Original Article

Breast cancer risk and night shift work in a case-control study in a Spanish population

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Conflict of Interest: The authors declare that they have no conflict of interest.

Abstract

Epidemiologic and animal data indicate that night shift work might increase the risk for breast cancer. We evaluated the association of night work with different clinical types of breast cancer in a population based case-control study (MCC-Spain study) taking into account chronotype, an individual characteristic that may relate to night shift work adaptation. Lifetime occupational history wasassessed by face-to-face interviews and shift work information was available for 1708 breast cancer cases and 1778 population controls from 10 Spanish regions, enrolled from 2008 to 2013. We evaluated 3 shift work domains, including shift work type (permanent vs rotating), lifetime cumulative duration and frequency. We estimated odds ratios (OR) for night work compared to day work using unconditional logistic regression models adjusting for confounders. Having ever worked permanent or rotating night shift was associated with an increased risk for breast cancer compared to day workers (Odds Ratio (OR) 1.18; 95% CI 0.97, 1.43). Chronotype was differentially associated with breast cancer depending on the duration of night shift work. Risk was higher in women with invasive tumors (OR 1.23; 95% CI 1.00, 1.51) and for estrogen and progestagen positive tumors among premenopausal women (OR 1.44; 95% CI 1.05, 1.99). Having ever performed night shift was associated with a small increased risk for breast cancer and especially in subgroups of women with particular hormone related characteristics.

Introduction

Breast cancer is the most common cancer in women worldwide and a leading cause of cancer death among females in most high income countries[1]. Despite the extensive research on the etiology of breast cancer, established or well-known risk factors (age at menarche, age at first birth, age at menopause and parity) explain <40% of the burden of the disease[2]. There is emerging evidence that exposure to light at night disrupts human biologic rhythms and night shift work has been suggested as a contributing risk factor for breast cancer[3]. Night work is one of the most widespread occupational exposures in the industrialized part of the world with 20% of the total active population working partly or entirely during the night[4].

In 2007 the International Agency for Research on Cancer evaluated the carcinogenicity of shift work concluding that "shift-work that involves circadian disruption is probably carcinogenic to humans" (Group 2A)[5]. This evaluation was primarily based on experimental evidence in animals for the carcinogenicity of light during night and limited evidence in humans. Recent systematic reviews, including studies published after the IARC´s evaluation, still describe the existing epidemiological evidence as limited or insufficient[6-9]. Combined risk estimates for breast cancer among female night shift workers vary from 1.01 to 1.21 across different meta-analyses. These differences are due to inconsistent definitions of night shift work often based on crude exposure assessment, potential confounding and the large heterogeneity in methods applied between studies. Most of the existing studies were performed among nurses or flight personnel, although night shift work is common in a wide variety of occupations. An IARC workshop report indicated that more studies were needed using detailed exposure assessment to capture aspects of shift schedules including type (permanent vs rotating shifts), duration (years) and frequency (nights per month) of

night shift work, that might be important for circadian disruption and cancer development[10].

Chronotype is an individual characteristic that describes the circadian phase and correlates with diurnal preference, the individual preference for morning or evening activity[11]. Chronotype has been associated to the capacity of night workers to adapt to non-day work schedules and it has been suggested that morning types working at night may be at a higher cancer risk than evening types[12]. Only a few studies have evaluated this hypothesis using basic chronotype information and showed inconsistent results[13-15].

It is unknown if night shift work, as other risk factors for breast cancer, has a differential effect depending on the tumor clinical subtype and hormone receptor status[16]. Night shift work may contribute to the increase in breast cancer risk through hormonal changes related with circadian disruption and it has been hypothesized that the risk of breast cancer might be higher if night work occurs before the first childbirth when the mammary gland is not completely differentiated[17]. Exposure to night shift work over critical exposure windows is still an understudied area[18].

We examined the association between night shift work and the risk for different clinical subtypes of breast cancer among women in the MCC-Spain study, a large population based case-control study. We assessed different types of night work in a variety of occupations and evaluated lifetime cumulative exposure but also focused on critical time windows of exposure and took into account individual chronotype.

Methods

MCC-Spain Study is a population based multi-case-control study on frequent tumors in Spain that includes 23 hospitals in 12 different regions and assesses 5 types of cancer (breast, colorectal, prostate, stomach and chronic lymphocytic leukemia) using the same series of population controls for all cases[19]. The main aim of this study is to investigate environmental and genetic factors related to the above cancer types in Spain. The MCC-Spain Study (www.mccspain.org) began in the year 2008 and the recruitment of incident cancer cases and population controls took place until the end of 2013.

We included 1708 incident breast cancer cases and 1778 population controls in ten participating centers (Asturias, Barcelona, Cantabria, Girona, Guipúzcoa, Huelva, León, Madrid, Navarra and Valencia). Cases were women, aged 20-85 with a new histological confirmed diagnosis of breast cancer living in the catchment area of each hospital for at least 6 months. Control subjects are women with no history of breast cancer living in the same catchment area as cases. Controls were selected randomly from the rosters of General Practitioners at the Primary Health Centers (PHC) participating in the study that cover nearly all the population living in the corresponding area. Controls were frequency matched to cases by age in 5-year age groups and study area. They were contacted on behalf of their General Practitioner and invited to participate in the study. Excluded were subjects incapable to participate in the interview due to communication difficulties (mental problems or speaking problems) or excess impairment of physical ability. Response rates varied by centre and on average were 72% among cases and 52% among controls with valid telephone numbers in the PHC rosters.

Data collection

Data was collected with face-to-face interviews performed by trained personnel. Lifetime occupational history was assessed for all jobs held for at least one year. For each job reported, detailed information was collected on job title, main activity or task performed, beginning and ending dates, shift type (day, night, rotating), exact time schedules, hours worked, and number of night shifts per month.

