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Circadian pathway genes in relation to glioma risk and outcome

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Abstract

Purpose—There is growing evidence that circadian disruption may alter risk and aggressiveness of cancer. We evaluated common genetic variants in the circadian gene pathway for associations with glioma risk and patient outcome in a US clinic-based case-control study.

Methods—Subjects were genotyped for 17 candidate single nucleotide polymorphisms (SNPs) in *ARNTL*, *CRY1*, *CRY2*, *CSNK1E*, *KLHL30*, *NPAS2*, *PER1*, *PER3*, *CLOCK* and *MYRIP*.

Unconditional logistic regression was used to estimate age and gender-adjusted odds ratios (OR) and 95% confidence intervals (CI) for glioma risk under three inheritance models (additive, dominant and recessive). Proportional hazards regression was used to estimate hazard ratios (HR) for glioma-related death among 441 patients with high grade tumors. Survival associations were validated using a TCGA (The Cancer Genome Atlas) dataset.

Results—A variant in *PER1* (rs2289591) was significantly associated with overall glioma risk (per variant allele OR: 0.80; 95% CI: 0.66–0.97; $p_{\text{trend}}=0.027$). The variant allele for *CLOCK* rs11133391 under a recessive model increased risk of oligodendroglioma (OR: 2.41; 95% CI: 1.31–4.42; $p=0.005$), though not other glioma subtypes (p for heterogeneity=0.0033). The association remained significant after FDR adjustment ($p=0.008$). Differential associations by gender were observed for *MYRIP* rs6599077 and *CSNK1E* rs1534891 though differences were not significant after adjustment for multiple testing. No consistent mortality associations were identified. Several of the examined genes exhibited differential expression in GBM versus normal brain in TCGA data (*MYRIP*, *ARNTL*, *CRY1*, *KLHL30*, *PER1*, *CLOCK*, *PER3*) and expression of *NPAS2* was significantly associated with a poor patient outcome in TCGA patients.

Conclusion—This exploratory analysis provides some evidence supporting a role for circadian genes in the onset of glioma and possibly the outcome of glioma.

Keywords

Glioma; Single Nucleotide Polymorphism; Genotype

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Introduction

The circadian “clock,” located in the suprachiasmatic nucleus in the hypothalamus, is known to drive numerous biologic processes in the body. Light-dark cycles influence genes along the circadian pathway that are linked to several cancer related biological pathways, including cell proliferation, apoptosis and regulation of cell cycle genes [1]. Environmental disruption or inherited mutations of genes in the circadian pathways may negatively affect cellular function, leading to an increased susceptibility to cancer. Population based studies have identified associations with variants in circadian pathways genes and risk of breast cancer [2–4], prostate cancer [5, 6] and non-Hodgkin’s lymphoma [7, 8]. Variants in these genes have also been associated with survival in hepatocellular carcinoma [9], breast [10], prostate [11] and colon [12] cancer.

Circumstantial evidence is consistent with a potential role for circadian genes in glioma. Melatonin, which regulates circadian rhythms, has been demonstrated to significantly reduce damage-induced apoptosis in astrocytoma cells [13]. In expression-based studies, *PER1* and *PER2* were underexpressed in glioma cells when compared with adjacent non-glioma cells, although no difference was noted between high and low grade gliomas [14]. A similar pattern was found for *CRY1* and *CRY2* [15].

In this exploratory analysis, we examined whether common genetic variants in the circadian pathway linked to different cancer phenotypes in previous investigations are related to glioma risk or patient outcome in a series of 622 newly diagnosed glioma cases and 628 healthy controls.

Methods

Study Population

The study population has been described previously [16]. Briefly, cases were Caucasian individuals aged 18 and above with a recent diagnosis (within 3 months) of primary, non-recurrent glioma and were identified in neurosurgery and neuro-oncology clinics at several major medical and oncology centers in the Southeastern US. Controls were comprised of non-blood related associates of the cases or were sampled from the communities giving rise to the cases using white page listings. Controls reporting a personal history of brain tumor were excluded from analysis. The study was approved by Investigational Review Committees at each participating center, and all subjects enrolled in the study provided written informed consent.

Biospecimen Collection and Genotyping

Genomic DNA samples for genotyping were obtained by oral rinse or the saliva method (www.dnagenotek.com). A total of 19 candidate SNPs in circadian-related genes were included in the present analysis. Genotyping was performed at the Center for Genome Technology at the Hussman Institute for Human Genomics, University of Miami using Illumina’s GoldenGate technology (Illumina, San Diego, CA) or Taqman. A total of 655 cases and 658 controls were submitted for genotyping. Laboratory personnel were blinded as to the case-control status of the samples.

