Primary Ovarian Insufficiency

Joop S. E. Laven, MD, PhD[1]

[1] Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Erasmus MC, Rotterdam, The Netherlands

Semin Reprod Med 2016;34:230–234

Address for correspondence Joop S. E. Laven, MD, PhD, Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Erasmus MC, PO Box 2040, 3000 CA Rotterdam, The Netherlands (e-mail: j.laven@erasmusmc.nl).

Abstract

Primary ovarian insufficiency (POI), also known as premature ovarian failure or premature menopause, is defined as cessation of menstruation before the expected age of menopause. Potential etiologies for POI can be divided into genetic, autoimmune, and iatrogenic categories. This review will try to summarize the genetic basis of POI focusing on recent data that are available using newer genetic techniques such as genome-wide association studies, whole-exome sequencing (WES), or next-generation sequencing techniques. By using these techniques, many genes have arisen that play some role in the pathophysiology of POI. Some of them have been replicated in other studies; however, the majority has not been proven yet to be unequivocally causative through functional validation studies. 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. Once additional variants are detected, it might become possible to predict the age of (premature) menopause in women at risk for POI. Women having certain perturbations of POI can be offered the option of oocyte cryopreservation, with later thawing and use in assisted reproductive technology at an appropriate age.

Keywords

- primary ovarian insufficiency
- ovarian failure
- genome wide association studies (GWAS)
- whole-exome sequencing (WES)
- next generation sequencing (NGS)
- genetic counseling
- fertility guidance

Primary ovarian insufficiency (POI), also known as premature ovarian failure (POF) or premature menopause, is defined as cessation of menstruation before the expected age of menopause. This age is traditionally defined to be before 40 years and diagnosis is confirmed by elevated serum follicle-stimulating hormone (FSH) levels ($>$ 40 IU/L). Although frequently stated that 1% of the population is affected with POI before the age of 40 years and 0.1% before 30 years, the prevalence is actually less certain.[1] Potential etiologies for POI can be divided into genetic, autoimmune, and iatrogenic categories. Unfortunately, for most patients presenting with POI, the cause will remain unexplained.[2] Confusion exists concerning nomenclature, namely, the use of POF or POI. It is the view of the authors that POI can be taken to encompass occult, biochemical, and overt stages, whereas POF is best considered as only the final stage of POI. The designation POI is thus best reserved as alluding to the entire gamut of disorders, having diminished ovarian reserve occult, subclinical, or iatrogenic.[1]

Genetics of Primary Ovarian Insufficiency

Chromosomal Abnormalities

Disorders that involve the X chromosome and loci that regulate germ cell development and viability are linked to POI. Chromosomal abnormalities have long been recognized as a cause of POI, but percentages vary widely among reported series. This clearly reflects biases of ascertainment, for example, reflecting whether a cohort was derived from a referral cytogenetic laboratory, a gynecologic practice, or a pediatric practice. Numerous different karyotypic anomalies have been found, ranging from numerical defects (monosomy X; X chromosomal mosaicism), X-deletions, X-autosome translocations, and X-isochromosomes and other rearrangements.[1]

Women who have phenotypic abnormalities in addition to ovarian insufficiency are likely to have syndromic, as opposed to nonsyndromic (pathology confined to ovarian insufficiency) type of POI. These syndromes can be due to chromosomal abnormalities such as Turner syndrome (monosomy X) or due

Issue Theme Lessons from Genome-Wide Association Studies in Reproduction; Guest Editor, Joop S. E. Laven, MD, PhD

DOI http://dx.doi.org/10.1055/s-0036-1585402.
ISSN 1526-8004.

to single gene mutations as is the case with galactosemia (*GALT*), pseudohypoparathyroidism type 1a (guanine nucleotide-binding protein, α stimulating; *GNAS1*), progressive external ophthalmoplegia (polymerase [DNA directed], gamma; *POLG*), autoimmune polyglandular syndrome type 1 (autoimmune regulator; *AIRE*), ovarian leukodystrophy (eukaryotic translation initiation factor 2B, subunit 2 β; *EIF2B2*), ataxia telangiectasia (*ATM*), Demirhan syndrome (bone morphogenetic protein receptor, type 1B; *BMPR1B*), and blepharophimosis–ptosis–epicanthus inversus syndrome (BPES) (*FOXL2*) among others. Women with nonsyndromic and idiopathic POI were evaluated for the presence of *EIF2B2* and *GALT* mutations; however, no significant associations were found. Mice lacking *ATM*, *AIRE*, or *BMPR1B* also have ovarian dysfunction, although mutations in these genes have not been evaluated in patients with nonsyndromic and idiopathic POI. A single nucleotide polymorphism (SNP) in *POLG* (rs2307449) associates with age at menopause.[3]

