

REVIEW ARTICLE

Primary ovarian insufficiency: Genetic etiology, *in vitro* follicle activation mechanisms, and clinical management perspectives

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Abstract

Introduction: Menstrual irregularities or amenorrhea affect at least 17.4 million women worldwide. Symptoms in young patients may be atypical, and imaging and serum hormone levels are sometimes insufficient to explain the condition.

Objectives: This review synthesizes current evidence to improve diagnostic and treatment strategies, reduce misdiagnosis of primary ovarian insufficiency (POI), facilitate timely treatment, and support earlier recognition and individualized follow-up to preserve reproductive function.

Methods: We conducted a narrative review of studies addressing the genetic basis, molecular mechanisms, follicular activation pathways, and clinical management of POI. Particular attention was given to single-gene variants, chromosomal abnormalities, phosphoinositide 3-kinase (PI3K), mechanistic target of rapamycin (mTOR), and Hippo signaling pathways, as well as acquired or functional conditions that may mimic genetic POI.

Results: POI is a heterogeneous reproductive endocrine disorder involving chromosomal abnormalities, X-linked factors, folliculogenesis-related genes, meiotic and chromosomal stability defects, and DNA damage repair pathways. Representative genes such as *FMR1*, *BMP15*, *GDF9*, *NR5A1*, *HFM1*, *STAG3*, and *BRCA2* illustrate the diverse mechanisms that impair ovarian reserve or cause follicular dysfunction. *In vitro* activation of dormant follicles is biologically supported by PI3K/phosphatase and tensin homolog/protein kinase B/FOXO3a, mTOR, and Hippo signaling pathways, but its clinical application remains limited and requires further validation. Functional hypothalamic amenorrhea, drug exposure, metabolic abnormalities, endocrine tumors, and autoimmune conditions should also be considered in the differential diagnosis.

Conclusion: POI in young women should not be simply attributed to stress, emotional factors, or menstrual irregularity. Early etiological evaluation, appropriate genetic testing, differential diagnosis, reproductive counseling, hormone replacement therapy when indicated, fertility preservation planning, and long-term multidisciplinary follow-up may improve clinical management. *In vitro* activation-related mechanisms provide a potential biological rationale for future fertility-preserving strategies, but broader clinical use requires stronger evidence.

Keywords: *In vitro* activation of dormant follicles; Primary ovarian insufficiency; Therapeutic mechanism; Genetic etiology; Clinical management

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1. Introduction

Menstrual irregularities or amenorrhea affect at least 17.4 million women worldwide.¹ Young women experiencing menstrual irregularities, however, may be less likely to seek regular medical care or undergo routine evaluation of their reproductive health. Since 2007, the prevalence of menstrual disorders among young women has gradually increased. The incidence of POI among females aged 15–19 years in Finland increased from 7% to 10% per 100,000 women-years after 2007.² A 30-year cohort study reported a crude mortality of 34.7% among women with POI compared with 19.3% among women without POI.³ When young women present with irregular menstruation or amenorrhea as their primary complaint, clinicians may underestimate the potential underlying causes because of the patients' young age. Consequently, once menstruation resumes, further investigation or retrospective assessment may be discontinued, potentially overlooking underlying reproductive or endocrine disorders.

In recent years, research on POI has gradually shifted from merely describing amenorrhea and hypergonadotrophinemia to studies on genetic heterogeneity, the regulation of follicular development, molecular pathological mechanisms, and long-term health management. Recent reviews indicate that clinical and genetic findings regarding POI continue to evolve; its etiology may involve chromosomal abnormalities, X-linked factors, genes related to folliculogenesis, genes associated with meiosis, and DNA damage repair, as well as the combined effects of complex polygenic and environmental factors.^{4–6} At the same time, the impact of POI on patients is not limited to reduced fertility, but also extends to bone health, cardiovascular risk, psychological burden, the need for genetic counseling, and long-term quality of life management. Therefore, the diagnosis and management of POI should not be confined to “regulating menstruation after amenorrhea” or simple hormone replacement therapy (HRT), but should instead establish a comprehensive approach ranging from the identification of genetic causes and elucidation of mechanisms to fertility preservation and long-term health management.⁷

This article aims to inspire clinicians to consider this when they encounter patients with poor treatment outcomes due to irregular menstruation. Heterogeneous genetic mutations disrupt core dormant follicle activation pathways, triggering early POI. Combined genetic screening, differential diagnosis, *in vitro* activation (IVA) fertility strategies, and long-term surveillance can improve young patients' reproductive and systemic health outcomes.

2. Methods

2.1. Aim and significance

In 2020, we encountered a case involving a young woman presenting with menstrual irregularities and work-related stress. We initially advised her to manage her emotional well-being and reduce work-related stress. Over time, the patient's condition gradually worsened, and her menstrual irregularities progressively developed into amenorrhea. In January 2023, we initiated a research investigation using a literature review approach to explore the treatment and clinical management of this condition. The initial literature included seed articles and articles from the *New England Journal of Medicine* identified through an initial keyword-based search. We then used these seed articles to identify additional literature through snowball sampling. This snowball sampling method has been described in our previous study.⁸

The primary aim of this study is to present the genetic mechanisms of POI and the therapeutic progress regarding IVA, as identified through our literature review. Crucially, this report uses this research process to remind clinicians not to attribute menstrual irregularities in young women to stress, emotions, or lifestyle alone; instead, incorporate POI, genetic mutations, immune and endocrine abnormalities, and fertility preservation into the clinical assessment process at the earliest opportunity. Only in this way can a definitive diagnosis be reached as quickly as possible, thereby preserving the fertility of young female patients. To this end, we have expanded this research into a project exploring the role of inflammation in mental health and the immune system.^{9–11}

2.2. Search strategy

The search strategy for this literature review identified studies investigating the genetic mechanisms of POI. Databases such as PubMed, Scopus, Web of Science, and Google Scholar were utilized, targeting publications up to 2026. Keywords used in the search included “primary ovarian insufficiency,” “POI,” “gene mutations,” “*in vitro* activation,” and related terms. Our selection criteria emphasized articles significantly contributing to understanding the genetics, pathophysiology, and therapeutic interventions relevant to POI. The inclusion of studies was determined based on substantial genetic evidence, elucidation of disease mechanisms, or presentation of novel treatment modalities.

Essential information on genetic mutations, associated pathophysiological mechanisms, and potential or existing treatment strategies was systematically gathered. The analysis focused on synthesizing these findings to identify

common genetic factors implicated in POI, the impact of these mutations on ovarian function, and the effectiveness of various therapeutic approaches. The selection process was meticulously documented to ensure reproducibility and to provide a comprehensive overview of the current state of research in the field.

3. Overview of primary ovarian insufficiency

Primary ovarian insufficiency is a condition where the ovaries stop functioning before the age of 40, causing infertility and hormonal imbalances.¹² Patients may present with menstrual irregularity, amenorrhea, hypoestrogenic symptoms, infertility, and psychological distress; metabolic or psychological factors may coexist or mimic POI (Figure 1). However, some patients may initially present with biochemical evidence of hypergonadotropic hypogonadism before overt clinical manifestations become apparent. Studies have shown that rare chromosomal mutations can cause POI.¹³ Clinical studies have confirmed that POI can reduce women's fertility and cause osteoporosis,¹⁴ cardiovascular disease, and other hazards,¹⁵ seriously affecting women's health.¹ Proper POI treatment during the first phase of the disease prevents deterioration in the patient's quality of life, reduces the risk of long-term sequelae, and improves fertility.¹⁶

According to epidemiological statistics, the prevalence of POI among women under 40 years old is 0.48%.² However, the incidence rises sharply in females under 40,¹⁷ while the average woman will experience menopause at 50–52 years,¹ indicating declining ovarian function. Available data show that POI incidence increases with age, while more cases are being recognized at younger ages.

