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

Hongyan Li[1], Mary Beth Terry[2,3], Antonis C. Antoniou[4], Kelly-Anne Phillips[5,6,7], Karin Kast[8,9,10], Thea M Mooij[11], Christoph Engel[12]; Catherine Noguès[13], Dominique Stoppa-Lyonnet[14,15], Christine Lasset[16], Pascaline Berthet[17], Veronique Mari[18], Olivier Caron[19], for the GENEPSO study; Daniel Barrowdale[4], Debra Frost[4], Carole Brewer[20], D. Gareth R. Evans[21], Louise Izatt[22], Lucy Side[23], Lisa Walker[24], Marc Tischkowitz[25], Mark T. Rogers[26], Mary E. Porteous[27], Katie Snape[28] for the EMBRACE study; Hanne E.J. Meijers-Heijboer[29], Jan JP Gille[29], Marinus J. Blok[30], Nicoline Hoogerbrugge[31], for the HEBON Investigators; Mary B. Daly[32], Irene L. Andrulis[33,34], Saundra S. Buys[35], Esther M. John[36]; Sue Anne McLachlan[37,38], Michael Friedlander[39,40] for the kConFab Investigators; Yen Y Tan[41], Ana Osorio[42], Trinidad Caldes[43], Anna Jakubowska[44,45], Jacques Simard[46], Christian F. Singer[41], Edith Olah[47], Marie Navratilova[48], Lenka Foretova[48], Anne-Marie Gerdes[49], Marie-José Roos-Blom[11], Brita Arver[50,51], Håkan Olsson[51], Rita K Schmutzler[52,53], John L. Hopper[54], Roger L. Milne[54,55,56], Douglas F Easton[4,57], Flora E van Leeuwen[11], Matti A. Rookus[11], Nadine Andrieu[58,59,60,61]*, David E. Goldgar[1,62]* .

*Joint senior authors

Correspondence to:
David E Goldgar, PhD, Cancer Control and Population Sciences, Huntsman Cancer Institute, 2000 Circle of Hope Drive, Salt Lake City, UT 84112, USA (e-mail: david.goldgar@hsc.utah.edu; telephone: (8015850337).

or

Nadine Andrieu, PhD, Cancer Genetic Epidemiology Team, INSERM Unit 900, Institut Curie, 26 rue d'Ulm, 75005 Paris, France (e-mail: nadine.andrieu@curie.fr; telephone: 0033 (0) 172 38 93 83).

[1] Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, UT USA

[2] Department of Epidemiology, Columbia University, New York, NY, USA

[3] Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY, USA

[4] Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care; Strangeways Research Laboratory, Worts Causeway, University of Cambridge, Cambridge, CBI 8RN, UK

[5] Centre for Epidemiology and Biostatistics, School of Population and Global Health, The University of Melbourne, VIC 3010, Australia

[6] The Sir Peter MacCallum Department of Oncology University of Melbourne, Parkville, Australia

[7] Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Victoria 3000, Australia

[8] Department of Gynecology and Obstetrics, Medical Faculty and University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany

[9] National Center for Tumor Diseases (NCT), Partner Site Dresden, Germany

[10] German Cancer Consortium (DKTK), Dresden and German Cancer Research Center (DKFZ), Heidelberg, Germany

[11] Department of Epidemiology, Netherlands Cancer Institute, P.O. Box 90203, 1006 BE Amsterdam, The Netherlands

[12] Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Germany

[13] Institut Paoli-Calmettes, Département d'Anticipation et de Suivi des Cancers, Oncogénétique Clinique and Aix Marseille Univ, INSERM, IRD, SESSTIM, Marseille, France;

[14] Institut Curie, Service de Génétique Médicale, Paris, France

[15] Inserm, U830, Université Paris Descartes, Paris, France

[16] Unité de prévention et Epidémiologie Génétique, Centre Léon Bérard - Lyon / UMR CNRS 5558, Université de Lyon – Lyon

