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ORIGINAL ARTICLE

Variants in circadian genes and prostate cancer risk: a population-based study in China

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Circadian genes influence a variety of biological processes that are important in prostate tumorigenesis including metabolism. To determine if variants in circadian genes alter prostate cancer risk, we genotyped five variants in five circadian genes in a population-based case–control study conducted in China (187 cases and 242 controls). These variants included *CRY2* rs1401417:G > C, *CSNK1E* rs1005473:A > C, *NPAS2* rs2305160:G > A, *PER1* rs2585405:G > C and *PER3* 54-bp repeat length variant. Men with the *cryptochrome* 2 (*CRY2*)-variant C allele had a significant 1.7-fold increased prostate cancer risk (95% confidence interval (CI), 1.1–2.7) relative to those with the GG genotype. This risk increased to 4.1-fold (95% CI, 2.2–8.0) in men who also had greater insulin resistance (IR) as compared to men with the GG genotype and less IR. In contrast, among men with less IR, the *NPAS2*-variant A allele was associated with decreased prostate cancer risk (odds ratio = 0.5, 95% CI, 0.3–1.0) as compared to the GG genotype. Our findings, although in need of confirmation, suggest that variations in circadian genes may alter prostate cancer risk and some biological processes may modify this effect.
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Introduction

Circadian rhythms are the daily oscillations of multiple biological processes driven by endogenous clocks with or without external cues. These rhythms not only maintain human sleep patterns but influence biological processes such as metabolism[1–4] and sex hormone biosynthesis and action;[5] both of which are important for prostate tumorigenesis. Epidemiologic data from occupational cohorts suggest that circadian rhythm disruptions increase the risk for prostate cancer. For example, rotating shift workers had a higher risk of prostate cancer as compared with only day- or only night-shift workers.[6,7] In addition, male airline pilots had an excess risk of prostate cancer[8–10] that increased with the increasing number of flight hours.[10] Although compelling, these data represent indirect associations between circadian rhythms and prostate cancer; no underlying molecular mechanism thus far has been identified.

Nine identified genes control endogenous circadian rhythms via a transcription–translation feedback loop and include *CLOCK*, *neuronal PAS domain protein 2* (*NPAS2*), *aryl hydrocarbon receptor nuclear translocator-like* (*ARNTL*), *cryptochrome 1* (*CRY1*), *cryptochrome 2* (*CRY2*), *period 1* (*PER1*), *period 2* (*PER2*), *period 3* (*PER3*) and *casein kinase 1-epsilon* (*CSNK1E*).[11] Three recent studies show that variants in circadian genes are associated with altered cancer risk. In breast cancer, a *PER3* repeat length polymorphism was associated with a 1.7-fold increased risk (95% confidence interval (CI), 1.0–3.0) among premenopausal women.[12] In contrast, women with the heterozygous genotype of *NPAS2* rs2305160:G > A single nucleotide polymorphism (SNP) had significantly reduced risk of breast cancer as compared to those with the common homozygous GG genotype (odds ratio (OR) = 0.61, 95% CI, 0.46–0.81, $P = 0.001$).[13] The same *NPAS2*-SNP was also linked to a reduced risk for non-Hodgkin's lymphoma (OR = 0.66, 95% CI, 0.51–0.85) with the association being the strongest with the B-cell lymphoma subtype (OR = 0.61, 95% CI, 0.47–0.80).[14] These findings suggest that the variation in circadian

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genes may affect the risk of a variety of cancers including that of the prostate.[15]

One possible mechanism by which circadian genes may influence prostate cancer risk is through their effect on metabolism and energy balance. In mice with mutated *Clock* or deleted *Bmal1* (a homologue of human *ARNTL*), gluconeogenesis was suppressed or abolished, respectively.[2] In addition, insulin injections elicited hypoglycemic responses in the mutant mice that were not seen in their wild-type counterparts. Furthermore, *Clock* mutant mice that had a greatly attenuated diurnal feeding rhythm, were hyperphagic and obese, and developed metabolic syndrome that included hypoinsulinemia.[3] Since many of the phenotypes described for *Clock* and *Bmal1* mutant mice are also the putative risk factors for prostate cancer,[16–19] it is important to determine if circadian genes can interact with indicators of obesity and insulin resistance in modifying prostate cancer risk.

