The Role of Long Noncoding RNA H19 in the Pathogenesis of Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is a common endocrine disorder accounting for ~70–80% of cases of female annovulatory infertility. Genetic factors, including non-coding RNAs, are involved in the etiology of PCOS. The aim of this paper is to systematize experimental and clinical data on the role of long non-coding RNA H19 in the pathogenesis of PCOS, to consider patterns of dysregulation and impact of H19 on steriogenesis in the blood, follicular fluid, cumulus and granulosa cells; to analyze associations of H19 polymorphisms with the risk of PCOS development; and to assess the potential of H19 as a diagnostic and therapeutic target. The obtained data indicate that H19 is stably dysregulated in the blood, follicular fluid, granulosa/cumulus cells in patients with PCOS. H19 integrates key links of pathogenesis: ovarian function (H19/let 7→AMH axis), inflammation and granulosa cell pyroptosis (H19/miR 29a 3p→NLRP3/HDAC1 axis), cell proliferation (H19/miR 19b→CTGF), steroidogenesis (CYP17A1 and testosterone), and STAT3 signaling. An association of the rs2067051 genetic variant of the H19 gene with the risk of developing PCOS in certain populations has been shown.

Keywords

PCOS, H19, insulin resistance, hyperandrogenism, SNP

Introduction

Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder, occurring in about 6–20% of females in reproductive age [1]. Prevalence rates depend on the studied population and the diagnostic criteria. PCOS is characterized by a range of clinical, metabolic, and hormonal features. PCOS accounts for about 80% of women with anovulatory infertility [2]. The most common and accepted diagnostic approach for PCOS is Rotterdam criteria (Figure 1) which involves at least two of the following features: hyperandrogenism, oligo- or an-ovulation, and polycystic ovaries [1].

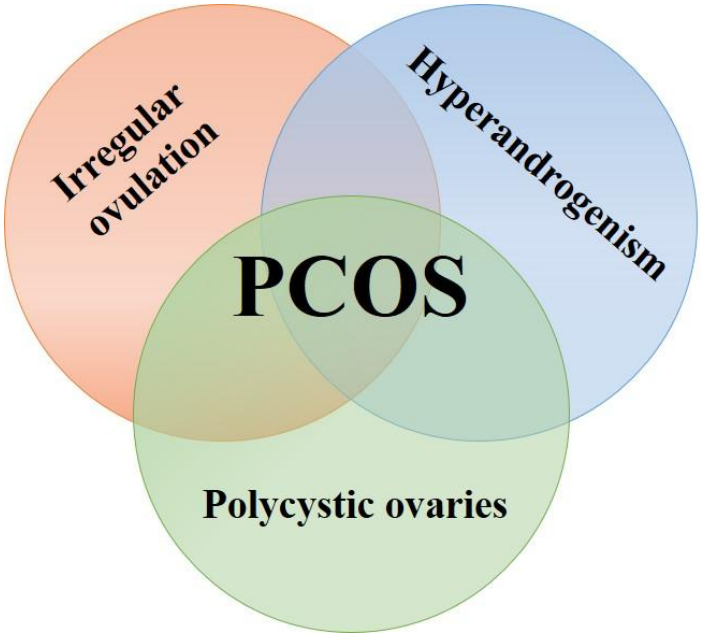


Fig.1 Rotterdam criteria for diagnosis of polycystic ovary syndrome (PCOS)

Both genetic and environmental factors are involved in the etiology of PCOS [3]. Environmental factors include unhealthy lifestyle, poor diet or infections that increase the risk of PCOS [4]. Genetic factors play an important role in the etiology of PCOS [3]. Several studies have been focused on the associations of dysregulated genes and genetic polymorphisms with PCOS in diverse populations [5-6]. Around 145 single nucleotide polymorphisms (SNPs) are associated with PCOS, according to PCOSKB (2016) [5].

