Inherited variation in circadian rhythm genes and risks of prostate cancer and three other cancer sites in combined cancer consortia

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Novelty & Impact: We found a significant association of circadian rhythm and melatonin pathway genes with prostate cancer risk, at the gene and pathway level, after taking multiple comparisons into account. The sample size is the largest to our knowledge, with a further replication in an independent data. This study provides evidence in support of a role for circadian rhythm and melatonin pathways in prostate carcinogenesis.

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ABSTRACT

Circadian disruption has been linked to carcinogenesis in animal models, but the evidence in humans is inconclusive. Genetic variation in circadian rhythm genes provides a tool to investigate such associations. We examined associations of genetic variation in nine core circadian rhythm genes and six melatonin pathway genes with risk of colorectal, lung, ovarian and prostate cancers using data from the Genetic Associations and Mechanisms in Oncology (GAME-ON) network. The major results for prostate cancer were replicated in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial, and for colorectal cancer in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO). The total number of cancer cases and controls was 15,838/18,159 for colorectal, 14,818/14,227 for prostate, 12,537/17,285 for lung and 4,369/9,123 for ovary. For each cancer site, we conducted gene-based and pathway-based analyses by applying the summary-based Adaptive Rank Truncated Product method (sARTP) on the summary association statistics for each SNP within the candidate gene regions. Aggregate genetic variation in circadian rhythm and melatonin pathways were significantly associated with the risk of prostate cancer in data combining GAME-ON and PLCO, after Bonferroni correction ($P_{pathway}<0.00625$). The two most significant genes were *NPAS2* ($P_{gene}=0.0062$) and *AANAT* ($P_{gene}=0.00078$); the latter being significant after Bonferroni correction. For colorectal cancer, we observed a suggestive association with the circadian rhythm pathway in GAME-ON ($P_{pathway}=0.021$); this association was not confirmed in GECCO ($P_{pathway}=0.76$) or the combined data ($P_{pathway}=0.17$). No association was observed for ovarian and lung cancer. These findings support a potential role for circadian rhythm and melatonin pathways in prostate carcinogenesis. Further functional studies are needed to better understand the underlying biologic mechanisms.

Keywords: circadian rhythm, melatonin, prostate cancer, cancer

INTRODUCTION

Circadian rhythm is driven by an internal biological clock, which enables humans to sustain an approximate 24-hour cycle of biological processes[1], and regulates diverse cancer-related biological functions such as metabolism, immune regulation, DNA repair and cell cycle control[2]. Disruption of circadian rhythm has been linked to carcinogenesis at the system, cell and molecular levels[2]. Based on sufficient evidence in experimental animals for the carcinogenicity of light exposure during the biological night, and limited epidemiological studies showing increased risk of breast cancer among female nightshift workers and flight attendants employed at least ten years, shift work with disrupted circadian rhythm has been categorized as a probable carcinogen to humans by the International Agency for Research on Cancer [3]. However, evidence for cancers other than breast is limited. Increased cancer risks in other organs have been observed in mouse models with ablated circadian rhythm genes, such as the blood[4], liver[4], ovary [4], intestine[5], colon [5] and skin [6], possibly due to constitutively elevated cell proliferation [6], impaired DNA repair [7] and apoptosis [8], and inefficient immune response [9, 10]. There is growing evidence from epidemiologic studies that other types of cancers including prostate [11-14], colon [15] and non-Hodgkin lymphoma[16] also may be associated with rotating and night shift work.

A few candidate gene studies have examined associations between genes involved in circadian processes and several cancer sites [17-29], especially breast [21, 24-26, 29]. In this study, we examined associations of the core genes involved in the circadian rhythm and melatonin pathways with the risk of prostate, colorectal, lung and ovarian cancer in population of European descent, taking advantage of the large study populations from the Genetic Associations and Mechanisms in Oncology (GAME-ON) GWAS consortia. We conducted a pathway-level

analysis, aggregating association evidence across multiple genes. Potentially interesting findings were further replicated in independent populations of European descent.

