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Altered Exression of Circadian Clock genes in Polyglandular Autoimmune Syndrome type III

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◆ Introduction

CLOCK system is a highly conserved, ubiquitous molecular ‘‘clock’’ which creates internal circadian rhythmicity under the influence of light/dark information. CLOCK system is regulated by the coordinated activation/inactivation of several transcription factors, including the *CLOCK,* the *BMAL1* and other essential regulators, such as the Pers, Crys and RORs. The present study aimed to evaluate the circadian rhythm of clock-related genes expressed in patients with polyglandular autoimmune syndrome type III (PASIII).

◆ Methods

Nineteen patients diagnosed with PASIII (5 males) and 12 healthy controls (4 males) were enrolled. The characteristics of the participants are shown in Table 1. All patients had normal response to Synacthen test. By performing real-time PCR , we analysed mRNA expression of CLOCK-related genes (*CLOCK, BMAL1, ROR, Per3* and *GILZ*) and glucocorticoid receptor (*GR*) gene in peripheral blood mononuclear cells (PBMCs) isolated by Lymphoprep density gradient centrifugation from blood samples drawn at 8 am and 8 pm. GR protein expression was analysed by Western Blot.

At the same time, serum cortisol and plasma ACTH were measured by chemiluminescence.

◆ Results

✧ No statistical differences were found in cortisol, ACTH and TSH plasma levels between patients and controls.

Characteristics	Patients				Controls				p*
Total (N)	19				12				**ns**
Age(median,yrs)	55				52				ns
Sex(m/f)	5/14				4/8				ns
	am	**pm**	**ΔCT pm/am**	**p(pm vs.am)**	**am**	**pm**	**ΔCT pm/am**	**p(pm vs.am)**	
Mean F(μg/dl)	19.4±4.4	5.2±3.4		**0.001**	16.5±3.5	5±2.9		**0.01**	ns
Mean ACTH(pg/ml)	14.7±5.3	10±7.5		**0.002**	17±9	11±9		0.1	ns
Mean TSH(U/ml)	2.7±1.4	-			1.8±1.2	-			ns
Genes (median ΔCT)									
-GR	-0.35	-0.6	1.04	0.1	-0.04	0.83	0.80	0.67	**0.05**
-CLOCK	5.6	4.0	0.74	**0.03**	3.91	4.40	0.96	0.52	**0.018**
-BMAL1	4.9	3.6	0.52	**0.04**	4.08	4.38	1.06	0.14	**0.033**
-ROR	0.6	0.35	0.27	0.8	0.16	1.26	0.478	0.12	0.1
-PER3	6.07	7.22	0.98	0.9	6.63	7.27	1.1	**0.03**	**0.05**
-GILZ	0.6	0.7	0.4	0.86	-1.8	-1	0.4	0.09	0.37

Table 1. Characteristics of the patients and controls. Differences in the biochemical markers and the mRNA expression (ΔCT) of the 6 CLOCK related genes between mesurement in the morning and the evening as well as differences between patients with PASS III and controls.

* patients vs. controls

✧ An overexpression of the evening *CLOCK* and *BMAL1* genes - compared to the morning- was observed in patients (Fig.1,2) .

