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Chronic insomnia: generation of iPSCs-derived neuronal disease model and characterization of peripheral circadian rhythms of patients

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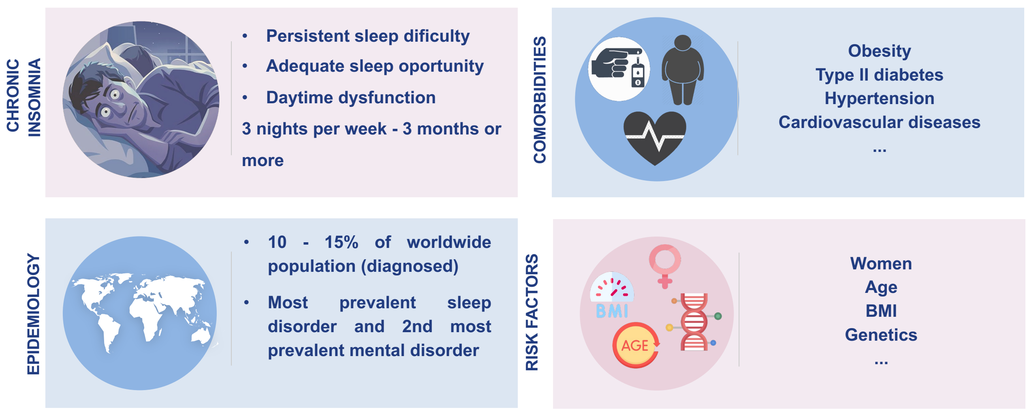
INTRODUCTION

- *Sleep is regulated through the sleep-wake homeostasis processes and by the circadian biological clock. The suprachiasmatic nucleus (SCN), located in the hypothalamus, contains the "master clock" regulator of sleep.*
- *Perturbations in circadian rhythms are associated with sleep disorders pathophysiology.*

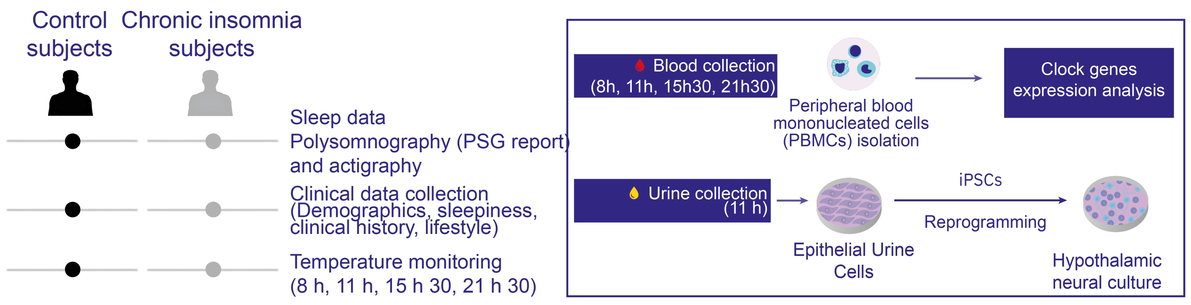


Thorpy ***International Classification of Sleep Disorders*** (2015); Van Someren et al. *Acta Neuropathologica* (2022)

AIM

- Evaluate clock genes expression between chronic insomnia patients relative to similar healthy controls and peripheal body temperature.
- Generate an in vitro human neuronal model to explore sleep regulation and evaluate biological clocks during sleep deprivation.

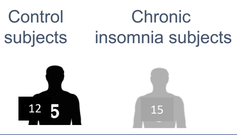
METHODS



RESULTS

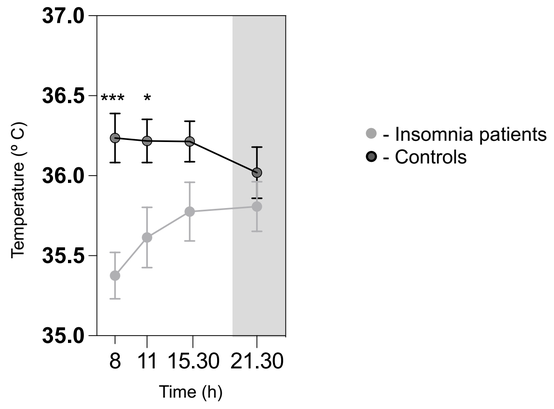
Peripheral circadian rhythms of Chronic Insomnia