A total of 33 cases (5.0%) and 30 controls (4.6%) were excluded due to low call rates leaving 622 cases and 628 controls in the final analysis. Among 17 successfully genotyped SNPs (see Supplementary Table 1), concordance of genotyping calls in 94 blinded duplicate pairs was observed in 85% for 2 SNPs (rs2289591, rs7698022), 88% in 2 SNPs (rs1401417, rs2305160), 91% in 2 SNPs (rs11133391, rs2585405), 95% in 1 SNP (rs1369481), 96% in 1 SNP (rs1005473), 97% in 1 SNP (rs6599077), and 100% in the remaining 8 SNPs

(rs1012477, rs12315175, rs1534891, rs17024926, rs2292912, rs7602358, rs7950226, rs7950226), for an average overall concordance of 95%. Genotyping success rates in individual subjects ranged from 88.2% to 99.8% (mean 96.7%). Well-established risk loci for glioma were confirmed in this case-control series [16, 17].

The Cancer Genome Atlas Data

In order to further investigate survival associations with SNPs in the circadian pathway, we obtained approval to access individual-level data from The Cancer Genome Atlas (TCGA) (<http://tcga-data.nci.nih.gov>) (study accession phs000178.v5.p5) which includes a genome-wide scan of approximately 505 GBM patients. Data for 8 of the successfully genotyped SNPs were available in TCGA, and 5 other SNPs had suitable proxies ($r^2 > 0.8$), whereas no counterpart could be identified for the four remaining SNPs genotyped (*PER1* rs2585405, *PER1* rs2289591, *CSNK1E* rs1005473, *CLOCK* rs11133391). In addition to the information of genotypes, gene expression data were downloaded for each of the 9 circadian-related genes. Gene expression values were defined as the log2 ratio of gene expression in tumor tissue from individual GBM patients to mean expression in a pool of control samples. Expression values were obtained using the Agilent Whole human Genome Microarray platform. Clinical data including vital status and survival time are also available for GBM patients in TCGA and were downloaded for the present analysis.

Statistical analysis

The association between each SNP and glioma risk was estimated with odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression. Three inheritance models were tested (additive, dominant and recessive) and regression models included terms for age (5 year categories) and gender. To test for linear trend, each SNP was modeled as an ordinal term coded 0, 1, 2 corresponding to the number of variant alleles. Multinomial logistic regression was used to examine associations between genotypes and histologic subtypes of glioma. A test for heterogeneity was performed in each multinomial model to evaluate whether odds ratios for each SNP were significantly different across histology subtypes.

Survival associations were examined among 441 patients with high grade tumors (GBM, anaplastic astrocytoma and high grade oligodendrogliomas). Cox proportional hazards regression was used to estimate hazard ratios (HR) and 95% CIs for the association between SNPs and mortality among patients with high grade tumors adjusting for age, gender, and histologic subtype of glioma (GBM; high grade astrocytoma (non-GBM); high grade oligodendroglioma).

Statistical analysis was performed using SAS Version 9.3 (SAS Institute, Inc., Cary, NC) and statistical significance was defined as a two-sided *P*-value < 0.05 . False discovery rate (FDR) was used to control for multiple testing according to the method of Storey et al [18, 19].

Functional impact of the examined noncoding variants were evaluated using the recently released *RegulomeDB* [20], a database that annotates SNPs with known and predicted regulatory elements in the intergenic regions of the *H. sapiens* genome, with source data including public datasets from GEO, the ENCODE project, and published literature.

Results

The analysis was based on a total of 622 cases and 628 controls. The median age was 52 (range: 18–88 years) among cases and 56 (range: 18–87 years) among controls. A

predominance of cases (63%) and controls (57%) were male. A total of 341 (55%) of cases had a diagnosis of glioblastoma multiforme (GBM), 146 (23%) had grade I, II, or III astrocytic lesions, 94 (15%) had oligodendroglial tumors (grades II–III) and 41 (6.6%) had another tumor subtype (ganglioglioma, subependymoma, etc.) or a glioma with no subtype specified in the pathology report.