Figure 1

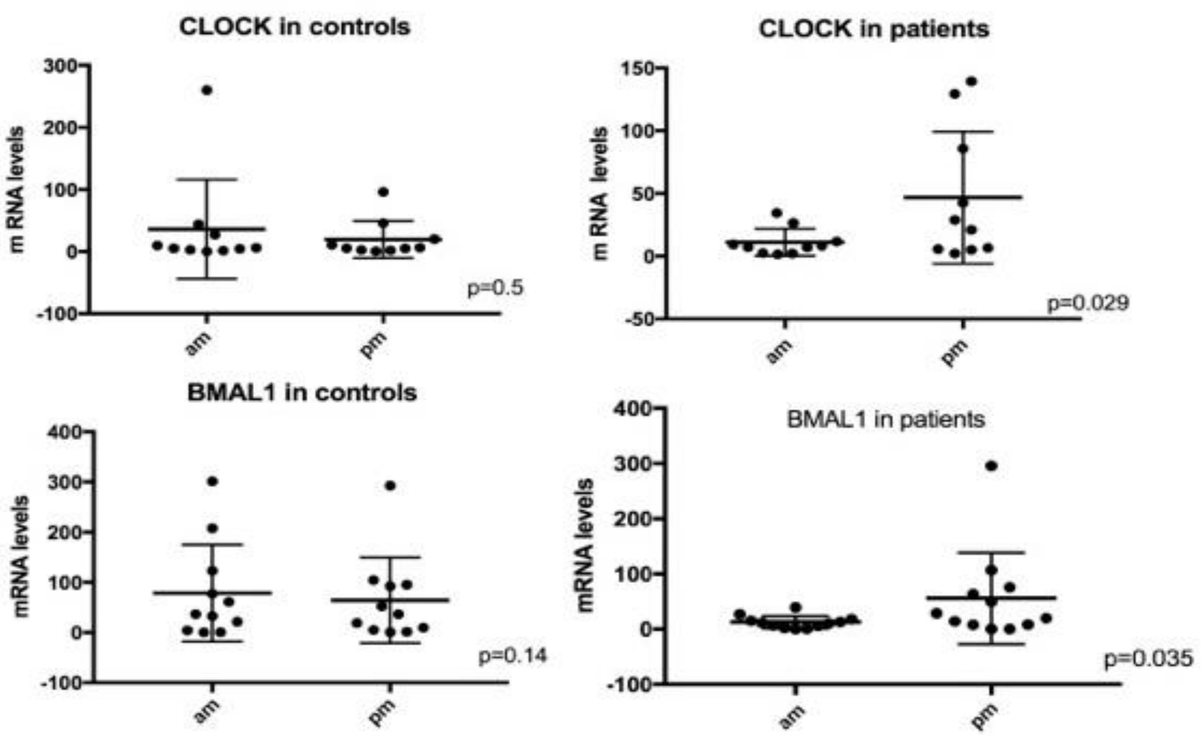


Figure 2

✧ Controls exhibited a significant overexpression of the *PER3* gene in the morning compared to the evening.

✧ Patients exhibited a significantly lower mRNA ratio ($R_{pm/am}$) of *GR, CLOCK*, *BMAL1*, and *PER3* compared to controls (Fig. 3,4, 5, 6).