Multiple genes have been associated with ovarian dysfunction in PCOS, including genes linked to gonadotropin action, sex hormone binding and insulin signaling [7, 8]. Furthermore, long non-coding RNAs (lncRNAs) have gained more attention recently as potential players in the pathophysiology of PCOS, regarding their interactions with mRNAs and microRNAs and influencing the hormonal and metabolic status of patients [9].

LncRNAs are non-coding RNAs with 200 nucleotides or more in length and can regulate gene expression at different levels. They are known to be involved in several vital processes such as cell differentiation, p53-mediated DNA damage response, glucose metabolism, inflammation and immune functions [10, 11]. Alterations in lncRNAs expression have been associated with several human diseases, including PCOS. For example, significant up- or downregulations of 623 lncRNAs and 260 mRNAs were noticed in patients with PCOS [12]. According to

LncRNADisease database [13], causal noncoding RNAs associated with PCOS, including lncRNAs such as MALAT1, BANCR, H19 and GAS5 are presented in figure 2.

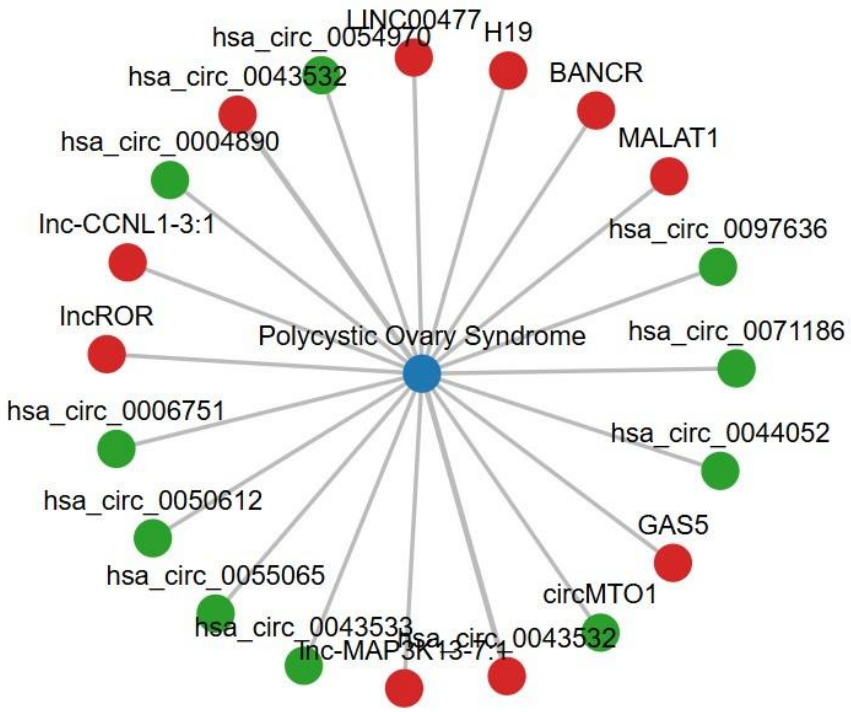


Fig. 2 Network of noncoding RNAs associated with polycystic ovary syndrome (source: LncRNADisease v3.0 database http://www.rnanut.net/lncrnadisease/index.php/home). hsa_circ: circular RNA