Recent medical technology has focused on improving survival for women with POI.¹² However, researchers have not paid enough attention to supporting short-term quality of life and avoiding long-term sequelae (e.g., osteoporosis) of premature menopause.¹⁸ The care plan for patients with POI should be multifaceted and include counseling, suggestions on diet and nutritional supplementation, different therapeutic approaches, reproductive healthcare, and mental support.¹⁹

There is no established treatment that reliably restores ovarian reserve; management focuses on HRT, reproductive counseling, fertility options, and long-term health protection. In China, treatment focuses on traditional Chinese medicine, such as acupuncture and Kuntai capsules, while in Europe, treatment primarily focuses on Western medicine, such as HRT, oocyte or embryo donation therapy, ovarian tissue cryopreservation, and stem cell therapy.^{18–20} Previous studies have shown that oocyte or embryo donation therapy is usually used

for patients with POI, as autologous *in vitro* fertilization is often limited by reduced follicular reserve and poor ovarian response, whereas donor oocyte or embryo donation remains an established option for many patients.²¹ However, IVA has recently attracted attention as a potential solution through physical and chemical methods, as it has the potential to continue to develop into mature follicles.²²

Many studies have demonstrated the mechanism and treatment of IVA. Previous studies have shown that the phosphoinositide 3-kinase (PI3K), Hippo, and mechanistic target of rapamycin (mTOR) pathways can explain the mechanism of dormant follicle activation. Most research has focused on the PI3K signaling pathway, one of the most critical pathways for activating primordial follicles.²³ However, few studies have examined IVA mechanisms and treatment methods in China. Given clinical needs and deepening research into related mechanisms, a systematic summary of IVA treatment mechanisms and methods is necessary. The diagnosis and treatment of POI in Europe and the United States are ahead of those in China. Although Chinese medicine generally applies to POI, the treatment cannot meet the needs of POI patients.^{20,24} Therefore, we aim to provide treatment inspiration for the following types of patients: (i) patients whose ovarian function does not recover significantly after HRT, (ii) patients with amenorrhea and childbirth needs, (iii) patients with clinical manifestations of unexplained low estrogen, and (iv) patients who experienced failure of assisted reproductive technology intervention.

4. Mechanisms of dormant follicle activation

The mechanisms linking folliculogenesis and follicular depletion have been extensively reviewed elsewhere.^{17,25,26} Accordingly, these mechanisms are not discussed further in the present section. The activation of dormant follicles is not the result of a single signaling molecule, but rather a continuous process jointly regulated by the oocyte, the granulosa cells of the primordial follicle, and the local ovarian microenvironment. Whilst the primordial follicle is in a dormant state, the oocyte and the surrounding flat granulosa cells remain relatively quiescent; once the activation signal is triggered, the granulosa cells of the primordial follicle are the first to undergo proliferation and morphological changes, gradually transforming from a flat to a cuboidal shape, and transmitting developmental signals to the oocyte. Subsequently, the oocyte increases in volume and secretes factors such as growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), which further promote granulosa cell proliferation, differentiation, and follicular maturation. Therefore,

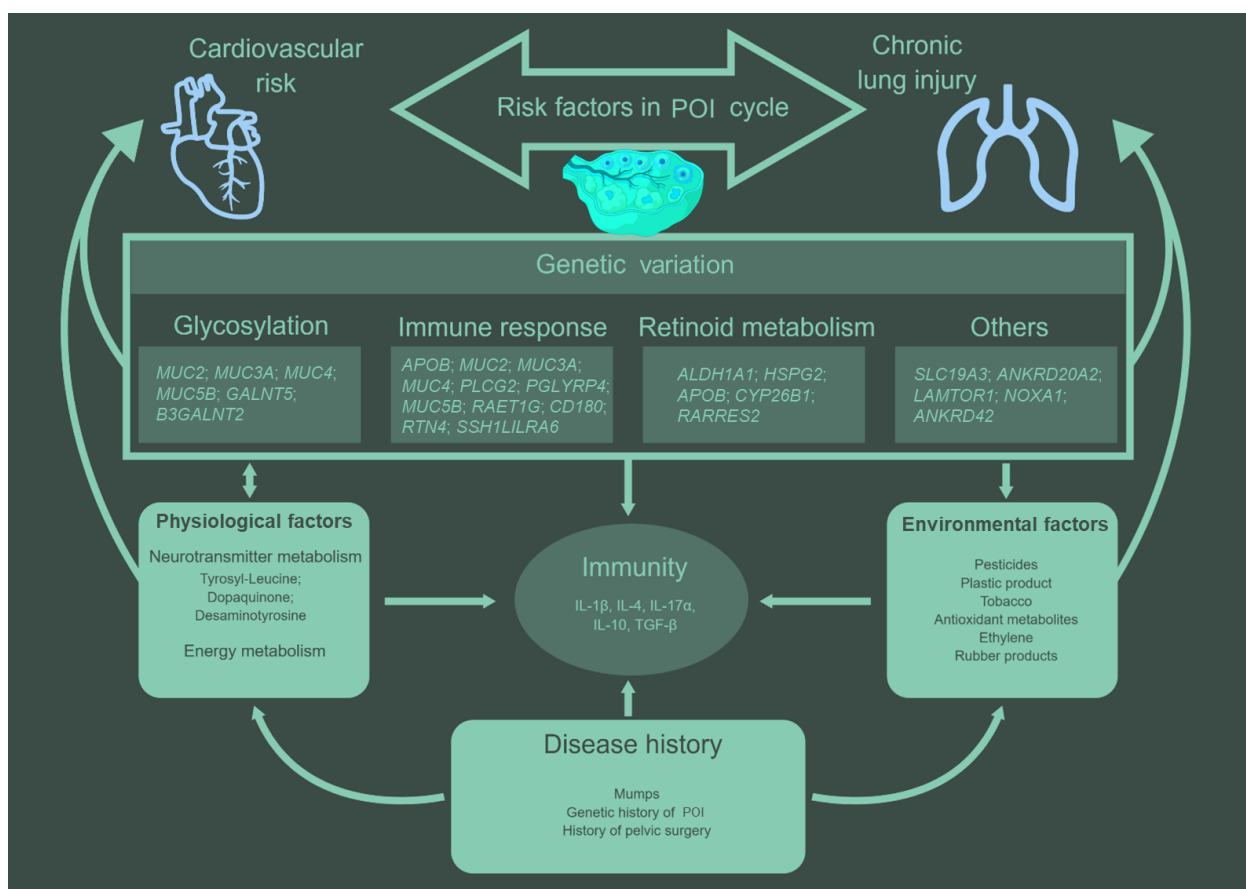


Figure 1. Multifactorial risk cycle of primary ovarian insufficiency (POI). The schematic illustrates the interconnected risk factors driving POI pathogenesis, structured around a core immune dysregulation hub.

the key to dormant follicle activation lies not merely in “awakening the oocyte,” but in re-establishing bidirectional communication between the oocyte and granulosa cells. Currently, the PI3K, mTOR, and Hippo signaling pathways are considered the primary mechanistic basis for the IVA of dormant follicles (Figure 2). This also provides a theoretical foundation for future fertility preservation and personalized treatment for patients with POI. We have currently identified three representative clinical trials of IVA.²⁷⁻²⁹ The clinical trial results for IVA are highly encouraging. For patients facing otherwise devastating circumstances, such as those undergoing cancer treatment, IVA may offer the possibility of preserving fertility and subsequently achieving a healthy pregnancy and live birth. Key data and results from the clinical trials are summarized in Table 1.

