Trends in Endocrinology & Metabolism

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Review

Molecular Genetics of Premature Ovarian Insufficiency

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Premature ovarian insufficiency (POI) is highly heterogeneous in genetic etiology. Yet identifying causative genes has been challenging with candidate gene approaches. Recent approaches using next generation sequencing (NGS), especially whole exome sequencing (WES), in large POI pedigrees have identified new causatives and proposed relevant candidates, mainly enriched in DNA damage repair, homologous recombination, and meiosis. In the near future, NGS or whole genome sequencing will help better define genes involved in intricate regulatory networks. The research into miRNA and age at menopause represents an emerging field that will help unveil the molecular mechanisms underlying pathogenesis of POI. Shedding light on the genetic architecture is important in interpreting pathogenesis of POI, and will facilitate risk prediction for POI.

Highlights

The traditional candidate gene approaches, although critical in establishing the field of POI genetics, have been proceeding slowly.

Recent advances of NGS, especially WES in large POI pedigrees, have revealed new causative genes, with a preponderance for DNA damage repair and homologous recombination.

The insights from GWAS on age of menopause and miRNA research will facilitate identification of new causative genes or signaling pathways for POI.

Targeted panels of causative genes, or WES/whole genome sequencing in the future are promising in risk prediction for POI; however, the causative role of identified variants should be elucidated.

Current Status of Premature Ovarian Insufficiency (POI) Genetics

POI, also termed premature ovarian failure (POF), is characterized by cessation of menstruation before the age of 40 years. Although no accurate epidemiological data exists, approximately 1% of women are affected by the age of 40 [1]. POI can be isolated (nonsyndromic), or as one component of a pleiotropic genetic syndrome. The disorder is highly heterogeneous in etiology. It can result from a small pool of primordial follicles, disturbances of follicle function, and premature follicle depletion due to accelerated atresia. A wide spectrum of causes has been considered, including genetic, autoimmune, infectious, or iatrogenic. Irrespectively, the majority remains to be elucidated. Genetic causes account for approximately 20–25% of patients [2]. Chromosomal abnormalities have long been considered as a component of POI etiology, and could explain 10–15% of POI cases [3]. Yet identifying precise causative genes for POI has been challenging. Over the past few decades, numerous candidate genes emerged but few were incorporated as causative with functional validation. Very recent approaches using **next generation sequencing (NGS)** (see Glossary), especially whole exome sequencing (WES) in large POI pedigrees, have led to new causatives being identified and candidates being proposed. Although lists are expanding, it is just the tip of the iceberg of POI genetics. In addition, the fact that no single underlying dominant gene deficiency could explain the disorder further supports the genetic heterogeneity of POI etiology. Here, we focus on NGS studies in nonsyndromic POI, and review the currently causative genes for POI. Moreover, we propose the future direction for new genes/mechanisms from the perspective of age at menopause and miRNA.

Causative Genes by Candidate Gene Approach

The hypothesis-driven candidate gene approach is based on the gene expression and function in **folliculogenesis** or ovarian development, or phenotypes of murine knockout models. Over the past decades, many genes have emerged as POI candidates, but only a minority have been proven causative by functional validation. These include genes involved in primordial germ cell

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migration and proliferation (*NANOS3*), in cell death (*PGRMC1* and *FMR1*), oocyte specific transcription factors (*FIGLA* and *NOBOX*), other transcription factors affecting folliculogenesis (*NR5A1*, *WT1*, and *FOXL2*), transforming growth factor-β superfamily (*BMP15* and *GDF9*) and hormone and receptors (*FSHR*, *AMH*, and *AMHR2)* [2,4,5] (Table 1 and Figure 1A). None,

Table 1. Causative Genes Identified by Candidate Gene Approach in Sporadic POI

Genes	Mutation rate (%)	Pathogenic mechanism of mutations
BMP15 (Xp11.2)	1.0–10.5	Impair transcriptional activity of *BMP15*, impair mature protein production and ability to synergize with *GDF9*, and inhibit granulosa cell proliferation.
PGRMC1 (Xq22-q24)	0.5–1.5	Attenuate ability to transduce progesterone's antiapoptotic action in granulosa cells, and reduce binding capacity to *CYP7A1*.
FMR1 (Xq27)	0.5–6.7 (permutation rate)	Lead to elevated *FMR1* mRNA levels but decreased protein level; increased levels of expanded mRNA may produce function toxicity and result in follicle impairment and atresia.
FIGLA (2p13.3)	0.5–2.0	Disrupt transcriptional activity on E-box-containing promoter and reduce binding capacity to TCF3 helix–loop–helix (HLH) domain.
FSHR (2p21-p16)	0.1–42.3	Reduce binding capacity and signal transduction, compromise response to FSH stimulation.
FOXL2 (3q23)	1.0–2.9	Impair transcriptional repression activity on target genes involved in granulosa cell steroidogenesis and proliferation (*CYP19, CYP11A1, StAR, CCND2*).
GDF9 (5q31.1)	0.5–4.7	Disrupt proper structure formation and mature protein production, and inhibit granulosa cell proliferation.
NOBOX (7q35)	1.0–8.0	Perturb nuclear localization and autophagosomal degradation, induce protein aggregates, impair transcriptional activity on target genes (*NOBOX* DNA-binding element, *GDF9, OCT4, KIT-L*, etc.), lose function on G2/M arrest induction.
NR5A1 (9q33)	0.3–2.3	Impair transcriptional activity on target genes (*CYP11A1*, *CYP19A1*, *AMH*, *INHIBIN-A*, etc.).
WT1 (11p13)	0.5	Decrease expression of *AMH* and *CDH1*, and increase expression of *FSHR* and *CYP19*. Impair granulosa cell differentiation and oocyte–granulosa cell interaction, resulting in loss of follicles.
AMHR2 (12q13)	1.0–2.4	Compromise protein stability and result in hypomorph, disrupt AMH signal transduction, and impair apoptosis repression.
NANOS3 (19p13.12)	1.0–2.4	Lead to rapid degradation of mutant protein and impair primordial germ cell maintenance.
AMH (19p13.3)	2.0	Impair transcriptional activity on *AMHR2*.