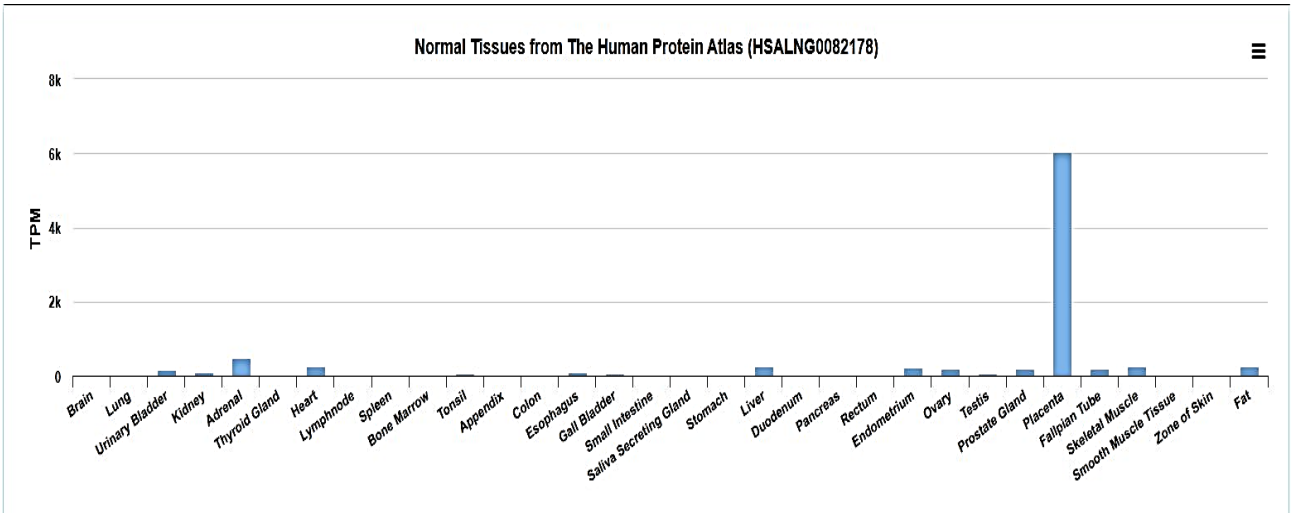
The aim is to systematize experimental and clinical data on the role of long non-coding RNA H19 in the pathogenesis of PCOS, to consider the patterns of dysregulation and the effect on steriogenesis of H19 in the blood, follicular environment, cumulus and granulosa cells; to analyze the associations of H19 polymorphisms with the risk of developing PCOS and to assess the potential of H19 as a diagnostic and therapeutic target.

Long noncoding RNA H19

Long noncoding RNA H19 is encoded by *H19* gene located on chromosome 11p15.5 with five exons and four introns. *H19* is transcribed as any other protein-coding gene passing through alternative splicing and polyadenylation, then the mature H19 (2.3 kb) is transferred to the cytoplasm [14, 15]. *H19* genetic locus also contains the highly conserved microRNAs, miR-675-3p and miR-675-5p [16]. In addition, scientists revealed that this locus includes protein coding gene *HOTS* (H19 opposite tumor suppressor), and long intergenic antisense transcript gene, called *91H* [14].

H19 genetic expression is mainly activated during embryonic stage (endoderm- and mesoderm-derived tissues) and it decreases after birth. According to LncExpDB database (https://ngdc.cncb.ac.cn/lncexpdb/), the highest level of H19 is in the placental tissue. It is also expressed in adrenal gland, skeletal muscles, heart, liver, endometrium and less in ovarian tissues and urinary bladder (figure 3).

Fig. 3 Expression level of *H19* in different normal tissues (source: LncExpDB database



https://ngdc.cncb.ac.cn/lncexpdb/)

H19 was the first discovered lncRNA along with genomic imprinting [17, 18]. This lncRNA has showed various regulatory functions, especially in cellular differentiation, proliferation and apoptosis [16]. Thus, it is intensively studied in different types of cancers and known to play a major role in fetal growth [19]. Moreover, H19 participates in regulating liver functions and hepatic insulin signaling cascade [20, 21]. Recently, it was studied in reproduction-related diseases and research showed that this lncRNA participate in the pathogenesis of endometriosis, diminished ovarian reserve, uterine fibroids, male factor infertility and PCOS [22].

Scientists suggest several mechanisms of action for H19 in embryonic and adult's tissues, first of which is genomic imprinting. *H19* locus is a highly-conserved, in which insulin-like growth factor 2 (*IGF2*) gene is also located (90 kb from *H19*). These two genes are co-regulated by an intergenic differentially methylated region (DMR) and downstream enhancers [23]. DMR of the paternal allele is methylated, inhibiting the promoter of *H19* (triggering *IGF2* promoter) while maternal allele is unmethylated and thus *H19* is only expressed in this allele. This

methylation balance is essential for *H19* functioning and its abnormalities are associated with pathological syndromes [24].

