THE INFLUENCE OF SHIFT WORK, LIGHT AT NIGHT AND CLOCK GENE POLYMORPHISMS ON MELATONIN LEVELS AND BREAST CANCER RISK

by

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Abstract

Background: Shift work has recently been identified as a breast cancer risk factor, where meta-analysis has indicated an approximately 50% increased risk among long-term shift workers. However, additional studies with more comprehensive methods of shift work exposure assessment are needed to capture the diversity of shift patterns. The hypothesized mechanism for this relationship involves chronodisruption (altered circadian rhythms), where increased exposure to light at night during night shifts may decrease production of the cancer-protective hormone melatonin. Further, coordination of circadian rhythms, including melatonin production, is governed by the interactions of a set of central clock genes. Recent studies have suggested that variants in clock genes are associated with cancer risk at multiple sites, including breast cancer, although few studies have considered potential interactions with shift work.

Methods: This thesis examined relationships of both shift work and clock gene polymorphisms (and their interactions) with breast cancer risk in a case-control study of 1,142 cases and 1,178 controls. The association between light exposure and melatonin production was also investigated in a longitudinal biomarker study conducted among 123 nurses working a two-day, two-night rotating shift pattern.

Results: In the case-control study, an association between breast cancer and ≥30 years of shift work (OR = 2.20, 95%CI = 1.13 – 4.28) was detected, although no relationship with short (0 – 14 years) or medium (15 – 29 years) term shift work was observed. As well, variants in 14 clock-related genes were not associated with breast cancer and there were no apparent interactions with shift work history. In the biomarker study, both peak melatonin levels and daily change in melatonin levels were similar when nurses were working their day and night shifts. Further, on

the night shift, a slight inverse relationship between light and change in melatonin was observed ($p = 0.04$).

Conclusions: Taken together, these results contribute to the understanding of both the association between shift work and breast cancer, and the biologic mechanisms underlying this relationship. Since shift work is required for many occupations, understanding the mechanisms through which it impacts breast cancer is important to the development of healthy workplace policy.

Co-Authorship

The manuscripts presented in this thesis are the work of Anne Grundy in collaboration with her supervisors, Kristan Aronson and Joan Tranmer, as well as co-authors. The case-control study from which analysis for manuscript 1 (Chapter 3) and manuscript 3 (Chapter 5) originated was designed and conducted by Kristan Aronson, John Spinelli, Chris Bajdik, Angela Brooks-Wilson, Harriet Richardson, Igor Burstyn, Pierre Ayotte, Caroline Lohrisch, Sandip SenGupta and Agnes Lai. The shift work and clock gene analyses were designed and conducted by Anne Grundy. The biomarker study from which manuscript 2 (Chapter 4) is based was designed and conducted by Kristan Aronson, Joan Tranmer, Harriet Richardson, Charles Graham and Anne Grundy.

Chapter 3, Manuscript 1: *Shift Work and Breast Cancer Risk: Results from a Case-Control Study in Canada.* This manuscript is presented formatted for submission to the Journal of the National Cancer Institute (JNCI). Co-authors on this manuscript are: Harriet Richardson, Igor Burstyn, Caroline Lohrisch, Sandip K. SenGupta, Agnes S. Lai, Derrick Lee, John J. Spinelli and Kristan J. Aronson. Interpretation of results and writing of the manuscript was performed by Anne Grundy with supervision from Kristan Aronson and John Spinelli, with feedback from Igor Burstyn, Harriet Richardson, Caroline Lohrisch, Sandip SenGupta, Agnes Lai and Derrick Lee.

Chapter 4, Manuscript 2: *The Influence of Light at Night Exposure on Melatonin Levels among Canadian Rotating Shift Nurses.* This manuscript is presented in the format that was published in Cancer Epidemiology, Biomarkers and Prevention in November 2011 (Citation: Grundy A, Tranmer J, Richardson H, Graham CH, Aronson KJ. The influence of light at night exposure on melatonin levels among Canadian rotating shift nurses. Cancer Epidemiol Biomarkers Prev 2011; 20(11):2404-2412.). Co-authors on this manuscript were Joan Tranmer, Harriet Richardson,

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Chapter 5, Manuscript 3: *Circadian Gene Variants Not Associated With Risk of Breast Cancer.* This manuscript is presented formatted for submission to Cancer Epidemiology, Biomarkers and Prevention. Co-authors on this manuscript are: Johanna Schuetz, Agnes S. Lai, Rozmin Janoo-Gilani, Stephen Leach, Igor Burstyn, Harriet Richardson, Angela Brooks-Wilson, John J. Spinelli and Kristan J. Aronson. Interpretation of results and writing of the manuscript was performed by Anne Grundy with supervision from Kristan Aronson, John Spinelli and Angela Brooks-Wilson, with feedback from Johanna Schuetz, Igor Burstyn, Harriet Richardson, Agnes Lai, Rozmin Janoo-Gilanai and Stephen Leach.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

BMI	Body Mass Index
DDNN	Two Day, two night rotating shift schedule
ER	Estrogen Receptor
FDR	False Discovery Rate
IARC	International Agency for Research on Cancer
IHC	Immunohistochemistry
LAN	Light at night
MCTQ	Munich Chronotype Questionnaire
MEQ	Horne-Ostberg Morningness-Eveningness Questionnaire
NSAIDs	Non-steroidal anti-inflammatory drugs
OR	Odds Ratio
PR	Progesterone Receptor
RR	Relative Risk
SCN	Suprachiasmatic Nucleus
SNP	Single Nucleotide Polymorphism

Chapter 1

General Introduction

1.1 Background and Rationale:

Shift work has been identified as a risk factor for a number of cancer sites (1–4) and in 2007 'shift work involving circadian disruption' was identified as a probable (Group 2A) carcinogen by the International Agency for Research on Cancer (IARC) (5). While epidemiologic studies have suggested associations between shift work and a number of cancer sites (4), the majority of evidence to date has been for breast cancer, where meta-analyses suggest a 40 – 50% increased risk among long-term (20 – 30 years) shift workers (1,2).

When classifying shift work as a 'probable carcinogen', IARC identified that evidence to support this relationship from studies in humans was 'limited', partially due to inconsistencies in the definitions and methods of assessment of shift work across studies (5,6). Two main strategies for shift work exposure assessment have been used. Some studies have used questionnaire-based methods to obtain information concerning shift work history (7–11), while others have utilized a job-exposure matrix-based approach (12–15). From a methodological perspective, questionnaire-based methods have benefitted from shift work information specific to individual study participants, although exposure misclassification may occur if the type of shift work participants have performed does not meet the definition offered in the specific questions. Conversely, studies that have used registry-based job classifications in a job-exposure matrix to classify shift work exposure will not have the same type of misclassification as questionnaires, since these methods are able to capture multiple patterns of shift work. However, there may still be issues of exposure misclassification if, in the absence of data obtained directly from individual study participants, some individuals within job classifications have not actually participated in the proportion of shift work assigned to their group (6). As such, a 2009 IARC Working Group on shift work exposure

assessment identified a need for additional research with more comprehensive methods of shift work exposure assessment (6).

In addition to limitations in shift work assessment in existing studies, the biological mechanism through which shift work influences cancer risk has not been definitively established. A number of factors related to chronodisruption (altered circadian rhythms) have been suggested as possible mechanisms, where, to date, the hypothesized pathway involving light at night exposure and disruption in melatonin has received the most attention in the literature (16). Melatonin is a hormone produced by the pineal gland according to a circadian rhythm, with peak levels observed at night when light is absent (17). Several anti-carcinogenic properties of melatonin have been demonstrated (17,18) and it has been suggested that increased light exposure during night shifts could reduce melatonin levels in shift workers, leading to an increase in cancer risk (19,20). A number of epidemiologic studies examining relationships between melatonin levels and breast cancer risk have been conducted, where results generally support an increased breast cancer risk with lower melatonin levels (21–25).

Investigating the light/melatonin association, experimental studies have indicated a dose-response relationship between light and melatonin, where increases in levels of nighttime light exposure decrease melatonin production (18,26–29). A much smaller number of observational studies of this relationship have been conducted, where some have used night work as a proxy for light exposure (30–35). Among studies where light has been measured directly, results have generally supported an inverse relationship between light and melatonin (30,33,36). However, these observations may be confounded by natural circadian melatonin variations as a result of comparisons of melatonin levels from samples taken at different times when comparing day and night shifts (30,33,36). Thus, additional studies that account for this potential source of confounding are needed to clarify relationships between light and melatonin in an observational setting.

At the molecular level, circadian rhythms are controlled by a set of circadian (clock) genes through a series of positive and negative feedback loops that fluctuate over a 24-hour period (37–39). The central circadian pacemaker is found in the suprachiasmatic nucleus, while the genes involved in circadian clockwork are found in all cells and there are a number of peripheral oscillators throughout the body coordinated through input from the central pacemaker (37). It has been demonstrated that these clock genes act as tumour suppressors and variations in gene expression have been hypothesized to be involved with cancer development and progression (37). Evidence from laboratory research has supported this idea, where clock gene function has been linked with cell cycle progression, the estrogen signaling system and the DNA damage response (40).

Epidemiologic studies have also begun to demonstrate links between clock gene polymorphisms and risk of breast cancer (41–44), prostate cancer (45,46) and non-Hodgkin's lymphoma (47,48). For breast cancer specifically, results from one Connecticut-based case-control study have reported associations with single nucleotide polymorphisms (SNPs) in *NPAS2*, *CLOCK*, *TIMELESS* and *CRY2* (41–44); however, no significant association between circadian gene SNPs and breast cancer was observed in recent analysis of the Nurses' Health Study (49). Given these differential results, and that associations have only been investigated in two distinct populations, further study is needed to clarify what, if any, relationships exist between clock gene variants and breast cancer risk. Further, as circadian disruption has been suggested as a mechanism that could explain the observed increased risk of breast cancer among shift workers (1,2,4), a role for circadian genes in the shift work-breast cancer relationship has been suggested (16,50). To date, only one breast cancer study has considered interactions between shift work and clock gene variants, where an interaction for one SNP (rs2305160 in *NPAS2*) with shift work was identified (49). As such, although these results provide preliminary evidence for a possible interaction, additional research is needed.

1.2 Overview of Thesis and Study Designs:

This thesis will address the potential relationship between shift work and breast cancer risk at several points along the proposed causal pathway, using data from two separate studies. The associations of both shift work and clock gene polymorphisms (and their interactions) with breast cancer risk will be examined in the context of a large case-control study, while a longitudinal biomarker study will be used to investigate associations of light at night exposure and shift work with melatonin production among rotating shift nurses.

The breast cancer case-control study collected information from both breast cancer cases and healthy controls in Vancouver, British Columbia and Kingston, Ontario. In Vancouver, women diagnosed with both *in situ* and invasive breast cancer were recruited from the British Columbia (BC) Cancer Registry and healthy controls were recruited from the Screening Mammography Program of BC through breast screening clinics. In Kingston, both cases and controls were recruited from the Hotel Dieu Breast Assessment Program, where cases were also women diagnosed with either *in situ* or invasive breast cancer and controls were women with normal mammogram results or benign breast disease. A total of 1,142 breast cancer cases and 1,178 controls from both Vancouver and Kingston were included in the final analysis. All study participants were asked to both complete a study questionnaire and provide a blood sample. Shift work history was obtained from the lifetime occupational history included in the questionnaire and DNA extracted from a portion of the blood sample was used for genotyping of clock gene variants.

The biomarker study was conducted among a group of rotating shift nurses at Kingston General Hospital working a two-day, two-night rotating shift pattern. Nurses participated in the study on both a day and a night shift, in each of the summer and winter seasons. Each participation session was 48 hours in length, covering either a day or a night shift. Light exposure data was collected using a light data logger worn by study participants for the entirety of each participation session. Melatonin data was collected from two urine and four saliva samples

collected over a 24-hour period covering either the day or night shift. Women also completed a study questionnaire at the beginning of the study and a diary during each participation session, where shift work history was obtained from the study questionnaire.

1.3 Thesis Objectives:

This thesis will address three main objectives. These are, to investigate:

1) The association of shift work history with breast cancer risk;
2) The relationships between light at night exposure, shift work history, and urinary and salivary melatonin levels among rotating shift nurses; and
3) The association of clock gene variants with breast cancer risk and to explore interactions between clock gene variants and shift work in this relationship.

1.4 Thesis Organization:

This thesis is structured as a manuscript-based thesis. The next chapter, prior to the manuscripts, contains a literature review which provides a general overview of the shift work-cancer relationship and potential mechanisms that may explain this association. Specifically, this chapter addresses the relationship between shift work and breast cancer, as well as briefly touches on other cancer sites; describes hypothesized mechanisms, with a detailed focus on the role of light exposure, melatonin and clock genes; and, addresses limitations of current research. Following the literature review, three manuscripts are included. The first manuscript addresses the relationship between shift work history and breast cancer risk, specifically examining the hypothesis that an increased risk of breast cancer will be observed among long-term shift workers. The second manuscript addresses the influence of nighttime light exposure and shift work history on melatonin levels among rotating shift nurses, where we hypothesized that both light and shift work history would be associated with decreases in melatonin production. The

third manuscript assesses the association of clock gene polymorphisms with breast cancer risk and explores potential interactions between clock gene polymorphisms and shift work history. Finally, the last chapter of this thesis is a general discussion of the main findings from each of the three manuscripts and includes a discussion of overall conclusions and future research directions suggested by the thesis as a whole.

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Chapter 2

Literature Review

2.1 Epidemiology of Breast Cancer:

Breast cancer is the leading cause of cancer incidence and second leading cause of cancer death among Canadian women, with an estimated 22,700 new cases and 5,100 deaths in Canada in 2012 (1). While the most well established risk factor for breast cancer is age, a number of other demographic, reproductive, genetic, lifestyle and environmental factors have also been suggested to be causal (2–5), which are covered in greater detail in Section 2.7.1.

Breast cancer has often been studied as a single disease, although advances in understanding of disease etiology and biology have indicated it may be more heterogeneous, with a number of specific subtypes (4). For example, it is now common to distinguish whether breast cancer is diagnosed before or after a woman has reached menopause, where premenopausal breast cancers are more likely to be aggressive and five year survival rates are lower for women diagnosed before age 40, compared to those diagnosed after age 60 (4). Breast cancers are also further categorized on the basis of tumour characteristics, including whether they display estrogen and progesterone receptors (4). Individuals with hormone receptor positive tumours are more likely to benefit from hormonal therapies such as tamoxifen and differences in etiology have also been suggested for hormone receptor positive and negative tumours, where the impact of several risk factors, such as parity and age at first birth, vary between tumour groups (4,6,7).

2.2 Shift Work and Cancer:

Shift work has been suggested as a risk factor for a number of cancer sites (8–11), where Statistics Canada surveys estimate the prevalence of shift work in the Canadian population to be between 11% and 28% (12,13). In 2007 long-term ‘shift work that involves circadian disruption’

was classified by the International Agency for Research on Cancer (IARC) as a probable (Group 2A) carcinogen, where this classification was based on 'sufficient evidence in experimental animals' and 'limited evidence in humans'(14). While the majority of existing epidemiologic studies have focused on relationships with breast cancer (15–27), there is more limited evidence linking shift work with prostate cancer (28–30), endometrial cancer (31), colon cancer (32), ovarian cancer (33) and non-Hodgkin's lymphoma (34).

2.2.1 Breast Cancer Studies:

Studies of breast cancer have generally supported an association with shift work, with results from two meta-analyses suggesting a 40 – 50% increased risk among long-term shift workers (8,9). However, the definition and methods of assessment of shift work across these studies have been less consistent. Several studies have used self-reported responses to specific questions to classify participants as shift workers (15–17,23,24). One study using data from the Nurses' Health Study found an increased risk among women who had worked rotating shifts (defined as at least three nights per month) for 30 or more years (RR=1.36, 95% CI=1.04 – 1.78) (16). This pattern remained following stratification by menopausal status, with an increased risk among postmenopausal women (≥30 years RR=1.36, 95% CI=1.04 – 1.78) and an elevated, although not statistically significant, risk among premenopausal women (≥20 years RR=1.66, 95% CI=0.81 – 3.40) (16). Similar results were seen among primarily premenopausal women from the Nurses' Health Study II cohort, with an increased risk of breast cancer among women who had worked 20 or more years of rotating shift work (RR=1.79, 95% CI=1.06 – 3.01) (17). In case-control analysis, Davis *et al.* observed an increased risk of breast cancer among women who reported ever having worked the 'graveyard shift' (OR=1.6, 95% CI=1.0 – 2.5) (15), while O'Leary *et al.* reported no association between any shift work and breast cancer and a protective relationship (OR=0.55, 95% CI=0.32 – 0.94) with overnight shift work among participants from the Electromagnetic Fields and Breast Cancer on Long Island study (23). More recently, results from

the German GENICA study did not demonstrate an association between ever having performed night work (any work between midnight and 5AM) and breast cancer, and although an elevated odds ratio was detected for 20 or more years of night work (OR=2.48, 95% CI=0.27 – 1.41), this relationship was not statistically significant (24). While measurement of shift work in all of these studies benefits from information specific to individual participants, exposure misclassification may occur if the shift work performed by individual study participants does not match the definition provided in the question.

In contrast, other studies have used job-exposure matrix-based methods with registry-based job classifications to define shift work history (18–20,25–27). An early case-control study among Norwegian radio and telegraph operators detected an exposure-response relationship between length of employment in shift work and breast cancer risk, where records of job histories were used to characterize shift work (26). Further, a Danish case-control study used employment data from the national pension fund to classify individuals as shift workers if in their trade at least 60% of respondents to a separate survey reported night work: in this study, an elevated risk of breast cancer was detected among women with a history of night work (OR=1.5, 95% CI=1.3 – 1.7) (18). However, a similar registry-based study conducted in Sweden found no association between shift work and breast cancer (20), and a Japanese cohort study using a job-exposure matrix to classify shift work found no increased risk of breast cancer for either ever or long duration night workers (20 years HR=1.0, 95% CI=0.8 – 1.2; 30 years HR=1.1, 95% CI=0.8-1.2) (25). Several studies have been conducted specifically among nurses, where employment in hospitals was used to define individuals as shift workers (19,27). While an increased risk of breast cancer was observed among nurses in Norway who worked 30 or more years of shift work (OR=2.21, 95% CI=1.10 – 4.45) (19), in a Danish cohort a tendency of increasing risk of breast cancer was seen for up to 25 years of work in a hospital, but at greater than 25 years the odds ratio was in the opposite direction (>25 years RR=0.7, 95% CI=0.5 – 1.1) (27). These registry-based studies are not subject to the same type of exposure misclassification as questionnaires

since the job-exposure matrix methods are able to capture multiple patterns of shift work. However, there may still be issues of exposure misclassification. For example, in the absence of data obtained from directly from study participants through the use of administrative datasets, some individuals within job classification groups may not have actually worked the proportion of shift work assigned to their group (35).

A number of studies have also considered breast cancer risk in flight personnel, where circadian disruption due to travel across time zones may influence cancer risk similarly to work at night among shift workers (36–44). However, a meta-analysis has grouped these studies separately from those of shift workers due to the potential for other occupation-specific exposures, such as cosmic radiation, in airline personnel (9). Most studies of airline workers compare rates of cancer to those of the general population using standardized incidence or mortality ratios (36–44). While some detected an elevated risk of breast cancer among flight personnel with risk increasing with longer duration of employment (37–39,42), in other studies this increased SMR/SIR was not-significant as the 95% confidence intervals included the null value (40,41,43), and two studies found no difference in breast cancer risk among flight attendants compared to the general population (36,44). However, despite these inconsistencies, when taken together in meta-analysis an increased risk of breast cancer (RR=1.7, 95% CI=1.4 – 2.1) among flight personnel was detected (9).

2.2.2 Other Cancer Sites:

In addition to breast cancer, a more limited number of studies have investigated associations between shift work and other cancer sites (20,27–29,31–34). Two separate studies of rotating shift work in Canada and Japan detected an elevated risk of prostate cancer (28,29), however, a third registry-based study in Sweden found no association between shift work and prostate cancer risk (20). A more recent Japanese study of prostate cancer risk also detected an

elevated, although not significant, increased risk with rotating shift work (RR=1.79, 95% CI=0.57 – 5.68), but was underpowered to detect effects with only 17 prostate cancer cases across both exposure groups (30). Analysis from the Nurses Health Study detected increased risks of both colorectal (≥15 years RR=1.35, 95% CI=1.03 – 1.77) (32) and endometrial cancer (≥20 years RR=1.47, 95% CI=1.03 – 2.10) (31) among long-term rotating shift workers, although no association was seen with ovarian cancer (33). Finally, registry-based analysis of Danish nurses detected an increased risk of skin melanoma, other skin cancers, and cancers of the brain and nervous system compared to the Danish general population (27), while a Finnish census-based study using the FINJEM job-exposure matrix detected an elevated risk of non-Hodgkin's lymphoma among men, but not women, with a history of night work (34).

2.2.3 Challenges in Shift Work Exposure Assessment:

When classifying 'shift work that involves circadian disruption' as a probable carcinogen, the IARC Monographs Working Group identified inconsistent definitions of shift work across studies as one of the main limitations of the existing epidemiologic evidence (14). To address this limitation, a 2009 IARC Working Group identified a need for more comprehensive methods of shift work exposure assessment (35). Shift system (type of shift, direction of rotation, regular or irregular schedule, work hours, number of consecutive nights); number of years on a specific shift schedule and cumulative exposure over a person's working life; and, shift intensity (number of days off between work days) were identified as the main exposure domains to be captured in epidemiologic research (35).

In response to this need, two more recent epidemiologic studies attempting to capture these shift work characteristics have examined breast cancer risk among nurses (21,22). While Lie *et al.* did not detect any relationship with breast cancer for previously used exposure metrics, they did detect an increased risk among nurses who had worked a minimum of 5 years in shift schedules with ≥6 (OR=1.8, 95% CI=1.1 – 2.8) and ≥7 (OR=1.7, 95% CI=1.1 – 2.8) consecutive

nights (21). Hansen and Stevens also detected elevated risks for specific shift systems among Danish nurses, where elevated risks were observed for ever having worked after midnight in rotating shifts without permanent nights (OR=1.8, 95% CI=1.2 – 2.8) and for ever having worked permanent nights in addition to rotating shifts (OR=2.9, 95% CI=1.1 – 8.0) (22). They also found an impact of duration of work on breast cancer risk, where elevated odds ratios were detected for work on the graveyard shift (work after midnight) for all durations greater than five years (22). These results demonstrate the importance of considering specific shift work characteristics in future epidemiologic studies, such that shift patterns that may have a greater impact on cancer risk can be identified.

2.3 Mechanisms Linking Shift Work and Cancer:

Despite having been classified as a 'probable carcinogen', the exact biological mechanism through which shift work influences cancer risk is still unknown. However, a number of mechanisms associated with chronodisruption (altered circadian rhythms) as a result of shift work that may lead to an increased risk of cancer have been proposed (45). To date, the mechanism that has received the most attention in the literature is the melatonin hypothesis, where it has been suggested that increased environmental light at night (LAN) exposure during night shifts could be responsible for observed increased risks of breast cancer through a reduction in nighttime melatonin (46). The details and evidence to date for this particular mechanism will be discussed in more detail in the next section of the literature review (Section 2.3).

Phase shift or phase desynchronization, in which the rhythms of peripheral functions are out of phase with central sleep/wake patterns, has also been suggested as a possible mechanism through which shift work may influence cancer risk (45,47). While details of the molecular coordination of circadian rhythms will be provided later in the literature review (Section 2.4.1), briefly, the timing of the central circadian oscillator is set primarily by the daily light/dark cycle

and to a lesser degree by social routine, physical exercise and food intake patterns (47). When an individual is required to adapt to a new activity-sleep schedule, such as when engaging in shift work, they must undergo a phase adjustment and during this time there will be a period of internal desynchronization where adaptation of central circadian oscillators will occur prior to adaptation in peripheral tissues (47). At the molecular level, links between circadian rhythms and the cell cycle (which are described in more detail in Section 2.5.1) have been suggested as one mechanism through which this internal desynchronization in circadian phase may contribute to cancer growth and development (45,47).

Sleep disruption is a third mechanism that has been suggested to help explain the shift work – cancer relationship (45,48). Several studies comparing individuals working day and night shifts have observed shorter sleep duration (49,50) and more sleep disturbances (50) on the night shift and there are several possible pathways through which sleep changes could influence cancer risk. Reduced melatonin production with shorter sleep duration has been suggested as one potential mechanism, as sleep laboratory studies have demonstrated duration of melatonin production is greater among longer sleepers (48). However, epidemiologic studies have failed to demonstrate a relationship between sleep duration and melatonin (45,49) and, in one study that did find one, samples from which melatonin was measured were not collected at a consistent time of day (51). Sleep may also influence cancer risk through changes in immune function, where sleep disturbances may lead to a shift in the balance of cytokine production from anti-cancer type 1 cytokines to type 2 cancer stimulatory cytokines (48). However, studies of sleep deprivation and immunity have been limited by inconsistent measures of immune function and, as sleep deprivation often occurs in combination with stress and changes in factors such as eating patterns, body temperature and hormone levels, isolating the specific influence of sleep disruption on immune function has been difficult (45,52). A third pathway through which sleep disturbances could influence cancer is through metabolic changes that may lead to obesity. Although several review articles indicate an association between short sleep duration and obesity in both cross-

sectional and prospective studies, the use of self-reported sleep duration in the majority of these studies is seen as a substantial limitation of the existing research (45,53–55).

Finally, lifestyle changes associated with shift work including poor diet, smoking and alcohol use, physical inactivity, obesity and reduced sun exposure have been suggested as mechanisms that may increase cancer risk (45). Recent results from the Nurses' Health Study have indicated that women with more years spent in rotating shift work were more likely to have a higher BMI and to be current smokers (56), supporting previous evidence from other cohorts (57). Further, it has been suggested that individuals working a nighttime schedule may spend less time outdoors and thus have reduced sun exposure, potentially leading to reductions in vitamin D levels (45). However, links between vitamin D and cancer have not been definitively established and there are no studies comparing vitamin D levels in shift workers and non-shift workers (45).

2.4 Light, Melatonin and Cancer Risk:

2.4.1 Melatonin and Cancer:

Melatonin is a hormone produced by the pineal gland in a pattern that varies within individuals over a 24-hour period, with peak levels observed at night when light is absent (58,59). Melatonin synthesis occurs through the conversion of tryptophan to serotonin, which is then converted to melatonin through a series of enzymatic reactions, the rate-limiting step of which is catalyzed by the enzyme arylakylamine-*N*-acetyl-transferase (58,59). The half life of melatonin in blood is quite short, in the range of 30 – 60 minutes, and most melatonin is cleared from circulation with one passage through the liver (58). Melatonin concentrations can be assessed directly from blood and saliva samples, while concentrations of 6-sulfatoxymelatonin, the primary melatonin metabolite, can be easily measured in urine (60). While there is considerable natural variation in melatonin levels between individuals, with peak levels of 18 – 40 pg/mL in low secretors and peaks of 54 – 75 pg/mL in high secretors, within individuals both the phase and

amplitude of the melatonin rhythm are fairly constant (58). Melatonin production is inhibited by light and environmental light at night exposure during night shifts is thought to decrease melatonin levels (46,61).

Laboratory-based studies have demonstrated several oncostatic properties of melatonin in both animal and cell culture models. In MCF-7 breast cancer cells, at physiological concentrations melatonin has been shown to decrease cell proliferation and invasiveness by decreasing cell attachment and cell motility (62,63). Human breast cancer xenografts perfused *in situ* with melatonin-rich blood collected from premenopausal women at night also exhibited suppressed signal-transduction and linoleic acid-dependent proliferation compared to controls perfused with melatonin-deficient blood collected during the day, while xenografts perfused with melatonin-defiicient blood collected from women exposed to light during the night were similar to controls (64). Animal models of chemically-induced carcinogenesis have also demonstrated decreases in both initiation and growth of several tumour types following melatonin administration (65–67). Further, in rats who had undergone pinealectomy (removal of the pineal gland), 88% of pinealectomized animals had tumours on the 240th day following tumour induction, compared to only 22% incidence in intact animals (67).

There are several mechanisms through which melatonin may inhibit cancer development. Melatonin is an antioxidant and as such, could reduce DNA damage that could lead to cancer (58). Experimental research has also demonstrated the anti-proliferative properties of melatonin, which in breast cancer cells appears to be mediated through the MT_1 melatonin receptor, a G-protein coupled receptor that is able to regulate signal transduction and gene expression (68). It has been further suggested that melatonin may inhibit tumour growth through enhancing immune function (69). Finally, melatonin may be linked to breast cancer through the estrogen-signaling pathway. Melatonin reduces activity of aromatases in MCF-7 breast cancer cells that convert androgens to estrogen and animals with pinealectomy or decreased melatonin levels have higher levels of serum estrogen, folliclar stimulating hormone and luteinizing hormone (70).

Several epidemiologic studies have also investigated links between melatonin and breast cancer risk. Case-control studies have compared melatonin production in breast cancer cases to that of healthy women (71,72). One study found that median levels of nocturnal 6-sulfatoxymelatonin, measured from overnight urine samples, were significantly lower among breast cancer cases compared to age-matched controls and that this pattern also corresponded to tumour stage, where $T_{3/4}$ and T_2 tumours had melatonin levels 71% and 40% lower compared to controls, while T_1 melatonin levels were 15% higher (71). Another earlier case-control study found that while both breast cancer cases and controls exhibited a characteristic diurnal melatonin rhythm, the mean day-night melatonin difference was significantly lower for estrogen (ER) and progesterone (PR) receptor positive breast cancer cases compared to ER/PR negative cases and controls (72). However, in both these studies the sample sizes were quite small and the case-control design prevented them from establishing a temporal relationship between changes in melatonin production and cancer development, as the measurement of melatonin levels following the breast cancer diagnosis meant that it was impossible to assess whether changes in melatonin production occurred prior to cancer development or as a result of it.

Prospective studies have also examined relationships between breast cancer and melatonin and have generally demonstrated an inverse relationship. In the Nurses' Health Study II cohort, where melatonin production was measured from morning spot urine samples, a marginally significant protective relationship with breast cancer risk was demonstrated in primarily premenopausal women in the highest quartile of melatonin production (OR=0.59, 95% CI=0.34 – 1.00) (73). A similar relationship was also seen for postmenopausal women in the highest quartile of melatonin production in both the Nurses' Health Study (OR=0.62, 95% CI=0.41 – 0.95) and the Euorpean ORDET cohort (OR=0.56, 95% CI=0.33 – 0.97) (74,75). However, an earlier British cohort found no relationship between melatonin and breast cancer where melatonin levels were similar for both pre and postmenopausal cases and controls (76) and no association between melatonin and breast cancer risk was detected among premenopausal

women in the ORDET cohort (OR=1.43, 95% CI=0.83 – 2.45) (77). The differential results from the British study may be partially explained by differences in exposure assessment methods, where the 24-hour urine samples used were unable to capture peak melatonin levels as can be done with the spot morning urine samples used in the other studies, making direct comparisons between studies difficult (78). Further, the authors of the study of premenopausal women in the ORDET cohort suggest the presence of preclinical disease may have influenced their findings, as the association became increasingly inverse when a lag time between melatonin assessment and breast cancer incidence was introduced (77). Therefore, while these results generally support an inverse relationship between melatonin production and breast cancer risk, the characterization of melatonin most relevant to cancer risk is still unclear and thus at this time the relationship cannot be considered causal (45).

2.4.2 Light and Melatonin:

The release of melatonin from the pineal gland is stimulated by darkness and inhibited by light (58) and experimental studies in humans have demonstrated a dose-response relationship between light exposure and melatonin levels (59,79–82). These studies have generally indicated that light levels of 250 – 400 lux were required to suppress melatonin (80,81), though more recent results found that although suppression of melatonin clearly occurred at illuminances greater than 200 lux, variable suppression was observed for illuminances of 80 – 200 lux (82). However, all these studies were conducted in a sleep laboratory setting and in many participants were required to look directly at the light source during periods of illumination (59,79–82), circumstances that are unlikely to be replicated outside of the laboratory setting. Thus, while these results demonstrate a clear inhibitory effect of light exposure on melatonin in humans, evidence from observational studies is required to determine if similar effects are seen among individuals exposed to light at night in the workplace.

Most epidemiologic studies of the light – melatonin relationship have used populations of shift workers, where multiple methods including questionnaires regarding shift work, as well as direct light intensity measurements have been used to assess light exposure (49,50,83–86). When night work is used as a proxy for light at night exposure, women in the Nurses Health Study who had worked at night in the previous two weeks displayed significant reductions in melatonin levels (83) and similar results were observed in a Danish study where nurses working the night shift had lower urinary melatonin levels during a workday compared to those on a day shift (85). However, long-term history of shift work was not associated with melatonin in the Nurses' Health Study (87) and ever having worked the 'graveyard shift' was not associated with melatonin in a Japanese cohort (86). These results are potentially limited by the use of shift work as a proxy for light exposure, as relationships may be confounded by other behaviours associated with shift work that are also related to melatonin production, thus studies with direct measurements of light intensity are required.

Among studies with direct measures of light exposure, results have been somewhat inconsistent, although several have supported the inverse relationship between light and melatonin observed in experimental settings. In a study of individuals on permanent day, evening and night shifts, Burch *et al.* observed higher 24-hour light exposure among individuals on the evening shift and also found that melatonin levels were lower after sleeping and higher after working for individuals on the night shift compared to the day shift (50). Similar to this, a Canadian study comparing rotating shift workers to permanent day workers found that individuals on rotating shifts had different patterns of light exposure and that when working at night, melatonin levels were higher upon arising and during work but lower during sleep compared to permanent day workers (84). More recently, a study among rotating shift nurses observed a statistically significant relationship between light and melatonin levels following sleep, however this relationship was no longer significant when results were stratified by shift type to account for the fact that urine samples were obtained at different times of day for individuals working day

and night shifts (49). This finding is relevant as it suggests that differences in melatonin levels observed between individuals sleeping during the day compared to those sleeping at night in previous studies (49,50,84) could be at least partially confounded by natural circadian variations in melatonin levels. As such, there is a need for further research that carefully considers the timing of melatonin assessment to account for this source of uncontrolled confounding in order to clarify the relationship between light and melatonin in an observational setting.