AMH, Anti-Mullerian hormone; *AMHR2*, anti-Mullerian hormone receptor, type II; *BMP15*, bone morphogenetic protein 15; *CCND2*, cyclin D2; *CDH1*, cadherin 1; *CYP7A1*, cytochrome P450 family 7 subfamily a member 1; *CYP11A1*, cytochrome p450 family 11 subfamily a member 1; *CYP19*, cytochrome P450 family 19; *CYP19A1*, cytochrome P450 family 19 subfamily a member 1; *FIGLA*, folliculogenesis-specific basic helix–loop–helix transcription factor; *FMR1*, fragile X mental retardation 1; *FOXL2*, forkhead box L2; FSH, follicle-stimulating hormone; *FSHR*, follicle-stimulating hormone receptor; *GDF9*, growth differentiation factor 9; *INHIBIN-A*, inhibin alpha subunit; *KIT-L*, kit ligand; *NANOS3*, nanos homolog 3; *NOBOX*, newborn ovary homeobox gene; *NR5A1*, nuclear receptor subfamily 5, group A, member 1; *OCT4*, octamer-binding protein 4; *PGRMC1*, progesterone receptor membrane component 1; *StAR*, steroidogenic acute regulatory protein; *WT1*, Wilms tumor 1.

Glossary

Cohesin: a complex holding sister chromatids together until the onset of anaphase. It is essential for mitosis and meiosis, and contributes to DNA repair and homologous recombination.

DNA repair: a process used to recognize and correct DNA damage induced by normal cellular processes or environmental factors, maintaining the integrity of genetic information. The mechanisms of DNA repair include direct, excision, mismatch, recombination, and SOS repair.

Early menopause (EM): permanent cessation of menses in women aged between 40 and 45 years.

Folliculogenesis: a process of ovarian follicle development and maturation from the primordial follicle to the preovulatory follicle.

Genome-wide association study (GWAS): a study of assessing a genome-wide set of genetic variants in different subjects to identify the associations between variations and a given trait.

Next generation sequencing (NGS): a high-throughput sequencing technique that enables millions or billions of DNA strands to be sequenced in parallel, yielding large quantities of data. When applied to genome sequencing, NGS includes whole exome sequencing (WES; sequencing all the protein-coding genes in a genome), and whole genome sequencing (WGS; sequencing the complete DNA sequence of a genome), simultaneously.

Noncoding RNAs (ncRNAs): functional RNA molecules that are not translated into proteins, and are important for gene expression regulation and epigenetic modifications. The epigenetic ncRNAs include miRNA, siRNA, long noncoding RNA (lncRNA), and circular RNA (circRNA).

Oogenesis: a differentiation process into a mature oocyte, competent to further development when fertilized. It begins when the primordial germ cells migrate into the embryonic gonad and become oogonia.

Primary amenorrhea: no spontaneous development of secondary sexual characteristics and menarche by the age of 14 years; or normal secondary sexual

however, has been consistently replicated due to limited sample size or ethnicity heterogeneity. No single underlying dominant deficiency could explain the disorder, further supporting its heterogeneity in etiology. In fact, no gene is implicated in more than 5% of cases, except for *BMP15*, *FMR1*, and *NOBOX* [6–8] (Table 1). In addition, inconsistent with homozygous pathogenicity in mice, most variants identified in human POI are heterozygous. In the future, replication in independent cohorts, inclusion of multiple family members, and functional validation are warranted to corroborate their causal relevance to POI and confirm the inheritance pattern. Although progressing slowly, the candidate gene approach has indeed contributed fruitful genetic variants and established the fundamental field of POI genetics. Nonetheless, it falls short of identifying novel or less well-known genes based on *a priori*

characteristics with no menarche by 16 years old.

Secondary amenorrhea: cessation of menstrual cycles for three periods or 6 months after spontaneous menarche.

Synaptonemal complex: a structure between homologous chromosomes to stabilize synapsis at meiotic prophase I. It consists of two lateral elements that align each homolog and the central element.