H19 is also known to act as a molecular sponge. Kallen et al. suggested it as let-7 antagonist, since H19 has a specific binding site on miRNA let-7 and their binding inhibits the function of this miRNA [25]. Till now, researchers mentioned many other miRNAs that are inhibited by H19 with molecular sponge mechanism, such as: miR-17–5p, miR-93–5p and miRNA-106 b-5p [26].

H19 in PCOS pathogenesis

The direct association of H19 level with PCOS risk was first indicated by Qin et al. In Chinese women, diagnosed with PCOS, H19 levels in peripheral blood were significantly higher comparing to controls [27]. The same results were obtained by other scientists showing that *H19* genetic expression is upregulated in peripheral blood, cumulus and granulosa cells [28, 29] and follicular fluid exosomes [30] of PCOS women. In addition, H19 levels showed a significant correlation with clinical (body mass index), metabolic (total cholesterol, LDL) and hormonal (FSH, LH) parameters in women suffering from PCOS [31]. The exact role of H19 in PCOS is not totally understood, but several studies have linked it to its effect on ovarian function, insulin resistance and hyperandrogenism.

H19 effect on ovarian function in PCOS

Qin et al. showed that H19 decreases the number of mature follicles that produce estradiol, and survive to ovulate. In mice with H19 knock-out, accelerated development of follicles and lower Anti-Mullerian hormone (AMH) levels were observed in comparison with controls [32]. The suggested mechanism of H19 effect was by regulating let-7. H19 acts as molecular sponge for let-7, which has a specific binding site on *AMH* gene. In other words, the absence of H19 will activates let-7 inhibition for *AMH*, while in the presence of H19, let-7 is inhibited and *AMH* is upregulated facilitating appropriate follicular growth. This suggestion was supported by the fact that let-7 expression is increased in women with poor ovarian reserve and premature ovarian insufficiency [33].

It was found that *H19* genetic expression is crucial in PCOS. Sun et al. suggested that overexpression of H19 leads to inhibition of miR-19b preventing its function on *CTGF*, which in turn is upregulated leading to higher granulosa cell proliferation. Thus, H19/miR-19b/CTGF axis is essential in the pathogenesis of PCOS [34]. Furthermore, Li et al. identified a binding site for STAT3 (Signal transducer and activator of transcription 3) within the promoter region of H19, suggesting that STAT3 may play a role in regulating *H19* expression. It was observed that H19 and STAT3 act synergistically in PCOS. The inhibition of STAT3 resulted in a significant reduction in H19 expression [28].

Another suggested mechanism for H19 role in PCOS is H19/miR-29a-3p/NLRP3 axis. *NLRP3* gene produces a key protein for triggering inflammation and apoptosis [35] and it's regulated by miR-29a-3p [36]. Chen al. showed that in PCOS animal model, H19 is overexpressed and competitively bound to miR-29a-3p, preventing the inhibition of *NLRP3*. This results in the downregulation of histone deacetylase 1 and causes ovarian damage and hormone disorders accompanied with PCOS [37].

H19 and Insulin Resistance in PCOS

Insulin resistance (IR) is commonly observed in women with PCOS and plays a crucial role in the reproductive and metabolic complications associated with this disorder [38, 39]. IR leads to hyperinsulinemia, which adversely affects ovarian function by increasing androgen production and halting the development of ovarian follicles [38]. Consequently, enhancing insulin sensitivity is considered one of the most effective treatment approaches for PCOS patients [40]. Although the available published studies do not specifically outline the precise molecular mechanisms through which lncRNA H19 influences each element of the insulin signaling pathway in PCOS granulosa cells, the overall significance of H19 in metabolism and its association with insulin resistance is well- established.