[17] Département de biopathologie, Oncogénétique clinique, Centre François Baclesse – Caen

[18] CLCC Antoine Lacassagne, Département d'Hématologie - Oncologie médicale, Nice, France

[19] Département de Médecine, Gustave Roussy Hôpital Universitaire – Villejuif, France

[20] Department of Clinical Genetics, Royal Devon & Exeter Hospital, Exeter, UK

[21] Genomic Medicine, NIHR Manchester Biomedical Research Centre, Manchester Academic Health Sciences Centre, Division of Evolution and Genomic Sciences, Manchester University, Manchester University Hospitals NHS Foundation Trust, Manchester, UK

[22] Clinical Genetics, Guy's and St. Thomas' NHS Foundation Trust, London, UK

[23] Wessex Clinical Genetics Service, The Princess Anne Hospital, Southampton, UK

[24] Oxford Regional Genetics Service, Churchill Hospital, Oxford, UK

[25] University of Cambridge Department of Medical Genetics, NIHR Cambridge Biomedical Research Centre, and Cancer Research UK Cambridge Center, Cambridge Biomedical Campus, Cambridge CB2 0QQ, UK

[26] All Wales Medical Genetics Services, University Hospital of Wales, Cardiff, UK

[27] South East of Scotland Regional Genetics Service, Western General Hospital, Edinburgh, UK

[28] Medical Genetics Unit, St George's, University of London, UK

[29] Department of Clinical Genetics, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands

[30] Department of Clinical Genetics, Maastricht University Medical Center, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands

[31] Department of Human Genetics, Radboud University Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

[32] Department of Clinical Genetics, Fox Chase Cancer Center, Philadelphia, PA, USA

[33] Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

[34] Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Ontario, Canada

[35] Department of Medicine, University of Utah Health Sciences Center, Huntsman Cancer Institute, Salt Lake City, UT, USA

[36] Stanford University School of Medicine, Department of Medicine, Division of Oncology, and Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA

[37] Department of Medicine, St Vincent's Hospital, The University of Melbourne, Parkville, Victoria, Australia

[38] Department of Medical Oncology, St Vincent's Hospital, Fitzroy, Victoria, Australia

[39] Department of Medical Oncology, Prince of Wales Hospital, Randwick, New South Wales, Australia

[40] Division of Cancer Medicine, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

[41] Dept of OB/GYN and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria, Waehringer Guertel 18-20, A 1090 Vienna, Austria

[42] Human Genetics Group, Spanish National Cancer Centre (CNIO) and Biomedical Network on Rare Diseases (CIBERER), Madrid, Spain

[43] Molecular Oncology Laboratory, Hospital Clinico San Carlos, IdISSC, CIBERONC (ISCIII) , Madrid, Spain

[44] Department of Genetics and Pathology, Pomeranian Medical University, Unii Lubelskiej 1, Szczecin, Poland

[45] Independent Laboratory of Molecular Biology and Genetic Diagnostics
Pomeranian Medical University, Szczecin, Poland

[46] Genomics Center, Centre Hospitalier Universitaire de Québec – Université Laval Research Center, 2705 Laurier Boulevard, Quebec City (Quebec), Canada

[47] Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary

[48] Department of Cancer Epidemiology and Genetics, Masaryk Memorial Cancer Institute, Zluty kopec 7, Brno, 65653, Czech Republic

[49] The Department of Oncology and Pathology, Karolinska Institute, 171 76 Stockholm, Sweden

[50] Department of Clinical Genetics, Rigshospitalet, Copenhagen University Hospital, Denmark

[51] Department of Oncology, Lund University Hospital, Lund, Sweden

[52] Center for Familial Breast and Ovarian Cancer, Center for Integrated Oncology (CIO), Medical Faculty, University Hospital Cologne, Cologne, Germany

[53] Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

[54] Centre for Epidemiology and Biostatistics, School of Population and Global Health, The University of Melbourne, VIC 3010, Australia

