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DIAGNOSTIC RADIATION EXPOSURE AND BREAST CANCER RISK IN BRCA1/2 MUTATION CARRIERS IN THE GENE-RAD-RISK STUDY

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ABSTRACT

Exposure to ionizing radiation is an established risk factor for breast cancer in the general population. BRCA1/2 mutation carriers might be more radiosensitive to ionizing radiation due to impaired DNA repair mechanisms. A retrospective European collaborative cohort study (GENE-RAD-RISK) of 1,993 female BRCA1/2 mutation carriers was performed using self-reported exposure to diagnostic radiation. Risk of breast cancer was estimated using a weighted Cox proportional hazards model with cumulative radiation exposure from diagnostic procedures as a time-dependent variable lagged by 5 years. A unique feature of this study is the individually estimated cumulative breast dose score. In the entire cohort, we observed no association between any of the diagnostic procedures and breast cancer risk. Subcohort analyses restricted cases diagnosed ≤5 years prior to questionnaire completion showed a non-significantly increased breast cancer risk after exposure to >2 fluoroscopies before age 20 (HR=2.01, 95%CI=0.71-5.71, P_{trend}=0.102) and a significant trend of increasing breast cancer risk with increasing number of X-rays before age 20 (P_{trend}=0.041) and before age 30 (P_{trend}=0.012) as compared to no exposure. A history of mammograms before age 30 was also associated with an increased risk of breast cancer (HR=1.43, 95%CI=0.85-2.40, Ptrend=0.040). For the cumulative dose score, we observed significant positive associations for both analytical cohorts. In the subcohort, a history of any diagnostic radiation before age 30 increased the risk of breast cancer (HR=1.90, 95%CI=1.20-3.00) and a dose-response association emerged: the risks for a cumulative dose <0.002 Gy, ≥0.002-0.0066 Gy, ≥0.0066-0.0174 Gy, and ≥0.0174 Gy were 1.63 (0.96-2.77), 1.78 (0.88-3.58), 1.75 (0.72-4.25), and 3.84 (1.67-8.79), respectively. In conclusion, exposure to diagnostic radiation before age 30 was associated with an increased breast cancer risk in BRCA1/2 mutation carriers at dose levels considerably lower than at which increases have been found in other radiation-exposed cohorts.

INTRODUCTION

Exposure to ionizing radiation is an established risk factor for breast cancer in the general population[1]. The risk appears to decrease with increasing age at exposure. Questions remain about the existence and possible magnitude of risk following low doses (i.e. <0.1 Gy) such as those used in diagnostic procedures[2]. Both BRCA1 and BRCA2 are involved in the repair of DNA double strand breaks, the most significant type of damage generated by ionizing radiation[3-7]. Therefore, it is possible that women carrying germline mutations in these genes might be more susceptible to radiation-induced breast cancer than non-carriers, or the general population. Recently, two studies[8,9] have reported an association between self-reported exposure to chest X-rays and breast cancer risk in BRCA carriers. Risks were particularly high among those exposed before age 20, and observed at dose levels considerably lower than at which increases have been found in other radiation-exposed cohorts. Two other carrier studies[10,11], focussing on mammography exposure and breast cancer risk, did not observe an association, but most of the exposure occurred after age 30. Limitations of studies published so far include the investigation of a single low dose ionizing radiation source (e.g. only chest X-rays or only mammograms), relatively small numbers, and a retrospective design (with potential recall and/or survival bias).

This chapter reports on the BRCA1/2 mutation carrier cohort in the GENE-RAD-RISK project, a large European study designed to examine whether mutations or polymorphisms in specific DNA repair genes increase the risk of radiation-induced breast cancer. A unique feature of the present study is the estimation of an individual age-specific cumulative breast dose score based on detailed, lifetime information on various diagnostic radiation exposures.

METHODS

STUDY POPULATION

The present retrospective analyses were based on a sample of 1,993 women tested in a clinical setting and proven to carry a BRCA1 or BRCA2 mutation. Women were recruited into the GENE-RAD-RISK study during the period 2006-2008 and were participants in three large ongoing national studies of BRCA carriers in France (GENEPSO; N=716 (35%)), the United Kingdom (EMBRACE[12]; N=688 (35%)), and the Netherlands (HEBON[13]; N=589 (30%)). These three cohort studies are part of the International BRCA1/2 Carrier Cohort Study (IBCCS)[14]. 417 (21%) of the participants in the current GENE-RAD-RISK study were also included in the earlier IBCCS X-ray paper[8].

Women were eligible for the GENE-RAD-RISK study if they were carrying a BRCA1 or BRCA2 mutation and were aged 18 or older at baseline questionnaire. Carriers were restricted to age <55 years at baseline questionnaire in France and the UK; in the Netherlands, carriers aged 55 years and older were also included. A standardized follow-up questionnaire on lifestyle and reproductive factors and the GENE-RAD-RISK study questionnaire were completed by each participant (response 78%, see Table 1).

TABLE 1. The GENE-RAD-RISK study: identification of the study population (N=1,993)

	Number
Eligible	**2,885**
Deceased	185
Too ill or old	4
Psychological burden	7
No MD approval	15
Living abroad	17
Lost to follow-up (unable to trace)	48
Did not want to be invited again after baseline	51
Other reason/unknown	2
Invited	**2,556**
Non-response	493
Refusal	63
Questionnaire received but consent not included	4
Address unknown	3
Questionnaires completed	**1,993**
Response 1 (vs. invited)	**78%**
Response 2 (vs. eligible)	**69%**

Nine percent (181/1,993) of carriers were recruited at baseline (response 70%) while the majority was recruited after they had completed a baseline questionnaire (response 79%) (data not shown). The study was approved by the medical ethics committees of all participating centres, and all participants provided written informed consent.

ASSESSMENT OF DIAGNOSTIC RADIATION EXPOSURE

Diagnostic radiation exposure history was self-reported by means of a detailed questionnaire containing indication-based questions on lifetime exposure to fluoroscopies, conventional radiograph of the chest/shoulders (X-rays), mammograms, chest/shoulder CT-scans, other diagnostic procedures using ionizing radiation (e.g. bone-scan) involving the chest and/or shoulders, and radiotherapy. Occupational exposure and exposure during pregnancy and breastfeeding was also reported. Each section of the questionnaire provided a detailed description of the procedure. The most common indications for each procedure (e.g. fluoroscopy screening for tuberculosis), together with other potential indications, were listed. For fluoroscopies, X-rays and mammograms, the following information was completed: ever/never exposure, age at first exposure, number of exposures before age 20, between age 20 and 29, between age 30 and 39, and age at last exposure. For each of the other exposure types, the indication, age at exposure, and number of exposures for each procedure was reported. A copy of the questionnaire is available upon request.