Information on other risk factors for breast cancer was collected such as age, educational level, family socioeconomic level, race, BMI, family history of breast cancer, age of menarche, parity, age at the first birth, menopausal status, smoking status, oral contraceptive use and history of hormonal replacement therapy. Leisure time physical activity information (type, frequency and duration) was available for all activities held over lifetime. Current sleep duration and sleep problems (waking up during the night, problems falling asleep, use of sleep medication) that persisted for at least 1 year were also assessed. Diet habits as well as current and past (at 30-40 years of age) alcohol consumption was reported for all cases and controls through a self-administered diet questionnaire. Individual chronotype was assessed through a follow-up phone interview and the use of the Munich Chronotype Questionnaire (MCTQ). In total 2854 subjects (1473 controls and 1381 cases) agreed to participate in this follow-up and completed the chronotype questionnaire. Clinical information was collected for most cases from medical records including tumor hormonal receptor status, differentiation grade and histological type.

The MCC-Spain Study followed the national and international directives such as the deontological code and declaration of Helsinki and the Spanish law on confidentiality of data (Ley Organica 15/1999 de 13 Diciembre de Proteccion de Datos de carácter personal -LOPD). All subjects that agreed to participate and fulfilled the eligibility criteria signed an informed consent form before participating in the study.

Statistical analysis

Night work was defined as a working schedule that involved partly or entirely working between 00:00 and 6:00 a.m. at least 3 nights per month. This definition included overnight, late evening (end after 00:00) and early morning (start before 6:00) shifts. The reference group consisted of women who had never worked at night including permanent day workers, shift workers with less than 3 nights per month and women who had never been employed (housewives). We compared permanent to rotating night shift schedules. A small group of permanent night workers had also performed rotating night shifts. For comparability we modeled the two exposures in a single model, and those subjects with both exposures were included in the permanent night shift group. Therefore subjects in the ever rotating night shift group had never worked in permanent night shift schedules.

Chronotype (MSF_{corr}) was estimated as the mid-sleep time on free days (MSF = [sleep onset on free day + (sleep duration on free day)/2)], corrected for oversleep on free days compared to working days (MSF_{corr} = [MSF - (sleep duration on free day-sleep duration on a working day)/2][11]. Chronotype was assessed using the continuous score expressed in local time, but also categorical variables with 3 categories (morning type: MSF_{corr} <04:00 h, neither type: MSF_{corr} =04:00-05:00 h, evening type: MSF_{corr} >05:00 h) and in alternative analyses with 5 or 7 categories. Women were considered premenopausal if during the last year they reported bleedings. Educational status referred to the highest grade completed. Daily alcohol (g ethanol/day), meat, vegetable and fruit consumption (g/day) and total energy intake (kcal/day) were estimated on the basis of the reported frequency of the different food items included in the diet questionnaire. The annual mean of physical activity was estimated (METS h/week) over the last 10 years and was used in the models as a categorical variable (inactive: 0 METS

h/week, slightly active: from >0 to 8 METS h/week, moderately active: >8 to 16 METS h/week and very active: greater than 16 METS h/week).

We evaluated the association of ever night shift work with breast cancer using unconditional logistic regression analysis and calculated Odds Ratios (OR) with 95% confidence intervals (CI). We assessed the type (permanent vs rotating), cumulative duration (total number of years worked at night) and cumulative frequency (total number of nights worked) of night shift work over the working life. Cumulative duration and frequency were categorized in groups of short, medium and long term exposure, using median cut-offs used in previous studies, as described in a systematic review of the literature[8]. We also used tertiles of exposure based on the distributions among exposed controls in our study. We tested possible interactions (Wald test) between night shift work and menopausal status at diagnosis, parity and chronotype and present the results in stratified analyses. We also examined the association between night shift work and breast cancer for parous women that started working at night before vs after their first full-term pregnancy. Finally, we used polytomous logistic regression models to analyze night shift work by different clinical/pathological characteristics of breast cancer: estrogen (ER) and progestagen (PG) receptors, c-Erb B2/Her-2 neu receptors, invasive vs in situ, differentiation grade (grade I: well differentiated, grade II: moderately differentiated, grade III: poorly differentiated, grade IV: undifferentiated) and histological type (ductal, lobular, others). We calculated Relative Risk Ratios (RRR) for each group of cases compared to controls (e.g. outcome variable: 0:control, 1:case ER+, 2:case ER-, 3:case ER not determined).

Variables initially considered for inclusion in multivariate analysis are shown in a directed acyclic graph (DAG) in Supplemental Figure 1. The selection of confounders was based on the DAG but also tested using statistical criteria (change-in-estimate,

forward and backward selection)[20]. No variable (family history of breast cancer, menopause status, BMI, alcohol consumption at the age of the 30-40, use of oral contraceptives) changed by more than 10% the risk estimates for night shift work when added individually in the models. In the final model, we kept those variables for which the respective risk estimates reached a significance level of $p<0.25$, in addition to age, study area and education. Most potential confounders had a small number of missing information (<1%) but chronotype had 18% and diet variables 15% of missing data. Cumulative frequency was missing in 35% of night workers while cumulative duration in <0.05%. The high percentage of missing values in cumulative frequency compared to duration was largely due to the fact that this question was only addressed to subjects that self reported night work while our definition was based on jobs´ time schedules and not self reports. To increase efficiency and minimize selection bias, we performed multiple imputation of missing values using chained equations[21] assuming the missing at random (MAR) hypothesis. To make the MAR assumption more plausible, we included in the imputation models a large number of variables including socio-demographic characteristics, pregnancies and menstrual cycle related variables, sunlight exposure, sleeping, diet and physical activity. Multiple imputations were done separately for cases and controls. We generated 30 complete data sets; which were analyzed individually to obtain a set of parameters and then combined into overall estimates using Rubin's rule[21]. In addition to the analysis of imputed missing data, a complete case analysis and a free floating approach were also performed.

Results

Table 1 shows the distribution of breast cancer risk factors in cases and controls. The study population was characterized by a high BMI (18.6% BMI≥30), low parity (19.8%

nulliparous) and use of oral contraceptives (47.7% ever used). Cases were younger (56.2; SD 0.3 vs 58.5; SD 0.3 years) and more often premenopausal (40.5% vs 34.1%) with a higher family history of breast cancer (30.8% vs 16.7%). They were more likely to smoke (44.6% vs 41.0%) and to report higher total energy (1861 vs 1754 kcal/day), red meat (59.4 vs 53.1 g/day) and past alcohol consumption. Smaller differences were observed for educational level, chronotype, number of full-term births among parous women, hormonal therapy and history of sleep problems.