METHODS

Study populations

Our initial analyses used data from 20 GWAS studies on four common cancer sites within the National Cancer Institute GAME-ON Network (http://epi.grants.cancer.gov/gameon/)[30], including 12,537 lung cancer cases and 17,285 controls from the Transdisciplinary Research for Cancer of Lung (TRICL) consortium; 5,100 colorectal cases and 4,831 controls from the ColoRectal Transdisciplinary Study (CORECT); 10,218 prostate cancer cases and 11,286 controls from the Elucidating Loci in Prostate Cancer Susceptibility (ELLIPSE) consortium; as well as 4,369 ovarian cancer cases and 9,123 controls from the Follow-up of Ovarian Cancer Genetic Association and Interaction Studies (FOCI) (Table 1). For colorectal and prostate cancer, potentially interesting findings were carried forward and replicated in additional independent data: 10,738 cases and 13,328 controls from the Genetics and Epidemiology of Colorectal Cancer Consortium for colorectal cancer (GECCO) [31]; 4,600 cases and 2,940 controls from the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial for prostate cancer [32]. All participants were of European descent, and most of the studies were conducted using Illumina genotyping platforms (Table 1). Details of the genotyping and quality control steps were published previously [30-32]. All participating studies obtained approval from the institutional ethics review board, and informed consents were obtained from each study participant by the individual study coordinating center.

Candidate genes

For the circadian rhythm pathway, we included nine well-established core circadian rhythm genes that generate the mammalian circadian rhythm[33] and were selected for a previous cancer study to represent the circadian rhythm pathway [24]: *CLOCK* and its paralogue *NPAS2* (neuronal PAS domain protein 2); *ARNTL* (aryl hydrocarbon receptor nuclear translocator-like; a.k.a. *Bmal1*); *CKIε* (casein kinase I ε; a.k.a. *CSNKIE*); Cryptochrome 1 (*CRY1*); *CRY2*; and three Period homologs (*PER1*, *PER2* and *PER3*).

Due to a close integration of melatonin to the circadian system, we also included four genes involved in melatonin biosynthesis (http://www.kegg.jp/kegg-bin/show_module?M00037) [34] and two melatonin receptor genes: arylalkylamine N-acetyltransferase (*AANAT*, a gene encoding the rate limiting enzyme in the melatonin biosynthesis*)*, *TPH1* (tryptophan hydroxylase 1), *TPH2*, and *DDC* (aromatic-L-amino-acid decarboxylase); *MTNR1α* (melatonin receptor 1α), and *MTNR1β*. Another gene involved in the melatonin biosynthesis, *ASMT* (Acetylserotonin O-methyltransferase) was not included because we have no access to the data of the x chromosome where this gene is located.

Statistical analyses

The analytical methods of original studies and the cancer-specific results have been described previously [31, 32, 35-38] and summarized in Table 1. Briefly each original study provided log odds ratios and standard errors on each SNP and each cancer risk, mostly adjusting for age, principal

components (PCs), and sex (if applicable). For each cancer site, fixed-effect meta-analyses were conducted to combine summary association statistics of participating studies by the cohort consortium. The genotypes were imputed based on data of European populations from the 1000 Genomes Project (March 2012 reference panel) [39], using either MaCH [40] or IMPUTE [41]. We extracted both the genotyped and imputed SNPs of the genetic regions from 20 kb upstream to 10 kb downstream of each candidate gene.

We conducted gene- and pathway-based meta-analyses using the summary based adaptive rank truncated product (sARTP) method, which combines SNP-level association evidence across SNPs in a gene or a pathway [42]. The sARTP method automatically adjusts for the size of the gene (i.e., number of SNPs in a gene) and the size of the pathway (i.e., number of genes in a pathway) through a resampling procedure. The final gene- and pathway-level p-values were estimated from the resampled null distribution through one million resampling steps. The sARTP method accounts for the linkage disequilibrium (LD) between SNPs to maintain proper type I error. The LDs between SNPs were estimated from the 503 European subjects (CEU, TSI, FIN, GBR, IBS) in the 1000 Genome Project (phase 3, v5, 2013/05/02) [39]. We excluded SNPs with MAF < 5% and applied LD filtering to highly correlated SNP pairs ($r^2 > 0.95$). We also conducted a sensitivity analysis using a more stringent threshold for LD pruning ($r^2 > 0.8$).

For prostate and colorectal cancer that have pathway p-values less than 0.05, we replicated our findings in PLCO and GECCO. We also repeated the gene- and pathway-based analyses on data combing the initial and replication studies.

To eliminate the impact of potential systematic biases in SNP-level association, we adjusted for the genomic control inflation factor (lambda=1.015) for data from the CORECT [37, 42]. The genomic control inflation factors for GECCO, ELLIPSE, PLCO, TRICL and FOCI were

close to or smaller than 1.0, thus were not adjusted in our analyses. To take potential false-positives from multiple-comparisons into account (two pathways, or 15 genes) for each of the four cancer sites, pathways with p-value < 0.00625 (0.05/ (2×4)) and genes with p-value < 0.00083 (0.05/ (15×4)) were considered significant.