DOSIMETRY OF DIAGNOSTIC RADIATION

We used the self-reported numbers and calendar time periods of the diagnostic procedures to calculate a cumulative breast dose score as an approximation of organ dose. The cumulative score was based on exposure to fluoroscopies, X-rays,

TABLE 2. Estimated nominal breast doses (in Gy) of diagnostic radiographic procedures by calendar time period

	Fluoroscopy[a]	Chest X-ray[b]	Mammogram[c]	Chest CT[b]
1930-1939	0.010	NA	NA	NA
1940-1959	0.010	0.0005	NA	NA
1960-1964	0.005	0.0005	0.0158	NA
1965-1969	0.005	0.0005	0.0186	NA
1970-1974	0.005	0.0005	0.0126	NA
1975-1979	0.005	0.0005	0.0090	0.02
1980-1984	0.005	0.0005	0.0066	0.02
1985-1989	0.005	0.0005	0.0042	0.02
1990-1994	0.005	0.0005	0.0042 (NL); 0.0036 (UK); 0.0039 (FR)	0.02
1995-1999	0.005	0.0005	0.0042 (NL); 0.0041 (UK); 0.0042 (FR)	0.02
2000-2004	0.005	0.0005	0.0035 (NL); 0.0043 (UK); 0.0039 (FR)	0.02
2005-2007	0.005	0.0005	0.0035 (NL); 0.0043 (UK); 0.0039 (FR)	0.02

NA, not applicable (procedure did not exist or nobody exposed)

a Based on the literature[15-20] and obtained through and estimated by AP and FvL *(personal communication)*

b Based on Sigurdson et al.[21]

c For period 1960-1989 doses obtained through ITC and AK *(personal communication)*; for period 1990-2007: NL[22,23], UK[24,25], and FR (average of dose NL and UK)

mammograms, and CT-scans and was calculated by the sum of the age-specific number of procedures multiplied by nominal estimates of breast dose (see Table 2). The estimates of the breast dose were derived from a literature review of published studies and institutional reports assessing radiation dose delivered to the breast from radiological examinations[15-25] and expert judgment by ITC, AK, FvL and AP. Where possible, the selected studies and reports were restricted to European studies performed on large patient samples, representative of patients and radiology services.

STATISTICAL ANALYSIS

The adjusted hazard ratios (HRs) of breast cancer and 95% confidence intervals (95%CI) were obtained using a Cox proportional hazards model with age (in years) as time scale and cumulative radiation exposure from diagnostic procedures as a time-dependent variable lagged by 5 years. All analyses were stratified for country (United Kingdom, France, and the Netherlands), birth cohort (<1955, 1955-1961, 1962-1968, >1968) and gene (BRCA1 and BRCA2), and clustered on family to account for potential within-family correlations in risk factors. Standard Cox regression leads to biased estimates of the HR because the women in this study were selected from high-risk families qualifying for genetic testing. The disease status may therefore have increased the likelihood of ascertainment leading to an oversampling of affected women. To correct for this potential bias, analyses were performed using the weighted regression approach described by Antoniou et al.[26]. By this procedure, HRs are typically shifted away from the null value (=1) at the cost of some power (wider 95%CIs).

Follow-up started at birth and ended at date of diagnosis of first breast cancer (N=848, including 11 incident cases that occurred after questionnaire completion), ovarian cancer (N=57), other cancer excluding basal cell carcinoma (N=39), date of bilateral prophylactic mastectomy (N=234), or questionnaire completion (N=815), whichever

occurred first. Five percent of breast cancer diagnoses were ductal carcinomas in situ, and for 7% the type was unknown. In total, there were 78,074 individual person-years of observation. Differences between characteristics were examined with the Pearson's $\chi 2$ test for discrete variables and the Student's t-test for continuous variables.

As diagnostic radiation exposure was reported in decades of age, we assumed that exposures were equally distributed across each decade, taking into account ages at first and last exposure. This resulted in the following categorization for cumulative number of exposures: 1=0.5≤x<1.5; 2=1.5≤x<2.5; 3-4=2.5≤x<4.5; >4=4.5 or more. Subsequently, procedures occurring 5 years prior to breast cancer diagnosis for cases (or censoring for unaffected carriers) were excluded from the analyses as a lag period, as procedures may have been performed because of a breast cancer diagnosis and exclusion of radiation dose that probably did not contribute to induction of breast cancer. We further examined this by excluding diagnoses occurring in the decade of exposure. If there was a difference between the results including and excluding these cases, then the results of the analysis excluding these cases were used. Categorization of the cumulative breast dose score was based on quartiles of cumulative dose at the end of follow-up.

Cox regression analyses were performed by type of exposure and for cumulative breast dose score in the following age-periods: before age 20, between age 20 and 29, before age 30, between age 30 and 39, and before age 40. HRs for exposure to X-rays and mammograms were adjusted for age at menarche, parity and menopausal status. Other potential confounding factors, including age at first full-term pregnancy and breastfeeding, did not change the log(hazard ratio) estimates by more than 10% and were omitted from final models. There were no confounding factors for fluoroscopies and CT-scans. The cumulative breast dose score was adjusted for parity and menopausal status. The proportional hazards assumption for each covariate was evaluated by inspecting the ln(-ln(survival)) curve, and using the goodness-of-fit test. No violation of the proportional hazards assumption was observed for any variable. Tests for trend were conducted by considering the median of the number of exposures in each category as a continuous variable in the model and were always unweighted. Missing values in ever/never exposure (<11%) and covariates (<1%) were coded as an additional category. Among carriers with any exposure to diagnostic radiation, missing values for age at first (<15% for fluoroscopies and X-rays, and <10% for mammograms) and last exposure (<5%) and number of exposures (<21% for fluoroscopies and X-rays, and <7% for mammograms), were imputed by age period, using the average age and number of exposures of women for whom complete data were available.

To investigate potential survival bias resulting from the women diagnosed with breast cancer a long time before the interview, a second set of analyses was performed on carriers diagnosed or censored within the 5 years prior to questionnaire completion. Follow-up was then counted only during this 5-year period and with a new set of period specific weights. This subcohort analysis contained a total of 1,122 participants among which 174 cases (mean age at diagnosis, 42.0 years) in 4,484 person-years.

In addition to the overall analysis, subgroup analyses were performed by gene (BRCA1 vs. BRCA2), attained age (<41 vs. ≥41 years), and year of birth (≤1961 vs. >1961).

Two-sided p-values ≤0.05 were considered statistically significant. The analyses were performed using STATA/SE 10.0 (StataCorp LP).

RESULTS

The characteristics of the study population are summarized in Table 3. In the entire cohort, 43% (N=848) of carriers had been diagnosed with breast cancer of whom the majority (88%) was diagnosed premenopausally. Eighty-nine percent of breast cancer cases were confirmed by medical records or linkage with national registries. There was no difference in age (mean ± standard deviation) at diagnosis and censoring for cases and unaffected carriers (39.5 ± 7.4 and 39.7 ± 7.4 years for cases and unaffected carriers, respectively; P=0.601). However, cases were older at questionnaire completion (49.7 ± 8.6 and 42.1 ± 10.5 years for cases and unaffected carriers, respectively; P<0.001). X-rays were the most common diagnostic procedure; 48% of carriers reported ever having had an X-ray while 33% ever had a mammogram. The median numbers of procedures before age 40 were 2.5 and 2.4 for X-rays and mammograms, respectively. The average age at first mammogram was 29.5 ± 5.8 years. Only a small proportion (<5%) of carriers was ever exposed occupationally, during pregnancy and/or breastfeeding, or to CT-scans or other diagnostic radiation procedures. None of the carriers had received radiotherapy prior to the end of follow-up because cancers other than breast cancer were censored.