Several studies showed that H19 can improve insulin sensitivity [41]. It plays an important role in regulating the hepatic insulin signaling cascade [34]. H19 circulating levels were significantly increased among patients with type 2 diabetes (T2D), which indicated its potential role as a biomarker of hepatic insulin resistance [42].

Goyal et al. showed that H19 inhibition by small interfering RNA (siRNA) led to insulin signaling dysfunction and increased nuclear localization of forkhead box O1 (FoxO1), a transcriptional regulator of gluconeogenic gene expression [43]. In addition, decreased levels of H19 have been observed in the skeletal muscle of T2D patients and in insulin-resistant rodents, where the absence of H19 resulted in impaired insulin signaling and reduced glucose uptake [44].

The PI3K/AKT pathway plays an important role in insulin signaling and glucose metabolism, and its dysregulation has been associated with IR [45, 46] and PCOS [47]. The activation of PI3K/AKT pathway results in the phosphorylation of DNA cytosine-5-methyltransferase 1 (DNMT1), which enhances its nuclear translocation and activity, consequently leading to methylation of the downstream genes [48]. Overexpression of DNMT1 downregulates H19 expression [49]. Inhibition of PI3K/AKT suppresses DNMT1 phosphorylation, which in turn suppresses H19 methylation and therefore upregulates *H19* expression [40]. This was indicated in a previous study that included combination therapy of PCOS, consists of Sitagliptin (TECOS) and metformin (DMBG) and this co-treatment induced the expression of H19 via inhibiting PI3K/AKT signaling pathway [40].

H19 and hyperandrogenism in PCOS

Increased serum testosterone, caused by hyperandrogenism, is a key clinical characteristic of PCOS [50]. Previous studies indicated that the elevated synthesis of testosterone observed in PCOS patients partially influenced by an increased activity of CYP17A1, a key enzyme in the steroidogenic pathway [29, 51]. CYP17A1 catalyzes the conversion of pregnenolone and progesterone into their products, which include testosterone, and plays a crucial role in adrenal and gonadal steroid biosynthesis [29]. Dysregulated expression of *CYP17A1* mRNA has been reported in ovarian theca cells from patients with PCOS [52]. H19 is also involved in the process, since it can modulate the production of steroid hormones through post-transcriptional regulation of the rate-limiting step of steroidogenesis [53]. A study on mouse models showed that deletion of H19 decreased steroid 17 alpha-monooxygenase (Cyp17) and serum testosterone [29], which indicates the impact of H19 on hyperandrogenism associated with PCOS.

Furthermore, as mentioned previously, H19 acts as a molecular “sponge" for let-7, a microRNA that regulates insulin metabolism [25]. Overexpression of let-7 causes hyperinsulinemia and contributes to ovarian hyperandrogenism [54]. H19 modulates let-7 availability and results in the derepression of its target genes. Thus, H19-mediated regulation of let-7 may be associated with adverse outcomes of PCOS [29].

A summary of discussed mechanisms and pathways, through which H19 is involved in PCOS pathogenesis is presented in figure 4.