In this study, we examined the relationships between five variants of five circadian genes and prostate cancer risk in a population-based case–control study conducted in Shanghai, China. Three of the five variants were chosen based on putative function and included *PER1* rs2585405:G>C (Ala962Pro), *NPAS2* rs2305160:G>A (Ala394Thr) and *PER3* 54-bp repeat length variant in exon 18; the *PER3* and *NPAS2* polymorphisms were associated with other cancers in previous studies.[12–14] Two other variants were chosen because they had minor allele frequencies greater than 5% and included *CRY2* rs1401417:G>C and *CSNK1E* rs1005473:A>C. In addition, we examined if body size and insulin resistance could influence the effect that circadian gene variation has on the prostate cancer risk.

Materials and methods

Study population

Details of this population-based case–control study conducted in Shanghai, China have been reported previously.[16–18,20,21] In brief, newly diagnosed primary prostate cancer cases (International Classification of Diseases 9 code 185) were identified through a rapid reporting system established between the Shanghai Cancer Institute and 28 collaborating hospitals in urban Shanghai, between 1993 and 1995. In-person interviews were conducted, using a structured questionnaire, to collect demographic characteristics information that included smoking history and alcohol use. Anthropometric measurements were also obtained as part of the interview. Of the 268 eligible cases (95% of the cases were diagnosed in Shanghai during the study period), 243 (91%) were interviewed. Male controls who were randomly selected from the general population, matched to the index case by age (within 5 years). Of the 495 eligible controls, 472 (95%) were interviewed. Neither cases nor controls had prior history of cancer. The study was approved by the Institutional Review Boards at the National Cancer Institute and the Shanghai Cancer Institute.

Biological specimen collection and genetic analysis

As part of the interview, 200 cases (82% of those interviewed) and 330 controls (70%) provided 20 ml of fasting blood for the study. For this study, biospecimens were available for 187 cases and 242 controls. The overnight fasting blood samples were used to measure insulin and glucose in the laboratory of FZ Stanczyk at the University of Southern California, as reported previously.[18]

Genomic DNA extracted from the buffy coat was used for genotyping five variants in five circadian genes in the laboratory of Y Zhu at Yale University. Approximately 5% of the samples were duplicated for quality control and two reviewers independently scored the genotypes to confirm the results. The *PER3* variant is a repeat polymorphism with four or five copies of a 54-bp repetitive sequence in exon 18 (GenBank accession no. AB047686)[22,23] and is located on chromosome 17p13.1. Details of the PCR-based sequence-length polymorphism analysis are described elsewhere.[12] The other four variants are SNPs of *CRY2* (rs1401417:G>C, chromosome 11p11.2), *CSNK1E* (rs1005473:A>C, chromosome 22q13.1), *NPAS2* (rs2305160:G>A, chromosome 2q11.2) and *PER1* (rs2585405:G>C, chromosome 17p13.1). TaqMan Assays-on-Demand primers and probes (Applied Biosystems, Foster City, CA, USA) were used according to the manufacturer and have been previously described.[12–14]

Statistical analyses

All statistical analyses were carried out using STATA statistical software (StataCorp LP, College Station, TX, USA). Allele frequencies for all gene variants in the controls were analysed for Hardy–Weinberg equilibrium. Unconditional logistic regression analyses were used to calculate ORs and 95% CIs. The OR and 95% CI are not given if either cases or controls have less than five individuals. All models were adjusted for age as a categorical variable with age categories of $\leqslant 65$, 66–75 and >75. Due to limited sample size, median values from controls were used to define categories of waist-to-hip ratio, body mass index and insulin resistance (IR) as defined by the homeostasis model assessment (HOMA) where appropriate. HOMA is calculated by the equation: HOMA = fasting insulin (μU ml^{-1}) $\times$ glucose (mmol l^{-1}) $\div$ 22.5. For interaction analyses, genotypes were dichotomized based on the presence or absence of the variant allele due to limited sample size. In addition, dummy variables representing the different combinations of genotype and IR were used in the models to determine ORs and 95% CIs. Furthermore, cross product terms were used to determine *P*-interaction. All tests for statistical significance were two-tailed with $\alpha = 0.05$.

Results

Selected characteristics of cases and controls are shown in Table 1. Cases and controls were of similar age and had similar body mass indices. Cases tended to be better educated (*P*-trend = 0.04), were less likely to be smokers (*P*-trend = 0.06), were less likely to use alcohol (*P*-trend < 0.01) and had higher median waist-to-hip ratio and HOMA (both $P < 0.01$) than controls. The cases had a median prostate specific antigen (PSA) level of 89.5 ng ml^{-1} and nearly two-thirds of them had advanced cancer (regional/remote).