Overall, in the procedure-specific analysis, there were no significant associations between having had any radiation and breast cancer risk in the entire cohort (Table 4). If any, exposure to CT-scans before age 30 seemed to be associated with non-significantly increased breast cancer risk (HR=2.36, 95%CI=0.71-7.88). In the subcohort, we observed a significant trend of increasing breast cancer risk with increasing number of X-rays before age 20 (P_{trend}=0.041) and a non-significantly increased risk of breast cancer after >2 fluoroscopies before age 20 (HR=2.01, 95%CI=0.71-5.71, P_{trend}=0.102) compared to no exposure. Furthermore, there was a non-significantly increased breast cancer risk after exposure to mammograms before age 30 (HR=1.43, 95%CI=0.85-2.40, P_{trend}=0.040). An almost 2-fold risk increase was observed for exposure to >4 X-rays before age 30 (HR=1.83, 95%CI=0.84-4.00, P_{trend}=0.012) and for >4 X-rays at ages 30-39 (HR=2.04, 95%CI=0.85-4.90, P_{trend}=0.101; data not shown), however, this latter category included only 6 cases. No other associations between exposure at ages 30-39 and breast cancer risk were observed.

The average cumulative breast dose score from fluoroscopies, X-rays, mammograms, and CT-scans combined was 0.014 Gy and ranged from 0.0005 to 0.613 Gy with an interquartile range of 0.002-0.017 Gy. In the entire cohort, a history of any exposure to fluoroscopies, X-rays, mammograms and/or CT-scans before age 30 significantly increased breast cancer risk (HR=1.39, 95%CI=1.12-1.73) but no dose-response emerged (Table 5). The association with ever exposure before age 20 was similar (HR=1.37, 95%CI=1.11-1.68); in this age-group there was some indication of a dose-response. In the subcohort, the observed associations were somewhat stronger. When compared to no exposure, a cumulative dose score of more than 0.0174 Gy before age 30 increased the

risk of breast cancer over 3-fold (HR=3.84, 95%CI=1.67-8.79). A similar risk increase was observed for exposure before age 20, but already after a dose of more than 0.0066 Gy (HR=3.16, 95%CI=1.19-8.39). There was no evidence of an increased breast cancer risk associated with exposure at ages 30-39, neither in the entire cohort nor in the subcohort.

As family history of breast cancer at an early age may be an indication for mammographic screening at a young age, we investigated this potential bias away from the null value by subgroup analyses in carriers who never had a mammogram before age 30 (Table 6). There was no difference in the association between cumulative dose score before age 30 and breast cancer risk in carriers without mammograms as compared to the complete model (including carriers with mammograms (Table 5)). In accordance, adding family history to the complete model (Table 5) as a covariate did not change the association between mammogram exposure and breast cancer risk either (data not shown).

Analysis by gene (Table 7) indicated that the association between diagnostic radiation and breast cancer risk was stronger for BRCA2 than for BRCA1 carriers in the entire cohort (e.g. HR=1.24, 95%CI=0.97-1.58 and HR=2.00, 95%CI=1.33-3.02 for exposure<20 for BRCA1 and BRCA2 carriers, respectively; $P_{interaction}$=0.034). However, in the subcohort, the risk estimates for BRCA1 carriers were higher than in the entire cohort, while for BRCA2 carriers the association observed in the entire cohort was attenuated in the subcohort ($P_{interaction}$=0.018). We observed no effect modification by attained age (Table 7) or by birth cohort (data not shown).

The risk estimates presented in Tables 4 and 5 were not materially affected by including the estimates for each age period (i.e. <20, 20-29, and 30-39) in the same model, or adjustment for occupational exposure. The results of the procedure-specific analyses did not change when including different exposure types in one model. Additionally, use of a 2 or 10 year time lag did not materially affect the results.

TABLE 3. Characteristics of the entire cohort (N=1,993) and the subcohort (N=1,122)

	Entire cohort				Subcohort			
	Cohort (N=1,993)		Cases (N=848)		Cohort (N=1,122)		Cases (N=174)	
Characteristic	**N[a]**	**%**	**N[a]**	**%**	**N[a]**	**%**	**N[a]**	**%**
Gene								
BRCA1	1,290	65%	575	68%	685	61%	114	66%
BRCA2	703	35%	279	32%	437	39%	60	34%
Birth cohort								
< 1955	462	23%	281	33%	127	11%	22	13%
1955 – 1961	517	26%	291	34%	224	20%	48	27%
1962 - 1968	508	26%	213	25%	315	28%	70	40%
> 1968	506	25%	63	7%	456	40%	34	20%
Study (country)								
GENEPSO (FR)	716	35%	257	30%	477	43%	37	21%
EMBRACE (UK)	688	35%	339	40%	408	36%	107	62%
HEBON (the Netherlands)	589	30%	252	30%	237	21%	30	17%
Age at menarche								
≤ 12 years	725	37%	311	37%	398	36%	57	33%
13 years	595	30%	247	29%	355	32%	59	34%
≥ 14 years	659	33%	286	34%	360	32%	58	33%
Parity								
Nulliparous	476	24%	152	18%	316	27%	34	20%
Parous	1,515	76%	695	82%	805	73%	140	80%
Number of children								
1 - 2 children	1,111	73%	519	75%	589	73%	104	74%
> 2 children	404	27%	176	25%	216	27%	36	26%
Age at first birth								
< 24 years	482	32%	248	35%	225	28%	41	29%
24 – 27 years	497	33%	224	32%	264	33%	46	33%
≥ 28 years	534	35%	223	32%	314	39%	53	38%
Breastfeeding								
Never	441	29%	202	29%	230	29%	34	25%
Ever	1,061	71%	487	71%	567	71%	104	75%
Menopausal status								
Premenopausal	1,573	79%	747	88%	791	71%	130	75%
Postmenopausal	417	21%	100	12%	329	29%	44	25%
Type								
Natural	151	36%	56	56%	90	27%	14	32%
Surgical, prophylactic	266	64%	44	44%	239	73%	30	68%
Fluoroscopy								
Never	1,512	84%	601	80%	900	90%	134	86%
Ever	280	16%	153	20%	100	10%	21	14%
X-ray								
Never	976	52%	392	49%	557	52%	79	49%
Ever	919	48%	403	51%	521	48%	83	51%
Mammogram								
Never	1,312	67%	624	74%	643	58%	100	58%
Ever	649	33%	214	26%	461	42%	73	42%
CT-scan								
Never	1,879	98%	789	99%	1,063	98%	164	98%
Ever	29	2%	10	1%	21	2%	3	2%
Other[b]								
Never	1,868	97%	794	98%	1,038	96%	157	96%
Ever	53	3%	19	2%	38	4%	6	4%
Occupational								
Never	1,886	96%	800	95%	1,056	95%	159	92%
Ever	88	4%	38	5%	57	5%	13	8%
Level[c]								
Low	44	50%	15	39%	31	54%	4	31%
Medium	39	44%	20	53%	22	39%	7	54%
High	5	6%	3	8%	4	7%	2	15%
Exposure during pregnancy/breastfeeding								
Never	1,871	96%	795	96%	1,061	96%	168	99%
Ever	81	4%	33	4%	41	4%	1	1%

a Numbers do not always add up to 100% due to missing values

b For example, DXA and isotope thyroid

c Category is based on a combination of dose (low or high) and duration (short or long) of occupational exposure; low: low dose and short duration, medium: low dose and long duration or high dose and short duration, high: high dose and long duration