The characteristics of night shift workers and day workers among controls are summarized in Supplemental Table 1. Night shift workers were younger (mean 55.6 years; SD 12.5 vs 58.9; SD 13.2 years) and thus more often premenopausal (43.2% vs 32.8%) compared to day workers. Night workers were more frequently nulliparous (23.7% vs 18.4%), ever smokers (49.5% vs 39.8%) and reported use of oral contraceptives (49.2% vs 39.8%), compared to day workers. Rotating shift workers had more frequently completed university studies (37.4%) compared to permanent night workers (13.4%) and day workers (21.5%). Permanent night workers were more frequently obese (26.8%) compared to rotating night (18.7%) and day workers (17.8%).

About 17% of cases and 14% of controls had ever worked in night shifts (Table 2). About 6% and 8.5% of the subjects had ever worked in permanent and rotating night shift respectively for more than 1 year. Having ever performed night shift work was associated with a higher breast cancer risk compared to subjects never engaged in night work (OR 1.18; 95% CI 0.97, 1.44) with similar estimates for permanent (OR 1.19; 95% CI 0.89, 1.60) and rotating (OR 1.17; 95% CI 0.91, 1.51) night work. Housewives were protected for breast prostate cancer compared to women that had never worked at night (OR 0.69 ; 95% CI 0.54, 0.88).We performed analyses removing housewives and rotating shift workers with no night shifts from the control group, creating a more clean

never shift work control group and results were similar (Table 2). We also removed subjects that had worked in jobs with 1 or 2 nights per month from the control group and results remained unchanged. Night shift work was found in different occupational sectors and was most common in personal services (such as nurse, child and home care assistants), restaurant services and health care related occupations (Supplemental Table 2). In sensitivity analysis we excluded overtime (>12h/day), part-time (<4/day) work as well as jobs that lacked some information on variables that were used for exposure classification such as time schedules, hours worked, nights worked per month and results were substantially the same.

The results of analyses by duration of night work and lifetime cumulative number of night shifts are presented in Table 3. Working for 15 or more years in permanent night shifts was associated with an OR of 1.49 (95% CI 0.88, 2.53). A higher number of cumulative permanent night shifts was also associated with a higher risk for breast cancer (>1800 night shifts: 1.48; 95% CI 0.81, 2.68). Among rotating night shift workers the highest OR was observed among those with the shorter period (<5 years) of exposure (OR 1.58; 95% CI 0.94, 2.66) and the smaller number of night shifts (<300 nights: OR 1.34; 95% CI 0.77, 1.67). Results were similar using tertiles of cumulative exposure among exposed controls.

Table 4 shows the association between night shift work and breast cancer risk for different chronotypes. Overall risk was only slightly higher among evening types (OR 1.27; 0.81, 2.00) compared to other chronotypes (p-interaction: 0.974). We found similar findings when using the 5 and 7 categories of chronotype (results not shown). Chronotype was differentially associated with breast cancer depending on duration of night shift work; longer exposure (>15 years) was associated with a higher risk in

evening types (OR 1.76; 0.85, 3.67) while short exposures increased risk two-fold in morning types (OR 2.09; 1.03, 4.22).

Results for ever night shift work compared to never night work was analyzed with respect to hormonal receptors and other clinical characteristics of breast tumors and are presented in Table 5 for all study population and also stratified by menopausal status. Night shift work was associated with a higher risk for tumors with EG+ PG+ receptors (RRR 1.44; 95% CI 1.04, 1.98) among premenopausal women. Night shift work resulted in an RRR of 1.47 (95% CI 0.98, 2.21) for ER+ PG- tumors in the full study population. The combined absence of estrogen and progestagen receptors resulted in no risk for breast cancer (RRR 1.07; 0.73, 1.58). Overall risk was higher for invasive (RRR 1.23; 95% CI 1.00, 1.51) and for lobular breast tumors (RRR 1.66; 95% CI 1.00, 2.75). Among postmenopausal women night shift work was associated with poorly differentiated or anaplastic tumors (Grade III & IV: 1.65; 95% CI 1.07, 2.54).

We also evaluated the association between night shift work and breast cancer risk stratified by menopausal status, parity and age of first exposure (before vs after first full term pregnancy) and found no statistically significant interaction (Supplemental Table 3). Night shift work was related with a slightly higher breast cancer risk among premenopausal (OR 1.33; 0.98, 1.79) than postmenopausal women (OR 1.08; 0.82, 1.42).. A slightly higher risk for breast cancer was found among women ever exposed to night shift work before their first full term pregnancy (OR 1.25; 95% CI 0.93, 1.67) compared to those exposed after their first full term pregnancy (OR 1.14; 95% CI 0.81, 1.60), however the difference was not statistically significant.

Discussion

In this population based case-control study in Spain having ever performed night work, either in a permanent or rotating schedule, in a variety of occupations, was associated with a small increase of breast cancer risk. The risk increased in subgroups with specific hormone related characteristics such as tumors with positive hormone receptors.

Our findings are in line with most previous studies on night shift work and breast cancer risk[6-9]. Among more recent studies, conducted after the IARC evaluation, seven showed positive associations between night shift work and breast cancer[14,17,22-27] and two found no association[28,29]. Night shift work definitions have largely varied across existing studies. We based the definition on jobs' start and end times and not on self-reports and evaluated jobs that take place over the dark period and can be disruptive for the human circadian clock. Apart from the definition, other shift work domains such as shift type, duration and frequency are considered important for the exposure assessment and have not been evaluated in most previous studies[10]. We found no difference in risk between permanent and rotating night work. It has been hypothesized that rotating shift work might provoke more circadian disruption than permanent night shifts[30]. One previous study showed that permanent night work in nurses was more disruptive than rotating night work, but it was performed in addition to rotating night shifts[24]. Permanent night workers are more likely to adapt by phase shifting their circadian rhythms but full entrainment is almost impossible to achieve in real life settings[31]. Furthermore a recent study showed a positive association between increasing number of consecutive night shifts and breast cancer risk which indicates that a higher degree of adaptation might not be safe if it is followed by a return on normal day habits on free days[25]. We found different effects of duration and frequency depending on whether night work was permanent or rotating, but our results are difficult

to explain. Specifically odds ratios increased as cumulative exposure to permanent night shift work increased while among rotating night shift workers the highest odds ratio was observed among those with the shortest exposure. Many other studies have failed to describe a clear dose-response pattern between the duration or frequency of night work and breast cancer risk[26,30,32,33]. It remains unknown how many nights per month and for how long can lead to a significant disruption of the circadian system.