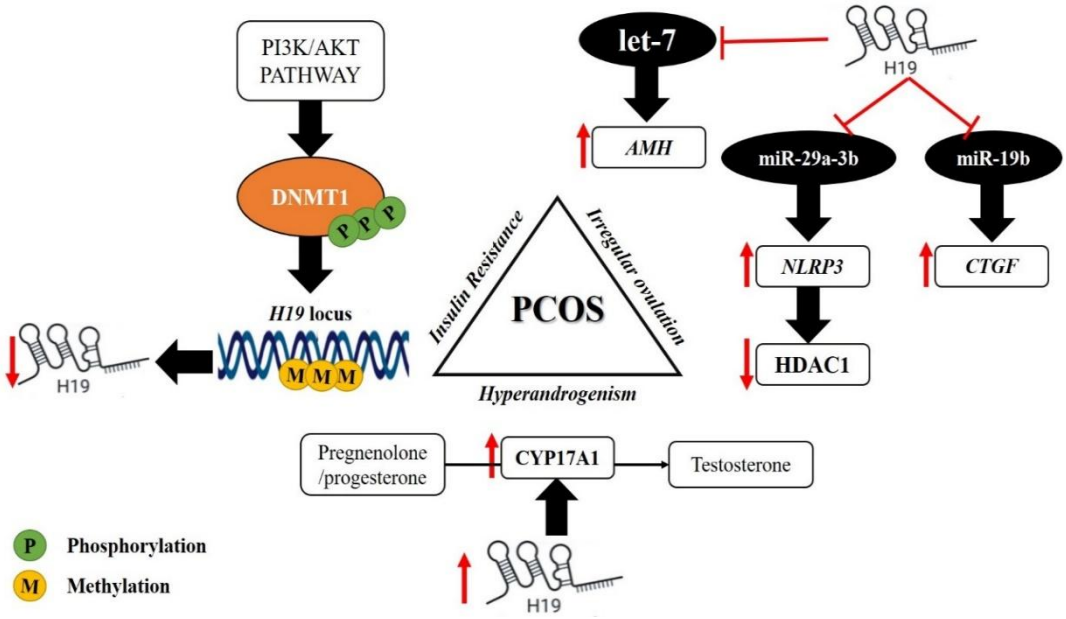


Fig.4 H19 suggested roles in PCOS pathogenesis. **PCOS**: Polycystic ovary syndrome. **DNMT1**: DNA (cytosine-5)-methyltransferase 1. **CYP17A1**: Cytochrome P450 17A1. **AMH**: Anti-Mullerian hormone. **NLRP3**: NLR family pyrin domain containing 3. **HDAC1**: Histone deacetylase 1. CTGF: Connective tissue growth factor

Genetic polymorphisms of *H19* and PCOS

According to the national human genome research institute (https://www.genome.gov/genetics-glossary/Polymorphism), genetic polymorphism is defined as the presence of two or more variant forms of a specific DNA sequence. Genetic polymorphisms of *H19* were mainly studied in different types of malignancies [55]. The association of *H19* genetic polymorphisms with PCOS was previously studied by Ghasemi et al. According to their research, minor allele of *H19* rs2067051, but not *H19* rs3741219, is associated with higher risk of developing PCOS in Iranian women [56].

As shown in figure 5, rs2067051 is located in the first exon of *H19* gene with a substitution of cytosine into thymine. Wang et al. showed that rs2067051 location is the flank region of the gene and it is very close to the imprinting control regions (ICRs). They suggested that the presence of minor allele of this variant is associated with alteration of ICRs binding ability, which is essential in the regulation of *H19* genetic expression [57]. Moreover, our analysis of rs2067051 genetic location in UCSC genome browser (https://genome.ucsc.edu/) showed that its region has a slightly high content of CpG sites, which indicates high possibility of DNA methylation and important role of this site in genetic expression regulation.