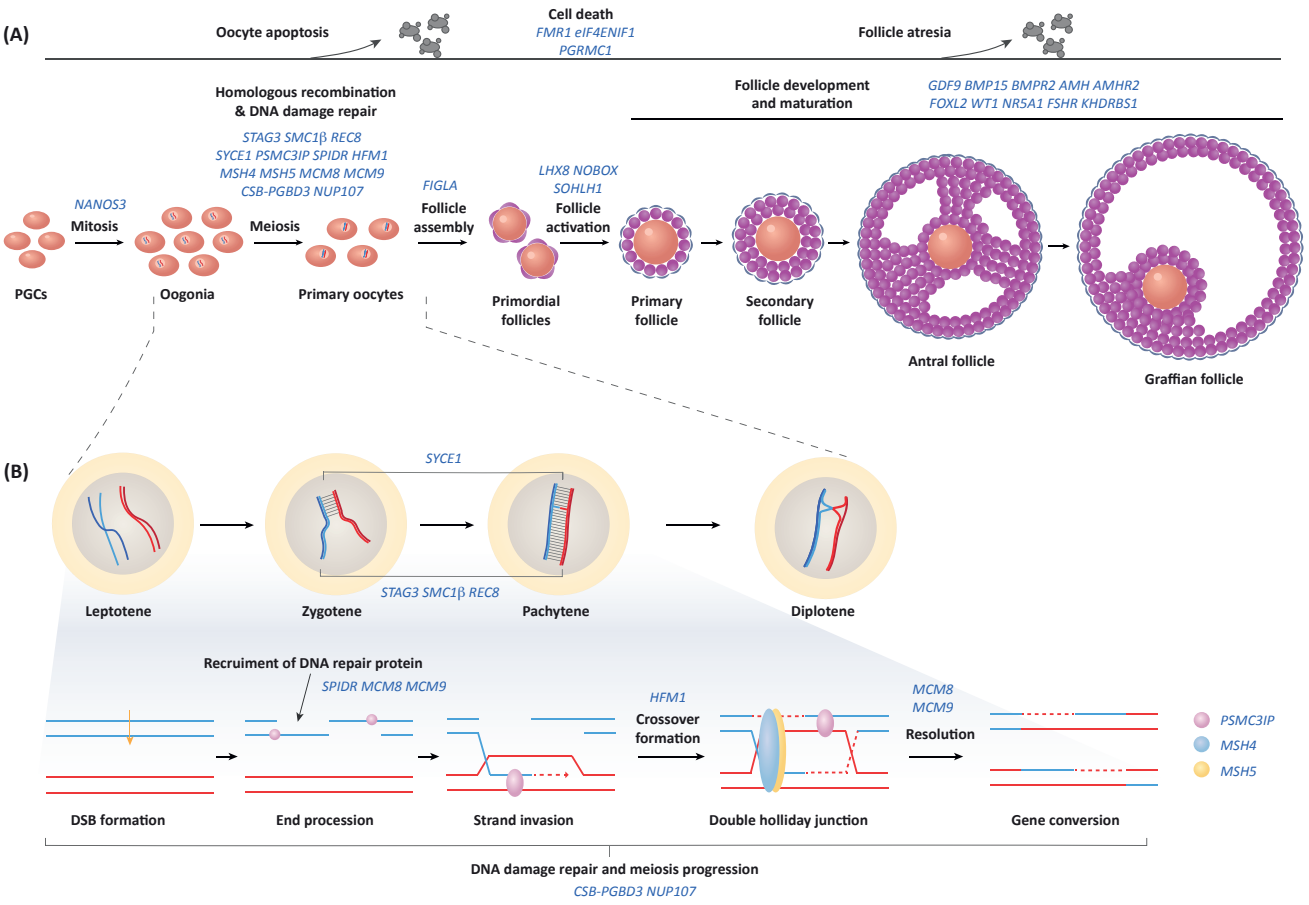

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Figure 1. (A) Schematic representation of premature ovarian insufficiency (POI) causative genes involved in oogenesis and folliculogenesis. The primordial germ cells proliferate by mitosis and differentiate into oogonia. Once meiosis initiates, primary oocytes are formed and subsequently arrest at the diplotene stage of meiotic prophase I. Then oocytes are enclosed by pregranulosa cells to assemble primordial follicles, which represent the quiescent follicle reserve in the ovary. Oocytes not enclosed in primordial follicles are lost by apoptosis. Most primordial follicles remain quiescent or undergo atresia, whereas a few are activated to primary follicle. Once initiated to grow, they either undergo atresia at a later stage of follicular development or by ovulation. POI causative genes participate and regulate oocyte and follicle development at, but not limited to, the above-defined stages throughout folliculogenesis. (B) Overview of POI causative genes clustered in DNA damage repair, homologous recombination and meiosis (Box 1). Meiosis prophase I (from oogonia to primary oocyte) includes four substages: leptotene, zygotene, pachytene, and diplotene. POI causative genes are involved in cohesion of sister chromatid, pairing, synapsis, and recombination between homologous chromosomes. Within this period, a series of events occur continuously, including DSB formation, end procession, strand invasion, crossover (double Holliday junction) formation and resolution, and the final gene conversion. DSB, double-strand break; PGCs, primordial granulosa cells.

hypothesis. Continued advance of NGS will overcome this drawback and also facilitate finding genes interrelated within pathways responsible for POI.