We found a complex pattern of risk of night shift work, breast cancer and chronotype. An overall higher breast cancer risk was observed among night shift workers with an evening type, compared to morning or neither chronotypes. Evening types have been hypothesized to better tolerate night shift work and morning types to be more sensitive to circadian disruption and thus at a highest cancer risk[12]. A polymorphism in the circadian PER3 gene has been associated with both evening preference and increased breast cancer risk, suggesting that evening types might be more susceptible to cancer[7,34]. One study that evaluated this hypothesis assessing self-reported diurnal preference, found a higher overall breast cancer risk for evening types although among night shift workers risk was higher for morning types[14]. In two other studies, neutral types were at the highest risk for breast cancer[15,22]. While previous studies used self-reports or a single-item to assess chronotype, we used a validated questionnaire that allowed a quantitative assessment and also comparison of more extreme chronotypes. Based on small numbers, we found that morning chronotypes had a two-fold risk for breast cancer after shorter exposures. If morning types are more susceptible to circadian disruption they might be more likely to drop out of shift work earlier. Chronotypes may be unequally distributed across different strata of cumulative exposure, which might partly explain the lack of dose-response relationship between night shift work and cancer risk in previous studies. Finally, high missingness

in chronotype information was due to non responders in our follow up phone interviews and might have affected our estimates. We therefore compared the characteristics of responders to non responders and they were similar for most characteristics tested including case-control status, age, educational level, bmi and shift work history.

We found an indication of a possible association between night shift work and hormone related parameters. Breast cancer risk was higher in the presence of ER and PR receptors, especially among premenopausal women, in line with one previous study[33]. ER+ and/or PR+ are expected to be more closely related with risk to night shift work since one of the main hypothesized underlying mechanisms involves a potential disruption of sex hormones. However other studies have showed mixed results[23,35,36]. Furthermore a higher, though not statistically significantrisk was observed among women that started to work at night before their full term pregnancy. One previous study has described a similar pattern of risk[17]. This is in line with what is observed for other risk factors such as smoking[37]. It is possible that during this exposure window the mammary gland is more susceptible to hormonal changes related to circadian disruption We also found that housewives, a group of women that typically performs domestic tasks during the day period were protected against breast cancer, after adjusting for reproductive history and other characteristics. This is an interesting finding that is in line with our hypothesis and provides us with a negative control group for the present analysis. Finally, we found a higher risk for invasive, lobular and anaplastic tumors, suggesting a possible association with tumors with worse prognosis. Invasive lobular cancer is less frequent than ductal, more difficult to detect mammographically and usually detected at later stages. This tumor has been associated with a better prognosis with early diagnosis but a worse prognosis with later detection[38]. This is partly in line with experimental evidence showing acceleration of

tumor development and growth after circadian disruption[39]. Furthermore recent evidence suggests decreased survival of breast cancer among night shift workers[40]. This finding is new and needs confirmation in other study populations.

This is the first study evaluating the association between night-shift and breast cancer risk in the south of Europe and Spain. Although the study is large, we had relatively small numbers in some of the subgroups, which may have resulted in limited power to assess possible effect modification. The sub analyses are interesting not in that we identify statistically significant results but in that we describe a pattern of risk. This pattern indicates differential risk of shift work and breast cancer by several hormonal dependent parameters. This analysis presents limitations inherent to all case-control study designs. Interview based case-control studies like the present are normally prone to information bias. The retrospective estimations of exposures based on self reported occupational history may lead to exposure misclassification. We based the classification of night work on time schedules provided for each job, which is probably more precise and reliable compared to self-reported data. Differential recall between cases and controls for shift work is unlikely given that the study had a more general orientation and the association of shift work and cancer is not yet widely discussed in Spain. Interviewer bias was minimised as interviews were done by well trained personnel. Selection bias might have occurred if non participants (controls or cases) were engaged more frequently in night shift work than participants. Among controls, current night shift workers may have been less likely to answer our phone calls due to daytime sleep as compared to day workers. However, phone calls were scheduled and performed repeatedly in different times during the day. Finally the analytic control for potential confounders was among the strengths of the study.

In conclusion our results suggest an association between night work and breast cancer risk, especially in subgroups with particular hormone related parameters. Differences were also observed by tumor clinical characteristics and by morning/evening chronotype. The magnitude of the observed risk in this and other studies is not high, but given the high prevalence of both the disease and the exposure in modern societies it may be associated with a considerable number of cancer cases.

Acknowledgements:

We would like to acknowledge the participants in the study, the data manager and all the interviewers and technicians involved in the data collection. We would also like to thank Juan Alguacil, Miguel Santibañez, Victor Moreno and Laura Costas for their valuable comments in the text.

Compliance with Ethical Standards

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Funding: The study was funded by the “Accion Transversal del Cancer", approved on the Spanish Ministry Council on the 11th October 2007, by the Instituto de Salud Carlos III-FEDER (PI08/1770, PI08/0533, PI08/1359, PI09/00773-Cantabria, PI09/01286-León, PI09/01903-Valencia, PI09/02078-Huelva, PI09/01662-Granada, PI11/01403, PI11/01889-FEDER, PI11/00226, PI11/01810, PI11/02213, PI12/00488, PI12/00265, PI12/01270, PI12/00715, PI12/00150), by the Fundación Marqués de Valdecilla (API 10/09), by the ICGC International Cancer Genome Consortium CLL (The ICGC CLL-Genome Project is funded by Spanish Ministerio de Economía y Competitividad (MINECO) through the Instituto de Salud Carlos III (ISCIII) and Red Temática de Investigación del Cáncer (RTICC) del ISCIII (RD12/0036/0036)), by the Junta de Castilla y León (LE22A10-2), by the Consejería de Salud of the Junta de Andalucía

(2009-S0143), by the Conselleria de Sanitat of the Generalitat Valenciana (AP_061/10), by the Recercaixa (2010ACUP 00310), by the European Commission grants FOOD-CT-2006-036224-HIWATE, by the Spanish Association Against Cancer (AECC) Scientific Foundation, by the Catalan Government DURSI grant 2009SGR1489, and by a predoctoral grant PFIS (FI09/00385).

