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Variation in breast cancer risk with mutation position, smoking, alcohol, and chest X-ray history, in the French National BRCA1/2 carrier cohort (GENEPSO)

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Abstract Germline mutations in BRCA1/2 confer a high risk of breast cancer (BC), but the magnitude of this risk varies according to various factors. Although controversial, there are data to support the hypothesis of allelic-risk heterogeneity. We assessed variation in BC risk according to the location of mutations recorded in the French study GENEPSO. Since the women in this study were selected from high-risk families, oversampling of affected women was eliminated by using a weighted Cox-regression model. Women were censored at the date of diagnosis when affected by any cancer, or the date of interview when unaffected. A total of 990 women were selected for the analysis: 379 were classified as affected, 611 as unaffected. For BRCA1, there was some evidence of a central region where the risk of BC is lower (codons 374–1161) (HR = 0.59, $P = 0.04$). For BRCA2, there was a strong evidence for a region at decreased risk (codons 957–1827) (HR = 0.35, $P = 0.005$) and for one at increased risk (codons 2546–2968) (HR = 3.56, $P = 0.01$). Moreover, we found an important association between radiation exposure from chest X-rays and BC risk (HR = 4.29, $P < 10^{-3}$) and a positive association between smoking more than 21 pack-years and BC risk (HR = 2.09, $P = 0.04$). No significant variation in BC risk associated

GENEPSO Collaborating Centers are listed in Appendix.

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with chest X-ray exposure, smoking, and alcohol consumption was found according to the location of the mutation in BRCA1 and BRCA2. Our findings are consistent with those suggesting that the risk of BC is lower in the central regions of BRCA1/2. A new high-risk region in BRCA2 is described. Taking into account environmental and lifestyle modifiers, the location of mutations might be important in the clinical management of BRCA mutation carriers.

Keywords Breast cancer · Risk factor · Genotype–phenotype correlation · BRCA1 · BRCA2 · Interaction

Introduction

Carriers of mutations in the *BRCA1* and *BRCA2* genes are at very high risk of developing breast cancer (BC) and ovarian cancer. Estimates of the lifetime risk of developing BC for *BRCA1* and *BRCA2* mutation carriers range, respectively, from 30 to 80% and from 9 to 84% [1]. Incomplete penetrance and the range of these risk estimates suggest the existence within families of genetic or shared environmental or lifestyle factors that modify the risk of BC.

Among studies that have attempted to identify environmental or lifestyle modifiers of *BRCA1* or *BRCA2*, few have examined smoking, alcohol consumption, or history of chest X-ray exposure, and they have often reached different conclusions, showing an overall lack of consistency. The effect of alcohol among *BRCA1/2* carriers has been investigated in only two studies [2, 3]. Studies on the effect of smoking on BC in *BRCA* mutation carriers have also yielded different results [4–10]. Among studies of the effect of exposure to low-dose radiation during mammography or chest X-ray [11–15], two show a significantly increased risk of BC [11, 12]. These inconsistencies between the studies may be due to heterogeneity in BC risk associated with environmental factors according to the location of the mutation in the gene. Genotype-phenotype correlations have been described in both *BRCA1* and *BRCA2* (e.g. [16–19]). In BRCA1, the risk of BC has been reported to increase from the 5′ to 3′ regions [20, 21] and to be lower in the central region [18]. In *BRCA2*, most studies have reported a central region named OCCR (for "ovarian cancer cluster region") where the risk of BC is decreased compared with outside this region [17, 20, 22, 23]. Therefore, we first studied the effect of mutation position on the risk of BC and identified homogeneous regions. Then for homogeneous BC risk regions, we studied the effects of smoking, alcohol consumption, and history of chest X-ray exposure on the risk of BC.

Materials and methods

Data

The GENEPSO study was initiated to estimate the risk of breast, ovarian, and other cancers in *BRCA1/2* mutation carriers and to assess potential risk-modifying factors. Subjects were ascertained from the family cancer clinics of the Genetic and Cancer Group of the Fédération Nationale des Centres de Lutte Contre le Cancer. Any woman who was known to carry a deleterious mutation in the *BRCA* genes was eligible, including those who had been diagnosed with cancer, as well as those who were currently unaffected. They had to be at least 18 years old, mentally capable of giving informed consent to study participation, and had been counseled about their mutation status. The research protocol was approved by the relevant ethics committees, and all participants provided written informed consent.

The study population was based on the women enrolled into the GENEPSO study from 2000 to 2010. A total of 1,337 women were recruited, 863 (65%) were *BRCA1* mutation carriers, and 474 (35%) were *BRCA2* mutation carriers. To assess variation in BC risk according to mutation position, a sample with one subject per family was randomly selected to avoid overmatching on the mutation.