2.5 Characterizing Circadian Rhythms:

2.5.1 Molecular Coordination of Circadian Rhythms:

Circadian rhythms, defined as "daily oscillations in physiological and behavioural processes" (88), are controlled by the suprachiasmatic nucleus (SCN) in the anterior hypothalamus (88,89). At the molecular level, circadian rhythms are coordinated through the interaction of a set of circadian (or clock) genes in a series of positive and negative feedback loops which fluctuate in a 24-hour cycle (88–90). The main genes involved in this mechanism are *CLOCK*, *BMAL1* (also called *ARNTL*), *CRY1, CRY2*, *PER1, PER2, PER3*, *NR1D1* (*Rev-Erbα)* and *CSNK1E* (Figure 2.1) (88). The negative feedback loop begins with the binding of CLOCK/BMAL1 heterodimers to promoters of *CRY*, *PER* and *Rev-Erbα*, leading to an increase in transcription of these genes (89). As PER and CRY proteins accumulate in the cytoplasm they come together and are phosphorylated by CSNK1E, before being translocated back into the nucleus where they inhibit the activity of CLOCK/BMAL1 heterodimers, leading to a decrease in *CRY* and *PER* transcription (89). The positive feedback loop involves the regulation of *BMAL1* transcription (88). *Rev-Erbα*, whose transcription is up-regulated by the binding of CLOCK/BMAL1 heterodimers to its promoter, acts as an inhibitor of *BMAL1* transcription, meaning increased production of Rev-Erbα, due to CLOCK/BMAL1, leads to a decrease in BMAL1 production (88,89). However, the inhibition of CLOCK/BMAL1 by PER and CRY

proteins also inhibits the production of Rev-Erbα, which removes the inhibition of *BMAL1* transcription, leading to an increase in BMAL1 production (88,89). Eventually this BMAL1 is able to combine with CLOCK to produce new CLOCK/BMAL1 heterodimers, which are then able to promote transcription of *CRY*, *PER* and *Rev-Erbα* (88,89). The interaction of these feedback loops ensures low levels of PER and CRY and high levels of BMAL1 at the beginning of the circadian day (88). In addition to these genes, other genes including *TIMELESS* (a mammalian homolog of the protein TIM from *Drosophila*) and *DEC1* and *DEC2* (transcriptional repressors for CLOCK-BMAL1 induced promoter activity) appear to have a role in the functioning of the molecular clock (91–94). However, although a role for *TIMELESS* in connecting the circadian and cell cycles has been suggested (91), the precise role of these components is still unclear (92–94).

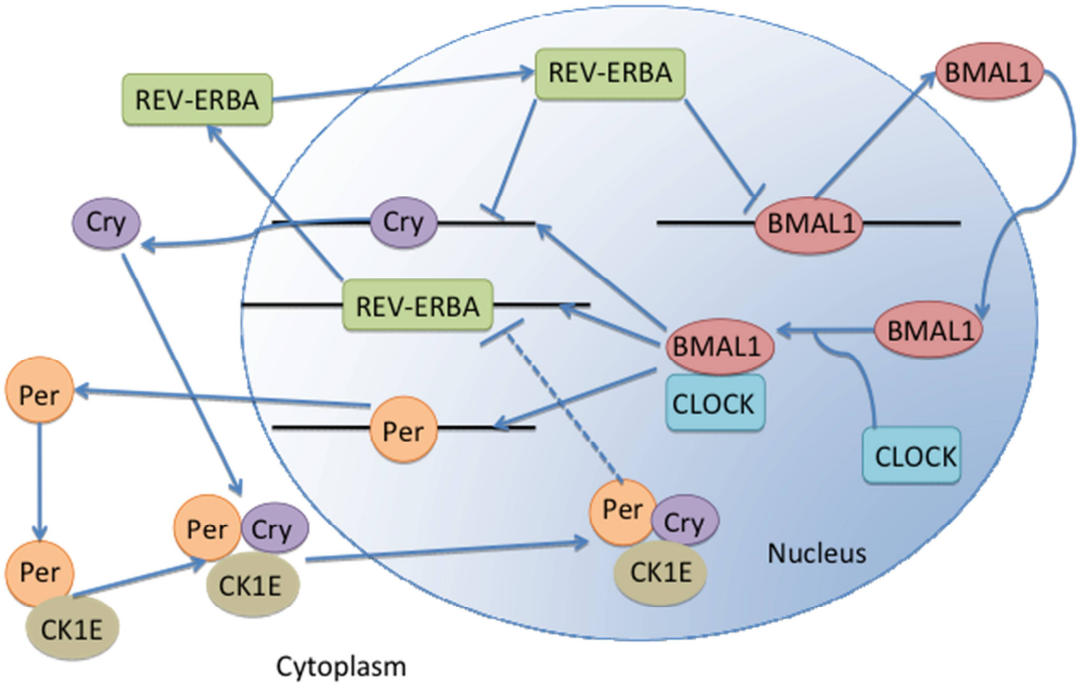

Figure 2.1: Interaction of Clock Genes.
Adapted from: Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. Nat Rev Cancer 2003; 3:350-361. (79)

The genes involved in the circadian clockwork are found in all cells, as there are several peripheral oscillators throughout the body, whose rhythms are synchronized by input from the central pacemaker in the SCN (89). The central clock in the SCN is synchronized to daily light fluctuations, where changes in lighting levels influence the output of the SCN-based circadian clock. This in turn impacts the physiological processes under circadian control, such as the production of melatonin by the pineal gland (95). Light on the retina is transmitted to the SCN via the retinohypothalamic tract and can affect expression patterns of circadian genes, leading to the entrainment of the SCN to light/dark patterns (89).

2.5.2 Melatonin as a Biomarker of Circadian Rhythms:

There are multiple functions, both behavioural and physiological, that are regulated by the SCN and thus follow distinct circadian rhythms (96). These include sleep-wake cycles, body temperature and multiple endocrine functions, including melatonin production (96). Studies that wish to characterize circadian rhythms use measurements of these circadian outputs to describe variations in circadian stage, with core body temperature and melatonin being the most commonly used measurements (97–100). While measurement of body temperature is generally less invasive than measurements of melatonin, for which saliva, urine or blood samples are required, temperature rhythms are influenced by multiple environmental factors, such as sleep, posture, exercise patterns and meal timing, as well as output from the SCN (98). Due to this potential for environmental influences, previous studies have recommended the use of a constant routine protocol for study subjects when circadian rhythms are characterized using temperature rhythms (97,98). Conversely, melatonin rhythms have been shown to be resistant to many of these influences, with output from the SCN, the central circadian pacemaker, being the primary

driving force behind melatonin rhythms and thus are considered a better measure of individual circadian rhythms (97–100). In observational studies where constant routine protocols are not possible, melatonin therefore represents the most appropriate measure of circadian rhythms.

2.5.3 Chronotype:

The synchronization of the central circadian clock in the SCN (described in section 2.4.1) to a 24-hour cycle by external cues or zeitgebers is a process known as entrainment (101). Individuals each have specific temporal relationships with zeitgebers and this relationship, the difference between internal and external time, is known as the phase of entrainment and differences in this trait between individuals are referred to as differences in chronotypes (101). Chronotype, which generally refers to human preferences in the timing of sleep and wake, is usually measured through questionnaires (101–103). The most common of these is the Horne-Ostberg Morningness-Eveningness Questionnaire (MEQ) (104), which evaluates timing of an individual's 'feeling best' rhythm, although a number of new questionnaires have been developed in recent years including the Munich ChronoType Questionnaire (MCTQ) which examines sleep and activity patterns separately on both work and free days to characterize chronotype (102). Chronotype generally becomes earlier with age (101) and is considered to be at least partly determined by genetics, as several clock gene polymorphisms have been linked with diurnal preference (105–111). It has recently been suggested that tolerance of shift work may be related to chronotype such that this factor should be considered in studies examining relationships between shift work and chronic disease outcomes such as cancer (103).

2.6 Clock Genes and Cancer:

2.6.1 Experimental Evidence:

It has been demonstrated that clock genes act as tumour suppressors and variations in gene expression may be involved development and progression of cancer (88). Laboratory studies

in cell line and animal models have linked cell cycle progression, the estrogen signaling system, and the DNA damage response to clock gene function (112–123). Specifically, one study demonstrated that down-regulation of *PER2* produced accelerated mammary cell proliferation in cell culture, as well as accelerated tumour growth in mice, where differences in the growth dynamics of tumour cells were apparent in both experimental models (119). Over-expression of *PER2* was also linked to decreased growth of Lewis lung carcinoma (LLC) and murine mammary carcinoma (EMT6) cell lines, with higher apoptotic peaks in *PER2* expressing cells compared to controls (120). This effect was also seen in MCF-7 breast cancer cells and became even stronger in cells expressing both *PER2* and *CRY2* (123). *PER2* over-expressing cells have also demonstrated up-regulation of tumour suppressor genes *p53* and *Bax* and downregulation of oncogenes genes *c-Myc*, *Bcl-*X_L and *Bcl-2* (120). In addition, *NPAS2* depleted cells do not exhibit the expected cell-cycle delay in response to mutagen treatment and knockdown of *NPAS2* significantly represses expression of several cell-cycle and DNA repair genes (114). Finally, over-expression of *PER1* makes colon cancer cells more susceptible to radiation-induced apoptosis, further supporting a role for the circadian clock in coordinating cell-cycle related events (121).

Studies of tissue samples from several cancers including breast have also demonstrated alterations in clock gene expression compared to normal tissue (124–128). In breast cancer, one study found that 48/55 pairs of breast cancerous and non-cancerous tissues showed differential expression of one or two PER proteins in cancer cells and that the *PER1* promoter was methylated in 56.1% of cancerous tissues compared with 29.1% of normal tissues, where *PER1* promoter methylation was correlated with *c-erbB2* (Her2/neu) expression (127). Further, another study demonstrated that 37/53 breast cancer tissues had hypermethylation of the promoters of at least one of *PER1*, *PER2*, *CRY1* and *BMAL1* (124). The study also found that expression of circadian genes in normal tissues obtained at similar clock times was relatively homogeneous, suggesting cells are at similar stages of circadian rhythms, while expression in cancerous tissues

was more heterogeneous, suggesting different circadian clocks in different populations of cancer cells (124). Different expression patterns between cancerous and non-cancerous tissues have also been observed in chronic myeloid leukemia and hepatocellular carcinoma (125,126). As well, the heterogeneous expression patterns of clock genes within cancer tissues observed in breast cancer were also seen in hepatocellular carcinoma samples (126). These results, taken together with those of studies characterizing the molecular role of clock genes (112–123), provide relatively persuasive evidence for a role for clock genes in cancer development.

2.6.2 Epidemiologic Evidence:

Epidemiologic studies have demonstrated associations of clock gene polymorphisms with breast cancer (128–132), prostate cancer (133,134) and non-Hodgkin's lymphoma (135,136). The earliest studies of these associations focused on two specific variants: a tandem repeat in *PER3* with four or five copies of a 54-bp repetitive sequence in exon 18 (131,133) and a non-synonymous A/G single nucleotide polymorphism (SNP) in *NPAS2* (rs2305160) which changes the Ala at codon 394 to Thr (132,133,136). The 5-repeat form of the *PER3* polymorphism has been associated with an increased risk of breast cancer among pre-menopausal women (OR = 1.7, 95% CI = 1.0-3.0) (131) and an increased risk of prostate cancer has been suggested (OR = 1.3, 95% CI = 0.9-2.1) (133). Conversely, the heterozygous form of the *NPAS2* Ala394Thr SNP has been shown to reduce risk of prostate cancer, breast cancer and non-Hodgkin's lymphoma (132,133,136).

Recent studies of non-Hodgkin's lymphoma, prostate and breast cancer have taken a broader candidate gene approach to investigating the association of clock gene variants with cancer, demonstrating potential associations for several variants (128–130,134,135). The major findings of these more recent studies are summarized in Table 2.1. The lymphoma study found significantly increased risks for three SNPs in *CRY2* (135) and the prostate cancer study found increased risk for at least one SNP in each of *PER1*, *PER2*, *PER3*, *CSNK1E*, *CRY1*, *ARNTL*, and

NPAS2 and protective relationships for SNPs in *PER1*, *CRY2*, *CLOCK* and *NPSAS2* (133). More recently, several studies have examined associations of SNPs in *CLOCK*, *CRY2* and *TIMELESS* with breast cancer among participants in a Connecticut case-control study (128–130). The *CLOCK* study found four SNPs associated with breast cancer risk; however, this relationship did appear to differ according to tumor hormone receptor status, as six of nine SNPs tested were significantly associated with risk of estrogen (ER) and progesterone (PR) receptor negative tumors, while none were associated with ER/PR-positive tumors (128). Differences in associations according to tumour ER and PR status were also observed for *CRY2* and *TIMELESS* (129,130). As with *CLOCK*, associations were seen for ER/PR negative but not positive tumours for *CRY2* SNPs , while the opposite pattern was observed for *TIMELESS* SNPs, where ER/PR positive but not negative tumours displayed associations with breast cancer (129,130).

While previous studies had examined only a small number of specific variants (131–133,136), these more recent studies have identified SNPs using a Tagging algorithm (128–130,134,135,137). Given that the molecular mechanism through which variations in clock genes may influence the carcinogenic process is unknown (88), this broader approach to SNP identification provides a greater opportunity to identify variants associated with cancer risk (138). However, particularly for breast cancer where most research has occurred within one case-control population (128–132), given the number of genetic variants investigated in these more recent studies and the potential for chance findings, further research in different populations is required. Recent analysis from the Nurses' Health Study investigating associations of nine circadian genes with breast cancer risk has begun to address this need for analysis in different populations and in contrast to previous findings, did not detect any significant relationships between circadian gene variants and breast cancer risk (137). However, this study did detect an interaction with history of shift work for rs2305160 in *NPAS2*, where the heterozygous form of the rs2305160 variant was protective among women with <2 years history of rotating shift work (OR=0.58, 95%CI=0.42 – 0.81) but not significantly associated with risk among those with ≥2 years of rotating shift work

(OR=0.76, 95%CI=0.53 – 1.09) (137). Further when shift work history was compared within genotypes, odds of breast cancer were higher for women with ≥2 years of rotating shift work for both the heterozygous (Ala/Thr) (OR=1.31, 95%CI=0.91 – 1.88) and homozygous (Thr/Thr) (OR=2.83, 95%CI=1.47 – 5.56) genotypes when compared to women with <2 years history of rotating shift work (137). This is the first study to investigate potential interactions with shift work and provides preliminary support for the hypothesis that there could be potential for gene-environment interactions between shift work and clock gene variants (139,140) in associations with cancer risk.

Table 2.1: Published Associations Between Circadian Gene Single Nucleotide Polymorphisms and Cancer

	Cancer Site		
	Breast Cancer	**Prostate Cancer**	**Non-Hodgkin's Lymphoma**
SNP	**OR (95%CI)**	**OR (95%CI)**	**OR (95%CI)**
CRY2			
rs1401417 (G>C)	Hoffman *et al.* (121): CC: 0.44 (0.21 – 0.92)[c] GC+CC: 0.72(0.52 – 1.00)[c] GC+CC: 0.49 (0.25 – 0.95)[c]	Chu *et al.* (124): GC: 1.7 (1.1 – 2.7) GC+CC: 1.7 (1.1-2.6)	Hoffman *et al.* (126): GG: 2.97 (1.57 – 5.63)
rs11038689 (A>G)	Hoffman *et al.* (121): AG+GG: 0.71(0.51 – 0.99)[c] AG+GG: 0.44(0.22 – 0.89)[b]		Hoffman *et al.* (126): GG: 2.34 (1.28 – 4.27)
rs7123390 (G>A)	Hoffman *et al.* (121): AA: 0.44 (0.22 – 0.86)[c] GA+AA: 0.48(0.25 – 0.93)[b]		Hoffman *et al.* (126): GG: 2.40 (1.39 – 4.13)
rs11605924 (A>C)	Hoffman *et al.* (121): CC: 2.49 (1.03 – 5.99)[b]		
rs2292912 (C>G)		Zhu *et al.* (125): CG+CC: 0.82 (0.69 – 0.99)	
ARNTL			
rs7950226 (G>A)		Zhu *et al.* (125): GA: 1.22 (1.01 – 1.48) GA+AA: 1.22 (1.02 – 1.46)	

		GA: 1.24 (1.01 – 1.54)[d]	
		GA+AA: 1.22 (1.00 – 1.49)[d]	
NPAS2			
rs2305160 (G>A)	Zhu *et al.* (123):	Chu *et al.* (124):	Zhu *et al.* (127):
	GA: 0.61 (0.46 – 0.81)	GA: 0.6 (0.4 – 1.0)	GA: 0.69 (0.53 – 0.90)
	GA: 0.44 (0.25 – 0.77)[e]	AA: 2.0 (0.9 – 4.4)	AA: 0.55 (0.36 – 0.85)
	GA: 0.65 (0.46 – 0.91)[c]		GA+AA: 0.66 (0.51 – 0.85)
rs895521 (G>A)		Zhu *et al.* (125):	
		GA: 0.85 (0.71 – 1.00)	
		GA+AA: 0.83 (0.70 – 0.93)	
rs1369481 (G>A)		Zhu *et al.* (125):	
		GA: 0.81 (0.68 – 0.96)	
		GA+AA: 0.81 (0.69 – 0.95)	
		GA: 0.81 (0.67 – 0.98)[d]	
		GA+AA: 0.80 (.67 – 0.96)[d]	
rs17024926 (T>C)		Zhu *et al.* (125):	
		TC: 1.26 (1.06 – 1.49)	
		TC+CC: 1.25 (1.07 – 1.47)	
		TC: 1.26 (1.04 – 1.53)[d]	
		CC: 1.34 (1.00 – 1.78)[d]	
		TC+CC: 1.28 (1.07 – 1.53)[d]	
TIMELESS			
rs7302060 (T>C)	Fu *et al.* (120):		
	TC+CC: 0.54 (0.54 – 0.99)		

	CC: 0.36 (0.17 – 0.78)[a]	
rs2291738 (A>G)	Fu *et al.* (120):	
	CC: 0.46 (0.22 – 0.97)[a]	
CLOCK		
rs7698022	Hoffman *et al.* (119):	
	OR_{dom}=1.34 (1.02 – 1.76)	
	OR_{hom}=2.87 (1.25 – 6.59)[b]	
rs11133391	Hoffman *et al.* (119):	
	OR_{dom}=0.75 (0.56 – 0.99)	
rs11932595	Hoffman *et al.* (119):	
	OR_{dom}=1.43 (1.07 – 1.91)	
	OR_{dom}=1.88 (1.06 – 3.31)[b]	
rs1048004	Hoffman *et al.* (119):	
	OR_{dom}=1.34 (1.02 – 1.76)	
	OR_{hom}=2.69 (1.18 – 6.13)[b]	
rs6850524	Hoffman *et al.* (119):	
	OR_{dom}=0.45 (0.27 – 0.76)[b]	
rs13102385	Hoffman *et al.* (119):	
	OR_{dom}=0.46 (0.27 – 0.76)[b]	
rs1801260	Hoffman *et al.* (119):	
	OR_{hom}=2.57 (1.14 – 5.82)[b]	
CSNK1E		
rs1534891 (C>T)		Zhu *et al.* (125):
		TT: 2.65 (1.16 – 5.95)

		TT: 3.09 (1.32 – 7.21)[d]	
PER3 rs1012477 (C>G)		Zhu *et al.* (125): CG: 1.28 (1.04 – 1.56) CG+GG: 1.25 (1.03 – 1.52)	

a. ER/PR+ tumours only (breast cancer)
b. ER/PR- tumours only (breast cancer)
c. Postmenopausal women only
d. Less aggressive disease (prostate cancer) only
e. Premenopausal women only

2.7 Potential Confounders:

There are a number of additional factors that need to considered when evaluating relationships of shift work, light exposure and clock gene polymorphisms with melatonin levels and breast cancer risk. There are a number of established risk factors for breast cancer and other determinants of melatonin, all of which need to be considered as potential confounders of the relationships of interest and these will be summarized in this section.

2.7.1 Known Breast Cancer Risk Factors:

In addition to age, the strongest established risk factor for breast cancer in women, there are a number of other demographic, reproductive, genetic, and lifestyle and environmental factors that have been associated with breast cancer risk (2–5). Other than age, demographic characteristics that have been linked to breast cancer risk include ethnicity and socioeconomic status (5). With respect to ethnicity, breast cancer incidence is generally highest among white women and lowest among Asians (5). Further, rates are higher among women from higher socioeconomic groups, when measured by both income and education (5).

A number of reproductive characteristics are also among the most well established breast cancer risk factors. These include age at menarche, parity, age at first pregnancy and age at menopause, where early age at menarche and late age at menopause are thought to increase risk and greater parity and earlier age at first pregnancy generally reduce risk (2,5). These factors are generally thought to influence breast cancer risk through their impact on a woman's exposure to endogenous estrogens, as the proliferative effects of estrogen have been implicated in both breast carcinogenesis and tumour promotion (141). The plausibility of this mechanism is supported by results of several prospective studies that have demonstrated increasing breast cancer risk with increasing blood concentrations of estradiol, estrone and estrone sulfate (141). Further, menopausal status has been increasingly recognized as an effect modifier of relationships between various etiologic factors and breast cancer, where premenopausal tumours are more

likely to have an aggressive biology, to be high grade, to be estrogen and progesterone receptor negative, overexpress Her2-neu and be familial (4).

Family history of breast cancer among first degree relatives has been associated with a relative risk of 1.5 – 3.0 and approximately 15% of breast cancers arise in women with a family history among their mother, sister or daughter, which has indicated a role for genetics in the disease (4,142). While a number of different genetic influences have been identified, the *BRCA1* and *BRCA2* gene mutations have been most strongly associated with breast cancer risk (2,142). However, although these mutations appear to account for a relatively high proportion of breast cancers in very high risk breast or breast-ovarian families, they account for only 20 – 25% of overall familial breast cancer risk and about 5% of all breast cancers (2,142).

As many established risk factors for breast cancer are not modifiable (ex. age, family history), the relationships of a number of lifestyle and environmental factors with breast cancer risk have also been investigated (2,5), of which alcohol consumption and physical activity are among the most well established. Physical activity has been associated with a 20 – 40% decreased risk of breast cancer, where risk reductions are generally larger among postmenopausal women (5,143). In contrast, alcohol consumption has been consistently associated with an increased breast cancer risk (5). Given the known role of estrogen in breast cancer etiology, the influence of exogenous hormones, specifically menopausal hormone replacement therapy and oral contraceptive use on risk has also been investigated. While there is strong evidence that combined estrogen and progestin menopausal hormone therapy increases breast cancer risk (particularly hormone receptor positive tumours), recent use of oral contraceptives has only been associated with a modest increase in breast cancer risk among premenopausal women and no association is seen among postmenopausal women (5,144). The relationships of other factors including non-steroidal anti-inflammatory drugs (NSAIDs), smoking, diet and vitamin D have also been investigated, however, results are less consistent (2,5).

2.7.2 Determinants of Melatonin:

In addition to light exposure, a number of lifestyle and reproductive factors, as well as age and several pharmacologic agents, are thought to influence melatonin production. Lifestyle factors thought to be associated with melatonin include alcohol and caffeine consumption, smoking and body mass index, which are all thought to decrease melatonin production (76,87,145–150). Along with a decrease in melatonin production with age (87,145,147,151), reproductive factors including menopausal status and parity are thought to influence melatonin levels (71,76,87,151,152). However, these effects appear to be in opposite directions, where menopause is thought to decrease melatonin while increases are observed among highly parous women (71,76,87,151,152). Finally, anti-depressants, beta-blockers, non-steroidal anti-inflammatory drugs (NSAIDs), hormone replacement therapy and sedatives are all thought to decrease melatonin while an increase in the hormone has been observed with use of oral contraceptives (153–160).

2.8 Summary of Rationale:

In 2007 IARC classified shift work involving circadian disruption as a 'probable carcinogen' (14). However, this classification was made based on 'sufficient evidence in experimental animals' and 'limited evidence in humans', where studies in humans were limited by inconsistent definitions of shift work (14). As a result of these limitations, a 2009 IARC Working Group identified a need for additional research in humans with more comprehensive methods of shift work exposure assessment (35). Further, while the influence of light at night on melatonin production is often cited as the mechanism linking shift work to cancer risk, evidence from observational studies in humans supporting this relationship is limited (49,50,84) and studies that carefully consider the timing of melatonin assessment when examining this association are needed. Given that shift work is a necessary component of many occupations, an

understanding of the mechanism responsible for the observed relationship between shift work and cancer is important to the development of shift work patterns that are best for health.

In addition, epidemiologic studies have begun to identify a potential role for clock genes in the development of cancer at several sites including breast (128–137). However, the majority of associations between clock gene variants and breast cancer have been investigated from one case-control study in Connecticut (128–132), and more evidence concerning the genetic effects of clock gene variants on breast cancer risk in different populations is needed in order to confirm observed associations. It is unknown whether the observed effects of shift work and chronodisruption on cancer risk are the result of genetic susceptibility alone, or whether there is an interaction between genetic variants and environmental factors, as only one recently-published study has investigated interactions between clock gene variants and shift work (137).

This thesis will examine both the association between shift work and breast cancer itself, as well as relationships at several points along the hypothesized causal pathway linking shift work to breast cancer (Figure 2.2). The manuscripts included in this thesis correspond directly to areas targeted for research by the 2009 IARC Working Group on shift work exposure assessment (35). By examining the influences of both shift work and clock gene polymorphisms (and their interactions) with breast cancer risk, as well as the relationship between light and melatonin, a potential biomarker of chronodisruption, the research presented here will contribute new knowledge to the understanding of shift work and breast cancer risk and the potential underlying mechanisms in this pathway.

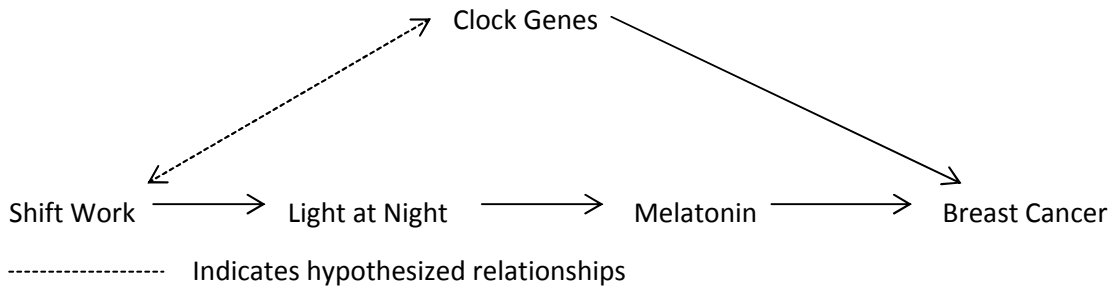

Figure 2.2: Conceptual Model

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Chapter 3

Shift Work and Breast Cancer Risk: Results from a Case-Control Study in Canada

3.1 ABSTRACT

Background: Long-term night shift work has been suggested as a risk factor for breast cancer; however, additional studies with more comprehensive methods of exposure assessment to capture the diversity of shift patterns are needed. As well, few studies have considered the role of hormone receptor subtype in this relationship.

Methods: The relationship between shift work and breast cancer risk was examined among 1,142 breast cancer cases and 1,178 controls, frequency-matched by age in Vancouver, British Columbia and Kingston, Ontario. Shift work history was measured through self-reported lifetime occupational history and associations with breast cancer quantified using multivariable logistic regression. Hormone receptor status was obtained from tumour pathology data.

Results: Approximately one third of both cases and controls had ever been employed in shift work. While no relationship between either short (0 – 14 years) or medium (15 – 29 years) term shift work and breast cancer was observed, an association was apparent for ≥30 years of shift work (OR = 2.20, 95% CI = 1.13 – 4.28). The association appears to be robust to alternative definitions of prolonged exposure to shift work. No interaction between estrogen/progesterone receptor positive (OR = 2.24, 95%CI = 1.12 – 4.49) and negative (OR = 1.04, 95%CI = 0.23 – 4.66) tumours was observed.

Conclusions: Long-term shift work is associated with increased breast cancer risk, consistent with previous studies, and most apparent among post-menopausal women. Considering hormone receptor status, increased risk of similar magnitude was detected for both tumour sub-groups.

3.2 INTRODUCTION

Shift work has been suggested as risk factor for several cancer sites (1–4) and in 2007 the International Agency for Research on Cancer (IARC) classified shift work involving circadian disruption as a probable carcinogen (5), on the basis of "sufficient evidence" in experimental models and "limited evidence" in humans. Most epidemiologic studies of this relationship to date have focused on relationships with breast cancer, where results from meta-analyses demonstrate a 40 – 50% increase in breast cancer risk associated with long-term (20 – 30 years) shift work (2,3). While the biological mechanism linking shift work with cancer risk remains unknown, the main hypothesized pathway involves melatonin (6). Melatonin is a cancer-protective hormone inhibited by light and a biomarker of circadian disruption, where increased exposure to light during night shifts is thought to decrease production of melatonin, thereby increasing cancer risk (6). It is unknown whether cumulative shift work or shift work during specific lifetime "exposure windows" is most relevant to cancer risk.

While the majority of studies have reported a modest increase in risk of some cancer sites among long-term shift workers (7–17), several definitions and methods of assessment of shift work have been used (18), making it challenging to synthesize evidence. Some studies have used responses to specific questions, such as history of rotating night shifts in the Nurses' Health Study, to classify whether an individual is a 'shift worker' (9,10,12,14,16,17). While these methods benefit from exposure information specific to individual participants, some may be limited by exposure misclassification if the shift work that has been performed does not meet the definition provided in the question (18). Other studies have used job-exposure matrix-based methods to assess shift work, where registry-based job classifications are used to define shift work exposure (8,11,13,15). Although these methods are not subject to the same type of misclassification issues as questionnaires, they may still suffer from exposure classification errors, as some individuals within job-classification groups may not have actually worked the

proportion of shift work assigned to their group, a potential source of differential misclassification (18,19).

Due to limitations in measurement of shift work in previous studies, a 2009 IARC Working Group identified a need for more comprehensive methods of exposure assessment to reduce misclassification and characterize the diversity of shift patterns (18). Further, most studies with individual-level measures of shift work have been limited to nurses (9–11,16,17) and such findings have to be tested in the general population to address possible confounding by specific characteristics of the nursing profession. Finally, few studies have considered a role for breast tumour estrogen/progesterone receptor status in relationships with shift work (9,12). Differences in etiology have been suggested for hormone receptor positive and negative tumours, as the effects of several breast cancer risk factors, such as parity and age at first birth, vary between tumour groups (20–22). Further, melatonin, a hypothesized intermediate between shift work and breast cancer (6), may influence risk through an increase in estrogen production (23), such that a stronger relationship for hormone-dependent tumours might be expected. As such, it is possible that the effects of shift work could vary by tumour hormone receptor status. The objective of this research was to test a hypothesis that an increased risk of breast cancer in women is associated with long-term shift work in the general population.

3.3 METHODS

3.3.1 Study Population:

A case-control study was conducted in Vancouver, British Columbia and Kingston, Ontario from 2005 – 2010. Ethical approval for this study was provided by both the University of British Columbia – BC Cancer Agency Research Ethics Board and the Queen's University Health Sciences Research Ethics Board.

Vancouver:

Incident breast cancer cases were recruited from the British Columbia (BC) Cancer Registry. Eligible cases were women ages 20 – 80 with a diagnosis of either in situ or invasive breast cancer with had no previous cancer history (except non-melanoma skin cancer) living in Vancouver, New Westminster, Richmond and Burnaby. Potential controls were cancer-free individuals from the Screening Mammography Program of BC recruited from breast screening clinics in the same geographic areas who consented to participate in research studies over the course of routine screening mammography (available to women in BC ages 40 – 79). Controls were frequency-matched to cases by five-year age group.

All potential participants were sent a study package including a letter describing the study, a consent form, and a study questionnaire. Study participation involved completing the questionnaire, either self-administered or collected by telephone interview in English, Cantonese, Mandarin or Punjabi, providing a blood sample and granting access to medical records concerning breast health. Response rates were 54% among cases and 57% among controls, with a total of 1,062 cases and 1,015 controls recruited. However, as the minimum age for screening mammography in BC is 40, all breast cancer cases diagnosed under age 40 were excluded from the analysis. Following this exclusion, 1,011 cases and 1,014 controls from Vancouver were included in the analysis.

Kingston:

Both cases and controls were recruited from the Hotel Dieu Breast Assessment Program in Kingston, Ontario. Women were eligible for the study if they were under 80 years of age, had no previous cancer history (except non-melanoma skin cancer), were not too ill to participate and were not taking cancer-preventative drugs. Those consenting to be contacted were called by the study coordinator to confirm eligibility and were sent a package including study information, questionnaire and consent form. Cases were women with a subsequent diagnosis of either in situ or invasive breast cancer, while controls, who were frequency matched by age to cases as in

Vancouver, were women with either normal mammogram results or who were diagnosed with benign breast disease. Among those consenting to be contacted, response rates were 59% among cases and 49% among controls, with a total of 131 cases and 164 controls included. Study participation involved the same process as in Vancouver, although in Kingston all participants self-administered the questionnaire.

3.3.2 Procedures:

The questionnaire (Appendix B) contained information regarding education; ethnicity; health, medical and reproductive history; family history of cancer; lifestyle characteristics including lifetime tobacco and alcohol consumption; lifetime physical activity and cooking habits; as well as lifetime occupational and residential histories.

Shift Work Exposure Assessment:

Lifetime exposure to shift work was obtained from the occupational history section of the questionnaire. For any job worked for at least six months, participants provided the industry and job title, start and end dates, average number of hours per week, percentage of time on day, evening and night shifts (as a continuous variable), as well as start and end times for each shift type. This information was used to categorize each job as either a 'shift work' or 'non-shift work' occupation. For the 'main' analyses, shift work jobs were classified as those where ≥50% of time was reported to have been spent on evening and/or night shifts, capturing both rotating and permanent night shift schedules. The total number of years spent employed in 'shift work' jobs was calculated for each individual. The proportion of evening and late night shifts required for a job to be considered 'shift work' was varied with definitions of 20%, 40%, 60%, 80% and 100%, compared to the 50% used in the main analysis, to investigate the effects of our definition of 'shift work', with stronger associations expected as the threshold increased. For the overall and postmenopausal analysis, duration of shift work was classified into four categories: none, >0 –

14, 15 – 29 and ≥30 years (9–11). Among premenopausal women, categories were: none, >0 – 9, 9 – 19 and ≥20 years (10). As well, duration of shift work was split into eight categories of 5-year increments to further investigate the impact of the broader categorizations used in the main analysis.