Risk associations for the examined SNPs are shown in Table 1. Carriers of the minor ('A') allele in *PER1* rs2289591 had a reduced risk of glioma under an additive (per allele OR: 0.80; 95% CI: 0.66–0.97; $p_{\text{trend}}=0.027$) and recessive (OR: 0.57; 95% CI: 0.35–0.93; $p=0.024$) model of inheritance. In addition, the minor ('A') allele in *MYRIP* rs6599077 was significantly associated with a reduced risk under both an additive (per allele OR: 0.79; 95% CI: 0.65–0.95; $p_{\text{trend}}=0.012$) and dominant (OR: 0.73; 95% CI: 0.57–0.93; $p=0.010$) model. None of these associations remained significant after adjustment for FDR. No other examined variant was associated with overall glioma risk.

We examined SNP associations according to glioma histology (Supplemental Table 2). Significant heterogeneity according to histology was demonstrated for *CLOCK* rs11133391 (test for heterogeneity under a recessive model $p=0.0033$). Persons carrying 2 variant alleles were at excess risk of oligodendroglioma (OR: 2.41; 95% CI: 1.31–4.42; $p=0.005$) though not GBM (OR: 0.75; 95% CI: 0.48–1.17; $p=0.206$) or lower grade astrocytic tumors (OR: 1.31; 95% CI: 0.75–2.31; $p=0.346$). The association for oligodendroglioma remained significant after FDR adjustment ($P=0.008$). Associations limited to specific glioma subtypes were observed for *CRY2* rs1401417, *CLOCK* rs7698022, *PER1* rs2289591, *MYRIP* rs6599077, and *NPAS2* rs1369481; however, tests for interaction were nonsignificant.

In models stratified by gender, a reduced risk of glioma was observed among variant 'A' allele carriers for *MYRIP* rs6599077 in males only; the test for interaction was significant under a recessive model (P for interaction = 0.043). *CSNK1E* rs1534891 also demonstrated heterogeneity by gender: the variant 'T' allele was associated with a nonsignificant excess risk in females (OR: 7.1; $P=0.077$) and a reduced risk in males (OR: 0.38; $P=0.110$) (P for interaction under a recessive model = 0.026).

Two of the examined variants, *CLOCK* rs7698022 and *PER1* rs2289591, were significantly associated with mortality among the patients with high grade tumors. Homozygous carriers of the *CLOCK* rs7698022 variant 'G' allele had reduced survival (HR: 1.85; 95% CI: 1.23–2.79; $p=0.003$). However, in TCGA, a proxy for this SNP (rs880358; $R^2 = 1.00$; located in an intron of *TMEM165* was not associated with GBM outcome (not shown). In the present data, the variant allele in *PER1* rs2289591 was also marginally associated with poorer patient outcome (per allele HR: 1.22; 95% CI: 1.00, 1.47; $P=0.044$); no proxy for this SNP was available in TCGA.

A number of the circadian genes were overexpressed (*MYRIP*, *ARNTL*, *CRY1*, *KLHL30*, *PER1*, and *CLOCK*) or underexpressed (*PER3*) in glioma tissue when compared to expression levels in normal brain. Increased expression of *NPAS2* was significantly associated with a poor patient outcome (Figure 1) (HR: 1.84; 95% CI: 1.32–2.56; $P=0.0003$); mortality was marginally elevated for tumors classified as having intermediate expression (HR: 1.22; 95% CI: 0.92–1.62; $P=0.1741$). Two of the examined SNPs (or their proxies) were significantly associated with gene expression in TCGA (Table 2); one of these SNPs, a proxy for the sleep duration SNP *MYRIP* rs6599077 [21] (*MYRIP* rs4234131; $r^2 = 0.80$) which was associated with a reduced glioma risk in the present data. An increasing number of variant alleles in *MYRIP* rs4234131 was associated with increased expression of *MYRIP* relative to normal brain (p for trend = 0.0026) in a dose dependent manner.

In order to explore possible mechanisms by which variants implicated in this analysis might influence glioma risk we sought to evaluate their functional impact. SNPs in non-coding regions may act through modulation of activity in transcriptional regulatory regions [22], and investigation of such mechanisms has been accelerated by the recent ENCODE data release [23, 24]. To interrogate the co-localization of SNPs in our study and biofeatures predictive of transcriptional activity we used the on-line tool *RegulomeDB* [20] assembled in part based on the ENCODE project. Among the implicated SNPs, only *CSNK1E* rs1534891 and *MYRIP* rs6599077 variants demonstrated any evidence of binding activity (eg. scores less than 6).