A standardized questionnaire on reproductive factors, lifestyle factors, and history of chest X-ray exposure was administered to the study subjects by mail. Tobacco history was assessed first as past, current, or never use. For subjects who ever smoke, information on age when starting (and when stopping for past smokers), the number of cigarettes, and total duration of smoking was required. Alcohol consumption was also first assessed as ever or never use. Detailed information was recorded on the number of glasses per week, categorized as 0, 1–5, 6–10, and more

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than 10 at the age of 20 and at the interview. Exposure to chest X-rays (excluding mammograms) was first assessed as ever or never exposed. For subjects who reported at least one exposure, more specific information was requested relating to the number of X-ray exposures before and after age 20 (0, 1–4, or ≥5 X-rays in each of the two age periods). These two variables were combined to create a measure related to age at first exposure (before or after age 20) and another measure related to level of X-ray exposure (only one period with ≤4 X-rays and no period with ≥5 X-rays; two periods with 1–4 X-rays; one or two periods with ≥5 X-rays).

Genotyping

The mutation screening strategy was similar across the clinics, i.e., the youngest living affected family member was tested first and, if a *BRCA1/2* mutation was found, affected and unaffected family members were offered testing.

The full coding sequences and the exon–intron junctions of the *BRCA* genes were screened for variants, based on pre-screening (DGGE, SSCP, PTA, dHPLC, HRM or EMMA) and sequencing. Several large rearrangements were identified by large cDNA sequencing, MLPA [24], QMPSF [25], qPCR [26], qPCR HRM [27], EMMA [28], bar code screening [29], or dedicated array CGH [30]. Mutation description was provided by each French laboratory, coded, and standardized according to the international nomenclature.

Statistical methods

The data presented here were analyzed using a modified Cox proportional hazards regression model. Standard Cox regression may lead to biased estimates of the hazard ratio (HR) because the women in this study were taken from high-risk families qualifying for genetic testing. The disease status may therefore have affected the likelihood of ascertainment and the selection leading to an over-sampling of affected women. To correct for this bias, the Cox regression analyses were performed using the weighted regression approach described by Antoniou et al. [31]. Individuals were weighted such that the observed BC incidence rates in the study sample were consistent with established BC risk estimates for *BRCA1* and *BRCA2* carriers [1]. The affected mutation carriers were underweighted (weights <1), and the unaffected mutation carriers were overweighted (weights >1). The weights were applied to all person-years of each subject in the modified Cox model.

Subjects were followed up from birth and censored for women who were affected by any cancer at the date of diagnosis, or the date of prophylactic bilateral mastectomy or interview for unaffected women.

Deleterious mutations were classified in two classes: nonsense mutation and other type (i.e., missense mutations, in-phase skipping, partial, or entire gene deletions).

To assess variation in BC risk according to the location of nonsense mutations, *BRCA1* and *BRCA2* were divided into 20 codon regions of variable length corresponding to the 20-quantiles of the distribution of the mutation position among unaffected women. The HR was then estimated for each region, and contiguous regions with similar HR point estimates were combined to determine homogeneous regions in BC risk, and 95% confidence intervals were calculated. To avoid tests biased by the step-by-step inference process that we used to identify homogeneous regions, we performed permutation analyses to estimate the *P* value. 10,000 samples were simulated under the null hypothesis of no variation in BC risk according to nonsense mutation position. The HRs associated with regions defined as homogeneous were calculated in the 10,000 samples. The *P* value equals the proportion of HRs estimated from simulated samples which were equal to or less probable than the observed HR.

Tobacco use changed over time, so it was analyzed as a time-dependent covariate. All analyses were stratified by the year of birth (before 1940, 1940–1949, 1950–1959, 1960 or later). In addition, because parity, menopausal status, BMI, and gene may substantially modify the risk of BC and thus may be potential confounders, analyses were adjusted for menopausal status (yes/no) and parity (0, 1, 2, 3, ≥4) both time-dependent variables; BMI (<18.5, 18.5–25, >25); and gene (*BRCA1* or *BRCA2*). Because alcohol and tobacco consumption are strongly associated, analyses of their effects were carried out separately: (1) for never-smokers and ever-smokers when the effect of alcohol use was assessed; and (2) for alcohol drinkers and non-drinkers when smoking effect was assessed.

All statistical analyses were two-sided and were performed using the STATA statistical package (version 10; Stata Corporation, College Station TX).