To further describe the types of jobs performed, the self-reported industry for the job that each individual held for the longest period of time was classified according the Statistics Canada National Occupational Classification 2006 (24) into one of ten categories. For women with a history of shift work, the job they had held for the longest period of time that met the criteria for a 'shift work' job was classified.

Breast Tumour Biomarker Assessment:

Pathologic data concerning tumour estrogen (ER) and progesterone (PR) receptor biomarker status was collected for all breast cancer cases. In Vancouver, this information was obtained from the BC Cancer Registry and BC Breast Cancer Outcomes Unit and in Kingston was obtained directly from electronic patient charts. ER status was determined from immunohistochemistry (IHC) results and classified into one of six categories: OZER = negative (0/3), OLOW = weakly positive (1/3), OMOD = moderately positive (2/3), OHIG = strongly positive (3/3), OXXX = receptors tested but not sufficient quantity for interpretation or borderline/equivocal and XXXX = not tested. Tumours were considered estrogen-receptor positive if they were classified as OLOW, OMOD or OHIG. PR status was also determined through IHC testing using the same categorizations as the estrogen receptor analysis.

Assessment of Menopausal Status

The relationship between shift work and breast cancer risk was examined in the full study population, as well as stratified by menopausal status. Women were classified as postmenopausal if they: stated their periods had stopped for greater than one year; periods stopped naturally and were over 50 years of age if time since last period was missing; had a bilateral oophrectomy; or they were over age 55 and periods stopped due to chemotherapy or other reasons, similar to

Friedenreich et al. (25). A total of 838 women were premenopausal and 1,436 were postmenopausal.

3.3.3 Statistical Analysis:

Multivariable logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals to estimate the relationship between shift work and breast cancer risk. Age (continuous) and centre (Vancouver/Kingston) were forced into the model and all other variables were selected using an all-possible-models manual backwards selection procedure (26), where potential confounders associated with breast cancer at $p < 0.25$ were initially included in the modeling process, and only variables that changed the OR by >10% were retained in the final model. Potential confounders were ethnicity; household income; education; menopausal status; use of fertility drugs, oral contraceptives, NSAIDs, antidepressants and hormone replacement therapy; reproductive factors including ever having been pregnant, number of pregnancies, age at first birth and age at first mammogram; family history of breast cancer among first degree relatives; lifestyle factors including smoking status, pack-years smoking and lifetime alcohol consumption; and body mass index. Tests for trend across shift work categories were calculated by treating levels of exposure as a continuous ordinal variable and possible interactions by menopausal status were assessed by including a shift work – menopausal status interaction term. Multivariable polytomous logistic regression was used to evaluate the breast cancer risk associated with shift work history by hormone receptor status in one of two categories, as either ER and/or PR positive or negative. Cases where both ER and PR status were either not tested or missing were excluded from this analysis (n=136). Among excluded cases, 95 (70%) were cases of ductal carcinoma *in situ*, in which testing of ER and PR tumour status is not routine. A total of 78 (82%) of *in situ* cases were explicitly classified as not tested, compared to 8 (20%) of non-*in situ* cases.

Since controls in Vancouver were obtained from a screening population while cases came from the population-based BC Cancer Registry, a sensitivity analysis was performed excluding cases that did not participate in screening. Cases from Vancouver were linked to the Screening Mammography Program of BC using provincial personal health numbers and those that had not been seen in the screening program were excluded (n = 258). Further, to examine whether specific exposure-time windows had an influence on risk, variables indicating whether individuals had ever performed shift work (based on the 50% evening/night shift definition previously described) during specific decades of life (20s, 30s, 40s, 50s) were analyzed.

3.4 RESULTS

Characteristics of cases and controls are described in Table 3.1. The case group included a smaller proportion of Europeans and a greater proportion of Asians than the control group. Further, cases had lower levels of household income and education, were less likely to have used NSAIDs and oral contraceptives, were more likely to have ever been pregnant, had an older average age of first mammogram, were more likely to have a family history of breast cancer among first degree relatives, and had lower levels of lifetime alcohol consumption compared to controls.

Approximately one-third of both cases and controls (Table 3.2) had a history of shift work. Proportions of cases and controls were similar in the 0 – 14 (minimum duration of exposure 3 months) and 15 – 29 years of shift work categories, while the proportion of cases in the ≥ 30 (maximum = 47) years shift work category was higher than in the control group. These patterns were maintained in multivariate analysis (Table 3.2) where no association between shift work and breast cancer risk was observed for either the 0 – 14 and 15 – 29 year categories, while an association with ≥ 30 years shift work was observed. However, no trend across categories of shift work was noted. Further, cumulative exposure to shift work appeared to be the relevant

exposure measure, as no association between shift work in any of four decades of life (20s, 30s, 40s, 50s) was observed (data not shown).

Among postmenopausal women results were similar to the overall analysis (Table 3.2). Among premenopausal women, no relationship between any shift work category and breast cancer was observed. Although no interaction by menopausal status was detected in the 15 – 29 years and ≥30 years shift work groups, there did appear to be a significant ($p = 0.02$) interaction in the 0 – 14 years shift work group, likely because estimated ORs in these groups were in opposite directions.

Results from sensitivity analysis excluding non-screened cases were similar to those from the full study population where no association was observed for either the 0 – 14 (OR = 0.94, 95% CI = 0.76 – 1.16) or 15 – 29 years (OR = 0.96, 95% CI = 0.67 – 1.37) shift work categories and a positive association was seen in the ≥30 years shift work category (OR = 2.22, 95% CI = 1.11 – 4.42).

Sensitivity analysis examining the influence of a priori exposure categorizations of shift work (9–11) on relationships with breast cancer risk was performed. When the influence of the proportion of evening and late night shifts required for a job to be considered 'shift work' was examined, for all, no association for either short (0 – 14 years) or medium (15 – 29 years) term shift work was observed, while the odds ratio between shift work and breast cancer in the long term shift work (≥30 years) category generally increased as the cut-point increased (Table 3.3). However, due to small numbers in the most extreme category, precision declined substantially for estimates of long-term shift work for thresholds over 50%. When duration of exposure to shift work was split into eight categories of 5-year increments, as in the main analysis, no association between any duration of shift work under 30 years and breast cancer was observed (Table 3.3).

When examining the influence of tumour ER/PR biomarker status on the relationship between shift work history and breast cancer, the proportions of women with no history of shift work were similar across subgroups (Table 3.4). As in the non-stratified analysis, no association

between 0 – 14 and 15 – 29 years of shift work and breast cancer risk was seen for either breast cancer subgroup. While the odds ratio for the association for ≥30 years of shift work was stronger in the ER/PR+ subgroup, no significant interaction by hormone receptor biomarker status was detected (Table 3.4).

The industry of the job held for the longest period of time was classified into one of ten categories for women in each of the four shift work history groups (Table 3.5) to classify the types of jobs held by women who did and did not engage in shift work. This analysis demonstrated that the largest proportion were employed in health occupations such as nursing in both the ≥30 years (44%) and 15 – 29 years (41%) shift work groups, while the proportions in this industry in the 0 – 14 years (19%) and never shift worker (10%) groups were substantially lower. Finally, to investigate the influence of job type, associations between shift work and breast cancer in the four main shift work duration categories were investigated for women employed in health occupations only. As in the main analysis, among those in health occupations no association was observed for the 0 – 14 years (OR = 0.82, 95% CI = 0.50 – 1.36) and 15 – 29 years (OR = 1.06, 95% CI = 0.58 – 1.92) shift work categories, while increased breast cancer risk was observed among women working ≥30 years of shift work (OR = 3.09, 95%CI = 1.10 – 8.71). Similar patterns were observed for non-health occupations with no association for either 0 – 14 years (OR = 1.04, 95%CI = 0.85 – 1.29) and 15 – 29 years (OR = 1.05, 95%CI = 0.68 – 1.61) of shift work, and a suggestion of increased risk in the ≥30 years of shift work category (OR = 2.24, 95%CI = 0.91 – 5.48).

3.5 DISCUSSION

The results of this study demonstrate an increased risk of breast cancer among women employed in shift work for ≥30 years, consistent with several previous studies (9–11,17), with no association seen for shorter durations. The level of 30 years of shift work was supported by the

sensitivity analysis examining the effect of duration of shift work in 5-year increments, where no increase in risk was observed in any category where women had worked in shift work for less than 30 years. Similar to other studies conducted in the general population (7,12,14), the majority of both cases and controls had not participated in any type of shift work and only a small proportion had been engaged in shift work for 30 years or more.

One strength of this study was its use of a lifetime occupational history including the proportion of day, evening and night shifts for each job - this allowed jobs with both rotating and permanent night shift patterns to be included in the definition of shift work. This is an improvement over some previous studies in which questions regarding shift work have focused on one specific pattern (9,10). One recent study found increased risk of breast cancer among nurses who worked at least five years in shift patterns that involved a minimum of six or seven consecutive night shifts (16). A limitation of the current study was the absence of information concerning the number of consecutive night shifts in each job, such that a similar analysis could not be performed.

When stratified by menopausal status, among postmenopausal women, an association was found with increased risk of breast cancer among long-term (≥30 years) shift workers. The Nurses' Health Study also reported increased risk of breast cancer among postmenopausal women working ≥30 years of rotating shift work (9), while results from postmenopausal women in a Norwegian nursing cohort suggested increased risk among those working ≥30 years shift work and a protective relationship among short-term shift workers (11). Among premenopausal women in our study, no relationship between shift work and breast cancer was observed, while previous studies have indicated an increased risk among long-term (≥20 years) premenopausal shift workers in the Nurses' Health Study (10), and an elevated odds ratio suggested for the relationship between long-term shift work and breast cancer risk among Norwegian nurses (11).

When stratified by ER/PR status, no significant interactions by ER/PR status were detected, although the odds ration for ER/PR+ tumours in the ≥30 years of shift work group was stronger.

Two previous studies also saw no differences in the effect of shift work when considering the effect of tumour ER status alone (9,12).

When classifying shift work as a "probable carcinogen" and categorizing the evidence from human studies as "limited", the IARC Working Group noted that a number of existing studies in humans were limited to nurses (5). While our study was not limited to nurses, the largest proportion of shift workers from both the ≥30 years and 15 – 29 years shift work groups were in health occupations. However, it is important to note that relationships observed between shift work duration and breast cancer risk were similar to those of the main analysis when restricted both to individuals employed in health occupations and those in other occupations. Other occupational exposures that could increase risk of breast cancer and might confound the shift work-breast cancer association are the subject of future analysis, and thus were not considered as potential confounders in this work.

As selection of longest-held job with shift work to classify women into occupational categories can be problematic if women change occupation but not pattern of shift work, the association between longest exposed job and total duration of shift work was examined. This analysis demonstrated a strong positive relationship that degraded for longer durations of exposure, but was strong for durations of exposure <30 years (Figure 3.1). However, a limitation of this analysis is that for individuals who changed jobs within industries, the ability of the longest exposed job to act as a proxy for total exposure is likely under-estimated, an effect which may be greater among long-term shift workers who had more opportunity to work in multiple jobs.

The recruitment of cases in Vancouver from the population-based BC Cancer Registry and controls from screening clinics may have introduced a source of selection bias, as some cases may not have participated in screening and would have been ineligible to be included as controls. However, a sensitivity analysis excluding all cases from Vancouver who were unlikely to have

participated in screening produced similar results to the overall analysis, suggesting the influence of any potential selection bias on study results would be small.

Although there were differences in the characteristics of cases and controls, for instance in ethnicity, education etc., none of these factors were identified as confounders of the shift work – breast cancer association. Further, modest response rates in both Vancouver (54% in cases, 57% in controls) and Kingston (59% in cases, 49% in controls) also create the possibility of response bias in this study. However, in order to bias results, study participation would need to be related to both shift work and breast cancer risk. Given that rates were relatively similar across both the case and control groups and that many factors in addition to shift work were included in the study questionnaire, it is anticipated that the potential for response bias would also have a minimal impact on the observed results.

While light at night and melatonin have been proposed as one pathway through which shift work may influence breast cancer and data from prospective studies has generally supported a protective effect of melatonin on breast cancer (27–31), studies of night work and melatonin are less consistent (32–35). These results suggest a role for other mechanisms such as sleep disturbances or lifestyle differences that may be intermediates or confounders of the night work – cancer relationship (6) and should be considered in future work. As well, chronotype has been suggested as a factor that may play a role in shift work-cancer relationships (36); but, since an assessment of chronotype was not included in the study questionnaire, it could not be considered here.

In summary, the results of this study support an association between ≥ 30 years of shift work and breast cancer, consistent with some previous studies (9–11,17), while accounting for several potential confounders. As shift work is necessary for many occupations, understanding of which shift patterns and specifically how shift work influences breast cancer risk is needed for the development of healthy workplace policy.

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3.9 TABLES AND FIGURES

Table 3.1: Characteristics of Study Population

	Cases (N=1142) Mean (SD) / N (%)	Controls (N=1178) Mean (SD) / N (%)
Age	57.31 (10.3)	56.72 (10.1)
Body Mass Index	25.67 (5.5)	25.27 (5.8)
Ethnicity		
European	713 (62.4%)	920 (78.2%)
Chinese	240 (21.0%)	114 (9.7%)
South Asian	34 (3.0%)	35 (3.0%)
Filipino	61 (5.3%)	38 (3.2%)
Japanese	24 (2.1%)	14 (1.2%)
Mixed	19 (1.7%)	12 (1.0%)
Other	51 (4.5%)	43 (3.7%)
Household Income		
< $30,000	203 (17.8%)	121 (10.3%)
$30,000 - $59,999	282 (24.7%)	269 (22.8%)
$60,000 - $99,999	248 (21.7%)	288 (24.5%)
> $100,000	239 (20.9%)	338 (28.7%)
Not stated	170 (14.9%)	162 (13.6%)
Education		
High school or less	395 (34.8%)	299 (25.5%)
College/trade certificate	346 (30.5%)	349 (29.7%)
Undergraduate degree	267 (23.5%)	302 (25.7%)
Graduate/Professional degree	126 (11.1%)	225 (19.2%)
Menopausal Status		
Pre-menopausal	394 (34.6%)	444 (37.8%)
Post-menopausal	744 (65.4%)	732 (62.2%)
Age at Menarche	12.88 (1.6)	12.84 (1.5)
Medication Use		
Fertility Drugs (Ever/Never)	63 (5.6%)	64 (5.4%)
# Years NSAID Use		
None	1002 (87.7%)	991 (84.1%)
< 2.34	55 (4.8%)	62 (5.3%)
2.34 – 8.50	48 (4.2%)	61 (5.2%)
≥ 8.51	37 (3.2%)	64 (5.4%)

# Years Oral Contraceptive Use		
None	563 (49.3%)	429 (36.4%)
< 4.50	192 (16.8%)	248 (21.1%)
4.50 – 10.00	216 (18.9%)	275 (23.3%)
≥ 10.01	171 (15.0%)	226 (19.2%)
# Years Antidepressant Use		
None	917 (80.3%)	915 (77.7%)
< 1.50	74 (6.5%)	86 (7.3%)
1.50 – 7.50	87 (7.6%)	87 (7.4%)
≥ 7.51	64 (5.6%)	89 (7.6%)
# Years HRT Use		
None	839 (73.5%)	853 (72.4%)
< 5.00	92 (8.1%)	99 (8.4%)
5.00 – 12.00	114 (10.0%)	120 (10.2%)
≥ 12.01	97 (8.5%)	106 (9.0%)
Reproductive History		
Age at Menarche	12.88 (1.6)	12.84 (1.5)
Ever Been Pregnant	943 (82.8%)	936 (79.6%)
Age at First Pregnancy	27.67 (5.4)	27.54 (5.5)
Number of Pregnancies	2.29 (1.7)	2.25 (1.7)
Age at First Mammogram	44.75 (8.8)	42.77 (7.5)
Family History of Breast Cancer	224 (19.6%)	168 (14.3%)
Lifestyle Characteristics		
Current Smoker	73 (6.4%)	71 (6.0%)
Pack-years Smoking	5.64 (12.1)	5.29 (11.2)
Lifetime Alcohol Consumption (# drinks/wk)		
Teen	1.05 (3.5)	1.63 (4.0)
20s	2.23 (3.9)	3.26 (6.1)
30s	2.90 (6.0)	3.61 (5.8)
40s	3.09 (6.0)	3.75 (6.1)
50s	2.74 (4.9)	3.71 (6.1)

Table 3.2: Shift Work History and Breast Cancer Risk

Years Shift Work History[a]	Cases N (%)	Controls N (%)	Odds Ratio (95% CI)	Interaction by Menopausal Status
Overall[b]				
None	756 (66.2%)	772 (65.5%)	-	
0 – 14	286 (25.0%)	312 (26.5%)	0.96 (0.79 – 1.16)	p = 0.02
15 – 29	72 (6.3%)	81 (6.9%)	0.92 (0.66 – 1.29)	p = 0.7
≥ 30	28 (2.5%)	13 (1.1%)	2.20 (1.13 – 4.28)	p = 0.2
			p-trend = 0.5	
Postmenopausal[c]				
None	534 (71.4%)	501 (68.3%)	-	
0 – 14	144 (19.3%)	175 (23.8%)	0.78 (0.61 – 1.01)	
15 – 29	48 (6.4%)	46 (6.3%)	0.98 (0.64 – 1.50)	
≥ 30	22 (2.9%)	12 (1.6%)	1.73 (0.85 – 3.54)	
			p-trend = 0.8	
Premenopausal[c]				
None	222 (56.4%)	271 (61.0%)	-	
0 – 9	127 (32.2%)	119 (26.8%)	1.31 (0.96 – 1.79)	
10 – 19	27 (6.9%)	35 (7.9%)	0.98 (0.57 – 1.68)	
≥ 20	18 (4.6%)	19 (4.3%)	1.28 (0.65 – 2.54)	
			p-trend = 0.3	

a. Using 50% of time definition
b. Model adjusted for age and centre
c. Model adjusted for age, centre and BMI

Table 3.3: Additional Shift Work Categorizations

Variable	Cases (N=1142) Mean (SD)	Controls (N=1178) Mean (SD)	OR (95% CI)[a]
20% Evening or Night Shifts			
None	612 (53.6%)	618 (52.5%)	-
<15	343 (30.0%)	352 (29.9%)	1.01 (0.84 - 1.22)
15 - 30	129 (11.3%)	160 (13.6%)	0.83 (0.64 - 1.07)
≥30	58 (5.1%)	48 (4.1%)	1.22 (0.82 - 1.82)
40% Evening or Night Shifts			
None	725 (63.5%)	751 (63.8%)	-
<15	301 (26.4%)	316 (26.8%)	1.01 (0.83 - 1.22)
15 - 30	86 (7.5%)	95 (8.1%)	0.95 (0.70 - 1.30)
≥ 30	30 (2.6%)	16 (1.4%)	1.95 (1.06 - 3.62)
60% Evening or Night Shifts			
None	881 (77.2%)	902 (76.6%)	-
<15	203 (17.8%)	228 (19.4%)	0.93 (0.75 - 1.16)
15 - 30	40 (3.5%)	42 (3.6%)	0.97 (0.62 - 1.51)
≥ 30	18 (1.6%)	6 (0.5%)	3.07 (1.21 - 7.80)
80% Evening or Night Shifts			
None	948 (83.0%)	973 (82.6%)	-
<15	163 (14.3%)	181 (15.4%)	0.95 (0.75 - 1.20)
15 - 30	20 (1.8%)	21 (1.8%)	0.97 (0.52 - 1.81)
≥ 30	11 (1.0%)	3 (0.3%)	3.70 (1.03 - 13.31)
100% Evening or Night Shifts			
None	983 (86.1%)	1028 (87.3%)	-
<15	137 (12.0%)	139 (11.8%)	1.05 (0.82 - 1.35)
15 - 30	17 (1.5%)	9 (0.8%)	1.92 (0.85 - 4.32)
> 30	5 (0.4%)	2 (0.2%)	2.61 (0.50 - 13.5)
5 Year Shift Work Segments (using 50% of time definition)			
None	756 (66.2%)	772 (65.5%)	-

0 - 4	162 (14.2%)	142 (12.1%)	1.19 (0.93 - 1.53)
5 - 9	86 (7.5%)	119 (10.1%)	0.75 (0.56 - 1.01)
10 - 14	38 (3.3%)	51 (4.3%)	0.79 (0.51 - 1.21)
15 - 19	30 (2.6%)	34 (2.9%)	0.93 (0.56 - 1.53)
20 - 24	26 (2.3%)	27 (2.3%)	1.01 (0.58 - 1.74)
25 - 29	16 (1.5%)	20 (1.7%)	0.81 (0.42 - 1.58)
30 - 34	14 (1.2%)	7 (0.6%)	2.03 (0.82 - 5.07)
≥ 35	14 (1.2%)	6 (0.5%)	2.39 (0.91 - 6.26)

a. Adjusted for age and centre

Table 3.4: Shift Work by Hormone Receptor Status

Years Shift Work History[a]	Controls (N=1178)	ER/PR+ (N=837)		ER/PR- (N=169)		Interaction by ER/PR Status[c]
	N (%)	N (%)	OR[b] (95% CI)	N (%)	OR[b] (95% CI)	
None	772 (65.5%)	544 (65.0%)	-	118 (69.8%)	-	-
0 – 14	312 (26.5%)	216 (38.2%)	1.00 (0.82 – 1.24)	38 (26.7%)	0.79 (0.53 – 1.17)	0.22
15 – 29	81 (6.9%)	55 (6.6%)	0.98 (0.68 – 1.40)	11 (6.5%)	0.89 (0.46 – 1.72)	0.79
≥ 20	13 (1.1%)	22 (2.5%)	2.38 (1.19 – 4.77)	2 (1.2%)	1.04 (0.23 – 4.66)	0.26

a. Using 50% of time definition
b. Model adjusted for age and centre
c. P-values calculated from case-only model comparing ER/PR+ and ER/PR- groups

Table 3.5: Industry of Shift Work Jobs

Industry	Years Shift Work History			
	≥ 30 N (%)	**15 – 29** N (%)	**0 – 14** N (%)	**None** N (%)
Management Occupations	-	1 (0.7%)	10 (1.7%)	11 (0.7%)
Business, Finance & Administrative Occupations	1 (2.4%)	10 (6.5%)	30 (5.0%)	244 (16.0%)
Natural and Applied Sciences & Related Occupations	-	3 (2.0%)	11 (1.9%)	64 (4.2%)
Health Occupations	18 (43.9%)	63 (41.2%)	111 (18.6%)	156 (10.2%)
Occupations in Social Science, Education, Government Science & Religion	9 (22.0%)	24 (15.7%)	82 (13.8%)	452 (29.7%)
Occupations in Art, Culture, Recreation & Sport	3 (7.3%)	11 (7.2%)	47 (7.9%)	81 (5.3%)
Sales & Service Occupations	7 (17.1%)	31 (20.3%)	258 (43.3%)	252 (16.5%)
Trades, Transport & Equipment Operators & Related Occupations	2 (4.9%)	4 (2.6%)	26 (4.4%)	79 (5.2%)
Occupations Unique to Primary Industry	-	3 (2.0%)	2 (0.3%)	15 (1.0%)
Occupations Unique to Processing, Manufacturing & Utilities	1 (2.4%)	3 (2.0%)	19 (3.2%)	81 (5.3%)
No Job	-	-	-	83 (5.5%)
Missing	-	-	2 (0.3%)	10 (0.7%)

Figure 3.1: Comparing Number of Years in Longest Shift Work Job to Total Years in Shift Work

Chapter 4

The Influence of Light at Night on Melatonin Levels Among Canadian Rotating Shift Nurses

4.1 ABSTRACT

Background: Shift work has been identified as a risk factor for several cancer sites in recent years, with melatonin as a potential intermediate on the proposed causal pathway. This study examined the influence of nighttime light exposure on melatonin levels among 123 rotating shift nurses.

Methods: Nurses working a rotating shift schedule (two 12-hour days, two 12-hour nights, and five days off) were recruited and participated on a day and night shift in both the summer and winter seasons. Over each 48-hour study period, nurses wore a light data logger and provided two urine and four saliva samples.

Results: Saliva measurements showed that the pattern of melatonin production did not differ between day and night shifts. Mean light exposure was significantly higher ($p < 0.0001$) when nurses were working at night, although peak melatonin levels ($p = 0.65$) and the daily change in melatonin levels ($p = 0.80$) were similar across day/night shifts. Multivariate analysis did not show an association between light exposure and melatonin levels when data from both shifts was combined; however, when data from the night shift was considered alone, a statistically significant inverse relationship between light and change in melatonin was observed ($p = 0.04$).

Conclusion: These results show that light exposure does not appear to be strongly related to reduced melatonin production among nurses on this rapidly rotating shift schedule.

Impact: Future research considering more extreme shift patterns or brighter lighting conditions could further clarify the relationship between light exposure and melatonin production in observational settings.

4.2 INTRODUCTION

Shift work has been increasingly recognized as a risk factor for several cancer sites (1–12) and, in 2007, was classified as a probable carcinogen by the International Agency for Research on Cancer (IARC) (13). The majority of epidemiologic studies of shift work and cancer have focused on relationships with breast cancer, in which results from meta-analyses demonstrate a 40 to 50% increase in risk with long-term shift work (14,15).

Chronodisruption (altered circadian rhythms) associated with shift work is thought to be responsible for observed increases in cancer risk (15,16). Although the exact biological pathway is unknown, melatonin, a hormone produced according to circadian rhythms with peak levels seen at night (17), has been suggested as a potential intermediate. It is hypothesized that light exposure during night shifts could be responsible for an increased risk of cancer through a reduction in nighttime melatonin production (18,19). Several anti-carcinogenic properties of melatonin have been suggested, including inhibition of tumour development and reduction in levels of reproductive hormones thought to play a role in cancer etiology (20–25). Although results are not entirely consistent, prospective epidemiologic studies have demonstrated an inverse association between melatonin and breast cancer risk (26–30).

Experimental studies in humans have shown a dose-response relationship between light exposure and melatonin levels, where increased light is associated with decreased melatonin production (31–35). Epidemiologic studies, primarily among shift workers, have used multiple methods to assess exposure (36–41) and results of these studies generally demonstrate an inverse relationship between light exposure and melatonin (36–41). However, several existing studies have been limited by issues related to the timing of melatonin assessment, where functional time points (e.g., after sleep), as opposed to chronologic (clock) times, have been compared between shift groups (37,39,40). This melatonin assessment strategy assumes that the timing of melatonin production among individuals working non-day shifts will be altered. However, if this assumption

is not met, comparisons of nighttime light exposure and melatonin levels across shift groups may be confounded by circadian rhythm, when comparing melatonin levels from samples obtained at different times of day (40). Therefore, observational studies that allow for comparisons of chronological time points between shift groups are needed to determine if the relationship between light and melatonin in experimental work (31–35) is also seen in the context of an observational study. Recent work also indicates a need to incorporate other factors related to melatonin production, such as chronotype, when investigating these relationships (42).

Because shift work is necessary for many occupations, understanding the mechanism linking it with cancer risk is important to develop healthy workplace policy. However, the IARC classification of shift work as a 'probable carcinogen' was based on only limited evidence from studies in humans, due to inconsistencies in the definitions of shift work in the existing literature (1–13,43). Furthermore, although the influence of light on melatonin production is often cited as the mechanism linking shift work and cancer, evidence from observational biomarker studies supporting this relationship is limited (37,39,40). Epidemiologic research with objective measurements of light exposure and that avoids confounding of melatonin measures by circadian rhythm through careful consideration of the timing of melatonin assessment is needed. Therefore, a longitudinal study among full-time nurses was conducted, the objectives of which were to investigate the influence of light at night exposure and shift work history on melatonin levels among a group of rotating shift nurses. It was hypothesized that both light exposure and history of long-term shift work would be associated with decreased melatonin production.

4.3 METHODS

4.3.1 Study Population:

Female full-time registered nurses working the two 12-hour days (7AM – 7PM), two 12-hour nights (7PM – 7AM), 5 days off (DDNN) shift pattern at Kingston General Hospital were

offered the opportunity to participate in this study. Nurses were not eligible if they had been pregnant or lactating in the previous six months or if they were taking melatonin supplements. Study participation, including these exclusion criteria, was advertised through posters and pamphlets sent to all full-time nursing staff, as well as through presentations about the study to specific hospital units, with those who were ineligible asked to self-exclude. There are approximately 700 full-time nurses at the hospital, however the number not working the DDNN shift schedule or who were ineligible for other reasons was unavailable, such that response rates could not be calculated. Data collection occurred from May 2008 to August 2009 and was approved by the Queen's University Health Sciences Research Ethics Board.

The study included 4 data collection periods, where nurses were asked to participate in the study twice (day and night shift) in both the summer and winter seasons. For logistical reasons, participants were recruited in 2 cohorts: one in 2008 starting in the summer and one in 2009 starting in the winter. A total of 123 nurses were enrolled in the study, with 118 completing both a day and a night shift in the first season and 103 completing the day shift and 96 completing the night shift in the second season. Reasons for loss to follow-up included changes in work schedule, pregnancy, illness/injury and being too busy to complete the study protocol.

4.3.2 Procedures:

Each participation session took place over a 48-hour time period covering either a day or a night shift. During each session participants were asked to wear a light data logger (Hoskin Scientific Ltd.) and provide four saliva and two urine samples over a 24-hour period (Figure 4.1). The light loggers, which measured ambient light intensity in lumens/m^2, began recording at the beginning of the first day or first night shift in the rotating shift pattern and took readings every minute for the duration of the study period. Participants wore the loggers around their neck for the entire 48-hours of study participation and placed the logger on the bedside table while sleeping.

Participants completed a study questionnaire prior to the first data collection period and a study diary during all 4 data collection periods. The questionnaire (Appendix C) collected personal information and a history of health, employment and lifestyle characteristics, including lifetime smoking and alcohol consumption patterns. The diary (Appendix D) collected information concerning physical activity, lighting conditions, smoking, alcohol and caffeine consumption, use of medications and sleep duration and timing during the 24-hours of melatonin collection. Participants' height and weight were measured by trained study personnel when nurses enrolled in the study. After study participation had begun, the Horne-Ostberg Questionnaire (44) about chronotype was added. Participants were sent a copy of the questionnaire and asked to return it by intrahospital mail.

4.3.3 Melatonin Laboratory Analysis:

Levels of the primary urinary melatonin metabolite, 6-sulfatoxymelatonin, were assessed from urine samples using the Bühlmann 6-sulfatoxymelatonin ELISA kit (ALPCO Diagnostics). Urinary 6-sulfatoxymelatonin levels in the first morning void represent approximately 70% of blood levels and are considered a good measure of overnight melatonin production (45). To account for differences in urine volume, creatinine levels were assessed using the Parameter Creatinine assay (R&D Systems, Inc.) and melatonin levels adjusted for creatinine. Salivary melatonin levels were directly assessed from saliva samples using the Bühlmann Saliva Melatonin EIA Kit (ALPCO Diagnostics). Salivary melatonin represents approximately 30% of blood levels and is not a good measure of absolute melatonin levels; however, saliva is considered a good marker of melatonin variability within an individual over a 24-hour period (46). Saliva levels were used to characterize production patterns over each 24-hour study period to facilitate comparisons between day and night shifts. Samples for all 3 assays were run in duplicate and median coefficients of variation were 9.7% for urinary melatonin, 12.3% for salivary melatonin, and 9.2% for creatinine measures. According to the manufacturer's

instructions, all melatonin assays were run with 6 standards, a blank and a high and low control of known concentration, and creatinine assays were run with 7 standards and a blank.

4.3.4 Statistical Analysis:

For characteristics measured through the study questionnaire, means and SDs were calculated for continuous variables, and percentages for categorical variables. For characteristics specific to each data collection period measured in the study diary, least squares means and standard errors in a mixed model with a random subject effect were calculated for continuous variables and percentages for categorical variables. Differences between characteristics on day and night shifts were compared within each season using difference in least squares means estimates for continuous variables and McNemar's test for categorical variables.

Light exposure was characterized as the average light intensity from 12AM – 5AM, the expected time of peak melatonin production for both day and night shifts. Peak melatonin levels were those from urine samples collected during the early morning for both day and night shifts, and the change in melatonin levels over the 24-hour period was characterized as the difference in 6-sulfatoxymelatonin levels measured from the two urine samples (Figure 4.1). For both melatonin characterizations, geometric least squares means (back transformed means of log-transformed variables) were calculated, as neither untransformed melatonin measure was normally distributed.

To characterize melatonin secretion patterns on day and night shifts, mean salivary melatonin levels from samples taken at similar times of day were compared between the day and night shifts within each of the 2 seasons using difference in least squares means estimates. These means were then graphed (Figure 4.2) to compare timing of melatonin secretion across shift types.

Multivariate associations between light exposure from 12AM to 5AM and both peak and change in 6-sulfatoxymelatonin levels were assessed using mixed multiple linear regression in a

random effects model to account for the repeated measures within. Models were built using an all-possible-models backwards selection procedure (47), in which potential confounders that were associated with the outcome at $P < 0.25$ were included in the modeling process, and only variables changing the parameter estimate by >10% were included in the final model. Variables considered as potential confounders were as follows: age, body mass index (BMI), total years of shift work, general lifestyle characteristics including smoking status, pack-years smoking and lifetime alcohol consumption patterns; reproductive characteristics including age at menarche, ever having been pregnant and number of pregnancies, number of days since previous menstrual period and menopausal status; season; regular use of antidepressants, betablockers, hormone replacement therapy or migraine medication; use of nonsteroidal anti-inflammatory drug (NSAID), sedatives, or oral contraceptives during the 24-hours of melatonin assessment; the number of alcoholic and caffeinated beverages consumed and smoking behaviour during the 24-hours of melatonin assessment; and sleep duration and wearing a mask for daytime sleep. Analyses stratified by shift type (day/night), season (summer/winter) and among premenopausal women only were also conducted using the same model building strategies.

The relationship between shift work history (total years of work in jobs in which at least 50% of shifts were night shifts to capture both rotating and permanent night shift patterns) and melatonin production on the night shift was investigated using multiple linear regression. Because lifetime history of shift work was assessed only once in the study questionnaire, melatonin levels measured during the first season of data collection were used as the outcome measure to maximize sample size. Shift work history was characterized both as a continuous variable and as a categorical variable (>20 vs ≤20 years shift work) (4). Multivariate models were built using the same process described above.