Genome-wide Studies in POI

Contemporary genetic strategies have extended beyond suspected candidate gene interrogations to genome-wide approaches. They discover genetic variants without the limitation of a single candidate or a panel of candidates, and are more promising for explanation of the genetic heterogeneity of POI. The **genome-wide association studies (GWAS)** and array comparative genomic hybridization have revealed multiple loci potentially associated with POI susceptibility in different ethnicities [2]. However, most studies failed to pinpoint precisely the causative genes. They lacked evidence of biological relevance even with plausible candidates proposed. Few positive results have been replicated in an independent cohort. Sample sizes were limited, hence lacking statistical power sufficient to detect any genome-wide significant signal. Moreover, quite a few loci were identified not in coding regions, making it difficult to further interpret functionally. Interestingly, very recent NGSs have led to new POI causative genes being identified and relevant candidates being proposed. Specifically, the WESs in large POI pedigrees have identified a growing list of genes not heretofore implicated in the past 4 years. These include causal genes important for DNA damage repair, homologous recombination (HR) and meiosis (*STAG3*, *SYCE1*, *SPIDR*, *PSMC3IP*, *HFM1*, *MSH4*, *MSH5*, *MCM8*, *MCM9*, *CSB-PGBD3*, *NUP107*), mRNA transcription and translation (*eIF4ENIF1*, *KHDRBS*), and known POI candidates (*SOHLH1*, *FSHR*) (Table 2 and Figure 1).

WES in POI Pedigree

Stromal Antigen 3 (*STAG3*)

STAG3 encodes a subunit of **cohesin**, and it is essential for the assembly of the meiotic cohesin ring and the **synaptonemal complex** during meiosis. Mice deficient in *Stag3* showed sterility and severe ovarian dysgenesis without oocytes. The fetal oocytes had early meiotic arrest and centromeric chromosomal cohesion defects [9]. To date, four distinctive truncating variants in *STAG3* have been identified in large consanguineous families [9–12]. All of four mutations were homozygous in recessive inheritance. All affected members manifested with **primary amenorrhea** and streak ovaries. These results emphasize the importance of correct architecture of the chromosome-spindle molecular machinery for a successful gametogenesis.

Synaptonemal Complex Central Element 1 (*SYCE1*)

The *SYCE1* gene encodes a component of the synaptonemal complex where paired chromosome homologs closely associate in meiosis (synapsis) before crossover. Disruption of *Syce1* in female mice resulted in infertility due to meiotic arrest in meiosis I [13]. Different microdeletions of *SYCE1* have been reported by copy number variation studies in POI [14–17]. In a consanguineous Israeli Arab pedigree, a protein-truncating homozygous mutation p.Q205* in *SYCE1* was identified in two sisters with primary amenorrhea in autosomal recessive inheritance [18].

Scaffolding Protein Involved in DNA Repair (*SPIDR*)

SPIDR encodes a scaffolding protein, most highly expressed in ovary and involved in HR repair. *SPIDR*-depleted cells have defects in HR and higher levels of genome instability [19]. In a consanguineous Israeli Arab pedigree, a homozygous nonsense mutation p.W280* in *SPIDR* was identified in two sisters with 46,XX gonadal dysgenesis (XX-GD) in autosomal recessive inheritance. This mutation altered SPIDR activity and compromised HR repair function [20]. Therefore, *SPIDR* is important for HR in **oogenesis**.

Table 2. Causative Genes Identified by Whole Exome Sequencing in POI Pedigrees

Gene	Phenotype	Mutation	Mechanism	Inheritance	Refs
HFM1 1p22.2	SA	c.1686-1G > C/p.I884S	Affect the canonical splicing site/predicted to be deleterious	AR	[26]
MSH4 1p31	SA	p.I743_K785del	Result in exon 17 skipping, ablation of the Walker B motif and inactivate MSH4	AR	[30]
KHDRBS1 1p35.2	SA	p.M154V	Alter mRNA expression level and alternative splicing	AD	[47]
FSHR 2p16.3	PA[a]	p.D408Y	Disrupt membrane trafficking and the second messenger cAMP signal	AR	[50]
	PA[a]	p.I418S	Disrupt cAMP second messenger response	AR	[49]
MSH5 6p21.3	SA	p.D487Y	Result in POI in *MSH5*$^{D486Y/D486Y}$ mice, disrupt DSBs DNA homologous recombination repair	AR	[31]
MCM9 6q22.31	PA[a]	c.1732 + 2T > C	Result in a truncated protein and impair DNA damage recruitment	AR	[35]
	PA[a]	p.R132*	Compromise repair of chromosome breaks in lymphocytes	AR	
	PA[a]	p.E495*	Result in a truncated protein or nonsense-mediated mRNA decay	AR	[38]
STAG3 7q22.1	PA[a]	p.F187fs*7	Result in a truncated protein	AR	[9]
	PA[a]	p.Y650Sfs*22	Result in a truncated protein or nonsense-mediated mRNA decay	AR	[10]
	PA[a]	p.S227*	Result in a truncated protein	AR	[12]
	PA[a]	p.L490Tfs*10	Result in exon 15 skipping and a truncated protein.	AR	[11]
SPIDR 8q11.21	PA[a]	p.W280*	Result in a truncated protein or nonsense-mediated mRNA decay and compromise HR repair function.	AR	[20]
SOHLH1 9q34.3	PA[a]	p.P235fs*4	Result in a truncated protein	AR	[48]
	PA[a]	p.Y9*	Result in a truncated protein	AR	
CSB-PGBD3 10q11.23	SA	p.G746D	Compromise DNA damage repair	AD	[42]
SYCE1 10q26.3	PA[a]	p.Q205*	Result in a truncated protein	AR	[18]
NUP107 12q15	PA[a]	p.D447N	Result in reduced fertility and defective oogenesis in *Drosophila* female flies	AR	[43]
PSMC3IP 17q21.2	PA[a]	p.E201del	Abolish activation of estrogen-driven transcription and impair estrogenic signaling	AR	[21]
	PA[a]	p.Y163*	Abolish interaction with ssDNA and proteins required for homologous recombination RAD51 and DMC1	AR	[22]
MCM8 20p12.3	PA[a]	p.P149R	Compromise repair of chromosomal breaks, inhibit recruitment to DNA damage sites, and impair DNA binding ability	AR	[36]
	PA[a]	c.1954-1G > A	Compromise repair of chromosome breaks	AR	[37]
	PA[a]	c.1469-1470insTA	Compromise repair of chromosome breaks	AR	
	PA[a]	p.H161P	Compromise repair of chromosomal breaks	AR	[39]
eIF4ENIF1 22q12.2	SA	p.S429*	Result in haploinsufficiency or abnormal truncated protein	AD	[45]