For prostate cancer, where we found significant associations with genetic variations of circadian and melatonin pathways after the Bonferroni correction, secondary analyses for aggressive prostate cancer were conducted at the gene and pathway level, using data combining six studies of ELLIPSE and PLCO (4,446 cases and 12,724 controls). For the SNPs with the smallest p-values in the genes with $P_{gene} \leq 0.05$ on the risk of overall prostate cancer, we also checked their SNP associations with aggressive prostate cancer.

RESULTS

We found suggestive associations between genetic variation in both circadian rhythm and melatonin pathways and prostate cancer risk based on data of GAME-ON, with ($P_{pathway}$=0.014 and 0.024, respectively (Table 2). These associations were not statistically significant in PLCO alone ($P_{pathway}$=0.28 and 0.21), but were enhanced in the combined data of GAME-ON and PLCO ($P_{pathway}$=0.0016 and 0.0060) (Table 2), both being significant after Bonferroni correction. *NPAS2* in the circadian rhythm pathway (P_{gene}=0.0062) and *AANAT* (P_{gene}=0.00078) in the melatonin pathway contributed the most to the association with the risk of prostate cancer, with *AANAT* survived Bonferroni correction (Table 3). Other genes with the gene-level p-values at borderline significance were *CLOCK* (P_{gene}=0.021), *CRY2* (P_{gene}=0.043), *DDC* (P_{gene}=0.050), *PER2* (P_{gene}=0.060), and *PER1* (P_{gene}=0.063) (Table 3). A sensitivity analysis with more

stringent threshold in LD pruning ($r^2 > 0.8$) produced consistent pathway-level and gene-level results (data not shown). SNPs with p-value < 0.01 in *NPAS2* and *AANAT* are presented in Table 4.

With a much smaller number of aggressive prostate cancer cases (4,446 cases, 12,724 controls), we did not observe significant association of aggressive prostate cancer with either pathway ($P_{pathway}$=0.29 and 0.66), but we observed a suggestive association with *PER3* (P_{gene}=0.03) (Supplementary Table 2). For SNPs that have the smallest p-values in genes *CLOCK*, *CRY2*, *NPAS2*, *AANAT*, and *DDC* ($P_{gene} \leq 0.05$ with overall prostate cancer), the log odds ratios (β) estimated for overall and aggressive prostate cancer are comparable and have the same direction (Supplementary Table 3).

For colorectal cancer (Table 2), we observed a suggestive association with circadian rhythm pathway in GAME-ON ($P_{pathway}$=0.021), but not in GECCO ($P_{pathway}$=0.76) or in the combined data ($P_{pathway}$=0.17) (Supplementary Table 4). No association was observed for ovarian cancer and lung cancer (Table 2, Supplementary Table 5).

DISCUSSION

We found common genetic variations in the circadian rhythm and melatonin pathways were associated with prostate cancer risk in the population of European descent. These associations were initially identified in the GAME-ON consortium, and further confirmed in the data combining the GAME-ON and PLCO studies. Our findings suggest that the circadian rhythm and melatonin pathways may be involved in prostate carcinogenesis.

Circadian disruption has been suggested as a prostate cancer risk factor based on epidemiological observation of increased prostate cancer risks among shift workers[11-14], and countries with more light exposure at night[43]. In support of this hypothesis, three genetic epidemiology studies found suggestive associations between SNPs in core circadian genes and prostate cancer [19, 23, 27] or aggressive prostate cancer [23] in Caucasian [23, 27] and Asian [19] populations, although these studies had limited power (sample sizes < 2600) to adjust for multiple comparisons. By taking advantage of the large study population from cancer consortia and using a novel analytical tool, our study provided further evidence that the circadian rhythm and melatonin pathways may be involved in prostate carcinogenesis in humans.

Although multiple genes are likely to contribute to pathway association signals, the most significant genes were *NPAS2* and *AANAT*. Previous functional studies suggest that *NPAS2* plays an important role in DNA damage response, cell cycle control and apoptosis by activating diverse downstream genes[44, 45], consistent with a role as a tumor suppressor. In line with our finding, the Thr allele of rs23051560 ($P=7.5\times10^{-4}$), a non-synonymous SNP (Ala394Thr) in the *NPAS2*, has been suggestively associated with lower risks of breast cancer[28], prostate cancer[19], and NHL[46], three tumors that have been linked with circadian disruption in epidemiologic studies.