TABLE 4. Diagnostic radiation exposures by age period and risk of breast cancer for the entire cohort (N=1,993) and subcohort (N=1,122)

	Entire cohort (N=1,993)				Subcohort (N=1,122)			
	Person-years	Cases	HR (95%CI)[a]		Person-years	Cases	HR (95%CI)[b]	
			Unweighted	Weighted			Unweighted	Weighted
Exposure before age 20								
Fluoroscopy								
Never	61,286	641	1.00	1.00	3,712	142	1.00	1.00
Ever	9,397	115	1.10 (0.89-1.36)	1.10 (0.82-1.47)	320	13	1.25 (0.66-2.36)	1.30 (0.59-2.88)
No. of fluoroscopies								
1-2	5,441	63	1.04 (0.80-1.36)	1.13 (0.79-1.60)	210	7	0.87 (0.35-2.17)	1.07 (0.38-3.00)
>2	3,956	52	1.19 (0.88-1.62)	1.05 (0.69-1.60)	110	6	2.46 (1.09-5.54)	2.01 (0.71-5.71)
X-ray								
Never	52,523	541	1.00	1.00	3,092	113	1.00	1.00
Ever	22,242	256	1.07 (0.91-1.24)	1.07 (0.86-1.33)	1,217	50	1.30 (0.90-1.88)	1.29 (0.84-1.98)
No. of X-rays								
1	10,845	132	1.17 (0.97-1.42)	1.16 (0.88-1.53)	568	24	1.16 (0.73-1.84)	1.30 (0.79-2.15)
2	7,666	81	0.89 (0.71-1.13)	0.90 (0.64-1.26)	425	17	1.43 (0.82-2.51)	1.17 (0.59-2.30)
>2	3,907	43	1.13 (0.84-1.54)	1.14 (0.73-1.78)	223	9	1.53 (0.74-3.17)	1.43 (0.58-3.57)
Mammogram								
Never	76,249	830	1.00	1.00	*	*	*	*
Ever	1,570	11	0.70 (0.40-1.23)	0.77 (0.40-1.50)				
Exposure between age 20 and 29								
Fluoroscopy								
Never	65,472	689	1.00	1.00	3,876	147	1.00	1.00
Ever	5,275	67	1.16 (0.92-1.47)	1.23 (0.87-1.73)	172	8	1.24 (0.63-2.44)	1.72 (0.74-2.96)
No. of fluoroscopies								
1-2	3,171	40	1.22 (0.89-1.67)	1.25 (0.79-1.96)	*	*	*	*
>2	2,103	27	1.10 (0.78-1.56)	1.21 (0.73-2.01)				
X-ray								
Never	51,571	539	1.00	1.00	3,036	117	1.00	1.00
Ever	23,403	259	1.06 (0.91-1.24)	1.01 (0.81-1.26)	1,276	46	1.23 (0.83-1.82)	1.14 (0.69-1.86)
No. of X-rays[c]								
1	9,403	99	0.99 (0.79-1.23)	0.89 (0.66-1.20)	570	22	1.16 (0.72-1.87)	0.98 (0.54-1.77)
2	4,200	48	1.17 (0.88-1.57)	1.20 (0.81-1.77)	572	15	1.06 (0.60-1.89)	1.14 (0.53-2.44)
3-4	6,421	73	1.19 (0.93-1.51)	1.10 (0.77-1.56)	123[c]	8[c]	1.72 (0.77-3.86)[c]	1.80 (0.68-4.71)[c]
>4	3,245	37	0.97 (0.69-1.39)	0.97 (0.58-1.60)				
Mammogram								
Never	67,889	752	1.00	1.00	3,692	147	1.00	1.00
Ever	9,928	89	1.11 (0.90-1.37)	1.08 (0.80-1.45)	709	27	1.48 (0.97-2.25)	1.31 (0.76-2.26)
No. of mammograms[c]								
1	4,956	44	1.07 (0.79-1.44)	1.08 (0.72-1.61)	377	14	1.32 (0.79-2.20)	1.17 (0.60-2.30)
2	1,783	16	1.06 (0.68-1.65)	0.94 (0.48-1.84)	235	7	1.40 (0.61-3.24)	1.08 (0.39-3.00)
3-4	1,782	17	1.31 (0.86-1.99)	1.36 (0.71-2.61)	96[c]	6[c]	2.44 (0.91-6.55)[c]	2.35 (0.64-8.60)[c]
>4	1,406	12	1.09 (0.63-1.88)	1.00 (0.47-2.16)				
Exposure before age 30								
Fluoroscopy								
Never	58,690	607	1.00	1.00	3,622	136	1.00	1.00
Ever	11,914	147	1.15 (0.95-1.40)	1.16 (0.88-1.52)	410	19	1.37 (0.82-2.29)	1.65 (0.89-3.08)
No. of fluoroscopies								

1-2	6,314	73	1.06 (0.83-1.36)	1.11 (0.79-1.57)	245	12	1.18 (0.62-2.25)	1.66 (0.77-3.57)
>2	5,599	74	1.27 (0.98-1.65)	1.21 (0.85-1.73)	165	7	1.93 (0.86-4.34)	1.64 (0.60-4.50)
X-ray								
Never	41,609	426	1.00	1.00	2,451	90	1.00	1.00
Ever	33,249	370	1.09 (0.94-1.27)	1.11 (0.90-1.37)	1,856	73	1.35 (0.95-1.93)	1.33 (0.84-2.08)
No. of X-rays								
1	10,877	122	1.16 (0.95-1.42)	1.24 (0.94-1.65)	636	28	1.32 (0.85-2.05)	1.40 (0.81-2.40)
2	7,398	79	0.95 (0.75-1.21)	0.96 (0.69-1.33)	446	18	1.20 (0.68-2.10)	1.08 (0.56-2.08)
3-4	7,841	91	1.22 (0.97-1.54)	1.15 (0.83-1.62)	424	11	1.23 (0.62-2.43)	1.14 (0.46-2.81)
>4	7,132	78	1.03 (0.79-1.33)	1.04 (0.72-1.52)	349	16	1.98 (1.06-3.71)	1.83 (0.84-4.00)
Mammogram								
Never	67,179	747	1.00	1.00	3,621	144	1.00	1.00
Ever	10,592	94	1.05 (0.86-1.30)	1.07 (0.81-1.43)	775	30	1.54 (1.03-2.28)	1.43 (0.85-2.40)
No. of mammograms[c]								
1	5,224	47	0.99 (0.74-1.33)	1.11 (0.76-1.63)	402	17	1.56 (0.98-2.46)	1.52 (0.83-2.79)
2	1,974	18	1.11 (0.72-1.71)	0.94 (0.49-1.80)	277	7	1.14 (0.47-2.75)	0.87 (0.30-2.56)
3-4	1,876	14	1.05 (0.65-1.69)	1.00 (0.50-2.00)	96[c]	6[c]	2.41 (0.90-6.49)[c]	2.36 (0.65-8.60)[c]
>4	1,517	15	1.22 (0.74-2.02)	1.19 (0.59-2.42)				
CT-scan								
Never	75,201	793	1.00	1.00	*	*	*	*
Ever	530	6	1.82 (0.91-3.63)	2.36 (0.71-7.88)				

