Outcome event

The outcome was defined as the incidence of primary breast cancer. The prevalent cases were defined as all cases both before and after genetic testing—so including (semi-) incident cases. Incident cases were defined as breast cancer cases occurring $\geq$3 months after a woman's DNA testing date. Semi-incident cases were defined as breast cancer cases occurring after the DNA testing date of the family's index carrier, which means that the semi-incident cases included both cases that occurred both before and after the woman's DNA testing date.

The subjects' breast cancer and ovarian cancer status was either registry-confirmed or self-reported. If a woman reported that a malignancy was detected at the time of the RRM or at risk-reducing salpingo-oophorectomy (RRSO) in a calendar year outside the coverage of the Dutch Cancer Registry, her breast cancer or ovarian cancer status was adapted accordingly.

Determinants of referral bias

Referral bias was operationalized by family history or by personal history of cancer. Referral bias by family history was evaluated by taking each of the following factors of this bias into account in separate models: (i) the year of the family's ascertainment (i.e., year of index carriers' genetic testing) because referral criteria have become slightly less stringent over time; (ii) referral criteria, which are based on multiple components of family history; and (iii) separate components of family history for each tumor type, age group, and type of relatedness. The referral criteria (ii) were as assessed as following: $\geq$1 FDR or SDR with breast cancer by the age of 35 years; $\geq$1 FDR or SDR with ovarian cancer; $\geq$1 male FDR or SDR with breast cancer; $\geq$2 FDRs with breast cancer of whom $\geq$1 case by the age of 50 years; or $\geq$3 FDRs or SDRs with breast cancer of whom $\geq$1 affected FDR by the age of 50 years or $\geq$1 affected SDR by the age of 60 years. The family history (iii) of breast cancer, ovarian cancer, or another cancer was assessed as follows with and without stratification by gender: having $\geq$1 parents, $\geq$1 siblings only, $\geq$1 siblings or children, $\geq$1 FDRs or $\geq$1 SDRs affected with cancer by the age of 40, 50, 60 years or any age. Data on the family history of breast cancer, ovarian cancer, and other cancers were available for both FDRs and SDRs. For some FDRs and SDRs, the family history of cancer for one or more family members was not reported. In those cases, the family history was assumed to be negative.

Referral bias by personal history of cancer was evaluated by considering only incident and semi-incident cases.

Statistical analysis

Descriptive statistics were used to give an overview of the study population characteristics, and appropriate tests were used to test for differences between mutation carriers with a paternal or maternal origin of the *BRCA1/2* mutation.

The effect of the parental origin of the *BRCA* mutation on the risk of developing breast cancer was estimated using Cox regression survival analyses. In all these analyses, the paternal group was compared with the maternal group (i.e., reference group). Robust SEs were calculated to account for the clustering of carriers within families. The assumption of proportional hazards was tested using Schoenfeld residuals and log-minus-log plots. Censoring was applied at the first moment any of the following events occurred: breast cancer, ovarian cancer, RRM, RRSO, or last date of information.

To assess whether the effect of parental origin of the *BRCA* mutation was independent of referral bias due to a family history of cancer, Cox regression analyses of prevalent cases were adjusted

Table 1. Clinical characteristics of carriers stratified by the parental origin of the *BRCA* mutation