This SNP has also been suggested to modify the association of night shift work and breast cancer risk, with Thr carriers more vulnerable to shift work effect[24]. AANAT (aka., serotonin N-acetyltransferase) is the rate limiting and originating enzyme for melatonin synthesis, through which the suprachiasmatic nucleus via a sympathetic multisynaptic pathway regulates rhythmic melatonin synthesis [47]. Melatonin acts as a chronobiotic molecule, optimizing phase relationships between oscillators in both central nervous system and peripheral organs, reinforcing circadian rhythms of body functions, and entraining body rhythms to the environmental light phase [48, 49].

A mechanism linking the circadian system, melatonin and prostate cancer may operate through the neuroendocrine gonadal axis. The pineal gland and melatonin have a role in the inhibition of the neuroendocrine gonadal axis[50]; while sex hormones, such as androgen, are essential on prostate development. Androgen has been a prostate cancer inducer in animals [51], and associated with increased prostate cancer risk in humans [52, 53]. Therefore, it is possible that an increase in androgen, subsequent to disrupted circadian rhythm and/or suppressed melatonin [54], may contribute to prostate carcinogenesis. Alternatively, melatonin may have a direct anti-tumor effect, by controlling the p53 pathway, or its antimitotic, antioxidant and immune-modulatory activities[1]. Both in vitro and in vivo studies provide evidence that melatonin inhibits prostate tumor growth[55, 56], whereas melatonin suppression in rats increases tumor growth in a dose-dependent manner[50]. In agreement with the melatonin hypothesis, lower urinary 6-sulfatoxymelatonin has been associated with an increased risk of advanced prostate cancer in a prospective study [57].

Apart from mechanisms related to melatonin, the circadian clock may control cell proliferation and apoptosis through regulating the expression of genes involved in these processes at the transcription or translation level, such as *c-Myc* and *Mdm2*, *Trp53* and *Gadd45*, *cyclins* etc. [2]

We did not find any significant association for the risk of aggressive prostate cancer at the gene or pathway level. Given a much smaller number of aggressive prostate cancer cases, and the fact that genetic effects are generally small on cancer risk, the statistical power of gene- and pathway-based analyses was limited. However, we observed a suggestive association with *PER3* (P_{gene}=0.03); a SNP (rs1012477) of this gene has been associated with prostate cancer aggressiveness in a previous report[27]. For SNPs with the smallest p-values associated with overall prostate cancer within *CLOCK*, *CRY2*, *NPAS2*, *AANAT,* and *DDC*, the estimated effect sizes for the risk of overall and aggressive prostate cancer are comparable and have the same direction. Given the poor prognosis and public health impact of aggressive prostate cancer, more focused study is needed for the role of circadian rhythm genes and prostate cancer aggressiveness.

Our study did not find associations in the circadian rhythm or melatonin pathway genes with colorectal, lung or ovarian cancer. Several important factors need to be considered before concluding that circadian rhythm has no effect on these cancer sites. First, gene functions differ by organs and although we studied the core genes in each pathway, there might be other critical circadian-related genes missed in this study. RORα, for example, suggested as an important regulator for homeostasis in intestinal epithelium[58], as well as newly identified circadian genes [59] are worthwhile to be evaluated in the future. Second, the statistical power of gene- and pathway-

based analyses for studying ovarian cancer may be limited by small sample size compared with other cancer sites considered in this paper. Third, for lung and colorectal cancer, where environmental and life style risk factors play a dominant role, the contribution of disrupted circadian rhythm might be small and/or may be indirectly associated with cancer through modifying the toxicity of environmental carcinogens [60], or altering the DNA damage response[6, 7]. Therefore, incorporating data on environmental carcinogens and measures of toxicity into the study of circadian rhythm and cancer may be important. Fourth, although genetic variation does not suffer from confounding bias by other life style factors, it may have a smaller impact on circadian rhythm disruption than light exposure at night and night shift work. Therefore, future studies of both environmental or life style inducers of circadian disruption coupled with mechanistic or genetic marker studies in circadian rhythm pathways are needed.