4.1. Phosphoinositide 3 kinase pathway

The PI3K pathway is key to the transition of dormant

primordial follicles from a quiescent to an activated state. For patients with POI, the theoretical premise of IVA is not follicle regeneration, but using molecular interventions to induce these follicles to re-enter the growth phase, based on the assumption that residual dormant primordial follicles may still be present in the ovarian tissue of some patients. In their review of IVA, Kawamura *et al.*¹⁷ noted that although natural menstrual cycles may have ceased in patients with POI, varying numbers of dormant follicles may still be present in the ovaries of some patients; by activating these residual follicles *in vitro*, patients may be able to achieve fertility using their own oocytes. In this subsection, we explain why dormant follicles may be reawakened.

The PI3K/protein kinase B (AKT)/mTOR pathway is not linear, but a complex network of interactions involved in cell growth, metabolism, proliferation, and survival. Ersahin *et al.*³⁰ conducted a systematic network integration of the PI3K/AKT/mTOR pathway, emphasizing that it is

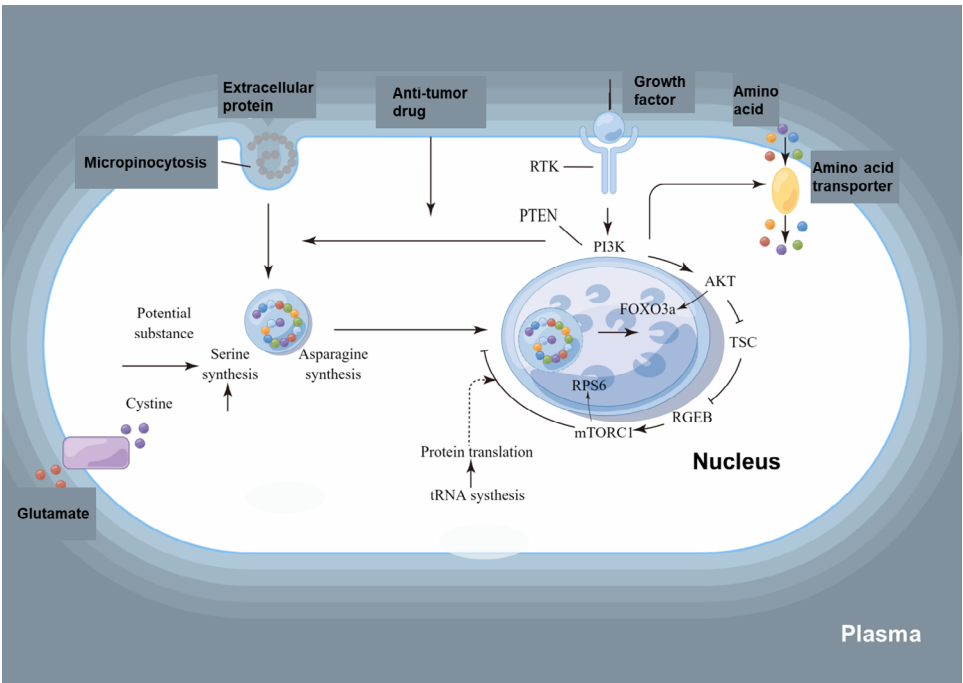


Figure 2. Mechanisms of dormant follicle activation. The schematic illustrates the core phosphoinositide 3-kinase (PI3K)–protein kinase B (AKT)–mechanistic target of rapamycin (mTOR) signaling pathway and its crosstalk with metabolic processes in primary ovarian insufficiency.

Table 1. Human clinical trial data of *in vitro* activation technology

Ref	Information	IVA type	Ovarian response	Pregnancy and live birth data
Grin <i>et al.</i> , 2025 ²⁷	Drug-free (mechanical fragmentation only)	POR (<i>n</i> = 7); POI (<i>n</i> = 14)	POR: 100% follicle growth, 71% AFC elevation, 57% AMH rise POI: 71% follicle growth, 50% AFC elevation, only 7% AMH improvement	11 FET cycles: 2 clinical pregnancies, 1 singleton live birth; fertilization rates were 72% in POR and 59% in POI
Zhang <i>et al.</i> , 2021 ²⁸	Shortened chemical IVA (PI3K/mTOR agonist 1 h incubation)	POI (<i>n</i> = 15); POR (<i>n</i> = 3)	9/15 POI patients developed follicular waves; large ovarian strips reduced follicle apoptosis	2 live births: 1 spontaneous conception, 1 IVF singleton; 4-year follow-up showed no acute adverse events
Lunding <i>et al.</i> , 2019 ²⁹	Drug-free fragmentation and autotransplant RCT	DOR (<i>n</i> = 20)	No significant increase in recruitable follicles at 10 weeks; transient AMH elevation (5–8 weeks) in 7/20 patients	12 clinical pregnancies (60%), cumulative live birth rate per IVF cycle: 18.4%

Abbreviations: AFC: Antral follicle count; AMH: Anti-Müllerian hormone; DOR: Diminished ovarian reserve; FET: Frozen embryo transfer; IVF: *In vitro* fertilization; IVA: *In vitro* activation; mTOR: Mechanistic target of rapamycin; PI3K: Phosphoinositide 3 kinase; POI: Primary ovarian insufficiency; POR: Poor ovarian response; RCT: Randomized controlled trial.

regulated by multiple upstream signals and extensively intersects with other compensatory pathways. This suggests that during follicular activation, the PI3K pathway not only plays a single initiating role but may also collaborate with mTOR, KIT–KITL, and other local ovarian signals to determine the fate of primordial follicles—whether they become activated or remain dormant. In primordial follicles, upon binding of the KIT ligand to its receptor c-KIT,

the PI3K signaling pathway is activated. Subsequently, PI3K converts phosphatidylinositol 4,5-bisphosphate into phosphatidylinositol (3,4,5)-trisphosphate (PIP3); PIP3 further activates phosphoinositide-dependent kinase 1 (PDK1) and promotes AKT activation. Activated AKT can enter the cell nucleus and phosphorylate forkhead box O3 (FOXO3a), causing FOXO3a to translocate from the nucleus to the cytoplasm, thereby losing its function

in maintaining follicular dormancy. As FOXO3a is a key factor in maintaining the dormant state of primordial follicles, a decrease in its nuclear activity signifies that the dormancy inhibition is lifted, and the primordial follicles begin to enter the activation and growth phases.^{17,31}

Phosphatase and tensin homolog (PTEN) is a key negative regulator in this pathway. Its primary function is to dephosphorylate PIP3 back to phosphatidylinositol 4,5-bisphosphate, thereby inhibiting the PI3K–AKT signaling pathway and maintaining the relative stability of the primordial follicle reserve. In a review on the regulation of primordial follicle activation, Kim³¹ summarized evidence from multiple transgenic mouse models. Oocyte-specific *Pten* deficiency led to excessive primordial follicle activation and premature depletion of the follicular pool. Mice lacking *Foxo3a* also exhibited similar phenotypes of premature primordial follicle activation and infertility, whilst PDK1–AKT–ribosomal protein S6 kinase beta-1 (S6K1)–ribosomal protein S6 (rpS6) signaling was closely associated with the survival and activation of primordial follicles.³¹ This evidence suggests that the PTEN/PI3K/PDK1/AKT/FOXO3a axis not only promotes follicular growth but also regulates the balance between dormancy and activation. The study by Li *et al.*²² provides more direct experimental evidence for the role of the PI3K pathway in IVA. The study involved short-term *in vitro* treatment of neonatal mouse ovaries with PTEN inhibitors and PI3K-activating peptides, revealing that this intervention enhances AKT activity and promotes the efflux of FOXO3 from the nuclei of primordial follicle oocytes, thereby activating dormant primordial follicles. Subsequently, when the treated ovarian tissue was transplanted into ovariectomized host mice, the primordial follicles continued to develop to the pre-ovulatory stage, yielding mature oocytes.²² This study suggests that short-term, targeted stimulation of the PI3K–AKT pathway may convert dormant follicles into follicles capable of continued development, providing important experimental evidence for fertility preservation in patients with POI or diminished ovarian reserve.