HR, hazard ratio; CI, confidence interval

a Time-varying Cox proportional hazards model, stratified for gene (BRCA1 and BRCA2), country (FR, UK, and NL) and birth cohort (<1955, 1955-1961, 1962-1968, >1968), and clustered on family (1,522 clusters). For X-rays and mammograms, the models were additionally adjusted for age at menarche (≤12;13;≥14), parity (no children; 1-2 children; >2 children; time-varying), and menopause (premenopausal; natural menopause; bilateral prophylactic oophorectomy; time-varying)

b The subcohort includes carriers diagnosed or censored within 5 years prior to questionnaire completion, with follow-up being counted only during this 5-year period, and with a new set of weights calculated only for this period. Familial clustering includes 930 clusters. Age at entry in the subcohort is included as a covariate

c For the subcohort analysis, the categories are: 1, 2-4, and >4

* power too low (<6 cases ever exposed or per category of number of exposures)

>> TABLE 5: see page 42 and 43.

TABLE 6. Cumulative breast dose score before age 30 and risk of breast cancer for carriers without a history of mammograms

	Entire cohort (N=1,993; 747 cases)			Subcohort (N=955; 144 cases)		
	Person-years	Cases	HR (95%CI)[a]	Person-years	Cases	HR (95%CI)[b]
Before age 30						
Never	27,160	263	1.00	1,679	57	1.00
Ever	28,110	333	1.33 (1.12-1.57)	1,412	58	1.65 (1.11-2.46)
Dose category						
<0.002	14,442	164	1.29 (1.06-1.57)	874	33	1.48 (0.94-2.33)
0.002-0.0066	6,031	73	1.35 (1.04-1.77)	280	12	1.55 (0.81-2.98)
0.0066-0.0174	3,965	45	1.22 (0.89-1.67)	147	6	1.90 (0.69-5.21)
≥0.0174	3,671	51	1.56 (1.15-2.11)	109	7	4.16 (2.01-8.62)

HR, hazard ratio; CI, confidence interval

a Unweighted time-varying Cox proportional hazards model, stratified for gene (BRCA1 and BRCA2), country (FR, UK, and NL) and birth cohort (<1955, 1955-1961, 1962-1968, >1968), clustered on family (1,522 clusters), and adjusted for parity (no children; 1-2 children; >2 children; time-varying) and menopause (premenopausal; natural menopause; bilateral prophylactic oophorectomy; time-varying)

b The subcohort includes carriers diagnosed or censored within 5 years prior to questionnaire completion, with follow-up being counted only during this 5-year period. Familial clustering includes 816 clusters. Age at entry in the subcohort is included as a covariate

TABLE 5. Cumulative breast dose score and risk of breast cancer for the entire cohort (N=1,993) and the subcohort (N=1,122)

	Entire cohort (N=1,993)				Subcohort (N=1,122)			
	Person-years	Cases	HR (95%CI)[a]		Person-years	Cases	HR (95%CI)[b]	
			Unweighted	Weighted			Unweighted	Weighted
Before age 20								
Never	39,908	380	1.00	1.00	2,576	91	1.00	1.00
Ever	24,584	294	1.28 (1.10-1.49)	1.37 (1.11-1.68)	1,211	49	1.42 (0.97-2.07)	1.62 (1.02-2.58)
Dose category[c]								
<0.002	13,429	158	1.24 (1.02-1.49)	1.30 (1.00-1.70)	748	31	1.30 (0.85-2.00)	1.47 (0.89-2.42)
0.002-0.0066	4,235	49	1.35 (1.02-1.80)	1.45 (0.97-2.17)	226	6	1.09 (0.48-2.51)	1.09 (0.41-2.91)
0.0066-0.0174	4,341	55	1.33 (1.02-1.74)	1.61 (1.12-2.33)	236[c]	12[c]	2.56 (1.28-5.12)[c]	3.16 (1.19-8.36)[c]
≥0.0174	2,579	32	1.30 (0.86-1.96)	1.13 (0.64-2.02)				
Between age 20 and 29								
Never	37,920	393	1.00	1.00	2,240	83	1.00	1.00
Ever	26,750	285	1.21 (1.03-1.41)	1.15 (0.93-1.43)	1,567	57	1.48 (1.04-2.09)	1.43 (0.93-2.21)
Dose category								
<0.002	11,820	130	1.18 (0.97-1.44)	1.14 (0.87-1.51)	690	21	1.21 (0.73-1.99)	1.13 (0.62-2.08)
0.002-0.0066	6,917	65	1.13 (0.86-1.48)	1.00 (0.69-1.46)	466	19	1.52 (0.91-2.53)	1.55 (0.76-3.17)
0.0066-0.0174	4,533	52	1.46 (1.12-1.90)	1.60 (1.10-2.35)	253	9	1.77 (0.88-3.56)	1.62 (0.68-3.83)
≥0.0174	3,479	38	1.18 (0.84-1.67)	1.00 (0.60-1.66)	157	8	2.12 (1.02-4.37)	2.11 (0.78-5.70)
Before age 30								
Never	27,160	263	1.00	1.00	1,679	57	1.00	1.00
Ever	37,332	411	1.30 (1.11-1.52)	1.39 (1.12-1.73)	2,108	83	1.73 (1.20-2.48)	1.90 (1.20-3.00)
Dose category								
<0.002	14,442	164	1.28 (1.05-1.56)	1.43 (1.09-1.87)	874	33	1.45 (0.92-2.27)	1.63 (0.96-2.77)
0.002-0.0066	9,042	93	1.32 (1.03-1.67)	1.33 (0.95-1.88)	574	22	1.70 (1.03-2.79)	1.78 (0.88-3.58)
0.0066-0.0174	7,460	74	1.20 (0.93-1.53)	1.31 (0.93-1.85)	413	14	1.74 (0.89-3.39)	1.75 (0.72-4.25)
≥0.0174	6,387	80	1.47 (1.13-1.92)	1.46 (1.00-2.12)	245	14	3.29 (1.83-5.93)	3.84 (1.67-8.79)
Between age 30 and 39								
Never	41,287	500	1.00	1.00	2,402	80	1.00	1.00
Ever	25,836	219	1.03 (0.85-1.24)	1.06 (0.83-1.36)	1,553	63	1.02 (0.70-1.51)	1.06 (0.66-1.71)
Dose category								
<0.002	4,862	55	1.01 (0.75-1.36)	1.02 (0.70-1.50)	194	8	0.83 (0.40-1.74)	0.88 (0.39-2.02)
0.002-0.0066	7,970	72	1.10 (0.86-1.41)	1.23 (0.89-1.71)	492	22	1.17 (0.71-1.96)	1.29 (0.70-2.36)
0.0066-0.0174	7,828	53	0.91 (0.68-1.21)	0.86 (0.59-1.26)	539	20	0.89 (0.52-1.51)	0.83 (0.42-1.64)
≥0.0174	5,275	39	1.12 (0.78-1.61)	1.13 (0.70-1.84)	327	13	1.20 (0.62-2.29)	1.30 (0.58-2.93)
Before age 40								
Never	19,329	212	1.00	1.00	1,148	37	1.00	1.00
Ever	45,163	462	1.23 (1.04-1.46)	1.35 (1.08-1.70)	2,639	103	1.59 (1.06-2.38)	1.91 (1.12-3.26)
Dose category								
<0.002	10,117	118	1.10 (0.88-1.38)	1.19 (0.88-1.62)	615	23	1.38 (0.78-2.44)	1.59 (0.78-3.22)
0.002-0.0066	11,171	120	1.37 (1.10-1.70)	1.51 (1.12-2.03)	666	23	1.50 (0.88-2.56)	2.01 (1.02-3.95)
0.0066-0.0174	11,727	100	1.11 (0.87-1.41)	1.21 (0.87-1.67)	733	33	1.74 (1.04-2.89)	1.80 (0.91-3.54)
≥0.0174	12,145	124	1.44 (1.13-1.82)	1.56 (1.13-2.16)	624	24	1.79 (1.04-3.07)	2.31 (1.16-4.59)