In this study, like other candidate pathway-based analyses [61], we assigned SNPs to each of the circadian genes based on genomic location. Approaches that assign SNPs to a gene based on functionality such as a genetic influence on gene expression or expression quantitative risk loci (eQTL) might reveal more signals, but this type of approach relies heavily on the known eQTL function of the SNPs in the tissue of interest and, in fact, the eQTL effects on gene expression are typically tissue-specific [62]. We attempted to evaluate the involvement of the top prostate cancer risk SNPs of *AANAT* and *NPAS2* as functional eQTLs using RNA-seq and SNP data from ten normal brain tissues (GTEx). We observed modest eQTL effects on *AANAT* and *NPAS2* mRNA levels by the top risk SNPs, but no risk eQTL survived correction for multiple comparisons (data not shown). Importantly, published data suggest that the target tissue for melatonin synthesis is the pineal gland, while for circadian rhythm it is the superchiasmatic nucleus (SCN) [1]. RNA-seq data for these normal brain tissues are not available in GTEx or to

our knowledge from any other publically available database. Thus, whether the observed prostate cancer risk SNPs of *AANAT* and *NPAS2* circadian genes are functional eQTLs, and whether the changes in mRNA levels in the pineal gland and SCN are associated with prostate cancer susceptibility remains to be determined.

Our study has many strengths. Using genetic markers to examine circadian hypotheses minimizes the bias due to potential confounders, and therefore is a valuable complement to traditional epidemiologic studies (e.g., in night shift workers). We used an analytical tool that combines signals across SNPs within genes and pathways, and therefore found significant results that would have been detectable by single SNP analysis. To our knowledge, the sample sizes in our study are the largest to date for colorectal, lung, and prostate cancer. The data quality of the included GWAS studies is well established. To control potential false positive findings, we adjusted for multiple comparisons, and replicated our findings in independent data.

In summary, our study suggests that common genetic variation in and around circadian rhythm and melatonin pathways may be involved in human prostate carcinogenesis, in support of circadian disruption as a potential human carcinogen.

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Table 1. Summary of study populations and designs for each cancer site

Consortium Name	Cancer Site	No. study*	Cases	Controls	Genotyping Platform	Reference Panel	Covariants
Initial data of GAME-ON							
CORECT	Colorectal	6	5100	4831	Affymetrix Axiom	1000 Genome†	age, sex, first 4 principal components (PCs) [37]
TRICL	Lung	6	12537	17285	Illumina 317K/550K/610K	1000 Genome†	age, sex, PCs [38]
FOCI	Ovary	3	4369	9123	Illumina 317K/370K/550K/610K/670K/2.5M	1000 Genome†	study, first 5 PCs [36]
ELLIPSE	Prostate	5	10218	11286	Illumina, Affymetrix	1000 Genome†	age, study, PCs [35]
Replication data							
PLCO	Prostate	1	4600	2940	Illumina HumanOmni2.5 Beadchip	1000 Genome†	age, 2 significant PCs [32]
GECCO	Colorectal	21	10738	13328	Illumina 550K/610K/CytoSNP/Omni; Affymetrix for one study	1000 Genome†	age, sex (when applicable), center/region (when applicable), batch (when applicable), smoking status (when applicable), first 3 PCs [31]

*Contributed studies are listed in the supplementary table 1; †1000 Genome March 2012 reference panel

CORECT: ColoRectal Transdisciplinary Study

TRICL: Transdisciplinary Research for Cancer of Lung

FOCI: Follow-up of Ovarian Cancer Genetic Association and Interaction Studies

ELLIPSE: Elucidating Loci in Prostate Cancer Susceptibility

PLCO: Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

GECCO: Genetics and Epidemiology of Colorectal Cancer Consortium

Table 2. Pathway results for each cancer site

		Circadian rhythm pathway		Melatonin pathway	
Cancer	Data	N.SNP	P-value	N.SNP	P-value
Prostate	GAME-ON	520	**0.014**	258	**0.024**
	PLCO	521	0.28	223	0.21
	Combined data	521	**0.0016***	263	**0.0060***
Colorectal	GAME-ON	653	**0.021**	352	0.24
	GECCO	670	0.76	376	0.066
	Combined data	842	0.17	459	0.091
Lung	GAME-ON	510	0.71	243	0.22
Ovary	GAME-ON	521	0.14	263	0.26

*Statistically significant after Bonferroni correction ($p < 0.05/8=0.00625$)

P-value <0.05 in bold

Table 3. Pathway-based and gene-based results between circadian rhythm-melatonin pathway genes and prostate cancer

