Circadian gene expression patterns on the periphery depend on genotype

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THE CIRCADIAN CLOCK AND MOUSE STRAINS

- CIRCADIAN CLOCKs
  - Evolutionary adaptation
  - Affect physiological processes
  - Hierarchical structure

- MOUSE STRAINS
  - Biomedical models
  - Many different strains
  - Genetic variability

Are there differences in circadian gene expression between strains.
CIRCADIAN GENE EXPRESSION IN MOUSE STRAINS 129SvPas AND C57BL/6

- Sampling of mice every 4h.
- Liver and adrenal glands.
- Measure expression of genes using qPCR.

CIRCADIAN EXPERIMENT

129SvPas
Liver
Adrenal gland
qPCR
Analysis and comparison of gene expression patterns

C57BL/6J
Liver
Adrenal gland
qPCR
MAJOR CORE CLOCK AND METABOLIC OUTPUT GENE EXPRESSION DIFFERENCES IN ADRENALS OF 129SvPas AND C57BL/6

- Liver differs in *Bmal1* and *Cry1*.

Genes of interest

**Core clock**
- *Bmal1*, *Per1*, *Per2*, *Per3*, *Cry1*, *Cry2*

**Transcription factors**
- *Dbp*, *Dec1*, *Dec2*, *Car*, *Pgc-1a*, *Ppar’s*, *RevERBa*

**Metabolic genes**
- *Cyp7*, *Cyp11*, *Cyp17*, *Cyp21*, *Cyp39*, *Cyp51*, *Por*, *Hmgcr*

Circadian expression profiles

![Graphs showing circadian expression profiles for Bmal1 and Dbp in adrenal glands under LD conditions.]
MAJOR CORE CLOCK AND METABOLIC OUTPUT GENE EXPRESSION DIFFERENCES IN ADRENALS OF 129SvPas AND C57BL/6

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**Metabolic genes**

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Circadian expression profiles
DIFFERENCES IN PEAK EXPRESSION (PHASE) ARE MOST PROMINENT FOR METABOLIC GENES

- Adrenal glands, LD

![Graphs showing gene expression patterns for Bmal1 and Dbp](image-url)
DIFFERENCES IN PEAK EXPRESSION (PHASE) ARE MOST PROMINENT FOR METABOLIC GENES

- Adrenal glands, LD

![Graphs showing differences in peak expression](image-url)
Could differences in peak expression be explained by genomic variation of 129 and C57BL/6?

To answer this:
- Data on structural variation of the three available 129 strains and comparison to the reference strain C57BL/6
- Database resources: Mouse Genomes Db (SANGER) and dbVar (NCBI)
- Deeper analysis on the subset of genes of interest
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- Core clock genes
- Steroid metabolism and regulation
- Light perception
- HPA axis (hormone synthesis, receptors)
- Light transduction to the SCN
- Light response in the SCN
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**Core clock genes**
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- Light response in the SCN

**Steroid metabolism and regulation**

**HPA axis (hormone synthesis, receptors)**

**201 genes**

- 129S1
- 129S5
- 129P2
- C57BL/6J
C57BL6 and 129 strains differ in over 20,000 SNVs and over 150 structural variants.

**Structural variation**

Genomic location of SV in 129 strains

- **129 strain**
  - P2OlaHsd
  - S1SvlmlJ
  - S5SvEvBrd

Number of SV

- Introns: 150
- Promoters: 50
- Splice sites: 10

% of GOI with SV = 24.71%

**SNV**

Number and location of SNP

- Coding: 1,000
- Five UTR: 500
- Introns: 1,500
- Promoters: 200
- Three UTR: 100

% of GOI with SNP = 76.12%
Several SNV variants reside in clock–dependent promoters of metabolic output and circadian genes

ChipSeq data:
Koike et al., Science 19 October 2012
DNA VARIATIONS IN GENES THAT SHOWED DIFFERENTIAL CIRCADIAN EXPRESSION IN C57BL/6 AND 129 MICE

<table>
<thead>
<tr>
<th>Gene</th>
<th>Promoter mutations</th>
<th>Structural variants</th>
<th>SNVs</th>
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- Adrenal clock
- Transcriptional factors
- Cyp51
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- **Adrenal clock**
  - **Transcriptional factors**
  - **Cyp51**
- **retina**
  - **Opn4**
- **SCN**
  - **Camk2b, Egr1, Grin2c, Ltpr3, Ssfa2**
- **Pituitary**
  - **Nr3c1 - GR**
- **ADRENAL GLAND**
  - **Tspo, Gnb4, Pbx1, Nr5a1 (SF-1), Chrb4, Nr1d1, Nr1d2**

Gene with discovered nonsynonymous mutations.
CONCLUSION

- Circadian expression of many core clock and metabolic output genes differs between 129 and C57Bl/6 mouse strains. Most variations were observed in adrenal glands under LD, where several CYPs exhibit crucial differences in peak expression (phase).

- Core clock and metabolic genes that vary in gene expression 129 to C57Bl/6 gene expression harbor numerous DNA variants in promoter, intron and coding regions.

- The genomes of the three 129 strains investigated are genetically very similar. Many SNVs and structural variants lie at the same location in the genome.

- These findings are relevant for future chronopharmacology studies since the genotype could crucially affect the circadian expression of drug metabolizing genes.
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Thank you for your attention!
Kosir et al, IUBMB Life 2013