AD, Autosomal dominant; AR, autosomal recessive; DSBs, double-strand breaks; PA, primary amenorrhea; POI, premature ovarian insufficiency; SA, secondary amenorrhea.
[a]Mutations identified in consanguineous families.

Proteasome 26S Subunit, ATPase, 3-Interacting Protein (*PSMC3IP*)

PSMC3IP is critical for homologous pairing and HR in meiosis, as indicated by its yeast ortholog *Hop2*. Female *Hop2/Psmc3ip*-deficient mice showed severe reduction in ovarian size and lack of follicles. In a consanguineous Palestinian family with five members affected by XX-GD, a shared homozygous mutation in *PSMC3IP* was identified. The deletion mutation abolished PSMC3IP activation of estrogen-driven transcription. Impaired estrogenic signaling can lead to ovarian dysgenesis both by affecting the follicular pool and by failing to counteract follicular atresia [21]. Recently, another homozygous stop mutation in *PSMC3IP* was reported in a consanguineous Yemeni family of four sisters with ovarian dysgenesis and a brother with azoospermia [22]. The C-terminal deletion in PSMC3IP abolished interaction with single-strand DNA and proteins required for HR (RAD51, DMC1) [23]. These results revealed a critical role of *PSMC3IP* in both male and female germ cell development.

ATP-Dependent DNA Helicase Homolog (*HFM1*)

HFM1 encodes the DNA helicase preferentially expressed in gonads, which is required for HR and synapsis between homologous chromosomes during meiosis [24]. *Hfm1* null mice were sterile and had small ovaries with decreased follicles [25]. Wang *et al*. [26] identified compound heterozygous mutations in the *HFM1* gene in two affected POI sisters and a sporadic POI woman. It suggested that biallelic mutations in *HFM1* cause recessive POI in humans. Subsequently, different *HFM1* heterozygous mutations have been found in Chinese with sporadic POI [27]. However, their pathogenicity needs further functional validation.

MutS Homolog 4 (*MSH4*) and 5 (*MSH5*)

As members of the DNA mismatch repair family, MSH4 and MSH5 form a heterodimeric complex and exert pivotal roles in chromosome synapsis and meiotic recombination. Disruption of both genes resulted in sterility secondary to defective chromosome synapsis during meiosis [28,29]. In a Colombian POI family with two sisters affected by **secondary amenorrhea**, a homozygous splice-site mutation p.I743_K785del in *MSH4* was identified. Exon trapping experiments showed that the mutation induced skipping of exon 17 and deletion within the highly conserved ATP-binding domain, thus inactivating MSH4 [30]. In a Chinese POI pedigree with two affected siblings, a homozygous mutation p.D487Y, located in DNA-binding domain of *MSH5*, was found in recessive inheritance. This mutant impaired DNA HR repair of *MSH5 in vitro*. Knock-in mice with homozygous D486Y were infertile and had atrophic ovaries [31]. These results imply an essential role of recessive *MSH4/MSH5* mutations in the pathogenesis of human POI.

Minichromosome Maintenance Complex Component 8 (*MCM8*) and 9 (*MCM9*)

As members of minichromosome maintenance protein family, MCM8 and MCM9 are implicated in HR and repair of double-stranded DNA breaks (DSB). Mice deficient for either gene were sterile due to defects in HR and gametogenesis [32]. Previously single nucleotide polymorphism rs16991615 in *MCM8* was found as the most associated locus for age at menopause [33,34]. Using WES approaches, different homozygous mutations in *MCM8* and *MCM9* were identified in consanguineous POI families in autosomal recessive inheritance [35–39]. These mutants had defects in recruitment at DNA damage sites and impairment in chromosomal breaks repair. All affected patients presented with primary amenorrhea, gonadal failure, and chromosomal instability in somatic cells. The heterozygous mutation carriers appeared healthy and fertile. However, novel heterozygous mutations have been identified in sporadic patients with POI [40,41]. Desai *et al*. [41] also examined the epistatic interaction between *MCM8* and *MCM9*. They found one participant with biallelic heterozygous variants, and two with multi-allelic heterozygous variants in genes related to DNA damage repair

pathways. Further functional characterization of these bi-/multi-allelic interactions might explain the complexity of POI etiology.

CSB-PGBD3

The fusion gene *CSB-PGBD3* encodes protein participating in transcription-coupled **DNA repair** of DNA damage. In a Chinese family with four affected members with POI, Qin *et al*. [42] identified a heterozygous mutation p.G746D in *PGBD3*. Another two heterozygous mutations (p.V1056I, p.E215X) in *CSB-PGBD3* were also found in sporadic POI cases. All three mutants impaired the response to DNA damage, as indicated by delayed or absent recruitment to cellular damage. This study provides the first evidence that mutations in the *CSB-PGBD3* fusion gene contribute to POI due to DNA repair impairment.