However, this technique for activating dormant follicles has not yet been widely adopted. The view that the PI3K pathway activates dormant follicles requires caution. On the one hand, activation of the PI3K–AKT signaling pathway may help residual dormant follicles re-enter the developmental process. On the other hand, prolonged or abnormal activation of this pathway *in vivo* may also lead to excessive mobilization of primordial follicles and premature depletion of the follicular pool. In other words, it serves both as a therapeutic target for IVA technology and as a potential mechanism underlying ovarian reserve

depletion in POI. Huhtaniemi *et al.*³² emphasize that POI exhibits genetic heterogeneity, with multiple genes and pathways already identified as being associated with its pathogenesis; future therapeutic strategies will need to integrate genetic diagnosis, assessment of residual follicular reserve, and individualized fertility preservation protocols.

4.2. Mechanistic target of rapamycin pathway

The mTOR pathway is a key signaling axis that supports cell growth, protein synthesis, and continued follicular development after activation of dormant follicles. The mTOR pathway emphasizes enabling oocytes and primordial follicle granulosa cells to acquire the metabolic and translational capacity required for continued growth. Therefore, within the mechanistic framework of IVA, the mTOR pathway can be understood as a key growth regulator for both “follicular awakening” and “follicular progression.”

Mechanistic target of rapamycin is a serine/threonine protein kinase that functions primarily in the form of two complexes: mTORC1 and mTORC2. mTORC1 is primarily involved in protein synthesis, cell growth, metabolic regulation, and cell proliferation, whilst mTORC2 is more involved in cytoskeletal remodeling, cell survival, and cell cycle regulation. Ersahin *et al.*³⁰ systematically integrated the PI3K/AKT/mTOR signaling network, a complex network that intersects with multiple upstream signals and compensatory pathways and can regulate cell survival, proliferation, growth, and metabolism.³⁰ This characteristic suggests that during follicular activation, the mTOR pathway is not only a downstream effector of PI3K–AKT but may also integrate stimulatory signals from the ovarian microenvironment via nutritional and energy status as well as local growth factor signaling. Within the oocyte, mTORC1 activity is negatively regulated by the TSC1/TSC2 complex. As inhibitors of mTORC1, TSC1/TSC2 help maintain primordial follicles in a relatively quiescent state. Upon activation of AKT, the TSC1/TSC2 complex is inhibited, thereby lifting its suppression of mTORC1 and enhancing mTORC1 activity. Subsequently, mTORC1 promotes phosphorylation of S6K1 and rpS6, enhancing ribosome biogenesis and protein translation capacity and providing the material basis for oocyte enlargement and continued development.^{30,31}

The review noted that mTORC1 is negatively regulated by TSC1/TSC2. When TSC1 or TSC2 is specifically deleted in oocytes, mTORC1 activity increases and promotes protein translation and ribosome synthesis via the S6K1–rpS6 pathway, leading to premature activation of primordial follicles and early depletion of the follicular pool.³¹ Consequently, the mTORC1–S6K1–rpS6 axis not

only supports follicular growth but also participates in maintaining the dynamic equilibrium of the primordial follicle reserve. Adhikari *et al.*³³ provided more direct experimental evidence using a mouse model with oocyte-specific *Tsc1* deficiency. The study found that *Tsc1* deficiency increased mTORC1 activity in oocytes, causing nearly the entire primordial follicle pool to activate prematurely around puberty, followed by follicular depletion and a premature ovarian failure phenotype in early adulthood. More importantly, the mTORC1 inhibitor rapamycin can, to some extent, reverse the excessive activation of primordial follicles caused by *Tsc1* deficiency, restoring the proportion of primordial follicles to levels close to those of the control group.³³ This result supports the view that moderate inhibition of mTORC1 activity helps maintain the dormancy of primordial follicles, whereas elevated mTORC1 activity drives their activation.

Another significant aspect of the mTOR pathway is that it facilitates bidirectional communication between primordial follicle granulosa cells and oocytes. Research by Zhang *et al.*³⁴ demonstrates that primordial follicle granulosa cells are not merely passive structures enveloping the oocyte, but can actively determine the fate of dormant oocytes. The study found that inhibiting mTORC1 signaling in primordial follicle granulosa cells prevents their differentiation into mature granulosa cells and keeps dormant oocytes quiescent. Conversely, overexpression of mTORC1 signaling in primordial follicle granulosa cells accelerates their differentiation and, via KIT ligand, awakens the oocyte, inducing premature activation of the dormant follicle.³⁴ Consequently, mTORC1–KITL signaling in primordial follicle granulosa cells and KIT–PI3K signaling in oocytes form a somatic–germ cell communication network that provides a crucial mechanistic basis for primordial follicle activation. Li *et al.*²² IVA study indirectly supports the continuous role of the PI3K–AKT–mTOR axis in the activation of dormant follicles. The study showed that short-term *in vitro* treatment of ovarian tissue promotes FOXO3 nuclear export from primordial oocytes and primordial follicle activation, resulting in follicles that undergo further development and mature oocytes following transplantation.²² The mTORC1–S6K1–rpS6 pathway, in turn, provides translational and metabolic support for subsequent oocyte growth and follicular development.

4.3. Hippo signaling pathway

The Hippo signaling pathway is key to explaining how “mechanical processing of ovarian tissue induces follicular growth” in IVA. The Hippo pathway places greater emphasis on the roles of ovarian tissue architecture, cell–cell contacts, the cytoskeleton, and mechanical signaling in

follicular activation. The Hippo pathway can be understood as the hub linking “ovarian tissue fragmentation” with the “initiation of follicular growth.”

In normal ovarian tissue, the Hippo signaling pathway helps maintain tissue homeostasis, limit excessive cell proliferation, and regulate organ size. Its core mechanism involves the phosphorylation of Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) via a kinase cascade involving mammalian Ste20-like protein kinase 1/2 and large tumor suppressor homolog 1/2, causing YAP/TAZ to remain in the cytoplasm or be degraded, thereby inhibiting the growth-promoting transcriptional activity they mediate upon nuclear entry. In other words, when the Hippo pathway is active, cell proliferation and tissue growth are restricted. However, when the Hippo pathway is disrupted, YAP/TAZ can enter the cell nucleus and, in collaboration with transcription factors such as TEAD, promote the expression of growth-related genes.

The study by Kawamura *et al.*³⁵ provides direct experimental evidence for the role of the Hippo signaling pathway in IVA. The study found that fragmentation of ovarian tissue induces the polymerization of actin from G-actin to F-actin, thereby disrupting Hippo signaling, reducing YAP phosphorylation levels, and promoting YAP nuclear translocation. Upon nuclear translocation, YAP upregulates CCN-family growth factors and baculoviral IAP repeat-containing-family apoptosis-inhibiting factors, thereby enhancing cell growth, survival, and proliferation and promoting continued follicular development.³⁵ The significance of the study lies in its interpretation of “ovarian tissue fragmentation” as an intervention that promotes follicular growth through a well-defined molecular mechanism. From an IVA perspective, the intervention primarily relies on fragmentation of ovarian cortical tissue. Once ovarian tissue is cut into smaller fragments, contact inhibition between granulosa cells weakens, altering cell morphology and the cytoskeleton; mechanical signals are then transmitted via actin polymerization to the Hippo–YAP/TAZ axis. Subsequently, YAP/TAZ translocates into the nucleus to promote the expression of downstream molecules such as connective tissue growth factor/CCN2, cysteine-rich angiogenic inducer 61/CCN1, and baculoviral IAP repeat-containing proteins, thereby facilitating granulosa cell maturation, cell survival, and follicular growth.³⁵ Consequently, the Hippo signaling pathway serves as the key mechanism by which cells convert mechanical stimuli into growth signals following structural alterations in the ovarian microenvironment.

