Correlation between Circadian Gene Variants and Serum Levels of Sex Steroids and Insulin-like Growth Factor-I

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Abstract

A variety of biological processes, including steroid hormone secretion, have circadian rhythms, which are influenced by nine known circadian genes. Previously, we reported that certain variants in circadian genes were associated with risk for prostate cancer. To provide some biological insight into these findings, we examined the relationship of five variants of circadian genes, including *NPAS2* (rs2305160:G>A), *PER1* (rs2585405:G>C), *CSNK1E* (rs1005473:A>C), *PER3* (54-bp repeat length variant), and *CRY2* (rs1401417:G>C), with serum levels of sex steroids and insulin-like growth factor (IGF)-I and IGF-binding protein 3 (IGFBP3) in 241 healthy elderly Chinese men (mean age of 71.5). Age-adjusted and waist-to-hip ratio–adjusted ANOVA followed by likelihood ratio tests (LRT) showed that the *NPAS2* variant A allele was associated with lower free and bioavailable testosterone (P_{LRT} = 0.02 and 0.01, respectively) compared with the GG genotype. In addition, the *PER1* variant was associated with higher serum levels of sex hormone-binding globulin levels (P_{trend} = 0.03), decreasing 5α-androstane-3α, 17β-diol glucuronide levels (P_{trend} = 0.02), and decreasing IGFBP3 levels (P_{trend} = 0.05). Furthermore, the *CSNK1E* variant C allele was associated with higher testosterone to dihydrotestosterone ratios (P_{LRT} = 0.01) compared with the AA genotype, whereas the longer *PER3* repeat was associated with higher serum levels of IGF-I (P_{LRT} = 0.03) and IGF-I to IGFBP3 ratios (P_{LRT} = 0.04). The *CRY2* polymorphism was not associated with any biomarkers analyzed. Our findings, although in need of confirmation, suggest that variations in circadian genes are associated with serum hormone levels, providing biological support for the role of circadian genes in hormone-related cancers. (Cancer Epidemiol Biomarkers Prev 2008;17(11):3268–73)

Introduction

Circadian rhythms are the daily oscillations of multiple biological processes driven by endogenous clocks with or without external cues (1). These rhythms help maintain human sleep patterns and influence certain biological processes, such as sex hormone secretion (2, 3). Nine identified genes control the endogenous circadian rhythms via a transcription-translation feedback loop and include *neuronal PAS domain protein 2* (*NPAS2*), *casein kinase 1, ε* (*CSNK1E*), *cryptochrome 1* (*CRY1*), *CRY2, period 1* (*PER1*), *PER2, PER3, clock homologue (mouse)* (*CLOCK*), and *aryl hydrocarbon receptor nuclear translocator-like* (*ARNTL*; ref. 4).

In an earlier report, we showed that certain circadian gene variants were associated with increased risk of prostate cancer (5). Although the exact mechanism is unclear, it is possible that the link between circadian genes and prostate cancer is mediated through hormones because steroid hormones play an important role in prostate cancer.

There is ample evidence that certain circulating hormone levels, such as testosterone, oscillate in a diurnal rhythm (2, 3). The central circadian clock located in the brain influences sex hormone secretion via the hypothalamic-pituitary-gonadal axis. Sleep fragmentation, in normal men and those with obstructive sleep apnea, resulted in elevated testosterone levels sustained throughout the night (6, 7). In mouse models, *CLOCK* mutants lacked the appropriate circadian signals required to coordinate hypothalamic hormone secretion, which resulted in reproduction disruptions in female mice (8). Because testosterone influences the growth hormone/insulin-like growth factor (IGF)-I pathway (9), IGF-I and its binding protein, IGFBP3, may also be influenced by circadian genes. Taken together with findings that variants in circadian genes are associated with delayed sleep phase syndrome and morning/evening preference (10-12), it is plausible that variants in these genes are associated with varying serum hormone levels.