Nucleoporin-107 (*NUP107*)

NUP107 encodes an essential nuclear pore complex protein expressed ubiquitously. Its precise role is yet to be confirmed. It relates to genes involved in mitotic/meiotic progression in *Drosophila*. In a consanguineous family of Palestinian origin, a recessive mutation p.D447N in *NUP107* segregated with the 46,XX-GD phenotype. To model human *NUP107* $^{p.D447N}$ in *Drosophila*, *Nup107*^{D364N} transgenic flies had defective oogenesis and infertility [43]. It thus demonstrated the tissue-specific importance of *Nup107* for ovarian development and function.

Genes related to mRNA transcription and translation were also associated with autosomal dominant POI in large families. *eIF4ENIF1* represses translation through *eIF4E* and plays an important role in ovarian germ cell development [44]. A heterozygous mutation (p.S429*) in *eIF4ENIF1* segregated with ovarian insufficiency in a French-Canadian family. Decreased mRNA degradation and increased mRNA stability by *eIF4ENIF1* haploinsufficiency might contribute to cell toxicity and follicle atresia [45]. *KHDRBS1* is involved in mRNA splicing and translation, cell cycle regulation, and apoptosis. The knockout mice were subfertile, with decreased secondary and preantral follicles [46]. Wang *et al*. [47] found a heterozygous mutation p.M154V in a Chinese pedigree. Further sequencing in sporadic POI identified another heterozygous mutation, p.P88L. Functional analysis identified 66 differentially expressed genes and 145 alternative splicing induced by M154V *KHDRBS1* expression. Therefore, gene deficiency related to mRNA splicing and translation might disrupt the development of normal oocyte complement and underlie POI pathogenesis.

In addition, WES in consanguineous families also found the known POI candidate genes *SOHLH1* and *FSHR*. Homozygous loss-of-function mutations p.P235fs*4 and p.Y9stop in *SOHLH1* and p.I418S and p.D408Y in *FHSR*, co-segregated with primary amenorrhea in the affected sisters of Turkish and Indian origin [48–50]. The heterozygous carriers had no recognizable pathology in sexual or gonadal development. Heterozygous variants of both genes have been reported in sporadic POI cases, which are likely not the cause or there are other variants in other genes that may interact.

WES approaches in consanguineous and large POI pedigrees have been very promising in identifying genetic variants not previously anticipated. Given the variants co-segregated with phenotype, WES does identify the causative gene and variants that cause the phenotype. Interestingly, it showed an overwhelming enrichment of genes involved in DNA damage repair, HR, and meiosis (Figure 1B and Box 1). Accumulated DNA damage due to repair deficiency within oocytes could induce meiotic arrest and promote apoptosis resulting in accelerated oocyte loss, thus causing ovarian failure. The stage at which DNA damage repair influences oocyte reserve is not yet known. Although it seems to be meiotic-specific repair during

Box 1. Meiosis and HR

Meiosis is required for gametogenesis and involves a single round of DNA replication followed by two rounds of chromosome segregation [71]. At the first stage of meiosis (meiotic prophase I), homologous chromosomes pair and undergo recombination, which is essential for exchange of genetic material between non-sister chromosomes. Based on chromosome configuration and structure, meiotic prophase I is divided into four substages (see Figure 1B in main text) [72]. At leptotene, chromatin condenses into a thin thread-like structure. Then homologous chromosomes begin to pair together (synapsis) and form synpatonemal complexes at zygotene. Synapsis completes once the pachytene stage starts, subsequently crossing over between the pairing of homologous chromosomes and formation of chiasma. At diplotene, the synpatonemal complex disassembles and homologous chromosomes are attached by chiasmata. Then meiosis arrests and oocytes remain dormant until meiosis resumption before ovulation [73].

During meiosis, large amounts of programmed DNA DSBs are introduced and repaired through HR [74]. HR is initiated by DSB formation. 5′ ends at the breaks are resected by exonucleolytic cleavage (end procession) to yield 3′ single-strand DNA (ssDNA). This ssDNA invades into a chromatid of the homologous chromosome (strand invasion). The disassembly of single-end strand invasion intermediates results in noncrossovers (NCOs). After the second end invades, recombination intermediates are processed into double Holliday junctions (dHJs). A majority of dHJs are resolved into crossovers with gene conversion, while a minority undergo dissolution to become NCOs [72]. Deficiency of genes involved in DSB repair and HR will block meiosis and result in oocyte loss and POI.

recombination, it is also possible that DNA damage during mitosis or acquired by environmental exposure could result in oocyte depletion.