Table 1 Selected characteristics of cases and controls

	Cases (N = *187*)		*Controls* (N = *242*)		P_{trend}
	n	%	n	%	
Education					
No formal education	17	9.1	33	13.6	0.04
Elementary school	64	34.2	87	36.0	
Middle school	42	22.5	58	24.0	
High or occupational school and some college	32	17.1	35	14.5	
College or above	32	17.1	28	11.6	
Other	0	0.0	1	0.4	
Smoking, n *(%)*					
Non-smoker	83	44.4	86	35.5	0.06
Former smoker	46	24.6	62	25.6	
Current smoker	58	31.0	94	38.8	
Alcohol use, n *(%)*					
Non-drinker	129	69.0	136	55.7	<0.01
Former drinker	24	12.8	16	6.6	
Current drinker	34	18.2	92	37.7	
Prostate cancer stage					
Unstaged	2	1.1	—		
Localized	68	36.4	—		
Regional	57	30.5	—		
Remote	60	32.1	—		
	Median	*Range*	*Median*	*Range*	P[a]
Age (years)	73	49–85	72	50–89	0.45
Body mass index (BMI; kg m^{-2})	21.5	15.9–32.1	21.5	14.9–36.1	0.46
Waist-to-hip ratio (WHR)	0.91	0.77–1.11	0.89	0.74–1.05	<0.01
HOMA	2.10	0.28–53.47	1.44	0.25–18.30	<0.01
Total PSA (ng ml^{-1})	89.5	0.3–19000	1.5	0.1–320	<0.01

Abbreviation: HOMA, Homeostasis Model Assessment.
[a]*t*-test.

Allele frequency distributions for the five circadian gene variants in the controls were in Hardy–Weinberg equilibrium ($P > 0.05$; data not shown). The ORs and 95% CIs for prostate cancer associated with variants of circadian genes are presented in Table 2. Men with the *CRY2*-variant C allele had a significant 1.7-fold increased risk (95% CI, 1.1–2.6) for prostate cancer as compared with those with the GG genotype. In addition, suggestive increase and decrease in risk are seen in men with variants of *PER3* ($OR_{4\text{-}/5\text{-}+5\text{-}/5\text{-repeat}} = 1.3$, 95% CI, 0.9–2.1 versus 4-/4-repeat genotype) and *NPAS2* ($OR_{GA+AA} = 0.8$, 95% CI 0.5–1.1 versus GG genotype) respectively. We also observed a decreased risk associated with the heterozygous *NPAS2* genotype ($OR_{GA} = 0.6$, 95% CI, 0.4–1.0 versus the GG genotype) but an increased risk associated with the homozygous variant genotype ($OR_{AA} = 2.0$, 95% CI, 0.9–4.4 versus the GG genotype); this difference in risks may be due to small sample size. Risk estimates for all gene variants were similar for advanced and non-advanced tumors (data not shown). Further adjustment for waist-to-hip ratio, body mass index, IR, or alcohol use did not materially change the results (data not shown).

Table 3 shows prostate cancer risk in relation to circadian gene polymorphisms by HOMA as a measure of IR; men with HOMA < 1.44 had less IR while those with HOMA ⩾ 1.44 had greater IR. In general, greater IR conferred an approximate 2-fold higher risk for prostate cancer, which is consistent with a previous study in the study population;[18] this effect was independent of circadian gene variants. However, men with greater IR and the *CRY2*-variant C allele had a 4.1-fold increased risk (95% CI, 2.2–8.0) for prostate cancer relative to men with less IR and the GG genotype. Excess risk for prostate cancer was also seen in the men with greater IR and variants of *CSNK1E* (OR = 2.7, 95% CI, 1.2–5.9) and *PER3* (OR = 3.1, 95% CI, 1.7–5.7) relative to those with the wild type and lower IR. In contrast, within the group of men with less IR those with the *NPAS2*-variant A allele had reduced prostate cancer risk (OR = 0.5, 95% CI, 0.3–1.0) as compared to men with GG genotype; this effect was not detected in the group of men with greater IR. However, interactions between IR and the circadian gene variants were not significant due to small numbers. No differences in risk were seen in stratification analyses using body mass index and waist-to-hip ratio as indicators of body size.