To provide biological insight into the relationship between circadian genes and cancer, we examined the association between five circadian gene variants, included in our earlier investigation (5), and serum

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sex steroid, IGF-I, and IGFBP3 levels in 241 healthy Chinese men. Three of the five variants examined were originally chosen based on putative function by amino acid conservation analysis [*PER1* rs2585405:G>C ($Ala^{962}Pro$), *NPAS2* rs2305160:G>A ($Ala^{394}Thr$), and *PER3* 54-bp repeat length variant], whereas two variants were chosen because they had minor allele frequencies >5% (*CRY2* rs1401417:G>C and *CSNK1E* rs1005473:A>C).

Materials and Methods

Study Population. Details of this population-based study conducted in Shanghai, China have been reported previously (13). Briefly, healthy men were randomly selected from the general population in Shanghai between 1993 and 1995 and recruited as controls for a prostate cancer case-control study. Four hundred ninety-five eligible subjects were identified and 472 (95%) were interviewed, using a structured questionnaire, to collect information on selected demographic characteristics, including smoking history and alcohol use. Anthropometric measurements were also obtained as part of the interview, permitting the calculation of usual adult body mass index [BMI; weight (kg) / height $(m)^2$] and waist-to-hip ratio (WHR). Of the 472 men with interview data, 330 men (70%) provided 20 mL of overnight fasting blood (collected between 7:00 a.m. and 10:00 a.m.) that was processed within 4 h of collection and stored at −70°C. Serum hormone and genotyping data were available for 241 (73%) of the 330 participants for this study. The study was approved by the Institutional Review Boards at the National Cancer Institute and the Shanghai Cancer Institute.

Serum Hormone and Genetic Analysis. Total testosterone, dihydrotestosterone (DHT), estradiol, sex hormone-binding globulin (SHBG), and 5α-androstane-3α, 17β-diol glucuronide (3α-diol G) were assayed at the University of Southern California by RIAs, as previously reported (13). Testosterone, DHT, and estradiol were quantified following organic solvent extraction and Celite column partition chromatography using ethylene glycol as the stationary phase (13). SHBG and 3α-diol G were measured directly in serum using commercial kits (Diagnostic Systems Laboratories); the intraassay and interassay coefficients of variation ranged from 4% to 8% and 10% to 13%, respectively. Total testosterone, DHT, and SHBG measurements were used to calculate free and bioavailable testosterone (14), as well as molar ratios of testosterone to DHT (T:DHT), using the molecular weights of testosterone (288.4 g/mol) and DHT (290.4 g/mol) as conversion factors.

Plasma IGF-I and IGFBP3 were assayed by Diagnostic Systems Laboratories using ELISA methods as previously described (15). The lower limits of detection of IGF-I and IGFBP3 were 0.03 and 0.04 ng/mL, respectively. For both analytes, each sample was assayed twice, and the mean of the two determinations was used for data analysis. Samples for which the relative difference between the two determinations exceeded 10% were repeated. Split samples ($n = 45$) from a single individual were included among the study samples and used to assess laboratory reproducibility. The coefficients of variation for these split samples were 11.2% for IGF-I and 17.3% for IGFBP3. Molar ratios of IGF-I to IGFBP3 were calculated by the following formula (16):

$$\text{IGF} - \text{I} : \text{IGFBP3} = [\text{IGF} - \text{I}(\text{ng/mL}) \times 0.130]/[\text{IGFBP3}(\text{ng/mL}) \times 0.036]$$

Genomic DNA extracted from buffy coat was used for genotyping circadian gene variants at Yale University as previously reported (5). Approximately 5% of the samples were duplicated for quality control and two reviewers independently scored the genotypes to confirm all results. The *PER3* repeat length variation has either four or five copies of a 54-bp repetitive sequence in exon 18 (Genbank accession no. AB047686). Details of the PCR-based sequence length variant analysis are described elsewhere (17). The other four variants were single nucleotide polymorphisms of *CRY2* (rs1401417:G>C), *CSNK1E* (rs1005473:A>C), *NPAS2* (rs2305160:G>A), and *PER1* (rs2585405:G>C). Taqman Assays-on-Demand primers and probes (Applied Biosystems) were used according to the manufacturer's instructions and have been previously described (18). Alleles for all single nucleotide polymorphisms are identified according to the SNPper online database (19).