		GAME-ON (10218 cases, 11286 controls)		PLCO (4600 cases, 2941 controls)		Combined data (14818 cases, 14227 controls)	
Gene	Chr	N.SNP	P-value	N.SNP	P-value	N.SNP	P-value
Circadian rhythm pathway							
ARNTL	11	80	0.41	80	0.40	80	0.29
CK1E	22	48	0.67	48	0.11	48	0.30
CLOCK	4	24	**0.013**	24	0.44	24	**0.021**
CRYI	12	35	0.27	35	0.87	35	0.55
CRY2	11	20	0.53	20	0.073	20	**0.043**
NPAS2	2	167	0.051	167	0.14	167	**0.0062**
PER1	17	29	0.24	30	0.12	30	0.063
PER2	2	50	0.090	50	0.57	50	0.060
PER3	1	67	**0.020**	67	0.94	67	0.24
Pathway-level		520	**0.014**	521	0.28	521	**0.0016***
Melatonin pathway							
AANAT	17	34	0.071	38	**0.043**	38	**0.00078***
DDC	7	84	**0.033**	77	0.63	84	**0.050**
MTNR1A	4	35	**0.041**	18	0.52	35	0.35
MTNR1B	11	23	0.94	7	0.92	23	0.96
TPH1	11	18	0.72	18	0.17	18	0.15
TPH2	12	64	0.081	65	0.12	65	0.21
Pathway-level		258	**0.024**	223	0.21	263	**0.0060***

*Statistically significant after Bonferroni correction ($p < 0.05/8=0.00625$ at pathway level; $p < 0.05/60=0.00083$ at gene level)

P-value<0.05 in bold

Table 4. SNPs in *AANAT* and *NPAS2* with prostate cancer with meta-analyses p-value < 0.01

		Allele			GAME-ON (ELLIPSE)		PLCO		Fixed-effect meta-analyses	
SNP	Loc	Ref	Effect	RAF*	β	P	β	P	β	P
Gene: *AANAT*										
rs150316415	74475409	G	A	0.94	0.34	4.33×10^{-3}	0.25	2.15×10^{-3}	0.28	3.41×10^{-5}
rs3744045	74475024	G	A	0.08	-0.27	5.04×10^{-3}	-0.21	2.85×10^{-3}	-0.23	4.80×10^{-5}
rs61742551	74472998	G	A	0.98	N/A	N/A	0.41	8.12×10^{-4}	0.41	8.12×10^{-4}
rs9894765	74456426	G	C	0.24	-0.07	0.16	-0.10	2.11×10^{-2}	-0.09	7.14×10^{-3}
rs12945905	74456758	C	T	0.80	0.13	1.67×10^{-2}	0.07	0.14	0.09	8.08×10^{-3}
Gene: *NPAS2*										
rs1542178	101595475	G	A	0.67	-0.08	6.50×10^{-4}	-0.09	9.88×10^{-3}	-0.08	2.03×10^{-5}
rs2305160	101591304	G	A	0.67	-0.08	7.70×10^{-4}	-0.09	1.52×10^{-2}	-0.08	3.47×10^{-5}
rs2305159	101591443	C	A	0.32	-0.08	4.84×10^{-4}	-0.04	0.24	-0.07	3.37×10^{-4}
rs1542179	101595235	G	A	0.32	-0.08	5.50×10^{-4}	-0.04	0.28	-0.07	4.55×10^{-4}
rs4851392	101581976	G	A	0.74	-0.07	2.26×10^{-3}	-0.06	8.68×10^{-2}	-0.07	4.71×10^{-4}
rs13019460	101461099	G	C	0.21	-0.06	0.18	-0.13	1.70×10^{-3}	-0.10	1.24×10^{-3}
rs6747874	101578489	G	A	0.74	0.08	2.77×10^{-3}	0.05	0.19	0.07	1.27×10^{-3}
rs6747755	101578458	G	A	0.74	0.08	3.18×10^{-3}	0.05	0.19	0.07	1.46×10^{-3}
rs12622050	101579454	G	A	0.76	0.08	2.47×10^{-3}	0.05	0.27	0.07	1.65×10^{-3}
rs12619710	101579487	C	T	0.26	-0.07	3.56×10^{-3}	-0.05	0.21	-0.07	1.73×10^{-3}
rs2278728	101598312	C	T	0.32	-0.07	2.02×10^{-3}	-0.04	0.33	-0.06	1.80×10^{-3}
rs876060	101576964	T	A	0.24	-0.08	2.47×10^{-3}	-0.04	0.31	-0.07	1.92×10^{-3}
rs13012930	101460947	G	A	0.82	0.04	0.18	0.15	9.93×10^{-4}	0.08	2.56×10^{-3}
rs4851391	101579811	G	C	0.24	-0.07	6.25×10^{-3}	-0.05	0.26	-0.06	3.60×10^{-3}
rs4851377	101522266	C	T	0.46	-0.05	5.54×10^{-2}	-0.07	3.33×10^{-2}	-0.06	4.98×10^{-3}
rs13017728	101481348	G	T	0.09	-0.10	0.1.8	-0.15	1.24×10^{-2}	-0.13	5.42×10^{-3}
rs965519	101470349	G	A	0.18	-0.04	0.22	-0.13	2.53×10^{-3}	-0.07	6.15×10^{-3}