Results

Characteristics of the whole cohort and of one-woman-per-family cohorts are listed in Table 1. A total of 563 women had been diagnosed with BC at the time of their interview; however, only 499 of these women were considered as affected in this analysis after censoring. The remaining 838 women were censored at age, at diagnosis of ovarian cancer ($N = 89$), at diagnosis of another cancer ($N = 16$), at prophylactic bilateral mastectomy ($N = 11$), or at interview ($N = 722$). The average age at censoring for the 838 participants without BC was 40.0 years (SD = 10.8),

Table 1 Characteristics of the cohort study of *BRCA1/2* mutation carriers

Characteristics	Whole cohort						One woman per family					
	All women ($N = 1337$)		With BC ($N = 499$)		Without BC ($N = 838$)		All women ($N = 990$)		With BC ($N = 379$)		Without BC ($N = 611$)	
	No	%	No	%	No	%	No	%	No	%	No	%
Gene mutated												
BRCA1	863	64.6	332	66.5	531	63.4	635	64.1	240	63.3	395	64.7
BRCA2	474	35.5	167	33.5	307	36.6	355	35.9	139	36.7	216	35.4
Age at interview, years												
Mean	44.1		49.4		41.0		43.7		48.6		40.7	
SD	12.7		10.8		12.7		12.3		10.5		12.3	
Age at diagnosis/censoring, years												
Mean	40.4		41.0		40.0		40.1		40.4		39.8	
SD	10.8		8.80		10.8		10.5		8.80		11.5	
<30	196	14.7	34	6.80	162	19.3	142	14.3	29	7.70	113	18.5
30–39	487	36.4	205	41.1	282	33.7	371	37.5	159	42.0	212	34.7
40–49	403	30.1	176	35.3	227	27.1	306	30.9	133	35.1	173	28.3
50–59	180	13.5	67	13.4	113	13.5	126	12.7	47	12.4	79	12.9
≥60	71	5.30	17	3.40	54	6.40	45	4.60	11	2.90	34	5.60
Year of birth												
<1950	354	26.5	201	40.3	153	18.3	237	23.9	139	36.7	98	16.0
1950–1960	324	24.2	165	33.1	159	19.0	248	25.1	128	33.8	120	19.6
1960–1970	351	26.3	119	23.9	232	27.7	282	28.5	99	26.1	183	30.0
≥1970	308	23.0	14	2.80	294	35.1	223	22.5	13	3.40	210	34.4
Consumption of tobacco												
Never	693	51.8	262	52.5	431	51.4	507	51.2	194	51.2	313	51.2
Ever	642	48.0	236	47.3	406	48.5	481	48.6	184	48.6	297	48.6
Missing	2	0.20	1	0.20	1	0.10	2	0.20	1	0.30	1	0.20
Consumption of alcohol												
Never	352	26.3	117	23.5	235	28.0	259	26.2	90	23.8	169	27.7
Ever	984	73.6	382	76.6	602	71.8	730	73.7	289	76.3	441	72.2
Missing	1	0.10	0	0.00	1	0.10	1	0.10	0	0.00	1	0.20
Chest X-ray exposure												
Never exposed	187	14.0	14	2.80	173	20.6	139	14.0	10	2.60	129	21.1
≥1 X-ray	1,121	83.8	481	96.4	640	76.4	826	83.4	365	96.3	461	75.5
Missing	29	2.20	4	0.80	25	3.00	25	2.50	4	1.10	21	3.40

which is similar to the age at diagnosis of the women with BC (41.0 years, SD = 8.8), although the age at interview was substantially greater for the BC patients, reflecting the pattern of genetic testing among participants. Sampling of one woman per family did not change any characteristic distribution or the average of age at censure (40.4 years, SD = 8.8 and 39.8 years, SD = 11.5, respectively, for women with and without BC). Year of birth, consumption of tobacco and alcohol, and chest X-ray exposure are also described. There were a total of 39,666 person-years of observation.

Variation of BC risk according to mutation type and location in *BRCA1* and *BRCA2* is shown in Table 2. First, classification of mutations as nonsense or other showed that there was no significant difference in BC risk for either *BRCA1* (HR = 1.06, 95% CI = 0.68–1.66) or BRCA2 (HR = 0.49, 95% CI = 0.18–1.29), even though the point estimate showed a 50% decrease in BC risk. The distribution of the nonsense mutations is shown in Figs. 1 and 2 for *BRCA1* and *BRCA2* carriers, respectively. To assess variation in BC risk according to the position of the nonsense mutations, we partitioned *BRCA1* and *BRCA2* according to the 20-quantiles of the distribution of mutation positions among unaffected women, and this led to regions of varying length (mean length = 101 codons and 165 codons for *BRCA1* and *BRCA2*, respectively). Three

Table 2 Variation of BC risk according to the type and location of the mutation in *BRCA1* and *BRCA2*