Statistical Analyses. All statistical analyses were carried out using STATA statistical software (StataCorp LP). Genotype frequencies for all gene variants were tested for Hardy-Weinberg equilibrium. ANOVA was used to compare differences in serum biomarkers and biomarker ratios among subjects with different genotypes for each single nucleotide polymorphism, and likelihood ratio tests (LRT) comparing models with and without genotyping data were done to determine if genetic variations contribute significantly to the model. Homozygous minor allele genotype categories that had less than five individuals were consolidated with corresponding heterozygous genotype categories with the understanding that for these markers, only a dominant effect could be detected; LRT comparing these models has 1 degree of freedom (*df*). If three genotype groups were available for analyses, LRT has 2 *df* and linear regression analysis was used to determine significance of monotonic dose-response relationships with the genotypes coded as a continuous variable (0, 1, and 2). Outcome variables were transformed (by $\log_{10}$, inverse, or square root) when necessary for normalization. All models were adjusted for categorical variables of age with age categories of ≤65, 66 to 75, and >75 and WHR or BMI using median values (0.89 and 21.5 kg/m^2, respectively) as cutoff due to limited sample size. WHR and BMI were also assessed (as continuous variables) to determine if associations existed between variants in circadian genes and markers of adiposity. The associated *P* values were calculated for the two-sided tests with $\alpha = 0.05$.

Results

Selected demographic and serologic characteristics of the participants are shown in Table 1. The mean age of the study participants was 71.5. The study population was lean, with a mean BMI of 22.0 kg/m^2 and WHR of 0.89.

Table 1. Selected demographic, serologic, and genetic characteristics of study participants

Characteristic	Participants (n = 241)	
	Mean	SD
Age	71.5	6.5
BMI (kg/m^2)	22.0	3.4
WHR	0.89	0.06
Testosterone (ng/dL)	645.3	228.1
Free testosterone (ng/dL)	12.8	4.2
Bioavailable testosterone (ng/dL)	302.7	103.7
DHT (ng/dL)	67.4	27.6
T:DHT (molar ratio)	10.3	3.3
3α-diol G (ng/dL)	573.5	380.1
SHBG (nmol/L)	40.8	20.1
Estradiol (ng/L)	5.0	1.4
IGF-I (ng/mL)	124.0	42.5
IGFBP3 (ng/mL)	2,764.9	840.4
IGF-I:IGFBP3 (molar ratio)	0.165	0.046
Variation in circadian genes	n	%
NPAS2 (rs2305160:G>A)		
GG	140	58.1%
GA	90	37.3%
AA	11	4.6%
PER1 (rs2585405:G>C)		
GG	62	28.4%
GC	106	48.6%
CC	50	22.9%
CSNK1E (rs1005473:A>C)		
AA	202	86.7%
AC	30	12.9%
CC	1	0.4%
PER3 [54-bp repeat polymorphism (Genbank AB047686)]		
4-/4-repeat	180	76.3%
4-/5-repeat	53	22.5%
5-/5-repeat	3	1.3%
CRY2 (rs1401417:G>C)		
GG	188	79.0%
GC	46	19.3%
CC	4	1.7%

Minor allele frequencies for each variant analyzed ranged from 6.9% for the *CSNK1E* rs1005473 C allele to 47.2% for the *PER1* rs2585405 C allele. Among participants, genotype frequency distributions for the five circadian gene variants were in Hardy-Weinberg equilibrium (P > 0.05; data not shown). No statistically significant associations were seen between markers of adiposity (WHR and BMI) and the five variants of circadian genes (data not shown).