Discussion

In this population-based case–control study, we found that an intronic *CRY2* variant was significantly associated with a 1.7-fold risk of prostate cancer and this risk was more pronounced for men who also had a greater IR. Men with a greater IR and with the variant alleles of *CSNK1E* or *PER3* also had excess prostate cancer risk relative to those with less IR and with the wild

Table 2 Odds ratios and 95% CIs for prostate cancer in relation to circadian gene polymorphisms

Circadian gene	*Cases* (N = *187*)		*Control* (N = *242*)		*OR*[a]	*(95% CI)*	P_{trend}
	n	%	n	%			
CRY2—rs1401417:G>C (intron 2)							
GG	128	69.2	189	79.1	1.0		0.03
GC	53	28.6	46	19.2	1.7	(1.1–2.7)	
CC	4	2.2	4	1.7	—	—	
GC+CC	57	30.8	50	20.9	1.7	(1.1–2.6)	
CSNK1E—rs1005473:A>C (intron 7)							
AA	161	88.0	203	86.8	1.0		0.70
AC	22	12.0	30	12.8	0.9	(0.5–1.7)	
CC	0	0.0	1	0.4	—	—	
AC+CC	22	12.0	31	13.2	0.9	(0.5–1.7)	
NPAS2—rs2305160:G>A (exon 13)							
GG	119	63.6	140	57.8	1.0		0.92
GA	49	26.2	91	37.6	0.6	(0.4–1.0)	
AA	19	10.2	11	4.6	2.0	(0.9–4.4)	
GA+AA	68	36.4	102	42.2	0.8	(0.5–1.1)	
PER1—rs2585405:G>C (exon 19)							
GG	51	28.5	63	28.8	1.0		0.60
GC	81	45.2	106	48.4	0.9	(0.6–1.5)	
CC	47	26.3	50	22.8	1.2	(0.7–2.0)	
GC+CC	128	71.5	156	71.2	1.0	(0.7–1.6)	
PER3—54 bp repeat polymorphism (exon 18; GenBank accession no. AB047686)							
4-/4-repeat	128	70.3	180	75.9	1.0		0.28
4-/5-repeat	53	29.1	54	22.8	1.4	(0.9–2.2)	
5-/5-repeat	1	0.6	3	1.3	—	—	
4-/5-repeat and 5-/5-repeat	54	29.7	57	24.0	1.3	(0.9–2.1)	

Abbreviation: CI, confidence intervals.
[a]Adjusted for age.

type alleles. In contrast, among men with less IR, a reduced prostate cancer risk was associated with the *NPAS2*-variant allele that was not seen among men with greater IR. These results provide the first evidence supporting the circadian gene hypothesis in prostate tumorigenesis.[15]

Findings from animal studies support the hypothesis that circadian genes may affect cancer susceptibility. For example, mice with the mutant *PER2* gene had impaired DNA damage responses to γ- irradiation and were more cancer-prone as compared to their wild type counterparts.[24] The impaired DNA damage response was due to the deregulation of circadian gene-controlled expression of cyclin D1, cyclin A2, Mdm2, Gadd45α and Myc, all of which are involved in the cell cycle regulation and tumor suppression. Interestingly, the human *MYC* gene resides close to the 8q24 region that was found to be strongly associated with prostate cancer risk in recent genome-wide association studies of prostate cancer.[25–28] Although no *in vivo* studies directly assessed the relationship between *CRY2* and cancer, it is plausible that variations or mutations of *CRY* genes may alter cancer risk in a manner similar to that of *PER2* because both are negative regulators of the circadian pathway. Since animal studies also suggest that circadian genes may be tumor suppressors, disruptions to human circadian genes might increase cancer risk by interfering with or inhibiting their tumor suppression activity.[11]

Data from the recently released and publicly available NCI genome-wide association study on prostate cancer also support our hypothesis that variants of circadian genes may alter prostate cancer risk. The NCI cancer genetic markers of susceptibility (CGEMS) project genotyped 550 000 SNPs on 1182 prostate cancer cases and 1174 controls from the prostate, lung, cervical and ovarian cancer screening trial.[29] A total of 155 SNPs from the nine circadian genes were included in the CGEMS project. Eight of the 155 SNPs, located on four genes, *CRY1* (2 SNPs), *CRY2* (1 SNP), *CSNK1E* (2 SNPs) and *NPAS2* (3 SNPs) were significantly associated with altered prostate cancer risk (global $P \leqslant 0.05$ for all SNPs) including three with global $P \leqslant 0.01$. One SNP was in common between this study and that of CGEMS (*NPAS2* rs2305160) and results from both studies show a reduction in the prostate cancer risk associated with this SNP; in CGEMS, this finding was borderline significant (global $P = 0.07$) as a main effect similar to our findings. Since we found that risk-estimates may be attenuated due to interactions with other potential risk factors (such as by IR), it is likely that the effects of the circadian gene variants on prostate cancer risk in the CGEMS study are also modified by similar interactions.