	***BRCA1* carriers**			***BRCA2* carriers**		
	Paternal $n = 330$	**Maternal** $n = 458$	***P***	**Paternal** $n = 222$	**Maternal** $n = 304$	***P***
Clinical characteristics						
Age at follow-up, median (IQR), y	47 (37–55)	45 (36–55)	0.061	49 (40–58)	47 (39–57)	0.463
Year of ascertainment family < 2005, *n* (%)	76 (33.4%)	124 (37.8%)	0.322	63 (33.6%)	93 (40.3%)	0.167
Age at DNA testing						
Missing, *n* (%)	105 (31.8%)	131 (28.6%)	0.331	35 (15.8%)	75 (24.7%)	0.013
Median (IQR)	40.0 (30.9–49.5)	38.9 (29.9–46.1)	0.146	42.7 (33.3–51.7)	41.6 (33.5–51.0)	0.743
Presymptomtic genetic testing, *n* (%)	200 (88.9%)	293 (89.6%)	0.899	167 (89.3%)	203 (88.6%)	0.955
Breast cancer						
Number of cancers (%)	137 (41.5%)	121 (26.4)	<0.001	61 (27.5%)	59 (19.4%)	0.029
Age at diagnosis, median (IQR), y	41.2 (35.5–50.3)	42.3 (35.0–48.0)	0.476	44.8 (38.7–51.6)	47.5 (41.0–51.9)	0.317
Incident breast cancer						
Number (% of women with DNA date)	19 (8.4%)	28 (8.6%)	0.961	15 (8.0%)	20 (8.7%)	0.795
Age at diagnosis, median (IQR), y	44.6 (36.2–49.4)	42.2 (33.0–47.5)	0.170	43.7 (37.7–52.1)	47.9 (41.6–52.6)	0.382
Ovarian cancer						
Number of cancers (%)	15 (4.5%)	17 (3.7%)	0.559	4 (1.8%)	6 (2.0%)	1.0
Age at diagnosis, median (IQR), y	49.6 (45.1–56.6)	55.3 (49.5–58.0)	0.230	54.4 (53.2–54.7)	57.3 (53.4–60.0)	0.352
RRM						
Number (%)	74 (22.4%)	123 (26.9%)	0.156	48 (21.6)	65 (21.4)	0.947
Age at surgery, median (IQR), y	36.0 (31.0–43.0)	36.0 (31.0–43.0)	0.822	42.0 (35.0–50.0)	39.0 (34.0–46.0)	0.088
RRSO						
Number (%)	220 (66.7%)	291 (63.5%)	0.364	148 (66.7)	190 (62.5%)	0.345
Age at surgery, median (IQR), y	45.0 (38.0–51.0)	43.0 (40.0–50.0)	0.649	48.0 (41.8–55.0)	47.0 (41.0–53.0)	0.281
Follow-up time, y						
Prevalent case analysis, median (IQR); total	40 (33–47); 13,440	39 (33–46); 18,363		43 (36–51); 9,700	42 (37–50); 13,249	
Semi-incident case analysis, median (IQR); total	3 (1–6); 616	4 (2–7); 1150		3 (1–7); 645	3 (1–7); 819	
Incident case analysis, median (IQR); total	2 (1–4); 287	2 (1–4); 454		2 (1–4); 277	2 (1–4); 370	

Abbreviation: IQR, interquartile range (i.e., 25th percentile–75th percentile).

for each factor separately. Factors that altered the parent-of-origin effect by 5% or more were considered to be relevant and are reported in Results. We also took the second-degree family history into account to evaluate the potential referral bias. Because this information was not available for the complete cohort, we used a sub cohort of 742 (94%) *BRCA1* carriers and 488 (93%) *BRCA2* carriers.

To assess whether the parental-origin effect of the *BRCA* mutation was affected by referral bias due to a personal history of cancer, Cox regression analyses were performed on semi-incident and incident cases. For the semi-incident case analyses, follow-up time and cases were counted from the date of the family index carrier's DNA test. For the incident case analyses, follow-up time and cases ($\geq$3 months after DNA test) were counted from the woman's date of DNA test onward. In these analyses, only mutation carriers who did not have breast or ovarian cancer, RRM, or RRSO before the start of the follow-up period were included as these were reasons for censoring.

Sensitivity analyses were performed for the unadjusted analyses by changing the outcome event to (i) prevalent registry-confirmed cases or (ii) all self-reported and registry-confirmed prevalent cases.

In addition, RRSO was included as a time-dependent covariate and no censoring was applied at this event. Other sensitivity analyses were performed only for the prevalent case analyses adjusted for family history of cancer. First, the analyses were additionally adjusted for the number of reported family members. Second, carriers with missing information for the family history factors were excluded. Third, analyses were performed with the family history as documented at the time of personal genetic testing because this might be the more optimal scenario to assess referral bias.

All analyses were performed with stratification by the *BRCA1/2* gene. The analyses were performed using R, and statistical significance was defined as $P < 0.05$ (17).

Results

Population characteristics

In total, 1,314 *BRCA* mutation carriers were included in this study, of whom 788 (60%) harbored a mutation in the *BRCA1* gene and 526 (40%) in the *BRCA2* gene. Of these, 236 (33%) of the *BRCA1* carriers and 120 (23%) of the *BRCA2* carriers developed breast cancer. For both *BRCA1* and *BRCA2* carriers, the percentage of ovarian cancer was relatively low, 2% to 4%, most likely due to the high rate of RRSO (~65%). Of all carriers with information on the date of genetic testing [$n = 968$ (74%)], the testing was performed presymptomatically in 493 (89%) of the *BRCA1* carriers and in 370 (89%) of the *BRCA2* carriers (Table 1).