Candidate Gene Studies

Again numerous genetic variants have been investigated in different populations using a candidate gene approach. Most of the identified variants were associated with POI in one study, whereas other studies failed to replicate these findings. For an extensive review, refer to the reviews by Qin et al[1] and Wood and Rajkovic.[3] Briefly, human *FIGLA* is expressed as early as 14 weeks of gestational age with a dramatic increase in transcripts by midgestation, suggesting a similarly conserved function of the human and mouse FIGLA proteins. Heterozygous mutations in *FIGLA* were found to be present in women with POI. Thus, haploinsufficiency of *FIGLA* likely causes accelerated loss of ovarian reserve in humans. *NOBOX* promotes primordial follicle activation. Human *NOBOX* expression within the ovary is oocyte specific and observed from the primordial follicle to the metaphase II oocyte. *NOBOX* mutations were identified in a population of Caucasian women with POI. Forkhead box O3 (*FOXO3*), a transcription factor, is an important oocyte-specific regulator of primordial follicle activation, and mutations in *FOXO3* were identified in women with POI. Growth differentiation factor-9 (GDF9) and bone morphogenic protein 15 are oocyte-secreted growth factors that affect granulosa cell differentiation function. Three missense mutations in GDF9 were found in POI patients of Chinese or Indian descent. Two additional mutations were present in Caucasian women with POI. However, none of these variants were identified in Japanese women with POI. Forkhead box L2 (*Foxl2*), a transcriptional regulator, can repress primordial follicle activation through upregulation of anti-Müllerian hormone (AMH). *FOXL2* activates the expression of AMH in granulosa cells of developing follicles, which, when secreted, can act in a paracrine manner to repress primordial follicle activation. Mutations in *FOXL2* cause BPES types I and II. Type I can present with POI. Multiple mutations in *FOXL2* have also been found in women with nonsyndromic POI suggesting that mutations in *FOXL2* may be a cause of idiopathic POI. SNPs in genes of the estrogen receptor (*ESR1*), inhibin A (*INHA*), the FSH receptor (*FSHR*), and aromatase (*CYP19A1*) did produce conflicting results among different ethnic populations.[3]

Genome-Wide Association Study and Primary Ovarian Insufficiency

Genome-wide association studies (GWASs) have revealed multiple loci potentially associated with POI in Chinese, Korean, and Dutch women. However, in each, it was difficult to identify genetic variants that were genome-wide significant. Moreover, quite a few of these regions were not in coding areas of the genome and were not replicated in other studies. Finally, none of the GWASs done so far had more than 1,000 patients in the analysis hence they were probably severely underpowered and therefore unable to detect any significant signal.