In studies of human ovarian tissue, De Roo *et al.*³⁶ further observed that the fragmentation of ovarian tissue

in vitro can promote the activation of primordial follicles via the Hippo signaling pathway, and that this process occurs primarily during the early stages of culture. This suggests that the cutting of ovarian tissue itself may alter the fate of follicles toward activation or dormancy, rather than merely improving culture medium permeation or tissue oxygenation.³⁶ This provides more direct human tissue evidence to explain follicular activation following clinical ovarian tissue cryopreservation, transplantation, and fragmentation. During follicular activation, mature granulosa cells are influenced not only by growth factors downstream of YAP/TAZ, but also continue to engage in bidirectional communication with the oocyte via signals such as KITL, GDF9, BMP15, and SMAD.

5. Genetic mechanism and differential diagnosis

To incorporate genetic testing into clinical assessment, it is essential to understand the genetic mechanisms underlying POI. In the following sections, we discuss the various genetic mechanisms and conclude by outlining the clinical implications of genetic testing, genetic counseling, fertility preservation, HRT, and long-term follow-up. We also added a brief summary table of POI pathogenic genes for clinical reference (Table 2).

5.1. Genetic heterogeneity

Primary ovarian insufficiency is a syndrome characterized by significant clinical and genetic heterogeneity. In

his review of clinical practice regarding POI, Nelson¹² emphasized that POI should not be simply understood as “complete ovarian failure” or “premature menopause,” but rather viewed as a continuum of impaired ovarian function. Some patients may still exhibit intermittent ovarian activity and unpredictable residual follicular function following diagnosis. Consequently, when faced with young women presenting with menstrual irregularities, amenorrhea, unexplained hypogonadism, or premature decline in fertility, clinicians should not make a definitive diagnosis based solely on a single serum hormone test or ultrasound scan, but should instead investigate the underlying causes further.

A review by Qin *et al.*²⁵ on the genetics of POI summarized evidence from chromosomal abnormalities, candidate genes, genome-wide association studies, cytogenetic methods, and whole-exome sequencing, concluding that genetic factors play a significant role in POI development. In addition to chromosomal abnormalities, monogenic variants in *FMR1*, *BMP15*, *GDF9*, *FIGLA*, *NOBOX*, *NR5A1*, and *NANOS3* have also been reported to be associated with POI; these genes are involved in key processes such as ovarian development, follicle formation, oocyte maturation, granulosa cell interactions, and endocrine regulation.²⁵ Much of the evidence for these candidate genes stems from small-sample studies, family studies, or specific population cohorts; their pathogenicity and general applicability still require validation through further research across different populations and clinical phenotypes. Consequently, the genetic mechanisms

Table 2. Primary ovarian insufficiency pathogenic genes

Gene category	Key genes	Mechanism	Clinical tip
Follicle development	<i>FMR1</i> , <i>BMP15</i> , <i>GDF9</i>	Disrupt follicle growth and oocyte-granulosa cell signaling	Young diminished ovarian reserve, familial POI
Gonadal steroidogenesis	<i>NR5A1</i>	Ovarian dysplasia, impaired estrogen synthesis	Primary amenorrhea, gonadal dysgenesis
Meiotic chromosome stability	<i>STAG3</i> , <i>HFM1</i>	Meiotic defects induced massive oocyte loss	Early-onset familial POI
DNA damage repair	<i>BRCA2</i>	Defective oocyte genome maintenance	POI or family cancer history
Follicle dormancy control	<i>PTEN</i> , <i>TSC1/2</i> , <i>FOXO3a</i>	Excessive primordial follicle activation	Candidates for IVA treatment

Note: Genetic screening of these genes is recommended for unexplained POI. Carriers of *PTEN*/*TSC* variants have a higher risk of excessive primordial follicle activation after IVA intervention, requiring personalized fertility management.

Abbreviations: IVA: *In vitro* activation; POI: Primary ovarian insufficiency.

underlying POI should be understood within a multi-level molecular pathological framework. In some patients, chromosomal abnormalities may be the primary cause; in others, monogenic mutations may cause the condition; and in others, it may result from the combined influence of polygenic susceptibility, environmental factors, immunological factors, and endocrine status.

Genetic factors associated with POI can be broadly categorized into several groups. The first group comprises chromosomal abnormalities and X-linked factors, such as *FMR1* premutation and X-chromosomal structural abnormalities; these factors may affect the maintenance of the follicular reserve and oocyte survival. The second group includes genes associated with follicular development, such as *BMP15*, *GDF9*, *FIGLA*, and *NOBOX*; these genes can influence signaling between oocytes and granulosa cells, thereby disrupting follicular maturation. The third category includes genes that regulate gonadal development and steroidogenesis, such as *NR5A1*; abnormalities in these genes may affect ovarian development, regulation of the hypothalamic–pituitary–gonadal axis, and hormone synthesis. The fourth category comprises genes associated with meiosis, chromosome stability, and DNA damage repair, such as *STAG3*, *HFM1*, *MCM8*, *MCM9*, and *BRCA2*. Abnormalities in these genes may lead to oocyte development failure, abnormal chromosome segregation, impaired DNA repair, and premature depletion of the follicular pool.

Huhtaniemi *et al.*³² further noted that, with advances in next-generation sequencing technologies and animal models, an increasing number of genes and molecular pathways associated with POI are being identified, suggesting that the genetic architecture of POI is far more complex than traditional clinical diagnosis can reveal. Certain genetic defects not only relate to ovarian function itself but may also influence patients' susceptibility to cancer, the rate of decline in residual follicular reserve, the timing of fertility preservation, and long-term health management.³² Therefore, genetic testing for POI should support etiological stratification, genetic counseling, reproductive planning, and personalized treatment decisions.

5.2. *NR5A1*

NR5A1 is one of the most representative genes involved in regulating gonadal development within the genetic mechanisms underlying premature ovarian insufficiency. *NR5A1*, also known as steroidogenic factor 1 (SF-1), encodes a nuclear receptor transcription factor that regulates the expression of genes related to the hypothalamic–pituitary–gonadal axis, gonadal development, and steroid hormone

synthesis. Consequently, variants in *NR5A1* may affect gonadogenesis, ovarian development, steroid production, sex hormone levels, and reproductive endocrine homeostasis.

The study by Lourenço *et al.*³⁷ represents one of the most significant pieces of original evidence regarding the relationship between *NR5A1* and POI. The study performed *NR5A1* sequencing in four families with a history of both disorders of sex development and POI, as well as 25 patients with sporadic POI. The results showed that affected members in the four families, as well as two of the 25 sporadic POI cases, carried *NR5A1* variants, including frameshift, missense, and in-frame deletion mutations. Functional assays further indicated that these variants significantly impair *NR5A1* transcriptional activation. The study therefore proposes that *NR5A1* mutations are associated not only with disorders of sex development but also with POI.³⁷ This finding has significant clinical implications. Firstly, it suggests that the etiology of some POI cases may not lie within the ovaries themselves, but rather in abnormalities in the regulation of gonadal development and steroidogenesis at a more upstream level. Second, *NR5A1*-associated phenotypes show marked variability: the same genetic variant may manifest as disorders of sex development, POI, gonadal dysgenesis, pubertal development abnormalities, primary amenorrhea, or secondary amenorrhea in different individuals. This phenotypic breadth indicates that *NR5A1* cannot be simply understood as a “disease-causing gene for a single, fixed condition,” but should instead be regarded as a key regulatory node influencing reproductive development and reproductive endocrine homeostasis.