BRCA1 carriers had more FDRs affected with breast cancer diagnosed before the age of 50 years (38% vs. 31%, $P = 0.010$) or ovarian cancer at any age (20% vs. 11%, $P < 0.001$) compared with *BRCA2* carriers but had fewer male FDRs with breast cancer (0.9% vs. 3.6%, $P < 0.001$) which is in line with known differences in cancer penetrance between *BRCA1* and *BRCA2* families (18–21).

The *BRCA* mutation was of paternal origin in 552 (42%) women: 330 (42%, $P < 0.001$) *BRCA1* and 222 (42%, $P < 0.001$) *BRCA2* carriers (Table 1). For both *BRCA1* and *BRCA2* carriers, women with a paternal origin of the *BRCA* mutation differed significantly from the women with a maternal origin in the following ways: they were more likely to have breast cancer themselves ($BRCA1_{pat}$ 42% vs. $BRCA1_{mat}$ 26%; $BRCA2_{pat}$ 28% vs. $BRCA2_{mat}$ 19%); less likely to have FDRs affected with breast or

Table 2. Family history of cancer of mutation carriers stratified by the parental origin of the *BRCA* mutations

	***BRCA1* carriers**			***BRCA2* carriers**		
	Paternal	**Maternal**		**Paternal**	**Maternal**	
	***n* = 330**	***n* = 458**	***P***	***n* = 222**	***n* = 304**	***P***
FDR family history						
Affected parents with breast cancer or ovarian cancer						
Mother with breast cancer (%)	16 (4.8%)	230 (50.2%)	<0.001	12 (5.4%)	152 (50.0%)	<0.001
Mother with breast cancer ≤ 40 years of age (%)	0 (0%)	73 (15.9%)	<0.001	1 (0.5%)	30 (9.9%)	<0.001
Mother with breast cancer ≤ 50 years of age (%)	3 (0.9%)	150 (32.8%)	<0.001	2 (0.9%)	77 (25.3%)	<0.001
Mother with breast cancer ≤ 60 years of age (%)	7 (2.1%)	190 (41.5%)	<0.001	6 (2.7%)	119 (39.1%)	<0.001
Mother with ovarian cancer (%)	4 (1.2%)	123 (26.9%)	<0.001	1 (0.5%)	42 (13.8%)	<0.001
Mother with ovarian cancer ≤60 years of age (%)	3 (0.9%)	92 (20.1%)	<0.001	0 (0%)	30 (9.9%).	<0.001
Father with breast cancer (%)	3 (0.9%)	2 (0.4%)	0.655	6 (2.7%)	5 (1.6%)	0.540
Parents with cancer other than breast cancer/ovarian cancer						
Mother with cancer (%)	36 (10.9%)	32 (7.0%)	0.055	24 (10.8%)	34 (11.2%)	0.893
Father with cancer (%)	108 (32.7%)	88 (19.2%)	<0.001	93 (41.4%)	86 (28.3%)	0.002
Affected FDRs with breast cancer or ovarian cancer						
≥1 siblings with breast cancer (%)	92 (27.9%)	96 (21.0%)	0.411	68 (30.6%)	52 (17.1%)	<0.001
≥1 male FDRs with breast cancer (%)	4 (1.2%)	3 (0.7%)	0.025	9 (4.1%)	10 (3.3%)	0.643
≥1 FDRs with breast cancer (%)	113 (34.2%)	286 (62.4%)	<0.001	80 (36.0%)	188 (61.8%)	<0.001
≥1 FDRs with breast cancer ≤ 50 years of age (%)	88 (26.7%)	212 (46.3%)	<0.001	56 (25.2%)	108 (35.5%)	0.012
≥1 FDRs with ovarian cancer (%)	22 (6.7%)	138 (30.3%)	<0.001	9 (4.1%)	51 (16.8%)	<0.001
≥1 FDRs with cancer other than breast cancer/ovarian cancer (%)	145 (43.9%)	131 (28.6%)	<0.001	115 (51.8%)	131 (43.1%)	0.048
SDR family history	*N = 313*	*N = 429*		*N = 208*	*N = 280*	
Affected SDRs with breast cancer or ovarian cancer						
≥1 SDRs with breast cancer (%)	190 (60.7%)	241 (56.0%)	0.204	126 (60.6%)	173 (61.8%)	0.786
≥1 SDRs with ovarian cancer (%)	83 (26.5%)	146 (34.0%)	0.030	33 (15.9%)	48 (17.1%)	0.708

