Alcohol consumption, cigarette smoking, and risk of breast cancer for *BRCA1* and *BRCA2* mutation carriers: results from The BRCA1 and BRCA2 Cohort Consortium

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Running title: Alcohol and smoking and breast cancer risk for *BRCA* carriers

Abbreviations used:

BC: breast cancer
DNA: Deoxyribonucleic acid
HR: Hazard Ratios
HR_R : Hazard Ratios for retrospective cohort
HR_P : Hazard Ratios for prospective cohort
CI: confidence interval
FFTP: first full-term pregnancy
RRM: risk-reducing mastectomy
RRSO: risk-reducing oophorectomy

Abstract:

Background: Tobacco smoking and alcohol consumption have been intensively studied in the general population to assess their effects on the risk of breast cancer (BC), but very few studies have examined these effects in *BRCA1* and *BRCA2* mutation carriers. Given the high BC risk for mutation carriers and the importance of *BRCA1* and *BRCA2* in DNA repair, better evidence on the associations of these lifestyle factors with BC risk is essential.

Methods: Using a large international pooled cohort of *BRCA1* and *BRCA2* mutation carriers, we conducted retrospective (5,707 *BRCA1* mutation carriers; 3,525 *BRCA2* mutation carriers) and prospective (2,276 *BRCA1* mutation carriers; 1,610 *BRCA2* mutation carriers) analyses of alcohol and tobacco consumption using Cox proportional hazards models.

Results: For both *BRCA1* and *BRCA2* mutation carriers, none of the smoking-related variables was associated with BC risk, except smoking for more than five years before a first full-term pregnancy (FFTP) when compared to parous women who never smoked. For *BRCA1* mutation carriers, the HR from retrospective analysis (HR_R) was 1.19 (95%CI:1.02,1.39) and the HR from prospective analysis (HR_P) was 1.36 (95%CI:0.99,1.87). For *BRCA2* mutation carriers, smoking for more than five years before a FFTP showed an association of a similar magnitude, but the confidence limits were wider (HR_R=1.25,95%CI:1.01,1.55 and HR_P=1.30,95%CI:0.83,2.01). For both carrier groups, alcohol consumption was not associated with BC risk.

Conclusions: The finding that smoking during the pre-reproductive years increases BC risk for mutation carriers warrants further investigation.

Impact: This is the largest prospective study of BRCA mutation carriers to assess these important risk factors.

Introduction

Carriers of pathogenic variants (mutations) in the *BRCA1* and *BRCA2* genes are at very high risk of developing breast cancer (BC) and ovarian cancer. We recently reported cumulative risks of BC to 80 years of 72% (95% CI, 65%-79%) for *BRCA1* mutation carriers and 69% (95% CI, 61%-77%) for *BRCA2* mutation carriers based on prospective follow-up of unaffected female mutation carriers (1). However, the associations of lifestyle risk factors on BC risk for *BRCA1* and *BRCA2 (BRCA1/2)* mutation carriers remain uncertain. The Oxford collaborative reanalysis of 53 epidemiological studies concluded that for women unselected for family history, alcohol consumption was associated with increased BC risk while there was no association between smoking and BC risk (2). However, some recent studies have found that BC risk may be increased if smoking starts early in life, i.e., before menarche or a first full-term pregnancy (FFTP) (3 - 5) . Of the studies that have attempted to identify lifestyle factors that modify BC risk for *BRCA* mutation carriers, few have examined associations with smoking or alcohol consumption and the results are inconsistent (6-15), possibly due to methodological limitations and small sample sizes. In view of the very high BC risk for *BRCA1/2* mutation carriers, together with the well-known carcinogenic and mutagenic activity of alcohol metabolites (16) and tobacco components (17) and the widespread consumption of alcohol and tobacco, it is important to derive reliable estimates of the associations of alcohol and tobacco consumption with BC risk for *BRCA1* and *BRCA2* mutation carriers. Moreover, given the role of BRCA1 and BRCA2 in DNA repair, it is plausible that smoking and alcohol consumption could have a disproportionate effect for mutation carriers at least in terms of absolute risk. Further, recent experimental data have shown a haplo-insufficiency for BRCA2 and a replication fork instability in *BRCA2* heterozygous cells induced by acetaldehyde, an endogenous product of alcohol catabolism (18).

To provide more reliable estimates of the associations of these lifestyle factors with BC risk for mutation carriers, we analyzed data from the largest available cohort of nearly 10,000 *BRCA1* and *BRCA2* mutation carriers (1) and compared the results from this prospective analysis with the results from the retrospective analysis from same cohort.

Materials and Methods

Study design

We harmonized risk factor and follow-up data from three prospective cohorts: The International BRCA1/2 Carrier Cohort Study (IBCCS) (19), the Kathleen Cuningham Foundation Consortium for Research Into Familial Breast Cancer (kConFab) Follow-Up Study (20,21), and the Breast Cancer Family Registry (BCFR) (22). The combined cohort ("The *BRCA1* and *BRCA2* Cohort Consortium") included data from 21 centres in Western countries (supplemental Table 1S). The total cohort enrolled 9,845 *BRCA1* and *BRCA2* mutation carriers aged 18-80 years (after excluding 14 carriers of a mutation in both genes) (19, 23). Sixty-six percent of the study participants were enrolled in one of the five ongoing nationwide studies in the United Kingdom and Ireland (Epidemiological Study of Familial Breast Cancer [EMBRACE]), France (Gene Etude Prospective Sein Ovaire [GENEPSO]), the Netherlands (Hereditary Breast and Ovarian cancer study Netherlands [HEBON]), Australia and New Zealand (kConFab) or Austria (Medical University of Vienna [MUV]). The other studies were based on regional clinical genetic centers or were population-based (3 centers of the BCFR).