Allali *et al.*³⁸ further expanded the spectrum of *NR5A1*-associated phenotypes. In this study, *NR5A1* mutation analysis was performed in 77 patients with disorders of sex development, and five cases carried heterozygous *NR5A1* variants, accounting for 6.5%. These patients had external genital malformation and hypospadias; functional experiments showed that some mutant proteins had reduced transcriptional activation capacity and impaired ability to co-activate the anti-Müllerian hormone (AMH) promoter with *GATA4*.³⁸ Although the study focused primarily on disorders of sex development and cannot be directly cited as core evidence for POI, it supports a key hypothesis. *NR5A1* variants are associated with a wide range of reproductive developmental phenotypes and can cause varying degrees of reproductive endocrine abnormalities by affecting gonadal development, AMH regulation, and steroidogenesis pathways.

The study by Fabbri-Scallet *et al.*³⁹ suggests that variants in the non-coding region of *NR5A1* also warrant

attention. Through Sanger sequencing, *in vitro* functional assays, and whole-exome sequencing, the study identified variants in the non-coding region of *NR5A1* in patients with disorders of sex development and observed that these variants can affect promoter activity.³⁹ This indicates that the pathophysiological mechanisms of *NR5A1*-related disorders are not limited to missense, frameshift, or truncation mutations in the coding region; variants in regulatory regions may also influence gonadal development and reproductive endocrine function by altering gene expression levels.

NR5A1 variants may contribute to POI through multiple mechanisms. First, *NR5A1* influences gonadal development, and pathogenic variants may lead to ovarian hypoplasia or abnormalities in gonadal tissue structure. Secondly, *NR5A1* regulates the expression of genes involved in steroidogenesis; variants may affect estrogen synthesis, the follicular microenvironment, and feedback within the hypothalamic–pituitary–ovarian axis. Third, *NR5A1* can influence the expression of reproductive endocrine-related molecules such as AMH, inhibin subunit alpha, cytochrome P450 family 19 subfamily A member 1, and steroidogenic acute regulatory protein, thereby disrupting follicular recruitment, follicular maturation, and the maintenance of ovarian reserve. Fourth, *NR5A1*-associated phenotypes exhibit incomplete penetrance and variable expression; consequently, patients may present solely with menstrual irregularities or diminished ovarian function, without necessarily exhibiting obvious abnormalities of the external genitalia or pubertal development. In young women with POI, *NR5A1* helps clinicians identify abnormalities in the regulation of gonadal development and steroidogenesis. For patients presenting with primary amenorrhea, abnormalities in pubertal development, early-onset secondary amenorrhea, ovarian hypoplasia, a family history of disorders of sex development or early-onset ovarian insufficiency, or cases where conventional hormonal and imaging investigations fail to fully explain the clinical presentation, consideration should be given to including *NR5A1* in the scope of genetic testing and genetic counseling.

5.3. *HFM1* and *STAG3*

Any genetic defect affecting homologous chromosome pairing, recombination, sister chromatid adhesion, DNA repair, or chromosome segregation may lead to early oocyte loss, insufficient follicle pool formation, or premature depletion of ovarian reserve. *HFM1* and *STAG3* are representative genes within this mechanistic module.

HFM1 is closely associated with meiotic homologous recombination and encodes an ATP-dependent DNA

helicase homolog. This gene is involved in homologous recombination and maintaining DNA structural stability during meiosis. If *HFM1* function is impaired, oocytes may be unable to complete homologous chromosome exchange and recombination normally, ultimately leading to impaired oocyte survival and reduced ovarian reserve. Zhe *et al.*⁴⁰ performed whole-exome sequencing on a Chinese POI family and validated candidate variants via Sanger sequencing. The study found that both the proband and her affected mother carried a heterozygous *HFM1* (c.3470G>A) variant, whereas unaffected family members did not carry this variant. Further splicing predictions and minigene experiments confirmed that this variant altered the splicing pattern of *HFM1* mRNA, producing abnormal transcripts distinct from the wild-type. The study suggests that *HFM1* variants may contribute to the pathogenesis of POI by affecting splicing and meiosis-related functions.⁴⁰ The value of this evidence lies in the fact that it not only reports a candidate variant but also supports the variant's impact on RNA splicing through functional experiments.

STAG3 represents another class of POI mechanisms associated with chromosome adhesion and separation. *STAG3* encodes a meiosis-specific subunit of the cohesin complex. The cohesin complex maintains sister chromatid adhesion during meiosis and is involved in homologous chromosome pairing, recombination, and correct segregation. If *STAG3* is functionally defective, chromosome structural stability during oocyte meiosis will be compromised, potentially leading to arrested oocyte development, cell death, and depletion of the follicular reserve. The study by Caburet *et al.*⁴¹ provides key primary evidence for the relationship between *STAG3* and POI. The study performed whole-exome sequencing on a patient with hereditary premature ovarian failure from a consanguineous large family and identified a homozygous 1-bp deletion in the *STAG3* gene, resulting in a frameshift mutation. As *STAG3* is a key component of the meiosis-specific cohesin ring, this mutation disrupts sister chromatid adhesion and chromosome stability, thereby explaining the mechanism underlying the premature ovarian failure observed in this family.⁴¹ In their review of the genetics of POI, Qin *et al.*²⁵ noted that with the application of whole-exome sequencing in families with non-syndromic POI, genes associated with meiosis, chromosome stability and DNA repair—such as *STAG3*, *SYCE1*, *MCM8*, *MCM9*, and *HFM1*, have been progressively identified.²⁵ The significance of the review lies in its placement of *HFM1* and *STAG3* within a broader genetic context. This implies that POI is not merely a result of abnormal follicular development signaling, but may also result from early failure in oocytes during meiosis and chromosome maintenance.

HFM1 and *STAG3* may represent two interrelated yet distinct molecular processes in POI. *HFM1* is more closely associated with homologous recombination and DNA unwinding, suggesting that oocytes require normal recombination and splicing regulation to maintain meiosis stability; *STAG3* is more closely associated with cohesin-mediated sister chromatid adhesion and chromosome segregation, suggesting that chromosomal structural stability is crucial for oocyte survival. These findings suggest that the genetic mechanisms underlying POI may occur earlier than follicular maturation, namely during oocyte formation, maintenance, and meiotic quality control. For patients with primary amenorrhea, early-onset secondary amenorrhea, familial clustering of POI, rapid decline in ovarian reserve, or cases unexplained by routine endocrine and imaging investigations, consideration should be given to the inclusion of *HFM1*, *STAG3*, and other genes associated with meiosis and chromosomal stability in the scope of genetic testing. If abnormalities in these genes are identified, the clinical interpretation should not be limited to “declining ovarian function,” but should further suggest that the patient may have underlying defects in oocyte quality control, chromosomal stability, or follicular reserve formation.

5.4. *BRCA2*

Defects in DNA damage repair represent a crucial yet often overlooked category within the genetic mechanisms underlying POI. Whilst *BRCA2* is typically the focus of attention due to its association with susceptibility to breast cancer, ovarian cancer, and other tumors, its biological functions are not limited to tumor suppression. *BRCA2* is also a key molecule in maintaining oocyte genomic stability, meiosis, recombination, and the repair of double-strand DNA breaks.

The study by Weinberg-Shukron *et al.*⁴² provides key original evidence for the relationship between *BRCA2* and ovarian development and function. The study reported on two sisters with ovarian hypoplasia who carried compound heterozygous truncating mutations in *BRCA2*. These mutations led to reduced *BRCA2* protein levels and impaired the DNA damage response. Functional experiments showed increased chromosome breaks in the patients' cells, and *RAD51* could not be recruited effectively to DNA double-strand break sites. As *RAD51* recruitment is a critical step in homologous recombination repair, these findings suggest that *BRCA2* defects may affect ovarian development and oocyte survival by disrupting the repair of DNA double-strand breaks and the process of meiotic recombination.⁴² The significance of this study lies in its expansion of the role of *BRCA2* from a traditional “tumor susceptibility gene” to a “gene regulating

ovarian development and oocyte quality control.” Oocytes must undergo complex processes of meiosis, DNA recombination, and maintenance of chromosomal stability during their formation and maintenance. If *BRCA2* function is impaired, oocytes may be unable to repair DNA damage effectively, leading to chromosomal instability, cell death, or insufficient follicle pool formation. Consequently, the etiology of some cases of POI or ovarian hypoplasia may not be solely due to hormonal abnormalities, but rather stem from earlier failures in germline genomic maintenance.