[55] Cancer Epidemiology and Intelligence Division, Cancer Council Victoria, Melbourne 3004, Australia

[56] Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, Victoria 3168, Australia

[57] Centre for Cancer Genetic Epidemiology, Department of Oncology, Strangeways Research Laboratory, Worts Causeway, University of Cambridge, Cambridge, CBI 8RN, UK

[58] INSERM, U900, Paris, France

[59] Institut Curie, Paris, France

[60] Mines Paris Tech, Fontainebleau, France

[61] PSL Research University, Paris, France

[62] Department of Dermatology, University of Utah School of Medicine, Salt Lake City, UT, USA

Keywords: BRCA1, BRCA2, breast cancer, alcohol, cigarette smoking

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.

Acknowledgments

The Breast Cancer Family Registry (BCFR), and authors Mary Beth Terry, Irene Andrulis, David Goldgar, Saundra Buys, Esther John, and Mary Daly, were supported by grant UM1CA164920 from the National Cancer Institute (USA). The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the BCFR, nor does mention of trade names, commercial products, or organizations imply endorsement by the USA Government or the BCFR.

This work was partially supported by the Spanish Ministry of Economy and Competitiveness (MINECO) SAF2014-57680-R and the Spanish Research Network on Rare diseases (CIBERER).

This work was supported by the Canadian Institutes of Health Research for the "CIHR Team in Familial Risks of Breast Cancer" program – grant # CRN-87521 and the Ministry of Economic Development, Innovation and Export Trade – grant # PSR-SIIRI-701.

The DKFZ study was supported by the German Cancer Research Center (DKFZ).

EMBRACE and Douglas Easton are supported by Cancer Research UK Grants C1287/A10118 and C1287/A11990. D. Gareth Evans is supported by an NIHR grant to the Biomedical Research Centre, Manchester (IS-BRC-1215-20007). The Investigators at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust are supported by an NIHR grant to the Biomedical Research Centre at The Institute of Cancer Research and

The Royal Marsden NHS Foundation Trust. Antonis C. Antoniou is funded by Cancer Research – UK grants C12292/A20861, C12292/A11174.

The German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC) is supported by the German Cancer Aid (grant no 110837, Rita K. Schmutzler). This work was supported by LIFE – Leipzig Research Center for Civilization Diseases, Universität Leipzig. LIFE is funded by means of the European Union, by the European Regional Development Fund (ERDF) and by means of the Free State of Saxony within the framework of the excellence initiative

The national French cohort, GENEPSO, had been supported by a grant from the Fondation de France and the Ligue Nationale Contre le Cancer and is being supported by a grant from INCa as part of the European program ERA-NET on Translational Cancer Research (TRANSCAN-JTC2012, n°2014-008).

HCSC was supported by a grant RD12/0036/0006 and 15/00059 from ISCIII (Spain), partially supported by European Regional Development FEDER funds.

The HEBON study is supported by the Dutch Cancer Society grants NKI1998-1854, NKI2004-3088, NKI2007-3756, the Netherlands Organisation of Scientific Research grant NWO 91109024, the Pink Ribbon grants 110005 and 2014-187.WO76, the BBMRI grant NWO 184.021.007/CP46 and the Transcan grant JTC 2012 Cancer 12-054.

The International Hereditary Cancer Centre (IHCC) was supported by Grant PBZ_KBN_122/P05/2004 and The National Centre for Research and Development (NCBR) within the framework of the international ERA-NET TRANSAN JTC 2012 application no. Cancer 12-054 (Contract No. ERA-NET-TRANSCAN / 07/2014).

This work was supported by grants to kConFab and the kConFab Follow-Up Study from Cancer Australia (809195, 1100868), the Australian National Breast Cancer Foundation (IF 17), the National Health and Medical Research Council (454508, 288704, 145684), the National Institute of Health U.S.A. (1RO1CA159868), the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia, and the

Cancer Foundation of Western Australia. Kelly Phillips is an Australian National Breast Cancer Foundation fellow.