Conflict of Interest: The authors declare that they have no conflict of interest.

RESEARCH INVOLVING HUMAN PARTICIPANTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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Supplemental Table 1. Characteristics of never night, permanent night and rotating night shift workers among controls in the MCC-Spain study (Numbers may differ due to missing values; SD Standard Deviation)

Factor	Never night (N= 1542)	Ever night (N=236)	Permanent night (N=97)	Rotating night (N=139)
	%	%	%	%
Age; mean (SD)	58.9 (13.2)	55.6 (12.5)	56.0 (12.9)	55.2 (12.3)
Age groups				
<50	26.7	35.2	35.1	35.3
50-59	24.1	26.3	23.7	28.1
60-69	23.2	22.0	21.7	22.3
>70	25.5	16.5	19.6	14.4
Family history of breast cancer				
No	82.9	85.5	88.5	83.5
Yes	17.1	14.5	11.5	16.6
Education				
Less than primary	16.2	14.8	23.7	8.6
Primary	30.9	23.3	25.8	21.6
High school	31.5	34.3	37.1	32.4
University	21.5	27.5	13.4	37.4
BMI (kg/cm^2)				
<22.5	24.5	22.9	13.4	29.5
22.5-25	26.7	26.3	24.7	27.3
25-30	31.1	28.8	35.1	24.5
≥30	17.8	22.0	26.8	18.7
Menopause status				
Premenopausal	32.8	43.2	40.2	45.3
Postmenopausal	67.3	57.8	59.8	54.7
Parity				
Nulliparous	18.4	25.0	23.7	25.9
Ever parous	81.7	75.0	76.3	74.1
Number of full-term births; mean (SD) [A]	2.0 (1.5)	1.7 (1.5)	1.7 (1.2)	1.7 (1.7)
Age first child; mean (SD) [A]	26.8 (4.7)	26.2 (5.2)	24.9 (4.7)	27.1 (5.4)
Breastfeeding (months); mean (SD) [A]	9.7 (21.1)	7.2 (11.3)	7.5 (12.3)	7.0 (10.5)
Alcohol consumption; mean (SD)[B]	5.4 (9.6)	5.4 (9.4)	6.6 (11.1)	4.6 (8.0)
Ever oral contraceptives	47.6	55.3	55.2	55.4
Ever hormonal therapy	7.4	8.4	5.5	10.4
Ever smoker	39.8	49.2	49.5	48.9
Ever sleep problems	44.4	44.9	43.3	46.0
Sun exposure 10 yrs ago				
<2 hour	55.7	60.2	57.7	61.9
2-4 hours	23.3	21.6	23.7	20.1
>4 hours	16.4	14.4	15.5	13.7
weekends	4.6	3.8	3.1	4.3
Chronotype				
Morning	38.3	39.8	40.9	39.2
Neither	40.4	36.1	29.6	40.0
Evening	21.3	24.1	29.6	20.8

[A]Estimated among parous women, [B]Alcohol consumption at 30-40 years old

Supplemental Table 2 The five most frequent occupational sectors that include night shift work based on the 2-digit international standard job classification (ISCO-88)[A]

Occupational sector	Jobs with night shift work (N=652)	Types of night shift work	
		Permanent (N=254)	Rotating (N=398)
	%	%	%
Personal services[B]	21	11	27
Restaurant services	16	29	8
Professions relate to natural sciences and health	16	6	22
Cleaning personnel working indoors	9	8	6
Fixed machine operators	6	13	7

[A]Table based on number of jobs; subjects may have more than one night job

[B]Examples of jobs: nurse assistants, home and child care assistants, flight attendants

Supplemental Table 3. Association between night shift work and breast cancer by menopausal status, parity and age of first exposure (before vs after the first full term pregnancy (FFTP) among parous women (OR=odds ratio, 95% CI=95% confidence interval).

	Controls (N=1229)	**Cases (N=1175)**	**OR (95% CI)[A]**	**OR (95% CI)[B]**
	N (%)	**N (%)**		
Premenopausal				
Never night work	505 (83.2)	552 (79.8)	1 (Ref)	1 (Ref)
Ever night work	102 (16.8)	140 (20.2)	1.26 (0.95, 1.67)	1.33 (0.98, 1.79)
Postmenopausal				
Never night work	1037 (88.6)	886 (87.2)	1 (Ref)	1 (Ref)
Ever night work	134 (11.4)	130 (12.8)	1.11 (0.85, 1.44)	1.08 (0.82, 1.42)
p-interaction				*0.642*
Nulliparous				
Never night work	283 (82.8)	289 (83.1)	1 (Ref)	1 (Ref)
Ever night work	59 (17.3)	59 (17.0)	0.96 (0.64, 1.45)	0.97 (0.63, 1.49)
Parous				
Never night work	1259 (86.7)	1149 (84.5)	1 (Ref)	1 (Ref)
Ever night work	177 (12.3)	211 (15.5)	1.21 (0.97, 1.52)	1.21 (0.96, 1.52)
p-interaction				*0.305*
First exposure before FFTP	102 (7.5)	130 (10.2)	1.30 (0.99, 1.72)	1.25 (0.93, 1.67)
First exposure after FFTP	75 (5.6)	81 (6.6)	1.10 (0.79, 1.53)	1.14 (0.81, 1.60)

[A]OR adjusted for age and centre and educational level

[B]OR adjusted for age, centre, educational level (less than primary, primary, high school, university), parity (nulliparous, 1-2, ≥3), menopausal status (premenopausal, postmenopausal), family history of breast cancer (yes/no), body mass index (<22.5, 22.5-25, 25-30, ≥30), smoking status (ever, never), oral contraceptive use (yes, no), leisure time physical activity (inactive, little active, moderately active, very active), alcohol consumption (quartiles), sleep duration (<7 hours, 7-8 hours, >8 hours)

Supplemental Figure 1. Directed acyclic graph (DAG) used for the selection of the confounders of the association between night shift work and breast cancer risk