Abbreviation: IQR, interquartile range (i.e., 25th percentile–75th percentile).

ovarian cancer (*BRCA1*$_{pat}$ 37% vs. *BRCA1*$_{mat}$ 82%; *BRCA2*$_{pat}$ 37% vs. *BRCA2*$_{mat}$ 74%); less likely to have a mother affected with any cancer (*BRCA1*$_{pat}$ 17% vs. *BRCA1*$_{mat}$ 83%; *BRCA2*$_{pat}$ 17% vs. *BRCA2*$_{mat}$ 73%); and more likely to have a father with any cancer (*BRCA1*$_{pat}$ 34% vs. *BRCA1*$_{mat}$ 20%; *BRCA2*$_{pat}$ 44% vs. *BRCA2*$_{mat}$ 30%). While breast cancer was the most common cancer among all mothers of mutation carriers, only a small fraction of all fathers was affected with breast cancer (Table 2).

BRCA1/2 mutation carriers with a paternal origin of the mutation were more often affected with cancer at the time of the index carrier's DNA test (*BRCA1*$_{pat}$ 20% vs. *BRCA1*$_{mat}$ 7%; *BRCA2*$_{pat}$ 10% vs. *BRCA2*$_{mat}$ 7%), and by the time of their personal DNA test, *BRCA1/2* mutation carriers with a paternal origin of the mutation were even more often affected (*BRCA1*$_{pat}$ 25% vs. *BRCA1*$_{mat}$ 13%; *BRCA2*$_{pat}$ 13% vs. *BRCA2*$_{mat}$ 13%). *BRCA1/2* mutation carriers with a paternal mutation who were unaffected at the time of DNA testing were more likely to have affected siblings, children, or SDRs at this time compared with unaffected mutation carriers with a maternal origin of the mutation (Supplementary Table S1).

The parent-of-origin effect and referral bias operationalized by family history of cancer

A woman's breast cancer risk was significantly increased when the *BRCA1* mutation was of paternal origin [HR, 1.54; 95% confidence interval (CI), 1.19–2.00]. For *BRCA2* carriers, this increase was of the same order of magnitude but not statistically significant (HR, 1.40; 95% CI, 0.95–2.06; Table 3).

When taking bias by family history into account in separate models, the risk increase associated with the paternal origin of the *BRCA* mutation varied from 1.41 to 1.83 in *BRCA1* carriers and 1.27 to 1.62 in *BRCA2* carriers (Fig. 1).

For *BRCA1* carriers, the effect of the paternal origin remained significantly increased irrespective of the adjustment. For *BRCA2* carriers, the effect of the paternal origin was only significantly increased when adjusted for maternal breast cancer up to the age of 60 years or for having a sibling with breast cancer. For both *BRCA1* and *BRCA2* carriers, adjustment for having FDRs with ovarian cancer mitigated the effect of paternal origin, whereas having FDRs with breast cancer increased the effect. Overall, adjustment for the second-degree family history had a similar but weaker impact on the paternal origin effect of the *BRCA* mutation.

The parent-of-origin effect and referral bias operationalized by personal history of cancer

The semi-incident case analyses included 46 *BRCA1*-related and 36 *BRCA2*-related breast cancers. The HRs of the parent-of-origin effects were insignificant: 1.02 (95% CI, 0.56–1.88) for *BRCA1* carriers and 0.94 (95% CI, 0.48–1.85) for *BRCA2* carriers (Table 3). The incident case analyses included 16 *BRCA1*-related and 15 *BRCA2*-related breast cancers. The HRs of the parent-of-origin effect were 0.77 (95% CI, 0.25–1.71) for *BRCA1* carriers and 1.14 (95% CI, 0.24–3.15) for *BRCA2* carriers, both being insignificant.

Sensitivity analyses

In the sensitivity analyses restricted to only registry-confirmed prevalent cases, as compared with the prevalent case analyses, the unadjusted effect of the paternal origin was 1.52 (−3%) in *BRCA1* carriers and 1.44 (+2%) *BRCA2* carriers (Table 3). For the analyses including both registry-confirmed and self-reported prevalent cases, these numbers were 1.56 (−1%) in *BRCA1* carriers and 1.28 (−8%) in *BRCA2* carriers.