Study participants and Data Collection

Women were eligible for this analysis if they were 18-80 years of age and had tested positive for a pathogenic *BRCA1* or *BRCA2* mutation. The total group with follow-up for a first BC and eligible for retrospective or prospective analyses consisted of 9,845 women (9,232 for retrospective and 3,886 for prospective analysis), including 6,032 *BRCA1* and 3,813 *BRCA2* mutation carriers (Figure 1). Women who were unaffected with breast cancer at baseline were excluded from prospective analyses if either: they had ovarian cancer (415 *BRCA1* carriers, 142 *BRCA2*); other cancer (*146 BRCA1*, 141 *BRCA2*; Risk-reducing mastectomy (RRM) (298 *BRCA1*; 139 *BRCA2*); or did not have follow-up data (360 *BRCA1*; 226 *BRCA2*). Participants provided written informed consent and each study was approved by the relevant ethical committee. Study participants were invited to complete a baseline questionnaire at enrolment and regular follow-up questionnaires. The questionnaires requested detailed information on known or suspected risk factors for breast and ovarian cancer. The primary sources of information on cancer occurrence were: self-report via questionnaire only (6 studies, 8% of the study group), self-report with medical record

validation (2 studies, 37%), medical records (4 studies, 18%), and linkage to cancer registries (4 studies, 37%), although some studies had a mix of these diagnostic sources. Information on vital status was obtained from municipal or death registries or from contact with family members.

Assessment of alcohol consumption and cigarette smoking

We collected information on ever smoking (defined as at least one pack of cigarettes per month for one year), current smoking intensity (average number of cigarettes per day), age started smoking, age stopped smoking, and total duration of smoking (in years) and average number of cigarettes per day during this period. Questionnaires also asked about ever alcohol use (at least one glass per month for one year), alcohol use in the last year (i.e., current use), and total years of consumption. In most studies separate questions were asked about types of alcohol and for each type the amount consumed per week. Some studies asked about alcohol use at age 20 years; women in studies without this information (e.g. BCFR) were treated as missing for variables related to alcohol consumption at age 20 years.

After data harmonization across studies, smoking variables were converted to ever/never smoking, number of cigarettes per day (current or past for ex-smokers) in five categories (0; 1-5, 6-10, 11-20, >20), years of smoking and estimated number of pack-years in 3 categories (<1, 1-20, >20), age started in 3 categories (<= 15 years, 16-19, >= 20 years) and timing relative to their FFTP. Alcohol variables were converted to ever/never, and total average number of standard drinks per day at age 20 years and in the year prior to completing the questionnaire.

Statistical analysis

To assess the association between alcohol and tobacco consumption and the risk of BC, we used Cox proportional hazards regression models. Women were eligible for prospective analyses if they were free of cancer and had no history of risk-reducing mastectomy (RRM) at the start of follow-up (enrolment/baseline questionnaire or mutation test, whichever came last); for participants recruited in a research setting, follow-up was considered to begin at enrolment. The primary endpoint was BC (invasive (n=393) or *in situ* (n=33) for the prospective analyses) diagnosed more than one month after enrolment. The censoring event was the first of diagnosis of primary BC (invasive or *in situ*), diagnosis of another cancer,

RRM, last questionnaire, last information from external source (e.g., linkage), loss to follow-up, age 80 years, or death. Alcohol and tobacco variables were analyzed as fixed in the models because timing of changes in consumption was too uncertain to generate time-dependent variables. Analyses were adjusted for alcohol consumption (ever vs. never) when tobacco consumption was analyzed and for smoking (ever vs. never) when alcohol consumption was analyzed. Because the consumption of alcoholic beverages and tobacco consumption might interact with other BC risk factors (24, 25), we also performed analyses adjusted on additional potential confounders like age at menarche (<12, ≥12 - <13, ≥13 - <14, ≥14 - <15, >=15, age missing or never had menstrual period), age at 1st full-term pregnancy (<30, ≥30+nulliparous), number of full-term pregnancies (0, 1, ≥2), body mass index (<18.5, 18.5–24.9, 25–29.9,30 or greater, missing), oral contraceptive use (ever, never, missing), bilateral oophorectomy (yes, no), and number of affected relatives with breast cancer (0, 1, ≥2, Unknown). We conducted separate analyses for *BRCA1* and *BRCA2* mutation carriers. We stratified for birth cohort and study and used robust variance estimation to account for familial clustering.

In addition to the prospective analysis, we conducted full-cohort retrospective analyses, in which follow-up was assumed to start at birth and women were followed until the first of diagnosis of primary BC (invasive or *in situ*), diagnosis of another cancer, RRM, start of prospective follow-up (baseline questionnaire or mutation test, whichever came last) or age 80 years. Thus, there was no overlap in follow-up period for individual women included in the retrospective and prospective analyses. Due to non-random sampling of prevalent cases of BC, all analyses of retrospective data were performed using the weighted regression approach described by Antoniou et al (26). Since changes in habits might occur after a BC diagnosis, the number of glasses per day consumed during the last year and the number of pack years at the date of baseline questionnaire were not included in the retrospective analyses.

To minimize potential survival bias, we also conducted an additional retrospective analysis of a pseudo-incident cohort, which was defined as follow-up starting five years before enrolment/baseline questionnaire, and thus only included cases diagnosed within the 5 years prior to enrolment.

Analyses were stratified by birth cohort into four groups (<=1950, 1951-1959, 1960-1968, >=1969 for retrospective analyses and <=1957, 1958-1966, 1967-1974, >=1975 for

prospective analyses). We also assessed associations by birth cohort and study. All statistical analyses were performed using STATA (version 14, StataCorp, College Station, TX).

Results

Tables 1 and 2 summarize the descriptive statistics of the two cohorts of *BRCA1* and *BRCA2* mutation carriers, respectively. Comparison of the retrospective and prospective analysis identified differences in distributions of cigarette consumption: we observed more ex-smokers and never smokers (i.e., non-current smokers) in the retrospective analysis than in the prospective analysis for both *BRCA1* (84.9% vs. 75.5% for cases, 81.1% vs. 79.0% for unaffected women) and *BRCA2* (85.0% vs. 84.1% for cases, 82.4 % vs. 81.3% for unaffected women) mutation carriers.