rs2309993	101499264	C	T	0.67	0.07	0.10	0.08	3.24×10^{-2}	0.07	7.25×10^{-3}
rs4851386	101566938	C	T	0.52	-0.05	3.58×10^{-2}	-0.06	9.42×10^{-2}	-0.05	7.48×10^{-3}
rs3739006	101566184	G	A	0.52	-0.04	4.22×10^{-2}	-0.06	8.14×10^{-2}	-0.05	7.91×10^{-3}
rs4851385	101566323	G	C	0.48	0.04	4.22×10^{-2}	0.06	8.14×10^{-2}	0.05	7.91×10^{-3}
rs3739005	101566070	C	T	0.48	0.05	3.46×10^{-2}	0.05	0.13	0.05	9.19×10^{-3}

[*]Reference allele frequency. The frequencies are calculated from 503 European subjects in the 1000 Genomes data.

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Supplementary Table 1. Population and design of each contributed study

Cancer	Study	Locations	Design
Initial analytical data in GAME-ON			
Colon & Rectum	MECC	US	Cohort
(CORECT)	CFR	US	Cohort
	Kentucky	US	Pop. CC
	CPS-II/ACS	US	Cohort
	Melbourne	Australia	Cohort
	Newfoundland	Canada	Pop. CC
Lung	MDACC	US	Hospital CC
(TRICL)	ICR	UK	Hospital CC
	Toronto	Canada	Clinic CC
	IARC	Europe	Hospital CC
	GLC	German	Pop. CC
	NCI	US	Pop. CC and nested CC
Ovary	UKGWAS	UK	CC
(FOCI)	USGWAS	US, Canada, Poland	CC
	U19	US	CC
Prostate	BPC3	US	CC, nested CC
(ELLIPSE)	CRUK1	UK	CC
	CRUK2	UK	CC
	CAPS1	Sweden	CC
	CAPS2	Sweden	CC
Replication data			
Prostate (PLCO)	PLCO	US	Nested CC

Colon & Rectum (GECCO)	ASTERISK	France	Hospital CC
	COLO23	US	Pop. CC
	DACHS1	Germany	Pop. CC
	DACHS2	Germany	Pop. CC
	DALS1	US	Pop. CC
	DALS2	US	Pop. CC
	HPFS1	US	Nested CC
	HPFS2	US	Nested CC
	HPFSad	US	Nested CC
	MEC	US	Nested CC
	NHS1	US	Nested CC
	NHS2	US	Nested CC
	NHSad	US	Nested CC
	OFCCR	Canada	Pop.CC
	PHS1P2	US	Nested CC
	PLCO1	US	Nested CC
	PLCO2	US	Nested CC
	PMH	US	Pop. CC
	VITAL	US	Nested CC
	WHI1	US	Nested CC
	WHI2	US	Nested CC

CC: case-control

Supplementary table 2. Gene- and pathway-based p-values for overall and aggressive prostate cancer

		Combined results (14818 cases, 14227 controls)		Aggressive prostate (up to 4446 cases, 12724 controls)	
Gene	Chr	N.SNPs	P-value	N.SNPs	P-value
Circadian rhythm pathway					
ARNTL	11	80	0.29	80	0.54
CK1E	22	48	0.30	48	0.58
CLOCK	4	24	**0.021**	24	0.093
CRYI	12	35	0.55	35	0.87
CRY2	11	20	**0.043**	20	0.57
NPAS2	2	167	**0.0062**	167	0.18
PER1	17	30	0.063	30	0.70
PER2	2	50	0.060	50	0.23
PER3	1	67	0.24	67	**0.030**
Pathway-level		521	**0.0016***	521	0.29
Melanotin pathway					
AANAT	17	38	**0.00078***	38	0.47
DDC	7	84	**0.050**	84	0.49
MTNR1A	4	35	0.35	35	0.22
MTNR1B	11	23	0.96	23	0.32
TPH1	11	18	0.15	18	0.96
TPH2	12	65	0.21	65	0.35
Pathway-level		263	**0.0060***	263	0.66