	Person-years	Without BC	With BC	HR	95% CI	*P*
BRCA1						
Mutation type						
Nonsense	22,057	353	209	1.00		
Other type	2,988	42	31	1.06	0.68–1.66	ns
Nonsense mutation location, codon						
0–373	4,327	71	43	1.00		
374–1161 (LR1)	5,367	89	43	0.59	0.36–0.97	0.04
1162–1851	12,363	193	123	0.87	0.58–1.30	ns
BRCA2						
Mutation type						
Nonsense	13,493	198	131	1.00		
Other type	1,128	18	8	0.49	0.18–1.29	ns
Nonsense mutation location, codon						
0–956	3,957	60	39	1.00		
957–1827 (LR2)	2,281	43	11	0.35	0.15–0.80	0.005
1828–2545	4,642	66	45	0.96	0.53–1.73	ns
2546–2968 (HR2)	1,137	10	19	3.56	1.49–8.50	0.01
2969–3418	1,476	19	17	1.49	0.70–3.19	ns

HR hazard ratio; *CI* confidence interval

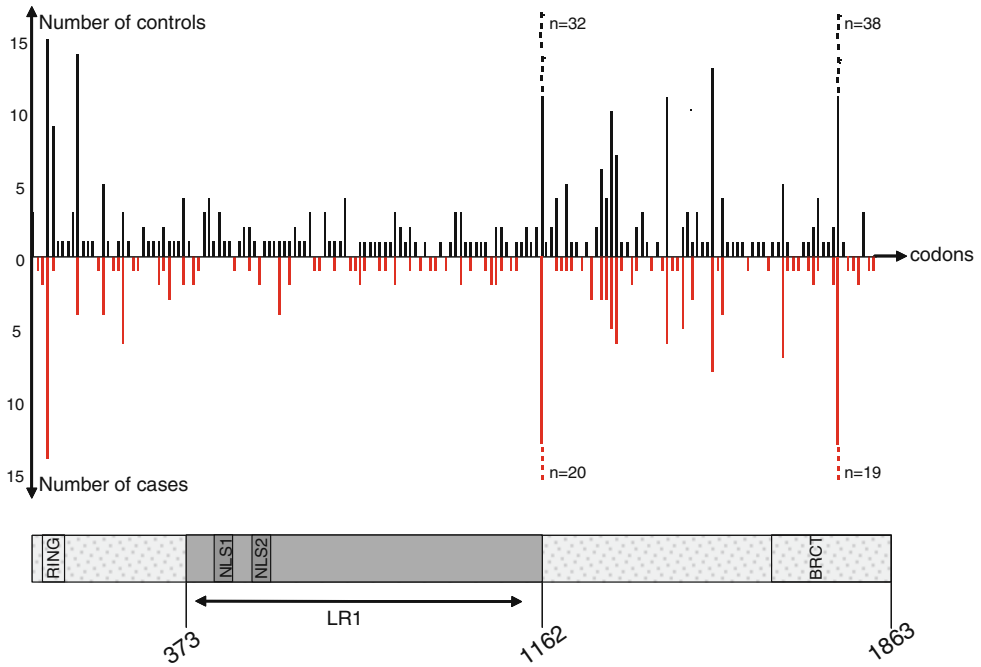


Fig. 1 Distribution of *BRCA1* mutations among the study subjects. *Ring* ring finger domain; *NLS* Nuclear Localization Signal; *BRTC* BRCA1 C-terminal domain; *LR1* lower risk region in BRCA1. ▬ Controls; ▬ Cases

regions homogeneous in BC risk were determined in *BRCA1* and four in *BRCA2*. In *BRCA1*, mutations between codons 374 and 1161 (LR1 for "lower risk region in *BRCA1*") were associated with a significant decrease in the risk of BC (HR = 0.59, 95% CI = 0.36–0.97, $P = 0.04$) as compared with mutations located before codon 373. The third homogeneous region located from codon 1161 to the end was associated with a nonsignificant HR equal to 0.87