BRCA1 mutation carriers:

For *BRCA1* mutation carriers, there were no associations between BC risk and the alcohol measures examined, except for reduced risk associated with higher alcohol consumption in the retrospective analysis, with an HR_R of 0.59 (95%CI:0.43,0.81; p=0.001) for more than two glasses of alcohol consumed at age 20 years when compared to 0 glasses of alcohol (Table 3). However, no associations were observed with alcohol consumption at age 20 years or at baseline in the prospective analyses.

There were no associations with ever smoking, number of cigarettes smoked, pack-years, or age at start smoking, in either the prospective or retrospective analysis. However, among parous women, increased BC risk was associated with more than 5 years of smoking before their FFTP in both prospective and retrospective analyses when compared to parous women who never smoked (HR_R=1.19, 95%CI 1.02,1.39; and HR_P=1.36, 95%CI:0.99,1.87, respectively). Among nulliparous women, there was no evidence of an association with ever smoking when compared to never smoking (HR_R=0.97, 95%CI: 0.74,1.27); and HR_P=1.20, 95%CI: 0.68,2.12). Figure 2 displays the cumulative risks of BC for women who smoked for more than 5 years before their FFTP compared to parous never smokers for *BRCA1* mutation carriers.

***BRCA2* mutation carriers:**

Both ever use of smoking and alcohol drinking were associated with increased BC risk when compared to women who neither smoked nor drank alcohol for *BRCA2* mutation carriers, but only in the retrospective analysis (ever alcohol consumption in non-smokers HR_R=1.32, 95%CI: 1.03,1.70; p=0.03; ever smoking in non-drinkers HR_R=1.37, 95%CI:1.00,1.89; p=0.05, ever alcohol and ever smoking (i.e., at least one glass per month for one year plus at least one pack of cigarettes per month for one year); HR_R=1.43, 95%CI:1.13,1.81; p=0.003) (Table 4). Similar to the findings for *BRCA1* mutation carriers, in both prospective and retrospective analyses we observed an increased BC risk associated with having smoked more than 5 years before a FFTP (HR_R=1.25, 95%CI:1.01,1.55; and HR_P=1.30, 95%CI:0.83,2.02, respectively), but the estimates were statistically significant only in the retrospective analysis.

Sensitivity Analyses

Results from retrospective analyses that used the pseudo-incident cohort were consistent with those from the full-cohort retrospective analyses except for *BRCA1* mutation carriers where smoking more than 5 years before a FFTP point estimate slightly lowered and significance disappeared (Supplementary Data, Table 2S). We observed no significant heterogeneity in the HRs for smoking more than 5 years before a FFTP (Supplemental Figures 1 and 2), with the exception of heterogeneity by birth cohort for the prospective analysis for *BRCA1* mutation carriers which is due to the most recent birth cohort where the HR_p was high, though bounded by a large confidence interval (see Supplemental Figure 2a). There was no significant heterogeneity by study group with regard to the association we observed for high alcohol consumption in the retrospective analysis for *BRCA1* or *BRCA2* mutation carriers (p=0.19 and 0.14, respectively) (data not shown).

In our primary analyses we did not adjust the analyses for other possible confounders since most of the other risk factors for BC are unlikely to be correlated with the primary alcohol and smoking exposures of interest. The multivariable-adjusted results are presented in

Supplementary Tables 3S and 4S. As expected, multivariable adjustment did not materially change the HR estimates and more importantly, the overall conclusions of the study.

We performed separate analyses for *BRCA1* and *BRCA2* cohorts based on the hypothesis that the role of the two genes may be different in response to carcinogens from alcohol and tobacco. However, we also performed a pooled analysis which, again, did not change drastically our initial findings nor our conclusions (Table 5S).

Discussion

Using data from the largest international cohort of *BRCA1* and *BRCA2* mutation carriers, we examined associations with alcohol consumption and smoking separately using both independent retrospective and prospective data. We found no evidence of an overall association between cigarette consumption with BC risk, except for the *BRCA2* mutation carriers in the retrospective analysis. However, among parous women, we observed that mutation carriers who smoked more than five years before their FFTP had a significantly increased risk of BC. This association was seen in both prospective and retrospective analyses, and was seen for both *BRCA1* and *BRCA2* mutation carriers, though the confidence limits for *BRCA2* mutation carriers were wider. The consistency of these findings for mutations carriers of either gene as well as similar point estimates between prospective and retrospective analyses support the overall conclusion that this time window prior to breast tissue differentiation from pregnancies may be a particularly sensitive window for environmental carcinogenesis.

Unlike in the general population (2, 27) and in accordance with other studies on *BRCA1* and *BRCA2* mutation carriers (6, 28), our findings do not support a positive association between alcohol intake and BC risk, although power was somewhat limited to detect the relatively modest association observed in prior studies (3, 27).

Findings from studies that have examined associations between smoking and BC risk for *BRCA1* and *BRCA2* mutation carriers have been inconsistent. Some reported a null association (12-15), two reported a negative association (9, 10), and two reported a positive association (8, 13), although the latter study showed this association only for *BRCA1* mutation carriers with a past history of smoking (13). While retrospective studies have the

advantage of larger size and full life history of smoking, prospective studies have the advantage that reporting of behaviors is not influenced by disease.

In our study, women with *BRCA1* mutations who drank more than two glasses of alcohol per day were at decreased BC risk, but only in the retrospective analyses. This discrepancy in results between the two designs for heavier consumers might be explained by survival bias. While tobacco consumption has been suggested as a poor prognostic factor, particularly for women with a diagnosis of triple negative and luminal A-like breast tumours (29), the association of alcohol with prognosis is less clear. Regular drinking of 0.5 standard drinks or more per day has been shown to be associated with higher risk of BC recurrence, particularly among postmenopausal women (30). Therefore, if women who are heavy consumers are more likely to die after a diagnosis of BC than non-drinking women with BC, the inclusion of prevalent cases in a retrospective analysis may bias results toward unity or even lead to an artifactual negative association (8).