COHORT CHARATERIZATION



	Control subjects	Chronic insomnia subjects
Age (years) (mean±SD)	38.08±8.59	44.62±12.56
Sex (F/M)	9 (F) /3 (M)	13 (F) /2 (M)
BMI (mean±SD)	22.7±2.9	22.8±3.2
Insomnia Severity Index (ISI) (mean±SD)	6.1 ± 5.7	17.3±3.9
Total Sleep Time (h) (mean±SD)	6.9±1.2	5.6±1.7

AXIAL BODY TEMPERATURE



CLOCK GENES EXPRESSION IN PBMCS

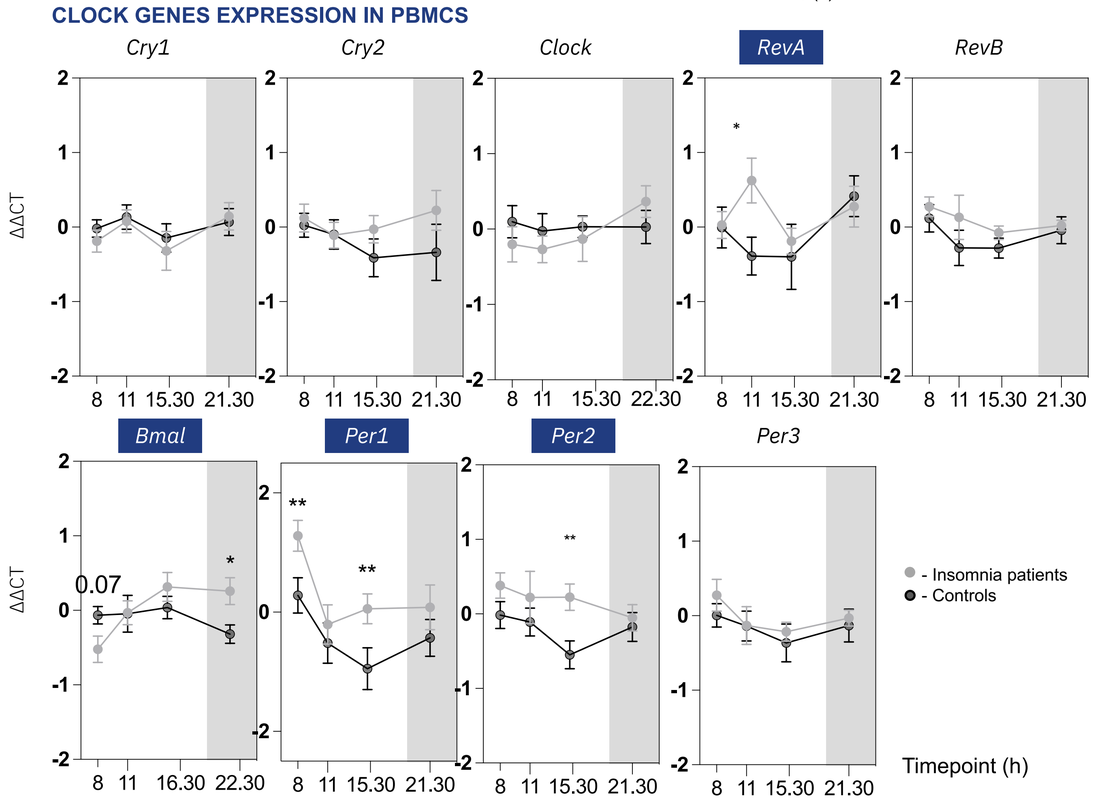
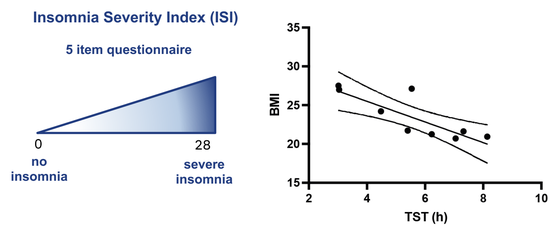


FIG 1 - (a) Mean temperature of each participant data is presented using the mean temperature values ± standard error of the mean (SEM). (b) Clock genes expression of each participant data using the mean ΔΔCT values ± standard error of the mean (SEM). Significant alterations were evaluated using Mann Whitney test between controls and insomnia patients at each timepoint. Significant alterations were evaluated using Mann whitney test between controls and insomnia patients. (c) Spearman correlations were evaluated between each two variables. * p < 0.05; ** p < 0.01; ***p < 0.001.