Weinberg-Shukron *et al.*⁴² further supported this mechanism using animal models. In their study, fruit fly models with a deletion of the *BRCA2* homolog exhibited infertility and gonadal dysgenesis, indicating that *BRCA2*-related DNA repair mechanisms play a conserved role in gonadal development. This evidence strengthens the causal link between *BRCA2* and ovarian developmental abnormalities and suggests that DNA repair-related genes should be incorporated into the key functional modules of the genetic mechanisms underlying POI. Similar to *HFM1* and *STAG3*, *BRCA2* is also a key gene involved in maintaining oocyte chromosomal stability. However, the three genes have different focuses: *HFM1* is more involved in homologous recombination and DNA unwinding processes, *STAG3* is more involved in cohesin-mediated sister chromatid adhesion and chromosome separation, whilst *BRCA2* is primarily involved in homologous recombination repair following double-strand breaks and the recruitment of *RAD51*. These findings suggest that the genetic basis of POI may lie in deeper mechanisms of oocyte development and quality control during meiosis, rather than solely in follicle recruitment or hormone secretion.

Although the study by Ludman *et al.*⁴³ does not directly prove that *BRCA2* causes POI, it provides valuable insights for *BRCA2*-related genetic counseling. The study reported recurrent pathogenic *BRCA2* variants in the Ethiopian Jewish population, including variants such as c.5159C>A (p.Ser1720Ter) and c.7579delG (p.Val2527Ter), and discussed the value of incorporating these recurrent variants into population-specific screening panels.⁴³ When pathogenic *BRCA2* variants are detected in patients with POI, clinical focus should not be limited to ovarian function and fertility, but should also consider familial cancer risk, genetic counseling, long-term monitoring, and screening of relatives.

BRCA2 testing has dual clinical significance. Firstly, it may help explain the etiology in some patients with primary amenorrhea, ovarian hypoplasia, or premature ovarian insufficiency. Second, it may reveal potential tumor

susceptibility risks in the patient and their family, thereby influencing long-term follow-up, risk management, and reproductive decisions. In particular, for patients presenting with ovarian hypoplasia, primary amenorrhea, a family history of cancer, a personal or family history of *BRCA2*-associated cancers, such as breast, ovarian, pancreatic, or prostate cancer, or unexplained premature ovarian failure, *BRCA2* and other DNA repair-related genes should be included in the scope of genetic evaluation.

5.5. Mimics of genetic primary ovarian insufficiency

When discussing the genetic mechanisms underlying POI, it is important to emphasize that not all cases of amenorrhea, oligomenorrhea, or low estrogen levels in young women are attributable to genetic POI. In some patients, clinical presentations may resemble POI, but the true etiology stems from drug exposure, nutritional or metabolic abnormalities, chronic stress, weight fluctuations, excessive exercise, suppression of the hypothalamic–pituitary–ovarian axis, endocrine tumors, or autoimmune diseases. Such conditions can clinically “mimic” genetic POI; if not distinguished, this can easily lead to misdiagnosis, overuse of genetic testing, or inappropriate treatment pathways.

Drug-induced ovarian dysfunction is a category of acquired factors that requires identification. Christopoulos *et al.*⁴⁴ reported a case of imatinib-associated POI, suggesting that tyrosine kinase inhibitors may affect ovarian function in specific contexts. However, such evidence is primarily derived from case reports and cannot be directly equated with universal genetic mechanisms. Roskoski's⁴⁵ review of the erythroblastic oncogene B/ human epidermal growth factor receptor protein tyrosine kinase family and cancer-related pathways illustrates the biological significance of tyrosine kinase signaling in cell proliferation, survival, and tumor therapy,⁴⁵ but it does not directly prove a genetic etiology for POI. Therefore, a more appropriate approach would be to place imatinib-associated POI within the differential diagnosis of “drug-induced or iatrogenic ovarian dysfunction,” rather than within the core evidence chain for genetic mechanisms.

Psychological factors also warrant attention. Menstrual irregularities in young women are often accompanied by anxiety, depression, mood swings, or work-related stress; consequently, in clinical practice, amenorrhea is frequently attributed simply to “excessive stress” or “emotional issues.” The review by Martinowich *et al.*⁴⁶ on brain-derived neurotrophic factor, depression, and anxiety helps to explain that emotional symptoms have a neurobiological basis, but it does not directly prove that emotional disorders lead to POI. Anxiety, depression, and stress may be associated with menstrual irregularities, inhibition of the

hypothalamic–pituitary–ovarian axis, or delayed medical consultation; however, they cannot replace a systematic assessment for POI, functional hypothalamic amenorrhea, endocrine disorders, and genetic causes.

Metabolic abnormalities represent another important category of acquired mechanisms. A study by Guo *et al.*⁴⁷ demonstrated that branched-chain amino acid deficiency can impair ovarian granulosa cell function through abnormal activation of the ceramide–reactive oxygen species axis and induce a POI phenotype in animal models. The study suggests that declining ovarian function does not necessarily stem solely from chromosomal abnormalities or monogenic mutations, but may also involve nutritional metabolism, oxidative stress, and granulosa cell damage. Compared with genetic POI, metabolic ovarian damage places greater emphasis on the influence of external nutritional status, energy metabolism and oxidative stress on the ovarian microenvironment. Therefore, in young patients, metabolic factors should be included in the differential diagnosis if dietary restrictions, significant weight changes, malnutrition, or metabolic abnormalities are present.

Functional hypothalamic amenorrhea is one of the conditions most frequently confused with POI. In a clinical practice review, Gordon⁴⁸ noted that functional hypothalamic amenorrhea is typically associated with stress, weight loss, inadequate energy intake, or excessive exercise; its core mechanism involves suppression of pulsatile gonadotropin-releasing hormone secretion from the hypothalamus, leading to reduced levels of luteinizing hormone, follicle-stimulating hormone, and estrogen.^{48,49} POI is typically characterized by compensatory elevation of pituitary gonadotropins following impaired ovarian function, whereas functional hypothalamic amenorrhea is more central in nature, often presenting with low estrogen levels accompanied by low or normal gonadotropin levels. Therefore, although both conditions may present as amenorrhea and a state of low estrogen, their etiological levels, treatment objectives, and fertility management strategies differ.

Germain *et al.*⁵⁰ conducted a study on patients with anorexia nervosa who continued to experience amenorrhea despite weight restoration. They found that these patients exhibited a higher estradiol response to pulsed gonadotropin-releasing hormone therapy, suggesting that their hypothalamic–pituitary–ovarian axis may still be reversible.⁵⁰ Treatment for functional hypothalamic amenorrhea typically focuses on restoring energy balance, body weight, psychological well-being, and pulsatile gonadotropin-releasing hormone function, whereas treatment for genetic POI focuses on stratifying etiology,

long-term management of low estrogen levels, fertility preservation, and genetic counseling. Confusing the two conditions can lead to misguided clinical management.

Endocrine tumors and systemic endocrine disorders may also present with clinical features similar to those of POI. A case report by Kimball *et al.*⁵¹ demonstrated that complex endocrine disorders, such as adrenal cortical tumors, may underlie fatigue, weight gain, and abdominal symptoms in young women. The case of chronic primary adrenal insufficiency reported by Yoshiji *et al.*⁵² also suggests that abnormalities in the adrenal axis may persist undetected for long periods and lead to non-specific symptoms. These cases do not demonstrate the genetic mechanisms of POI, but remind clinicians that when young women present with amenorrhea, fatigue, weight changes, mood disturbances, or hypohormonemia, they should be assessed from a more comprehensive endocrine perspective rather than focusing solely on the ovaries.

