Circadian rhythm gene expression in cluster headache

IHC-DP-005

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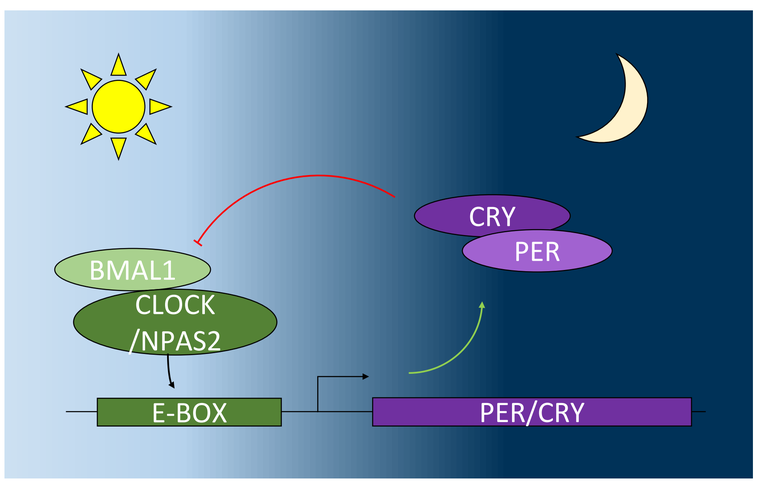


NPAS2 and PER2
- Differentially expressed in cluster headache

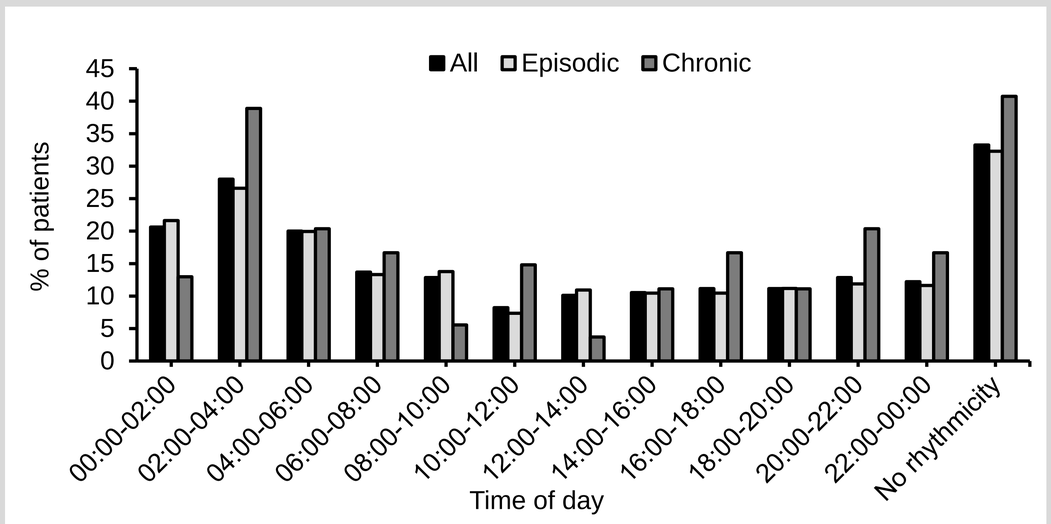
Both NPAS2 and PER2 are **core clock genes**, essential to the maintenance of circadian rhythm in mammals. These results strongly support the hypothesis of a **circadian component in the pathophysiology of cluster headache**.

Aim

Characterize circadian rhythm in cluster headache (CH) by studying gene expression of key players of the molecular clock in primary fibroblasts from patients and controls.

Introduction

Cluster headache is a trigeminal autonomic cephalalgia with a striking circadian pattern. Several genes linked to circadian rhythm have previously been investigated in relation to CH; HCRTR2, CLOCK, PER3, CRY1 and CRY2.



Attack distribution per 24 hours in episodic (n = 421) and chronic (n = 54) CH patients. DOI: (10.1177/0333102417731773)