Although our results showing an effect of the interaction between IR and variants in circadian genes on prostate cancer risk should be considered merely suggestive, it is possible that metabolism acts as a link between circadian genes and IR.[4] *In vitro* findings show that cell-redox flux, an indicator of a cell's energy state, can alter the core circadian machinery[1] but *in vivo* studies indicate that alterations in this molecular clock may alter

Table 3 Odds ratios and 95% (CIs) for prostate cancer in relation to circadian gene polymorphisms by insulin resistance level

Circadian gene	*HOMA*	*Cases* (N = 187)		*Control* (N = 242)		*OR*[a]	*(95% CI)*	P[b]
		n	%	n	%			
CRY2—rs1401417: G>C (intron 2)								
GG	<1.44	40	21.9	89	38.2	1.0		0.48
GC+CC	<1.44	18	9.8	28	12.0	1.5	(0.7–2.9)	
GG	⩾1.44	86	47.0	95	40.8	2.0	(1.3–3.3)	
GC+CC	⩾1.44	39	21.3	21	9.0	4.1	(2.2–8.0)	
CSNK1E—rs1005473: A>C (intron 7)								
AA	<1.44	54	29.8	96	42.1	1.0		0.04
AC+CC	<1.44	3	1.7	18	7.9	—	—	
AA	⩾1.44	105	58.0	101	44.3	1.9	(1.2–2.9)	
AC+CC	⩾1.44	19	10.5	13	5.7	2.7	(1.2–5.9)	
NPAS2—rs2305160:G>A (exon 13)								
GG	<1.44	40	21.6	63	26.7	1.0		0.13
GA+AA	<1.44	18	9.7	55	23.3	0.5	(0.3–1.0)	
GG	⩾1.44	78	42.2	73	30.9	1.7	(1.0–2.8)	
GA+AA	⩾1.44	49	26.5	45	19.1	1.7	(1.0–3.0)	
PER1—rs2585405:G>C (exon 19)								
GG	<1.44	12	6.7	29	13.6	1.0		0.46
GC+CC	<1.44	45	25.3	79	37.1	1.4	(0.6–3.0)	
GG	⩾1.44	38	21.3	32	15.0	2.9	(1.2–6.5)	
GC+CC	⩾1.44	83	46.6	73	34.3	2.7	(1.3–5.8)	
PER3—54 bp repeat polymorphism (exon 18; GenBank accession no. AB047686)								
4-/4-repeat	<1.44	43	23.9	88	38.1	1.0		0.34
4-/5-repeat and 5-/5-repeat	<1.44	14	7.8	29	12.6	1.0	(0.5–2.1)	
4-/4-repeat	⩾1.44	84	46.7	88	38.1	1.9	(1.2–3.1)	
4-/5-repeat and 5-/5-repeat	⩾1.44	39	21.7	26	11.3	3.1	(1.7–5.7)	

Abbreviations: CI, confidence intervals; HOMA, Homeostasis Model Assessment; less insulin resistance is defined as HOMA < 1.44 and greater insulin resistance is defined as HOMA ⩾ 1.44.
[a]Adjusted for age.
[b]$P_{interaction}$ between HOMA and circadian gene polymorphism.

cell metabolism as well. The *Clock* mutant mice that had attenuated diurnal feeding rhythms were hyperphagic and obese, and developed several metabolic syndrome phenotypes including hyperglycemia and hypoinsulinemia.[3] In addition, mice with mutant *Clock* or deleted *Bmal1* (a homologue of human *ARNTL*) had impaired gluconeogenesis which resulted in altered daily fluctuations of plasma glucose and triglycerides.[2] Although a chronic high-fat diet amplified the daily oscillations in glucose tolerance and insulin sensitivity, these mutant mice were protected against the development of frank diabetes. Because obesity is a major hypothesis for prostate tumorigenesis,[16–19,30,31] it is plausible that the interplay between circadian genes and metabolic processes such as insulin-action may also contribute to altering prostate cancer risk.