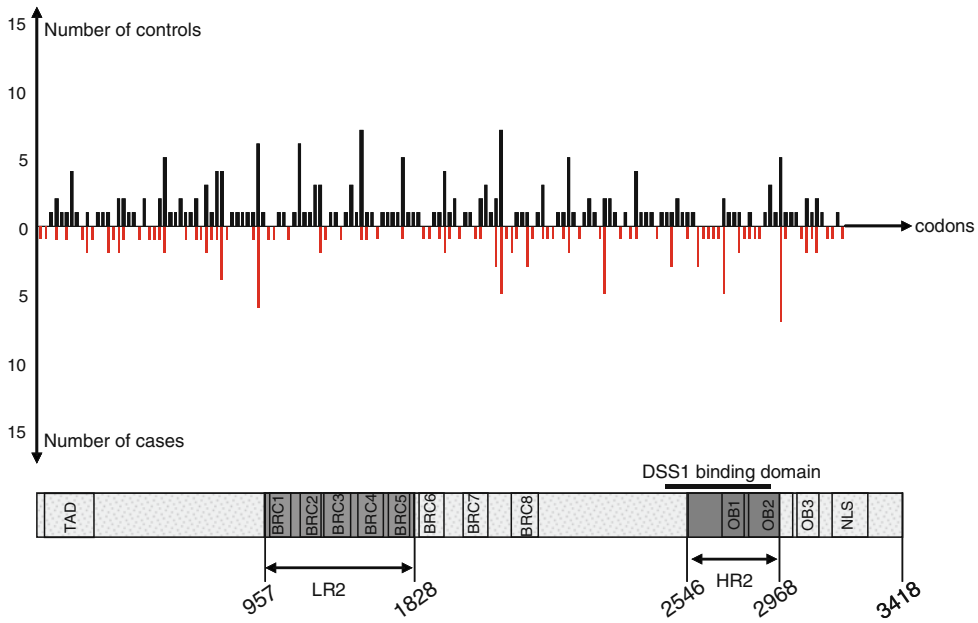


Fig. 2 Distribution of *BRCA2* mutations among the study subjects. *TAD* Transcription activation domain; *BRC* eight ~35 amino acid motifs repeats; *OB* oligonucleotide-binding folds; *NLS* Nuclear Localization Signal; *DSS1* deleted in split hand/split foot protein 1; *LR2* lower risk region in BRCA2; *HR2* higher risk region in BRCA2. ▬ Controls; ▬ Cases

Table 3 Variation of BC risk according to chest X–ray exposure

	No. of person-years[a]	No. of cases[a]	HR	95% CI	*P* value
X-ray exposure[b]					
Never exposed	4,726	10	1.00		
≥1 X-ray	33,670	365	4.29	2.09–8.81	$<10^{-3}$
Age at 1st X-ray exposure[b]					
Never exposed	4,726	10	1.00		
Before age 20 years	30,979	331	4.16	2.03–8.56	$<10^{-3}$
After age 20 years	2,691	34	6.45	2.86–14.6	$<10^{-3}$
Level of X-ray exposure[b]					
Never exposed	4,726	10	1.00		
Only one period with 1–4 X-rays	1,520	12	4.83	1.83–12.8	$<10^{-3}$
Two periods with 1–4 X-rays	20,348	276	6.22	2.94–13.1	$<10^{-3}$
At least one period with 5 + X-rays	9,139	62	2.80	1.30–6.05	0.01

HR hazard ratio; *CI* confidence interval

[a] Not including missing data

[b] Adjusted for parity, menopausal status, and gene

(95% CI = 0.58–1.30). Among *BRCA2* mutation carriers, mutations between codons 957 and 1827 (LR2 for "lower risk region in *BRCA2*") were associated with a significant decrease in the risk of BC (HR = 0.35, 95% CI = 0.15–0.80, $P = 0.005$), whereas mutations between codons 2546 and 2968 (HR2 for "higher risk region in BRCA2") were associated with a significantly increased BC risk (HR = 3.56, 95% CI = 1.49–8.50, $P = 0.01$), as compared with mutations located before codon 956.

The estimated risks of BC associated with chest X-ray exposure (excluding mammograms), tobacco consumption, and alcohol consumption are summarized in Tables 3, 4, 5, and 6.

Very few subjects, particularly those with BC, had never been exposed to chest X-ray at censure (2.8% of cases). Overall, any exposure to chest X-ray was associated with a significantly increased risk of BC (HR = 4.29, 95% CI = 2.09–8.81) (cf. Table 3). Chest X-ray exposure was significantly associated with an increased risk of BC whenever the first exposure was before or after age 20 and whatever the level of exposure. No difference was found when stratified by gene. Exposure to chest X-ray was associated with a significantly increased risk of BC among *BRCA1* carriers (HR = 4.12, 95% CI = 1.82–9.35) and among *BRCA2* carriers (HR = 5.43, 95% CI = 1.36–21.7) (data not shown).