CLOCK GENES EXPRESSION AND SLEEP PARAMETERS



correlation	r	p value
*Per1*_8h00 and Sleep Efficiency (%))	-0,447	*
*Per2*_15h30 and Sleep Efficiency (%)	-0.492	*
*Per1*_8h and ISI	0.529	***
*Per1*_15h30 and ISI	0.529	****
*Per2*_15h30 and ISI	0.529	*
*Clock*_21h30 and sleep latency	-0.468	*
*Cry1*_21h30 and body temperature	-0.420	*
*Cry2*_21h30 and body temperature	-0.425	*
RevB and body temperature	-0.530	***

Generation of hypothalamic-like neurons

HUMAN DERIVED EPITHELIAL CELL CULTURE

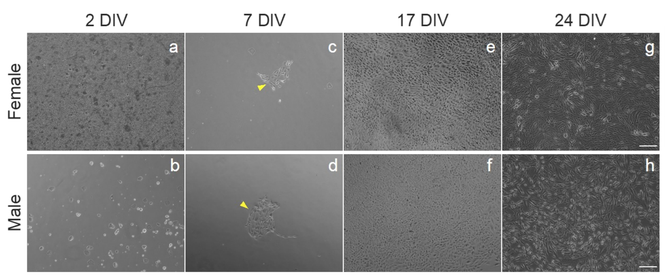


FIG 2 - Renal epithelial cells morphology at different timepoints in culture after collection and isolation. (a) Urine samples from a healthy donor after 2 days in vitro (DIV) (b) urinary cell colonies after 7 DIV (c) urinary renal epithelial cells culture after 17 DIV (d) urinary renal epithelial cells culture after 24 DIV. Images were obtained with a 10x objective. Scale Bar 200 µm, EVOS XL Invitrogen.

HUMAN IPSCS COLONIES

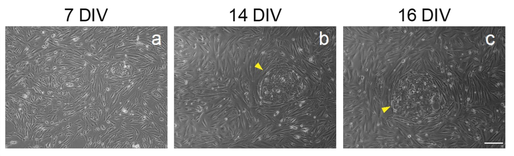


FIG 3 - Expansion of human iPSCs colonies obtained with a 4x and 10x objective, respectively. Colonies of undifferentiated hPSCs should have cells that are uniform in morphology. Images were obtained with a 4x and 10x objective. Scale bar, 200 µm,