Major strengths of our study include the large sample size for both retrospective and prospective cohorts with very good follow-up and the largest number of *BRCA1* and *BRCA2* prospective mutation carrier BC cases studied to date. Potential weaknesses include the fact that information on alcohol intake and tobacco consumption was self-reported with accompanying potential exposure misclassification and the potential for the retrospective analyses to be affected by survival bias due to the inclusion of prevalent cases. However, the prospective part of our study minimized recall and survival biases.

As in the general population (5), we found a consistent association of increased BC risk with cigarette smoking for mutation carriers who smoked for more than 5 years before their FFTP. The period preceding a FFTP has been shown to be a critical period for breast carcinogenesis (31, 32), particularly for women with a mutation in BRCA1 or BRCA2 (33), and potentially even more so for women who accumulated DNA defects during the years before a FFTP because of smoking (Figure 2).

With the exception of the association with smoking for more than five years before a FFTP, no associations were found for most smoking-related variables for either *BRCA1* or *BRCA2* mutation carriers. Similarly, no association with alcohol consumption was found in the

prospective analysis. However, associations with these two lifestyle factors might be complex and need more detailed information on consumption (e.g., quantities and calendar years of starting and stopping) and timing to be able to prospectively investigate them as time-dependent exposures and extended follow-up might shed further light upon associations of smoking and alcohol with BC risk for *BRCA1* and *BRCA2* mutation carriers.

In summary, we found no substantial association of BC risk with alcohol consumption or smoking except for women who smoked for more than five years before their FFTP. These findings suggest that smoking during the pre-reproductive years may increase BC risk for mutation carriers, warranting further investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' contributions

DEG and NA drafted the initial manuscript, while the complete writing group consisted of DEG, NA, MBT and FVL. DEG and HL performed and with NA are responsible for the statistical analyses, while the complete analysis group additionally consists of KAP, JLH, HO, ACA, MBT, DFE, CE, KK, RM and MAR. TMM and MRB are the database managers. MAR coordinated the collaborative study, while DEG, DFE, NA, CN, MBT, JLH, MAR, KAP initiated and coordinated the original studies. All authors read and approved the final manuscript. The funders had no role in the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication.

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Table 1. Characteristics of the *BRCA1* mutation carriers

	Women with Breast Cancer		Unaffected Women	
	retrospective (N=2537)	prospective (N=269)	retrospective (N=3170)	prospective (N=2007)
	N(%) or Mean±SD	N(%) or Mean±SD	N(%) or Mean±SD	N(%) or Mean±SD
Age at Entry		40.7 ± 10.3		37.5 ± 11.8
Age at Censure	40.1 ± 8.8	44.9 ± 10.3	39.3 ± 11.6	43.1 ± 12.3
Year of Birth				
<1950	804 (31.7)	35 (13.0)	527 (16.6)	205 (10.2)
1950 - 1959	842 (33.2)	76 (28.3)	647 (20.4)	347 (17.3)
1960 - 1969	662 (26.1)	104 (38.7)	946 (29.8)	586 (29.2)
≥1970	229 (9.0)	54 (20.1)	1050 (33.1)	869 (43.3)
Study Group				
EMBRACE	743 (29.3)	41 (15.2)	817 (25.8)	432 (21.5)
GENEPSO	324 (12.8)	46 (17.1)	692 (21.8)	442 (22.0)
HEBON	337 (13.3)	40 (14.9)	465 (14.7)	202 (10.1)
KConFab		55 (20.4)		270 (13.5)
BCFR	456 (18.0)	50 (18.6)	433 (13.7)	277 (13.8)
Others[§]	677 (26.7)	37 (13.8)	763 (24.1)	384 (19.1)
Smoking/alcohol status				
Never	567 (22.3)	64 (23.8)	698 (22.0)	457 (22.8)
Ever, alcohol only	773 (30.5)	65 (24.2)	976 (30.8)	618 (30.8)
Ever, smoking only	281 (11.1)	29 (10.8)	306 (9.7)	193 (9.6)
Ever, smoking and alcohol	891 (35.1)	104 (38.7)	1146 (36.2)	713 (35.5)
missing	25 (1.0)	7 (2.6)	44 (1.4)	26 (1.3)
Smoking status				
Never	1344 (53.0)	132 (49.1)	1688 (53.2)	1081 (53.9)
Past smoker	809 (31.9)	71 (26.4)	883 (27.9)	504 (25.1)
Current smoker	364 (14.3)	63 (23.4)	565 (17.8)	398 (19.8)
missing	20 (0.8)	3 (1.1)	34 (1.1)	24 (1.2)
Cigarettes per day (current or past)				
0	1344 (53.0)	132 (49.1)	1688 (53.2)	1081 (53.9)
≤ 5	266 (10.5)	32 (11.9)	366 (11.5)	237 (11.8)
6-10	311 (12.3)	34 (12.6)	414 (13.1)	259 (12.9)
11-20	417 (16.4)	49 (18.2)	482 (15.2)	294 (14.6)
> 20	86 (3.4)	14 (5.2)	80 (2.5)	58 (2.9)
missing	113 (4.5)	8 (3.0)	140 (4.4)	78 (3.9)
Number of pack-years				
<1	1387 (54.7)	138 (51.3)	1774 (56.0)	1147 (57.1)
1-20	722 (28.5)	96 (35.7)	976 (30.8)	628 (31.3)
>20	281 (11.1)	26 (9.7)	238 (7.5)	132 (6.6)
missing	147 (5.8)	9 (3.3)	182 (5.7)	100 (5.0)