Lenka Foretova was supported by MH CZ - DRO (MMCI, 00209805) and by MEYS - NPS I - LO1413 to LF, MN.

Edith Olah and The Hungarian Breast and Ovarian Cancer Study was supported by Hungarian Research Grants KTIA-OTKA CK-80745, NKFI OTKA K-112228 and the Norwegian EEA Financial Mechanism HU0115/NA/2008-3/ÖP-9.

Hakan Olsson and Lund-BRCA collaborators are supported by the Swedish Cancer Society, Lund Hospital Funds, and European Research Council Advanced Grant ERC-2011-294576. Stockholm-BRCA collaborators are supported by the Swedish Cancer Society.

BCFR wish to thank members and participants in the Breast Cancer Family Registry from the New York, Northern California, Ontario, Philadelphia and Utah sites for their contributions to the study. BCFR-Australia wish to acknowledge Maggie Angelakos, Judi Maskiell, Gillian Dite, Helen Tsimiklis.

CNIO thanks Alicia Barroso, Rosario Alonso and Guillermo Pita for their assistance.

We acknowledge the GENEPSO Centers: the Coordinating Center: Institut Paoli-Calmettes, Marseille, France: Catherine Noguès, Lilian Laborde, Pauline Pontois, Emanuelle Breysse, Margot Berline and the Collaborating Centers: Institut Curie, Paris: Dominique Stoppa-Lyonnet, Marion Gauthier-Villars, Bruno Buecher, Chrystelle Colas ; Institut Gustave Roussy, Villejuif: Olivier Caron; Hôpital René Huguenin/Institut Curie, Saint Cloud: Catherine Noguès, Emmanuelle Mouret-Fourme, Claire Saule, Chrystelle Colas; Centre Paul Strauss, Strasbourg: Jean-Pierre Fricker; Centre Léon Bérard, Lyon: Christine Lasset, Valérie Bonadona, Sophie Dussard; Centre François Baclesse, Caen: Pascaline Berthet; Hôpital d'Enfants CHU Dijon – Centre Georges François Leclerc, Dijon: Laurence Faivre; Centre Alexis Vautrin, Vandoeuvre-les-Nancy: Elisabeth Luporsi; Centre Antoine Lacassagne, Nice: Véronique Mari; Institut Claudius Regaud, Toulouse: Laurence Gladieff; Réseau Oncogénétique Poitou Charente, Niort: Paul Gesta, Stéphanie Chieze-Valéro; Institut Paoli-Calmettes, Marseille: Catherine Noguès, Jessica Moretta Hagay Sobol, François

Eisinger,Cornel Popovici; Institut Bergonié, Bordeaux: Michel Longy; Centre Eugène Marquis, Rennes: Louise Grivelli; GH Pitié Salpétrière, Paris: Chrystelle Colas, Florent Soubrier, Patrick Benusiglio; CHU Arnaud de Villeneuve, Montpellier: Isabelle Coupier, Pascal Pujol, Carole Corsini; Centres Paul Papin/ICO, Angers :Marie-Emmanuelle Morin-Meschin ; CliniqueCatherine de Sienne, Nantes: Alain Lortholary; Centre Oscar Lambret, Lille: Claude Adenis, Audrey Maillez; Institut Jean Godinot, Reims: Tan Dat Nguyen; Centre René Gauducheau, Nante s: Capucine Delnatte, Caroline Abadie; Centre Henri Becquerel, Rouen: Julie Tinat, Isabelle Tennevet; Hôpital Civil, Strasbourg:, Christine Maugard; Centre Jean Perrin, Clermont-Ferrand: Yves-Jean Bignon, Mathilde Gay Bellile; Polyclinique Courlancy, Reims: Clotilde Penet; Clinique Sainte Catherine, Avignon: Hélène Dreyfus; Hôpital Saint-Louis, Paris: Odile Cohen-Haguenauer; CHRU Dupuytren, Limoges: Brigitte Gilbert, Laurence Venat-Bouvet; CHU de Grenoble: Dominique Leroux, Clémentine Legrand, Hélène Dreyfus; Hôpital de la Timone, Marseille: Hélène Zattara-Cannoni ; Hôpital Jacques Monod, le Havre : Valérie Layet, Elodie Lacaze ; CHU Chambéry, Chambéry : Sandra Fert-Ferrer ; Hôpital Clarac, Fort de France : Odile Bera ; CHU la Milétrie, Poitiers : Brigitte Gilbert-Dussardier, David Tougeron, Stéphanie Chieze-Valéro ; Hôpital Saint Louis, la Rochelle : Hakima Lallaoui ; CH Pellegrin, Bordeaux : Julie Tinat ;