Most mutations are inherited in autosomal recessive fashion in consanguineous families with primary amenorrhea, suggesting their deleterious effect on the underlying germ cell pool. Of note, mutations in most genes (12/15, including all meiotic genes) were in bialleic state (homozygous or compound heterozygous), and heterozygous carriers exhibited normal fertility (Table 2), which questions the causality of heterozygous variants identified in sporadic POI. Considering the technological and historical limitation, genes/variants identified by candidate gene approaches were considered pathogenic if with deleterious functional validation. Nevertheless, these previously deemed causative heterozygous cases now might be considered incorrect or false correlation. Hypothesis of digenecity or polygenecity, discussed later, might be another explanation. Further functional validation and multiple family members are warranted to further decipher their true pathogenicity in sporadic POI. In addition, distinct mutations in the same gene (e.g., *STAG3*, *MCM8*, *MCM9*, *PSMC3IP*) identified from different families result in same clinical manifestation. Defects in HR and DSB repair genes are more likely to cause meiotic arrest and primary amenorrhea; while various clinical phenotypes have been observed when mutations occurred in genes affecting folliculogenesis.

NGS in Sporadic POI

To date, four studies using NGS have been performed in sporadic women with POI to propose new putative candidates (Figure 1). A recent WES in 95 sporadic POI women revealed a heterozygous mutation p.A17E in *AMHR2*. However, this mutant showed no defects in AMH signal transduction [51]. The remaining three studies were sequencing or filtering data based on a panel of target candidate genes. Fonseca *et al*. [52] sequenced 70 candidate genes in 12 Colombian POI women. Four mutations in *ADAMTS19*, *BMPR2*, and *LHCGR* were identified in three cases (25%). The *BMPR2* mutation p.S987F showed significant endoplasmic reticulum retention, which might perturb BMP15/BMPR2/SMAD signaling and granulosa cell proliferation [53]. Bouilly *et al*. [54] screened for variants in 19 genes in 100 POI patients. At least one rare variant was identified in 19 patients, including missense mutations in new candidates (*SMC1β*, *REC8*, and *LHX8*). Novel or recurrent deleterious mutations were also detected in the known POI candidates *NOBOX*, *FOXL2*, *SOHLH1*, *FIGLA*, *GDF9*, and *BMP15*. Seven patients (7/19, 36%) harbored mutations in two different loci, suggesting a digenic nature of POI pathogenesis.

A synergistic effect of several mutations may underlie the POI phenotype. Interestingly, another WES in a cohort of Caucasian patients concluded similar oligogenicity/polygenicity of POI. Based on a panel of 420 POI candidate genes, 48% of patients carried potentially deleterious variants while 42% had at least two mutations in distinct genes. As all mutations in meiotic genes responsible for POI have been biallelic, it is interesting that 64% of patients having a heterozygous mutation in a meiotic gene were carriers of at least one further variant in the same or a distinct gene [55].

NGS holds great promise for identifying new molecular variants, alone or in combination, in signaling regulatory networks involved in POI. Various genes interacting and complementing might affect several mechanisms and pathways that contribute to POI phenotype. Whether the cumulative effect of different genes/variants supports the polygenicity of POI remains to be elucidated. Furthermore, how to separate the 'wheat' of causative alterations from the 'chaff' of the vast amount of noise variants is challenging. Hence variants of unknown significance should be further routinely prioritized and classified, and their clinical relevance should also be considered.

GWAS Involving Age at Menopause

The age of menopause is an inheritable trait and has a strong genetic component. Insights from shared genetic susceptibility between POI and age at natural menopause (ANM) or **early menopause** (EM) represent another path for unraveling the genetic mechanism involved in POI. Among the vast number of susceptible candidate loci unearthed by GWAS on ANM/EM, 16 (*ASH2L*, *BAT2/PRRC2A*, *BRSK1/TMEM150B*, *C11orf46/PPED2*, *ESR1*, *HK3*, *NLRP11*, *POLG/FANCI*, *PRIMI*, *TDRD3*, *TLK1*) were validated and shared by ANM, EM, and POI. Genetic determinants of menopause were increased substantially by large-scale GWASs in Caucasian ancestry [56]. A meta-analysis of 53 GWASs with 70 000 women identified 44 loci associated with ANM [57]. Interestingly, two-thirds harbored genes involved in DNA damage repair pathway, including *EXO1*, *HELQ*, *MCM8*, *MSH5*, *FAM175A*, *FANCI*, *TLK1*, *POLG*, and *BRCA1*. Subsequent replications in additional ethnicities corroborated the functional importance of these genes in human gonadal development [58]. The knockout mice of *EXO1*, *HELQ*, *MCM8*, *MSH5*, and *POLG* exhibit infertility, ovarian dysgenesis, and premature failure [59–62]. Furthermore, mutations in *MCM8*, *EIF2B4*, *POLG*, *DMC1*, and *MSH5* were reported in human POI [31,36]. These results convincingly indicated that some genes associated with ANM are also causative for POI. The roles of other genes associated with ANM/EM, such as *NLRP11*, *SLCO4A1*, and *RHBDL2*, are unknown and deserve further exploration. Of note, EM and POI represent the tail of the menopause age distribution. A concomitant presence of POI and EM in the same pedigree might indicate a similar genetic pathogenesis but with a variable expressivity. Compared with ANM/EM, POI might have genetic generality and specificity. The additive effect of environmental factors cannot be ruled out either. The hypothesis that overlapping polygenic etiology, with individuals carrying more ANM variants having increased risk of EM and POI, also seems possible.