The first GWAS in POI was reported in 2008 in a small number discovery sample of 24 women with POI and a similar number of controls. These authors identified a SNP in the parathyroid hormone responsive-B1 (*PTHB1*) gene that was strongly associated with POI. Moreover, this variant confers susceptibility to POI. Although causative SNPs were not identified, the polymorphism of this nonsynonymous SNP and the repeated association of one haplotype with POI suggested that PTHB1 may contribute to POI pathogenesis.[4] A second GWAS followed in 2009 in a larger group of affected women. This GWAS involving 309,158 SNPs was performed in 99 unrelated idiopathic Caucasian patients with POI and 235 unrelated Caucasian female controls. A replication study on the most significant finding was performed. These authors focused a priori specifically on chromosomal areas and candidate genes previously implicated in POI. They observed a possible association between POI and a SNP in a biologically plausible candidate gene ADAMST19. Although limited by sample size, this proof-of-principle study revealed ADAMTS19 as a possible candidate gene for POI.[5] A third GWAS was done in 2012 in a small discovery sample constituting 24 POI patients and 24 matched controls. A strongly associated region was retested to confirm the association with POI in a replication sample using 98 patients and 218 matched controls. These authors showed that *LAMC1* was significantly associated with POI. Moreover, they also showed that specifically one haplotype was associated with susceptibility to POI. This implies that this specific haplotype may coexist with causative variant for susceptibility to POI in linkage disequilibrium and that the *LAMC1* may be involved in POI pathogenesis.[6] Another study in 2012, using Affymetrix (Affymetrix, Santa Clara, CA) SNP 6.0 chip, was conducted in an initial discovery set of 391 well-documented Chinese Han POI patients, compared with 895 unrelated Chinese female controls. A replication study on the most significant loci was then performed in an independent set of 400 cases and 800 controls. Suggestive significant associations were observed at 8q22.3. Replication of eight SNPs, all marginally (10^{-6}) genome-wide significant, was confirmed in verification sets. No specific candidate gene was found in the immediate region of 8q22.3. This GWAS, involving by far the largest sample of POI cases accumulated to date, revealed heretofore-unrecognized association between POI and a novel genetic locus or region of unknown nature on 8q22.3. These authors speculate existence of a long-distance

regulatory region that has relevance to the control of ovarian differentiation or oogenesis. Given failure to find association with any of the other autosomal regions known to harbor genes causing ovarian failure, their findings also underscore the likelihood of considerable genetic and etiologic heterogeneity in POF and the need for additional approaches such as whole-genome sequencing.[7]

Genome-Wide Association Study and Family Studies

Another approach to use genome-wide linkage is the use of GWAS in restricted families with multiple affected and unaffected family members. The first study was performed within a relatively large Dutch family with seven patients suffering from POI, showing a dominant pattern of inheritance. A genome-wide analysis, using 50K SNP arrays, was combined with conventional parametric linkage analysis. These authors identified three genomic regions on chromosomes 5, 14, and 18 yielding suggestive linkage with multipoint Logarithm (base 10) Of oDds score (LOD) score of 2.4 for each region. After extending the number of family members with one older unaffected family member, only the region on chromosome 5 remains as a putative POI locus. In addition, they investigated a second family consisting of three living patients spanning three generations for the regions on chromosomes 5, 14, and 18. Haplotype analysis supported only the locus on chromosome 5q14.1-q15, being a region, which might harbor a novel POI susceptibility gene.[8] A second family study using a whole-exome sequence approach of a large consanguineous family with inherited POI. This study identified a homozygous 1-bp deletion inducing a frameshift mutation in the *STAG3* gene located on chromosome 7. *STAG3* encodes a meiosis-specific subunit of the cohesin ring, which ensures correct sister chromatid cohesion. Female mice devoid of *STAG3* are sterile, and their fetal oocytes are arrested at early prophase I, leading to oocyte depletion at 1 week of age. However, sequencing the three most plausible candidate genes in this region—DLX5, DLX6, and DSS1—failed to reveal mutations.[9]

Copy Number Variation

There are several studies that have assessed differences in copy numbers of stretches of DNA, which are commonly detected throughout the human genome. These might involve duplications of genes or microdeletions and microinsertions of specific part of the genome. Studies were dealing with different ethnicities and generally dealing with limited numbers of women affected by POI. They all detected areas of the genome that were apparently critical for the regulation of different genes, which impacted on ovarian function leading to POI.[10–15]

Early Menopause and Primary Ovarian Insufficiency

The genetic etiology of early menopause (EM) is largely unknown in the majority of cases. Recently, a GWAS, a meta-analysis of several genome-wide association studies, in 3,493 women with EM and 13,598 controls from 10 independent studies has been performed. No novel genetic variants were discovered, but the 17 variants previously associated with normal age at natural menopause as a quantitative trait were also associated with EM as well as with POI. Thus, POI and EM do substantially overlap with normal menopause and is at least partly explained by the additive effects of the same polygenic variants. The combined effect of the common variants captured by the SNP arrays was estimated to account for approximately 30% of the variance in EM. Moreover, the distribution of risk alleles was similar in POI and EM individuals. The association between the combined 17 variants and the risk of EM was greater than the best validated nongenetic risk factor being smoking. Genetic markers of ovarian aging are present throughout life and thus may be superior to current best predictors, for example, AMH, inhibin B, and FSH levels, which are only reliable indicators toward the end of a woman's reproductive period, which precedes actual menopause. As more genetic components of this trait are discovered, we will be able to include additional genetic data in predictive models for menopause age, giving women information about potential reproductive lifespan and enabling them to make informed reproductive choices.[2]