HCSC acknowledge Alicia Tosar and Paula Diaque for their technical assistance.
The Hereditary Breast and Ovarian Cancer Research Group Netherlands (HEBON) consists of the following Collaborating Centers: Netherlands Cancer Institute (coordinating center), Amsterdam, NL: M.A. Rookus, F.B.L. Hogervorst, F.E. van Leeuwen, M.A. Adank, M.K. Schmidt, N.S. Russell, J.L. de Lange, R. Wijnands, D.J. Jenner; Erasmus Medical Center, Rotterdam, NL: J.M. Collée, A.M.W. van den Ouweland, M.J. Hooning, C. Seynaeve, C.H.M. van Deurzen, I.M. Obdeijn; Leiden University Medical Center, NL: C.J. van Asperen, J.T. Wijnen, R.A.E.M. Tollenaar, P. Devilee, T.C.T.E.F. van Cronenburg; Radboud University Nijmegen Medical Center, NL: C.M. Kets, A.R. Mensenkamp; University Medical Center Utrecht, NL: M.G.E.M. Ausems, R.B. van der Luijt, C.C. van der Pol; Amsterdam Medical Center, NL: C.M. Aalfs, H.E.J. Meijers-Heijboer, T.A.M. van Os; VU University Medical Center, Amsterdam, NL: K. van Engelen, J.J.P. Gille, Q. Waisfisz; Maastricht University Medical Center, NL: E.B. Gómez-Garcia, M.J. Blok; University of Groningen, NL: J.C. Oosterwijk, A.H. van der Hout, M.J. Mourits, G.H. de Bock; The Netherlands Comprehensive Cancer Organisation (IKNL): S. Siesling, J.Verloop; The nationwide network and registry of histo- and cytopathology in The Netherlands (PALGA):

L.I.H. Overbeek. HEBON thanks the study participants and the registration teams of IKNL and PALGA for part of the data collection.

INHERIT would like to thank Dr Martine Dumont for sample management and skillful assistance.

We thank Stephanie Nesci, Sandra Picken, Lucy Stanhope, Sarah O'Connor, Heather Thorne, Eveline Niedermayr, all the kConFab research nurses and staff, the heads and staff of the Australian and New Zealand Family Cancer Clinics and the many families who contribute to kConFab for their contributions to this resource.

Czech Republic, MMCI, Brno - many thanks to Dita Hanouskova, research nurse, Jitka Berkovcova, research technician, for data collection and management.

We wish to thank the Hungarian Breast and Ovarian Cancer Study Group members (Maria Balogh, Janos Papp, Matrai Zoltan, Judit Franko Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary) and the clinicians and patients for their contributions to this study.

Swedish scientists participating as SWE-BRCA collaborators are: from Lund University and University Hospital: Håkan Olsson, Carolina Ellberg ; from Stockholm and Karolinska University Hospital: Brita Arver.

References

(1) Kuchenbaecker KB, Hopper JL, Barnes DR, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA 2017;317(23):2402-16.

(2) Hamajima N, Hirose K, Tajima K, et al. Alcohol, tobacco and breast cancer--collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. Br J Cancer 2002;87(11):1234-45.