An alternative mechanism by which circadian genes may affect the prostate cancer risk (that was not explored in this study) is through their effect on sex hormone levels in serum, in particular androgens,[15] because prostate cancer is a hormone-dependent malignancy.[19] The central circadian clock in the brain influences steroid hormone secretion via the hypothalamic–pituitary–gonadal axis. In a mouse model, *Clock* mutants lacked the appropriate circadian signals that are required to coordinate the hypothalamic–pituitary–gonadal axis, which resulted in disrupted reproduction.[32] In men with normal sleep patterns and those with obstructive sleep apnea, sleep fragmentation resulted in significant reductions in nocturnal testosterone;[33,34] similar disruptions in testosterone rhythm are also seen in prostate cancer patients.[35] Since variants in *PER* genes are associated with delayed sleep phase syndrome and extreme diurnal preference,[22,23,36,37] it is plausible that variants in *PER* genes are associated with different serum steroid hormone levels thus affecting risk for hormone-related cancers. Interestingly, the *PER3* structural variant that was associated with an increased prostate cancer risk in a subgroup of men in this study was also linked to an increased risk for breast cancer among premenopausal women.[12] In addition, *NPAS2*, the homolog to *Clock*, was recently shown to interact with the androgen receptor *in vitro*.[38] Taken together, these data support the hypothesis that circadian genes may modify the risk of hormonally mediated cancers through regulating sex hormones in serum and their action on cells.[39] Therefore, future studies should also examine the relationship between circadian genes and sex hormones.

On a chromosomal level, several circadian genes reside on regions of chromosomes that are frequently altered or near the genes that are related to certain genetic events in prostate cancer progression. For example, *PER1* resides on chromosome 17p (at band 17p13), which is one of the most frequently lost regions of the genome found in prostate tumors.[40] Chromosome 17p13 also harbors the gene encoding the tumor suppressor p53, sex hormone binding globulin and aurora kinase B, all of which have been associated with

prostate cancer.[41–43] Two other circadian genes reside near the genes that encode UDP glucuronosyltransferases (UGT), which are involved in clearance of steroid hormones. *PER2* and three UGT genes (*UGT1A1*, *UGT1A8* and *UGT1A9*) reside on chromosome 2q37 while *Clock* (on 4q12) is near *UGT2B15* and *UGT2B17* on 4q13. The proximity of circadian genes to genes important in prostate cancer progression suggests that cancer risk variants of the genes may have high linkage disequilibrium with each other and/or the genes may share common transcriptional regulatory elements. Thus, future studies should also investigate the relationship between circadian genes and their neighboring genes.

Strengths of our study include minimal selection and survival biases because over 90% of eligible cases participated in the study and most of the cases were interviewed within 21 days of diagnosis. In addition, close to 75% of the study participants gave blood for the study and thus it is unlikely that observed allele frequencies are related to response status among cases and controls. Furthermore, quality control measures were taken to minimize misclassification of genotyping including the confirmation of genotyping results by two independent reviewers.

Limitations of the study should be noted. Since the Shanghai population is relatively homogeneous, we have limited generalizability. We also do not have data on sleep patterns and light exposure, which could also affect circadian rhythmicity and interact with genetic susceptibility. Future studies should include these variables in data collection and analyses. In addition, this study population is rather lean and may not be ideal for addressing the link between obesity and prostate cancer although age-adjusted prevalence of IR or metabolic syndrome in Chinese men is notable at 10.1%.[44] Furthermore, the variants analyzed in this study provided very limited gene coverage. Based on data from the International HapMap Consortium,[45] approximately 275 tag SNPs are needed adequately to cover the nine circadian genes. In addition, there are about 40 putatively functional SNPs that may also be of interest. Therefore, to characterize the role of circadian gene variants in prostate cancer risk more precisely, future studies will need to examine over 300 SNPs in these nine genes.

In conclusion, our population-based study conducted in a low-risk population suggests that polymorphisms in circadian genes may affect prostate cancer risk and this risk may be modified by some biological processes such as IR. Future studies with larger sample size and more complete gene coverage are needed to confirm our findings. In addition, biological processes such as hormone biosynthesis and action should be examined for their possible roles in modifying the relationship between circadian genes and prostate cancer risk.