	Women with Breast Cancer		Unaffected Women	
	retrospective (N=2537)	prospective (N=269)	retrospective (N=3170)	prospective (N=2007)
	N(%) or Mean±SD	N(%) or Mean±SD	N(%) or Mean±SD	N(%) or Mean±SD
Age started smoking (years)				
Never	1344 (53.0)	132 (49.1)	1688 (53.2)	1081 (53.9)
≤ 15	233 (9.2)	33 (12.3)	364 (11.5)	240 (12.0)
16-19	487 (19.2)	69 (25.7)	576 (18.2)	385 (19.2)
≥20	190 (7.5)	15 (5.6)	194 (6.1)	106 (5.3)
missing	283 (11.2)	20 (7.4)	348 (11.0)	195 (9.7)
Glasses of alcohol per day in past year £				
0	1017 (40.1)	108 (40.1)	1222 (38.5)	772 (38.5)
<1	830 (32.7)	72 (26.8)	955 (30.1)	560 (27.9)
1-2	430 (16.9)	48 (17.8)	588 (18.5)	391 (19.5)
>2	93 (3.7)	12 (4.5)	217 (6.8)	143 (7.1)
missing	167 (6.6)	29 (10.8)	188 (5.9)	141 (7.0)
Glasses of alcohol per day at age 20 years				
0	804 (31.6)	65 (24.2)	992 (31.3)	520 (25.9)
<1	670 (26.4)	41 (15.2)	785 (24.8)	421 (21.0)
1-2	292 (11.5)	22 (8.2)	470 (14.8)	269 (13.4)
>2	64 (2.5)	9 (3.4)	183 (5.8)	111 (5.5)
missing	707 (27.9)	132 (49.1)	740 (23.3)	686 (34.2)

§ Others included the following studies (total number): MUV-Austria (261), MODSQUAD (228), GC-HBOC (178), Lund-BRCA (160), OUH (105), HCSC (84), INHERIT (66), NIO-Hungry (98), IHCC (97), Stockholm-BRCA (71), CNIO (40), Milan Italy (33), HSP (9), DKFZ (4), Belgium (3), Dusseldorf Germany (3).
£ Year preceding completion of last questionnaire

Table 2. Characteristics of the *BRCA2* mutation carriers

	Women with Breast Cancer		Unaffected Women	
	retrospective (N=1555)	Prospective (N=157)	retrospective (N=1970)	prospective (N=1453)
	N(%) or Mean±Std	N(%) or Mean±Std	N(%) or Mean±Std	N(%) or Mean±Std
Age at Start		45.1 ± 10.1		40.0 ± 12.6
Age at Censure	43.4 ± 9.1	49.0 ± 10.3	41.4 ± 12.4	45.0 ± 13.0
Year of Birth				
<1950	563 (36.2)	42 (26.8)	386 (19.6)	200 (13.8)
1950 - 1959	510 (32.8)	44 (28.0)	388 (19.7)	259 (17.8)
1960 - 1969	385 (24.8)	55 (35.0)	572 (29.0)	433 (29.8)
≥1970	97 (6.2)	16 (10.2)	624 (31.7)	561 (38.6)
Study Group				
EMBRACE	611 (39.3)	42 (26.8)	744 (37.8)	441 (30.4)
GENEPSO	161 (10.4)	18 (11.5)	437 (22.2)	307 (21.1)
HEBON	90 (5.8)	4 (2.5)	147 (7.5)	71 (4.9)
KConFab		38 (24.2)		250 (17.2)
BCFR	359 (23.1)	33 (21.0)	322 (16.3)	222 (15.3)
Others[§]	334 (21.5)	22 (14.0)	320 (16.2)	162 (11.1)
Smoking/alcohol status				
Never	321 (20.6)	44 (28.0)	440 (22.3)	361 (24.8)
Ever, alcohol only	486 (31.3)	41 (26.1)	632 (32.1)	459 (31.6)
Ever, smoking only	134 (8.6)	13 (8.3)	154 (7.8)	110 (7.6)
Ever, smoking and alcohol	597 (38.4)	56 (35.7)	722 (36.6)	512 (35.2)
missing	17 (1.1)	3 (1.9)	22 (1.1)	11 (0.8)
Smoking status				
Never	808 (52.0)	85 (54.1)	1079 (54.8)	824 (56.7)
Past smoker	514 (33.1)	47 (29.9)	544 (27.6)	357 (24.6)
Current smoker	218 (14.0)	21 (13.4)	334 (17.0)	264 (18.2)
missing	15 (1.0)	4 (2.5)	13 (0.7)	8 (0.6)
Cigarettes per day (current or past)				
0	808 (52.0)	85 (54.1)	1079 (54.8)	824 (56.7)
≤ 5	158 (10.2)	15 (9.6)	212 (10.8)	154 (10.6)
6-10	202 (13.0)	24 (15.3)	232 (11.8)	173 (11.9)
11-20	262 (16.8)	19 (12.1)	316 (16.0)	214 (14.7)
> 20	55 (3.5)	7 (4.5)	59 (3.0)	49 (3.4)
missing	70 (4.5)	7 (4.5)	72 (3.7)	39 (2.7)
Number of Pack-years				
<1	846 (54.4)	90 (57.3)	1126 (57.2)	862 (59.3)
1-20	430 (27.7)	44 (28.0)	578 (29.3)	434 (29.9)
>20	197 (12.7)	16 (10.2)	179 (9.1)	111 (7.6)
missing	82 (5.3)	7 (4.5)	87 (4.4)	46 (3.2)

	Women with Breast Cancer		Unaffected Women	
	retrospective (N=1555)	Prospective (N=157)	retrospective (N=1970)	prospective (N=1453)
	N(%) or Mean±Std	N(%) or Mean±Std	N(%) or Mean±Std	N(%) or Mean±Std
Age at start smoking (years)				
Never	808 (52.0)	85 (54.1)	1079 (54.8)	824 (56.7)
≤ 15	136 (8.7)	19 (12.1)	228 (11.6)	148 (10.2)
16-19	302 (19.4)	23 (14.6)	358 (18.2)	290 (20.0)
≥20	153 (9.8)	20 (12.7)	154 (7.8)	102 (7.0)
missing	156 (10.0)	10 (6.4)	151 (7.7)	89 (6.1)
Glasses of alcohol per day past year£				
0	596 (38.3)	68 (43.3)	754 (38.3)	568 (39.1)
<1	574 (36.9)	41 (26.1)	605 (30.7)	420 (28.9)
1-2	280 (18.0)	30 (19.1)	375 (19.0)	264 (18.2)
>2	57 (3.7)	7 (4.5)	166 (8.4)	118 (8.1)
missing	48 (3.1)	11 (7.0)	70 (3.6)	83 (5.7)
Glasses of alcohol per day at age 20 years				
0	424 (27.3)	31 (19.7)	592 (30.1)	345 (23.7)
<1	439 (28.2)	32 (20.4)	489 (24.8)	283 (19.5)
1-2	201(12.9)	15 (9.6)	337 (17.1)	219 (15.1)
>2	56 (3.6)	3 (1.9)	132 (6.7)	89 (6.1)
missing	435 (28.0)	76 (48.4)	420 (21.3)	517 (35.6)