Whole-Exome Sequencing

Because of the low prevalence and impaired fecundity resulting in limited numbers of POI pedigrees without associated somatic anomalies (nonsyndromic) has led to increasing application of whole-exome sequencing (WES), another agnostic genetic approach. WES has identified several genes in POI not previously anticipated. For an extensive review, refer to the review by Qin et al.[1]

In POI associated with somatic features, causative perturbations have been found by WES for HSB17B4, LARS2, CLPP, and C10orf2 in Perrault syndrome very elegantly reviewed by Qin et al. Up to the present, there have been six WES conducted in nonsyndromic POI pedigrees. The whole-exome studies in familial POI mentioned earlier mainly involve genes crucial during meiosis, such as generating and repairing double strand breaks in DNA, chromosome synapsis and recombination, and sister chromatid cohesion. This implies that perturbation of genes encoding proteins that regulate meiosis can result in autosomal recessive POI in humans.[1] Interestingly, almost all plausible candidate genes identified were involved in meiosis and DNA repair again indicating that aging of germ cell lines as well as somatic cell aging are intertwined with each other. Indeed, biological as well as epidemiological data seem to indicate that reproductive performance, age at menopause, and longevity are interlinked through common genetic factors involved in DNA repair and maintenance. In case these systems fail, cell death and accelerated aging occur. Consequently, it seems that the aging of the soma, as a result of dysfunctional DNA repair, is responsible for failure to reproduce and the subsequent occurrence of menopause. Hence, reproductive performance constitutes a good predictor for general health in later life.[16]

Next-Generation Sequencing

Concerning POI, as mentioned earlier, three studies using next-generation sequencing (NGS) technologies have been performed in family-related cases of POI. One study identified a homozygous mutation in *SYCE1* gene in humans. They used a family-based genetic approach in two daughters of consanguineous parents (first cousins) from a 13-member family who were diagnosed with POI. Genotyping was performed in the index patients, their parents, and four unaffected siblings. The genotypes of interest were confirmed and genotypes of the additional family members were determined by Sanger sequencing. Genotyping was also performed in 90 ethnically matched control individuals. A nonsense homozygous mutation (c.613C > T) was identified in the *SYCE1* gene in both affected sisters. The parents and three brothers were heterozygous for the mutation, and an unaffected sister did not carry the mutation. The mutation was not identified in the DNA samples from the 90 control subjects. Given the known function of the *SYCE1* gene, these authors suggest that the nonsense mutation identified accounts for the POI phenotype. These results highlight the importance of the synaptonemal complex and meiosis in ovarian function.[17]

A second recent study from a Colombian group of researchers used NGS to assess genetic alterations in POI patients. The complete coding regions of 70 candidate genes were sequenced in 12 women affected by POI. Bioinformatics and genetic analysis led to the identification of mutations in *ADAMTS19* and *BMPR2* that are potentially related to POI pathophysiology. *LHCGR* mutations that might have contributed to the phenotype were also detected. NGS would thus seem to be a powerful tool for identifying new molecular actors involved in POF and for diagnostic/prognostic purposes.[18]

Future Directions and Research

Future directions would involve testing a significant number of isolated (nonfamily-related) nonsyndromic POI cases. This approach could be facilitated by designing custom microarrays including coding and regulatory regions for a large number of POI candidate genes. It would also be possible to perform exome sequencing assays in which a subset of genes (the POI candidates) might be specifically analyzed. Furthermore, WES might reveal further disease pathogenesis-related regions. NGS technology will certainly involve a key tool for mapping genome variations participating in ovarian-related physiological and pathological conditions. It could be used in the near future regarding a variety of ovarian dysfunctions for diagnostic and predictive purposes.[19]

Moreover, many genes that currently appear isolated in function actually may be interrelated within yet to be defined pathways. It is logical to stratify by gene function in ostensibly distinct systems: endocrine, folliculogenesis, cell cycle, meiosis, mitochondrial, as examples. More difficult are gene–gene or protein–protein interactions, acting in ways not yet evident.[1]

Finally, after detection of genes that might be involved, functional studies should be performed in animal models and cell culture systems using human tissue to elucidate the exact pathophysiological mechanism behind the causative gene. Only by doing so, the real important genetic contributors to either nonsyndromic or syndromic POI will be discovered. The last step, however, must involve the relationship between causative genes and their environment.

