

The role of circadian clock in the Polycystic Ovary Syndrome

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Abstract. The Polycystic Ovary Syndrome (PCOS) is a worldwide disease related to infertility. This intrinsic syndrome affects 5-15% of females under 18-44 age globally. There is still no exact explanation to the cause of PCOS on molecular and genetic level. PCOS has been proved to have a strong genetic predisposition. One of the symptoms of PCOS is the sleep disturbances related to circadian disorder. A numerous research has already been done on relationship between the genetic pathway and other factors in PCOS. Androgen can act as a modulator exert to effect on the receptors in SCN to change circadian rhythms. CYP17A1, one of the most important gene in the aetiology of PCOS, has also been shown as a direct CLOCK-BMAL1 target in peripheral blood mononuclear cells (PBMCs). The circadian rhythm and clock genes have already been taken in account to be related with PCOS. The circadian disorder often synergize with the loss of metabolic disorder, the steroidogenesis, to intensify the compromised fertility. This review introduces the physiological links between circadian clocks and Polycystic Ovary Syndrome. It highlights the present current researches on the molecular level associations between the circadian disorders and PCOS, which introduce a potential cause of it.

Keywords: Circadian rhythm, PCOS, BMAL1, SCN, peripheral clock.

1. Introduction

The Polycystic Ovary Syndrome (PCOS) is a worldwide disease related to infertility. This intrinsic syndrome affects 5-15% of females under 18-44 age globally. Patients with PCOS suffer the infertility while face an assortment of complications. The infertility is often caused by the numerous cysts in the sac of ovary, which eliminates the releasing of Primary oocyte and brings deregulation of the menstrual cycle. The level of androgen also excessively elevates in the plasma. Hyperandrogenism has already been one of the clinical diagnosis standards for PCOS. The abnormal functioning of hormones can cause hirsutism and acne and leads to long lasting anovulation and infertility. PCOS can also increase the risk of serious complications among females. Type 2 diabetes, obesity, hypertension and obstructive sleep apnoea are the typical types of complications. While these complications can ultimately cause the cardiovascular disorders and gynecological problems, such as oligomenorrhea, which can further exacerbate into endometrial cancer [1]. There have been numerous researches on the genetic pathway and variation in PCOS. Genes like Cytochrome family p450, insulin gene, FSHR, CAPN10 have already been proved to have effects in the pathology of PCOS. For environmental factors, stress, obesity, irregularity of life routine, shift work, chronic jet lag is also the cause worldwide.

Human's biological system has an intrinsic clock that exhibits oscillation in 24h which is adapted to the 24h solar day. The system can be divided as central and peripheral clocks. The suprachiasmatic nuclei (SCN), located in anterior hypothalamus, is the control of the endogenous circadian rhythm. While the non-SCN brain structures and the other tissue throughout body combine as the peripheral clock. The circadian rhythm exerts its function by molecular oscillations consisted of regulatory feedback circuits. The circadian genes serve roles in activating transcription as the positive loop, which happens to the repressor genes or to the other clock-controlled genes. Examples include CLOCK, BMAL1, and others. While the transcription is being inhibited by repressor circadian genes like CRY1 and CRY2 and PER1, PER2 and PER3 as the negative loop.

The feedback loop is the reason of the rhythmical transcription of some genes. The circadian clock can be entrained by the environmental light-dark cycles and align clock-controlled metabolism with external factors.

Circadian misalignment can be driven by the mistiming of environmental factors, including excessive light exposure, irregular food intake, sleep disturbance. The derailed clock genes may cause assorted of metabolic, neurodegenerative diseases. Some observational studies have proved polymorphisms in CRY2 is associated with high level fasting glucose. In 2014, Hassan S. Dashti et al. tested the effects of carbohydrate diet and CRY1 polymorphism to insulin resistance using linear regression interaction models. There is a significant correlation between those factors for insulin resistance [2]. Body temperature is an important parameter in the circadian oscillation. In a study on the circadian dysfunction in Parkinson's disease, the lower nocturnal core body temperature and a reduction in the amplitude of temperature is observed in the patients [3].

One of the symptoms of PCOS is the sleep disturbances related to circadian disorder [4]. Numerous research has already been done on relationship between the genetic pathway and other factors in PCOS. Androgen can act as a modulator exert to effect on the receptors in SCN to change circadian rhythms. CYP17A1, one of the most important gene in the aetiology of PCOS, has also been shown as a target of CLOCK-BMAL1 in human peripheral blood mononuclear cells (PBMCs) [5].

The paper introduces the physiological links between circadian clocks and Polycystic Ovary Syndrome. It highlights the present current researches on the molecular level associations between the circadian disorders and PCOS, which introduce a potential cause of pathogenesis of PCOS.

2. Sleep disorder and PCOS risk

2.1. Work in night increased risk of PCOS

There are some controversial results on the relationship between PCOS and work in night. From the research conducted by Wang et al. using the statistical analyses, they find there is the women who have night shift work, even the work is not permanent, are under higher risk to suffer PCOS. What they find is a prominent correlation between PCOS and night shift work [6]. While in a cross-sectional study, it shows that there is no typical association between the PCOS and night shift work. The insufficiency of sleep can increase the risk of abnormal menstrual disturbance and insulin resistance [7].