Table 2 shows the age-adjusted means and SDs of serum androgens in relation to genotypes of circadian genes. LRT comparing models with and without the genotyping data shows that the *NPAS2* rs2305160 variant A allele (or genotypes GA + AA) was associated with decreased levels of free and bioavailable testosterone (P_{LRT} = 0.02 and 0.01, respectively) but not with other markers when compared with the GG genotype; comparisons between different genotype groups of *NPAS2* variation also show significant differences in free and bioavailable testosterone levels (P_{LRT} = 0.04 and 0.03, respectively). The *PER1* rs2585405 C allele was associated with lower 3α-diol G (P_{LRT} = 0.02) and higher SHBG (P_{LRT} = 0.04) levels; the relationship between the analytes and genotype categories seems to be linear (P_{trend} = 0.04 and 0.03, respectively). Furthermore, although no associations were seen between the five genetic variants analyzed and levels of total testosterone or DHT, the C allele of the *CSNK1E* variant was associated with a higher value of T:DHT (11.7) compared with men with the AA genotype (10.0; P_{LRT} = 0.01); this variant was also borderline associated with 3α-diol G (P_{LRT} = 0.06). No associations were seen for the *CRY2* variant with any of the markers analyzed. These results did not change materially after adjustment for BMI instead of WHR (data not shown).

Similar to the patterns observed for sex steroids, associations were seen between variants of circadian genes and age-adjusted mean serum levels of IGF-I and IGFBP3 (Table 3). A borderline significant decreasing trend in IGFBP3 levels was seen across genotypes of the *PER1* variant (P_{LRT} = 0.05), although no significant associations were seen when comparing GC or CC with the GG genotype. In addition, men who had the longer *PER3* repeat length variant had significantly higher serum IGF-I levels (mean, 133.7 ng/mL) and IGF-I:IGFBP3 ratios (mean, 0.173) compared with those with the homozygous four-repeat genotype [mean, 120.9 ng/mL (P_{LRT} = 0.03) and mean, 0.158 ng/mL (P_{LRT} = 0.04), respectively].

Discussion

Results from this population-based study suggest that variations in circadian genes are associated with serum levels of androgens and IGF markers. In particular, *NPAS2* rs2305160:G>A (Ala^{394}Thr), *PER1* rs2585405:G>C (Ala^{962}Pro), and *CSNK1E* rs1005473:A>C are associated with increased levels of serum androgens, whereas there is a suggestive association between *PER* variants and IGF markers. Although in need of confirmation, these findings are consistent with observations that some circulating hormones oscillate in a circadian rhythm.

These findings support our previous report that selected variants of circadian genes are associated with altered prostate cancer risk (5). For example, the *NPAS2* rs2305160 A allele, which was associated with lower free and bioavailable testosterone levels in this study, conferred a 20% reduced risk of prostate cancer (95% confidence interval, 0.5-1.1; ref. 5). *In vivo* studies have shown that androgens are important in the growth of normal and cancerous prostate cells (20, 21). However, epidemiologic studies of serum androgens have been inconclusive. Several studies report suggestive associations of androgens with increased prostate cancer risk, whereas others report null or even inverse associations (22, 23). One reason for these mixed results may be that serum androgen levels are indirect indicators of intraprostatic androgenicity and may not truly reflect androgen metabolism and action within the prostate (22). It is also unclear if androgen measurements taken at time of the study reflect causative androgen exposure because serum androgen levels are known to decrease with age (3) and are influenced by time of day and sleep patterns (6, 7).

It is noteworthy that the *PER1* and *SHBG* genes reside ~500 kb apart on chromosome 17p12-p13, given our finding that there was an association between *PER1* rs2585405 and serum SHBG. Because of the proximity of these genes, it is plausible that there may be some degree

Table 2. Serum levels of sex steroid hormones and SHBG among study participants in relation to variants of circadian genes