HR, hazard ratio; CI, confidence interval. Categorization based on quartiles of cumulative dose at age 40. Interquartile ranges by category: <0.002: 0.0005-0.0006; 0.002-0.0066: 0.0035-0.0054; 0.006-0.0174: 0.0092-0.0142; ≥0.0174: 0.0222-0.0435.

a Time-varying Cox proportional hazards model, stratified for gene (BRCA1 and BRCA2), country (FR, UK, and NL) and birth cohort (<1955, 1955-1961, 1962-1968, >1968), clustered on family (1,522 clusters), and adjusted for parity (no children; 1-2 children; >2 children; time-varying) and menopause (premenopausal; natural menopause; bilateral prophylactic oophorectomy; time-varying)

b The subcohort includes carriers diagnosed or censored within 5 years prior to questionnaire completion, with follow-up being counted only during this 5-year period and with a new set of weights calculated only for this period. Familial clustering includes 930 clusters. Age at entry in the subcohort is included as a covariate

c For the subcohort analysis, the categories are: <0.002, 0.002-0.0066, and ≥0.0066

TABLE 7. Cumulative breast dose score and risk of breast cancer stratified by gene mutation (BRCA1 and BRCA2) and by attained age (<41 years)

	Entire cohort				Subcohort			
	Person-years	Cases	HR (95%CI)[a]		Person-years	Cases	HR (95%CI)[b]	
			Unweighted	Weighted			Unweighted	Weighted
BRCA1								
Before age 20[c]								
Ever vs. never	16,161	193	1.12 (0.94-1.34)	1.24 (0.97-1.58)	787	37	1.94 (1.23-3.08)	2.33 (1.34-4.06)
<0.002	8,752	103	1.10 (0.87-1.38)	1.20 (0.87-1.64)	476	21	1.71 (1.02-2.87)	2.09 (1.15-3.81)
0.002-0.0066	2,811	33	1.17 (0.83-1.67)	1.29 (0.82-2.04)	311[c]	16[c]	2.39 (1.27-4.49)[c]	2.78 (1.20-6.41)[c]
0.0066-0.0174	2,916	40	1.26 (0.94-1.69)	1.60 (1.07-2.40)				
≥0.0174	1,681	17	0.90 (0.51-1.60)	0.80 (0.40-1.59)				
Between age 20 and 29								
Ever vs. never	16,749	185	1.18 (0.98-1.43)	1.09 (0.84-1.40)	937	37	1.63 (1.04-2.56)	1.58 (0.92-2.70)
<0.002	7,032	83	1.19 (0.93-1.53)	1.12 (0.81-1.56)	382	10	1.08 (0.52-2.25)	1.11 (0.49-2.49)
0.002-0.0066	4,297	47	1.24 (0.90-1.73)	1.01 (0.65-1.57)	263	13	1.81 (0.95-3.48)	1.85 (0.76-2.47)
0.0066-0.0174	3,174	34	1.23 (0.88-1.71)	1.42 (0.93-2.18)	182	8	2.09 (0.96-4.55)	1.94 (0.76-4.97)
≥0.0174	2,244	21	0.99 (0.62-1.58)	0.80 (0.44-1.45)	110	6	2.16 (0.85-4.45)	2.06 (0.65-6.58)
Before age 30								
Ever vs. never	23,871	273	1.22 (1.01-1.48)	1.32 (1.02-1.70)	1,290	57	2.44 (1.50-3.96)	2.83 (1.59-5.04)
<0.002	8,835	108	1.26 (0.99-1.60)	1.44 (1.05-1.97)	504	19	1.96 (1.09-3.54)	2.46 (1.27-4.77)
0.002-0.0066	5,678	64	1.30 (0.97-1.74)	1.28 (0.86-1.92)	318	14	2.30 (1.20-4.43)	2.45 (1.02-5.90)
0.0066-0.0174	4,821	49	1.10 (0.81-1.47)	1.26 (0.85-1.86)	269	12	2.51 (1.12-5.61)	2.72 (0.99-7.44)
≥0.0174	4,535	52	1.21 (0.88-1.66)	1.22 (0.80-1.85)	198	12	4.26 (2.07-8.79)	5.00 (1.96-12.7)
BRCA2								
Before age 20								
Ever vs. never	8,423	101	1.72 (1.29-2.30)	2.00 (1.33-3.02)	422	12	0.83 (0.41-1.70)	0.62 (0.26-1.45)
<0.002	4,677	55	1.58 (1.12-2.22)	1.75 (1.08-2.84)	*	*	*	*
0.002-0.0066	1,423	16	1.92 (1.20-3.09)	2.63 (1.27-4.45)				
0.0066-0.0174	1,425	15	1.72 (0.93-3.18)	1.75 (0.70-4.39)				
≥0.0174	897	15	2.83 (1.47-5.45)	5.42 (2.25-13.0)				
Between age 20 and 29[c]								
Ever vs. never	10,001	99	1.26 (0.96-1.64)	1.41 (0.94-2.10)	629	20	1.17 (0.64-2.14)	0.98 (0.45-2.15)
<0.002	4,787	47	1.18 (0.85-1.64)	1.22 (0.75-1.99)	309	11	1.21 (0.59-2.51)	1.02 (0.41-2.54)
0.002-0.0066	2,620	18	0.88 (0.53-1.44)	0.91 (0.46-1.78)	320[c]	9[c]	1.10 (0.52-2.34)[c]	0.92 (0.35-2.45)[c]
0.0066-0.0174	1,358	18	2.21 (1.45-3.38)	3.53 (1.61-7.72)				
≥0.0174	1,234	17	1.64 (1.01-2.65)	2.71 (1.22-6.02)				
Before age 30[c]								
Ever vs. never	13,461	138	1.50 (1.13-2.00)	1.66 (1.10-2.51)	817	26	1.02 (0.58-1.81)	0.76 (0.36-1.61)
<0.002	5,606	56	1.35 (0.96-1.90)	1.37 (0.84-2.24)	370	14	0.99 (0.49-1.99)	0.73 (0.31-1.69)
0.002-0.0066	3,363	29	1.38 (0.90-2.11)	1.56 (0.83-2.94)	447[c]	12[c]	1.08 (0.55-2.12)[c]	0.82 (0.30-2.21)[c]
0.0066-0.0174	2,639	25	1.51 (0.96-2.38)	1.68 (0.84-3.34)				
≥0.0174	1,851	28	2.53 (1.58-4.05)	4.88 (2.40-9.92)				
Attained age <41 years[d]								
Before age 20[c]								
Ever vs. never	11,347	165	1.25 (1.03-1.53)	NA	5,215	20	1.54 (0.88-2.72)	NA
<0.002	7,204	97	1.23 (0.98-1.58)		3,729	13	1.55 (0.83-2.90)	
0.002-0.0066	2,107	31	1.32 (0.91-1.91)		1,485[c]	7[c]	1.54 (0.67-3.52)[c]	
0.0066-0.0174	1,357	25	1.21 (0.79-1.83)					
≥0.0174	678	12	1.34 (0.70-2.57)					

