31

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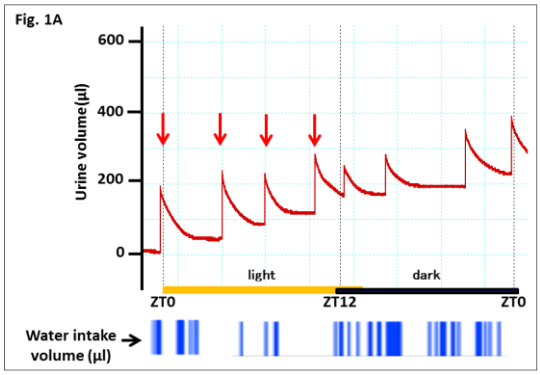
CLOCK MUTANT MOUSE IS A NOVEL EXPERIMENTAL MODEL FOR NOCTURIA/NOCTURNAL POLYURIA

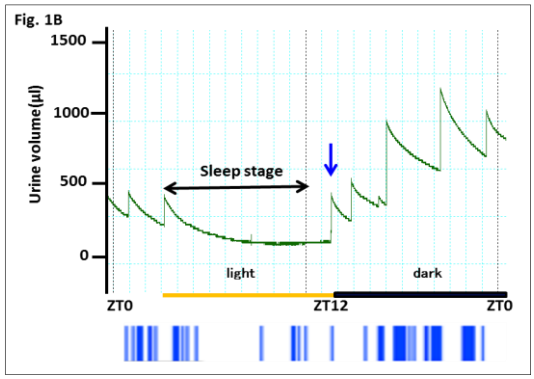
Hypothesis / aims of study

The pathophysiology of nocturnal polyuria (NP) is multifactorial, complex, and its cause remains unclear in a great number of patients. Besides, there is no ideal animal model to examine and to evaluate the clinical efficacy of therapeutic modalities for NP. Clock genes exist in almost all of the cells and organs, whose products regulate the oscillations of sleep-awake rhythm, expression rhythm of various metabolic enzymes, channels, and receptors. Among these clock genes, *Clock* is one of the most important genes to regulate circadian rhythm. It has been reported that total urine volume and urine volume/void change between day and night, and clock genes contribute to these diurnal variation [1][2]. In the present study, we measured 24hours' urination behaviour of *Clock* mutant mice using metabolic cages and compared with wild-type mice.

Study design, materials and methods

Male C57BL/6 mice aged 8-12 weeks (WT) and male C57BL/6 *Clock* mutant mice aged 8 weeks ($Clock^{\Delta 19/\Delta 19}$) were bred under 12 h light and 12 h dark conditions for 2 week (n=10 for WT, n=8 for $Clock^{\Delta 19/\Delta 19}$). The light period started from 6 a.m. and that time was set as Zeitgerber time (ZT) 0. $Clock^{\Delta 19/\Delta 19}$ mice have an A to T mutation in the 5' splice site of intron19, as a consequence, an in-frame deletion of entire exson19, results in loss of normal transcriptional activity. For the analysis of urination behaviour, we used urination metabolic cages [3]. Mice were individually placed in the cages and after an acclimation period of 2 days the following parameters were measured for 2 days: water-intake-volume (WIV), voiding frequency (VF), urine volume (UV) and urine volume/void (UV/V). Urination during the light period was counted as a nocturia (red arrows in Fig. 1A). When urination was not observed for more than 8 hours during the light period, it was defined as the sleeping stage (Fig. 1B). The first urination in the dark period after the sleeping stage was counted as urination in the light period (blue arrow in Fig. 1B). Data were analysed using t-test, Mann-Whitney U-test, Welch's test and a one-way ANOVA (*P<0.05, **P<0.01; n.s., not significant), if applicable, and all values were expressed as means ± S.E.M.





Results

The body weight (BW) and WIV for 24 hours showed no difference between WT group and $Clock^{\Delta 19/\Delta 19}$ group (BW: 23.68 ± 0.26 vs. 23.95 ± 0.33 g, n.s.; and total WIV: 3497 ± 134 vs. 3537 ± 417 µl, n.s.). Either WT or $Clock^{\Delta 19/\Delta 19}$ group showed time-dependent change of WIV every 4 hours (P<0.01 by one way ANOVA for each). There were no differences in WIV on each time point between 2 groups when compared by two-way measures ANOVA with Tukey's test (Fig. 2). $Clock^{\Delta 19/\Delta 19}$ group voided more frequently than WT group in the light period, whereas in the dark period, there was no difference in VF between the 2 groups (Fig. 3A). In WT group, UV/V was higher in the light period than that in the dark period. In contrast, there was no difference in UV/V between dark and light periods in $Clock^{\Delta 19/\Delta 19}$. In the both periods, UV/V in WT was higher than that in $Clock^{\Delta 19/\Delta 19}$ (Fig. 3B). Both groups showed same diurnal pattern in total UV for 24 hours. This suggests that UV in the dark period was higher than that in the light period. In the total UV during the light period, $Clock^{\Delta 19/\Delta 19}$ group showed a larger volume than WT group. There were no differences between WT and $Clock^{\Delta 19/\Delta 19}$ in the total UV for 24 hours and UV during the dark period (Fig. 3C) (total UV for 24 hours: 2084.83 ± 129.85 for WT and 2319.16 ± 94.79 for $Clock^{\Delta 19/\Delta 19}$, n.s.). Nocturnal Polyuria Index (NPI) was significantly higher in $Clock^{\Delta 19/\Delta 19}$ than in WT group (Table. A).