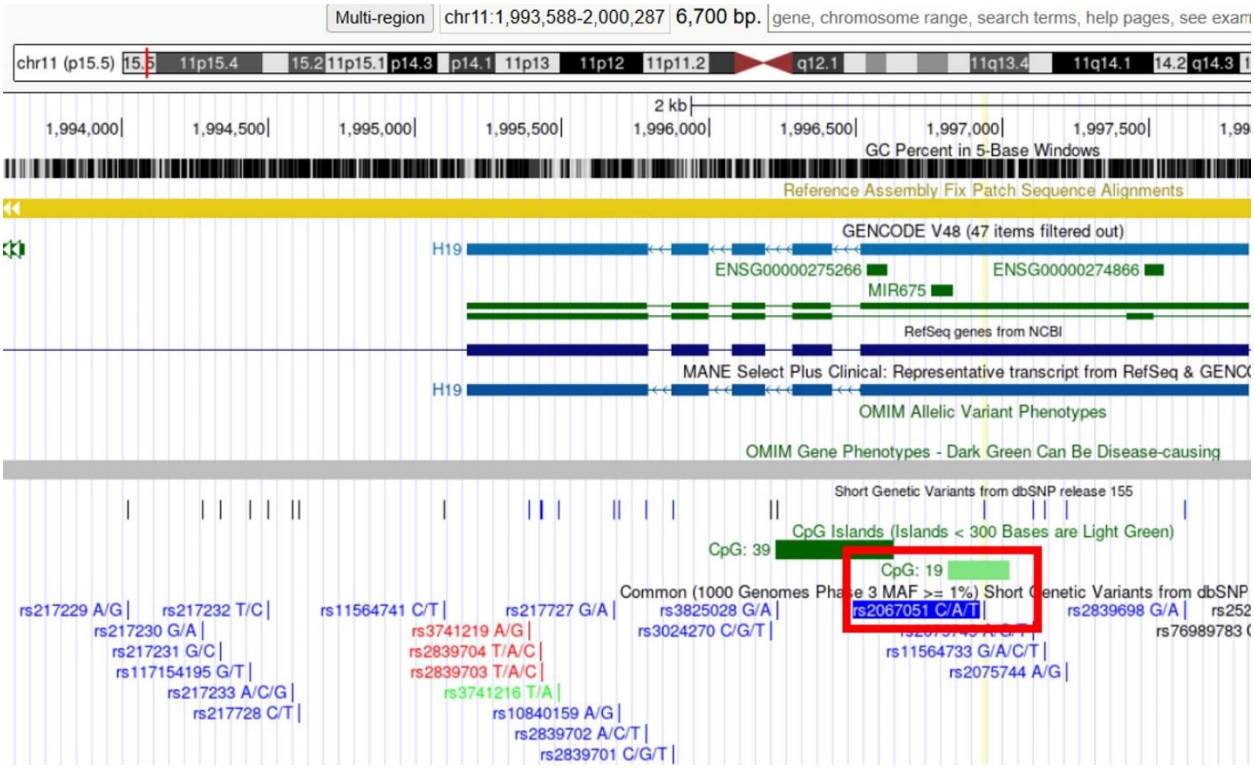


Fig. 5 Location of rs2067051 genetic polymorphism in *H19* gene (source: UCSC browser https://genome.ucsc.edu/)

rs3741219 is the second polymorphism of *H19* studied by Ghasemi et al. in PCOS patients. No association was found between this polymorphism and PCOS risk [56]. UCSC browser showed that rs3741219 is located in the fifth exon of *H19* and represents exonic variant of lncRNA H19 (rs3741219, A/G), consequences - structural-regulatory, not encoding amino acid substitution (figure 6). This is consistent with the previous study by Xia et al. noted that minor allele of rs3741219 is associated with structural change of H19 [55].