Chronotype was categorized using the Horne-Ostberg questionnaire (44) into one of 5 categories (Table 4.1). Because chronotype was assessed only once, geometric means and 95% CIs for both peak and change in melatonin levels were calculated for each chronotype category

using melatonin measures from the first season to maximize sample size. All statistical analyses were conducted using SAS, Version 9.2.

4.4 RESULTS

Characteristics of the study population and comparisons between day and night shifts in the first season are described in Table 4.1. Comparisons across shift types were also conducted for the second season and results were similar (Table E.1). Participants reported significantly longer sleep duration and consuming a greater number of alcoholic beverages during the 24-hours of melatonin collection when working their day shift. There were also differences in the number of days since the beginning of the previous menstrual period between day and night shifts in both seasons. However, because these were in opposite directions across seasons, they were likely due to factors related to scheduling of study participation sessions.

Mean light intensity levels from 12AM to 5AM were significantly higher when nurses were working their night shift (Table 4.1), although actual light exposure on the night shift was much lower (maximum 37.2 lux) than the approximately 200 lux found to decrease melatonin production in experimental research (34,35). However, there were no significant differences in either peak urinary melatonin or in the change in melatonin levels when nurses were working day and night shifts (Table 4.1).

Mean salivary melatonin levels from saliva samples taken at similar times of day (Figure 4.1) were compared for nurses working their day and night shifts. Figure 4.2 (comparing day and night shifts in the first season) confirms that peak melatonin production occurred at night when nurses were working both day and night shifts (40), suggesting the absence of a major phase shift in melatonin production with night work. Thus, in our study population, early morning urine samples from both the day and night shifts are the best to capture peak melatonin production, as found in our previous study (40).

Mixed multiple linear regression was used to examine the relationship between light exposure and both melatonin measures for data from both seasons combined. Only observations with complete data for both light exposure and melatonin were included in this analysis. Of 435 eligible observations, 27 were missing light data, 20 were missing morning urinary melatonin levels and 35 were missing values for the change variable. In the full study population, neither peak melatonin nor change in melatonin were associated with light exposure (Table 4.2). In the model examining change in melatonin levels, five overly influential individuals were removed from the analysis to improve model fit, although neither model (full or reduced) demonstrated a significant relationship between light exposure and melatonin production.

An analysis stratified by shift was conducted to account for the observed wide range of interindividual variability in melatonin levels. On the day shift, there was little variation in light exposure from 12AM to 5AM but large interindividual variability in melatonin levels, such that lack of exposure variability in this group could be hiding a potential relationship between light and melatonin, when data from both shift types was examined together. On the night shift, in which an effect of light exposure on melatonin would be expected, there was more variability in both light exposure and melatonin values. When working the night shift, a slight inverse relationship between light exposure and peak melatonin levels was suggested (Table 4.2), and a statistically significant small inverse association between light exposure and change in melatonin levels was observed (Figure 4.3).

In bivariate analysis, the number of days since previous period was associated with melatonin levels. Therefore, a restricted analysis among premenopausal women (both shift types) was conducted to allow this potential confounder to be considered; however, no relationship between light and either melatonin measure was detected (Table 4.2). An analysis stratified by season was also conducted to investigate potential differences across seasons; however, no relationship with either melatonin variable was observed in summer (peak = 0.018, $P = 0.79$; change = 0.03, $P = 0.68$)) or winter (peak = -0.07, $P = 0.37$; change = -0.12, $P = 0.14$).

The relationship between the number of years of shift work, defined as the number of years spent working a job that included 50% or more nights, and melatonin levels was also examined. When a continuous representation of years of shift work was used with melatonin levels following the night shift, a statistically significant positive relationship was observed, in which shift work history was associated with an increase in peak melatonin levels and a borderline positive relationship with the change in melatonin levels was also observed (Table 4.3). Furthermore, studies of shift work history and breast cancer suggest that it is long-term shift work that increases cancer risk (14,15); therefore, an analysis to investigate the effects of long-term shift work on melatonin levels was also conducted using a cut point of 20 years (4). A positive relationship (although not significant) in which long-term shift work was associated with increased melatonin levels was also observed (Table 4.3).

Finally, relationships between melatonin and chronotype were explored. Eighty-four nurses (71%) returned the chronotype questionnaire. Three were classified as extreme morning types, 18 as moderate morning type, 8 as moderate evening type, and 55 as neither type. No relationship between mean melatonin values on the day or night shift from the first season and chronotype was observed (Table E.2). Furthermore, the influence of chronotype as a confounder of the shift work – melatonin relationship was explored. Whereas the distribution of chronotypes differed by long-term shift work status (Table 4.3), chronotype had little effect on the shift work – melatonin relationship and thus was not included in the final statistical models.

4.5 DISCUSSION

These results show that the pattern of melatonin production was similar when rotating shift nurses were working days or nights. Furthermore, light exposure was not strongly related to reduced melatonin production, although a slight inverse relationship with change in melatonin (but not peak) was seen when nurses were working at night. If replicated, these findings may

imply that the rapidly rotating shift schedule examined here (or exposure to low light levels while working at night) is not sufficiently disruptive to produce changes in melatonin production. Alternatively, these results may also point to a role for other biologic pathways, in addition to melatonin, in the relationship between shift work and cancer risk (48).

Main strengths of this study are its objective measures of both light intensity and melatonin while investigating a commonly worked shift schedule. Although light exposure and melatonin are often cited as intermediates between shift work and cancer, few studies with objective measures of light intensity examining relationships with melatonin in an observational setting have been published (37,39,40). In epidemiologic studies, associations between shift work history, a proxy for light exposure, and melatonin may be confounded by other behaviours associated with this work schedule that are related to melatonin production. Thus, the use of an objective exposure measure in our study allowed the role of nighttime light in melatonin production to be specifically examined in an observational setting.

As well, by comparing melatonin levels from biological samples collected at similar times of day, this study accounts for potential confounding by natural circadian variations in melatonin production when comparing individuals on day and night shifts, a feature that has been absent from most published observational studies of the light-melatonin relationship (37,39,40). Specifically, one study found melatonin levels were lower after sleeping and higher after working among permanent night shift workers compared to day workers (39), whereas another found similar results among rotating shift workers (37). Our previous study observed an inverse relationship between light exposure and melatonin levels following sleep; however, this relationship was no longer significant when results were stratified by shift type to account for differences in the timing of urine sampling across shift groups, and salivary melatonin analysis revealed that timing of melatonin production was similar across shift types (40). These results suggest that differences in melatonin levels among individuals sleeping during the day compared to those sleeping at night observed in previous studies (37,39) could be partially confounded by

natural circadian variations in melatonin (40). Thus, uncontrolled confounding in other studies could explain why our findings differ.

Given that previous studies have examined more extreme shift schedules (permanent nights, different rotation patterns) (37–41) and the potential for residual confounding by circadian rhythms in biomarker studies, differences in the results of this study compared with previously published work are understandable. Furthermore, nighttime lighting conditions in the hospital in our study may have been too dark to reach the threshold required to produce a strong effect on melatonin. Although light levels during the night observed here were significantly higher on the night shift, mean light levels were much lower than those used in experiments to produce changes in melatonin production (34,35). Future studies among individuals with a wider range of light exposure variability will have greater power to detect relationships between light exposure and melatonin in an observational setting. Additional study strengths included the use of urine and saliva samples, which allowed us to measure validated biomarkers of melatonin production (45,46) and adjustment of urinary melatonin measures for creatinine allowed for differences in urine diluteness to be controlled.

The study also investigated the association between shift work history and melatonin production. When examined as a continuous variable, a small positive relationship between shift work history and melatonin production on the night shift was seen; however, no statistically significant relationships between long-term shift work history and melatonin production were found. Two reports from the Nurses Health Study found decreased melatonin levels among women with recent (previous two weeks) shift work history (36,49), although only one was significant (36). Furthermore, long-term history of shift work was not associated with melatonin levels in the Nurses' Health Study (49) and ever having worked the 'graveyard' shift was not associated with melatonin among Japanese women (41). Although we found that individuals with less than 20 years of shift work experience were more likely to be evening types, this difference is likely age related, as chronotype becomes earlier with age (50). Nurses in the shift work

experience group of more than 20 years were substantially older and the addition of chronotype to models looking at shift work history and melatonin that already included age had little impact on observed relationships. Previous studies (41,49) have not considered the role of chronotype in the shift work – melatonin relationship, therefore, future studies that include chronotype are needed to confirm our results.

Although the inclusion of nurses working only the DDNN rotating shift pattern allowed for a sample collection scheme accounted for the influence of circadian melatonin variations, the use of a single shift schedule limits generalizability to other patterns of shift work. More extreme shift schedules, such as more consecutive nights, may be associated with a greater degree of circadian disruption, and there may be a greater effect of light exposure on melatonin. Several previous studies have focused on populations working fixed shifts and differences in shift schedules between these studies and ours could partially explain observed differences in the influence of light exposure on melatonin production (38,39). Furthermore, although the use of an objective measure of light was a strength of this study, light loggers were worn by nurses around their neck, such that there may have been small differences between logger-measured light intensity and actual intensity perceived by the retina. However, such differences would be quite small and unlikely to substantially impact study results.

We also observed a wide range of interindividual variability in melatonin levels measured on both day and night shifts, which may have limited power to detect small relationships with light exposure, particularly given the low lighting levels when working at night. In addition, whereas first morning urine has been shown to reflect overnight melatonin production during sleep, because urine was not collected over the entire night during the night shift, morning urine samples collected following the night shift may represent an underestimate of melatonin production. However, because peak melatonin levels measured following both shifts were similar, the potential underestimation of melatonin production on the night shift is unlikely to have influenced study findings. Finally, it has been suggested that phase shifts (altered timing) of

melatonin production may be important to cancer risk (48,51); however, given the timing of both urine and saliva sampling (Figure 4.1), this study was unable to detect small phase shifts in melatonin production across shift types.

This study observed no difference in melatonin production between the day and night shifts, and only a small inverse relationship between light exposure and change in melatonin on the night shift. Future studies of different shift schedules with brighter light exposure are needed to provide context to these results. If future work also fails to find strong relationships between light exposure and melatonin production, this could suggest that other mechanisms (48) are responsible for the observed link between shift work and cancer (1–12,52). Alternatively, if melatonin is indeed the pathway through which shift work influences cancer risk, our results suggest the prevalent rotating shift pattern of two 12-hour days, two 12-hour nights with relatively low levels of nighttime light exposure followed by five days off is minimally disruptive to melatonin production.

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4.9 TABLES AND FIGURES

Table 4.1: Characteristics of Study Population

Variable	Day Shift[a]	Night Shift	p-value[b]
	Mean (SE) / N (%)	Mean (SE) / N (%)	
Age	40.5 (1.02)	-	-
Body Mass Index (kg/m^2)	28.4 (0.82)	-	-
# Years of Shift Work	14.0 (1.01)	-	-
Ethnicity			
White	113 (95.76%)	-	-
Other	5 (4.24%)	-	-
Reproductive Characteristics			
Age at Menarche	12.55 (0.13)	-	-
Number of pregnancies	1.58 (0.18)	-	-
Ever Been Pregnant			
Yes	69 (58.47%)	-	-
No	49 (51.53%)	-	
Menopausal Status			
Pre-menopausal	89 (75.42%)	-	-
Post-menopausal	29 (24.58%)	-	
Number of days since previous period	13.83 (1.73)	19.58 (1.71)	0.009
Sleep Characteristics			
Sleep Duration	6.91 (0.15)	5.23 (0.15)	<0.0001
Sleep interrupted	15 (12.71%)	19 (16.10%)	0.45
Lights on for >1hr if interrupted	3 (2.54%)	8 (6.78%)	0.09
Experience sleep problems	69 (58.47%)	-	-
Diagnosed with sleep disorder	4 (3.49%)	-	-
Medication Use			
Antidepressants	12 (10.17%)	-	-
Beta-blockers	2 (1.69%)	-	-
Hormone Replacement Therapy	7 (5.93%)	-	-
Migraine Medication	6 (5.08%)	-	-
Pain medication (NSAIDs)	29 (24.58%)	28 (23.73%)	0.86
Sedatives or muscle relaxants	6 (5.08%)	7 (5.93%)	0.74

Oral contraceptives	19 (16.10%)	15 (12.70%)	0.25
Lifestyle Characteristics			
Pack-years Smoking	2.77 (0.57)		
Smoked during 24h of melatonin collection	10 (8.47%)	11 (9.32%)	0.56
Caffeine consumption (# drinks during 24 hours melatonin collection	2.61 (0.22)	2.97 (0.22)	0.10
Alcohol consumption (# drinks during 24 hours melatonin collection)	0.33 (0.07)	0.06 (0.07)	0.008
Lifetime Alcohol Consumption (# drinks/wk)			
Teen	2.52 (0.33)	-	-
20s	4.30 (0.38)	-	-
30s	2.83 (0.34; n = 95)	-	-
40s	2.71 (0.36; n = 66)	-	-
50s	2.75 (0.74; n = 26)	-	-
Chronotype			
Definite morning type	3 (3.57%)	-	-
Moderate morning type	18 (21.43%)	-	-
Neither type	55 (65.48%)	-	-
Moderate evening type	8 (9.52%)	-	-
Definite evening type	0	-	-
Light Exposure			
Log-transformed mean light intensity (log lumens/m^2)	-2.14 (0.06)	-0.06 (0.06)	<0.0001
Urinary 6-sulfatoxymelatonin[c]			
Morning 6-sulfatoxymelaotnin (ng/mg creatinine)	27.25 (1.11)	25.49 (1.11)	0.65
Change in 6-sulfatoxymelatonin (ng/mg creatinine)	23.48 (1.14)	24.53 (1.14)	0.80

a. Characteristics assessed in the study questionnaire (administered once) are shown in the 'Day Shift' column
b. Differences between day and night shifts are compared using difference of least squares means estimates in a mixed model with a random subject effect for continuous variables and using McNemar's test for categorical variables
c. Geometric means (calculated by back-transforming log-transformed variables) are presented here

Table 4.2: Association between light and urinary melatonin

Model	Regression Coefficient	p-value
Light Exposure:		
Full Population		
Peak Urinary Melatonin[a]	-0.03301	0.49
Change in Urinary Melatonin[b]	-0.03128	0.58
Night Shift Only		
Peak Urinary Melatonin[c]	-0.04037	0.07
Change in Urinary Melatonin[d]	-0.05494	0.04
Day Shift Only		
Peak Urinary Melatonin[e]	-0.08371	0.47
Change in Urinary Melatonin[f]	0.04392	0.75
Premenopausal Women Only		
Peak Urinary Melatonin[g]	0.04535	0.50
Change in Urinary Melatonin[h]	-0.07648	0.27

a. Adjusted for use of antidepressant medication and the number of caffeinated beverages consumed during the 24-hours of melatonin assessment.
b. Adjusted for use of antidepressants, oral contraceptives and the numbers of caffeinated beverages consumed during the 24-hours of melatonin assessment.
c. Adjusted for total number of years shift work and use of antidepressant medication.
d. Adjusted for use of antidepressant medication.
e. Adjusted for menopausal status and the number of caffeinated beverages consumed during the 24 hours of melatonin collection.
f. Adjusted for menopausal status, antidepressant and migraine medication use, the number of caffeinated beverages consumed during the 24 hours of melatonin assessment and total number of years of shift work.
g. Adjusted for total number of years of shift work, number of days since previous menstrual period, smoking, and both number of alcoholic beverages and number of caffeinated beverages consumed during the 24 hours of melatonin assessment. Four overly influential individuals removed to improve model fit.
h. No variables changed the parameter estimate by more than 10%, therefore no confounders included in model.

Table 4.3: Influence of shift work history on melatonin

Model	Regression Coefficient		p-value
Number of Years Shift Work (continuous)			
Peak Urinary Melatonin[a]	0.03251		0.02
Change in Urinary Melatonin[b]	0.03373		0.05
>20 vs ≤20 Years Shift Work			
Peak Urinary Melatonin[b]	0.55187		0.07
Change in Urinary Melatonin[b]	0.63605		0.08
Chronotype[c]	≤20 Years Shift Work	> 20 Years Shift Work	
Definite Morning Type	2 (3.64%)	1 (3.45%)	0.04
Moderate Morning Type	7 (12.73%)	11 (37.93%)	
Neither Type	39 (70.91%)	16 (55.17%)	
Moderate Evening Type	7 (12.73%)	1 (3.45%)	

a. Adjusted for age.
b. Adjusted for age and light exposure between 12AM and 5AM during 24-hours of melatonin assessment.
c. Chronotype frequency by long-term shift work status

Day Workers:

Functional Time Point:	*Upon Awakening* Saliva Sample 1	*Mid-Shift* Saliva Sample 2	*Before Sleep* Saliva Sample 3	*Upon Awakening* Saliva Sample 4
Time of Day:	5 - 7 AM	3 - 5 PM Urine Sample	11PM - 1AM	5 - 7AM Urine Sample

Night Workers:

Functional Time Point:	*Upon Awakening* Saliva Sample 1	*Mid-Shift* Saliva Sample 2	*Before Sleep* Saliva Sample 3	*Upon Awakening* Saliva Sample 4
Time of Day:	3 - 5 PM	11PM - 1AM	5 - 7 AM Urine Sample	3 - 5PM Urine Sample

Figure 4.1: Urine and saliva sample collection timeline

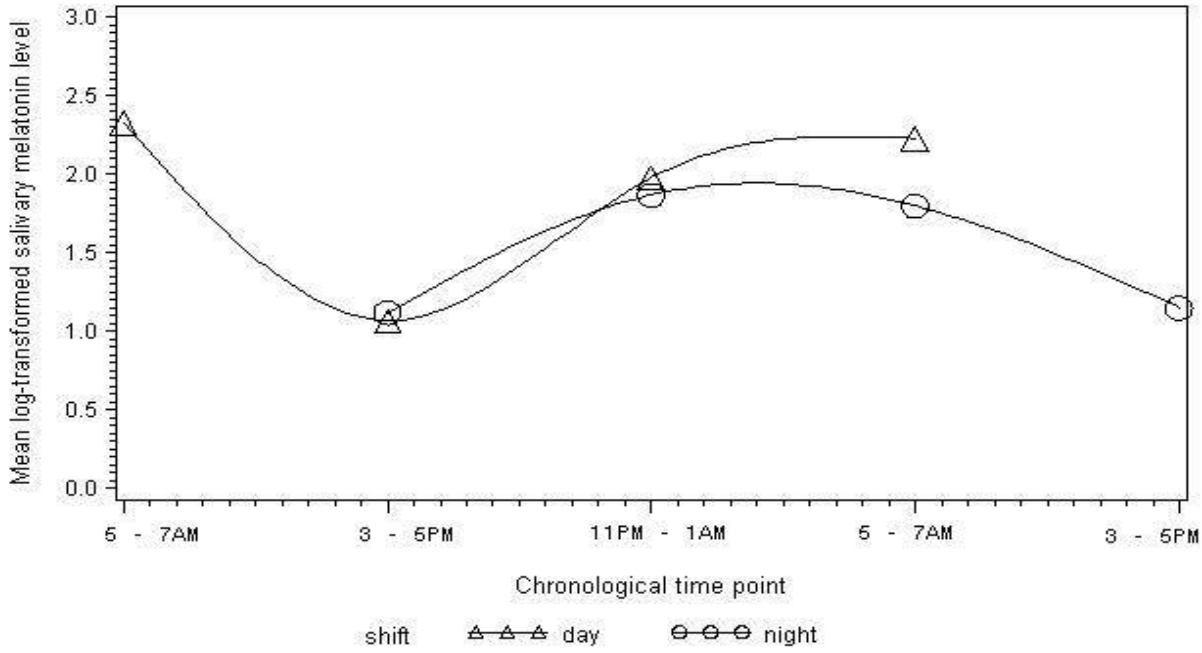

Figure 4.2: **Salivary melatonin levels comparing chronological time points in season 1.**
For comparisons between day and night workers: 3PM – 5PM: p-value = 0.99; 11PM – 1AM: p-value = 0.84; 5AM – 7AM: p-value = 0.04.

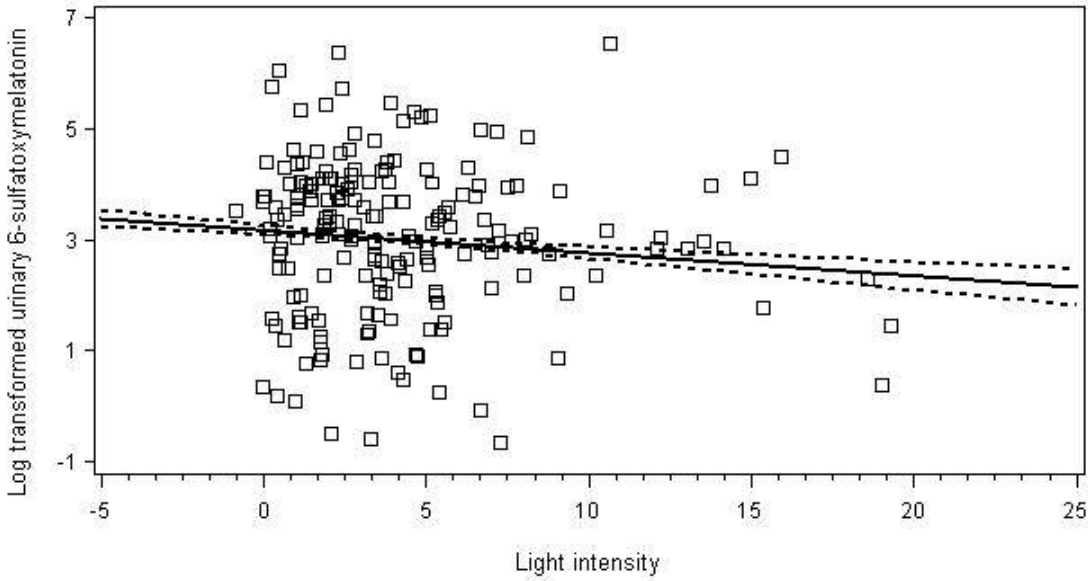

Figure 4.3: Association between light intensity (lumens/m2) and log-transformed change in urinary melatonin (lg ng/mg creatinine). Change in melatonin is calculated as the difference in melatonin values from the 2 urine samples. Parameter estimate = -0.05494 (*P*=0.04). Model adjusted for use of antidepressants.

Chapter 5

Circadian Gene Variants and Risk of Breast Cancer

5.1 ABSTRACT

Background: Circadian (clock) genes have been linked with several functions relevant to cancer development and progression, and epidemiologic research has suggested relationships with breast cancer risk for variants in *NPAS2*, *CLOCK*, *CRY2* and *TIMELESS*. Increased breast cancer risk has also been observed among shift workers, suggesting potential interactions in relationships of circadian genes with breast cancer.

Methods: Relationships with breast cancer of 100 SNPs in 14 clock-related genes, as well as potential interactions with shift work history, were investigated in a case-control study (1,050 cases, 1,050 controls) conducted in Vancouver, British Columbia and Kingston, Ontario. Odds ratios in an additive genetic model for European-ancestry participants (651 cases, 805 controls) were calculated, using a two-step correction for multiple testing: within each gene through permutation testing (10,000 permutations), and correcting for the false discovery rate across genes. Interactions with ethnicity and shift work (<2 years vs ≥2 years) were evaluated.

Results: Following permutation analysis, two SNPs (rs11113179 in *CRY1* and rs3027188 in *PER1*) displayed significant associations with breast cancer and one SNP (rs3816360 in *ARNTL*) was on the margin of significance; however, none were significant following adjustment for the false discovery rate. No significant interaction with shift work history was detected.

Conclusions: In this study, clock gene variants were not associated with breast cancer risk and interactions with shift work were not apparent.

5.2 INTRODUCTION

Circadian rhythms, "daily oscillations in physiological and behavioural processes" (1), are controlled by the suprachiasmatic nucleus (SCN) (1,2) and at the molecular level are coordinated through the interactions of a set of circadian (clock) genes (1–3). The main genes involved in this mechanism, *CLOCK*, *ARNTL* (also called *BMAL1*), *CRY1, CRY2*, *PER1, PER2, PER3*, *NR1D1* (*Rev-Erbα*) and *CSNK1E*, are organized into a set of positive and negative feedback loops that fluctuate in a 24-hour cycle (1–3). In addition to these genes, three others (*TIMELESS*, *DEC1* and *DEC2*), also appear to have a role in the functioning of the molecular clock (4–6). Although the central circadian clock is located in the SCN, clock genes are found in all cells and there are a number of peripheral oscillators throughout the body, synchronized by input from the central circadian pacemaker (2).

Clock genes act as tumour suppressors, such that variations in gene expression could have a role in cancer development and progression (1). Experimental work has suggested links between clock gene function and cell cycle progression, apoptosis, the estrogen signalling system and the DNA damage response (7–17). Epidemiologic studies have also begun to identify relationships between clock gene polymorphisms and risk of breast cancer (18–23), prostate cancer (24,25) and non-Hodgkin lymphoma (25,26). For breast cancer in particular, results from a Connecticut-based case-control study have demonstrated links between single nucleotide polymorphisms (SNPs) in *NPAS2 (*a homolog of *CLOCK)*, *CLOCK*, *CRY2* and *TIMELESS* (19–22), although recent analysis from the Nurses' Health Study cohort was not able to replicate these findings (23). Given the potential for chance findings, and that these variants have only been investigated in two separate case-control studies, additional research is required to clarify the relationship of clock gene variants with breast cancer risk.

Further, an increased risk of breast cancer has been observed among shift workers (27–34), where circadian disruption is the main mechanism hypothesized to explain the association and a role for circadian genes in this relationship has been suggested (35,36). To date, only one

study has investigated potential interactions between shift work and clock gene variants with respect to breast cancer risk (23). This study found the heterozygous genotype at rs2305160 in *NPAS2* was protective among women with less than two years shift work history, but not related to breast cancer risk among those who had worked shift work for two or more years (23). As well, when risk of breast cancer with shift work was examined within genotypes (i.e. Ala/Ala, Ala/Thr. Thr/Thr), the odds of breast cancer were higher among women who had done two or more years of shift work in both the Ala/Thr and Thr/Thr genotype groups (23). These results provide preliminary support for an interaction between shift work and clock gene variants. The objectives of our study were to investigate associations of clock genes with breast cancer risk and to explore possible interactions between clock gene variants and shift work in a large breast cancer case-control study.

5.3 METHODS

5.3.1 Study Population:

A population-based case-control study was conducted in Vancouver, British Columbia (BC), Canada and Kingston, Ontario (ON), Canada to identify genetic, lifestyle and environmental risk factors for breast cancer. The methods have been previously described (Chapter 3). Briefly, in Vancouver, cases (n=1,062) were women, aged 20 – 80, with either *in situ* or invasive breast cancer living in Vancouver or surrounding communities, and recruited from the BC Cancer Registry. Controls (n=1,015) were cancer-free individuals from the Screening Mammography Program of BC recruited from breast screening clinics in the same geographic areas, and frequency matched to cases by age. In Kingston, both cases and controls were recruited from the Hotel Dieu Breast Assessment Program, where cases (n=131) were individuals with a diagnosis of either *in situ* or invasive breast cancer and controls (n=164) were women with normal mammogram results or benign breast disease, also frequency matched to cases by age.

Study participation in both Vancouver and Kingston involved completing a study questionnaire, either self-administered (BC and ON) or by telephone interview (BC only). Women were also asked to provide a blood (BC and ON) or saliva (BC only) sample and grant access to medical records to allow information concerning breast health to be collected. DNA was extracted from blood (n=1,986) or saliva (n=205) samples; 2,190 participants (92%) had sufficient DNA for genotyping. Ethical approval for this study was provided by both the Joint University of British Columbia/BC Cancer Agency Research Ethics Board and the Queen's University Health Sciences Research Ethics Board.

5.3.2 Selection of Genetic Variants:

Thirteen genes (*CLOCK*, *NPAS2*, *ARNTL*, *CRY1, CRY2*, *PER1*, *PER2, PER3*, *NR1D1* *CSNK1E*, *TIMELESS*, *DEC1* and *DEC2*) related to the molecular control of circadian rhythms were identified from the literature (1,5,6,37). Further, given that melatonin production is controlled by circadian rhythms set by clock genes (38–41), and melatonin's suggested role as an intermediate between shift work and cancer risk (35,42), two genes controlling the expression of the melatonin receptor (*MTNR1A* and *MTNR1B*) were also included.

For each gene, a set of HapMap tagSNPs were selected using data from the CEU population from HapMap release 28 using Tagger (43) in Haploview (44). The minimum minor allele frequency was set at 0.10 and the r^2 threshold set to 0.8. Clock gene SNPs identified from the literature as having been previously associated with risk of cancer at any site were force included in the set. This process generated a list of 114 SNPs in fourteen of the clock genes described above. No tagSNPs that met the inclusion criteria for minor allele frequency could be found for *DEC2*, so this gene was excluded from further analysis.

5.3.3 Genotyping:

The SNPs in this analysis were analyzed as part of larger 768 SNP Golden Gate (Illumina) assay that included SNPs related to other research questions. SNPs that failed assay design were replaced where possible with an equivalent tagSNP; SNPs for which no equivalent could be found were excluded (n = 4 for clock genes). Genotyping was performed by the McGill University and Genome Quebec Innovation Centre, Montreal, Canada.

5.3.4 Quality Control of Genotype Data:

Genotype quality control steps for the full 768 SNP set were performed in Genome Studio v2011.1 (Illumina, San Diego, CA, USA), PLINK v1.07 (45), GRR (46) and Excel 2007 (Microsoft, Redmond, WA, USA).

Data quality control for SNPs

SNPs with the following criteria were excluded: according to Illumina's recommendation for GoldenGate genotype data (Illumina User Guide, Genome Studio Genotyping Module v.10 User Guide, Illumina, Part#11319113), SNPs with GenCall Scores < 0.25, SNPs with GenTrain scores < 0.4; SNPs with poor clustering (more than three clusters or poor inter-cluster separation); mono-allelic SNPs; SNPs with any genotype discrepancies in 126 pairs of replicate samples; SNPs with call rate < 95%; SNPs with unexpectedly low minor allele frequency (MAF) in Caucasian controls compared to HapMap CEU data (i.e. examining the distribution of relative MAF differences in all SNPs and removing outliers; this removes SNPs for which the minor allele was likely under-called); and SNPs out of Hardy-Weinberg equilibrium (HWE) (p<0.001) in European-ancestry controls. SNPs with a call rate <95% in saliva samples only were excluded for subjects with saliva samples, but included for subjects with blood samples.

Following an iterative design process (as described above), 114 clock-related SNPs were selected for genotyping. Fourteen SNPs were excluded: 2 SNPs were rejected by the genotyping

centre upon initial inspection, 1 had a low GenTrain score, 9 displayed poor clustering, 1 had a low call rate, and 1 SNP failed HWE. 100 SNPs remained for analysis and are listed in Appendix F.

Data quality control for samples

Samples were excluded for the following reasons: samples with excess heterozygosity (more than 3 standard deviations from the mean) when compared to other samples of the same ethnicity, samples with call rate <0.95, samples for which the genotypes at three Y chromosome markers indicated that the sample was from a male, unrelated samples having identical genotypes, and discrepancies between estimated ethnicity identified from genotype data and self-reported ethnicity (see below). 2,318 DNA samples from 2,190 individuals were genotyped, 215 being saliva samples. 126 sample pairs (5.8%) were included as duplicates for quality control purposes. The following samples were removed: 16 had a call rate <95%; 2 samples (from one individual) had a gender discrepancy; 5 samples (from 4 individuals) had excess heterozygosity; 3 pairs of samples had identical genotypes; and in 5 samples predicted ethnicity disagreed with self-reported one. Following the exclusions described, when genotypes were merged the concordance rate between pairs was one.

61/68 Ancestral Informative Markers (AIMs) were available after quality control. The calculated inflation factor using AIMS was λ=16.99, indicating overall population stratification between the case and control groups (47–49). When Caucasian samples only were analyzed with AIMs, however, no population stratification was detected (λ=1.0). Thus, the population stratification detected in the overall study was due to known differences in the proportion of women of different ethnicities in the case and control groups.

Self-reported ethnicity was then compared to data-predicted ethnicity by calculating identity by state and then creating multi-dimensional scaling plots (45) with HapMap samples in the CEU, CHB, CHD, JPT, YRI and TSI populations as reference groups. One individual who self-reported as “European” plotted similarly to individuals of mixed ethnicity and was excluded.

Nine pairs of samples from relatives were also identified and verified using questionnaire data. As this study is population-based, one member of each pair was excluded, where when the pairs were both cases the individual with the earlier diagnosis date was included and when both were controls, the older individual was included.

Following quality control procedures, 2,275 samples (2,151 individuals) remained. After the genotypes of duplicate samples were merged, 1,099 cases and 1,052 controls were available for analysis. Since the minimum age for screening mammography in British Columbia is 40 and this was the source for controls, all samples with age <40 from British Columbia were excluded, leaving 1,050 cases and 1,050 controls in the final clock gene analysis. Reasons for which samples were excluded from the final analysis are summarized in Figure 5.1.

5.3.5 Statistical Analysis:

As ethnicity was suspected as a potential confounder *a priori* due to the risk of population stratification (50,51), all analyses were stratified by ethnicity. As it was the largest, comprising nearly 70% of the sample, the European subgroup was used for the main analysis. Relationships between individual SNPs and breast cancer were investigated using multivariable logistic regression in an additive genetic model, with all models adjusted for age and centre. Analyses within other ethnicities (Asian, Mixed/Other and South Asian) were conducted to compare results to those observed among Europeans. Individuals of Filipino ancestry were included in the "Asian" subgroup as these subgroups clustered together in the multi-dimensional scaling analysis. To investigate potential interactions by ethnicity, a second model including both the European and Asian groups with a genotype-ethnicity interaction term was created. Other ethnicity groups were excluded from the interaction analysis due to sample size. All analyses were conducted using SAS, Version 9.2 and PLINK (45).

The influence of multiple testing was assessed using a two-step process (52). Within each of the 14 clock genes, set-based permutation testing with 10,000 permutations was used in PLINK to identify the SNP with the most extreme p-value, where each of the 14 genes represented one set. For each permutation, the program randomly assigns case status to genotypes and calculates the number of times a more extreme p-value could be observed by chance in permutations of all SNPs within a set (gene) and reports an adjusted p-value for the set. The single SNP identified for each gene in permutation testing was then taken to represent that gene and the Benjamini-Hochberg (53) adjustment was applied to control the false discovery rate (FDR) across all genes (n = 14) and provide a corrected p-value. SNPs with $p < 0.05$ following FDR adjustment were considered to have a statistically significant association with breast cancer risk.