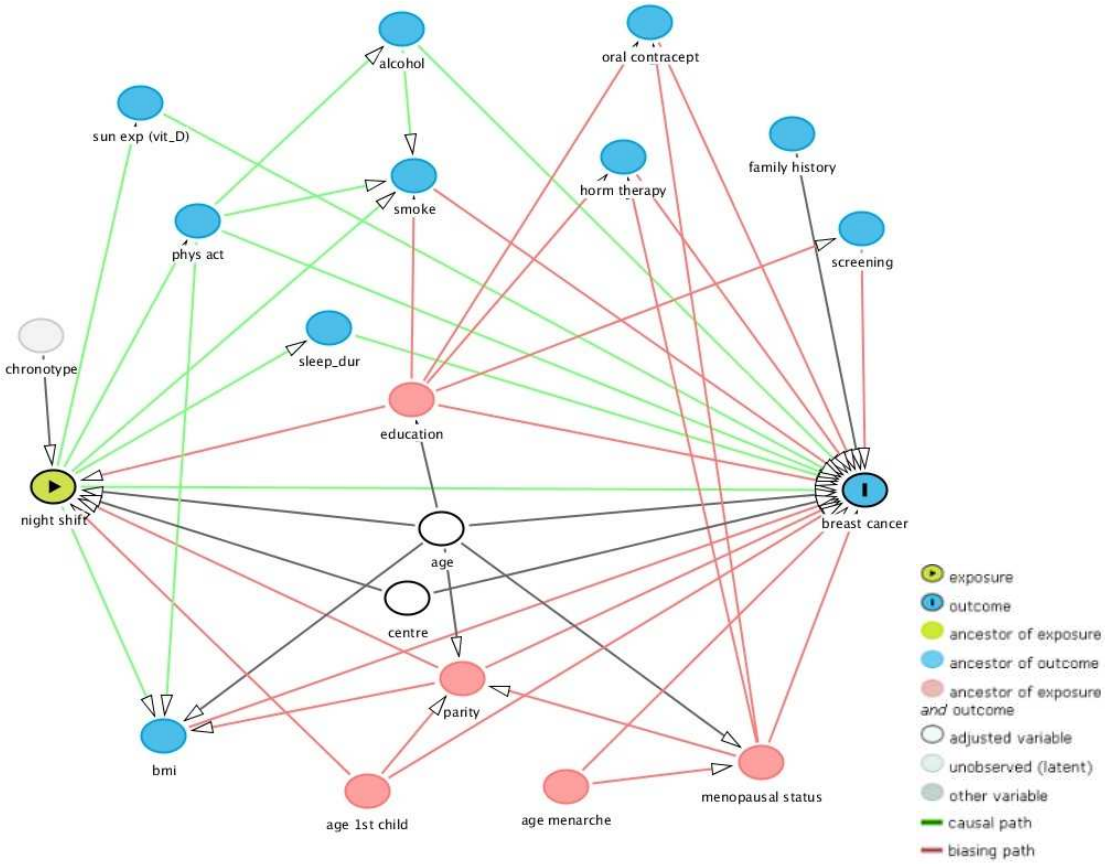

Table 1. Potential breast cancer risk factors among participants in the MCC-Spain study (Numbers may differ due to missing values; SD Standard Deviation)

Factor	**Controls (N=1778) N (%)**	**Cases (N=1708) N (%)**
Age (years); mean (SD)	58.5 (0.3)	56.2 (0.3)
Age groups		
<50	496 (27.9)	555 (32.5)
50-59	433 (24.4)	487 (28.5)
60-69	411 (23.1)	407 (23.5)
≥70	438 (24.6)	259 (15.2)
Participating centres		
Madrid	365 (20.5)	341 (20.0)
Barcelona	309 (17.4)	290 (17.0)
Navarra	177 (10.0)	227 (13.3)
Guipuzcoa	252 (14.2)	220 (12.9)
Leon	175 (9.8)	205 (12.0)
Cantabria	185 (10.4)	140 (8.2)
Huelva	76 (4.3)	110 (6.4)
Asturias	119 (6.7)	68 (4.0)
Valencia	65 (3.7)	61 (3.6)
Girona	55 (3.1)	46 (2.7)
Family history of breast cancer		
No	1476 (83.3)	1176 (69.3)
Yes	297 (16.8)	521 (30.7)
Education		
Less than primary	284 (16.0)	261 (14.7)
Primary	531 (29.9)	553 (32.4)
High school	567 (31.9)	567 (33.2)
University	396 (22.3)	327 (19.2)
Marital status		
Not married	215 (12.1)	222 (13.0)
Married	1169 (65.8)	1147 (67.4)
Divorced	150 (8.4)	142 (8.3)
Widow	243 (13.7)	192 (11.3)
BMI (kg/cm^2)		
<22.5	431 (24.2)	400 (23.4)
22.5-25	474 (26.6)	409 (24.0)
25-30	547 (30.8)	582 (34.1)
≥30	326 (18.3)	317 (18.9)
Tobacco smoking		
Ever smoker	729 (41.0)	761 (44.6)
Physical activity[A]		
Inactive or a little active	1023 (66.1)	1030 (68.7)
Moderately or very active	525 (33.9)	469 (31.3)
Sleep habits		
Sleep duration (hrs/day) ; mean (SD)	7.0 (0.1)	7.0 (0.1)
Ever sleeping problems	789 (44.5)	700 (41.2)
Chronotype, MSF; mean (SD)	04:18 (2´)	04:24 (2´)

Morning type	567 (38.5)	514 (37.2)
Neither type	587 (39.9)	536 (38.8)
Evening type	319 (21.7)	331 (23.9)
Diet habits		
Total energy consumption (kcal/day)	1754 (14.2)	1861 (17.1)
Past alcohol consumption (g ethanol /day)[B]	5.4 (0.2)	6.2 (0.3)
Red meat consumption (g/day)	53.1 (0.9)	59.4 (1.0)
Fruit consumption	360.6 (5.7)	363.1 (6.3)
Menopausal status		
Premenopausal	607 (34.1)	692 (40.5)
Postmenopausal	1171 (65.9)	1016 (59.5)
Parity		
Nulliparous	342 (19.2)	348 (20.4)
1-2 children	973 (54.7)	944 (55.3)
≥3 children	463 (26.0)	416 (24.4)
Age first full-term birth; mean (SD)[C]	26.7 (0.1)	26.8 (0.1)
Breastfeeding (months); mean (SD)[C]		
Ever oral contraceptives	863 (48.7)	795 (46.7)
Ever hormonal therapy	129 (7.5)	115 (6.9)
Past sun exposure		
<2 hour	699 (56.0)	941 (55.4)
2-4 hours	410 (23.1)	430 (25.3)
>4 hours	286 (16.1)	257 (15.1)
Weekends/vacations	80 (5.5)	73 (4.3)

[A]Assessed over the last 10 years excluding 2 years prior to diagnosis, [B]Assessed at 30-40 yrs, [C]Estimated among parous women

Table 2. Association between shift work and night work and breast cancer risk in MCC-Spain study (OR=odds ratio, 95%CI=95% confidence interval).