Figure 3

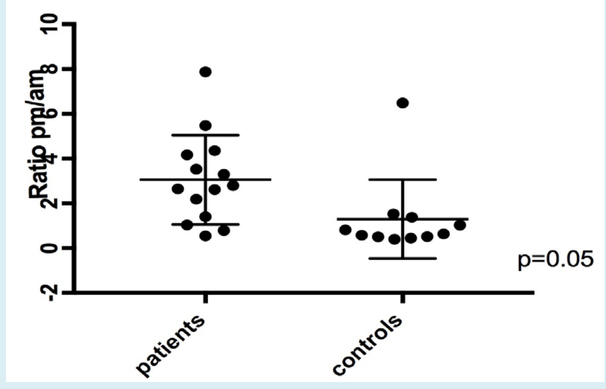


Figure 4

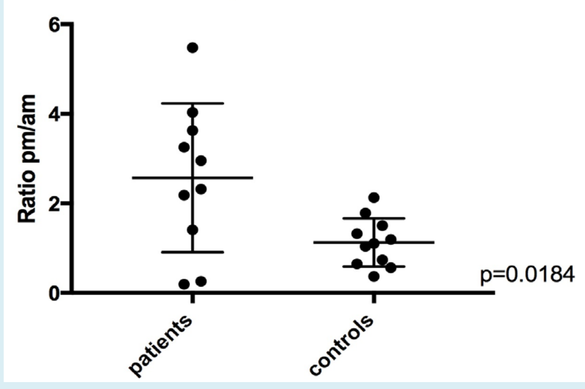


Figure 5

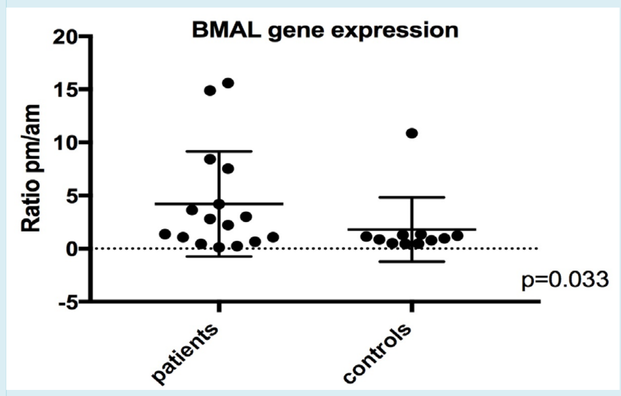
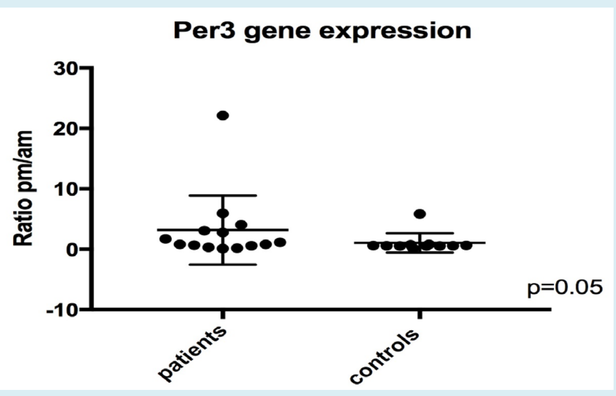


Figure 6

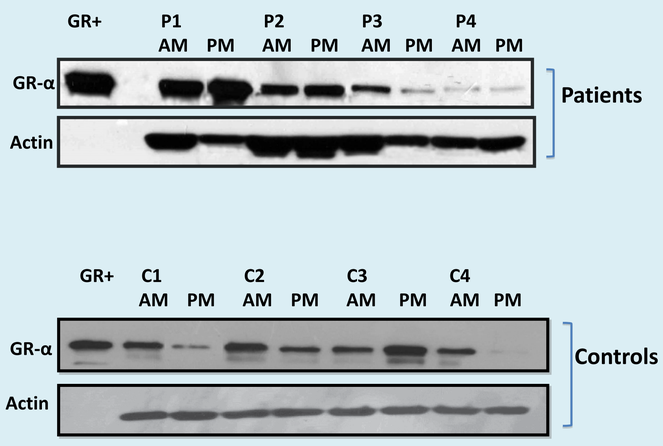


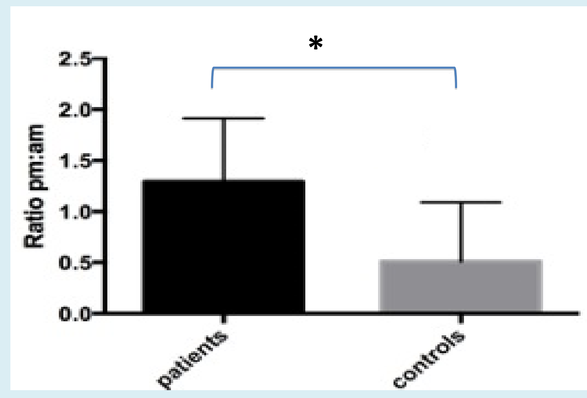
✧ Cortisol circadian variation ($\Delta F_{pm/am}$) demonstrated a significant positive correlation with the mRNA ratio ($R_{pm/am}$) of *GILZ*. Table 2.

	Δ CT pm/am GR	Δ CT pm/am CLOCK	Δ CT pm/am BMAL1	Δ CT pm/am Per3	Δ CT pm/am ROR	Δ CT pm/am GILZ
Patients						
F pm/am	r=0.12(-0.4-0.6) p=0.6	r=0.34(-0.22-0.7) p=0.2	r=0.29(-0.2-0.6) p=0.2	r=0.21(-0.29-0.6) p=0.4	r=0.46, p=0.06 p=0.06	r=0.7(0.34-0.9) p=0.002
Controls						
F pm/am	r=-0.7(-0.9-0.19) p=0.53	r=0.18(-0.45-0.69) p=0.5	0.2(-0.4-0.75) p=0.43	r=0.41(0.22-0.8) p=0.18	r=0.03(-0.57-0.6) p=0.95	r=-0.4(-0.79-0.23) p=0.2

Table 2. Correlations among mRNA levels (ratio of ΔCTpm/am) of *GR, CLOCK, BMAL1, PER3, ROR, GILZ* with the ratio pm/am of cortisol (F).

◆ Western Blot analysis





Western blot analysis revealed a significant greater slope of the GR-α protein level in the evening in control group compared to patient group.

◆ Conclusions

These findings suggest that there is an abberant expression of clock-related genes in patients with PASIII compared to healthy controls. Daily pattern expression of the 6 circadian clock genes was disrupted in patients with PAS III indicating a possible association with the pathogenesis of the disease.