Noncoding RNAs

Causative genes of POI have been extensively studied to date in coding regions, presuming plausible protein function disruption. However, the protein-coding region only accounts for 1.5% of the whole human genome. The **noncoding RNAs (ncRNAs)**, as epigenetic regulators, have recently begun to be explored in ovaries; this has added another complexity to the etiology of POI. Profiling analyses have shown miRNA widely expressed in mammalian ovaries [63]. Their abundance and expression pattern changes during ovarian development and

folliculogenesis [64]. These studies implicate the functional roles of miRNAs throughout the ovarian cycle. Association studies revealed that miRNA polymorphisms miR-146aC > G, miR-196a2T > C, miR-499A > G, and miR-449bA > G, were associated with POI in Korea [65]. MiR-146a C > G has been implicated in differential expression of POI-related genes *FOXO3* and *CCND2* in granulosa cells [66]. Additionally, miR-146a upregulation contributed to granulosa cells apoptosis via toll-like receptor signaling and caspase cascades [67]. Therefore, miR-146a might act as a plausible candidate for POI. Using microarray chip techniques, distinct plasma miRNA signatures have been reported in Chinese POI cases [68,69], indicating a promising role for miRNAs serving as noninvasive diagnostic tools in clinic. Recently, with miRNA and mRNA microarrays in granulosa cells from biochemical POI, miR-379-5p was identified significantly upregulated, which suppressed cell proliferation and impaired DNA repair function through directly targeting *PARP1* and *XRCC6* [70]. It corroborated the significance of DNA repair pathway for POI and brought up an epigenetic explanation for the disease.

The discovery of stable and reproducible miRNAs supports their potential use as novel biomarkers. More reliable miRNA profiles in ovarian microenvironment (follicular fluid, granulosa cells) in larger sample sizes are warranted. Transcriptome sequencing is a powerful approach for exploring the interaction of miRNA and target genes. In addition, these findings raise the intriguing possibility that miR-dependent epigenetic regulation could underlie new pathogenesis of POI when no mutations are found. Nevertheless, the challenge remains to determine the potential roles of specific miRNA or miRNA-signaling networks in physiological ovarian function and pathogenesis of POI. Future research on other uncovered ncRNA (long noncoding RNA, circular RNA) in POI also represents an emerging field.

Concluding Remarks and Future Perspectives

Elucidating the genetic and molecular basis of POI is of paramount importance, not only in understanding ovarian physiology but also in providing genetic counseling and fertility guidance. This perspective is becoming increasingly important, as an increasing number of women prefer to conceive into their thirties and forties. To date, what we know is just the tip of the iceberg of POI genetics, despite the fact that causative lists are expanding. The traditional approaches, although critical in establishing the field of POI genetics, have been proceeding slowly. Given the contribution of fruitful novel genes, new strategies with NGS and whole genome sequencing in the near future will certainly revolutionize this field and give more precise insight into the complex gene network involved in POI. Recent advances of WES on POI pedigrees and GWAS on age of menopause revealed a preponderance of genes enriched in DNA damage repair and HR, which corroborates their functional importance in reproduction aging and deserves further exploration. The insights from miRNA research will facilitate identification of new causative gene or signaling pathways.

Yet, characterizing the potential causative gene for POI also brings to the forefront some key questions (see Outstanding Questions) and challenges. Currently, the underlying genetic architecture of POI is equally heterogeneous with mutations in several genes, without predominance in any single one. Its genetic complexity is also reflected in different inheritance patterns. Whereas most sporadic POI harbor heterozygous mutations, analysis of pedigrees reveals an autosomal recessive, or less commonly dominant inheritance. In addition to these Mendelian modes of inheritance, an even more complex genetic architecture for POI has been documented, wherein mutations in two or more POI genes are found in a single case. These coexisting mutations could therefore play the role of 'second hits' or act as 'modifier' genes involved in POI. In addition, the ambiguous nomenclature and definition of POI complicates phenotypic variability, further exacerbating the heterogeneity of POI genetics and vice versa.

Outstanding Questions

Do causative genes identified in familial POI account for sporadic POI? How can we explain normal phenotype in POI pedigree but pathogenic phenotype in sporadic POI for heterozygous mutations?

Could causative genes identified in POI explain male oligo/asthenospermia or azoospermia, especially those critical in early stages of oogenesis? Future researches should also pay attention to male siblings in POI pedigrees.

Is the hypothesis of the oligogenic/polygenic nature of POI correct? If so, how much of a cumulative effect of variants/genes is enough for pathogenesis of POI?

Vast numbers of genetic variants, with or without functional significance, will emerge in the omics era. How can we efficiently leverage this knowledge base clinically?

Will gene editing be feasible for improving ovarian function after validation of causative variants/genes?

Whether distinct phenotypes, such as ovarian dysgenesis, primary amenorrhea, secondary amenorrhea, or early or late onset of POI share the same genetics with different cumulative effects remains unclear. In the era of NGS, a surprising number of variants of unknown significance will emerge. Data filtering and analysis will be considerably challenging, but extremely important. Causative relevance should be validated with transformation experiments or mutation-directed transgenic models. Nonetheless, since many of the known POI genes seem to act synergistically, while individually displaying features of incomplete penetrance or variable expressivity, it is becoming ever more difficult to label a single genetic change in any individual POI patient as 'causative'. Further research on genotype–phenotype correlation needs to be elucidated.

Identification of individuals with POI risk remains a challenge. NGS technologies have enormous potential when implemented in clinical practice. Targeted panels of hotspot mutations and/or validated causative genes, or wider analysis such as whole-exome and whole genome sequencing in the future will add important value in a context of risk prediction, routine diagnosis, and earlier intervention.

Supplemental Information

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