Autoimmune-related endocrine syndromes should also be considered. Some patients with POI may have concomitant autoimmune thyroid disease, adrenal dysfunction, or other autoimmune endocrine disorders. In cases where conventional hormonal, imaging, and genetic testing fail to provide an explanation, thyroid autoantibodies, adrenal-related antibodies, and other autoimmune markers may offer additional value.³² The significance of such assessments lies in identifying systemic causes that are monitorable, amenable to intervention, or requiring long-term follow-up, rather than simply classifying all cases of POI as irreversible ovarian failure.

5.6. Clinical implications

Based on the above analysis of the genetic heterogeneity of POI, representative pathogenic genes, and acquired and functional disease models, amenorrhea or diminished ovarian reserve in young women cannot be attributed to a single cause. Genes such as *NR5A1*, *HFM1*, *STAG3*, and *BRCA2* suggest that POI may arise from gonadal dysgenesis, meiosis defects, compromised chromosomal stability, or defects in DNA damage repair; whereas drug exposure, nutritional and metabolic abnormalities, functional hypothalamic amenorrhea, endocrine tumors, and autoimmune factors suggest that, in some patients, clinical presentations may resemble those of genetic POI, yet the etiology and treatment pathways are entirely different. Based on this understanding, the clinical management of POI in young women should shift from traditional health management toward a comprehensive approach. Specifically, clinicians must first determine whether the patient truly has ovarian-derived functional decline. Second, they must distinguish between genetic POI

and functional, acquired, or systemic endocrine disorders; and subsequently, based on the patient's age, menstrual history, family history, hormone levels, ovarian reserve, fertility needs, and genetic testing results, decide whether to proceed with genetic counseling, fertility preservation, referral for assisted reproduction, HRT, and long-term follow-up. The clinical significance of this approach lies in avoiding the simple attribution of menstrual irregularities in young women to stress or emotional factors, which could lead to a missed diagnosis of POI; it also prevents the over-interpretation of all cases of amenorrhea as hereditary ovarian failure, thereby overlooking reversible or treatable causes.

First, genetic testing should stratify causes rather than serve as routine screening for all patients. Genetic evaluation should be prioritized for patients with primary amenorrhea, early-onset secondary amenorrhea, familial clustering of POI, rapid decline in ovarian function, cases not explained by routine hormonal and imaging tests, and those with abnormalities in pubertal development or concomitant atypical endocrine manifestations. Testing may include karyotyping, *FMR1* premutation testing with CGG repeat expansion analysis, chromosomal microarray analysis, and targeted gene panel sequencing or whole-exome sequencing for POI-associated genes. Huhtaniemi *et al.*³² emphasize that the molecular pathology and genetic background of POI are highly complex; whilst next-generation sequencing technologies have expanded our understanding of POI-associated genes and pathways, a significant proportion of patients remain without a clear aetiology.³²

Second, genetic counseling should be provided both before and after genetic testing. Before testing, patients need to understand the types of results genetic testing may yield, including pathogenic variants, likely pathogenic variants, variants of uncertain significance, and negative results. A negative result does not completely rule out a genetic cause, as current testing technologies and the ability to interpret genetic data remain limited. Following testing, if mutations in *FMR1*, *NR5A1*, *HFM1*, *STAG3*, *BRCA2*, *MCM8*, *MCM9*, or other POI-associated genes are identified, the implications for the patient, fertility plans, risk to relatives, and long-term health management should be further assessed. For patients with a clear genetic etiology or non-iatrogenic POI, female relatives may face a higher risk.

Third, genetic testing results can influence fertility preservation strategies. Fertility management for patients with POI should not be limited to the question of "whether natural conception is possible," but should involve an early assessment of residual ovarian reserve and the

fertility window. For patients whose follicular reserve has not yet been completely depleted, who still have residual follicular activity, or who are at high risk but have not yet developed POI, options such as oocyte cryopreservation, embryo cryopreservation, ovarian tissue cryopreservation, or assisted reproductive techniques may be discussed on a case-by-case basis. For high-risk groups of iatrogenic POI, such as patients about to undergo gonadotoxic chemotherapy, radiotherapy, or ovarian surgery, fertility preservation should be completed prior to treatment wherever possible. For patients with confirmed POI and significantly depleted follicular pools, opportunities for autologous oocyte retrieval may be limited; oocyte donation, embryo donation, or other reproductive options may represent more realistic pathways.

Fourth, HRT is a fundamental treatment that cannot be overlooked in the long-term management of POI. The low estrogen status in POI patients may also increase the risk of bone loss, osteoporosis, cardiovascular complications, and a reduced quality of life. Machura *et al.*⁵³ emphasize that HRT in POI patients is not merely symptomatic treatment, but also a crucial management strategy for preventing the long-term consequences of chronic estrogen deficiency.

Fifth, a clear distinction must be made between HRT and fertility management. Whilst HRT can improve low estrogen levels and reduce long-term health risks, it cannot restore an already depleted ovarian reserve, nor can it replace assisted reproductive technology. Patients who still wish to conceive should be referred to a reproductive medicine specialist at the earliest opportunity to assess residual ovarian function, the likelihood of natural conception, the response to ovarian stimulation, the possibility of oocyte or embryo cryopreservation, and whether options such as oocyte donation are required. Furthermore, if a patient carries mutations in DNA repair-related genes such as *BRCA2*, cancer risk assessment, family screening, and fertility decisions should be integrated within the same genetic counseling framework. In other words, fertility management should not focus solely on ovarian function itself, but should also take into account genetic risk, family history, and the patient's long-term health.

Sixth, the management of POI should emphasize a multidisciplinary approach and long-term follow-up. Patients with genetic POI may require the joint involvement of gynecology, reproductive medicine, endocrinology, genetic counseling, psychological support, and, where necessary, assessment of genetic cancer risk. Follow-up should include monitoring menstrual and symptomatic changes, HRT adherence and safety, bone density, cardiovascular risk factors, lipid and glucose levels,

thyroid- and adrenal-related autoimmune risks, and mental and sexual health. For younger patients, communication regarding the diagnosis is particularly important.

Autoimmune and systemic endocrine disorders should also be incorporated into the long-term management framework. Some patients with POI may present with concomitant autoimmune thyroid disease, adrenal dysfunction, or other autoimmune endocrine disorders. In cases that cannot be explained by routine hormonal, imaging, or genetic testing, assessment of thyroid function and autoantibodies, adrenal markers, and other autoimmune indicators may provide additional value. The significance of such assessments lies in identifying systemic causes that are monitorable, amenable to intervention, or requiring long-term follow-up, rather than simply classifying all cases of POI as irreversible ovarian failure. Particularly in younger patients, if amenorrhea is accompanied by fatigue, weight changes, skin pigmentation changes, hypotension, thyroid symptoms, or other systemic manifestations, further endocrine and immunological evaluations should be undertaken.

Finally, the clinical management of POI should also prioritize psychological support and patient education. When young women are diagnosed with POI, they often face concurrent anxieties regarding fertility, a sense of physical aging, relationship pressures, and uncertainty about the future. If clinicians focus solely on hormonal markers whilst neglecting patients' concerns about fertility, marriage, body image, and long-term health, both treatment adherence and the quality of follow-up care may be compromised.

6. Conclusion

Primary ovarian insufficiency is a heterogeneous condition that should not be simply attributed to stress or menstrual irregularity in young women. Genetic factors, follicular activation pathways, and functional or acquired mimics should be considered during clinical assessment. Early diagnosis, appropriate differential evaluation, reproductive counseling, HRT when indicated, and long-term follow-up are important for improving patient management.

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