Altered Expression 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.

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

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

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).

Cortisol circadian variation (ΔF_{pm/am}) demonstrated a significant positive correlation with the mRNA ratio (R_{pm/am}) of GILZ. Table 2.

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 patients.

Conclusions
These findings suggest that there is an aberrant 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.