Between age 20 and 29[e]								
Ever vs. never	13,315	169	1.23 (1.01-1.51)	NA	7,353	27	1.39 (0.85-2.28)	NA
<0.002	5,587	78	1.23 (0.95-1.59)		2,765	7	0.90 (0.39-2.11)	
0.002-0.0066	3,692	46	1.37 (0.99-1.88)		2,345	12	1.66 (0.92-2.99)	
0.0066-0.0174	2,413	27	1.14 (0.79-1.66)		2,242[e]	8[e]	1.67 (0.75-3.73)[e]	
≥0.0174	1,622	18	1.10 (0.67-1.82)					
Before age 30[e]								
Ever vs. never	18,630	241	1.27 (1.04-1.55)	NA	10,037	40	1.87 (1.13-3.10)	NA
<0.002	7,908	103	1.24 (0.97-1.58)		4,275	15	1.68 (0.87-3.24)	
0.002-0.0066	4,986	63	1.45 (1.09-1.94)		2,878	13	2.02 (1.17-3.48)	
0.0066-0.0174	3,298	39	1.08 (0.77-1.51)		2,884[e]	12[e]	2.07 (0.99-4.30)[e]	
≥0.0174	2,436	36	1.35 (0.92-1.98)					

HR, hazard ratio; CI, confidence interval; NA, not applicable (no cohort specific weights were available)

a Time-varying Cox proportional hazards model, stratified for country (FR, UK, and NL) and birth cohort (<1955, 1955-1961, 1962-1968, >1968), clustered on family, and adjusted for parity (no children; 1-2 children; >2 children; time-varying) and menopause (premenopausal; natural menopause; bilateral prophylactic oophorectomy; time-varying)

b The subcohort includes carriers diagnosed or censored within 5 years prior to questionnaire completion, with follow-up being counted only during this 5-year period and with a new set of weights calculated only for this period. Age at entry in the subcohort is included as a covariate.

c For the subcohort analysis, the categories are: <0.002 and ≥0.002

d Weighted analysis not applicable because no subgroup-specific weights were available

e For the subcohort analysis, the categories are: <0.002, ≥0.002-0.0066, and ≥0.0066

* power too low

DISCUSSION

This study indicates that exposure to diagnostic radiation is associated with increased breast cancer risk in BRCA1/2 mutation carriers. A unique feature of our study is the estimation of the cumulative breast dose score from various diagnostic radiation exposures. We observed a significant risk increase for exposure before age 30, even for a relatively low dose category (i.e. below 0.007 Gy). These results appear to support the non-threshold model. No association with breast cancer risk was apparent for exposure at ages 30-39.

The IBCCS[8] reported a 1.8-fold increased risk for ever versus never exposure to X-rays in carriers in the subcohort while we observed a 1.4-fold non-significantly increased risk for exposure to X-rays before age 40 (HR=1.38, 95%CI=0.87-2.20; data not shown). This difference may be explained by the fact that we included prediagnostic X-rays only while the IBCCS had included all X-rays lifetime. A case-case study in BRCA1 carriers and non-carriers observed that the odds ratio for ever having had a chest X-ray below age 30, given a BRCA1 mutation, was 1.8 (P=0.01), suggesting gene-radiation interaction[9]. Two other carrier studies on exposure to mammograms observed no association with breast cancer risk[10,11]. This may be due to the relatively high age at first mammogram which was on average 35 years, while in our study it was 29.5 ± 5.8 years. We observed a 1.5-fold increased risk of breast cancer after mammogram exposure before age 30 with a significant dose-response trend (Table 4). We were concerned that this latter association might be attributed to confounding by indication, i.e. self-selection to have early mammography by carriers with a mother and/or sister with early breast cancer. This was not the case as the association holds with diagnostic radiation after exclusion of carriers with mammograms (Table 6).

Our study is the first to investigate other types of diagnostic exposures than mammograms and X-rays in carriers. For fluoroscopies alone, we observed a significantly increased risk of breast cancer after exposure to more than 2 fluoroscopies before age 20. No meaningful analyses could be conducted for fluoroscopy exposure in other age periods, CT-scans and other procedures, for exposure during pregnancy/breastfeeding, and occupational exposure, because of very small numbers of exposed carriers in these categories (Table 3).

Our results may support the linear non-threshold model, which is widely accepted to apply to low-dose risk estimation, and used in radiation protection[27,28]. However, for both the procedure-specific and cumulative dose score results, dose-response trends were not always present. Linear non-threshold extrapolation may not apply to groups with a genetic susceptibility for increased radio-sensitivity. Also, a few studies appear to reveal some differences in the biological responses to high- and low-dose radiation[2]. The associations between the dose score and breast cancer risk were almost always strongest for the highest dose category, e.g. we observed a 3-fold increase in risk for a cumulative dose score of 0.0174 Gy or higher before age 30. We wondered whether this high risk could partly be attributed to outliers. However, sensitivity analyses excluding carriers with a cumulative dose higher than 0.1 Gy (N=8; 3 cases) did not affect our results.

In the general population, a minimal induction time for breast cancer after exposure to radiation of 10 to 15 years is generally accepted[29]. Since we hypothesized that BRCA carriers may have increased radiosensitivity due to impaired DNA repair mechanisms, we used a 5 year time lag in our analyses. Analyses using a 2 or 10 year time lag showed very similar results (data not shown). We kept the 5 year time lag to minimize potential contamination by diagnostic procedures related to breast cancer diagnosis.