	Controls (N=1778)	**Cases (N=1708)**	**OR (95% CI)[A]**	**OR (95% CI)[B]**
	N (%)	**N (%)**		
Never night work	1542 (86.7)	1438 (84.2)	1 (Ref)	1 (Ref)
Ever night work	236 (13.3)	270 (15.8)	1.18 (0.97, 1.43)	1.18 (0.97, 1.43)
Permanent night	97 (5.5)	114 (6.7)	1.17 (0.87, 1.55)	1.19 (0.89, 1.60)
Rotating night	139 (7.8)	156 (9.1)	1.18 (0.93, 1.51)	1.17 (0.91, 1.51)
Never shift work	1195 (67.2)	1190 (69.7)	1 (Ref)	1 (Ref)
Permanent night	97 (5.5)	114 (6.7)	1.10 (0.83, 1.47)	1.13 (0.84, 1.51)
Rotating night	139 (7.8)	156 (9.1)	1.12 (0.87, 1.43)	1.11 (0.86, 1.43)
Rotating no night	118 (6.6)	93 (5.4)	0.77 (0.57, 1.03)	0.78 (0.57, 1.05)
Housewives	229 (12.9)	155 (9.1)	0.70 (0.55, 0.88)	0.69 (0.54, 0.88)

[A]OR adjusted for age and centre and educational level

[B]OR adjusted for age, centre, educational level (less than primary, primary, high school, university), parity (nulliparous, 1-2, ≥3), menopausal status (premenopausal, postmenopausal), family history of breast cancer (yes/no), body mass index (<22.5, 22.5-25, 25-30, ≥30), smoking status (ever, never), oral contraceptive use (yes, no), leisure time physical activity (inactive, little active, moderately active, very active), alcohol consumption (quartiles), sleep duration (<7 hours, 7-8 hours, >8 hours)

Table 3. Association between years of shift work and number of night shifts with breast cancer risk in MCC-Spain study (OR=odds ratio, 95% CI=95% confidence interval).

	Controls (N=1778)	Cases (N=1708)	OR (95% CI)[A]	OR (95% CI)[B]	p-trend
	N (%)	N (%)			
Never night work	1542 (86.7)	1438 (84.2)	1 (Ref)	1 (Ref)	
Cumulative years of total night work					
1-5 years	58 (3.2)	67 (3.9)	1.19 (0.82,1.72)	1.21 (0.83, 1.76)	
5-15 years	85 (4.8)	103 (6.0)	1.16 (0.86, 1.57)	1.13 (0.83, 1.53)	
>15 years	91 (5.1)	97 (5.7)	1.18 (0.87, 1.59)	1.21 (0.89, 1.65)	0.176
Cumulative years of permanent night work					
1-5 years	34 (2.1)	32 (2.1)	0.92 (0.56, 1.52)	1.00 (0.59, 1.66)	
5-15 years	36 (2.2)	46 (3.0)	1.23 (0.79, 1.94)	1.17 (0.74, 1.87)	
>15 years	27 (1.7)	34 (2.2)	1.37 (0.82, 2.31)	1.49 (0.88, 2.53)	0.109
Cumulative years of rotating night work					
1-5 years	26 (1.6)	40 (2.5)	1.64 (0.98, 2.73)	1.58 (0.94, 2.66)	
5-15 years	57 (3.4)	56 (3.5)	0.96 (0.65, 1.41)	0.96 (0.65, 1.41)	
>15 years	54 (3.2)	59 (3.7)	1.23 (0.84, 1.80)	1.22 (0.82, 1.81)	0.369
Cumulative number of night shifts					
36-600 nights	53 (3.1)	62 (3.9)	1.15 (0.81, 1.64)	1.15 (0.80, 1.64)	
600-1800 nights	48 (2.8)	53 (3.3)	1.21 (0.86, 1.71)	1.20 (0.85, 1.70)	
>1800 nights	53 (3.1)	56 (3.5)	1.17 (0.82,1.65)	1.18 (0.83, 1.69)	0.248
Cumulative number of permanent night shifts					
36-600 nights	12 (0.8)	14 (0.9)	0.93 (0.49, 1.76)	0.96 (0.50, 1.85)	
600-1800 nights	11 (0.7)	16 (1.1)	1.13 (0.65, 1.95)	1.15 (0.65, 2.04)	
>1800 nights	15 (0.9)	20 (1.3)	1.43 (0.80, 2.53)	1.48 (0.81, 2.68)	0.149
Cumulative number of rotating night shifts					
36-600 nights	12 (0.8)	14 (0.9)	1.14 (0.78, 1.67)	1.34 (0.77, 1.67)	
600-1800 nights	11 (0.7)	16 (1.1)	1.38 (0.88, 2.16)	1.32 (0.83, 2.08)	
>1800 nights	15 (0.9)	20 (1.3)	1.07 (0.65, 1.74)	1.08 (0.66, 1.79)	0.519

[A]OR adjusted for age and centre and educational level

[B]OR adjusted for age, centre, educational level (less than primary, primary, high school, university), parity (nulliparous, 1-2, ≥3), menopausal status (premenopausal, postmenopausal), family history of breast cancer (yes/no), body mass index (<22.5, 22.5-25, 25-30, ≥30), smoking status (ever, never), oral contraceptive use (yes, no), leisure time physical activity (inactive, little active, moderately active, very active), alcohol consumption (quartiles), sleep duration (<7 hours, 7-8 hours, >8 hours)