§ Others included the following studies (total number): MUV-Austria (100), MODSQUAD (80), GC-HBOC (105), Lund-BRCA (58), OUH (62), HCSC (65), INHERIT (74), NIO-Hungry (31), IHCC (0), Stockholm-BRCA (13), CNIO (44), Milan Italy (12), HSP (10).
£ Year preceding completion of last questionnaire

Table 3. Association of alcohol and tobacco consumption with breast cancer risk for *BRCA1* mutation carriers, retrospective (weighted) and prospective analyses

	retrospective	P value	prospective	P value
	HR[a] (95% CI)		HR[b] (95% CI)	
Smoking/alcohol status				
Never	1.00		1.00	
Ever, alcohol only	1.04 (0.89,1.22)	0.63	0.89 (0.61,1.28)	0.52
Ever, smoke only	1.13 (0.92,1.38)	0.25	0.89 (0.57,1.38)	0.59
Ever, smoke and alcohol	1.04 (0.89,1.22)	0.59	1.16 (0.81,1.65)	0.41
Cigarettes per day* (current or past)				
0	1.00		1.00	
≤ 5	1.01 (0.85,1.21)	0.91	1.25 (0.85,1.85)	0.26
6-10	1.01 (0.85,1.20)	0.91	1.06 (0.73,1.54)	0.75
11-20	1.11 (0.95,1.29)	0.20	1.19 (0.86,1.66)	0.30
> 20	1.16 (0.87,1.55)	0.32	1.26 (0.70,2.27)	0.44
Continuous (missing excluded)*	1.00 (1.00, 1.01)	0.15	1.01 (1.00, 1.02)	0.22
Number of Pack-Years*				
<1			1.00	
1-20			1.21 (0.92,1.58)	0.17
>20			1.20 (0.78,1.85)	0.40
Continuous (missing excluded)*			1.01 (1.00, 1.02)	0.12
Age at start smoking (years)*				
Never	1.00		1.00	
≤ 15	1.10 (0.90,1.34)	0.34	1.11 (0.75,1.63)	0.60
16-19	1.09 (0.94,1.27)	0.25	1.27 (0.93,1.72)	0.13
≥20	1.02 (0.83,1.25)	0.88	1.10 (0.65,1.86)	0.73
Age at start for parous women (years)*				
Never	1.00		1.00	
≤ 15	1.10 (0.89,1.36)	0.37	1.18 (0.79,1.76)	0.42
16-19	1.12 (0.96,1.32)	0.16	1.24 (0.90,1.73)	0.19
≥20	1.03 (0.83,1.29)	0.77	1.04 (0.57,1.89)	0.90
Age at start for nulliparous women (years)*				
Never	1.00		1.00	
≤ 15	1.28 (0.75,2.19)	0.37	1.22 (0.40,3.68)	0.73
16-19	0.94 (0.64,1.39)	0.76	1.15 (0.48,2.71)	0.76
≥20	0.86 (0.50,1.49)	0.59	1.80 (0.61,5.31)	0.29
Smoking and Parity*				
Never smoke & parous	1.00		1.00	
Never smoke & nulliparous	1.31 (1.08,1.60)	0.01	0.70 (0.46,1.07)	0.10
Ever smoke & nulliparous	1.31 (1.05,1.62)	0.02	0.80 (0.49,1.31)	0.38
Ever smoke & parous				
5 yrs or less before F FTP	1.01 (0.85,1.20)	0.91	1.11 (0.75,1.66)	0.60
> 5 yrs before FFTP	1.19 (1.02,1.39)	0.03	1.36 (0.99,1.87)	0.06

	retrospective	P value	prospective	P value
	HR[a] (95% CI)		HR[b] (95% CI)	
Glasses of alcohol last year per day§				
0			1.00	
<1			0.99 (0.73,1.35)	0.95
1-2			1.06 (0.76,1.49)	0.74
>2			0.93 (0.50,1.72)	0.82
Continuous (per glass)§			1.02 (0.86,1.21)	0.84
Glasses of alcohol per day§ at age 20				
0	1.00		1.00	
<1	1.00 (0.87,1.16)	0.95	0.93 (0.62,1.39)	0.71
1-2	0.89 (0.74,1.07)	0.21	0.92 (0.57,1.51)	0.75
>2	0.59 (0.43,0.81)	0.001	1.35 (0.67,2.72)	0.40
Continuous (per glass)§	0.88 (0.80,0.96)	0.004	1.04 (0.84,1.28)	0.71

* Adjusted for alcohol consumption (ever vs. never)
§ Adjusted for tobacco consumption (ever vs. never).
[a] Stratified on birth cohort and 5 study groups for the retrospective analyses
[b] Stratified on birth cohort and 6 study groups for the prospective analyses