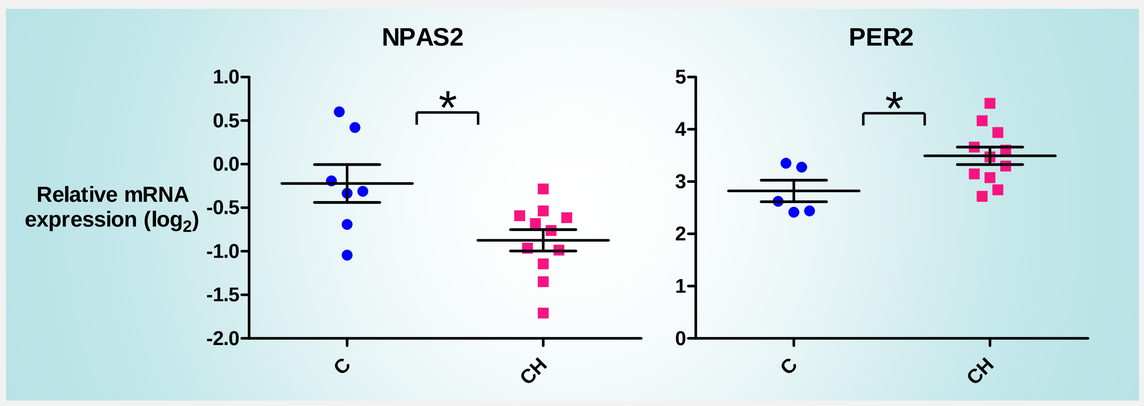
Material & Methods

cDNA was synthesized from RNA extracted from fibroblast cell pellets from 11 CH patients and 9 controls using the QIAGEN RNeasy Mini Kit. Cells were exposed to a serum shock 6 hours prior to harvest in order to reset their circadian clock. We used BioRad pre-designed 96-well panels; Neurophysiological process-Circadian rhythm H96. qPCR was run with iTaq SYBR green mastermix on a 7500 Fast ABI cycler, and analyzed with the BioRad Prime PCR Analysis software. Verification of qPCR was performed with our own designed primers in a regular RT-qPCR run with iTaq SYBR green mastermix on a CFX 348-well system. Analysis was made using the BioRad CFX- Manager software, and GraphPad Prism v.5.04. mRNA levels were normalized to two reference genes; TBP (TATA-binding protein) and IPO8 (Importin 8), and to a reference sample consisting of pooled cDNA from all control individuals. The normalized relative expression levels were analyzed for outliers (Grubb's test) then $\log_2$ transformed and compared using an unpaired t-test, two tailed p-values.

Results

We screened the expression of 40 genes involved in circadian rhythm in 11 CH patients and 9 controls. 5 genes showed differential expression in CH patients compared to controls; BMAL1, LDHA, NPAS2, NR1D1 and RAF1. 8 genes were excluded due to elevated C_T -values and 25 genes didn't express differently in the two groups.

Target	C n	Mean	Mean	+/- SEM	CH n	Mean	Mean	+/- SEM	unpaired t-test p-value	
BMAL1	9	-2.273	-2.273	0.1116	11	-2.827	-2.827	0.137	0.007	**
BHLHE40	9	-2.102	-2.102	0.219	11	-2.353	-2.353	0.24	0.458	ns
BHLHE41	9	-7.316	-7.316	0.203	11	-7.215	-7.215	0.201	0.731	ns
CALM1	9	-1.897	-1.897	0.217	11	-2.390	-2.390	0.202	0.115	ns
CALM2	9	3.406	3.406	0.198	11	3.081	3.081	0.17	0.227	ns
CALM3	9	0.143	0.000	0.14	11	0.146	0.000	0.132	0.986	ns
CAMK2A	9	-8.347	-8.347	0.106	11	-8.592	-8.592	0.31	0.5	ns
CAMK2B	9	-8.517	-8.517	0.322	11	-8.586	-8.586	0.298	0.877	ns
CAMK2D	8	-2.688	-2.688	0.075	10	-2.694	-2.694	0.062	0.948	ns
CAMK2G	9	-3.193	-3.193	0.155	11	-3.146	-3.146	0.135	0.82	ns
CLOCK	9	-5.054	-5.054	0.206	11	-5.284	-5.284	0.249	0.499	ns
CREB1	9	-1.963	-1.963	0.206	11	-2.217	-2.217	0.161	0.336	ns
CRY1	9	-1.812	-1.812	0.125	11	-1.982	-1.982	0.169	0.444	ns
CRY2	9	-4.107	-4.107	0.164	11	-3.963	-3.963	0.196	0.589	ns
CSNK1D	9	-2.478	-2.478	0.159	11	-2.810	-2.810	0.186	0.202	ns
CSNK1E	9	-0.735	-0.001	0.162	11	-0.788	-0.001	0.164	0.822	ns
GUCY1B3	9	-7.333	-7.333	0.265	11	-7.236	-7.236	0.253	0.794	ns
LDHA	9	3.522	3.522	0.117	11	3.109	3.109	0.087	0.01	**
MAP2K1	9	-1.240	-1.240	0.228	11	-1.756	-1.756	0.159	0.072	trend
MAPK1	9	-1.704	-1.704	0.163	11	-1.962	-1.962	-1.962	0.321	ns
MAPK3	9	-1.715	-1.715	0.118	11	-1.787	-1.787	0.122	0.681	ns
NPAS2	9	-1.950	-1.950	0.201	11	-2.825	-2.825	0.236	0.013	*
NR1D1	9	-8.205	-8.205	0.264	11	-7.444	-7.444	0.209	0.034	*
PER1	9	-6.779	-6.779	0.455	11	-6.392	-6.392	0.379	0.517	ns
PER2	9	-6.117	-6.117	0.301	11	-5.701	-5.701	0.218	0.266	ns
PER3	9	-6.335	-6.335	0.18	11	-6.110	-6.110	0.172	0.381	ns
RAF1	8	-1.694	-1.694	0.081	11	-2.088	-2.088	0.099	0.01	**
RASD1	9	-5.928	-5.928	0.518	11	-5.428	-5.428	0.46	0.595	ns
RORA	9	-4.318	-4.318	0.278	11	-4.809	-4.809	0.402	0.349	ns
RPS6KA1	9	-8.843	-8.843	0.249	11	-8.829	-8.829	0.145	0.959	ns
RPS6KA2	9	-3.260	-3.260	0.156	11	-3.711	-3.711	0.214	0.121	ns
RPS6KA3	9	-1.309	-1.309	0.19	11	-1.546	-1.546	0.204	0.413	ns



Verification of the 7 most interesting genes using a more stringent experimental design revealed that NPAS2 (Neuronal PAS domain protein 2) was downregulated (p=0.01) and PER2 (Period 2) was upregulated (p=0.05) in CH patients vs. controls (C).

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