Subsequently, the analyses were adjusted for family history but in addition also included the reported number of affected *and* unaffected FDRs and SDRs. In addition to our already reported factors, adjustment for having any FDRs with breast cancer or

Table 3. The *BRCA* mutation parent-of-origin effect (paternal vs. maternal) on the breast cancer risk in prevalent, semi-incident, and incident case analyses

Breast cancer events				Prevalent case analyses[b]		Semi-incident case analyses[c]		Incident case analyses[d]	
Registry confirmed	Self-reported: Selected[a]	Self-reported: All	Time-dependent effect	*N* (events)	HR (95% CI)	*N* (events)	HR (95% CI)	*N* (events)	HR (95% CI)
A) *BRCA1* parent-of-origin effect: paternal vs. maternal									
x	x		—	788 (228)	1.54 (1.19–2.00)	387 (46)	1.02 (0.56–1.88)	272 (19)	0.66 (0.25–1.71)
x	x		RRSO	788 (255)	1.53 (1.21–1.95)	387 (57)	1.23 (0.75–2.03)	272 (27)	1.10 (0.59–2.08)
x			—	788 (216)	1.53 (1.18–1.99)	387 (43)	1.17 (0.63–2.16)	272 (16)	0.77 (0.29–2.05)
x		x	—	788 (237)	1.53 (1.19–1.98)	386 (47)	0.98 (0.54–1.79)	271 (19)	0.65 (0.25–1.70)
B) *BRCA2* parent-of-origin effect: paternal vs. maternal									
x	x		—	526 (110)	1.40 (0.96–2.06)	321 (36)	0.94 (0.48–1.85)	229 (15)	1.14 (0.42–3.15)
x	x		RRSO	526 (120)	1.41 (0.96–2.04)	321 (41)	0.87 (0.45–1.66)	229 (17)	0.90 (0.36–2.23)
x			—	526 (107)	1.44 (0.97–2.14)	321 (35)	0.98 (0.50–1.94)	229 (15)	1.14 (0.42–3.15)
x		x	—	526 (116)	1.28 (0.89–1.86)	320 (38)	0.97 (0.52–1.82)	229 (15)	1.14 (0.42–3.15)

[a]Selected cancers: self-reported cancer in the years outside the coverage of the National Cancer Registry.
[b]Prevalent cases: all breast cancer cases before and after genetic testing until the moment of censoring, so including (semi-)incident cases.
[c]Semi-incident cases: breast cancer cases and follow-up time after the family's index carriers DNA test, so including incident cases.
[d]Incident cases: breast cancer cases and follow-up time after personal DNA test.

having any (male) FDRs with any other cancer (not breast or ovarian) increased the effect of paternal origin of *BRCA1* by 5% or more and adjustment for having any female FDRs with any other cancer by the age of 60 years increased the effect of paternal origin of *BRCA2* by 5% or more.

When women with missing data were excluded, the impact of family factors on the paternal origin effect was slightly stronger than in the main analyses. For *BRCA2* carriers, in addition to the already reported factors, adjustment for having any male FDRs with any cancer other than breast cancer reduced the paternal origin effect by 5%.

In our analyses, we used family history at time of the questionnaire. We wondered whether the family history at time of genetic testing would be more optimal for assessing referral bias because this is the family history that contributed to a person's ascertainment. Therefore, these analyses were performed on a subcohort of 550 (70%) *BRCA1* carriers and 416 (79%) *BRCA2* carriers for whom this data were available; however, no differences with the other prevalent case analyses were observed (data not shown).

Discussion

The results of the prevalent case analyses showed that paternal inheritance of a *BRCA* mutation increases the breast cancer risk for both *BRCA1* carriers (HR, 1.54; 95% CI, 1.19–2.00) and *BRCA2* carriers (HR, 1.40, 95% CI, 0.95–2.06). This risk increase was present, irrespective of the referral criteria because the HRs adjusted separately for several factors of family history ranged from 1.41 to 1.83 in *BRCA1* carriers and 1.27 to 1.62 in *BRCA2* carriers, and they were associated with a similar level of significance as the unadjusted effect. However, no parent-of-origin effect was observed when referral bias by personal history of cancer was taken into account. These HRs were 0.66 (95% CI, 0.25–1.71) for *BRCA1* and 1.14 (95% CI, 0.42–3.15) for *BRCA2*.