To facilitate comparisons of our results with previous studies, associations of seven SNPs previously associated with breast cancer risk were also examined among Europeans (19–22). The Benjamini-Hochberg (53) adjustment for the false discovery rate was applied to these p-values for both the full 100 SNP set and within each gene. Previous studies have also noted differences in relationships of clock genes with breast cancer by menopausal status and tumour hormone receptor (estrogen and progesterone receptor) status (19–22). As such, analysis stratified by menopausal status, as well as polytomous logistic regression for estrogen and/or progesterone receptor positive and estrogen and progesterone receptor negative tumours, was conducted.

Interactions between shift work history, which has been associated with breast cancer risk in this study (Chapter 3), and the SNP with the most extreme p-value identified for each of the 14 clock genes in the permutation analysis were also explored. To ensure sufficient numbers within shift work-genotype groups, and similar to the cutoff used in the most recent publication from the Nurses' Health Study (23), individuals were classified as having worked <2 years or ≥2 years in a job including shift work, where shift work was defined as a job in which 50% or more of time was spent on evening or late night shifts. Interactions were modeled by including both the shift

work main effect and shift work-genotype product terms in the logistic regression model and interaction p-values were corrected for the FDR (n = 14) (53). One additional SNP, rs2305160 in *NPAS2*, which recently displayed an interaction with shift work history in the Nurses' Health Study (23), was also investigated for interaction using the same methods.

5.4 RESULTS

Characteristics of the women included in the genetic analysis are described in Table 5.1. Cases had a greater proportion of individuals of Asian ethnicity than controls. As well, cases had lower levels of household income, an older average age at first mammogram and were more likely to have a family history of breast cancer among first degree relatives than controls.

The main genetic analysis was conducted in the European subgroup to address the *a priori* risk of confounding by ethnicity. Results of the two-step analysis process are found in Table 5.2. Following permutation analysis, two SNPs (rs11113179 in *CRY1* and rs3027188 in *PER1*) displayed significant associations with breast cancer and one SNP (rs3816360 in *ARNTL*) was on the margin of significance, however, none were significant following adjustment for the false discovery rate. To examine the possibility of a genotype-ethnicity interaction with respect to breast cancer risk, the most significant SNP for each gene in the European sample was tested in a model including both Europeans and Asians with a genotype-ethnicity interaction term. One SNP (rs3027188 in *PER1*) displayed a significant interaction with ethnicity (Table 5.2). In the Asian subgroup, associations with breast cancer risk for three SNPs (rs11037696 in *CRY1*, rs4388843 in *MTNR1B* and rs356642 in *NPAS2*) could not be calculated due to insufficient frequency of the minor allele (Table 5.2). Odds ratios for all ethnicity groups (including South Asian and Mixed/Other) are displayed in Table E.3. No difference was found when European data was stratified by either menopausal or tumour hormone receptor status (Tables E.5 and E.6). Associations with genotype for each of the 14 SNPs in Table 5.2 were assessed separately in

Europeans and Asians for variables significantly associated with breast cancer (household income, ever having been pregnant, family history, age at first mammogram, BMI and lifetime alcohol consumption) not initially included as confounders. When a significant association with genotype was observed, the impact of the potential confounder on the odds ratio between that SNP and breast cancer was assessed. One SNP (rs883871 in *NR1D1* with household income) displayed a change in the odds ratio >10% (54), with an adjusted odds ratio of 0.85 (0.65 – 1.11).

To facilitate comparisons with previous clock gene – breast cancer studies, the associations of six SNPs (rs1401417 in *CRY2*, rs2305160 in *NPAS2*, rs7302060 in *TIMELESS*, and rs7698022, rs11932595 and rs6850524 in *CLOCK*) that had previously displayed associations with breast cancer (19–22) were tested for replication in the European subset of our study. None displayed a statistically significant association with breast cancer risk (Table E.4). As well, no difference was seen by either menopausal or tumour hormone receptor status (Tables E.5 and E.6).

Finally, as shift work has been previously associated with breast cancer risk in this study (Chapter 3) and since one hypothesized mechanism for the influence of shift work on breast cancer is through circadian disruption (35), interactions between shift work and clock gene polymorphisms with respect to breast cancer risk were explored. Interactions were modeled by including a shift-work (<2 vs. ≥2 years)-genotype product term in logistic regression models for each of the 14 SNPs identified most significant within gene in the European subgroup. Marginally significant interactions with shift work were detected for rs4388843 in *MTNR1A* and rs11894535 in *PER2*, where the odds ratios for breast cancer for women with <2 years and ≥2 years history of shift work were in opposite directions (Table 5.3). However, following correction of the interaction p-values for the false discovery rate (53), no significant interaction between clock gene variants and shift work history was detected. The interaction between rs2305160 in *NPAS2* and shift work recently reported by the Nurses' Health Study (23) was also tested for replication in our study. While the odds ratios for breast cancer for women with <2 years

(OR=1.23, 95%CI=1.02 – 1.49) and ≥2 years (OR=0.92, 95%CI=0.71 – 1.18) shift work history were in opposite directions, the p-value for the interaction with shift work was just above the margin of statistical significance (p=0.068).

5.5 DISCUSSION

In this relatively large population-based case-control study we did not observe any association between clock gene variants and breast cancer risk after the potential false discovery rate was taken into account. While these results are consistent with a recent analysis from the Nurses' Health Study (23), a breast cancer case-control study among women in Connecticut reported a relationships for variants in *CRY2*, *NPAS2*, *TIMELESS* and *CLOCK* with breast cancer risk (19–22). While some of these previously observed associations were only apparent when the data was stratified by menopausal status (*NPAS2*, *CRY2*) or estrogen/progesterone receptor status (*CRY2*, *TIMELESS*, *CLOCK*) (19–22), similar stratification in our study did not modify the SNP-breast cancer relationships.

Further, results from the Connecticut case-control study (20–22) have demonstrated epigenetic differences in *CRY2*, *CLOCK* and *TIMELESS* between breast cancer cases and controls. In DNA from peripheral blood lymphocytes, hypermethylation of *CRY2* was associated with an increased breast cancer risk, while hypomethylation of *TIMELESS* increased risk of stage II, III and IV breast cancers and hypomethylation of the *CLOCK* promoter reduced breast cancer risk (20–22). If these epigenetic differences are driving associations between clock gene variants and breast cancer, differences in the distribution of epigenetic changes between populations could explain inconsistencies between studies that did observe an effect of circadian gene variants (19–22) and our study and the Nurses' Health Study analysis (23) that did not detect such effects. The case-control design of the study from Connecticut (20–22) makes it impossible to determine whether changes in methylation occurred before or after breast cancer development, even when

women who had undergone radiotherapy and chemotherapy were excluded from methylation analysis, such that the epigenetic results of these studies should be interpreted with caution.

This study, to our knowledge, is the first to explicitly examine the relationship between circadian gene variants and breast cancer in multiple ethnic groups, as previous studies have been conducted in samples where either all (23) or most (19–22) participants are Caucasians. In this study, only one SNP (rs3027188 in *PER1*) displayed a significant interaction with ethnicity (European vs. Asian) with respect to breast cancer risk. However, additional studies that include greater sample sizes in non-Caucasian ethnicities are needed to confirm this observation.

We did not observe an interaction between clock gene SNPs and shift work history. Only one previous study of circadian gene variants has investigated interactions with shift work (23). The investigators observed that the Thr variant (Ala/Thr and Thr/Thr genotypes combined) of rs2305160 in *NPAS2* displayed a protective effect compared to the Ala/Ala genotype among women with little to no (<2 years) history of rotating shift work, while among women with the Thr/Thr genotype, those with a history of rotating shift work (≥2 years) had a greater risk of breast cancer compared to women with <2 years (23).

The reasons for the difference in results of the shift work interaction analysis between our study and those of the Nurses' Health Study are not clear. As both studies restricted analysis to the European subgroup, differences in ethnic composition between our study and the Nurses' Health Study cannot explain the differences in observed relationships for rs2305160 (23). Although our study was conducted in the general population, such that individuals who participated in shift work did so in a variety of occupations including nursing (24% of women who had ever worked shifts were in health occupations), differences in the type or schedules of shift work between nurses and non-nurses seem unlikely to fully explain the divergent results. In analysis of the main effect of shift work on breast cancer in our study (Chapter 3), risks were similar for women who were and were not employed in health occupations and the magnitude of breast cancer risk associated with shift work was similar to that observed in the Nurses' Health

Study (28,30). As well, the results of the main effects of circadian gene variants were similar, as neither study reports a significant relationship with breast cancer for any clock gene SNP (23).

An alternative explanation for the absence of an observed interaction between shift work and clock gene variants may be the characterization of shift work. Previous analysis of the main effect of shift work on breast cancer in this study demonstrated a relationship for ≥30 years of shift work (Chapter 3), a cutoff of +/- 2 years was chosen to ensure there were sufficient numbers within shift work-genotype categories, even though no relationship between <2 vs. ≥2 years shift work and breast cancer was seen. Thus, this study would not have been able to demonstrate an interaction between shift work and clock gene variants if long-term shift work is the relevant exposure. However, the Nurses' Health Study also used the same short term dichotomization of shift work in analyzing potential shift work-circadian variant interactions and was able to demonstrate differences in risk, despite the absence of a main effect of ≥2 years of rotating shift work on breast cancer risk (23,30). The plausibility of long-term shift work as the relevant exposure in gene-environment interactions with clock gene variants is supported by recent pilot results from a Danish cohort that demonstrated differences in methylation of both *CRY2* and *CLOCK* in DNA from blood samples in long-term (>10 years) shift workers (55), although given the small number of shift workers included (n=19), these results are still preliminary.

In summary, this large study did not observe a relationship between clock-related genetic variants and breast cancer risk. While this study did not detect an interaction between shift work and clock gene variants with respect to breast cancer, these results are not consistent with those of the only other study that has considered such interactions (23). Therefore, at this time, there is insufficient evidence to either support or refute the existence of a shift work-clock gene interaction. Moving forward, studies able to stratify on the basis of longer periods of shift work will be useful in clarifying the relevant exposure for shift work-clock gene interactions, as both existing studies have only considered interactions with short durations (2 years) of shift work (23). If an interaction between shift work and clock gene variants with respect to breast cancer

risk does exist, such that some individuals are more susceptible than others to the effects of circadian disruption associated with working at night, identifying susceptible subgroups may have implications for occupational health.

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5.9 TABLES AND FIGURES

Table 5.1: Characteristics of Study Population

	Cases *Mean (SD) / N (%)*	**Controls** *Mean (SD) / N (%)*	**p-value**[a]
Age	57.3 (10.3)	56.9 (10.1)	0.34
Body Mass Index	25.6 (5.4)	25.1 (5.7)	0.02
Ethnicity			
European	651 (62.0%)	805 (76.7%)	<0.0001
Asian	252 (24.0%)	130 (12.4%)	
South Asian	31 (3.0%)	32 (3.1%)	
Filipino	58 (5.5%)	38 (3.6%)	
Mixed/Other	58 (5.5%)	45 (4.3%)	
Household Income			
< $30,000	183 (17.4%)	105 (10.0%)	<0.0001
$30,000 - $59,999	261 (24.9%)	230 (21.9%)	
$60,000 - $99,999	232 (22.1%)	260 (24.8%)	
> $100,000	223 (21.2%)	310 (29.5%)	
Not stated	151 (14.4%)	145 (13.8%)	
Menopausal Status			
Pre-menopausal	358 (34.2%)	390 (37.2%)	0.15
Post-menopausal	689 (65.8%)	659 (62.8%)	
Reproductive History			
Age at Menarche	12.9 (1.6)	12.8 (1.5)	0.41
Ever Been Pregnant	864 (82.6%)	824 (78.6%)	0.02
Age at First Pregnancy	27.8 (5.4)	27.7 (5.3)	0.70
Number of Pregnancies	2.3 (1.7)	2.2 (1.7)	0.57
Age at First Mammogram	44.6 (8.7)	42.7 (7.4)	<0.0001
Family History of Breast Cancer	208 (19.8%)	142 (13.5%)	0.0001
Lifestyle Characteristics			
Pack-years Smoking	5.7 (12.2)	5.1 (11.0)	0.77
Lifetime Alcohol Consumption (# drinks/wk)			
Teen	1.1 (3.6)	1.6 (4.0)	<0.0001
20s	2.2 (3.8)	3.3 (6.2)	<0.0001

30s	2.9 (6.0)	3.5 (5.4)	<0.0001
40s	3.1 (6.1)	3.7 (5.6)	<0.0001
50s	2.7 (4.9)	3.8 (6.2)	<0.0001
Shift Work History			
None	689 (65.6%)	695 (66.2%)	0.78
0 – 14 years	267 (25.4%)	276 (26.3%)	0.65
15 – 29 years	69 (6.6%)	68 (6.5%)	0.93
≥30 years	25 (2.4%)	11 (1.1%)	0.02

a. p-values calculated using T-tests and Wilcoxon Rank Sum tests for continuous variables and Chi-Square tests for categorical variables

Table 5.2: Analysis with European-based Permutations for Europeans and Asians[a]:

		EUROPEAN (651 cases, 805 controls)			ASIAN (310 cases, 168 controls)			
Gene	**SNP**	**Minor Allele Frequency (Controls)**	**OR (95% CI)**	**p-value[b]**	**Minor Allele Frequency (Controls)**	**OR (95% CI)**	**p-value[b]**	**p-value (Interaction)**
ARNTL	rs3816360	0.31	1.24 (1.06- 1.45)	0.081	0.36	1.04 (0.79 – 1.38)	1	0.12
CLOCK	rs2035691	0.32	1.15 (0.98 – 1.35)	0.23	0.41	0.99 (0.76 – 1.29)	1	0.43
CRY1	rs11113179	0.092	1.36 (1.07 – 1.73)	0.083	0.13	1.05 (0.69 – 1.58)	1	0.29
CRY2	rs11038696	0.086	0.84 (0.64 – 1.10)	0.29	0	-	NA	NA
CSNK1E	rs135757	0.25	0.88 (0.74 – 1.05)	0.29	0.20	0.85 (0.60 – 1.19)	0.94	0.82
DEC1	rs908078	0.16	0.88 (0.72 – 1.08)	0.29	0.19	1.13 (0.81 – 1.56)	1	0.20
MTNR1A	rs11728777	0.41	1.17 (1.01 – 1.36)	0.12	0.38	1.07 (0.82 – 1.41)	1	0.15
MTNR1B	rs4388843	0.11	1.11 (0.88 – 1.40)	0.42	0	-	NA	NA
NPAS2	rs356642	0.19	0.82 (0.68 – 0.99)	0.12	0	-	NA	NA
PER1	rs3027188	0.17	0.78 (0.64 – 0.96)	0.083	0.30	0.86 (0.65 – 1.15)	0.94	0.027

PER2	rs11894535	0.19	1.10 (0.91 – 1.32)	0.38	0.25	0.96 (0.71 – 1.29)	1	0.45
PER3	rs1012477	0.15	0.87 (0.71 – 1.08)	0.29	0.027	1.69 (0.77 – 3.68)	0.94	0.11
NR1D1	rs883871	0.15	0.86 (0.69 – 1.06)	0.29	0.38	1.14 (0.87 – 1.50)	0.094	0.91
TIMELESS	rs774036	0.45	1.04 (0.90 – 1.21)	0.59	0.27	0.85 (0.63 – 1.14)	0.94	0.48

a. Reported odds ratios for European and Asian groups from models stratified by ethnicity, p-interaction from combined model with ethnicity*genotype product term
b. False Discovery Rate adjusted p-value

Table 5.3: Interactions with Shift Work History[a]:

Gene	SNP	Genotype	<2 YEARS SHIFT WORK			≥2 YEARS SHIFT WORK			p-interaction (unadjusted)	p-interaction (FDR)
			Cases (n=442)	Controls (n = 555)	OR (95% CI)[b]	Cases (n=201)	Controls (n=246)	OR (95% CI)[b]		
ARNTL	rs3816360	AA	55	62	1.24 (1.03 – 1.50)	31	28	1.26 (0.96 – 1.65)	0.93	0.93
		AG	209	221		90	103			
		GG	178	272		80	115			
CLOCK	rs2035691	AA	47	49	1.18 (0.97 – 1.43)	17	21	1.06 (0.78 – 1.43)	0.58	0.84
		AG	210	249		103	121			
		GG	185	257		81	104			
CRY1	rs11113179	AA	6	2	1.27 (0.95 – 1.71)	3	1	1.50 (0.98 – 2.30)	0.52	0.84
		AG	87	98		47	45			
		GG	349	455		151	200			
CRY2	rs11038696	AA	3	2	0.90 (0.65 – 1.26)	3	1	0.71 (0.44 – 1.16)	0.45	0.84
		AG	59	87		22	45			
		GG	380	466		176	200			
CSNK1E	rs135757	AA	22	26	0.86 (0.69 – 1.06)	10	13	0.94 (0.68 – 1.29)	0.66	0.84
		AG	157	225		73	94			
		GG	263	304		118	139			
DEC1	rs908078	AA	318	391	0.87 (0.68 – 1.11)	145	168	0.92 (0.63 – 1.33)	0.79	0.91
		AG	118	144		51	75			
		GG	6	20		5	3			
MTNR1A	rs11728777	AA	83	88	0.80 (0.67 – 0.96)	35	48	0.98 (0.76 – 1.28)	0.22	0.76
		AG	231	268		102	113			
		GG	128	199		64	85			

MTNR1B	rs4388843	AA	4	7	1.32 (1.00 – 1.74)	3	2	0.78 (0.50 – 1.21)	0.044	0.41
		AG	109	103		31	52			
		GG	329	445		167	192			
NPAS2	rs356642	AA	15	23	0.75 (0.59 – 0.94)	10	11	0.97 (0.70 – 1.35)	0.21	0.84
		AG	105	169		50	66			
		GG	322	363		141	169			
PER1	rs3027188	CC	13	16	0.81 (0.64 – 1.04)	1	9	0.71 (0.49 – 1.04)	0.54	0.84
		CG	96	150		50	67			
		GG	333	389		150	170			
PER2	rs11894535	AA	21	23	0.98 (0.78 – 1.22)	9	2	1.48 (1.04 – 2.11)	0.058	0.41
		AG	137	182		68	74			
		GG	284	350		124	170			
PER3	rs1012477	CC	333	405	0.91 (0.70 – 1.17)	154	179	0.81 (0.55 – 1.19)	0.64	0.84
		GC	100	138		45	61			
		GG	9	12		2	6			
NR1D1	rs883871	AA	12	9	0.86 (0.67 – 1.11)	2	3	0.90 (0.60 – 1.35)	0.85	0.91
		AG	95	152		47	63			
		GG	335	393		150	79			
		Missing	0	1		2	1			
TIMELESS	rs774036	AA	92	113	1.01 (0.84 – 1.20)	52	48	1.13 (0.86 – 1.47)	0.46	0.84
		GA	214	274		93	131			
		GG	136	168		56	67			

a. Reported odds ratios for <2 years and ≥2 years shift work history from models stratified by shift work history (<2 vs ≥2 years), p-interaction from combined model with shift work*genotype interaction term

b. Model adjusted for age and centre

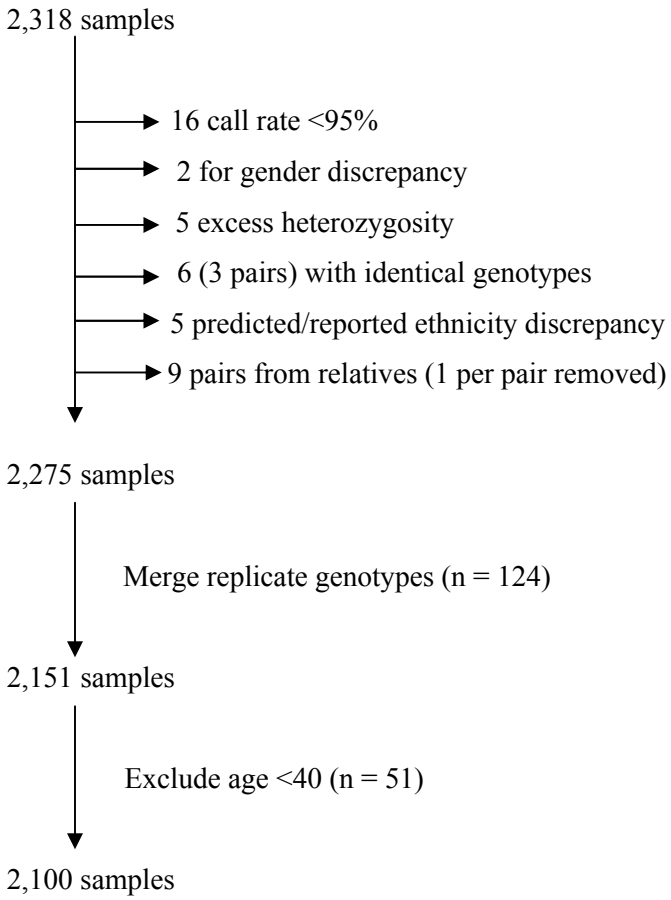

Figure 5.1: Flow Chart of Clock Gene Samples Quality Control

Chapter 6

General Discussion

6.1 Summary of Main Findings:

Results from the three manuscripts included in this thesis contribute to the understanding of the relationship between shift work and breast cancer risk, at several points along the proposed causal pathway. The associations of shift work history and clock gene variants with breast cancer risk, as well as potential shift work-clock gene interactions, were investigated in a large breast cancer case-control study. Associations between shift work history, light exposure and melatonin, hypothesized intermediates on the shift work-breast cancer causal pathway (1), were also explored in a longitudinal biomarker study among rotating shift nurses.

Consistent with some previous studies (2–5), an association between 30 or more years of shift work, but not shorter durations, and breast cancer was observed. As well, there were no significant interactions in the risk of breast cancer with shift work for ER/PR+ and ER/PR- tumours. Only two previous studies have considered ER-status in relationships between shift work and breast cancer (2,6), however, they also do not observe differences in risk for ER+ and ER- tumours, suggesting risk of breast cancer with shift work does not vary by tumour hormone receptor status. Further, the use of a single occupational group (nursing) has been identified as limitation of previous shift work – breast cancer studies (7,8). However, in this case-control study based in the general population, the relationship between shift work and breast cancer was similar for women whose longest 'shift work' job was in health vs. non-health occupations, suggesting that the shift work-breast cancer relationship cannot be entirely accounted for by characteristics specific to being a nurse. Taken together, these results provide support for a relationship between long-term shift work and elevated breast cancer risk.

Relationships between light at night exposure, shift work and melatonin, hypothesized intermediates on the causal pathway linking shift work with breast cancer risk (9), were also examined in a longitudinal biomarker study among rotating shift nurses working in a two-day, two-night rotating shift pattern. Light exposure during night shifts was relatively dim and no differences in melatonin production, measured from urine samples either as peak melatonin or change in melatonin during the day, were observed when nurses were working their day compared to their night shift. As well, patterns of melatonin production measured from saliva samples were also similar on both shift types, such that nurses appeared to still produce most of their melatonin at night while working a night shift, supporting results from our pilot work that this rotating shift pattern does not alter the timing of melatonin production (10).

In contrast with the results of some earlier studies which have observed associations between light exposure and melatonin production (10–12), only a small relationship between light exposure and change in melatonin on the night shift was observed. However, the association between light and melatonin in previous studies may be confounded by having measured melatonin at different times of day for day and night workers, such that natural circadian melatonin variations could explain some observed differences in melatonin levels between individuals on day and night shifts (10). One recent study published after ours found that women had reduced urinary melatonin levels both during night work and during nighttime sleep following night work, when compared to nighttime melatonin levels of women sleeping at night following a day shift (13). However, there are several differences in the protocol of this study compared to ours that may be responsible for the different results. Specifically, the shift pattern worked by night workers in this study is not mentioned and thus may be different than what we examined (13). As well, the comparison group involves day-only workers who were not included in our study, and light levels were not measured directly, such that they could have been brighter than those in our study (13). Finally, there was also no association between long-term (20 years)

shift work history and melatonin levels in our study, results which are similar to other studies that have examined associations between shift work and melatonin (14,15).

Lastly, associations of single nucleotide polymorphisms (SNPs) in 14 clock genes with breast cancer risk, as well as potential interactions between clock gene variants and shift work, were examined. We did not detect relationships between any clock-related SNPs and breast cancer, similar to recent results from the Nurses' Health Study (16), but in contrast to associations for SNPs in *CRY2*, *NPAS2*, *TIMELESS* and *CLOCK* with breast cancer identified in a Connecticut case-control study (17–20). We did, however, observe an interaction with ethnicity for rs3027188 in *PER1* with respect to breast cancer risk for Europeans compared to Asians. To our knowledge, no previous study has investigated potential interactions by ethnicity for clock gene-breast cancer relationships. However, further research is needed in samples with greater numbers of non-Caucasian participants to confirm these observations and to provide greater power to investigate potential interactions with other clock gene SNPs. As well, this study did not detect any significant interaction between shift work and clock gene variants on breast cancer risk. These results differ from those of the Nurses Health Study cohort, the only previous study to investigate potential interactions with shift work, which observed a reduced risk of breast cancer among women with less than two years of rotating shift work history for rs2305160 in *NPAS2*, but no association among those who worked two or more years rotating shift work (16).

6.2 Strengths and Limitations:

This thesis has a number of strengths and limitations. First, the exposure assessment of shift work provided an improvement upon methods used in previous questionnaire-based studies of shift work and breast cancer (2,3). The potential for exposure misclassification if the type of shift work an individual has performed does not meet the definition provided in the question has been identified as a limitation of previous work in which shift work history is obtained through

self-reported questionnaire responses (8). The lifetime occupational history included in our study questionnaire captured information concerning the proportion of time spent on day, evening and night shifts for each job reported, such that the definition of 'shift work' jobs, as those which involved ≥50% of time on evening and night shifts, was able to capture jobs both rotating and permanent shift schedules. Therefore, unlike other studies which have focused on specific types of shift work when asking these questions (2,3), in this thesis it is less likely that individuals who had a history of shift work were misclassified as unexposed.

However, despite this improved exposure assessment of shift work, since the questionnaire used in this study was designed in 2004, there were some domains of shift work that have only more recently been identified as relevant to cancer risk that could not be captured. For instance, the 2009 IARC Working Group on shift work exposure assessment identified several characteristics of shift work, including direction of rotation, number of consecutive nights, and number of days off between work days, as items that should be captured in future shift work studies (8). Recent studies have begun to include some of these characteristics. A study among nurses in Norway found an increased breast cancer risk among women who had worked five or more years in shift schedules with ≥6 or ≥7 consecutive nights (21). As specific information related to shift patterns, such as the number of consecutive nights, was not included in our questionnaire, similar analysis could not be performed in this thesis.

The objective measurement of both light and melatonin in the biomarker study is another strength of this thesis. While light at night exposure and melatonin are often cited as the mechanism linking shift work with cancer risk, very few studies conducted in an observational setting have used objective measurement of both light and melatonin levels (10–12). A number of studies have used night shift work as a proxy for light at night exposure (13–15,22,23); however, in the absence of specific information with respect to light levels on night shifts, it is difficult to determine whether these associations might be confounded by some other characteristic of night

work that is also linked with melatonin production. Thus, objective measurement of light in this study allowed its specific effect on melatonin to be isolated. As well, the timing of melatonin assessment in this study allowed for comparison of melatonin levels from both urine and saliva samples obtained at similar times of day during both day and night shifts. This is an improvement over previous studies, which have often compared melatonin levels from samples obtained at different times of day from workers on their day and night shifts, and as such, observed associations between light exposure and melatonin production may have been confounded by natural circadian melatonin variations (10–12). The melatonin assessment strategy used in our study was able to eliminate this potential source of confounding.

The examination of the influence of SNPs in 14 clock genes on breast cancer risk together in one analysis, using a two-step process to control for the influence of multiple comparisons, was another strength. In the Connecticut-based case-control study in which associations with breast cancer have been observed for SNPs in *CRY2*, *NPAS2*, *TIMELESS* and *CLOCK* (17–20), effects for each gene have been examined in separate publications and, within studies in which multiple SNPs were considered, none have explicitly stated that SNP-based analyses were controlled for multiple comparisons (18–20). In contrast, the Nurses' Health Study where, as in this thesis, circadian genes were considered in one analysis and a similar permutation-based method of multiple comparison adjustment was utilized, did not detect any significant relationships for clock gene SNPs with breast cancer risk (16), similar to our results. This suggests that some previously observed associations could have been the result of false-positive associations. This conclusion is supported by the observation in our study that prior to multiple comparison adjustment, SNPs in *ARNTL*, *CRY1*, *MTNR1A*, *NPAS2* and *PER1* displayed statistically significant associations with breast cancer risk, all of which were no longer significant following adjustment for multiple comparisons.

A further strength of this thesis was the use of a general population sample in which to analyze the relationship between shift work and breast cancer risk, since the use of nursing as a single occupational group in previous studies was identified as a limitation of studies in humans when IARC classified shift work as a Group 2A carcinogen (7). However, given that risks of breast cancer associated with shift work in this study were similar among workers in health and non-health industries and to those seen previously in studies of nurses (2–5,21), the use of nursing as a single occupational group may not be as substantial a limitation as previously suspected. As well, the use of a general population sample meant that in our study long-term shift work was an uncommon exposure, such that the ability of this study to detect relationships with breast cancer at all was itself remarkable. However, the small number of 'exposed' individuals limited the statistical power for sub-group analysis (ie. by menopausal status, hormone receptor status), and also meant that it was not possible to specifically look at interactions between clock gene variants and long-term shift work. Given that long-term shift workers were the group in which an elevated risk of breast cancer was detected, it is possible that the absence of interactions between clock gene variants and shift work in this thesis was the result of the necessity of using a more crude categorization of shift work.

Among the key limitations of this thesis is the potential for both selection and response bias in the case-control study. In Vancouver, as cases were recruited from the population-based BC Cancer Registry, while controls were recruited from screening clinics, there were some cases included in the study who did not participate in breast screening and as such, would not have been eligible to be included as controls. Associations between clock genes and breast cancer are not likely to be influenced by this potential bias, as screening participation is unlikely to be related to clock gene variants. However, given the observed differences in both income and education levels between cases and controls, and that participation in shift work could be related to socioeconomic status, it is possible that this selection bias could influence shift work – breast

cancer relationships. Nonetheless, in sensitivity analysis when cases who did not participate in screening were excluded, relationships between shift work and breast cancer were similar to those observed in the full study population. While there remain differences in demographic characteristics (ex. ethnicity, income, education) between cases and controls following these exclusions (Table E.7), such that screening behaviour cannot fully explain these differences, the similarity in relationships between shift work and breast cancer when unscreened cases are and are not included indicates this potential bias did not have a major influence on observed relationships.

As well, the modest response rates for both cases and controls in both Vancouver (54% in cases, 57% in controls) and Kingston (59% in cases, 49% in controls) create the possibility of response bias. However, in order to bias results, study participation would need to be related to both exposure (either shift work exposure or clock gene variants) and outcome. Given that this study investigated a number of potential breast cancer risk factors and that participants would not have been aware of their circadian genotypes prior to study participation, it is unlikely that these exposures would be related to study participation. As well, response rates were reasonably similar between the case and control groups in both Vancouver and Kingston. Thus, in this study, it seems unlikely that response bias would have a major impact on observed results.

Another limitation of this thesis is that the inclusion of only a single rotating shift pattern at one hospital in our biomarker study means that results may not be generalizable to alternative shift schedules (ex. permanent night shifts, slower rotation patterns) or other workplaces with different nighttime lighting levels. Specifically, while no difference in melatonin production was observed on the night shift compared to the day shift in our study, it is postulated that rotation schedules with more consecutive nights may be associated with a greater degree of circadian disruption, which could have a greater impact on melatonin levels. As well, all participants in this study were shift workers, such that greater differences in melatonin production may have been

observed if a comparison group of day-only workers was used. For example, Davis *et al.* recently found melatonin levels were lower both during night shifts and during nocturnal sleep following night work for women working night shifts when compared to those who only worked during the day (13). However, the schedule of shift work for women working night shifts who were included in the study was not described and it was not clear whether the pattern of shift work was the same for all night workers (13). As well, light exposure during night shifts was not assessed (13). Thus, it is possible that differences in the number of consecutive nights worked by women in the study or brighter light levels during night shifts could partially explain why differences were observed between melatonin production on day and night shifts in the Davis study, but not in ours. Future biomarker studies with objective measurements of both light and melatonin examining alternate shift patterns or workplaces with brighter nighttime lighting conditions will be useful in providing context for our results.

6.3 Future Research Directions:

The results of this thesis demonstrated an increased risk of breast cancer among women who had worked 30 or more years of shift work. However, as the information was not included in the study questionnaire, we were unable to assess the impact of several shift work domains in addition to duration, such as direction of rotation and number of consecutive nights. These shift work characteristics have been identified as priorities for future research (8) and recent results from Norwegian nurses have supported the idea that the number of consecutive nights in a rotation pattern may have an influence on cancer risk (21). Studies capturing these additional shift work characteristics will contribute to the understanding of whether some patterns of shift work have a greater impact on breast cancer risk than others. This information has implications for workplace policy, as there are some occupations from which night work cannot be eliminated. Thus, the identification of shift patterns that are best for health (ie. those which have less of an

impact on cancer risk) will be useful in the design of future shift patterns for those who must work at night.

This thesis also found that, among nurses working a two-day, two-night rotating shift pattern with exposure to low light levels when working at night, there was only a small inverse relationship between light exposure during the night shift and change in melatonin levels. While the low levels of nighttime light exposure may have been partially responsible for the absence of a strong relationship between light and melatonin in this study, the weak relationship may also suggest a role for other mechanisms besides melatonin in the shift work-breast cancer relationship. Sleep disturbances, phase shift (de-synchrony between central and peripheral circadian rhythms) and lifestyle changes such as diet and physical activity patterns, as well as obesity, have been suggested as possible alternative pathways through which shift work may influence cancer risk (1). Future research that explores some of these potential pathways is needed in order to provide a more detailed picture of the biological mechanisms through which shift work influences cancer risk.