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Table 4. Association of alcohol and tobacco consumption with BC risk for *BRCA2* mutation carriers, retrospective (weighted) and prospective analyses

	retrospective	P value	prospective	P value
	HR[a] (95% CI)		HR[b] (95% CI)	
Smoking/alcohol status				
Never	1.00		1.00	
Ever, alcohol only	1.32 (1.03,1.70)	0.03	0.77 (0.49,1.20)	0.25
Ever, smoking only	1.37 (1.00,1.89)	0.05	0.77 (0.39,1.50)	0.44
Ever, smoking and alcohol	1.43 (1.13,1.81)	0.003	0.98 (0.64,1.52)	0.94
Cigarettes per day*				
0	1.00		1.00	
≤ 5	1.08 (0.85,1.37)	0.52	1.06 (0.60,1.85)	0.85
6-10	1.22 (0.97,1.54)	0.08	1.75 (1.11,2.75)	0.02
11-20	1.21 (0.98,1.51)	0.08	0.85 (0.51,1.43)	0.55
> 20	0.97 (0.65,1.43)	0.87	0.74 (0.33,1.69)	0.48
Number of Pack-Years*				
<1			1.00	
1-20			1.20 (0.82,1.76)	0.34
>20			0.92 (0.53,1.60)	0.76
Continuous (missing excluded)*			1.00 (0.98,1.01)	0.75
Age at start smoking (years)*				
Never	1.00		1.00	
≤ 15	1.15 (0.87,1.50)	0.32	1.23 (0.74,2.06)	0.43
16-19	1.29 (1.06,1.58)	0.01	0.79 (0.50,1.26)	0.33
≥20	0.97 (0.76,1.25)	0.84	1.73 (1.06,2.85)	0.03
Age at start for parous women years)*				
Never	1.00			
≤ 15	1.22 (0.92,1.63)	0.17	1.31 (0.75,2.27)	0.34
16-19	1.27 (1.02,1.58)	0.03	0.78 (0.48,1.27)	0.32
≥20	0.93 (0.71,1.22)	0.61	1.71 (1.01,2.90)	0.05
Age at start for nulliparous women (years)*				
Never	1.00		1.00	
≤ 15	0.83 (0.26,1.91)	0.66	1.20 (0.28,5.05)	0.81
16-19	1.67 (1.00,2.77)	0.05	0.88 (0.16,4.90)	0.88
≥20	1.76 (0.91,3.39)	0.09	1.19 (0.24,5.76)	0.83
Smoking and Parity*				
Never smoke & parous	1.00		1.00	
Never smoke & nulliparous	1.13 (0.87,1.48)	0.37	0.82 (0.45,1.49)	0.51
Ever smoke & nulliparous	1.37 (1.02,1.83)	0.04	0.76 (0.35,1.69)	0.51
Ever smoke & parous				
5 yrs or less before FFTP	1.04 (0.84,1.29)	0.72	0.97 (0.59,1.59)	0.89
> 5 yrs before FFTP	1.25 (1.01,1.55)	0.04	1.30 (0.83,2.01)	0.25

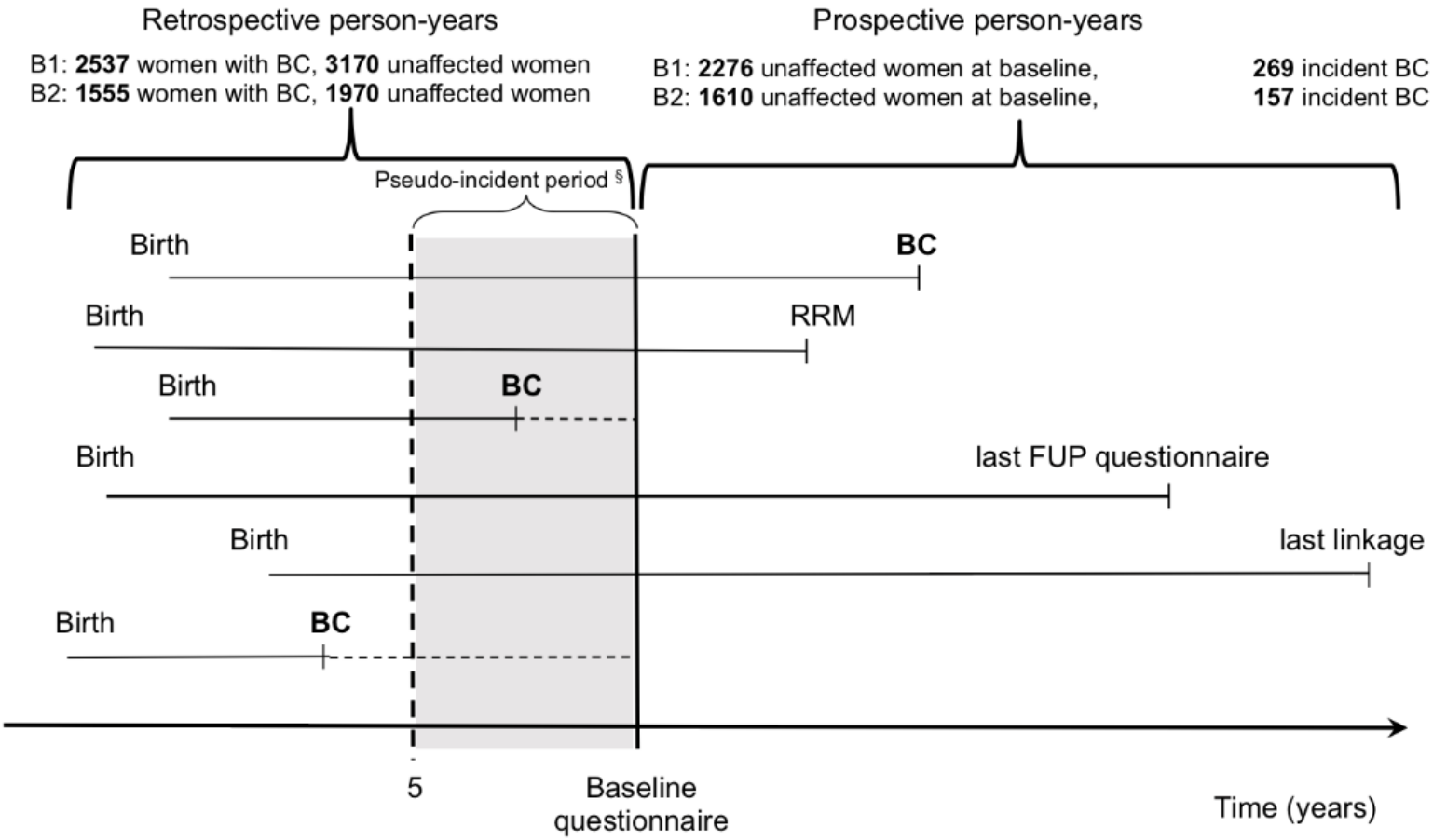
	retrospective	P value	prospective	P value
	HR[a] (95% CI)		HR[b] (95% CI)	
Glasses of alcohol last year per day§				
0			1.00	
<1			0.86 (0.57,1.29)	0.46
1-2			1.03 (0.66,1.60)	0.91
>2			0.99 (0.46,2.16)	0.98
Continuous (per glass)§			0.93 (0.75,1.17)	0.55
Glasses of alcohol at age 20 years per day§				
0	1.00		1.00	
<1	1.19 (0.98,1.44)	0.08	1.17 (0.70,1.95)	0.55
1-2	0.97 (0.76,1.23)	0.80	1.09 (0.57,2.08)	0.79
≥2	0.95 (0.65,1.39)	0.79	0.62 (0.18,2.13)	0.45
Continuous (per glass)§	1.02 (0.91,1.14)	0.76	0.95 (0.72,1.26)	0.73