		Testosterone (ng/dL)		Calculated free testosterone (ng/dL)		Calculated bioavailable testosterone (ng/dL)		T:DHT (molar ratio)		DHT (ng/dL)		3α-diol G (ng/dL)		SHBG (nmol/L)		Estradiol (ng/dL)	
Circadian gene variation	*n*	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD
NPAS2 (rs2305160:G>A)																	
GG	140	656.3	226.5	13.3	3.9	312.6	92.4	64.8	25.0	10.1	3.2	485.6	298.1	35.2	14.8	5.0	1.4
GA	90	621.5	225.0	12.0	3.9	279.9	92.9	64.2	25.2	10.1	3.2	462.7	281.7	38.6	16.0	4.8	1.4
AA	11	635.0	226.0	13.1	3.9	306.9	92.2	71.9	25.4	8.8	2.9	548.6	294.7	36.2	13.8	4.6	1.3
		P_{LRT}† = 0.50		P_{LRT}† = 0.04		P_{LRT}† = 0.03		P_{LRT}† = 0.68		P_{LRT}† = 0.42		P_{LRT}† = 0.50		P_{LRT}† = 0.27		P_{LRT}† = 0.39	
		P_{trend}‡ = 0.30		P_{trend}‡ = 0.06		P_{trend}‡ = 0.05		P_{trend}‡ = 0.74		P_{trend}‡ = 0.32		P_{trend}‡ = 0.89		P_{trend}‡ = 0.25		P_{trend}‡ = 0.18	
GA + AA	101	623.0	224.7	12.1	3.9	282.9	92.9	65.0	25.3	10.0	3.2	471.5	287.8	38.3	15.9	4.8	1.4
		P_{LRT}§ = 0.24		P_{LRT}§ = 0.02		P_{LRT}§ = 0.01		P_{LRT}§ = 0.97		P_{LRT}§ = 0.50		P_{LRT}§ = 0.78		P_{LRT}§ = 0.14		P_{LRT}§ = 0.18	
PER1 (rs2585405:G>C)																	
GG	62	603.4	230.5	13.0	4.0	304.7	93.5	61.7	24.7	9.8	3.2	542.0	322.0	33.0	13.6	5.0	1.4
GC	106	649.7	227.0	12.7	4.0	297.0	94.2	65.7	25.8	10.2	3.3	448.7	275.3	37.1	15.6	4.9	1.4
CC	50	653.2	226.7	12.7	4.0	296.8	92.7	67.2	25.5	9.8	3.2	421.4	250.2	38.9	15.9	5.0	1.4
		P_{LRT}† = 0.36		P_{LRT}† = 0.88		P_{LRT}† = 0.85		P_{LRT}† = 0.45		P_{LRT}† = 0.80		P_{LRT}† = 0.06		P_{LRT}† = 0.08		P_{LRT}† = 0.81	
		P_{trend}‡ = 0.21		P_{trend}‡ = 0.64		P_{trend}‡ = 0.64		P_{trend}‡ = 0.23		P_{trend}‡ = 0.99		P_{trend}‡ = 0.02		P_{trend}‡ = 0.03		P_{trend}‡ = 0.80	
GC + CC	156	650.9	226.3	12.7	4.0	296.9	93.5	66.2	25.8	10.1	3.3	439.7	271.7	37.7	15.9	4.9	1.4
		P_{LRT}§ = 0.16		P_{LRT}§ = 0.61		P_{LRT}§ = 0.58		P_{LRT}§ = 0.23		P_{LRT}§ = 0.71		P_{LRT}§ = 0.02		P_{LRT}§ = 0.04		P_{LRT}§ = 0.59	
CSNK1E (rs1005473:A>C)																	
AA	202	643.0	225.2	12.9	4.0	302.8	93.3	65.5	25.3	10.0	3.3	461.4	285.5	36.4	15.5	4.9	1.3
AC + CC	30	662.5	226.1	12.7	4.0	298.5	93.1	58.2	23.4	11.8	3.4	588.0	341.4	35.9	15.2	5.2	1.4
		P_{LRT}§ = 0.64		P_{LRT}§ = 0.78		P_{LRT}§ = 0.81		P_{LRT}§ = 0.13		P_{LRT}§ = 0.01		P_{LRT}§ = 0.06		P_{LRT}§ = 0.90		P_{LRT}§ = 0.21	
PER3 [54-bp repeat polymorphism (Genbank AB047686)]																	
4-/4-repeat	202	642.1	227.0	12.9	4.0	301.4	92.5	66.3	25.6	9.9	3.2	484.3	297.2	36.6	15.3	5.0	1.4
4-/5- and 5-/5-repeat	31	650.1	226.5	12.9	4.0	300.2	94.0	61.7	24.5	10.5	3.3	486.4	292.4	35.4	14.5	4.8	1.4
		P_{LRT}§ = 0.86		P_{LRT}§ = 0.95		P_{LRT}§ = 0.94		P_{LRT}§ = 0.21		P_{LRT}§ = 0.22		P_{LRT}§ = 0.72		P_{LRT}§ = 0.51		P_{LRT}§ = 0.31	
CRY2 (rs1401417:G>C)																	
GG	188	641.7	227.1	12.7	4.0	298.1	94.2	65.0	25.1	10.0	3.3	482.7	299.7	36.5	15.5	5.0	1.4
GC + CC	50	648.8	226.0	13.2	4.0	308.5	93.3	65.7	25.0	10.2	3.4	459.2	274.0	36.6	15.0	4.7	1.4
		P_{LRT}§ = 0.84		P_{LRT}§ = 0.50		P_{LRT}§ = 0.48		P_{LRT}§ = 0.86		P_{LRT}§ = 0.78		P_{LRT}§ = 0.65		P_{LRT}§ = 0.96		P_{LRT}§ = 0.15	