2.2. Sleep difficulties and PCOS

The patients with PCOS are more potent to experience the sleep difficulties. Women with PCOS are said to be more prone to experience trouble falling asleep, restless sleep, and extreme fatigue frequently or occasionally, according to the multivariate regression model [8]. There are also another research shows that the possibility of sleep disorder was twice in women with PCOS compared to the same age women without PCOS [4]. It also shows that the difficulties in maintaining sleep is mediated by obesity and depression, which are normal complications of PCOS.

3. Changes of circadian rhythm in PCOS

3.1. Oscillation controlled by SCN in female reproductive system

Suprachiasmatic nucleus (SCN) is a central circadian pacemaker determining the rhythm and translating phobic cues and passing temporal cues. Under the 24-hour light-dark cycle, the SCN collects information from retina to GnRH neurons. Then, it will stimulate the pituitary gland to secrete gonadotropin, which establishes the circadian oscillation in ovulation-inducing surge of LH. SCN will also directly regulate the clock in ovary via autonomic nervous system. The cyclic production of melatonin is also under control of SCN. The ovarian follicles have melatonin receptors, which indirectly regulate peripheral oscillators in turn [9]. Also, this substance safeguards the oocyte away from oxidative stress and delays aging of ovary [10]. Additionally, studies suggest that the malfunctioning feedback regulation of the rhythmic GnRH neuron, which results in the release of

anterior pituitary gonadotropins, is what causes follicular stoppage and excessive androgen secretion [11].

Androgen, acting on SCN, can regulate the circadian rhythm. Compared with surrounding hypothalamus, SCN packed astrocytes more densely. The density of astrocytes can directly and indirectly strengthen neuronal connectivity and are sensitive to androgens. By knockout neuron-specific androgen receptor (AR) in mice, the effects on reproductive system induced by excess androgens can be prevented. Besides, after androgen treatment, the expression of androgen receptor in ovariectomized female rats elevates to the level like the expression in the male SCN [12]. The kisspeptin neurons of third ventricle (RP3VKISS1) is in the upstream of GnRH neurons, which is under regulation of arginine vasopressin-expressing SCN neurons and express androgen receptor [13]. In primates, estrogen relays to GnRH neurons driving to preovulatory surge. But in research done by Jamieson et al. shows that the high androgen cannot regulate SCN and RP3VKISS1 [14].

3.2. The change in hypothalamo-pituitary-ovarian (HPO) axis

The hypothalamus controls the pulsatile release of GnRH, which is released into the blood and acts directly on the pituitary regulating the secretion of LH and FSH. With the increase in the ratio of LH/FSH, the follicular growth and production of estrogen in ovary is stimulated, which also act as a feedback loop to further facilitate the function of HPO axis. The confusion of HPO axis is regarded as core mechanism for PCOS.

Epilepsy is a persistent condition marked by aberrant brain neuronal discharge, particularly in the limbic system. It was found a relationship between the epilepsy and PCOS. Specifically, patient with epilepsy is likely to develop this disorder. The epileptiform discharges will affect hormone levels in the HPO axis because of their strong anatomical ties, including some hypothalamic regions that control the frequency of GnRH release [15]. The enhanced pulse of GnRH increases the concentration of LH. In addition, it will improve the ratio of LH/FSH. While the latter will make the androgen secretion high in the ovary. Androgen may lead negative feedback on estrogen, so it may finally lead to PCOS. Li et al. examined the negative effects on reproductive endocrine system caused by two kinds of anti-seizure medications, VPA and ASMs. They found that the VPA and ASMs cause dysregulation on HPO axis by affecting the level of sex hormones and γ -aminobutyric acid (GABA) [16].

3.3. Melatonin derailment

In human, the rhythmic synthesis of melatonin has already proved to have correlation with reproduction system. With the discovery of melatonin receptor in ovary granulosa cells and high level of melatonin in ovarian follicular fluids, it may also enable to modulate the circadian rhythm locally. Although the function of melatonin is controversial in some reports, the antigonadotrophic effect of melatonin has been abundantly reported. 100 μ M melatonin is observed to increase the concentration of androgen and progesterone while have no effect on estrogen [17]. In women with PCOS, the disruption of melatonin is reported. A study led by Jain et al. reports a significant increase in mean melatonin level observed in patients with PCOS than in controls. They also found that melatonin level is positively associated with increased testosterone level by using regression analysis [18]. In a study based on female juveniles found that patients with PCOS secreted melatonin later and last longer, which may explain the deficiency and inefficiency of sleep in all participants [19].