Although this study did not observe significant interactions between shift work and clock gene variants with respect to breast cancer risk, future studies that have sufficient numbers of long-term shift workers such that they are powered to look at interactions between long-term shift work and clock gene variants will be useful in further understanding this relationship. In our study, and in several previous studies (2–5,21), long durations of shift work were the exposure associated with elevated breast cancer risk, such that it is plausible that this could be the relevant exposure for potential interactions with clock genes. However, including the results described here, there have only been two studies looking at interactions between clock gene variants and breast cancer risk, neither of which have examined interactions with long-term shift work. Thus, studies that are large enough to stratify on the basis of long-term shift work will be useful in characterizing the etiologically relevant exposure for shift work – clock gene interactions, should

these in fact exist. As well, studies of relationships between clock gene variants and breast cancer risk in populations of non-European ethnicity will be useful in clarifying whether the clock gene-ethnicity interaction for rs3027188 in *PER1* observed here represents a true difference in the impact of clock gene variants on breast cancer risk across ethnic groups. Further, larger sample sizes of non-Caucasian ethnicities will provide greater power to investigate potential interactions between clock gene SNPs and ethnicity with respect to breast cancer risk, in addition to the interaction detected here.

Finally, a strength of this thesis was the evaluation of relationships between shift work and breast cancer risk at multiple points along the proposed causal pathway linking shift work with breast cancer. As such, the results are able to contribute knowledge regarding both the association between shift work and breast cancer, as well as potential mechanisms through which shift work may influence breast cancer risk. However, as one study population was used to evaluate relationships between shift work, clock gene variants and breast cancer risk and another used to look at light exposure and melatonin, analysis that examined all these factors simultaneously was not possible. As such, we could not evaluate whether the long-term shift workers in whom breast cancer risk was elevated also had reduced melatonin levels. Future studies in a cohort setting that allow for the evaluation of shift work, breast cancer risk and several of the potential mechanisms linking these in the same group of individuals will be better able to answer this type of question. Further, the inclusion of multiple potential intermediates in this type of study would allow the relative impact of potential mechanisms responsible for the shift work-cancer relationship to be evaluated. This is important as understanding of the underlying biological pathways may facilitate the development of interventions that could mitigate the risk of cancer among those who must work at night.

6.4 Candidate's Contribution:

I made contributions to a number of aspects of this thesis. The results of the three manuscripts included in this thesis addressed identified gaps in the literature in the area of shift work and breast cancer. I synthesized and critically assessed the literature in order to identify these gaps, formulated these as research objectives in new studies, and designed analyses to address specific research questions using multiple study designs. For the genetic inquiry specifically, I developed the rationale for investigating clock genes and selected the genes to be included in this analysis.

The biomarker study provided me with experience in both study design and primary data collection. I designed the urine sample collection scheme to measure melatonin so that the timing of sampling accounted for confounding by circadian melatonin variations, addressing a major methodological limitation of previous observational studies examining relationships between light exposure and melatonin among shift workers (10–12). I also conducted a portion of the laboratory analysis measuring both melatonin and creatinine from urine and saliva samples, experience useful in understanding the precision of biomarker measures.

Throughout this thesis I was also able to demonstrate an understanding of a number of core principles and methodological issues in epidemiology. I was able to adapt information from the lifetime occupational history to obtain a more detailed exposure measure of shift work than several previous studies, addressing issues of exposure misclassification (2,3). As well, as described above, the melatonin assessment strategy in the biomarker study addressed a source of previously uncontrolled confounding (10–12). Through the use of a multi-step analysis process in the genetic analysis, I also developed an understanding of issues related to multiple comparisons and the risk of chance findings, addressing a potential source of spurious associations from previous work (18–20). The analysis presented in each manuscript also provided an opportunity to employ advanced analytic approaches, including polytomous logistic and mixed regression

models. Finally, the genetic analysis also allowed me to gain exposure to techniques outside of those of traditional epidemiology in order to understand and appropriately utilize genotyping quality control data.

6.5 Contribution of Research and Conclusions:

The manuscripts included in this thesis make several contributions to the field of shift work and breast cancer research. First, the lifetime occupational history included in the study of shift work and breast cancer risk was able to include both rotating and permanent shift schedules in the definition of shift work, an improvement in exposure assessment over previous studies that have asked about specific types of shift work (2,3). As well, in contrast to studies that used job-exposure matrix based methods to evaluate shift work exposure and may have been subject to exposure misclassification if individuals did not actually work the proportion of shift work that is assigned to their job classification group (4,24–26), the exposure assessment method used in this study allowed shift work information specific to individual study participants to be obtained. The increased breast cancer risk observed among women who worked thirty or more years of shift work is consistent with other studies of shift work and breast cancer risk (2,3,5,21,23) and contributes to the growing body of evidence supporting a relationship between long-term shift work exposure and breast cancer.

The comparison of melatonin levels from urine and saliva samples obtained at similar times of day when making comparisons between day and night shifts in our biomarker study meant the potential confounding of light-melatonin relationships by natural circadian melatonin variations, a limitation of previous studies (10–12), was eliminated. The study demonstrated that the two-day, two-night rotating shift pattern with low levels of light exposure during night shifts was not disruptive to circadian rhythms as measured by melatonin. There was no difference in nighttime melatonin production when nurses were working day and night shifts and only a small

inverse relationship between light exposure and change in melatonin production on the night shift. Thus, if melatonin is an intermediate on the causal pathway between shift work and breast cancer, the rapidly rotating shift pattern in this study that was minimally disruptive to melatonin may be better for health. However, there was no association between long-term shift work and melatonin. Thus, combined with the weak association observed between light and melatonin, these results may suggest a role for mechanisms other than melatonin in relationships between shift work and breast cancer risk.

While no association between any clock gene SNPs and breast cancer risk was detected, this was the first study to present results stratified by ethnicity. As a significant interaction with ethnicity (European vs. Asian) was detected for one SNP in *PER1*, future studies with greater numbers of participants from non-European populations are required to determine if this represents a true difference in the relationship between clock gene variants and breast cancer risk between Europeans and Asians. As well, this was only the second study to consider interactions between shift work and clock gene variants with respect to breast cancer risk (16). Although our study did not detect interactions between shift work and clock gene variants, the other study that has considered this relationship did observe an interaction with shift work for rs2305160 in *NPAS2* (16). Thus, future studies that also consider these interactions are required to clarify this relationship.

Overall, the results from this thesis contribute to the understanding of both the association between shift work and breast cancer, and the biologic mechanisms underlying this relationship. Since shift work is a necessary component of many occupations, the chronodisruption associated with working these schedules can be conceptualized as a workplace hazard, and understanding of the mechanisms through which shift work influences breast cancer risk is important in the development of shift work patterns that are optimal for health.

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Appendix A

Ethics Approval

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

November 29, 2010

This Ethics Application was subject to:

☐ Full Board Review
Meeting Date:
☒ Expedited Review

Ms. Anne Grundy
Department of Community Health & Epidemiology
Division of Cancer Care and Epidemiology
Queen's Cancer Research Institute
10 Stuart Street
Queen's University

Dear Ms. Grundy,

Study Title: **The influence of shift work, light at night exposure and clock gene polymorphisms on melatonin levels and breast cancer risk**
Co-Investigators: **Dr. Kristan Aronson, Dr. Joan Tranmer**

I am writing to acknowledge receipt of your recent ethics submission. We have examined the protocol for your project (as stated above) and consider it to be ethically acceptable. This approval is valid for one year from the date of the Chair's signature below. This approval will be reported to the Research Ethics Board. Please attend carefully to the following list of ethics requirements you must fulfill over the course of your study:

- **Reporting of Amendments**: If there are any changes to your study (e.g. consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval. (see http://www.queensu.ca/vpr/reb.htm).

- **Reporting of Serious Adverse Events**: Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information.

- **Reporting of Complaints**: Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

- **Annual Renewal**: Prior to the expiration of your approval (which is one year from the date of the Chair's signature below), you will be reminded to submit your renewal form along with any new changes or amendments you wish to make to your study. If there have been no major changes to your protocol, your approval may be renewed for another year.

Yours sincerely,

Albert Clark
Chair, Research Ethics Board

Nov 29, 2010
Date

ORIGINAL TO INVESTIGATOR - COPY TO DEPARTMENT HEAD- COPY TO HOSPITAL - BINDER COPY - FILE COPY

Study Code: EPID-329-10

- **Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete**

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards as defined by the Tri-Council Policy Statement; Part C Division 5 of the Food and Drug Regulations, OHRP, and U.S DHHS Code of Federal Regulations Title 45, Part 46 and carries out its functions in a manner consistent with Good Clinical Practices.

Federalwide Assurance Number : #FWA00004184
#IRB00001173

Current 2010 membership of the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board

Dr. A.F. Clark	**Emeritus Professor, Department of Biochemistry, Faculty of Health Sciences, Queen's University (Chair)**
Dr. H. Abdollah	**Professor, Department of Medicine, Queen's University**
Dr. M. Evans	**Community Member**
Dr. S. Horgan	**Manager, Program Evaluation & Health Services Development, Geriatric Psychiatry Service, Providence Care, Mental Health Services Assistant Professor, Department of Psychiatry**
Dr. L. Keeping-Burke	**Assistant Professor, School of Nursing, Queen's University**
Ms. D. Morales	**Community Member**
Dr. W. Racz	**Emeritus Professor, Department of Pharmacology & Toxicology, Queen's**
Dr. B. Simchison	**Assistant Professor, Department of Anesthesiology, Queen's University**
Dr. A.N. Singh	**WHO Professor in Psychosomatic Medicine and Psychopharmacology Professor of Psychiatry and Pharmacology Chair and Head, Division of Psychopharmacology, Queen's University Director & Chief of Psychiatry, Academic Unit, Quinte Health Care, Belleville General Hospital**
Dr. E. Tsai	**Associate Professor, Department of Paediatrics and Office of Bioethics, Queen's University**
Rev. J. Warren	**Community Member**
Ms. K. Weisbaum	**LL.B. and Adjunct Instructor, Department of Family Medicine (Bioethics)**
Dr. S. Wood	**Director, Office of Research Services (Ex-Officio)**

Appendix B

Case-Control Questionnaire

STUDY OF ENVIRONMENT, GENES AND BREAST HEALTH RESEARCH QUESTIONNAIRE

This questionnaire is part of a research study to understand the relationship between a woman's environment, her genes and breast health. The specific objectives are to investigate the association between exposure to certain environmental factors, including fossil fuels from vehicle exhaust, and light exposure at night, and breast diseases. We also wish to determine if some women are more genetically susceptible to exposures that would put them at higher risk for breast diseases.

Please prepare answers to the following questions to the best of your ability. If you choose to respond by telephone, we expect that it will take approximately one hour to collect your answers. If you are able to prepare your answers ahead of time, the interview should take less time. Alternatively, you may return this completed questionnaire by mail in the prepaid envelope provided.

The answers that you share with us will be strictly confidential and identified by an encrypted code, known by selected members of our research team only. Your honesty is important for the success of this research, and any answer is better than no answer.

We appreciate your cooperation tremendously.

Thank you!

GENERAL INFORMATION

Please answer each question as completely as possible. If you are unsure of an exact answer,

give your best estimate.

Today's Date: _____

Month / day / year

1. When were you born? _____

Month / day / year

2. What is the highest grade of school you have completed?

- ❑ Some elementary (grade) school
- ❑ Completed elementary (grade) school
- ❑ Some secondary (high) school
- ❑ Completed secondary (high) school
- ❑ Trade certificate or diploma from a vocational school or apprenticeship training
- ❑ Certificate or diploma from a community college or CEGEP
- ❑ University degree (bachelor's degree)
- ❑ Graduate or professional school degree (above bachelor's degree)

3. What is your current employment status?

- ❑ employed (**full-time**)
- ❑ employed (**part-time**)
- ❑ self-employed (**full-time**)
- ❑ self-employed (**part-time**)
- ❑ homemaker
- ❑ student
- ❑ retired
- ❑ unemployed

4. Were you born in Canada?

- ❑ Yes
- ❑ No, I was born in

_____

5. How would you best describe you and your grandparent's race, ethnicity or colour? Please specify as many as applicable:

Race, ethnicity or colour	Yourself	Your Maternal Grandmother	Your Maternal Grandfather	Your Paternal Grandmother	Your Paternal Grandfather
White	❑	❑	❑	❑	❑
Chinese	❑	❑	❑	❑	❑
South Asian ***(e.g. East Indian, Pakistani, Punjabi, Sri Lankan)***	❑	❑	❑	❑	❑
Black	❑	❑	❑	❑	❑
Native/Aboriginal peoples of North America	❑	❑	❑	❑	❑
Arab/West Asian ***(e.g. Armenian, Egyptian, Iranian, Lebanese,***	❑	❑	❑	❑	❑

Moroccan)					
Filipino	❑	❑	❑	❑	❑
South East Asian *(e.g. Cambodian, Indonesian, Laotian, Vietnamese)*	❑	❑	❑	❑	❑
Latin America	❑	❑	❑	❑	❑
Japanese	❑	❑	❑	❑	❑
Korean	❑	❑	❑	❑	❑
Other *(Specify)*					

HEALTH AND MEDICAL BACKGROUND

6. What is your height? _____ (feet and inches) **or** _____ (cm)

7. What is your current weight? __ (lbs) **or** _____ (kg)

8. a) What was your weight 2 years ago? _____ (lbs) **or** _____ **(**kg)

   b) What was your weight when you were 25 years old?_____ (lbs) **or** _____(kg)

9. Not including pregnancy, what is the most you have weighed? ____ (lbs) **or**__ (kg)

10. How old were you when you had your first menstrual period? ____ years of age

    ❑ Have never menstruated (*go to question #13*)

11. Are you still menstruating?

    ❑ Yes (*go to question #14*)

    ❑ No

    How many years has it been since your last menstrual period? _____ years

12. How did your menstrual periods stop?

    ❑ Naturally (through onset of menopause)

    ❑ As a result of a hysterectomy

    ❑ As a result of radiation or chemotherapy

    ❑ Other – please specify**:** _____

13. Have you ever had a hysterectomy (that is, an operation to remove the womb/uterus)?

    ❑ No

    ❑ Yes - at what age? _ years

14. Have you ever had an oopherectomy (that is, an operation to remove one or both of your ovaries which is sometimes done at the same time as removal of the womb/uterus)?

    ❑ No

    ❑ Yes, one ovary removed: → at what age? _____ years

    ❑ Yes, second or both ovaries removed: → at what age? __ years

    ❑ Don't know

15. Have you ever had a tubal ligation (that is, sterilization by having your "tubes tied")?

❑ No

❑ Yes - at what age? __ Years

16. Have you ever taken fertility drugs (*e.g. Clomiphene, Clomid, Serophene, etc.*)

❑ No (*go to question #17*)

❑ Yes

↓

Please provide details. If you don't remember the name, fill in the type, date started, date stopped and duration if possible. If you don't remember the month, please fill in the year. In calculating total duration, please include only the time periods that you used the specific medication.

Brand Name	Medication Type	Date Started (Month-Year)	Date Stopped (Month-Year)	Total Duration
Example: Clomiphene	*Pill*	*Sept-1999*	*Oct-2002*	*3 yrs*

17. Have you ever taken prescribed birth control medication for birth control or any other medical reason for 6 months or more? (*e.g. Norplant, Norinyl, Demulen, Depo-Provera, Tri-Cyclen, Alesse, etc.*)

❑ No (*go to question #18*)

❑ Yes

↓

Please provide details. If you don't remember the name, fill in the type, date started, date stopped and duration if possible. If you don't remember the month, please fill in the year. In calculating total duration, please include only the time periods that you used the specific medication.

Brand Name	Medication Type	Date Started (Month-Year)	Date Stopped (Month-Year)	Total Duration
Example: Norinyl	*Pill*	*Sept-1990*	*Oct-1998*	*8 yrs*

18. Have you ever been prescribed antidepressants? (e.g. Fluoxetine (sold as Prozac), Nortriptyline (sold as Allegron), etc.)

❑ No (*go to question #19*)

❑ Yes

↓

Please provide details. If you don't remember the name, fill in the type, strength, date started, date stopped and duration if possible. If you don't remember the month, please fill in the year. In calculating total duration, please include only the time periods that you used the specific medication.

Brand Name	Medication Type	Strength (milligrams)	Date Started (Month-Year)	Date Stopped (Month-Year)	Total Duration
Example: Prozac	*Pill*	*20*	*Nov-1990*	*Feb-1994*	*4 yrs*

19. Have you ever taken aspirin, ibuprofen or other nonsteroidal anti-inflammatory (NSAIDs) pain medication or tylenol or other acetaminophen pain medication for at least once per week for **6 months or longer**?

❑ No (*go to question #20*)

❑ Yes

↓

Please provide details. If you don't remember the name, fill in the type, strength, number of tablets per week, date started, date stopped and duration if possible. If you don't remember the month, please fill in the year. In calculating total duration, please include only the time periods that you used the specific medication.

Brand Name	Medication Type	Strength (mg)	Number of Tablets/Week	Date Started	Date Stopped	Total Duration
Example: Tylenol	*Pill*	*200*	*28*	*Jan-1995*	*Nov-1995*	*11 months*

20. Have ever taken any type of female replacement hormones (presently known as hormone therapy or HT and previously called hormone replacement therapy or HRT? (*e.g. Estrace, Premarin, etc.*)

❑ Yes ❑ No (*go to question# 22*)

↓

Please provide details. If you don't remember the name, fill in the type, date started, date stopped and duration if possible. In calculating total duration, please include only the time periods that you used the specific medication.

	Medication Type	Date	

Brand Name	Indicate if estrogen and/or progesterone	Indicate method of use (*oral, patch, etc.*)	Started (Month-Year)	Stopped (Month-Year)	Total Duration
Example: Premarin	*Estrogen only*	*Oral*	*Feb-1963*	*Mar-1995*	*29 yrs*

21. Are you currently taking any type of hormone therapy or HT (*e.g. Estrase, Premarin, etc.*)?

❑ Yes ❑ No

The following questions are about screening for breast disease.

22. Have you ever had a mammogram (i.e. a breast x-ray)?

❑ Yes ❑ No (*go to question #23*)

↓

How old were you the first time you went for a mammogram?_____ years

What was the reason? _____

_____

How many times have you had a mammogram since the first time? _____

When was the last time? (i.e. 6 months ago? 5 or more years ago?) _____

23. Have you ever examined your own breasts for lumps?

❑ Yes ❑ No (*go to question #24*)

↓

How old were you when you first started? _____ years

How often do you examine your breasts for lumps?

❑ Weekly ❑ Monthly ❑Quarterly (every 3 months) ❑ Yearly

❑ Bi-weekly ❑ Bi-monthly ❑ Twice per year ❑ Unsure

24. These questions are about breast lumps or cysts that you had **more than a year ago**.

Left breast	Right breast

Have you ever had a lump or cyst in your breast? (*if no to both left and right breast, go to question #25*)	Yes ❑ No ❑	Yes ❑ No ❑
How old were you when the first lump/cyst appeared?	Age_____ (years)	Age_____ (years)
Did you have any of the lumps/cysts examined by a doctor?	Yes ❑ No ❑	Yes ❑ No ❑
Did you have a biopsy or fine needle aspiration for any of the lumps/cysts?	Yes ❑ No ❑	Yes ❑ No ❑
Did a doctor diagnose any of the lumps/cysts as breast cancer?	Yes ❑ No ❑	Yes ❑ No ❑

PREGNANCY

25. Have you ever been pregnant? (include live births, still births, miscarriages and abortions)

❑ Yes ❑ No (*go to question #26*)

↓

What is the total number of pregnancies? (include live births, still births, miscarriages and abortions) _____

Please fill in the following information for each of your pregnancies:

	Age at beginning of pregnancy (years)	Outcome	Weeks pregnancy lasted (weeks)	Number of months breast feeding
1st Pregnancy		❑ Single live birth ❑ Multiple live births ❑ Stillbirth ❑ Miscarriage ❑ Abortion		❑ Not applicable ❑ Did not breast feed ❑ 1 - 2 months ❑ 3 - 4 months ❑ 5 - 6 months ❑ 7 – 12 months ❑ >12 months

	Age at beginning of pregnancy (years)	Outcome	Weeks pregnancy lasted (weeks)	Number of months breast feeding
2nd Pregnancy		❑ Single live birth ❑ Multiple live births ❑ Stillbirth ❑ Miscarriage ❑ Abortion		❑ Not applicable ❑ Did not breast feed ❑ 1 - 2 months ❑ 3 - 4 months ❑ 5 - 6 months ❑ 7 – 12 months ❑ >12 months

	Age at beginning of pregnancy (years)	Outcome	Weeks pregnancy lasted (weeks)	Number of months breast feeding
3rd Pregnancy		❑ Single live birth ❑ Multiple live births ❑ Stillbirth ❑ Miscarriage ❑ Abortion		❑ Not applicable ❑ Did not breast feed ❑ 1 - 2 months ❑ 3 - 4 months ❑ 5 - 6 months ❑ 7 – 12 months ❑ >12 months

Note: If more than 3 pregnancies, please use an additional page or the blank space on the following page. The interviewer will inquire about more, if applicable.

FAMILY

The next six questions will be asking about your family members and their history of cancer.

The first five questions (#26 - #30) are about your:

- parents
- full brothers
- full sisters
- children.

(*A full sibling is one who has both the same mother and father as you*.)

The sixth question (#31) concerns your other relatives and their history of cancer.

Please do not include relatives who joined your family by marriage or adoption.

Please answer the following questions to the best of your knowledge and complete the next 3 pages attached here for all relatives in each of the listed categories – regardless of whether they have or had cancer, they're alive, or you haven't seen or spoken to them for a while.

26. How many full brothers do you have? _____

27. How many full sisters do you have? _____

28. How many children do you have? _____
    a. Number of sons? _____
    b. Number of daughters? _____

FAMILY (Cont'd)

29. Please answer the following questions about your parents and siblings.

Relative	Year of birth	Have they ever been diagnosed with cancer?	Type(s) of cancer	Year of diagnosis	Are they alive?	If they're deceased, what year did they die?	If you're not sure they're alive, what year did you last hear from (or hear of) them?
Mother		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		
Father		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		
Full Sister 1		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		
Full Sister 2		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		

Full Brother 1		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		
Full Brother 2		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		

FAMILY (Cont'd)

30. This question is about your children. Please provide details on each of them.

Relative	Year of birth	Have they ever been diagnosed with cancer?	Type(s) of cancer	Year of diagnosis	Are they alive?	If they're deceased, what year did they die?	If you're not sure they're alive, what year did you last hear from (or hear of) them?
Son 1		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		
Son 2		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		
Daughter 1		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		
Daughter 2		❑ Yes ❑ No ❑ Don't know			❑ Yes ❑ No ❑ Don't know		

FAMILY (Cont'd)

31. This question is asking if **your father's parents, mother's parents, uncles, aunts, half-brothers, half-sisters, nephews or nieces** have ever had cancer. (A half-sibling is a brother or sister who has the same mother or father as you, but the other parent is different)

Are you aware of any such family members who have been diagnosed with cancer? Yes ❑ No❑

If yes, please provide details on each of these relatives and whether they come from your mother's or father's side of your family. Please do not include relatives who joined your family by marriage or adoption.

Relative	**Mother's Side**	**Father's Side**	**Year of Birth**	**Year of Cancer Diagnosis**	**Type of Cancer**	**If deceased, year of death**
	❑	❑				
	❑	❑				
	❑	❑				
	❑	❑				

LIFESTYLE HABITS (TOBACCO and ALCOHOL)

32. Have you ever smoked more than 100 cigarettes in your lifetime?
❑ Yes ❑ No (*go to question #37*)

33. How old were you when you STARTED smoking? _____ years of age

34. Are you currently smoking?
❑ Yes ❑ No - If no, at what age did you quit? _____ years

35. How many years in total have you smoked cigarettes? (excluding the years that you quit)
_____ years

36. For the entire time you smoked, on average, how many cigarettes a day did you usually smoke?
_____ cigarettes/day OR _____ cigarettes/week

37. This question asks about your family's smoking habits when you were 19 or younger.

FAMILY'S SMOKING HABITS	**FATHER / GUARDIAN**	**MOTHER / GUARDIAN**	**OTHER MEMBER**
Did your parent(s) or other household member(s) ever smoke in your presence when you were 19 or younger? (*go to question #38 if "no" for all*)	❑ Yes ❑ No	❑ Yes ❑ No	❑ Yes ❑ No
For the entire time that s/he smoked, on average, how many cigarettes a day did s/he usually smoke? **(cigarettes/day)**	_____ cigarettes/day	_____ cigarettes/day	_____ cigarettes/day
What age were you when first exposed to your father's &/or mother's tobacco smoke? **(years)**	_____ years old	_____ years old	_____ years old
What age were you when no longer exposed to your father's &/or	_____ years old	_____ years old	_____ years old

mother's tobacco smoke? **(years)**			

38. During this time period (19 or younger), on average, how many hours per week were you exposed to someone else's tobacco smoke?

	Hours per week exposed to "second-hand" tobacco smoke						
	0	**<1**	**1-2**	**3-4**	**5-6**	**7-9**	**>9**
Age: 19 years and younger							

39. In the past (during different decades of your life), on average, **how many hours per week**, *outside of the workplace*, were you exposed to someone else's tobacco smoke?

	Hours per week exposed to "second-hand" tobacco smoke						
Age (decades)	**0**	**<1**	**1-2**	**3-4**	**5-6**	**7-9**	**>9**
20-29 years							
30-39 years							
40-49 years							
50-59 years							
2 years ago (if >60)							

40. This question asks about your alcohol consumption habits.

		Beer	**Wine**	**Spirits**
Have you ever drank the following more than twice a year? *(if "no" to all 3, i.e. beer, wine **and** spirits, go to question #41)*	Yes No	❑ ❑	❑ ❑	❑ ❑
On average, how many drinks per week did you consume during different decades of your life? ***One drink=1 glass of wine or 1 bottle of beer or 1 oz of spirits***				
Teens (15-19)?	Drinks/week OR < 1 time/month Never	___per wk ❑ ❑	___per wk ❑ ❑	___per wk ❑ ❑
20-29 years	Drinks/week OR < 1 time/month Never	___per wk ❑ ❑	___per wk ❑ ❑	___per wk ❑ ❑
30-39 years	Drinks/week OR < 1 time/month Never	___per wk ❑ ❑	___per wk ❑ ❑	___per wk ❑ ❑
40-49 years	Drinks/week OR < 1 time/month Never	___per wk ❑ ❑	___per wk ❑ ❑	___per wk ❑ ❑
50-59 years	Drinks/week OR < 1 time/month Never	___per wk ❑ ❑	___per wk ❑ ❑	___per wk ❑ ❑
2 years ago (if over 60 years of age)	Drinks/week OR	___per wk	___per wk	___per wk

	< 1 time/month	❑	❑	❑
	Never	❑	❑	❑

LIFESTYLE HABITS (PHYSICAL ACTIVITY - HOUSEHOLD)

The next question (#41) refers to the frequency, duration and intensity of household activities.

The **minimum** number of hours for household activity to be included is:

- **2 hours per week, per year, or**
- **7 hours per week for 4 months, if seasonal**

Household activities (housework, yard work and home repair)

The three categories of physical intensity level for household activities are:

Light: Activities that require minimal physical effort such as:

- **Home Activities** (sweeping, vacuuming, dusting, washing dishes, cooking, food preparation standing or sitting, putting away groceries, shopping, ironing, laundry)
- **Home Repair** (automobile repair, wiring, plumbing, carpentry, workshop)
- **Lawn and Garden** (watering lawn, fertilizing or seeding lawn, standing or walking in garden, mowing lawn on a rider mower)

Moderate: Activities that are not exhausting, that increase the heart rate slightly and may cause some light perspiration such as:

- **Home Activities** (general house cleaning, food shopping with grocery cart, standing packing/unpacking boxes, occasional lifting of household items, child care – light effort)
- **Home Repair** (automobile body work, finishing or refinishing cabinets or furniture, caulking, laying tile or carpet, painting, papering, plastering, scraping, sanding floors, washing/waxing/painting a car or boat, washing fence).
- **Lawn and Garden** (mowing lawn by walking with a power mower, trimming shrubs or trees, operating a snow blower, planting seedlings, shrubs, trees, weeding, cultivating a garden, general gardening, sacking leaves**,** grass).

Heavy: Activities that increase the heart rate and cause heavy sweating such as:

- **Home Activities** (major cleaning e.g. wash car, windows, mop, clean garage, sweeping sidewalk, scrubbing floors vigorous effort, moving household items, furniture, boxes), child care – moderate to heavy effort (e.g. walk/run-playing with children).
- **Home Repair** (outside carpentry, installing gutters, roofing, sawing hardwood, spreading dirt with a shovel, painting outside house).
- **Lawn and Garden** (carrying, stacking wood, lumber, chopping wood, splitting logs, clearing land, hauling branches, digging, spading, filling garden, laying sod, rock, mowing lawn with a push mower, shoveling snow by hand).

41. Please report household activities (housework, yard work and home repair) that you have done over your lifetime. It may help you to consider what a typical day is for you. Then think about how many hours of household and gardening or yard work you do in a typical day. Sedentary activities like sewing or bookkeeping are not included. ***You may list activities individually or group them as in the examples listed on page 15***.

No.	**Description of Household Activity**	**Age Started**	**Age Ended**	**Frequency of Activity**				**Time per activity**		**Intensity of Household Activity* (Please check one for each activity)**
				Days /week	**Weeks /month**	**Months /year**	**Years**	**Hours**	**Minutes**	
eg. 1	*Home Activities*	*12*	*18*	*3*	*4*	*12*	*6*	*1*		■ Light ❑ Moderate ❑ Heavy
eg. 2	*Childcare*	*24*	*32*	*7*	*4*	*12*	*8*	*10*		❑ Light ■ Moderate ❑ Heavy
1										❑ Light ❑ Moderate ❑ Heavy

* For definition of Intensity of household activity, please see list on previous page.
* Intensity of household activities defined as:
Light = activities that require minimal effort,
Moderate = activities that are not exhausting, that increase the heart rate slightly and that may cause some light perspiration,
Heavy = activities that increase the heart rate and cause heavy sweating.

LIFESTYLE HABITS (PHYSICAL ACTIVITY – SPORTS AND EXERCISE)

The next question (#42) refers to the frequency, duration and intensity of exercise and sports activities.

The minimum number of hours for an exercise and sports activity to be included is:

- **32 hours total per year, or**
- **40 minutes per week, per year, or**
- **2 hours per week for 4 months, if seasonal**

The three categories of physical intensity level for exercise and sports activities are:

Light: Activities that require minimal physical effort such as those activities that are done standing or with slow walking
Moderate: Activities that are not exhausting, that increase the heart rate slightly and may cause some light perspiration
Heavy: Activities that increase the heart rate and cause heavy sweating.

If you have multiple episodes of the same activity over the years, record each episode separately. If there is a change in the frequency (months or days) or duration (hours) of the activity without actually discontinuing the activity for a certain length of time, a new line should be started because of the change in pattern.

An example of how to work with the table:
Activities:
from 8 yrs to 16 yrs played soccer- 1.5 hours per day, 2 days per week, 4 weeks per month, 4 months per year
from 25 yrs to 29 yrs played soccer - 2 hours per day, 2 days per week, 4 weeks per month, 8 months per year
from 18 to 49 played golf - 3 hours per day, 1 day per week, 4 weeks per month, 4 months per year

No.	Description of Exercise / Sports Activity	Age Start	Age End	Frequency of Activity				Time per activity		Intensity of Leisure Activity* (Please check one for each activity)
				Days /week	Weeks /month	Months /year	Years	Hours	Minutes	
1	Soccer	8	16	2	4	4	8	1	30	❑ Light ❑ Moderate ■ Heavy
2	Soccer	25	29	2	4	8	4	2		❑ Light ❑ Moderate ■ Heavy
3	Golf	18	49	1	4	4	31	3		■ Light ❑ Moderate ❑ Heavy

LIFESTYLE HABITS (PHYSICAL ACTIVITY – SPORTS AND EXERCISE) (Cont'd)

42. This question asks about exercise or sports activities that you did during your lifetime starting with childhood. Please report the activities that you have done at **least 2 hours per week for at least 4 months** of the year. Please begin by entering the activities that you did during your school years.

No.	Description of Exercise/Sports Activity	Age Started	Age Ended	Frequency of Activity				Time per activity		Intensity of Leisure Activity* (Please check one for each activity)
				Days /week	Weeks /month	Months /year	Years	Hours	Minutes	
eg.	*Soccer*	*9*	*18*	*3*	*4*	*4*	*9*	*1*		❑ Light ❑ Moderate ■ Heavy
1										❑ Light ❑ Moderate ❑ Heavy
2										❑ Light ❑ Moderate ❑ Heavy
3										❑ Light ❑ Moderate ❑ Heavy
4										❑ Light ❑ Moderate ❑ Heavy
5										❑ Light ❑ Moderate ❑ Heavy
6										❑ Light ❑ Moderate ❑ Heavy

* Intensity of exercise/sports activity defined as:

Light = activities that require minimal effort,

Moderate = activities that are not exhausting, that increase the heart rate slightly and that may cause some light perspiration,

Heavy = activities that increase the heart rate and cause heavy sweating.

LIFESTYLE HABITS (SMOKED/GRILLED FOODS)

43. During different decades of your life, how often did you usually eat meat or fish that had been smoked or that had a strong smoky taste?

Age (decades)	Times/week OR	Times/month OR	Times/year OR	Never/Almost never
Teen's (12-19)				❑
20-29 years				❑
30-39 years				❑
40-49 years				❑
50-59 years				❑
2 years ago				❑

44. During different decades of your life, how often did you usually eat pickles or other pickled foods?

Age (decades)	Times/week OR	Times/month OR	Times/year OR	Never/Almost never
Teen's (12-19)				❑
20-29 years				❑
30-39 years				❑
40-49 years				❑
50-59 years				❑
2 years ago				❑

45. During different decades of your life, how often did you usually eat charcoal-grilled foods in the **summer**?

Age (decades)	Times/week OR	Times/month OR	Times/year OR	Never/Almost never
Teen's (12-19)				❑
20-29 years				❑
30-39 years				❑
40-49 years				❑
50-59 years				❑
2 years ago				❑

46. During different decades of your life, how often did you usually eat charcoal-grilled foods in the **winter**?

Age (decades)	Times/week OR	Times/month OR	Times/year OR	Never/Almost never
Teen's (12-19)				❑
20-29 years				❑
30-39 years				❑
40-49 years				❑
50-59 years				❑
2 years ago				❑

Congratulations!!

You are over halfway there!!