HYPOTHALAMIC CULTURES DIFFERENTIATION

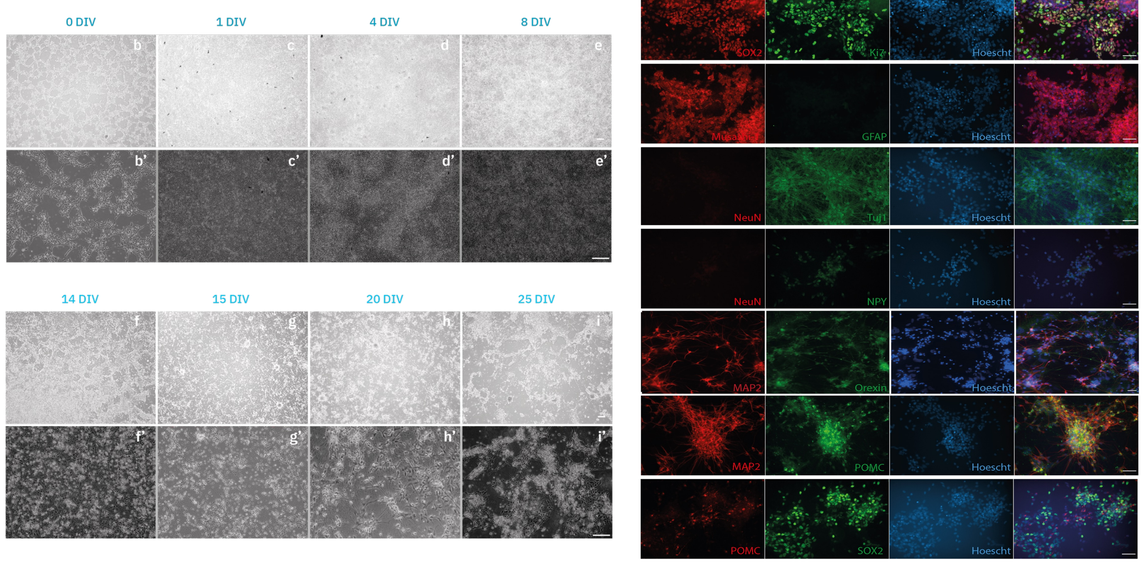
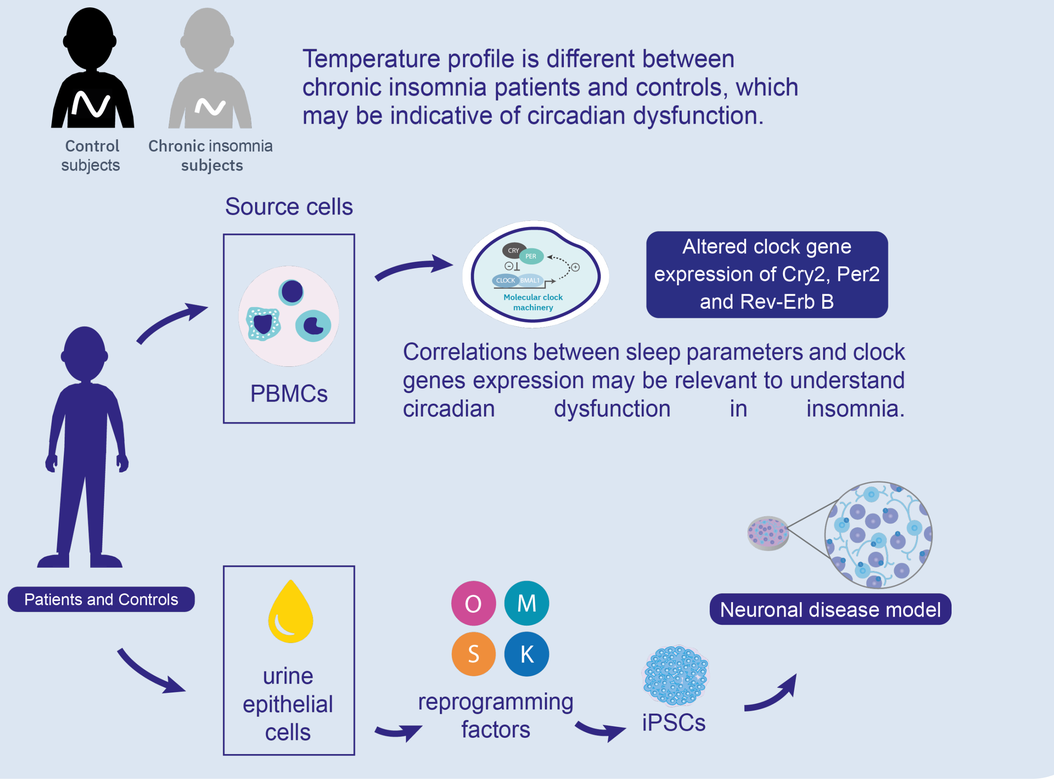


FIG 4- (a) Schematic of hypothalamic differentiation and maturation protocol. Differentiation of hPSCs to hypothalamic neurons (b) Dissociated hPSCs (c) Cultures should reach confluence and adopt a uniform appearance. (f) On Day 14, cultures have become overconfluent with progenitors and newborn neurons, and are passaged. (g) On Day 15, cells are changed to maturation media. (h) most cells should have a neuronal morphology. Images were obtained with a 10x objective (b-i). Scale bar, 200 µm, EVOS XL Invitrogen. (j) Characterization of iPSCs differentiation to hypothalamic neurons by immunocytochemistry. Images were obtained with a 10x objective. Scale bar, 200 µm, Cell Discover Zeiss.

CONCLUSION



ACKNOWLEDGMENTS

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