(3) Gaudet MM, Gapstur SM, Sun J, et al. Active smoking and breast cancer risk: original cohort data and meta-analysis. J Natl Cancer Inst 2013;105(8):515-25.

(4) Liu Y, Colditz GA, Rosner B, et al. Alcohol intake between menarche and first pregnancy: a prospective study of breast cancer risk. J Natl Cancer Inst 2013;105(20):1571-8.

(5) Gaudet MM, Carter BD, Brinton LA, et al. Pooled analysis of active cigarette smoking and invasive breast cancer risk in 14 cohort studies. Int J Epidemiol. 2017;46(3):881-893.

(6) McGuire V, John EM, Felberg A, et al. No increased risk of breast cancer associated with alcohol consumption among carriers of BRCA1 and BRCA2 mutations ages <50 years. Cancer Epidemiol Biomarkers Prev 2006;15(8):1565-7.

(7) Moorman PG, Iversen ES, Marcom PK, et al. Evaluation of established breast cancer risk factors as modifiers of BRCA1 or BRCA2: a multi-center case-only analysis. Breast Cancer Res Treat 2010;124(2):441-51.

(8) Breast Cancer Family Registry; Kathleen Cuningham Consortium for Research into Familial Breast Cancer (Australasia); Ontario Cancer Genetics Network (Canada). Smoking and risk of breast cancer in carriers of mutations in BRCA1 or BRCA2 aged less than 50 years. Breast Cancer Res Treat 2008;109(1):67-75.

(9) Brunet JS, Ghadirian P, Rebbeck TR, et al. Effect of smoking on breast cancer in carriers of mutant BRCA1 or BRCA2 genes. J Natl Cancer Inst 1998;90(10):761-6.

(10) Colilla S, Kantoff PW, Neuhausen SL, et al. The joint effect of smoking and AIB1 on breast cancer risk in BRCA1 mutation carriers. Carcinogenesis 2006;27(3):599-605.

(11) Friebel TM, Domchek SM, Rebbeck TR. Modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: systematic review and meta-analysis. J Natl Cancer Inst 2014;106(6):dju091.

(12) Ghadirian P, Lubinski J, Lynch H, et al. Smoking and the risk of breast cancer among carriers of BRCA mutations. Int J Cancer 2004;110(3):413-6.

(13) Ginsburg O, Ghadirian P, Lubinski J, et al. Smoking and the risk of breast cancer in BRCA1 and BRCA2 carriers: an update. Breast Cancer Res Treat 2009;114(1):127-35.

(14) Gronwald J, Byrski T, Huzarski T, et al. Influence of selected lifestyle factors on breast and ovarian cancer risk in BRCA1 mutation carriers from Poland. Breast Cancer Res Treat 2006;95(2):105-9.

(15) Nkondjock A, Robidoux A, Paredes Y, Narod SA, Ghadirian P. Diet, lifestyle and BRCA-related breast cancer risk among French-Canadians. Breast Cancer Res Treat 2006;98(3):285-94.

(16) Allen NE, Beral V, Casabonne D, et al. Moderate alcohol intake and cancer incidence in women. J Natl Cancer Inst 2009;101(5):296-305.

(17) IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Cancer IAfRo. Allyl compounds, aldehydes, epoxides, and peroxides. France: World Health Organization, International Agency for Research on Cancer, c1985; 1985;vol. 36.

(18) Tan SLW, Chadha S, Liu Y, et al. A Class of Environmental and Endogenous Toxins Induces BRCA2 Haploinsufficiency and Genome Instability. Cell 2017;169(6):1105-18.

(19) Goldgar D, Bonnardel C, Renard H, Yaqoubi O, Group TIC. The International BRCA1/2 Carrier Cohort Study: purpose, rationale, and study design. 2000. p. E10.

(20) Thorne H, Mitchell G, Fox S. kConFab: a familial breast cancer consortium facilitating research and translational oncology. J Natl Cancer Inst Monogr 2011;2011(43):79-81.