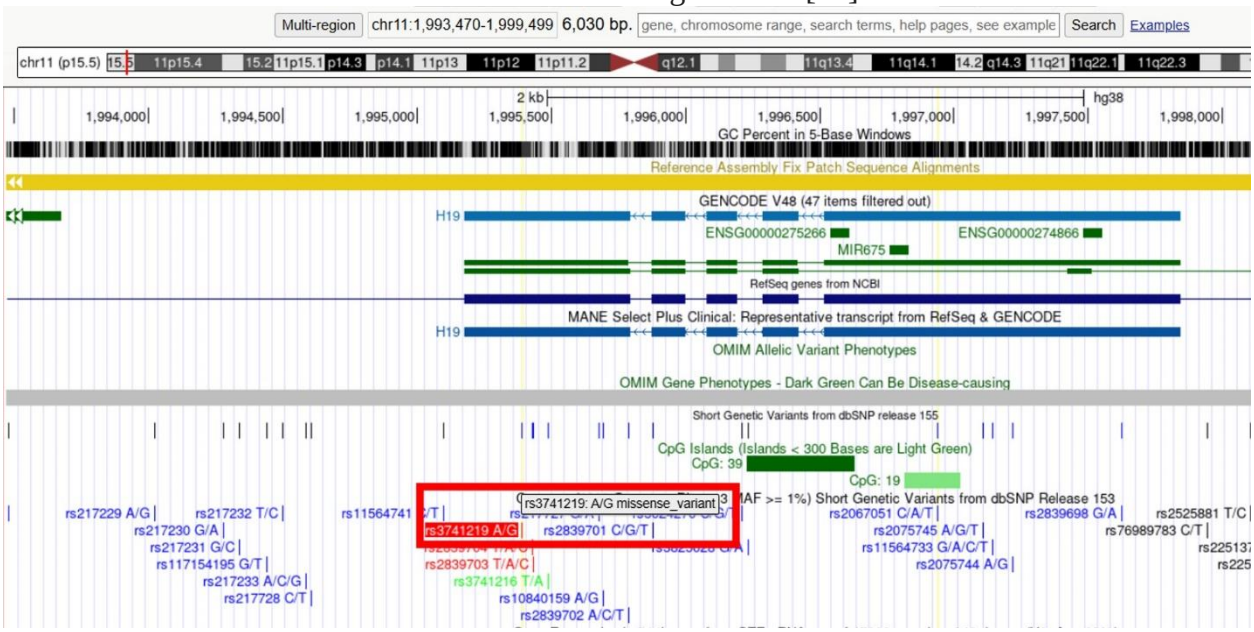


Fig. 6 Genetic location of rs3741219 genetic polymorphism in *H19* gene (source: UCSC browser https://genome.ucsc.edu/)

Although they were not studied in association with PCOS, several SNPs in the promoter region of *H19* gene are known to cause structural changes and affect the interaction of this lncRNA with targets and regulators. For example, minor allele of *H19* rs11752942 is significantly associated with decreased H19 levels. In addition, *H19* rs12325489 can alter the transcriptional activity of this non-coding RNA [58]. However, the most studied polymorphism of *H19* in different pathologies is rs217727 (C2992T).

rs217727 is located in the fifth exon of *H19*, near *MIR675* gene. H19 increases the expression of miR-675, which in turn reduces the expression of apoptotic genes and promotes proliferation, but suppresses apoptosis [59]. Minor allele (T) of rs217727 locus is known to increase cell apoptosis by inhibiting H19 effect on miR-675 level and affecting the binding sites of various microRNAs with H19. Presence of this allele causes the formation of miR-4804-5p and miR-8071 binding sites on *H19*, and the loss of miR-3960 binding sites [60].

The role of *H19* genetic polymorphisms in PCOS pathophysiology is not studied enough in scientific literature and since many of these variations revealed a functional effect on H19, they are suggested as perspective targets for future trials in PCOS patients.

Conclusion

The obtained data indicate persistent dysregulation of the H19 gene in the blood, follicular fluid and granulosa/cumulus cells in patients with PCOS. The H19 gene combines key links in pathogenesis: ovarian function (H19/let 7→AMG axis), inflammation and granulosa cell pyroptosis (H19/miR 29a 3p→NLRP3/HDAC1 axis), cell proliferation (H19/miR) 19b→CTGF), steroidogenesis (CYP17A1 and testosterone) and the STAT3 signaling pathway. An association of the rs2067051 genetic variant of the H19 gene with the risk of developing PCOS in certain populations was shown, but the obtained results require confirmation and functional validation. The H19 gene has potential as a biomarker and therapeutic target, however, large prospective cohorts with standardized phenotyping and proper adjustment for cofactors (obesity, insulin resistance, ethnicity) and imprinting/methylation context of the H19/IGF2 locus are lacking. Future research could focus on multicenter tissue-to-blood-to-exosome studies with replication; studying H19 miRNA axes to assess the impact on PCOS phenotype; and early clinical trials of safe modulation of the H19/let 7 and H19/inflammation pathways.

Statements and Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this article

Declaration of competing interest

The author declares no conflict of interests.

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