*Age-adjusted means.
† *P* value from LRTs comparing models with and without genotype data (*df*, 2).
‡ *P* trend from genotype from linear regression analysis, adjusted for age and WHR.
§ *P* value from LRTs comparing models with and without genotype data (*df*, 1).

Table 3. Serum levels of IGF-I and IGFBP3 among study participants in relation to variants of circadian genes

Circadian gene variation		IGF-I (ng/mL)		IGFBP3 (ng/mL)		IGF-I:IGFBP3 (molar ratio)	
	n	Mean*	SD	Mean*	SD	Mean*	SD
NPAS2 (rs2305160:G>A)							
GG	140	126.1	41.3	2,673.9	768.1	0.165	0.046
GA	90	120.0	41.3	2,662.6	761.8	0.156	0.045
AA	11	130.2	41.5	2,843.1	766.0	0.166	0.045
		P_{LRT} † = 0.44		P_{LRT} † = 0.72		P_{LRT} † = 0.31	
		P_{trend} ‡ = 0.59		P_{trend} ‡ = 0.71		P_{trend} ‡ = 0.30	
GA + AA	101	121.1	41.3	2,681.9	764.9	0.157	0.045
		P_{LRT}§ = 0.36		P_{LRT}§ = 0.92		P_{LRT}§ = 0.17	
PER1 (rs2585405:G>C)							
GG	62	130.8	40.8	2,836.6	799.5	0.161	0.045
GC	106	124.2	40.8	2,633.3	753.2	0.165	0.046
CC	50	118.6	40.8	2,560.7	737.2	0.163	0.045
		P_{LRT} † = 0.25		P_{LRT} † = 0.12		P_{LRT} † = 0.89	
		P_{trend} ‡ = 0.10		P_{trend} ‡ = 0.05		P_{trend} ‡ = 0.84	
GC + CC	156	122.4	40.8	2,609.9	749.9	0.164	0.046
		P_{LRT}§ = 0.16		P_{LRT}§ = 0.05		P_{LRT}§ = 0.67	
CSNK1E (rs1005473:A>C)							
AA	202	126.1	41.4	2,703.2	769.0	0.163	0.045
AC + CC	30	113.5	41.8	2,565.2	738.9	0.155	0.044
		P_{LRT}§ = 0.11		P_{LRT}§ = 0.33		P_{LRT}§ = 0.34	
PER3 [54-bp repeat variant (Genbank AB047686)]							
4-/4-repeat	202	120.9	40.8	2,676.9	768.4	0.158	0.045
4-/5- + 5-/5-repeat	31	133.7	41.0	2,682.2	759.5	0.173	0.047
		P_{LRT}§ = 0.03		P_{LRT}§ = 0.92		P_{LRT}§ = 0.04	
CRY2 (rs1401417:G>C)							
GG	188	125.4	40.9	2,708.1	765.3	0.162	0.046
GC + CC	50	120.9	40.9	2,584.9	744.0	0.162	0.045
		P_{LRT}§ = 0.49		P_{LRT}§ = 0.31		P_{LRT}§ = 0.96	