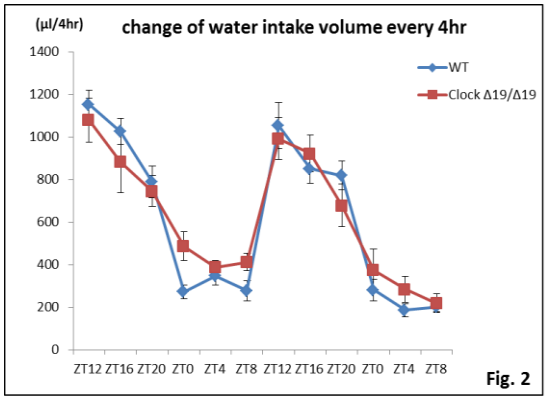
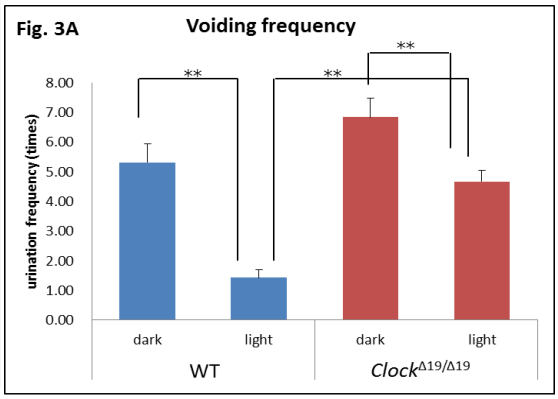
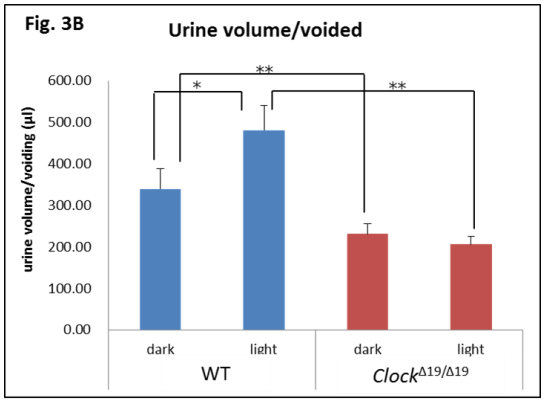


Fig. 2





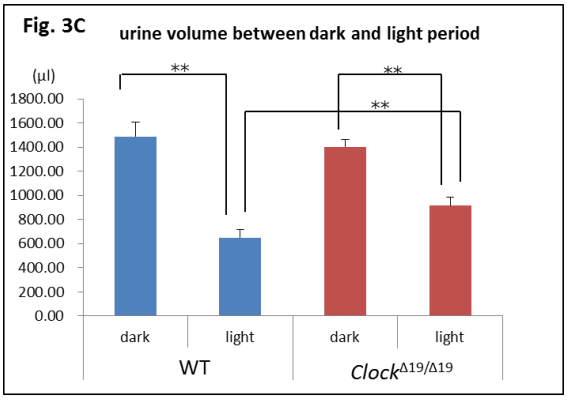


Table. A	NPI (%)
WT	30.71 ± 2.57
Clock$^{\Delta 19/\Delta 19}$	39.09 ± 2.25**

Interpretation of results

Although *Clock* has a very important role in producing and regulating circadian rhythm, water drinking behaviour rhythm in both 2 groups were almost similar because WIV showed same circadian rhythm in both 2 groups. According to these data, we hypothesized that WT mice but not *Clock* mutant mice show circadian rhythm in UV/V. NPI was also higher in the *Clock*$^{\Delta 19/\Delta 19}$ group. These data suggest that *Clock*$^{\Delta 19/\Delta 19}$ might be a key player in nocturnal polyuria without displaying abnormal behaviours such as polydipsia, polyuria and abnormal sleep-awake rhythm.

Concluding message

Clock mutant mouse has a potential to be a model animal of NP. To explore cellular mechanisms underlying the disappearance of diurnal urination rhythm in *Clock* mutant mouse, further investigations about the circadian change of bladder function and the regulation system of clock genes are needed.

References

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Disclosures

Funding: This work was supported by grants from Astellas Pharma Inc. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** mouse **Ethics Committee:** Yamanashi Institutional Animal Care andUse Commitee