*Statistically significant after Bonferroni correction ($p < 0.05/8=0.00625$ at pathway level; $p < 0.05/60=0.00083$ at gene level)

P<0.05 in bold

Supplementary Table 3. Comparison of SNP-based results between overall and aggressive prostate cancer*

		Allele		Overall			Aggressive		
Gene	SNP*	Ref**	Eff**	log(OR)	SE	P-value	log(OR)	SE	P-value
Circadian rhythm pathway									
CLOCK	rs62309758	T	C	-0.09	0.03	1.45E-03	-0.09	0.04	7.57E-03
CRY2	rs7108730	T	C	0.08	0.03	3.66E-03	0.06	0.04	1.05E-01
NPAS2	rs2305160	A	G	0.08	0.02	3.47E-05	0.06	0.03	3.00E-02
Melatonin pathway									
AANAT	rs150316415	G	A	0.28	0.07	3.41E-05	0.16	0.08	6.49E-02
DDC	rs12718611	G	A	-0.11	0.04	1.72E-03	-0.07	0.05	1.12E-01

*SNPs with the smallest p-value in the genes with $P_{gene} \leq 0.05$, based on association with overall prostate cancer.

**reference and effect alleles

Supplementary table 4. Gene- and pathway-based p-values for colorectal cancer in GAME-ON and replication samples

		Game-ON (CORECT) (5100 cases, 4831 controls)		GECCO (10738 cases, 13328 controls)		Combined results (15838 cases, 18159 controls)	
Gene	Chr	N.SNPs	P-value	N.SNPs	P-value	N.SNPs	P-value
Circadian rhythm pathway							
ARNTL	11	114	**0.0044**	113	0.78	140	**0.028**
CK1E	22	38	0.14	55	0.18	68	0.24
CLOCK	4	47	0.18	35	0.34	53	0.11
CRYI	12	56	0.81	47	0.83	73	0.95
CRY2	11	35	0.64	32	0.85	41	0.91
NPAS2	2	202	**0.011**	212	0.82	245	0.51
PER1	17	47	0.60	38	0.44	53	0.55
PER2	2	54	0.63	54	0.40	68	0.59
PER3	1	60	0.68	84	0.15	101	**0.047**
Pathway-level		653	**0.021**	670	0.76	842	0.17
Melatonin pathway							
AANAT	17	53	0.59	52	0.85	61	0.91
DDC	7	119	0.89	115	0.58	147	0.74
MTNR1A	4	60	0.18	61	0.86	72	0.30
MTNR1B	11	33	0.92	34	0.87	45	0.96
TPH1	11	20	**0.029**	22	0.27	27	0.068
TPH2	12	67	0.77	92	**0.0064**	107	**0.013**
Pathway-level		352	0.24	376	0.066	459	0.091

P<0.05 in bold. None of gene based or pathway based p values reached Bonferroni corrected significance

Supplementary table 5. Gene- and pathway-based p-values for lung and ovarian cancers in GAME-ON

		Lung cancer (12537 cases, 17285 controls)		Ovarian cancer (4369 cases, 9123 controls)	
Gene	Chr	N.SNP*	P-value	N.SNP*	P-value
Circadian rhythm pathway					
ARNTL	11	78	0.18	80	0.58
CK1E	22	47	0.35	48	**0.024**
CLOCK	4	24	0.19	24	0.20
CRYI	12	33	0.40	35	0.29
CRY2	11	18	0.52	20	0.13
NPAS2	2	165	0.56	167	**0.046**
PER1	17	29	0.35	30	0.87
PER2	2	50	0.87	50	0.54
PER3	1	66	0.90	67	0.68
Pathway-level		510	0.71	521	0.14
Melatonin pathway					
AANAT	17	30	0.63	38	0.14
DDC	7	82	0.089	84	0.10
MTNR1A	4	35	0.93	35	0.20
MTNR1B	11	21	0.85	23	0.64
TPH1	11	17	0.23	18	0.21
TPH2	12	58	**0.048**	65	0.75
Pathway-level		243	0.22	263	0.26

*SNP numbers after the LD pruning, using $r^2>0.95$

P<0.05 in bold. None of gene- or pathway-level p-values reached the Bonferroni correction threshold of significance.