An increase in breast cancer risk in case of a paternal origin of the mutation has been reported for *BRCA1*, whereas for *BRCA2* mutations, nonsignificant risk-increasing as well as risk-decreasing trends have been published (7–9). For both genes, we observed a risk increase in case of paternal transmission of the mutation, even when the effect was adjusted for possible referral bias by family history. This is in line with the results of a previous study in which an increased risk was observed in case of a paternal mutation when the analyses were adjusted for the year of birth, year of referral, and for oral contraceptive use (7). They observed a similar risk increase in the unadjusted and adjusted analyses [*BRCA1*: HR = 1.50 ($P = 0.02$) and HR = 1.53 ($P = 0.02$), respectively; *BRCA2*: HR = 1.23 ($P = 0.37$) and HR = 1.21 ($P = 0.41$), respectively]. Another study adjusted all analyses for referral bias by personal cancer history and included only women who were unaffected at baseline. In addition, the analyses were adjusted for referral bias by family history—the number of FDRs with breast cancer, and breast cancer in the mother—breast feeding, age at menarche, and country of residence (8). For *BRCA1*, they reported also an increased unadjusted and adjusted risk in case of paternal transmission of the mutation [HR = 1.46 ($P = 0.06$) and HR = 1.36 ($P = 0.19$), respectively]. For *BRCA2* carriers, they observed a risk decreasing trend in both the unadjusted and adjusted analyses in case of paternal transmission [HR = 0.81 ($P = 0.65$) and HR = 0.88 ($P = 0.82$), respectively]. These findings are similar to our results from the incident cases analyses with RRSO as a time-dependent variable (*BRCA1*: HR = 1.10, *BRCA2*: HR = 0.90). However, when the RRSO was incorporated as a censoring event, the risk ratios changed from risk increasing to decreasing and vice versa (*BRCA1*: HR = 0.66, *BRCA2*: HR = 1.14). Although none of these parent-of-origin effects were statistically significant.

Our study is the first to adjust the parent-of-origin analyses for referral bias by incorporating a family history of cancer in both male and female FDRs and SDRs and by including a history of cancer other than breast cancer. We show that having a family history of ovarian cancer in any FDRs and/or SDRs mitigates the paternal origin effect, whereas a family history of breast cancer increases this effect. However, we also addressed the impact of referral bias by a personal history of cancer. More mutation carriers with a paternally derived mutation were affected with breast cancer at the time of ascertainment of the family and at the time of their personal DNA test, as compared with mutation carriers with a maternally derived mutation, especially for *BRCA1*, which might suggest referral bias and genetic testing bias. When this was taken into account in the semi-incident and incident case analyses, no parent-of-origin