3.4. The change in clock genes under PCOS

Constant darkness induced PCOS in female rats. Acyclic menstrual cycles, polycystic ovaries, hyperinsulinemia and increased apoptosis of GCs are observed under constant darkness, which caused constant disorder on circadian clock. With this rat model, Lin et al. found that constant darkness reduced mRNA expression of BMAL1 in HepG2 cells and promoted insulin resistance [20]. In mature adipocytes, the decreased expression of BMAL1, Nampt, and Sirt1 was found in a dose-dependent

manner following with testosterone treatment, while the CLOCK, PER1, PER2 expression was found to stay unchanged [21].

The reduced BMAL1 expression is proved to directly promote the apoptosis in granulosa cells. By reduced the transcription of hormone receptor genes and decreased the key enzymes involved in hormone synthesis [14]. The reduced expression of BMAL1 may also indirectly act on the apoptosis in granulosa cells, which is proved to promote the insulin resistance in PCOS. In a study based on human volunteers, there found 2 genes responsible for negative feedback are upregulated in PCOS, which consists of PERs and CRYs [5].

4. CYP genes in PCOS affected by circadian rhythm

4.1. Cyp17a1, Cyp19a1, Cyp11a1

Proteins of CYP genes are subsets of steroidogenesis enzymes in the cytochrome P450. CYP17A1 located in chromosome 10, which only have a single species of mRNA. The activity of both 17-hydroxylase and C17,20lyase was combined in this enzyme to form two unique catalytic functions. CYP19A1 is located on the short arm of chromosome 15, which is encoded aromatase, a crucial enzyme in estrogen synthesis. CYP11A1 encoded enzyme that catalyses the first step of steroid hormone and converts cholesterol to pregnenolone, which located in the inner membrane of mitochondria.

4.2. Function of CYP genes

CYP17A1 catalyzes the production of all endogenous androgens. These atypical monooxygenases are expressed in the endoplasmic reticulum and function in the reactions for steroid formation [22]. Through its 17-hydroxylase activity, CYP17A1 catalyses the conversion of Preg to 17-OHPreg. After then, the CYP17A1 continues to transform 17-OH Preg into DHEA, a precursor to a variety of androgens like androstenedione (A) and testosterone (T). Prog can also be converted by CYP17A1 to 17-OH Prog, which can then be converted to A, via a different pathway. In the metabolism of androgens, A will be reduced to T through the activity of 17-hydroxysteroid dehydrogenase and T can be followed to be reduced to DHT. It is widely accepted that in humans, DHT is the most effective androgen receptor ligand and nuclear translocator. Because androgen is the precursor for estrogen, CYP17A1 make sense in the pathophysiology of PCOS because it is necessary for appropriate estrogen synthesis and metabolism.

The androgen is transformed into estrogen through the activity of aromatase encoded by CYP19A1. By FSH activation, the concentration of aromatase increases to produce estradiol, which promote the formation of follicles and increase the sensitivity of gonadotropin in the ovary. The normal expression of CYP19A1 is also essential in the apoptosis of granulosa cells, with the fact that it is the direct target of miR-146b, a micro-RNA that regulates the apoptosis [23].

4.3. CYP17A1 is the direct CLOCK-BMAL1 target

CYP17A1, one of the most important gene in the aetiology of PCOS, has also been shown as a direct target of CLOCK-BMAL1 in human peripheral blood mononuclear cells (PBMCs) [5]. In the recent research of Betsy et al. an untypical E box was found, which is identified as CACATG at 155 bp upstream differing from the typical E box sequence (CACGTG). By employing real-time PCR, the antibodies for CLOCK and BMAL1 later revealed CYP17A1 amplification, indicating that the promoter region of CYP17A1 was bind with CLOCK and BMAL1 both, which trigger the expression. The same study also demonstrates that higher CYP17A1 mRNA expression is accompanied by lower level of Bmal1 and Clock and a lower amplitude.

4.4. CYP19A1 and clock genes are influenced by NR1D1

The response elements ROREs in the promoter of CYP19A1 is directly regulated by NR1D1 in estradiol synthesis in granulosa cells. By attaching to ROREs in the Bmal1 promoter, Nr1d1 can also inhibit Bmal1 expression and alter the frequency of circadian oscillation [24]. After activation of NR1D1, the expression of BMAL1 and CYP19A1 is significantly decreased, the rhythm of CYP19A1 gene transcription is disordered and the amplitude of BMAL1 is reduced. In this research, it also found that the gene expression of CYP19A1 and NR1D1 has a rhythmic change [23]. It was tested that the transcription of NR1D1 has a significant increase in women with PCOS. After the knockdown of BMAL1, the mRNA level of CYP19A1 also decreased and a reduction in estradiol production [5].

5. Conclusion

Polycystic Ovary Syndrome is a very extensive and complex productive disease. Its pathogenesis has not been clearly studied so far. Under the modern routine, females are experiencing more stress, anxiety, irregular life styles, which may disturb the circadian rhythm and cause a series of physiological problem. Some studies have already proved that the derailment of circadian genes in the peripheral clock can worsen the progress of PCOS. It is of great significance to further explore the relationship of the pathways integrated between the circadian rhythm and PCOS.

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