Table 4 Variation of BC risk according to alcohol and tobacco consumption

	Never user of alcohol					Ever user of alcohol				
	Person-years[a]	No. of cases[a]	HR	95% CI	*P* value	Person-years[a]	No. of cases[a]	HR	95% CI	*P* value
Tobacco consumption[b]										
Never use	8,742	62	1.00			20,863	139	1.00		
Current use	1,037	18	2.09	1.04–4.19	0.04	5,630	85	0.97	0.66–1.44	ns
Past use	334	9	0.74	0.31–1.79	ns	1,917	61	1.21	0.83–1.77	ns
Pack-years[b]										
0–5	9,192	64	1.00			23,423	158	1.00		
6–20	846	17	1.03	0.52–2.05	ns	4,276	87	1.16	0.83–1.63	ns
≥21	67	8	3.21	1.24–8.31	0.02	689	40	1.13	0.68–1.86	ns
Time since last use[b]										
Never stopped	1,037	18	1.00			5,630	85	1.00		
≤10 years	230	8	0.70	0.23–2.14	ns	1,236	33	1.35	0.80–2.29	ns
>10 years	104	1	0.12	0.02–0.77	0.03	681	28	1.15	0.67–1.98	ns
Never used	8,742	62	0.48	0.24–0.96	0.04	20,863	139	1.02	0.69–1.51	ns
	Never smoker					Ever smoker				
	Person-years[a]	No. of cases[a]	HR	95% CI	*P* value	Person-years[a]	No. of cases[a]	HR	95% CI	*P* value
Alcohol consumption[b]										
Never use	8,742	62	1.00			1,371	27	1.00		
Current use	20,863	139	1.10	0.76–1.61	ns	7,547	146	0.89	0.53–1.52	ns
Alcohol consumption at age 20 years (glasses/week)[b]										
Never use	18,579	137	1.00			4,306	93	1.00		
1–5	5,246	29	0.82	0.50–1.34	ns	1,837	29	0.74	0.44–1.26	ns
6–10	4,164	24	1.02	0.56–1.87	ns	1,832	36	1.18	0.72–1.93	ns
>10	1,235	9	1.26	0.53–2.96	ns	669	12	0.89	0.40–1.96	ns

HR hazard ratio; *CI* confidence interval

[a] Not including missing data

[b] Adjusted for parity, menopausal status, gene, and number of years of smoking interruption

Because of a borderline negative interaction between the alcohol and tobacco consumption ($P = 0.058$), analyses were performed stratified by tobacco use when alcohol consumption effect was assessed and vice versa (Table 4). Among alcohol drinkers, none of the HRs associated with tobacco is significantly different from unity. Inversely, among those who have never drunk alcohol, a significantly increased risk of BC was found among current smokers (HR = 2.09, 95% CI = 1.04–4.19), with a maximum risk among those who smoked more than 21 pack-years (HR = 3.21, 95% CI = 1.24–8.31); among past smokers, a significant decrease in risk of BC was found for those who had stopped smoking more than 10 years before (HR = 0.12, 95% CI = 0.02–0.77). For alcohol consumption, there was no increased risk of BC among current smokers, past smokers, or never smokers. When stratified by gene, results for alcohol and tobacco consumption for *BRCA1* carriers were similar to those found for *BRCA1* and *BRCA2* mutation carriers taken together (cf. Table 5). Among BRCA2 carriers, there was no significant interaction between alcohol and tobacco consumption ($P = 0.75$), so analyses of tobacco and alcohol use were not stratified (Table 6). Results for tobacco use led to an increase in BC risk similar to that found for *BRCA1* and *BRCA2* mutation carriers taken together. For alcohol use, the HR point estimates are higher than those found among *BRCA1* carriers (e.g., ever vs. never, HR = 1.21, 95% CI = 0.68–2.15) and equals 1.78 among those who consumed more than five glasses per week at age 20 (95% CI = 0.97–3.27, $P = 0.07$) (data not shown).

Estimated risks of BC associated with tobacco or alcohol consumption and chest X-ray exposure (excluding mammograms) according to the mutation location were assessed by the regions defined as homogeneous. As described above, analyses by mutation location were performed stratified by tobacco use when alcohol consumption effect was assessed and vice versa for *BRCA1*, and not stratified for *BRCA2*.

Table 5 Variation of BC risk according to alcohol and tobacco consumption in *BRCA1* carriers

	Never user of alcohol					Ever user of alcohol				
	Person-years[a]	No. of cases[a]	HR	95% CI	*P* value	Person-years[a]	No. of cases[a]	HR	95% CI	*P* value
Tobacco consumption[b]										
Never use	5,136	38	1.00			13,562	92	1.00		
Current use	702	13	2.09	0.94–4.65	0.07	3,523	48	0.87	0.52–1.43	ns
Past use	240	5	0.56	0.19–1.62	ns	1,165	41	1.25	0.81–1.93	ns
Pack-years[b]										
0–5	5,453	40	1.00			15,116	103	1.00		
6–20	586	11	0.84	0.38–1.87	ns	2,711	60	1.23	0.83–1.82	ns
≥21	39	5	3.29	1.09–9.95	0.04	401	18	0.87	0.45–1.68	ns
Time since last use[b]										
Never stopped	702	13	1.00			3,523	48	1.00		
≤10 years	166	4	0.44	0.10–1.89	ns	773	25	1.64	0.87–3.10	ns
>10 years	74	1	0.13	0.02–0.80	0.03	392	16	1.26	0.64–2.46	ns
Never smoked	5,136	38	0.47	0.21–1.06	0.07	13,562	92	1.15	0.70–1.90	ns
	Never smoker					Ever smoker				
	Person-years[a]	No. of cases[a]	HR	95% CI	*P* value	Person-years[a]	No. of cases[a]	HR	95% CI	*P* value
Alcohol consumption[b]										
Never use	5,136	38	1.00			942	18	1.00		
Current use	13,562	92	1.02	0.65–1.60	ns	4,688	89	0.90	0.49–1.68	ns
Alcohol consumption at age 20 years (glasses/week)[b]										
Never use	11,560	86	1.00			2,906	63	1.00		
1–5	3,364	21	0.79	0.43–1.46	ns	1,029	16	0.70	0.35–1.38	ns
6–10	2,677	16	0.89	0.44–1.80	ns	1,032	19	1.10	0.59–2.03	ns
>10	866	6	1.16	0.42–3.19	ns	464	7	0.78	0.31–1.96	ns