(21) Phillips KA, Butow PN, Stewart AE, et al. Predictors of participation in clinical and psychosocial follow-up of the kConFab breast cancer family cohort. Fam Cancer 2005;4(2):105-13.

(22) John EM, Hopper JL, Beck JC, et al. The Breast Cancer Family Registry: an infrastructure for cooperative multinational, interdisciplinary and translational studies of the genetic epidemiology of breast cancer. Breast Cancer Res 2004;6(4):R375-R389.

(23) Terry MB, Phillips KA, Daly MB, et al. Cohort Profile: The Breast Cancer Prospective Family Study Cohort (ProF-SC). Int J Epidemiol 2016;45(3):683-92.

(24) Chen WY, Rosner B, Hankinson SE, Colditz GA, Willett WC. Moderate Alcohol Consumption During Adult Life, Drinking Patterns, and Breast Cancer Risk. JAMA. 2011;306(17):1884–1890.

(25) Terry MB, Zhang FF, Kabat G, et al. Lifetime alcohol intake and breast cancer risk. Ann Epidemiol. 2006;16(3):230–240.

(26) Antoniou AC, Goldgar DE, Andrieu N, et al. A weighted cohort approach for analysing factors modifying disease risks in carriers of high-risk susceptibility genes. Genet Epidemiol 2005;29(1):1-11.

(27) Feigelson HS, Calle EE, Robertson AS, Wingo PA, Thun MJ. Alcohol consumption increases the risk of fatal breast cancer (United States). Cancer Causes Control 2001;12(10):895-902.

(28) Dennis J, Ghadirian P, Little J, et al. Alcohol consumption and the risk of breast cancer among BRCA1 and BRCA2 mutation carriers. Breast 2010;19(6):479-83.

(29) Seibold P, Vrieling A, Heinz J, et al. Pre-diagnostic smoking behaviour and poorer prognosis in a German breast cancer patient cohort - Differential effects by tumour subtype, NAT2 status, BMI and alcohol intake. Cancer Epidemiol 2014;38(4):419-26.

(30) Kwan ML, Chen WY, Flatt SW, et al. Postdiagnosis alcohol consumption and breast cancer prognosis in the after breast cancer pooling project. Cancer Epidemiol Biomarkers Prev 2013;22(1):32-41.

(31) Russo J, Russo IH. DNA labeling index and structure of the rat mammary gland as determinants of its susceptibility to carcinogenesis. J Natl Cancer Inst 1978;61(6):1451-9.

(32) Russo J, Tay LK, Russo IH. Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res Treat 1982;2(1):5-73.

(33) Terry MB, Liao Y, Kast K, et al. The Influence of Number and Timing of Pregnancies on Breast Cancer Risk for Women With BRCA1 or BRCA2 Mutations. JNCI Cancer Spectr. 2018;2(4):pky078.

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 HR[a] (95% CI)	P value	prospective HR[b] (95% CI)	P value
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

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 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