Discussion

To our knowledge, this is the first study to consider whether genetic variation in the circadian pathway is associated with risk or outcome of glioma. In this exploratory analysis, a variant in *PER1* was significantly associated with overall glioma risk, and associations in *MYRIP* and *CSNK1E* emerged in gender-stratified analyses. A variant in *CLOCK* was associated with tumors arising along nerve axons (oligodendroglioma) though not with other glioma subtypes. The latter association remained significant after adjustment for FDR. Two of the examined SNPs (*CLOCK* rs7698022 and *PER1* rs2289591) were associated with patient mortality, though the association in *CLOCK* rs7698022 for which a suitable proxy SNP was available, could not be validated in TGA data. Expression of one examined gene in the circadian pathway (*NPAS2*) was associated with glioma-related mortality in the TCGA dataset. Taken together, these findings provide some limited support for the hypothesis that circadian disruption plays a role in gliomagenesis and may alter patient outcomes.

The present findings suggesting risk associations in glioma with variants in circadian genes is in line with studies showing association of variants in these genes with other cancers. Variants in the circadian genes *ARNTL*, *CLOCK*, *CRY2*, *CSNK1E*, *NPAS2*, *PER1* and *PER3* have been linked to risk of breast cancer [2–4], prostate cancer [5, 6], and non-Hodgkins lymphoma [7, 8]. In the present data, an intronic SNP in *PER1* (rs2289591) associated with overall glioma risk has also been linked with prostate cancer [6]. Variation in *PER1* has also been associated with sex hormone-binding globulin concentrations in serum [25], and with neurocognitive disorders including attention deficit hyperactivity [26] and autism [27].

A variant in circadian locomotor output cycles kaput (*CLOCK*) (rs11133391), located in the intronic region of the gene, was associated with the development of oligodendrogliomas, though not with other glioma subtypes. *CLOCK* is a key component of the mammalian circadian oscillator that transcriptionally regulates many critical circadian genes [28]. A haplotype encompassing the *CLOCK* rs11133391 SNP was associated with ER-negative breast cancer in one study [4]. In addition, a polymorphism in the 3'-untranslated region of *CLOCK* (3111T/C; rs1801260) was linked with sleep patterns [29], body weight [30, 31], and psychiatric disorders in several studies [32]. The *CLOCK* 3111C/T polymorphism is in modest linkage disequilibrium ($r^2 = 0.28$) with the *CLOCK* rs11133391 variant associated with oligodendroglioma risk in the present study.

In the present data, a variant in *CSNK1E* (rs1534891) exhibited differential associations by gender. *CSNK1E* rs1534891 has been linked to prostate cancer [6], as well as neurological diseases including heroin addiction [33] and susceptibility to bipolar disorder [34].

The SNP examined in *MYRIP* (rs6599077) was first identified in a GWAS of sleep duration [21]. The variant allele of this intronic SNP was associated with reduced glioma risk in the present data in males only (nonsignificant after adjustment for FDR). Of interest, a proxy for this SNP (rs4234131) was significantly associated with *MYRIP* expression in TCGA.

Variants in *MYRIP* have been implicated in genome-wide association studies of age-related macular degeneration [35] and cognitive function [36], however, these variants were not linked with the *MYRIP* rs6599077 variant examined in the current study.

The present results also suggest a potential impact of circadian genes on glioma survival. Genetic variation in the circadian system has been associated with tumor aggressiveness or patient survival in hepatocellular carcinoma [9], as well as prostate [11], colon [12] and breast [10] cancer. Similar to the association of *NPAS2* with GBM survival observed in TCGA, Yi et al reported a significant association between *NPAS2* expression and disease-free survival in breast cancer [10]. None of the 4 examined SNPs in this gene were associated with altered GBM outcome in the present study. Of 2 significant survival associations noted in our data, *CLOCK* rs7698022 was unrelated to outcome in TCGA and no suitable proxy was available for *PER1* rs2289591. Of interest, the *PER1* rs2289591 variant was also associated with glioma risk in the present data.

The large amount of data deposited by the ENCODE project and now in the public domain has been instrumental to the identification of regulatory regions, and was utilized here to gain insights on identified associations. The data available (with a limited number of experiments from cells derived from brain) is suggestive of functionality for 2 of the implicated SNPs in the present study (*CSNK1E* rs1534891 and *MYRIP* rs6599077).