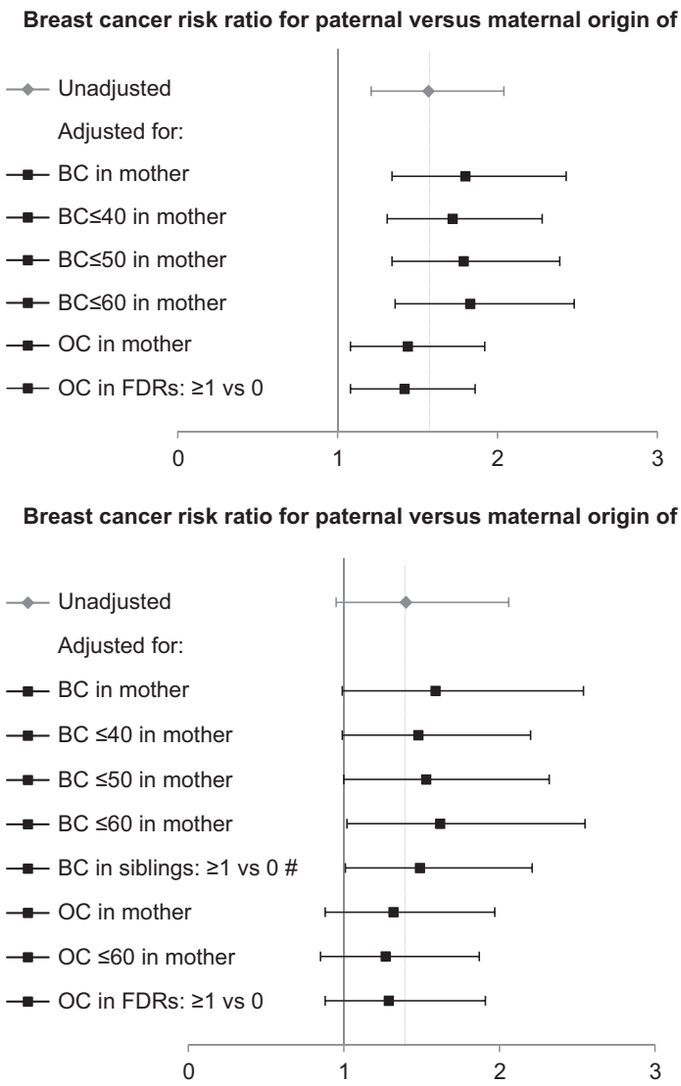

Figure 1.
The effect of the parent-of-origin on breast cancer risk: comparing *BRCA1/2* mutations of paternal origin to those of maternal origin (HR, 95% CI), without and with taking into account the indicated factors of family history*. **A,** *BRCA1* carriers. **B,** *BRCA2* carriers. #, Breast cancer in siblings: breast cancer in male and female siblings. *, Factors that altered the parent-of-origin effect by 5% or more were considered to be relevant and are reported in the figure. Abbreviations: BC, breast cancer; OC, ovarian cancer.

effect could be observed anymore, although numbers were small and thus statistically insignificant.

Sensitivity analyses showed similar results for the unadjusted prevalent and (semi-)incident case analyses using only registry-confirmed cases and/or all self-reported breast cancer cases. Moreover, in the adjusted analyses, similar results were found when missing family history was considered as missing instead of a negative family history.

For this study, we used a well-structured national cohort of *BRCA1/2* mutation carriers, and breast cancers were confirmed by linkage with the national cancer registry whenever possible. Only the impact of the parent-of-origin on breast cancer was assessed because the number of ovarian cancer cases was too small for risk assessment. A minor limitation is that the family history and risk-reducing surgeries were self-reported, but studies have shown that individuals report their family history of breast cancer quite accurately for FDRs and fairly accurately for SDRs (22–24). The self-reported family history of ovarian cancer is less accurate, especially in SDRs. However, results adjusted for SDR family history were in line with what was expected on the basis of the results of the FDR history. Information on the parental origin of the *BRCA* mutation and on second-degree family history was not available for the complete cohort. However, comparison of the selected study population with the complete cohort showed that our study population was a representative sample including a somewhat younger cohort with more risk-reducing surgeries and fewer cancers.

Both retrospective and prospective cohort studies can be used to assess risk factors, but biases should be addressed carefully in either study design, as there will always be some form of selection of the study population. In this study, the referral bias by family history was assessed by excluding the index cases and accounting for possible confounding by family history. However, our risk factor of interest, parent-of-origin, seems to be related to referral bias due to a personal history of cancer, and this could not be disentangled with retrospective analyses alone. The referral bias due to a personal history of cancer could only be addressed prospectively

without any further adjustment for family history due to small numbers.

Another selection bias that could have affected the observed parent-of-origin effect is a possible difference in genetic fitness between paternal and maternal mutation carriers (25). This might imply that a female carrier with a more severe genetic make-up will have an earlier disease onset, a worse prognosis, and be therefore less reproductive. However, we found no difference in the number of siblings between paternal and maternal mutation carriers.

Although the results from our epidemiologic study make the parent-of-origin effect seem less likely, research on the possible biologic mechanisms underlying this effect, like difference in maternal and paternal imprinting of the *BRCA* genes and their modifier genes, may help resolve the issue (7, 8).

In conclusion, the existence of a parent-of-origin effect depends on how researchers correct for referral bias. Correction of referral bias as defined by family history did not substantially impact this effect, whereas bias correction for the personal cancer history made the parent-of-origin effect disappear. This bias, when uncorrected for, may have produced the positive association between paternal origin of the *BRCA1/2* mutation and an increased risk of breast cancer reported in earlier studies. As the prospective cohort was relatively small, a larger prospective cohort study should address the combined impact of referral bias by family and personal history. Currently, there is no evidence for incorporating the parent-of-origin effect in risk prediction models for breast cancer in *BRCA1/2* mutation carriers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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