HR hazard ratio; *CI* confidence interval

[a] Not including missing data

[b] Adjusted for parity, menopausal status, gene, and number of years of smoking interruption

Among *BRCA1* mutation carriers, the number of mutation carriers in LR1 was too small to assess the effect of tobacco and alcohol consumption. The BC risk associated with chest X-ray exposure did not significantly differ between LR1 (HR = 3.75, 95% CI = 1.28–11.0) and outside LR1 (HR = 2.39, 95% CI = 0.67–8.48).

Among *BRCA2* mutation carriers, the number of subjects with a mutation in LR2 and HR2 was also too small to analyze variation in the effect of chest X-ray exposure, and in particular there were almost no unexposed subjects per region of interest. However, for consumption of alcohol and tobacco, despite the small number of subjects, HR point estimates seemed different between regions. Ever versus never smoking was associated with a HR point estimate greater than unity, albeit not significantly, in LR2 (LR2: HR = 2.84, 95% CI = 0.61–13.3; HR2: HR = 0.67, 95% CI = 0.17–2.56; reference region: HR = 1.12, 95% CI = 0.62–2.01; $P_{\text{heterogeneity}}$ = ns). For alcohol consumption, a HR point estimate greater than unity was found only in the reference zone (reference zone: HR = 1.82, 95% CI = 0.95–3.48; LR2: HR = 0.38, 95% CI = 0.07–1.90; HR2: HR = 0.28, 95% CI = 0.05–1.44; $P_{\text{heterogeneity}}$ = ns) (data not shown).

Discussion

Our results confirm the existence of a central low BC risk region in *BRCA1* (LR1) and one in *BRCA2* (LR2), the latter located in the same area as the OCCR region described previously [16–18, 23]. In addition, a new high BC risk region (HR2) is found in the 3′ region of *BRCA2* between the codons 2546 and 2968. No significant variation in BC risk associated with chest X-ray exposure is found according to the location of the mutation. Although not statistically significant, point estimates suggest a possible variation in BC risk associated with alcohol and tobacco consumption according to the location of the mutation in *BRCA2*.

Since our data used prevalent cases with some women being interviewed a long time after their BC diagnosis, we

Table 6 Variation of BC risk according to alcohol and tobacco consumption in *BRCA2* carriers

	Person-years[a]	No. of cases[a]	HR	95% CI	*P* value
Tobacco consumption[b]					
Never use	10,907	71	1.00		
Current use	2,442	42	1.39	0.73–2.63	ns
Past use	846	24	1.18	0.60–2.33	ns
Pack-years[b]					
0–5	12,046	79	1.00		
6–20	1,825	33	1.07	0.58–1.98	ns
≥21	316	25	2.25	1.05–4.82	0.04
Time since last use[b]					
Never stopped	2,442	42	1.00		
≤10 years	527	12	0.84	0.37–1.90	ns
>10 years	319	12	0.71	0.30–1.70	ns
Never smoked	10,907	71	0.65	0.37–1.14	ns
Alcohol consumption[c]					
Never use	4,035	33	1.00		
Current use	10,447	106	1.21	0.68–2.15	ns
Alcohol consumption at age 20 years (glasses/week)[c]					
Never use	8,510	81	1.00		
1–5	2,854	23	0.87	0.47–1.60	ns
6–10	2,319	25	1.85	0.89–3.86	ns
>10	574	8	1.64	0.66–4.02	ns

HR hazard ratio; *CI* confidence interval

[a] Not including missing data

[b] Adjusted for parity, menopausal status, gene, number of years of smoking interruption and alcohol use

[c] Adjusted for parity, menopausal status, gene, smoking and number of years of smoking interruption

cannot exclude that our findings on mutation position, chest X-ray exposure, and tobacco and alcohol consumption are affected by a potential survival bias. The negative interaction between tobacco and alcohol use found in our data could be due to such survival bias [32–36]. However, we could not detect it in our data by performing extra analyses on subsamples of individuals diagnosed or censured within the 5-year period before or at their interview, with a follow-up being counted only for this period. We do not observe differences in our results using this pseudo-incident cohort.