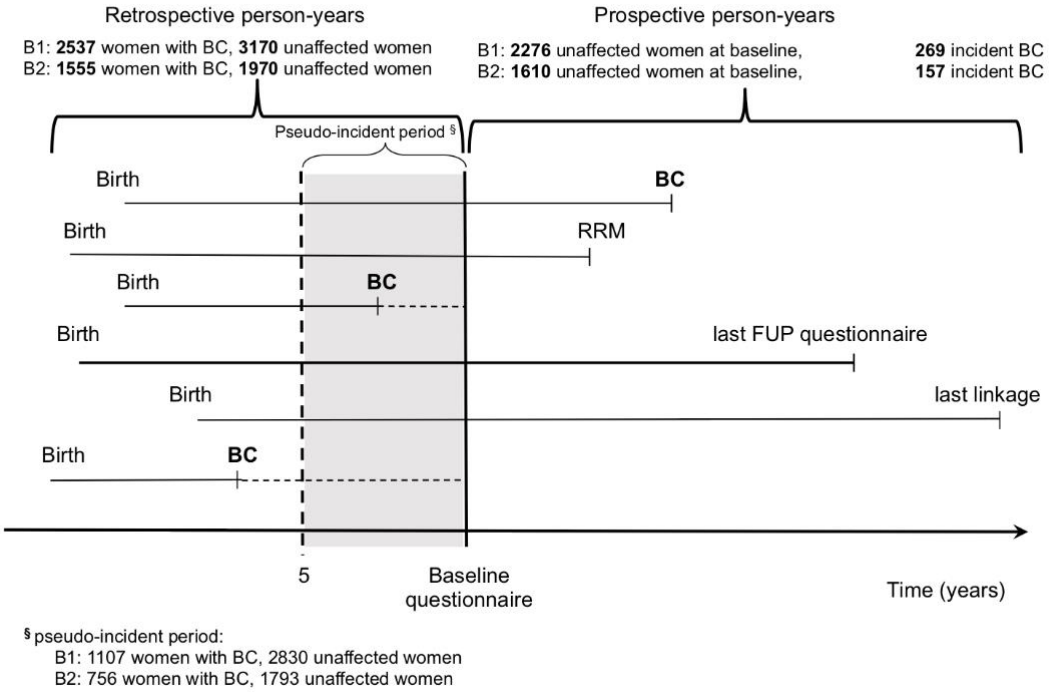

Fig.1

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)

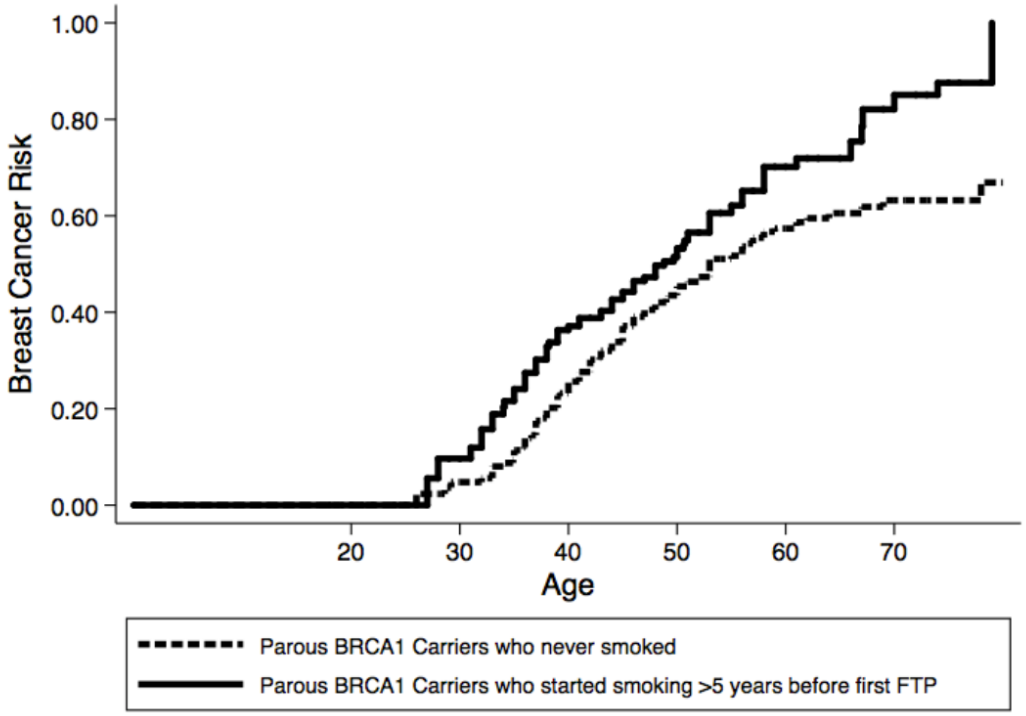

Fig. 2