The next portion of the questionnaire relates to residential and occupational history. Please take a moment to stretch your legs and pour a cup of tea if you wish before beginning this section.

… and please also remember that by completing this questionnaire you are contributing to very important research and your generous gift of time is very much appreciated!

RESIDENTIAL HISTORY (GENERAL)

47. Please list the addresses in which you have lived for 1 year or more, throughout your **lifetime**, ending with your current address.
We understand that it may be difficult to recall detailed information for all residences throughout your lifetime and would appreciate it if you would enter as much information as you are able.

	What was the address of the (first/next) place you lived in for 1 year or more? (If residence is outside of Canada, city and country will be fine)	What year did you start living there?	What year did you move from there?	How many years did (have) you lived there?	Type of Residence?
1st	_____ Street, apt # City/Town _____ Province (or Country if outside Canada) Postal code	_____ Date	_____ Date	_____ Years	❑ City ❑ Suburb ❑ Town ❑ Rural Other
2nd	_____ Street, apt # City/Town _____ Province (or Country if outside Canada) Postal code	_____ Date	_____ Date	_____ Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
3rd	_____ Street, apt # City/Town _____ Province (or Country if outside Canada) Postal code	_____ Date	_____ Date	_____ Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
4th	_____ Street, apt # City/Town _____ Province	_____ Date	_____ Date	_____ Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other

	(or Country if outside Canada) Postal code				
5th	Street, apt # City/Town Province (or Country if outside Canada) Postal code	Date	Date	Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
6th	Street, apt # City/Town Province (or Country if outside Canada) Postal code	Date	Date	Years	❑ City ❑ Suburb ❑ Town ❑ Rural Other
7th	Street, apt # City/Town Province (or Country if outside Canada) Postal code	Date	Date	Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
8th	Street, apt # City/Town Province (or Country if outside Canada) Postal code	Date	Date	Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
9th	Street, apt # City/Town	Date	Date	Years	❑ City ❑ Suburb ❑ Town ❑ Rural

	Province (or Country if outside Canada) Postal code				❑ Other
10th	Street, apt # City/Town Province (or Country if outside Canada) Postal code	Date	Date	Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
11th	Street, apt # City/Town Province (or Country if outside Canada) Postal code	Date	Date	Years	❑ City ❑ Suburb ❑ Town ❑ Rural Other
12th	Street, apt # City/Town Province (or Country if outside Canada) Postal code	Date	Date	Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
13th	Street, apt # City/Town Province (or Country if outside Canada) Postal code	Date	Date	Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
14th	Street, apt # City/Town				❑ City ❑ Suburb ❑ Town

	_____ Province (or Country if outside Canada) Postal code	Date	Date	Years	❑ Rural ❑ Other
15th	_____ Street, apt # City/Town _____ Province (or Country if outside Canada) Postal code	_____ Date	_____ Date	_____ Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
16th	_____ Street, apt # City/Town _____ Province (or Country if outside Canada) Postal code	_____ Date	_____ Date	_____ Years	❑ City ❑ Suburb ❑ Town ❑ Rural Other
17th	_____ Street, apt # City/Town _____Pr ovince (or Country if outside Canada) Postal code	_____ Date	_____ Date	_____ Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
18th	_____ Street, apt # City/Town _____Pr ovince (or Country if outside Canada) Postal code	_____ Date	_____ Date	_____ Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other
19t	_____				❑ City ❑ Suburb

h	Street, apt # City/Town Province (or Country if outside Canada) Postal code	_____ Date	_____ Date	_____ Years	❑ Town ❑ Rural ❑ Other
20th	_____ Street, apt # City/Town Province (or Country if outside Canada) Postal code	_____ Date	_____ 6.6.1.1.1 Date	_____ Years	❑ City ❑ Suburb ❑ Town ❑ Rural ❑ Other

Note: If more than 20 residences, please use an additional page or the back of this questionnaire. Interviewer will inquire about more residences, if applicable.

RESIDENTIAL HISTORY (SOURCES OF ENERGY)

48. Now I would like to know about the sources of energy available at each of these residences (place a check mark in those boxes that apply).

			1st Res	2nd Res	3rd Res	4th Res	5th Res	6th Res	7th Res	8th Res	9th Res	10th Res
A	What is (was) the major source of energy for the **oven or appliance used for cooking** at this address?	Electricity?										
		Natural gas?										
		Wood fire?										
		Other? *(Please specify)*										
		Not sure?										
B	What is (was) the major source of energy for the **furnace or the major source of heat** at this address?	Electricity?										
		Natural gas?										
		Oil?										
		Fireplace?										
		Wood/Gas Stove?										
		Other? *(Please specify)*										
		Not sure?										
C	If answer to B was fireplace or wood/gas stove, what materials did you usually burn?	Wood?										
		Coal?										
		Gas?										
		Other? *(Please specify)*										

		Not sure?										
D	If you use(d) a fireplace or wood/gas stove for additional heat or other purpose at this address, what materials did you usually burn? (Check all that apply)	Wood?										
		Coal?										
		Gas?										
		Synthetic logs?										
		Other? *(Please specify)*										
		Not sure?										

RESIDENTIAL HISTORY (SOURCES OF ENERGY)

If more than 10 residences apply, please continue:

48. (Cont'd)

			11th Res	**12th Res**	**13th Res**	**14th Res**	**15th Res**	**16th Res**	**17th Res**	**18th Res**	**19th Res**	**20th Res**
A	What is (was) the major source of energy for the oven or appliance used for cooking at this address?	Electricity?										
		Natural gas?										
		Wood fire?										
		Other? *(Please specify)*										
		Not sure?										
B	What is (was) the major source of energy for the	Electricity?										
		Natural gas?										

	furnace or the major source of heat at this address**?**	Oil?											
		Fireplace?											
		Wood/Gas Stove?											
		Other? *(Please specify)*											
		Not sure?											
C	If answer to B was fireplace or wood/gas stove, what materials did you usually burn?	Wood?											
		Coal?											
		Gas?											
		Other? *(Please specify)*											
		Not sure?											
D	If you use(d) a fireplace or wood/gas stove for additional heat or other purpose at this address, what materials did you usually burn? (Check all that apply)	Wood?											
		Coal?											
		Gas?											
		Synthetic logs?											
		Other? *(Please specify)*											
		Not sure?											

Note: If more than 20 residences, please note this information on the additional page. Interviewer will inquire about more residences, if applicable.

RESIDENTIAL HISTORY (GENERAL ENVIRONMENT)

49. Now I would like to know a little about the indoor and outdoor environment of each of these residences. Place a check mark in those boxes that apply.

❑ Please check here if these options do not apply to any of your residences

OUTDOOR ENVIRONMENT **Were any of these residences situated within one kilometer (~6 blocks) of:**										
	1st Res	**2nd Res**	**3rd Res**	**4th Res**	**5th Res**	**6th Res**	**7th Res**	**8th Res**	**9th Res**	**10th Res**
An airport?										
A railroad?										
An industrial site?										
A multi-lane highway (two lanes or more)?										
If more than 10 residences, please continue:										
	11th Res	**12th Res**	**13th Res**	**14th Res**	**15th Res**	**16th Res**	**17th Res**	**18th Res**	**19th Res**	**20th Res**
An airport?										

A railroad?										
An industrial site?										
A multi-lane highway (two lanes or more)?										

Note: If more than 20 residences, please note this information on the additional page. Interviewer will inquire about more residences, if applicable.

RESIDENTIAL HISTORY (INDOOR ENVIRONMENT)

50. Which best describes the ambient light in your bedroom, when you were sleeping at each of these residences?

Dark=could not see hand in front of face or wore a mask in bed

Medium=could see to the end of the bed

Light=could almost read without a light

	1st Res	2nd Res	3rd Res	4th Res	5th Res	6th Res	7th Res	8th Res	9th Res	10th Res
Ambient light in bedroom at each residence	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑

If more than 10 residences, please continue:

	11th Res	12th Res	13th Res	14th Res	15th Res	16th Res	17th Res	18th Res	19th Res	20th Res
Ambient light in bedroom at each residence	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light	Dark ❑ Med ❑ Light	Dark ❑ Med ❑ Light	Dark ❑ Med ❑ Light ❑	Dark ❑ Med ❑ Light	Dark ❑ Med ❑ Light	Dark ❑ Med ❑ Light ❑

				❑	❑	❑		❑	❑	

Note: If more than 20 residences, please note this information on the additional page. Interviewer will inquire about more residences, if applicable.

51. Now I would like to know about your usual sleeping habits throughout different decades of your life.

6.6.1.1.2	**12 - 19**	**20- 29**	**30 - 39**	**40 - 49**	**50 - 59**	**In the last 2 years**
Average time the lights were turned off for bed						
Average time when you woke-up						
If sleep was interrupted, were lights usually turned on?	❑ YES ❑ NO	❑ YES ❑ NO	❑ YES ❑ NO	❑ YES ❑ NO	❑ YES ❑ NO	❑ YES ❑ NO
Were lights usually turned on for more than 1 hour?	❑ YES ❑ NO	❑ YES ❑ NO	❑ YES ❑ NO	❑ YES ❑ NO	❑ YES ❑ NO	❑ YES ❑ NO

EMPLOYMENT HISTORY

52. Please tell us about EACH job or occupation you had for **at least** 6 months, including volunteer and military service, but not including schooling or homemaker. Include only seasonal or part-time work that is equivalent to 6 months or more. Begin with your most recent job and continue back to your first job. Include any absences from the work force and jobs you have done outside of Canada.

Job No.	**Time Period**		**Type of Industry, Business or Service**	**Company Name and Location**	**Job Title**	**Rate of Intensity*** (Please check one for each job)
	Start (Month-Year)	**End** (Month-Year)				
Eg.	*Nov-1993*	*Feb-2003*	*Hairdressing*	*Suki's Hair Salon, Vancouver, BC*	***Colour Specialist***	❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
1						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
2						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
3						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
4						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
5		6.6.1.1.3				❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy

* Intensity of job or occupation defined as:
Sedentary = work that involves sitting only, with minimal walking,
Light = work that involves minimal physical effort such as standing and slow walking with no increase in heart rate and no perspiration,
Moderate =work that is not exhausting, that increases the heart rate slightly and may cause some light perspiration, such as those that require carrying light loads (5-10 lbs) or that have continuous walking,
Heavy = work that is vigorous, increases the heart rate substantially and causes heavy sweating such as those that involve lifting, carrying heavy loads (>10 lbs), brisk walking, or climbing.
Note: Space for more jobs is on the next page.

EMPLOYMENT HISTORY (Cont'd)

52. (Cont'd) *Note: If more than 12 jobs, please use an additional page. Interviewer will inquire about more jobs, if applicable.*

Job No.	Time Period		Type of Industry, Business or Service	Company Name and Location	Job Title	Rate of Intensity* (Please check one for each job)
	Start (Month-Year)	End (Month-Year)				
6						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
7						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
8						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
9						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
10						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
11		6.6.1.1.4				❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
12		6.6.1.1.5				❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy

* Intensity of job or occupation defined as:

Sedentary = work that involves sitting only, with minimal walking,

Light = work that involves minimal physical effort such as standing and slow walking with no increase in heart rate and no perspiration,

Moderate =work that is not exhausting, that increases the heart rate slightly and may cause some light perspiration, such as those that require carrying light loads (5-10 lbs) or that have continuous walking,

Heavy = work that is vigorous, increases the heart rate substantially and causes heavy sweating such as those that involve lifting, carrying heavy loads (>10 lbs), brisk walking, or climbing.

EMPLOYMENT HISTORY (Cont'd)

53. Please tell us about the corresponding work shift for each job you listed

Job No.	Average number of hours worked per week	Percentage of time worked at each shift			Usual hours worked at each shift					
		Day shifts	Evening shifts	Late-night shifts (work through midnight)	Day shifts		Evening shifts		Late-night shifts	
					Start	End	Start	End	Start	End
eg.	35	80%	20%		10:00 am	5:30 pm	5:30 pm	9:00 pm		
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										

Note: If more than 12 jobs, please use an additional page. Interviewer will inquire about more jobs, if applicable.

EMPLOYMENT HISTORY (Cont'd)

54. Please tell us about the corresponding exposures to passive smoking and engine exhausts for each job you listed. In addition, could you tell us the mode of transport used to commute to each job listed

Job No.	At this job, on average, about how many people around you smoked?	While on this job, did you ever work near diesel engines or other types of engines?	While on this job, did you ever smell diesel exhaust or other types of engine exhaust?	How did you usually commute to this job?
1	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
2	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
3	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
4	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
5	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
6	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure

7	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
8	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
9	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
10	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
11	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure
12	❑ 0 smokers ❑ <10 ❑11 –19 ❑ 20 or more ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Yes ❑ No ❑ Not sure	❑ Bus/Tramway ❑Foot ❑ Metro/Train ❑ Other ❑ Car ❑ Truck ❑ Bicycle ❑ No Commute ❑ Motorcycle ❑ Not sure

Note: If more than 12 jobs, please use an additional page. Interviewer will inquire about more jobs, if applicable.

EMPLOYMENT HISTORY (Cont'd)

55. Referring to the jobs you listed in question #52, we would like to know if you ever worked for **more than 6 months** in any of the following specific jobs. If your work in any of these industries involved *primarily* office and administrative tasks, please indicate this by checking the box in the far right column.

Industry		Job number(s) from Question 52	Office/Admin.
Aircraft maintenance	Yes ❑ No ❑		❑
Building construction	Yes ❑ No ❑		❑
Fire-fighting	Yes ❑ No ❑		❑
Maritime industry	Yes ❑ No ❑		❑
Food services	Yes ❑ No ❑		❑
Landscaping	Yes ❑ No ❑		❑
Agriculture	Yes ❑ No ❑		❑
Gas distribution as station attendant	Yes ❑ No ❑		❑
Postal services as mail carrier	Yes ❑ No ❑		❑
Mining	Yes ❑ No ❑		❑
Oil refining industry	Yes ❑ No ❑		❑
Police detachment	Yes ❑ No ❑		❑
Plumbing	Yes ❑ No ❑		❑
Road construction and maintenance	Yes ❑ No ❑		❑
Roofing	Yes ❑ No ❑		❑
Waterproofing	Yes ❑ No ❑		❑
Rubber industry	Yes ❑ No ❑		❑
Metalworking	Yes ❑ No ❑		❑
Traffic/warehousing/shipping	Yes ❑ No ❑		❑
Production of coke	Yes ❑ No ❑		❑
Manufacture of electrodes	Yes ❑ No ❑		❑
Gas works	Yes ❑ No ❑		❑
Tar distillery	Yes ❑ No ❑		❑
Production of aluminum	Yes ❑ No ❑		❑

EMPLOYMENT HISTORY (Cont'd)

56. In any of your jobs we have already asked about, did you carry out any of the following tasks?

Tasks		Job number(s) from Question 52
Operating a boat engine	Yes ❑ No ❑	
Metal working (grinding, cutting, extruding, machining)	Yes ❑ No ❑	
Furnace work	Yes ❑ No ❑	
Fire fighting	Yes ❑ No ❑	
Cooking	Yes ❑ No ❑	
Baking bread products or pastries	Yes ❑ No ❑	
Operating coke oven	Yes ❑ No ❑	
Chimney sweeping	Yes ❑ No ❑	
Brick-laying	Yes ❑ No ❑	
Masonry	Yes ❑ No ❑	
Carpentry	Yes ❑ No ❑	
Repair electrical equipment or fixtures	Yes ❑ No ❑	
Driving a forklift	Yes ❑ No ❑	
Bartending	Yes ❑ No ❑	
Waitressing	Yes ❑ No ❑	
Gardening	Yes ❑ No ❑	
Waste incineration	Yes ❑ No ❑	

57. In any of your jobs we have already asked about, did you handle any of the following materials?

Materials		Job number(s) from Question 52
Coal tar	Yes ❑ No ❑ Don't Know ❑	
Pitch	Yes ❑ No ❑ Don't Know ❑	
Asphalt	Yes ❑ No ❑ Don't Know ❑	
Bitumen	Yes ❑ No ❑ Don't Know ❑	
Creosote	Yes ❑ No ❑ Don't Know ❑	
Soot	Yes ❑ No ❑ Don't Know ❑	
Anthracene oil	Yes ❑ No ❑ Don't Know ❑	
Cutting oils	Yes ❑ No ❑ Don't Know ❑	

INCOME

The following 2 questions are related to your household income. This information is very important and will only be used for the purposes of this research study. Please be assured that, like all other information you have provided, these answers will be kept strictly confidential.

58. **Thinking back to 2 years ago**, how many people were living in your household at that time? _____

59. **Thinking back to 2 years ago**, what was the **total income** for all people living in your household **from all sources, before taxes**? Sources include income from all earnings (wages and salaries), income from all government sources and all investment income (such as retirement funds).

- ❑ No income
- ❑ Less than $15,000
- ❑ $15,000 to $19,999
- ❑ $20,000 to $29,999
- ❑ $30,000 to $39,999
- ❑ $40,000 to $59,999
- ❑ $60,000 to $79,999
- ❑ $80,000 to $99,999
- ❑ $100,000 or more
- ❑ Not Stated

Thank you very much for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you did not skip any pages.

In the space below, please add any comments you wish, and thank you again for the information you have provided!

_____

_____

_____

Appendix C

Biomarker Study Questionnaire

STUDY OF LIGHT AT NIGHT, PHYSICAL ACTIVITY AND MELATONIN IN NURSES QUESTIONNAIRE

This questionnaire is part of our research study to understand the relationship between a woman's environment, behavioural patterns and melatonin production. The specific objectives are to investigate the association between melatonin levels produced by the body and exposure to certain environmental factors, including light exposure and physical activity.

The following questions should be completed on _____, the first day of study participation.

The answers that you share with us will be kept strictly confidential and identified by a study ID number, known only by selected members of our research team. Your honesty is important for the success of this research, and a partial answer is better than no answer at all.

We appreciate your cooperation.

Thank you

Please answer each question as completely as possible. If you are unsure of an exact answer, give your best estimate.

Today's Date: _____

Month / day / year

1) What is your date of birth? _____
Month / day / year

2) What was your weight at age 30? _____ (kg) **or** _____ (lbs)

3) At age 14 what was your height? _____ (feet and inches) **or** _____ (cm)

4) Relative to your peers at age 14 (grade 8 or 9), were you:
❑ Taller ❑ Shorter ❑ Average ❑ Don't remember

5) How would you best describe you and your grandparents' race, ethnicity or colour? Please specify as many as applicable:

Race, ethnicity or colour	Yourself	Maternal Grandmother	Maternal Grandfather	Paternal Grandmother	Paternal Grandfather
White	❑	❑	❑	❑	❑
Black	❑	❑	❑	❑	❑
Native/Aboriginal peoples of North America	❑	❑	❑	❑	❑
Filipino	❑	❑	❑	❑	❑
Chinese	❑	❑	❑	❑	❑
Japanese	❑	❑	❑	❑	❑
Korean	❑	❑	❑	❑	❑
South Asian *(e.g. East Indian, Pakistani, Punjabi, Sri Lankan)*	❑	❑	❑	❑	❑
South East Asian *(e.g. Cambodian, Indonesian, Laotian, Vietnamese)*	❑	❑	❑	❑	❑

Arab/West Asian *(e.g. Armenian, Egyptian, Iranian, Lebanese, Moroccan)*	❑	❑	❑	❑	❑
Latin America	❑	❑	❑	❑	❑
Other *(Specify)*					

6) What is the highest degree or diploma you have obtained?
   - ❑ High school
   - ❑ Postsecondary (certificate/diploma)
   - ❑ University undergraduate degree
   - ❑ Graduate degree (Master's, PhD)
   - ❑ Other _____

HEALTH BACKGROUND

7) How old were you when you had your first menstrual period?

_____ years of age ❑ Have never menstruated (*go to question #11)*

8) Are you still menstruating?

❑ No → How many years has it been since your last menstrual period? _____ years

❑ Yes (*go to question #8*)

9) How did your menstrual periods stop?

❑ Naturally (through onset of menopause) ❑ As a result of radiation or chemotherapy

❑ As a result of a hysterectomy ❑ Other – please specify: _____

8) Have you ever been pregnant?

❑ No (*go to question #10)* ❑ Yes → Please indicate the total number of times you have been pregnant (please include any live births, miscarriages or abortions). _____

9) Do you have any biological children?

❑ No (*go to question #10*) ❑ Yes → Please indicate how old you were when you gave

birth to your first child._____

10) Have you ever had a mammogram (i.e. a breast x-ray?)

❑ Yes ❑ No (*go to question #13*)

↓

How old were you the first time you went for a mammogram?_____ Years

What was the reason? _____

How many times have you had a mammogram since the first time? _____

_____

When was the last time? (ie. 6 months ago? 5 or more years ago?) _____

11) Have you ever been told that you have high breast (mammographic) density?

❑ Yes ❑ No (*go to question #13*)

HEALTH BACKGROUND (Cont'd)

12) These questions are about breast lumps or cysts that you may have had.

Have you ever had a lump or cyst in your breast?	No ❑ (*go to question #14*) Yes ❑
How old were you when the first lump/cyst appeared?	Age (years) _____
Did you have any of the lumps/cysts examined by a doctor?	No ❑ Yes ❑
Did you have a biopsy or fine needle aspiration for any of the lumps/cysts?	No ❑ Yes ❑ If yes, how many? _____
Did a doctor diagnose any of the lumps/cysts as *atypical hyperplasia?*	No ❑ Yes ❑

Did a doctor diagnose any of the lumps/cysts as *carcinoma in situ?*	No ❑ Yes ❑
Did a doctor diagnose any of the lumps/cysts as breast cancer?	No ❑ Yes ❑

13) These questions are about your sleep patterns.

Do you experience problems sleeping?	No ❑ Yes ❑ If yes, how often/when? _____ _____
Have you ever been diagnosed with a sleep disorder?	No ❑ Yes ❑ If yes: Name: _____ Date of diagnosis: _____

FAMILY

14) Have any of the following relatives been diagnosed with cancer? *(A full sibling is one who has both the same mother and father as you)*

Relative	Have they ever been diagnosed with cancer?	Type(s) of cancer
Mother	❑ Yes ❑ No ❑ Don't know	
Father	❑ Yes ❑ No ❑ Don't know	
Full Sister 1	❑ Yes ❑ No ❑ Don't know	
Full Sister 2	❑ Yes ❑ No ❑ Don't know	
Full Brother 1	❑ Yes ❑ No ❑ Don't know	
Full Brother 2	❑ Yes ❑ No ❑ Don't know	
Son 1	❑ Yes ❑ No ❑ Don't know	
Son 2	❑ Yes ❑ No ❑ Don't know	
Daughter 1	❑ Yes ❑ No ❑ Don't know	
Daughter 2	❑ Yes ❑ No ❑ Don't know	

If more space is required please use an additional page.

LIFESTYLE HABITS

15) Have you ever smoked more than 100 cigarettes in your lifetime?

❑ Yes ❑ No (*go to question #18*)

16) How old were you when you STARTED smoking? _____ years of age

17) Are you currently smoking?

❑ Yes ❑ No – If no, at what age did you quit? _____ years of age

18) How many years in total have you smoked cigarettes? (excluding the years that you quit)

_____ years

19) For the entire time you smoked, on average, how many cigarettes a day did you usually smoke?

_____ cigarettes/day OR _____ cigarettes/week

20) This question asks about your family's smoking habits when you were 19 or younger.

FAMILY'S SMOKING HABITS	**FATHER / GUARDIAN**	**MOTHER / GUARDIAN**	**OTHER MEMBER**
Did your parent(s) or other household member(s) ever smoke in your presence when you were 19 or younger? (*go to question #22 if "no" for all*)	❑ Yes ❑ No	❑ Yes ❑ No	❑ Yes ❑ No
For the entire time that s/he smoked, on average, how many cigarettes a day did s/he usually smoke? **(cigarettes/day)**	_____ cigarettes/day	_____ cigarettes/day	_____ cigarettes/day
What age were you when first exposed to your father's &/or mother's tobacco smoke? **(years)**	_____ years old	_____ years old	_____ years old
What age were you when no longer exposed to your father's &/or mother's tobacco smoke? **(years)**	_____ years old	_____ years old	_____ years old

LIFESTYLE HABITS (Cont'd)

21) During this time period (19 or younger), on average, how many hours per week were you exposed to someone else's tobacco smoke?

	Hours per week exposed to "second-hand" tobacco smoke						
	0	**<1**	**1-2**	**3-4**	**5-6**	**7-9**	**>9**
Age: 19 years and younger							

22) In the past (during different decades of your life), on average, how many hours per week, outside of the workplace, were you exposed to someone else's tobacco smoke?

	Hours per week exposed to "second-hand" tobacco smoke						
Age (decades)	**0**	**<1**	**1-2**	**3-4**	**5-6**	**7-9**	**>9**
20-29 years							
30-39 years							
40-49 years							
50-59 years							
2 years ago (if >60)							

23) This question asks about your alcohol consumption habits.

		Beer	**Wine**	**Spirits**
Have you ever drank the following more than twice a year? *(if "no" to all 3, i.e. beer, wine **and** spirits, go to question #41)*	Yes No	❑ ❑	❑ ❑	❑ ❑
On average, how many drinks per week did you consume during different decades of your life? ***One drink=1 glass of wine or 1 bottle of beer or 1 oz of spirits***				
Teens (15-19)?	# Drinks/week	____per wk	__per wk	__per wk
20-29 years	# Drinks/week	____per wk	____per wk	____per wk
30-39 years	# Drinks/week	____per wk	____per wk	____per wk
40-49 years	# Drinks/week	____per wk	____per wk	____per wk
50-59 years	# Drinks/week	____per wk	____per wk	____per wk
2 years ago (if over 60 years of age)	# Drinks/week	____per wk	____per wk	____per wk

EMPLOYMENT HISTORY

24) Please tell us about EACH job or occupation you had for **at least** 6 months, including volunteer and military service, but not including schooling or homemaker. Include only seasonal or part-time work that is equivalent to 6 months or more. Begin with your most recent job and continue back to your first job. Include any absences from the work force and jobs you have done outside of Canada.

Job No.	**Time Period**		**Type of Industry, Business or Service**	**Company Name and Location**	**Job Title**	**Intensity*** (Please check one for each job)
	Start (Month-Year)	**End** (Month-Year)				
Eg.	*Nov-1993*	*Feb-2003*	*Hairdressing*	*Suki's Hair Salon, Vancouver, BC*	*Colour Specialist*	❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
1						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy
2						❑ Sedentary ❑ Moderate ❑ Light ❑ Heavy

Intensity of job or occupation defined as:
Sedentary = work that involves sitting only, with minimal walking,
Light = work that involves minimal physical effort such as standing and slow walking with no increase in heart rate and no perspiration,
Moderate = work that is not exhausting, that increases the heart rate slightly and may cause some light perspiration, such as those that require carrying light loads (5-10 lbs) or that have continuous walking,
Heavy = work that is vigorous, increases the heart rate substantially and causes heavy sweating such as those that involve lifting, carrying heavy loads (>10 lbs), brisk walking, or climbing.

EMPLOYMENT HISTORY (Cont'd)

25) Please tell us about the corresponding shift work for each job you listed.

Job No.	Average number of hours worked per week	Percentage of time worked at each shift		Usual hours worked at each shift			
		Day Shifts	Night Shifts	Day Shifts		Evening Shifts	
				Start	End	Start	End
Eg.	35	80%	20%	10:00 am	5:30 pm	5:30 pm	9:00 pm
1							
2							
3							
4							
5							

Note: If more than 5 jobs, please use an additional page.

Thank you very much for completing this questionnaire

Appendix D

Biomarker Study Diary

STUDY OF LIGHT AT NIGHT, PHYSICAL ACTIVITY AND MELATONIN IN NURSES

ONE-DAY DIARY

This one-day diary is part of our research study to understand the relationship between a woman's environment, behavioural patterns and melatonin production. This one-day diary should be completed over a twenty-four hour period, beginning _____ and ending _____. **All questions included in this one-day diary pertain to activities completed and conditions experienced within this twenty-four hour period only.**

The answers that you share with us will be kept strictly confidential and identified by a study ID number, known only by selected members of our research team. Please note that although there are questions in this one-day diary that bear some similarity to those found in the questionnaire completed on the first day of study participation, it is imperative that you answer all questions. Your honesty is important for the success of this research, and a partial answer is better than no answer at all.

We appreciate your cooperation.

Thank you!

SAMPLE TIMES AND PEDOMETER VALUES

Please record the exact time and date of collection for all urine and saliva samples. Please also record the value on your pedometer at the time of each saliva sample collection.

Saliva Samples:

Time and date of saliva sample #1 collection: _____

Time and date of saliva sample #2 collection: _____

Time and date of saliva sample #3 collection: _____

Time and date of saliva sample #4 collection: _____

Urine Samples:

Time and date of urine sample #1 collection: _____

Time and date of urine sample #2 collection: _____

Pedometer Values:

Value at time of saliva sample #1 collection: _____

Value at time of saliva sample #2 collection: _____

Value at time of saliva sample #3 collection: _____

Value at time of saliva sample #4 collection: _____

Today's Date: ____ / _____ / _____ Shift worked during specified 24-hr period (day/night): _____
Day Month Year Shift worked yesterday (day/night/off): _____

HEALTH BACKGROUND

1) Are you still menstruating?
❑ Yes → what was the date of the first day of your last menstrual period? _____
❑ No

2) Have you taken aspirin, ibuprofen, other nonsteroidal anti-inflammatory (NSAIDs) pain medication or Tylenol/other acetaminophen pain medication in the 24-hour period specified on the front cover of this one-day diary??

❑ No (*go to question #3*) ❑ Yes → Please provide details. If you do not remember the brand name, fill in the type, dose and number of tablets taken.

Brand Name	***Dose (milligrams)***	***Number of Tablets***
Example: Tylenol	*200*	*1*

3) Have you used sedatives or muscle relaxants in the 24-hour period specified on the front cover of this one-day diary??

❑ No (*go to question #4*) ❑ Yes → Please provide details. If you do not remember the brand name, fill in the type, dose and number of tablets taken.

Brand Name	***Dose (milligrams)***	***Number of Tablets***
Example: Methocarbamol (Robaxin)	*200*	*1*

HEALTH BACKGROUND (cont'd)

4) During the 24-hour period specified on the on the front cover of this one-day diary, did you suffer from a migraine?

❑ No (*go to question #5*) ❑ Yes → Please provide details of the medication taken (including brand name, dose and number of tablets)

Brand Name	***Dose (milligrams)***	***Number of Tablets***

5) Have you taken prescribed birth control medication in the 24-hour period specified on the front cover of this diary? *(eg. Norplant, Norinyl, Demulen, Depo-Provera, Tri-Cyclen, Alesse, etc.)*

❑ No (*go to question #6*) ❑ Yes → Please provide details of the medication taken (including brand name, dose and number of tablets)

Brand Name	***Dose (milligrams)***	***Number of Tablets***

LIFESTYLE HABITS - Smoking

6) Did you smoke during the 24-hour period specified on the cover of this one-day diary?

❑ No (*go to question #7*) ❑ Yes → Please estimate the number of cigarettes you smoked today. _____

7) On average, how many hours, during the 24-hour period specified on the cover of this one-day diary, were you exposed to *someone else's tobacco smoke*?

The number of hours exposed to "second-hand" tobacco smoke						
0	**<1**	**1-2**	**3-4**	**5-6**	**7-9**	**>9**
❑	❑	❑	❑	❑	❑	❑

LIFESTYLE HABITS – Alcohol and caffeinated products consumption

8) Did you drink any of the following in the 24-hour period specified on the front cover of this one-day diary?

	Beer	Wine	Spirits	Coffee	Tea	Other caffeinated beverages
Yes	❑	❑	❑	❑	❑	❑
No	❑	❑	❑	❑	❑	❑
On average, how many drinks did you consume? ***One drink=1 glass of wine or 1 bottle of beer or 1 oz of spirits. For caffeinated beverages, specify size (e.g. small/single shot) and type (e.g. espresso).*** *(If "no" to all, i.e. beer, wine, spirits, coffee, tea, other caffeinated beverages, go to question #9)*						
Number of Drinks	_____	_____	_____	_____ *Specify average size and type* _____	_____ *Specify average size and type* _____	_____ *Specify average size and type* _____

LIGHTING CONDITIONS

9) Please answer the following questions:

What time did you wake-up at the start of the 24-hour period specified on the front cover of this one-day diary?	
What time did you go to sleep?	
What time did you wake-up at the end of the 24-hour period specified on the front cover of this one-day diary?	
If sleep was interrupted, were lights turned on? *(Please choose N/A if sleep was not interrupted.)*	❑ NO ❑ YES ❑ N/A
If the lights were on, were they on for more than 1 hour? *(Please choose N/A if sleep was not interrupted.)*	❑ NO ❑ YES ❑ N/A

10) When you sleep at night do you usually wear a sleep mask?
❑ Yes ❑ No

11) When you sleep during the day do you usually wear a sleep mask?
❑ Yes ❑ No

PHYSICAL ACTIVITY (Past 24 hours)

12) In the table below, please record which physical activities that you have participated in during the **24-hour period** specified on the front cover of this one-day diary. It is important to indicate the duration you performed each of these activities in the morning-afternoon (7:00 AM - 3:00 PM), afternoon-evening (3:00 PM – 11:00 AM), or evening-morning (11:00 PM – 7:00 AM). It is also important to indicate whether the intensity of the physical activity was light, moderate, and heavy. These activities include both *sports/exercise* and active forms of *transportation* (e.g., walking to work).

The 3 intensity categories can be defined as follows:

Light: Require minimal physical effort such as slow walking

Moderate: Activities that are not exhausting, but that increase heart rate and breathing rate slightly and may cause some light sweating

Heavy: Activities that substantially increase heart rate and breathing and cause heavy sweating.

Type of Activity	**Duration Performed (in minutes) at Different Times of the Day**			**Typical Intensity of Activity (Please check only one for each activity)**		
	Morning-Afternoon (07:00-15:00)	Afternoon-Evening (15:00-23:00)	Evening-Morning (23:00-07:00)	Low	Moderate	High
Walking (at least 10 minutes)				❑	❑	❑
Bicycling (stationary or outdoor)				❑	❑	❑
Elliptical Trainer				❑	❑	❑
Jogging or running (outdoors or treadmill)				❑	❑	❑
Aerobics class				❑	❑	❑

Yoga				❑	❑	❑
Strength training (including lifting weights)				❑	❑	❑
Rowing				❑	❑	❑
Tennis				❑	❑	❑
Squash/racquetball				❑	❑	❑
Calisthenics (sit-ups, push-ups, etc.)				❑	❑	❑
Hiking				❑	❑	❑
Swimming				❑	❑	❑
Golfing				❑	❑	❑
Dancing				❑	❑	❑
OTHER (please list below)				❑	❑	❑
				❑	❑	❑
				❑	❑	❑
				❑	❑	❑

In the above box be sure to indicate up to 3 additional activities that were not listed but which you performed in the specified 24 hour period. For these activities also list the duration and intensity of the physical activity session.