A puzzling finding was a difference in the association between cumulative dose score and breast cancer risk for BRCA1 and BRCA2 carriers (Table 7): the associations for BRCA1 carriers were stronger in the subcohort, while for BRCA2 carriers, the strongest associations were observed in the entire cohort. BRCA1 and -2 proteins both play a role in the repair of DNA damage of double strand breaks, which can be caused by ionizing radiation. However, their functions are quite distinct, and germline mutations in the two genes predispose to different age-specific risks of breast cancer and to different subtypes of breast cancer (BRCA1 mutations predominately predisposing to triple negative "Basal" disease). It is possible that the effects of ionizing radiation on breast cancer risk differ between BRCA1 and BRCA2 mutations. However, for these age-specific exposure effects the power in the BRCA1/2 groups is rather low. Thus, larger studies will be required to determine whether there exists a difference in response to ionizing radiation between BRCA1 en BRCA2 carriers.

Several strong and weak points of our study should be considered in the interpretation of these results. The strengths of our study include the sample size and the detailed information on all diagnostic procedures using ionizing radiation in different age periods. The high response rate to the radiation questionnaire suggests that selection bias due to non-response is not likely in our study. The weighted cohort approach[26] was used as a way to overcome potential testing bias (i.e. preferential testing of affected women). However, the retrospective character of our cohort and the type of study population, consisting of carriers tested in the clinical setting, may have caused some biases in our results. An important concern in a cohort study including prevalent cases is potential recall bias and survival bias. Recall bias implies that a history of breast cancer may have affected subjects' recall of their diagnostic radiation exposure. We relied on self-reports rather than medical record review because of the difficulties in accessing medical records with regard to the various diagnostic procedures which occurred in many different contexts, especially when occurred in the distant past. Nevertheless, in the Dutch part of the cohort, two methodological studies on test-retest reliability of several diagnostic procedures[30] and validity of self-reported mammograms (*submitted for publication*, see chapter 4 of this thesis) were conducted. The results showed that the extent of the observed misclassification of self-reported diagnostic radiation history was small and mainly non-differential by disease status, consistent with other studies[31,32]. The results of the present study may therefore have been biased towards unity. Additionally, as exposure before age 10 is unlikely to have been recalled by women, associations between breast cancer risk and exposure before age 20 may have been more prone to non-differential misclassification than exposure at ages 20-29. This might explain why the risk of breast cancer in carriers exposed before age 20 was

not stronger increased than among those exposed at ages 20-29, in contrast to what was observed in other radiation exposed cohorts that did not rely on self-reports[1,29]. Furthermore, our follow-up may not have been long enough to detect an association between radiation exposure at ages 30-40 and breast cancer risk as the average age at end of follow-up was 39 years and in the general population the induction period of radiation is estimated to be at least 8 years.

The stronger associations observed in the subcohort as compared to the entire cohort analyses are intriguing and suggest survival bias. Apparently, prevalent cases who were exposed to a greater extent did not survive until the start of the cohort. Little is known about the influence of exposure to ionizing radiation, low or high dose, on overall survival and breast cancer-specific survival in carriers. The question actually is whether radiation-induced breast cancers have a worse prognosis than non-radiation-induced breast cancers. A recent Dutch study among Hodgkin's lymphoma patients showed that radiation-induced breast cancers have a distinct gene expression profile and may have a less favorable prognostic profile[33]. An additional explanation for the difference in results between the entire and subcohort may be the presence of more non-differential misclassification in the entire cohort. There was a significant difference in age at questionnaire completion between the entire and the subcohort (50.7 ± 8.8 and 41.1 ± 9.7 years, respectively, P<0.001). Older age at questionnaire completion was a significant predictor of decreased proportion agreement in the test-retest reliability study[30]. However, adjustment for or stratifying on age at questionnaire completion did not materially affect the risk estimates (data not shown). Because the subcohort analysis is restricted to relatively recent diagnoses and is unlikely to suffer from survival bias, we consider the results of the subcohort to be the most valid, although it was based on smaller numbers. A prospective analysis was not yet possible because the number of incident cases was too small (N=11). Incident case numbers are not expected to increase rapidly due to the increasing uptake of prophylactic surgery (bilateral mastectomy and/or oophorectomy) among carriers.

The assumptions underlying the calculation of the cumulative dose score may have influenced the dosimetry results. First, the dose estimates for fluoroscopies were based on limited information from the (primarily Dutch) literature and personal communications. Secondly, no indication-specific dose estimates for fluoroscopies and X-rays were used. However, the majority (>95%) of fluoroscopies before age 20 were chest fluoroscopies for tuberculosis screening and originated from the Dutch cohort (where mass population screening for tuberculosis in young people was performed 1940-1960[15]). For X-rays before age 20, the majority (>90%) were chest X-rays for which the dose (0.0005 Gy) differed from the dose of a shoulder X-ray for exposures before 1974 only (0.001 Gy)[21]. Thirdly, we assumed that differences in dose estimates between the three European countries would be small as the recent country-specific mammogram dose estimates for the UK and the Netherlands were very similar. Additionally, the dose scores for mammograms were based on a 2-view mammogram. The doses used are typical doses for an average woman but there are large variations depending on several factors including the characteristics of the patient (breast size, shape, breast

composition), parameters of the X-ray machine (kVp, mA, exposure time, target/filter combination and Half Value Layer, film type and technology) and conditions of exposure (characteristics of the protocols and repetition of exposure in case of poor image quality). Because carriers are screened from a relatively young age onwards when in general breast tissue has a higher density, this may result in more than one set of 2-view mammograms and/or adjusted parameters of the X-ray machine, resulting in a higher absorbed dose. Therefore, our mammogram dose score may underestimate the true dose, and, as a consequence, the risk estimates presented may correspond to a higher dose category. However, sensitivity analysis of the cumulative dose score without women exposed to mammograms resulted in similar associations. The assumptions on the exposure- and calendar-specific dose estimates, together with the previously discussed non-differential misclassification in reporting exposure, may have led to a lack of consistent dose-response trends in both the procedure-specific and dose score analyses. In conclusion, exposure to diagnostic radiation before age 30 increased breast cancer risk in BRCA1/2 mutation carriers. Risks were observed at dose levels considerably lower than at which increases have been found in other radiation-exposed cohorts. Our findings may support a non-threshold dose-response relationship. In the recent past, carriers often started mammographic screening before age 30[10,11]. We recommend that physicians follow current recommendations[34-36], avoiding mammographic screening before age 30 and using magnetic resonance imaging (MRI) as the main tool for surveillance at young ages in BRCA1/2 mutation carriers. As a result of having a 50% chance of being a carrier, untested daughters of carriers are potentially increased radiosensitive as well. In an era of increasing collective dose from medical radiation, mostly due to increased use of CT scans[37,38], the medical need for diagnostic procedures using ionizing radiation must be balanced against the potential radiation risk, especially during childhood and adolescence. Future studies should focus on prospective follow-up and address modifying effects by genotype, for both low and high dose ionizing radiation exposure.

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APPENDIX

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