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References

1 Rutter J, Reick M, Wu LC, McKnight SL. Regulation of clock and NPAS2 DNA binding by the Redox state of NAD cofactors. *Science* 2001; **293**: 510–514.
2 Rudic RD, McNamara P, Curtis AM, Boston RC, Panda S, Hogenesch JB *et al.* BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLoS Biol* 2004; **2**: e377.
3 Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E *et al.* Obesity and metabolic syndrome in circadian clock mutant mice. *Science* 2005; **308**: 1043–1045.
4 Zvonic S, Floyd ZE, Mynatt RL, Gimble JM. Circadian rhythms and the regulation of metabolic tissue function and energy homeostasis. *Obesity* 2007; **15**: 539–543.
5 Lejeune-Lenain C, Van Cauter E, Desir D, Beyloos M, Franckson JR. Control of circadian and episodic variations of adrenal androgens secretion in man. *J Endocrinol Invest* 1987; **10**: 267–276.
6 Kubo T, Ozasa K, Mikami K, Wakai K, Fujino Y, Watanabe Y *et al.* Prospective cohort study of the risk of prostate cancer among rotating-shift workers: findings from the Japan collaborative cohort study. *Am J Epidemiol* 2006; **164**: 549–555.
7 Conlon M, Lightfoot N, Kreiger N. Rotating shift work and risk of prostate cancer. *Epidemiology* 2007; **18**: 182–183.
8 Band PR, Le ND, Fang R, Deschamps M, Coldman AJ, Gallagher RP *et al.* Cohort study of air Canada pilots: mortality, cancer incidence, and leukemia risk. *Am J Epidemiol* 1996; **143**: 137–143.
9 Irvine D, Davies DM. British airways flightdeck mortality study, 1950–1992. *Aviat Space Environ Med* 1999; **70**: 548–555.
10 Pukkala E, Aspholm R, Auvinen A, Eliasch H, Gundestrup M, Haldorsen T *et al.* Cancer incidence among 10,211 airline pilots: a Nordic study. *Aviat Space Environ Med* 2003; **74**: 699–706.
11 Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. *Nat Rev Cancer* 2003; **3**: 350–361.
12 Zhu Y, Brown HN, Zhang Y, Stevens RG, Zheng T. Period3 structural variation: a circadian biomarker associated with breast cancer in young women. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 268–270.
13 Zhu Y, Stevens R, Leaderer D, Hoffman A, Holford T, Zhang Y *et al.* Non-synonymous polymorphisms in the circadian gene NPAS2 and breast cancer risk. *Breast Cancer Res Treat* (e-pub ahead of print).
14 Zhu Y, Leaderer D, Guss C, Brown HN, Zhang Y, Boyle P *et al.* Ala394Thr polymorphism in the clock gene *NPAS2*: a circadian modifier for the risk of non-Hodgkin's lymphoma. *Int J Cancer* 2007; **120**: 432–435.
15 Zhu Y, Zheng T, Stevens RG, Zhang Y, Boyle P. Does 'clock' matter in prostate cancer? *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 3–5.
16 Hsing AW, Deng J, Sesterhenn IA, Mostofi FK, Stanczyk FZ, Benichou J *et al.* Body size and prostate cancer: a population-based case–control study in China. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 1335–1341.
17 Hsing AW, Chua Jr S, Gao Y-T, Gentzschein E, Chang L, Deng J *et al.* Prostate cancer risk and serum levels of insulin and leptin: a population-based study. *J Natl Cancer Inst* 2001; **93**: 783–789.
18 Hsing AW, Gao YT, Chua Jr S, Deng J, Stanczyk FZ. Insulin resistance and prostate cancer risk. *J Natl Cancer Inst* 2003; **95**: 67–71.
19 Hsing AW, Chokkalingam AP. Prostate cancer epidemiology. *Front Biosci* 2006; **11**: 1388–1413.
20 Hsing AW, Gao YT, Wu G, Wang X, Deng J, Chen YL *et al.* Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case–control study in China. *Cancer Res* 2000; **60**: 5111–5116.