*Age-adjusted means.
† *P* value from LRTs comparing models with and without genotype data (*df*, 2).
‡ *P* trend from genotype from linear regression analysis, adjusted for age and WHR.
§ *P* value from LRTs comparing models with and without genotype data (*df*, 1).

of linkage between these two genes and/or that they have gene expression regulatory elements in common. However, SHBG does not seem to be expressed in a circadian pattern (2, 3), suggesting that the relationship between *PER1* and SHBG cannot be explained by proximity of the genes alone and requires further investigation.

Because serum testosterone influences the growth hormone/IGF-I pathway (9), the suggestive association between variants in circadian genes and IGF-I, IGFBP3, and IGF-I:IGFBP3 (an indicator of free IGF-I) is not entirely unexpected. IGF-I is secreted primarily by the liver and is mostly bound by IGFBP3. Some but not all previous studies have shown that serum levels of IGF-I and IGFBP3 vary throughout the day in a circadian rhythm (24-29). In men with hypopituitarism, growth hormone treatment not only increased gene expression of IGF-I but also increased *CLOCK* expression and decreased *PER1* expression in muscle cells (30). Our observation of a suggestive relationship between circadian genes (specifically that of *PER* genes) and the IGF pathway is consistent with these previous findings. Because altered risks for cancers of the prostate and breast have been linked to variants in circadian genes (5, 17, 31) and IGF-I (32), the potential interaction between circadian genes and IGF-I on cancer risk warrants further examination.

The unique strength of our study includes population-based samples with minimal selection bias. Because close to 70% of the study participants gave fasting blood for the study, it is unlikely that observed allele frequencies are related to response status. Quality control measures taken to minimize misclassification of genotyping included confirmation of genotyping results by two independent reviewers and assessment of duplicate samples, which showed high concordance. In addition, we had high-quality serum assays of sex hormones as well as IGF markers, with low intraassay and interassay variation (coefficients of variation, <18%).

Limitations of the study should be noted. First, we have limited gene coverage, with only one variant genotyped in each of the five circadian genes. Based on data from the International HapMap Consortium (33), ~275 tag single nucleotide polymorphisms would be needed to cover the nine circadian genes adequately. There are also ~45 putatively functional variants that need to be typed to capture any risks related to function of the circadian genes. Therefore, to fully capture the variations of these nine genes, ~320 variants need to be genotyped. Second, we do not have data on sleep patterns and light exposure, which could also affect circadian rhythmicity of some hormones. Third, we have serum marker data from only one time point in the day (between 7 and 10 a.m.) and thus cannot determine how diurnal variations of the analytes will be affected by the genotypes studied; within the day, variations in serum steroid levels between early morning blood draws and other blood draw times have been observed (34, 35) but do not influence our results because we only collected blood in the early morning hours. Fourth, because the

Shanghai population is relatively homogeneous, we have limited generalizability to other populations. In addition, prostate cancer screening in Shanghai is relatively uncommon during our study period (1990-1995), and thus, prostate cancer is detected at a later age, with a mean age of 71, which is slightly older than most prostate cancer case patients in the United States. This limits our ability to generalize our results to younger men, although relationships between serum hormones and circadian genes in younger men also exist in our study, but the number of men in this age group is small.

In conclusion, our population-based study of healthy Chinese men suggests that variants in circadian genes may be related to varying serum hormone levels. Future studies with larger sample sizes and more complete gene coverage are needed to confirm our findings. In addition, genes that are related to the metabolism and expression of these hormones should also be studied to determine if an interaction exists between circadian genes and hormone-related genes that would affect serum hormone levels.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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