The locations of low or high BC risk regions in *BRCA1* and *BRCA2* differ a little from those previously published [17, 18, 20–23]. We may have missed other variations in BC risk along the genes because of our a priori cut-point which may not be fine enough. However, our a priori number of quantiles was dictated by sample size limitation. We can not exclude that these variations may be due to confounders like modifiers (either genetic or environmental) resulting in a strong family history of BC or of ovarian cancer. Unfortunately, the pedigree data were not available in this study. Our approach though allows us to describe a new region of high BC risk in *BRCA2* in the C-terminal region. HR2, which corresponds to the longest conserved region in the BRCA2 protein [37], is of major importance for the BRCA2 protein stability because of DSS1 (for "deleted in split hand/split foot protein 1") protein binding region [38]. The strong physical interaction between DSS1 and BRCA2 seems to be crucial for BRCA2 function in DNA double-strand break repair. It has been postulated that DSS1 may regulate the accessibility of the DNA binding sites on BRCA2 [38]. Therefore, a mutation in that region with even partial conservation may lead to a truncated but potentially stable protein. This stable truncated protein might have a dominant negative effect on the wild-type protein.

Our results on the effect of exposure to low-dose radiation due to chest X-ray are consistent with two [11, 12] of the five studies [11–15] where a significantly increased risk of BC was found.

The limitations in interpreting our results are as discussed in Andrieu et al. [12], since the study design and methodology are the same. In spite of the fact that our findings and the data set of Andrieu et al. overlap for 319 subjects and thus HRs are not independent, the BC risk associated with chest X-ray exposure in our report is about 2.5 times higher than that given by Andrieu et al. [12]

(HR = 1.56, 95% CI = 1.00–2.40). This difference may be due to the fact that our study subjects were more often exposed to chest X-ray (at least one period ≥5 X-rays: 57 vs. 45% in Andrieu et al.) and more often exposed before age 20 (81 vs. 59% in Andrieu et al.). In addition, the risk of BC associated with chest X-rays exposure in GENEPSO *BRCA1/2* mutation carriers is about 3 fold higher than in the general population (e.g. in women under age 50 years, HR = 2.1 when nine or more chest X-rays exposures [39]). More detailed information on level and on age at exposure is needed to specify the risk of BC associated with chest X-ray exposure in the French population of *BRCA1/2* mutation carriers, a population which appears to be particularly exposed.

Unlike in the general population [40], and in accordance with the two studies on *BRCA1/2* mutation carriers [2, 3], our findings do not support a positive association between alcohol intake and BC risk. Although there is no obvious hypothesis to observe an association between alcohol intake and BC risk limited to some mutation positions, our findings suggest such an association for mutations located outside LR2 or HR2. Nevertheless, this observation may be due to chance and should first be replicated in other and larger data sets.

Unlike in the general population, where no consistency in association between smoking and BC risk was found (e.g. [40]), we found a twofold increased risk of BC among those who do not drink alcohol. Studies that have examined the effect of smoking on BC in *BRCA* mutation carriers gave different results. Some reported a null association [4–7], two reported a protective association [8, 9], and two reported a positive association [5, 10], only among *BRCA1* carriers with a past history of smoking in Ginsburg et al. [5]. Boyd et al. [10] have suggested that using prevalent cases may have biased results toward a null association or even toward a protective association if women who smoke are more likely to die after a diagnosis of BC than women with BC who do not smoke. An association between smoking and BC risk among *BRCA1/2* mutation carriers is supported by our findings. We found that BC risk increases in subjects smoking more than 21 pack-years and decreases as time since last use increases. Moreover, because of the importance of *BRCA1* and *BRCA2* in DNA repair, *BRCA1/2* mutation carriers may be more sensitive to carcinogens contained in cigarette smoke than the general population.

Conclusions

This study describes a new region in *BRCA2* with a potential high risk of BC. The identification of this region may have implications for the clinical management of *BRCA2* mutation carriers. This study also indicates an important association between the radiation exposure from chest X-rays and BC risk in French *BRCA1/2* mutation carriers that is of greater magnitude than the association seen in the general population and in other *BRCA1/2* mutation carrier populations. These results have implications regarding the appropriate use of medical imaging in carriers. Furthermore, our findings point to a positive association between the tobacco use and BC risk. If confirmed, these results would provide a practical way for carriers to reduce their risk. This is the first time that the effects of chest X-ray, alcohol use, and tobacco use have been assessed according to the location of the mutation. The power to detect differential effects according to region was low, but may generate hypotheses to be tested on other datasets. Taking into account environmental and lifestyle modifiers, mutation position might be important for the clinical management of *BRCA* carriers and may be helpful in understanding the biology of *BRCA1* and *BRCA2* in BC etiology.

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Appendix

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