21 Hsing AW, Chen C, Chokkalingam AP, Gao Y-T, Dightman DA, Nguyen HT *et al*. Polymorphic markers in the *SRD5A2* gene and prostate cancer risk: a population-based case–control study. *Cancer Epidemiol BiomarkersPrev* 2001; **10**: 1077–1082.
22 Ebisawa T, Uchiyama M, Kajimura N, Mishima K, Kamei Y, Katoh M *et al*. Association of structural polymorphisms in the human period3 gene with delayed sleep phase syndrome. *EMBO Rep* 2001; **2**: 342–346.
23 Archer SN, Robilliard DL, Skene DJ, Smits M, Williams A, Arendt J *et al*. A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* 2003; **26**: 413–415.
24 Fu L, Pelicano H, Liu J, Huang P, Lee CC. The circadian gene period2 plays an important role in tumor suppression and DNA damage response *in vivo*. *Cell* 2002; **111**: 41–50.
25 Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA *et al*. A common variant associated with prostate cancer in European and African populations. *Nat Genet* 2006; **38**: 652–658.
26 Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A *et al*. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat Genet* 2007; **39**: 631–637.
27 Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A *et al*. Multiple regions within 8q24 independently affect risk for prostate cancer. *Nat Genet* 2007; **39**: 638–644.
28 Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S *et al*. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet* 2007; **39**: 645–649.
29 Core Genotyping Facility, Office of Cancer Genomics, Division of Cancer Epidemiology and Genetics NCI, NIH. The cancer genetic markers of susceptibility project. http://cgems.cancer.gov/. Date accessed: February 5, 2007.
30 Hammarsten J, Hogstedt B. Hyperinsulinaemia: a prospective risk factor for lethal clinical prostate cancer. *Eur J Cancer* 2005; **41**: 2887–2895.
31 Gong Z, Neuhouser ML, Goodman PJ, Albanes D, Chi C, Hsing AW *et al*. Obesity, diabetes, and risk of prostate cancer: results from the prostate cancer prevention trial. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1977–1983.
32 Miller BH, Olson SL, Turek FW, Levine JE, Horton TH, Takahashi JS. Circadian clock mutation disrupts estrous cyclicity and maintenance of pregnancy. *Curr Biol* 2004; **14**: 1367–1373.
33 Luboshitzky R, Zabari Z, Shen-Orr Z, Herer P, Lavie P. Disruption of the nocturnal testosterone rhythm by sleep fragmentation in normal men. *J Clin Endocrinol Metab* 2001; **86**: 1134–1139.
34 Luboshitzky R, Aviv A, Hefetz A, Herer P, Shen-Orr Z, Lavie L *et al*. Decreased pituitary–gonadal secretion in men with obstructive sleep apnea. *J Clin Endocrinol Metab* 2002; **87**: 3394–3398.
35 Bartsch C, Bartsch H, Fluchter SH, Mecke D, Lippert TH. Diminished pineal function coincides with disturbed circadian endocrine rhythmicity in untreated primary cancer patients. Consequence of premature aging or of tumor growth? *Ann N Y Acad Sci* 1994; **719**: 502–525.
36 Carpen JD, Archer SN, Skene DJ, Smits M, von Schantz M. A single-nucleotide polymorphism in the 5′-untranslated region of the hPER2 gene is associated with diurnal preference. *J Sleep Res* 2005; **14**: 293–297.
37 Carpen JD, von Schantz M, Smits M, Skene DJ, Archer SN. A silent polymorphism in the PER1 gene associates with extreme diurnal preference in humans. *J Hum Genet* 2006; **51**: 1122–1125.
38 Mukhopadhyay NK, Ferdinand AS, Mukhopadhyay L, Cinar B, Lutchman M, Richie JP *et al*. Unraveling androgen receptor interactomes by an array-based method: discovery of proto-oncoprotein c-Rel as a negative regulator of androgen receptor. *Exp Cell Res* 2006; **312**: 3782–3795.
39 Chokkalingam AP, Stanczyk FZ, Reichardt JKV, Hsing AW. Molecular epidemiology of prostate cancer: hormone-related genetic loci. *Front Biosci* 2007; **12**: 3436–3460.
40 Saramaki O, Visakorpi T. Chromosomal aberrations in prostate cancer. *Front Biosci* 2007; **12**: 3287–3301.
41 Dong J-T. Prevalent mutations in prostate cancer. *J Cell Biochem* 2006; **97**: 433–447.
42 Chieffi P, Cozzolino L, Kisslinger A, Libertini S, Staibano S, Mansueto G *et al*. Aurora B expression directly correlates with prostate cancer malignancy and influence prostate cell proliferation. *Prostate* 2006; **66**: 326–333.
43 Berndt SI, Chatterjee N, Huang W-Y, Chanock SJ, Welch R, Crawford ED *et al*. Variant in sex hormone-binding globulin gene and the risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 165–168.
44 Jia WP, Xiang KS, Chen L, Lu J, Wu YM. Epidemiological study on obesity and its comorbidities in urban Chinese older than 20 years of age in Shanghai, China. *Obes Rev* 2002; **3**: 157–165.
45 The International HapMap Consortium. A haplotype map of the human genome. *Nature* 2005; **437**: 1299–1320.