Table 4. Association of night shift work and breast cancer stratified by chronotype. (OR=odds ratio, 95% CI=95% confidence interval)[A]

	Morning chronotype		Neither chronotype		Evening chronotype	
	Controls / Cases (N)	**OR (95% CI)[B]**	**Controls / Cases (N)**	**OR (95% CI)[B]**	**Controls / Cases (N)**	**OR (95% CI)[B]**
Never night work	491/425	1 (Ref)	518/459	1 (Ref)	273/275	1 (Ref)
Ever night work	76/89	1.17 (0.83, 1.65)	69/77	1.17 (0.82, 1.69)	46/56	1.27 (0.81, 2.00)
Types of night work						
Permanent night work	29/37	1.26 (0.76, 2.09)	21/31	1.24 (0.70, 2.19)	21/25	1.11 (0.59, 2.12)
Rotating night work	47/52	1.11 (0.71, 1.74)	48/46	1.13 (0.72, 1.79)	25/31	1.43 (0.79, 2.59)
Lifetime cumulative duration of night work (years)						
<5 years	13/24	2.09 (1.03, 4.22)	19/19	0.97 (0.49, 1.89)	17/13	0.95 (0.44, 2.03)
5-15 years	26/32	1.14 (0.66, 1.98)	27/34	1.18 (0.68, 2.03)	14/20	1.17 (0.55, 2.48)
>15 years	36/31	0.91 (0.54, 1.51)	23/24	1.38 (0.76, 2.51)	14/23	1.76 (0.85, 3.67)
Lifetime cumulative frequency of night work (night shifts)						
<600 nights	9/23	2.10 (1.00, 4.42)	19/18	0.92 (0.50, 1.68)	14/9	0.80 (0.37, 1.72)
600-1800 nights	22 /19	1.00 (0.57, 1.80)	13/13	1.22 (0.64, 2.35)	6/14	1.90 (0.86, 4.22)
>1800 nights	24/17	0.90 (0.50, 1.59)	13/16	1.54 (0.80, 2.98)	7/10	1.38 (0.59, 3.24)

[A]Numbers may differ due to missing values

[B]OR adjusted for age, centre, educational level (less than primary, primary, high school, university), parity (nulliparous, 1-2, ≥3), menopausal status (premenopausal, postmenopausal), family history of breast cancer (yes/no), body mass index (<22.5, 22.5-25, 25-30, ≥30), smoking status (ever, never), oral contraceptive use (yes, no), leisure time physical activity (inactive, little active, moderately active, very active), alcohol consumption (quartiles), sleep duration (<7 hours, 7-8 hours, >8 hours)

Table 5. Relative risk ratios (RRR) for night shift work compared to never night shift work according to the tumors´ clinical characteristics (CI=confidence intervals)[A]

	All		Premenopausal		Postmenopausal	
	Cases (N)	**Adjusted RRR[B] (95% CI)**	**Cases (N)**	**Adjusted RRR[B] (95% CI)**	**Cases (N)**	**Adjusted RRR[B] (95% CI)**
Estrogen (ER) receptors						
ER+	1343	1.18 (0.96, 1.46)	552	1.38 (1.00, 1.89)	791	1.05 (0.78, 1.41)
ER-	284	1.11 (0.77, 1.61)	103	1.01 (0.56, 1.82)	181	1.20 (0.75, 1.94)
Progestagen (PG) receptors						
PG+	1150	1.16 (0.93, 1.45)	498	1.44 (1.05, 1.99)	652	0.95 (0.70, 1.31)
PG-	463	1.22 (0.91, 1.65)	154	0.90 (0.54, 1.51)	309	1.43 (0.99, 2.1)
Estrogen-progestagen combinations						
ER+ PG+	1125	1.15 (0.92, 1.43)	485	1.44 (1.04, 1.98)	640	0.94 (0.68, 1.29)
ER+ PG-	199	1.47 (0.98, 2.21)	61	0.87 (0.40, 1.89)	138	1.81 (1.11, 2.95)
ER- PG+	19	1.73 (0.58, 5.18)	9	2.56 (0.49, 13.29)	10	1.15 (0.18, 7.32)
ER- PG-	262	1.07 (0.73, 1.58)	93	0.91 (0.48, 1.72)	169	1.20 (0.73, 1.97)
Her-2-neu receptors						
Her-2-neu+	290	1.31 (0.93, 1.85)	116	1.56 (0.94, 2.59)	174	1.07 (0.65, 1.79)
Her-2-neu-	1234	1.16 (0.97, 0.99)	501	1.25 (0.90, 1.73)	733	1.10 (0.82, 1.48)
Invasive vs In situ						
Invasive	1470	1.23 (1.00, 1.51)	607	1.35 (0.99, 1.83)	1470	1.15 (0.87, 1.53)
In situ	170	0.94 (0.59, 1.52)	58	1.37 (0.67, 2.79)	170	0.68 (0.35, 1.34)
Differentiation grade						
I-II	899	1.07 (0.84, 1.36)	359	1.27 (0.88, 1.81)	540	0.90 (0.64, 1.27)
III-IV	359	1.19 (0.86, 1.67)	159	0.86 (0.51, 1.45)	200	1.65 (1.07, 2.54)
Histological Type						
Ductal	1265	1.22 (0.98, 1.50)	524	1.37 (1.00, 1.89)	741	1.10 (0.82, 1.47)
Lobular	111	1.66 (1.00, 2.75)	46	1.74 (0.82, 3.70)	65	1.62 (0.80, 3.28)

[A]Numbers may differ due to missing values; the same set of controls were used for all the analyses

[B]RRR adjusted for age, centre, educational level (less than primary, primary, high school, university), parity (nulliparous, 1-2, ≥3), menopausal status (premenopausal, postmenopausal), family history of breast cancer (yes/no), body mass index (<22.5, 22.5-25, 25-30, ≥30), smoking status (ever, never), oral

contraceptive use (yes, no), leisure time physical activity (inactive, little active, moderately active, very active), alcohol consumption (quartiles), sleep duration (<7 hours, 7-8 hours, >8 hours)