13) The following are questions about your household activity levels.

a) In the **24 hour period** specified on the cover of this one-day diary, about how many hours did you watch television (including videos and DVDs)? *Please mark one box for each of the 3 times of day listed*

Morning – Afternoon (07:00 – 15:00 hours)	Afternoon – Evening (15:00 – 23:00 hours)	Evening – Morning (23:00 – 07:00 hours)
❑ None at all	❑ None at all	❑ None at all
❑ About half an hour	❑ About half an hour	❑ About half an hour
❑ About 1 hour	❑ About 1 hour	❑ About 1 hour
❑ About 2 hours	❑ About 2 hours	❑ About 2 hours
❑ 3 or more hours	❑ 3 or more hours	❑ 3 or more hours

PHYSICAL ACTIVITY (Cont'd)

13) (cont'd)

b) In the **24 hour period** specified on the cover of this one-day diary, about how many hours did you use the computer (including Internet, email, chatting, etc.)? *Please mark one box for each of the 3 times of day listed*

Morning – Afternoon (07:00 – 15:00 hours)	Afternoon – Evening (15:00 – 23:00 hours)	Evening – Morning (23:00 – 07:00 hours)
❑ None at all	❑ None at all	❑ None at all
❑ About half an hour	❑ About half an hour	❑ About half an hour
❑ About 1 hour	❑ About 1 hour	❑ About 1 hour
❑ About 2 hours	❑ About 2 hours	❑ About 2 hours
❑ 3 or more hours	❑ 3 or more hours	❑ 3 or more hours

c) In the **24 hour period** specified on the cover of this one-day diary, about how many hours did you sit quietly around the home doing things such as reading, knitting, playing board games, etc?. This does not include time spent watching television or on the computer. *Please mark one box for each of the 3 times of day listed*

Morning – Afternoon (07:00 – 15:00 hours)	Afternoon – Evening (15:00 – 23:00 hours)	Evening – Morning (23:00 – 07:00 hours)
❑ None at all	❑ None at all	❑ None at all
❑ About half an hour	❑ About half an hour	❑ About half an hour
❑ About 1 hour	❑ About 1 hour	❑ About 1 hour
❑ About 2 hours	❑ About 2 hours	❑ About 2 hours
❑ 3 or more hours	❑ 3 or more hours	❑ 3 or more hours

d) In the **24 hour period** specified on the cover of this one-day diary, about how many hours did you perform light chores around the home such as cooking and cleaning? *Please mark one box for each of the 3 times of day listed*

Morning – Afternoon (07:00 – 15:00 hours)	Afternoon – Evening (15:00 – 23:00 hours)	Evening – Morning (23:00 – 07:00 hours)
❑ None at all	❑ None at all	❑ None at all
❑ About half an hour	❑ About half an hour	❑ About half an hour
❑ About 1 hour	❑ About 1 hour	❑ About 1 hour
❑ About 2 hours	❑ About 2 hours	❑ About 2 hours
❑ 3 or more hours	❑ 3 or more hours	❑ 3 or more hours

PHYSICAL ACTIVITY (Cont'd)

14) The following questions are about activities you performed while at work in the **24 hour period** specified on the front of this diary.

a) About how much time did you spend on your feet while at work?

❑ None at all
❑ About half an hour
❑ About 1 hour

- ❑ About 2 hours
- ❑ About 3 hours
- ❑ About 4 hours
- ❑ About 5 hours
- ❑ About 6 hours
- ❑ 7 or more hours

b) About how much time do you spend doing heavier activities such as lifting or bathing patients?

- ❑ None at all
- ❑ About half an hour
- ❑ About 1 hour
- ❑ About 2 hours
- ❑ About 3 hours
- ❑ About 4 hours
- ❑ About 5 hours
- ❑ About 6 hours
- ❑ 7 or more hours

PHYSICAL ACTIVITY (Past Month)

15) Please indicate which of the following physical activities you have performed **in the last 30 days** (1 month). For each activity you performed, indicate the number of times you have participated in the past 30 days, the average length/duration of participation for a given session, and whether the typical intensity was light, moderate, and heavy. These activities include both *sports/exercise* and active forms of *transportation* (e.g., walking to work).

The 3 intensity categories can be defined as follows:

Light: Require minimal physical effort such as slow walking
Moderate: Activities that are not exhausting, but that increase heart rate and breathing rate slightly and may cause some light sweating
Heavy: Activities that substantially increase heart rate and breathing and cause heavy sweating.

Type of Activity	**Number of times performed in past 30 days**	**Average duration of physical activity session**	**Typical Intensity of Activity (Please check only one for each activity)**		
			Low	**Moderate**	**High**
Walking (at least 10 minutes)			❑	❑	❑
Bicycling (stationary or outdoor)			❑	❑	❑
Elliptical Trainer			❑	❑	❑
Jogging or running (outdoors or treadmill)			❑	❑	❑
Aerobics class			❑	❑	❑
Yoga			❑	❑	❑
Strength training (including lifting weights)			❑	❑	❑
Rowing			❑	❑	❑
Tennis			❑	❑	❑
Squash/racquetball			❑	❑	❑
Calisthenics (sit-ups, push-ups, etc.)			❑	❑	❑
Hiking			❑	❑	❑
Swimming			❑	❑	❑
Golfing			❑	❑	❑
Dancing			❑	❑	❑
Hiking			❑	❑	❑
OTHER (please list below)			❑	❑	❑
			❑	❑	❑
			❑	❑	❑

			❑	❑	❑

In the above box be sure to indicate up to 3 additional activities that were not listed but which you performed in the past month. For these activities also list the number of times you have participated, the average duration, and the typical intensity.

16) Was the amount of physical activity you performed in the **past month** typical for you? Please check the correct response option.

- ❑ Yes
- ❑ No, I usually perform more physical activity
- ❑ No, I usually perform less physical activity

17) The following questions are about your household activity levels.

a) About how many hours a day in the **past 30 days** did you usually watch television (including videos and DVDs) in your free time? *(Please mark one box for days that you work and one box for days that you have off)*

Days that you work	Days that you have off
❑ None at all	❑ None at all
❑ About half an hour a day	❑ About half an hour a day
❑ About 1 hour a day	❑ About 1 hour a day
❑ About 2 hours a day	❑ About 2 hours a day
❑ About 3 hours a day	❑ About 3 hours a day
❑ About 4 hours a day	❑ About 4 hours a day
❑ About 5 hours a day	❑ About 5 hours a day
❑ About 6 hours a day	❑ About 6 hours a day
❑ About 7 hours or more a day	❑ About 7 hours or more a day

PHYSICAL ACTIVITY (Cont'd)

17) (cont'd)

a) About how many hours a day in the past 30 days did you use the computer (including Internet, email, chatting, etc.)? (*Please mark one box for days that you work and one box for days that you do not work)*

Days that you work	Days that you have off
❑ None at all	❑ None at all
❑ About half an hour a day	❑ About half an hour a day
❑ About 1 hour a day	❑ About 1 hour a day
❑ About 2 hours a day	❑ About 2 hours a day

❑ About 3 hours a day	❑ About 3 hours a day
❑ About 4 hours a day	❑ About 4 hours a day
❑ About 5 hours a day	❑ About 5 hours a day
❑ About 6 hours a day	❑ About 6 hours a day
❑ About 7 hours or more a day	❑ About 7 hours or more a day

b) About how many hours a day in the **past 30 days** do you sit quietly around the home doing things such as reading, knitting, playing board games, etc. This does not include time spent watching television or on the computer? *(Please mark one box for days that you work and one box for days that you do not work)*

Days that you work	Days that you have off
❑ None at all	❑ None at all
❑ About half an hour a day	❑ About half an hour a day
❑ About 1 hour a day	❑ About 1 hour a day
❑ About 2 hours a day	❑ About 2 hours a day
❑ About 3 hours a day	❑ About 3 hours a day
❑ About 4 hours a day	❑ About 4 hours a day
❑ About 5 hours a day	❑ About 5 hours a day
❑ About 6 hours a day	❑ About 6 hours a day
❑ About 7 hours or more a day	❑ About 7 hours or more a day

c) About how many hours a day in the **past 30 days** do you perform light chores around the home such as cooking and cleaning? *(Please mark one box for days that you work and one box for days that you do not work)*

Days that you work	Days that you have off
❑ None at all	❑ None at all
❑ About half an hour a day	❑ About half an hour a day
❑ About 1 hour a day	❑ About 1 hour a day
❑ About 2 hours a day	❑ About 2 hours a day
❑ About 3 hours a day	❑ About 3 hours a day
❑ About 4 hours a day	❑ About 4 hours a day
❑ About 5 hours a day	❑ About 5 hours a day
❑ About 6 hours a day	❑ About 6 hours a day
❑ About 7 hours or more a day	❑ About 7 hours or more a day

PHYSICAL ACTIVITY (Cont'd)

17) (cont'd)

a) About how many hours a day in the **past 30 days** do you perform light chores around the home such as cooking and cleaning? *(Please mark one box for days that you work and one box for days that you do not work)*

Days that you work	Days that you have off
❑ None at all	❑ None at all
❑ About half an hour a day	❑ About half an hour a day
❑ About 1 hour a day	❑ About 1 hour a day
❑ About 2 hours a day	❑ About 2 hours a day
❑ About 3 hours a day	❑ About 3 hours a day

❑ About 4 hours a day	❑ About 4 hours a day
❑ About 5 hours a day	❑ About 5 hours a day
❑ About 6 hours a day	❑ About 6 hours a day
❑ About 7 hours or more a day	❑ About 7 hours or more a day

b) About how many hours a day in the **past 30 days** do you perform heavy chores around the home such as gardening, shoveling snow, etc.? *(Please mark one box for days that you work and one box for days that you do not work)*

Days that you work	Days that you have off
❑ None at all	❑ None at all
❑ About half an hour a day	❑ About half an hour a day
❑ About 1 hour a day	❑ About 1 hour a day
❑ About 2 hours a day	❑ About 2 hours a day
❑ About 3 hours a day	❑ About 3 hours a day
❑ About 4 hours a day	❑ About 4 hours a day
❑ About 5 hours a day	❑ About 5 hours a day
❑ About 6 hours a day	❑ About 6 hours a day
❑ About 7 hours or more a day	❑ About 7 hours or more a day

PHYSICAL ACTIVITY (Cont'd)

18) The following questions are about activities you performed while at work in the **past 30 days**.

a) About how much time do you spend on your feet while at work?

- ❑ None at all
- ❑ About half an hour a day
- ❑ About 1 hour a day
- ❑ About 2 hours a day
- ❑ About 3 hours a day
- ❑ About 4 hours a day
- ❑ About 5 hours a day
- ❑ About 6 hours a day
- ❑ About 7 hours or more a day

b) About how much time do you spend doing heavier activities such as lifting or bathing patients?

- ❑ None at all
- ❑ About half an hour a day
- ❑ About 1 hour a day
- ❑ About 2 hours a day
- ❑ About 3 hours a day
- ❑ About 4 hours a day
- ❑ About 5 hours a day
- ❑ About 6 hours a day
- ❑ About 7 hours or more a day

CHANGES MADE TO LIGHTING CONDITIONS

19) By participating in this study, have you changed the lighting conditions in your home and/or bedroom?

❑ No ❑ Yes → Please provide details in the space provided specifying what type of changes you have made.

20) By participating in this study, will you change the lighting conditions in your home and/or bedroom?

❑ No ❑ Yes → Please provide details in the space provided specifying what type of changes you will make.

Because we want to be able to use all the information you have provided, please take a moment to review each page, making sure that you did not skip any pages.

If you have any additional comments, please provide them in the space provided below.

Thank you again for the information you have provided!
Your input is very valuable to us

Appendix E

Additional Data Tables

Table E.1: Descriptive Characteristics of Biomarker Study Participants in Season 2

Variable	SEASON 2		
	Day Shift	Night Shift	p-value[a]
	Mean (SE) / N (%)	Mean (SE) / N (%)	
Light Exposure			
Log-transformed mean light intensity (log lumens/m^2)	-2.10 (0.08)	-0.11 (0.08)	<0.0001
Urinary 6-sulfatoxymelatonin[b]			
Morning 6-sulfatoxymelatonin (ng/mL/mg/mL creatinine)	19.30 (1.15)	17.93 (1.15)	0.61
Change in 6-sulfatoxymelatonin (ng/mL/mg/mL creatinine)	16.76 (1.19)	16.70 (1.19)	0.99
Number of days since previous period	19.32 (2.32)	15.41 (2.34)	0.03
Sleep Characteristics			
Sleep duration (hours)	6.94 (0.14)	4.95 (0.15)	<0.0001
Sleep interrupted	10 (9.7%)	10 (10.4%)	1.00
Lights on for >1hr if interrupted	0 (0%)	3 (3.1%)	-
Medication Use			
Pain medication (NSAIDs)	23 (22.3%)	25 (26.0%)	0.44
Sedatives or muscle relaxants	7 (6.8%)	7 (7.3%)	0.71
Oral Contraceptives	17 (16.5%)	12 (12.5%)	0.27
Lifestyle Characteristics[c]			
Alcohol consumption (# drinks)	0.32 (0.06)	0.05 (0.07)	0.0008
Caffeine consumption (# drinks)	2.86 (0.22)	3.18 (0.22)	0.14
Smoking	11 (10.7%)	10 (10.4%)	0.32

a. Differences between day and night shifts compared using difference of least squares means estimates in a mixed model with a random subject effect for continuous variables and using McNemar's test for categorical variables.
b. Geometric means (calculated by back-transforming log-transformed variables) are presented here
c. Represents 24h of melatonin assessment

Table E.2: Chronotype and Melatonin in Season 1 of Biomarker Study

	DAY SHIFT			NIGHT SHIFT		
Chronotype	**N**	**Mean (Std Dev)[a]**	**95% Confidence Interval**	**N**	**Mean (Std Dev)[a]**	**95% Confidence Interval**
Extreme Morning Type	3	179.47 (7.37)	1.26 – 25,591.10	3	37.21 (1.16)	25.84 – 53.57
Moderate Morning Type	18	22.65 (3.22)	12.68 – 40.45	18	25.32 (3.03)	14.59 – 43.94
Neither Type	50	20.29 (3.97)	13.74 – 29.96	50	27.57 (3.25)	19.92 – 38.17
Moderate Evening Type	8	22.87 (3.29)	8.50 – 62.18	8	16.95 (1.94)	9.73 – 29.55

a. Geometric means (calculated by back-transforming log-transformed variables) are presented here

Table E.3: Analysis with European-based Permutations Across Ethnicities[a]:

Gene	SNP	EUROPEAN (651 cases, 805 controls)				ASIAN (310 cases, 168 controls)			MIXED/OTHER (57 cases, 45 controls)			SOUTH ASIAN (31 cases, 32 controls)		
		OR (95% CI)	p-value[b]	p-value[c]	p-value[d]	OR (95% CI)	p-value[b]	p-value[d]	OR (95% CI)	p-value[b]	p-value[d]	OR (95% CI)	p-value[b]	p-value[d]
ARNTL	rs3816360	1.24 (1.06 – 1.45)	0.0058	0.056	0.081	1.04 (0.79 - 1.38)	0.77	1	1.05 (0.61 - 1.79)	0.86	0.93	0.59 (0.26 - 1.33)	0.2	0.77
CLOCK	rs2035691	1.15 (0.98 – 1.35)	0.097	0.35	0.23	0.99 (0.76 - 1.29)	0.94	1	1.41 (0.80 - 2.49)	0.24	0.66	3.28 (1.35 - 8.00)	0.0088	0.12
CRY1	rs11113179	1.36 (1.07 – 1.73)	0.014	0.038	0.083	1.05 (0.69 - 1.58)	0.83	1	1.96 (0.97 - 3.96)	0.061	0.45	0.80 (0.26 - 2.44)	0.7	0.89
CRY2	rs11038696	0.84 (0.64 – 1.10)	0.21	0.56	0.29	-	NA	NA	0.95 (0.27 - 3.31)	0.93	0.93	0.80 (0.10 - 6.34)	0.83	0.89
CSNK1E	rs135757	0.88 (0.74 – 1.05)	0.16	0.22	0.29	0.85 (0.60 - 1.19)	0.33	0.94	1.09 (0.60 - 1.95)	0.79	0.93	1.29 (0.58 - 2.88)	0.54	0.89
DEC1	rs908078	0.88 (0.72 – 1.08)	0.23	0.23	0.29	1.13 (0.81 - 1.56)	0.47	1	0.87 (0.47 - 1.59)	0.65	0.93	0.83 (0.29 - 2.33)	0.72	0.89
MTNR1A	rs11728777	1.17 (1.01 – 1.36)	0.044	0.13	0.12	1.07 (0.82 - 1.41)	0.61	1	0.64 (0.36 - 1.14)	0.13	0.61	1.25 (0.63 - 2.48)	0.52	0.89
MTNR1B	rs4388843	1.11 (0.88 – 1.40)	0.39	0.74	0.42	-	NA	NA	0.53 (0.17 - 1.68)	0.28	0.66	0.95 (0.24 - 3.71)	0.94	0.94

NPAS2	rs356642	0.82 (0.68 – 0.99)	0.037	0.63	0.12	-	NA	NA	1.17 (0.47 - 2.93)	0.73	0.93	2.63 (0.56 - 12.3)	0.22	0.77
PER1	rs3027188	0.78 (0.64 – 0.96)	0.018	0.045	0.083	0.86 (0.65 - 1.15)	0.31	0.94	1.22 (0.73 - 2.05)	0.45	0.9	2.44 (0.71 - 8.38)	0.16	0.77
PER2	rs11894535	1.10 (0.91 – 1.32)	0.33	0.78	0.38	0.96 (0.71 - 1.29)	0.77	1	0.65 (0.35 - 1.21)	0.17	0.61	1.20 (0.48 - 2.99)	0.7	0.89
PER3	rs1012477	0.87 (0.71 – 1.08)	0.21	0.65	0.29	1.69 (0.77 - 3.68)	0.19	0.94	1.09 (0.39 - 3.09)	0.87	0.93	0.57 (0.08 - 3.83)	0.56	0.89
NR1D1	rs883871	0.86 (0.69 – 1.06)	0.17	0.45	0.29	1.14 (0.87 - 1.50)	0.34	0.094	1.13 (0.66 - 1.94)	0.66	0.93	0.74 (0.22 - 2.49)	0.63	0.89
TIMELESS	rs774036	1.04 (0.90 – 1.21)	0.59	0.7	0.59	0.85 (0.63 - 1.14)	0.29	0.94	0.59 (0.33 - 1.03)	0.065	0.45	0.90 (0.43 - 1.89)	0.77	0.89
						*includes Filipino			*includes SE Asian					

a. Contains all ethnicities from study including those found in Table 5.2
b. Unadjusted p-value
c. Permutation adjusted p-value
d. False Discovery Rate adjusted p-value

Table E.4: Replication Clock Gene SNPs from Previous Breast Cancer Studies (In Europeans):Table: Replication Clock Gene SNPs from Previous Breast Cancer Studies (In Europeans)

SNP	Cases (n=651)	Odds Ratio (95% CI)[a]	p-value (unadjusted)	p-value (Full FDR)	p-value (FDR per gene)
CRY2:					
rs1401417	390	1.08 (0.91 – 1.29)	0.38	0.81	0.47
CC					
CG	226				
GG	35				
NPAS2:					
rs2305160	81	1.11 (0.95 – 1.30)	0.18	0.68	0.71
AA					
AG	302				
GG	268				
TIMELESS:					
rs7302060	221	0.99 (0.85 – 1.15)	0.86	0.95	0.86
AA					
GA	318				
GG	112				
CLOCK:					
rs7698022	342	0.96 (0.81 – 1.14)	0.62	0.89	0.77
AA					
CA	272				
CC	37				
rs11932595	216	1.03 (0.89 – 1.20)	0.66	0.89	0.77

AA					
GA	324				
GG	111				
rs6850524					
CC	101	0.92 (0.79 – 1.07)	0.27	0.68	0.47
CG	319				
GG	230				
Missing	1				

a. Model adjusted for age and centre

Table E.5: Associations Between Clock Gene SNPs and Breast Cancer Stratified by Menopausal Status:

			PREMENOPAUSAL			POSTMENOPAUSAL			
Gene	SNP	Genotype	Cases (n=186)	Controls (n=273)	OR (95% CI)[a]	Cases (n=456)	Controls (n=527)	OR (95% CI)[a]	p-value (interaction)
ARNTL	rs3816360	AA	29	26	1.41 (1.07 – 1.86)	57	64	1.18 (0.98 – 1.42)	0.29
		AG	83	112		215	215		
		GG	74	135		184	184		
C LOCK	rs2035691	AA	16	25	1.34 (1.00 – 1.81)	48	45	1.07 (0.88 – 1.30)	0.21
		AG	109	127		204	243		
		GG	61	121		204	239		
CRY1	rs11113179	AA	3	1	1.31 (0.85 – 2.02)	6	2	1.34 (1.00 – 1.80)	0.95
		AG	38	50		95	93		
		GG	145	222		355	432		
CRY2	rs11038696	AA	3	1	0.90 (0.56 – 1.44)	3	2	0.81 (0.56 – 1.14)	0.72
		AG	25	48		56	84		
		GG	158	224		397	441		
CSNK1E	rs135757	AA	12	9	1.07 (0.77 – 1.47)	20	30	0.82 (0.66 – 1.01)	0.18
		AG	61	101		169	218		
		GG	113	163		267	279		
DEC1	rs908078	AA	130	184	0.87 (0.61 – 1.24)	333	374	0.88 (0.68 – 1.13)	0.97
		AG	52	79		116	140		
		GG	4	10		7	13		
MTNR1A	rs11728777	AA	33	48	1.04 (0.79 – 1.35)	85	87	0.78 (0.65 – 0.94)	0.10
		AG	89	136		243	245		
		GG	64	89		128	195		

MTNR1B	rs4388843	AA	4	4	1.23 (0.82 – 1.85)	3	5	1.07 (0.81 – 1.43)	0.57
		AG	38	48		102	107		
		GG	144	221		351	415		
NPAS2	rs356642	AA	8	11	0.99 (0.70 – 1.38)	17	23	0.75 (0.60 – 0.94)	0.20
		AG	46	71		109	163		
		GG	132	191		330	341		
PER1	rs3027188	CC	4	7	0.79 (0.54 – 1.14)	10	18	0.78 (0.61 – 1.00)	0.96
		CG	42	76		104	141		
		GG	140	190		342	368		
PER2	rs11894535	AA	4	10	1.04 (0.74 – 1.47)	26	15	1.10 (0.88 – 1.38)	0.77
		AG	62	79		142	177		
		GG	120	184		288	335		
PER3	rs1012477	CC	143	195	0.76 (0.52 – 1.11)	343	388	0.94 (0.73 – 1.22)	0.37
		GC	40	70		105	129		
		GG	3	8		8	10		
NR1D1	rs883871	AA	3	5	0.71 (0.48 – 1.06)	11	7	0.95 (0.74 – 1.23)	0.23
		AG	40	79		102	136		
		GG	143	189		341	382		
TIMELESS	rs774036	AA	41	57	1.13 (0.87 – 1.47)	103	104	0.89 (0.75 – 1.07)	0.15
		GA	82	143		224	262		
		GG	63	73		129	161		
CRY2	rs1401417	CC	114	166	0.99 (0.71 – 1.38)	270	327	1.10 (0.89 – 1.36)	0.62
		CG	65	97		159	173		
		GG	7	10		27	27		
NPAS2	rs2305160	AA	28	30	1.27	53	53	1.04	0.27

		AG	87	120	(0.96 – 1.67)	209	246	(0.86 – 1.26)	
		GG	71	123		194	228		
TIMELESS	rs7302060	AA	72	91		147	174		
		GA	81	139	0.93 (0.71 – 1.22)	232	260	1.01 (0.84 – 1.21)	0.61
		GG	33	43		77	93		
CLOCK	rs7698022	AA	94	136		245	277		
		CA	84	120	0.92 (0.68 – 1.27)	183	212	0.96 (0.78 – 1.17)	0.88
		CC	8	17		28	37		
CLOCK	rs11932595	AA	54	87		160	186		
		GA	102	146	1.11 (0.83 – 1.47)	216	243	1.00 (0.84 – 1.19)	0.53
		GG	30	40		80	98		
CLOCK	rs6850524	CC	20	46		79	88		
		CG	95	131	0.83 (0.63 – 1.09)	221	268	0.97 (0.81 – 1.16)	0.36
		GG	70	96		156	171		

a. Odds ratios calculated from an additive genetic model.

Table E.6: Associations Between Clock Gene SNPs and Breast Cancer in ER/PR+ and ER/PR- Tumours:

			ER/PR+			ER/PR-			
Gene	SNP	Genotype	Cases (n=477)	Controls (n=801)	OR (95% CI)[a]	Cases (n=100)	Controls (n=801)	OR (95% CI)[a]	p-value (interaction)[b]
ARNTL	rs3816360	AA	63	90	1.24 (1.05 – 1.46)	12	90	1.09 (0.81 – 1.48)	0.43
		AG	222	324		43	324		
		GG	192	387		45	387		
C LOCK	rs2035691	AA	51	70	1.16 (0.97 – 1.39)	8	70	1.14 (0.83 – 1.58)	0.93
		AG	229	370		53	370		
		GG	197	361		39	361		
CRY1	rs11113179	AA	5	3	1.33 (1.01 – 1.73)	2	3	1.51 (0.95 – 2.40)	0.57
		AG	100	143		22	143		
		GG	372	655		76	655		
CRY2	rs11038696	AA	3	3	0.81 (0.60 – 1.11)	2	3	0.93 (0.54 – 1.60)	0.69
		AG	62	132		12	132		
		GG	412	666		86	666		
CSNK1E	rs135757	AA	24	39	0.93 (0.77 – 1.13)	4	39	0.84 (0.58 – 1.05)	0.63
		AG	177	319		36	319		
		GG	276	443		60	443		
DEC1	rs908078	AA	336	559	0.93 (0.75 – 1.17)	78	559	0.69 (0.44 – 1.07)	0.18
		AG	133	219		20	219		
		GG	8	23		2	23		
MTNR1A	rs11728777	AA	88	136	0.84 (0.71 – 0.99)	18	136	0.87 (0.65 – 1.17)	0.83
		AG	251	381		52	381		
		GG	138	284		30	284		

MTNR1B	rs4388843	AA	7	9	1.13 (0.87 – 1.45)	0	9	1.13 (0.71 – 1.78)	0.98
		AG	100	155		24	155		
		GG	370	637		76	637		
NPAS2	rs356642	AA	20	34	0.83 (0.67 – 1.02)	3	34	0.70 (0.46 – 1.06)	0.48
		AG	114	235		22	235		
		GG	343	532		75	532		
PER1	rs3027188	CC	9	25	0.77 (0.61 – 0.96)	3	25	0.78 (0.51 – 1.20)	0.94
		CG	109	217		21	217		
		GG	359	559		76	559		
PER2	rs11894535	AA	23	25	1.13 (0.93 – 1.39)	4	25	1.08 (0.75 – 1.57)	0.82
		AG	157	256		33	256		
		GG	297	520		63	520		
PER3	rs1012477	CC	364	584	0.84 (0.66 – 1.07)	70	584	1.12 (0.75 – 1.68)	0.18
		GC	107	199		28	199		
		GG	6	18		2	18		
NR1D1	rs883871	AA	9	12	0.89 (0.71 – 1.13)	1	12	0.52 (0.31 – 0.87)	0.05
		AG	111	215		15	215		
		GG	356	572		84	572		
TIMELESS	rs774036	AA	108	161	0.96 (0.82 – 1.13)	21	161	0.97 (0.80 – 1.44)	0.48
		GA	225	405		45	405		
		GG	144	235		34	235		
CRY2	rs1401417	CC	292	494	1.01 (0.83 – 1.23)	55	494	1.21 (0.86 – 1.71)	0.29
		CG	164	270		40	270		
		GG	21	37		5	37		
NPAS2	rs2305160	AA	62	83	1.11	10	83	1.10	0.97

		AG	216	367	(0.94 – 1.32)	51	367	(0.80 – 1.51)	
		GG	199	351		39	351		
TIMELESS	rs7302060	AA	163	266		37	266		
		GA	231	399	0.99 (0.84 – 1.17)	49	399	0.87 (0.64 – 1.18)	0.41
		GG	83	136		14	136		
CLOCK	rs7698022	AA	255	414		52	414		
		CA	195	332	0.94 (0.78 – 1.13)	41	332	1.01 (0.72 – 1.41)	0.67
		CC	27	54		7	54		
CLOCK	rs11932595	AA	155	274		37	274		
		GA	240	389	1.05 (0.89 – 1.24)	46	389	0.95 (0.71 – 1.29)	0.52
		GG	82	138		17	138		
CLOCK	rs6850524	CC	68	134		16	134		
		CG	243	400	0.91 (0.77 – 1.08)	46	400	0.89 (0.66 – 1.20)	0.86
		GG	165	267		38	267		

a. Odds ratios calculated from an additive genetic model.
b. p-values calculated from case-only model comparing ER/PR+ and ER/PR- groups

Table E.7: Case-control Study Population Excluding Non-Screened Cases

	Cases	Controls
	Mean (SD) / N (%)	**Mean (SD) / N (%)**
Age	58.8 (9.9)	56.7 (10.0)
Body Mass Index	26.0 (5.5)	25.3 (5.8)
Ethnicity		
European	587 (66.4%)	920 (78.2%)
Chinese	167 (18.9%)	114 (9.7%)
South Asian	25 (2.8%)	35 (3.0%)
Filipino	39 (4.4%)	38 (3.2%)
Japanese	18 (2.0%)	14 (1.2%)
Mixed	14 (1.6%)	12 (1.0%)
Other	34 (3.9%)	43 (3.7%)
Household Income		
< $30,000	142 (16.1%)	121 (10.3%)
$30,000 - $59,999	222 (25.1%)	269 (22.8%)
$60,000 - $99,999	202 (22.9%)	288 (24.5%)
> $100,000	193 (21.8%)	338 (28.7%)
Not stated	125 (14.1%)	162 (13.6%)
Education		
High school or less	322 (36.7%)	299 (25.5%)
College/trade certificate	264 (30.1%)	349 (29.7%)
Undergraduate degree	191 (21.8%)	302 (25.7%)
Graduate/Professional degree	101 (11.5%)	225 (19.2%)
Menopausal Status		
Pre-menopausal	249 (28.3%)	444 (37.8%)
Post-menopausal	632 (71.7%)	732 (62.2%)
Reproductive History		
Age at Menarche	12.9 (1.5)	12.8 (1.5)
Ever Been Pregnant	734 (83.3%)	936 (79.6%)
Age at First Pregnancy	27.6 (4.7)	27.7 (4.4)
Number of Pregnancies	2.3 (1.7)	2.3 (1.7)
Age at First Mammogram	44.6 (8.4)	42.8 (7.3)
Family History of Breast Cancer	190 (21.5%)	168 (14.3%)

Appendix F

List Of Clock Gene SNPs

Table F.8: SNPs Included in Genetic Analysis

Gene	SNP	Gene	SNP
PER3	rs2288727	PER2	rs2304673
PER3	rs1012477	DEC1	rs908078
PER3	rs1891217	CLOCK	rs17777927
PER3	rs2172563	CLOCK	rs6824955
PER3	rs2640908	CLOCK	rs11932595
PER3	rs228665	CLOCK	rs12510681
NPAS2	rs3849380	CLOCK	rs6850524
NPAS2	rs3849381	CLOCK	rs2035691
NPAS2	rs7587470	CLOCK	rs7698022
NPAS2	rs13012930	MTNR1A	rs1800884
NPAS2	rs7598826	MTNR1A	rs6553010
NPAS2	rs2043534	MTNR1A	rs2165666
NPAS2	rs17024926	MTNR1A	rs11728777
NPAS2	rs6740935	MTNR1A	rs2375801
NPAS2	rs4851377	MTNR1A	rs1800885
NPAS2	rs13394520	ARNTL	rs1481892
NPAS2	rs4851379	ARNTL	rs7950226
NPAS2	rs7570190	ARNTL	rs6486116
NPAS2	rs12989454	ARNTL	rs10832020
NPAS2	rs356642	ARNTL	rs11022762
NPAS2	rs17655330	ARNTL	rs6486122
NPAS2	rs356653	ARNTL	rs7937060
NPAS2	rs17717414	ARNTL	rs3816360
NPAS2	rs3754674	ARNTL	rs1868049
NPAS2	rs3768984	ARNTL	rs11022778
NPAS2	rs895521	ARNTL	rs4757151
NPAS2	rs17025005	ARNTL	rs11022781
NPAS2	rs12712085	ARNTL	rs12364562
NPAS2	rs12612424	CRY2	rs10838524
NPAS2	rs10191450	CRY2	rs2292912
NPAS2	rs13409032	CRY2	rs11038696
NPAS2	rs895520	CRY2	rs1401417
NPAS2	rs17020663	CRY2	rs2292910
NPAS2	rs12622050	MTNR1B	rs4388843

NPAS2	rs7581886	MTNR1B	rs10830963
NPAS2	rs7340468	MTNR1B	rs3781638
NPAS2	rs2305160	TIMELESS	rs774036
NPAS2	rs4851394	TIMELESS	rs7302060
NPAS2	rs2278727	CRY1	rs10746075
NPAS2	rs3754680	CRY1	rs11113179
NPAS2	rs3768991	CRY1	rs11113181
NPAS2	rs11123857	PER1	rs2735611
NPAS2	rs17662394	PER1	rs3027188
NPAS2	rs6726870	PER1	rs3027178
NPAS2	rs9223	REVERBA	rs883871
NPAS2	rs2305158	REVERBA	rs2071427
PER2	rs934945	REVERBA	rs2269457
PER2	rs11894535	REVERBA	rs4795424
PER2	rs2304674	CSNK1E	rs6001093
PER2	rs10462023	CSNK1E	rs135757

Appendix G

Multidimensional Scaling Graph

Figure G.1: Multidimensional Scaling Plot for all Ethnicities