* Adjusted for alcohol consumption (ever vs. never)
§ Adjusted for tobacco consumption (ever vs. never)
[a] Stratified on birth cohort and 5 study groups for the retrospective analyses
[b] Stratified on birth cohort and 5 study groups for the prospective analyses

Figure Legends

Figure 1 : Design of the *BRCA1* and *BRCA2 c*ohort consortium.
Each line represents a sample IBCCS-BCFR-KConFab participant from birth to censure: a diagnosis of primary breast cancer (BC); a Risk Reduction Mastectomy (RRM); a last FUP questionnaire; and the most recent information from an external source (last linkage).
B1*: BRCA1* B2: *BRCA2*
BC: Breast Cancer

Figure 2: Cumulative risk of breast cancer for never smoking parous women and those who smoked for more than five years before the first full-term pregnancy among *BRCA1* mutation carriers (prospective analysis)



§ pseudo-incident period:
B1: 1107 women with BC, 2830 unaffected women
B2: 756 women with BC, 1793 unaffected women

Fig.1

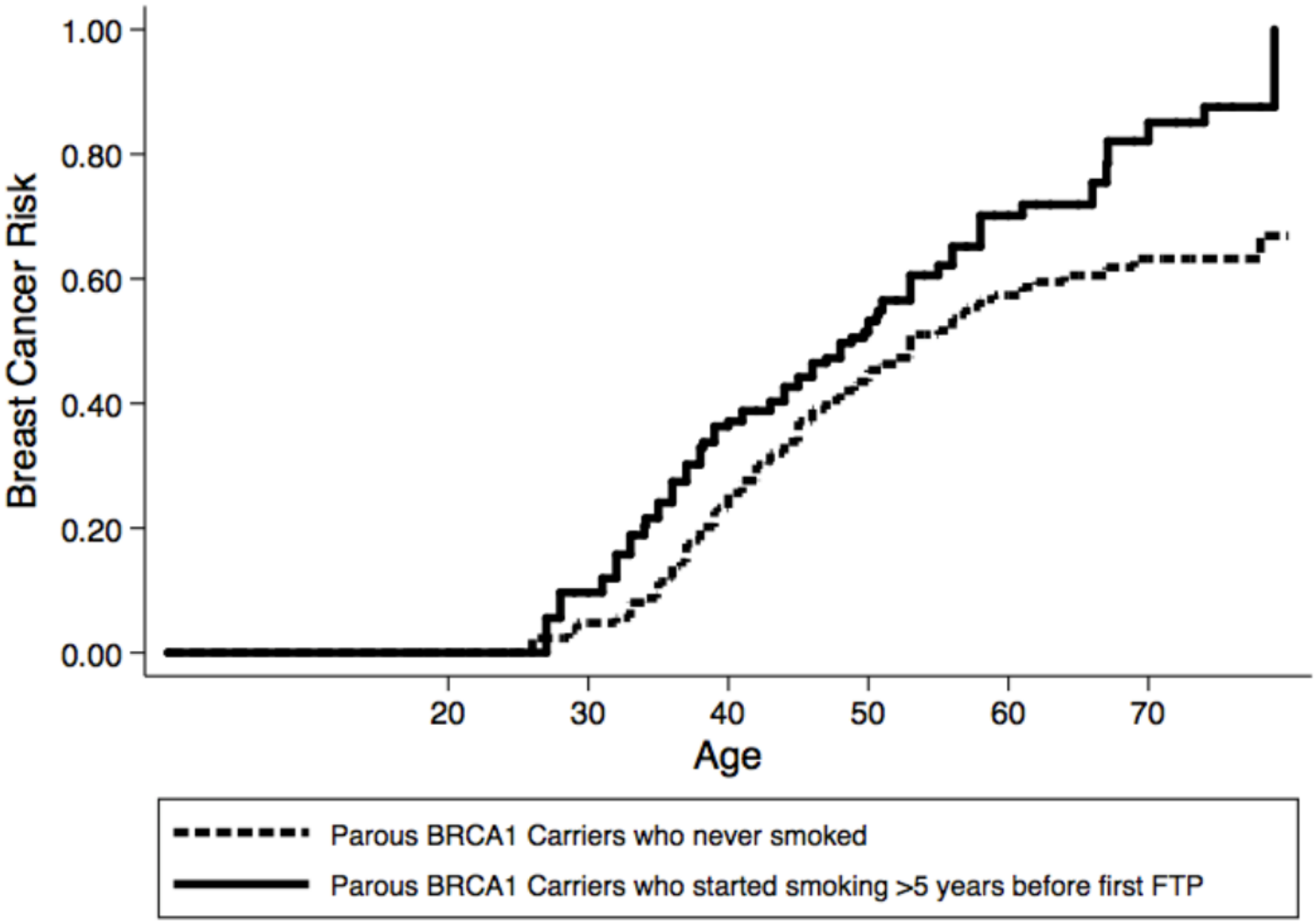


Fig. 2

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

Alcohol consumption, cigarette smoking, and risk of breast cancer for BRCA1 and BRCA2 mutation carriers: results from The BRCA1 and BRCA2 Cohort Consortium

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