In summary, the present study provides some limited evidence supporting a role of circadian pathway genes in gliomagenesis and the outcome of affected patients. Strengths of our study include a relatively large sample considering the rarity of glioma, rapid case ascertainment, and pathological confirmation of cases. However, many associations were examined and suggestive findings in the study may be attributable to chance. Results should also be interpreted with caution as reliability of genotyping was lower than optimal for several of the examined SNPs. On the basis of the present results, further research is warranted to determine the role of circadian genes in the development and outcome of brain tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Expression of NPAS2 and GBM survival

Table 1

Associations between variants in circadian pathway genes and glioma risk.

Gene	SNP ID	Minor Allele	Additive Model			Dominant Model			Recessive Model		
			OR (95% CI)	P		OR (95% CI)	P		OR (95% CI)	P	
PER3	rs1012477	G	0.97 (0.77, 1.22)	0.790		0.99 (0.76, 1.28)	0.935		0.79 (0.38, 1.66)	0.530	
PER1	rs2585405	C	1.16 (0.86, 1.57)	0.336		1.24 (0.90, 1.70)	0.191		0.22 (0.03, 1.90)	0.169	
PER1	rs2289591	A	0.80 (0.66, 0.97)	0.027		0.82 (0.64, 1.04)	0.104		0.57 (0.35, 0.93)	0.024	
ARNTL	rs7950226	A	1.05 (0.89, 1.23)	0.574		1.07 (0.83, 1.38)	0.578		1.05 (0.81, 1.37)	0.721	
CSNK1E	rs1534891	T	1.01 (0.79, 1.30)	0.926		1.03 (0.78, 1.36)	0.813		0.82 (0.33, 2.02)	0.668	
CSNK1E	rs1005473	C	0.91 (0.63, 1.32)	0.632		0.95 (0.64, 1.40)	0.789		0.30 (0.03, 2.78)	0.291	
MYRIP	rs6599077	A	0.79 (0.65, 0.95)	0.012		0.73 (0.57, 0.93)	0.010		0.77 (0.50, 1.19)	0.237	
CRY2	rs2292912	G	0.87 (0.71, 1.07)	0.194		0.86 (0.68, 1.09)	0.225		0.78 (0.42, 1.47)	0.448	
CRY2	rs1401417	G	0.94 (0.77, 1.14)	0.510		0.91 (0.71, 1.16)	0.443		0.98 (0.59, 1.63)	0.931	
CLOCK	rs11133391	C	1.08 (0.91, 1.29)	0.375		1.15 (0.90, 1.46)	0.264		1.03 (0.72, 1.47)	0.870	
CLOCK	rs7698022	G	0.86 (0.71, 1.03)	0.106		0.84 (0.66, 1.06)	0.145		0.78 (0.51, 1.21)	0.269	
NPAS2	rs17024926	C	0.87 (0.73, 1.03)	0.114		0.83 (0.66, 1.04)	0.107		0.86 (0.60, 1.24)	0.428	
NPAS2	rs1369481	A	1.13 (0.93, 1.38)	0.215		1.16 (0.92, 1.47)	0.199		1.12 (0.67, 1.88)	0.669	
NPAS2	rs895521	A	0.97 (0.80, 1.17)	0.728		0.98 (0.78, 1.24)	0.878		0.85 (0.51, 1.44)	0.555	
NPAS2	rs2305160	A	1.10 (0.92, 1.32)	0.284		1.16 (0.91, 1.48)	0.241		1.09 (0.76, 1.57)	0.649	
CRY1	rs12315175	C	1.02 (0.83, 1.24)	0.883		1.04 (0.82, 1.31)	0.763		0.90 (0.48, 1.66)	0.732	
KLHL30	rs7602358	G	0.97 (0.81, 1.17)	0.764		0.92 (0.74, 1.16)	0.496		1.15 (0.73, 1.82)	0.541	

* Models are adjusted for age and gender.

Table 2

Association between genotypes and gene expression in the Cancer Genome Atlas (N=417).

Gene	SNP	Genotype	Mean differential gene expression *	p for trend
KLHL30	rs7602358	AA	0.1224	0.0002
		AB	0.1625	
		BB (variant)	0.2714	
MYRIP	rs4234131 (proxy for rs6599077)	AA	2.0734	0.0026
		AB	2.2933	
		BB (variant)	2.6720	

* Gene expression is the log₂ transformed ratio of expression in GBM tumor tissue over the mean expression in a pool of normal brain tissue samples.