Conclusion

Taken together, recent old as well as newer genetic approaches have revealed numerous genetic causes of POI. Up till now, however, they only explain the genetic background in approximately 20 to 25% of POI cases. There are notable differences among populations as well as between different ethnicities. Some of the causative genes are not only expressed in the ovary but also play a role in other pathways. Moreover, many genes that currently appear isolated in function actually may be interrelated within yet to be defined pathways. It is logical to stratify by gene function in ostensibly distinct systems: endocrine, folliculogenesis, cell cycle, meiosis, mitochondrial, as examples. Uncovering the remainder of the causative genes will be facilitated not only by whole-genome approaches involving larger cohorts in multiple populations but also incorporating environmental exposures and exploring signaling pathways in intragenic and intergenic regions that point to perturbations in regulatory genes and networks.

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. Once additional variants are detected, it might become possible to predict the age of (premature) menopause in women at risk for POI. Women having certain perturbations of POI can be offered the option of oocyte cryopreservation, with later thawing and use in assisted reproductive technology at an appropriate age.

References

1 Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. Hum Reprod Update 2015;21(6):787–808

2 Perry JR, Corre T, Esko T, et al; ReproGen Consortium. A genome-wide association study of early menopause and the combined impact of identified variants. Hum Mol Genet 2013;22(7): 1465–1472

3 Wood MA, Rajkovic A. Genomic markers of ovarian reserve. Semin Reprod Med 2013;31(6):399–415

4 Kang H, Lee SK, Kim MH, et al. Parathyroid hormone-responsive B1 gene is associated with premature ovarian failure. Hum Reprod 2008;23(6):1457–1465

5 Knauff EA, Franke L, van Es MA, et al; Dutch POF Consortium. Genome-wide association study in premature ovarian failure patients suggests ADAMTS19 as a possible candidate gene. Hum Reprod 2009;24(9):2372–2378

6 Pyun JA, Cha DH, Kwack K. LAMC1 gene is associated with premature ovarian failure. Maturitas 2012;71(4):402–406

7 Qin Y, Zhao H, Xu J, et al; China POF Study Group. Association of 8q22.3 locus in Chinese Han with idiopathic premature ovarian failure (POF). Hum Mol Genet 2012;21(2):430–436
8 Oldenburg RA, van Dooren MF, de Graaf B, et al. A genome-wide linkage scan in a Dutch family identifies a premature ovarian failure susceptibility locus. Hum Reprod 2008;23(12):2835–2841
9 Caburet S, Arboleda VA, Llano E, et al. Mutant cohesin in premature ovarian failure. N Engl J Med 2014;370(10):943–949
10 Aboura A, Dupas C, Tachdjian G, et al. Array comparative genomic hybridization profiling analysis reveals deoxyribonucleic acid copy number variations associated with premature ovarian failure. J Clin Endocrinol Metab 2009;94(11):4540–4546
11 Knauff EA, Blauw HM, Pearson PL, et al; Dutch Primary Ovarian Insufficiency Consortium. Copy number variants on the X chromosome in women with primary ovarian insufficiency. Fertil Steril 2011;95(5):1584–8.e1
12 Liao C, Fu F, Yang X, Sun YM, Li DZ. Analysis of Chinese women with primary ovarian insufficiency by high resolution array-comparative genomic hybridization. Chin Med J (Engl) 2011;124(11):1739–1742
13 McGuire MM, Bowden W, Engel NJ, Ahn HW, Kovanci E, Rajkovic A. Genomic analysis using high-resolution single-nucleotide polymorphism arrays reveals novel microdeletions associated with premature ovarian failure. Fertil Steril 2011;95(5):1595–1600
14 Quilter CR, Karcanias AC, Bagga MR, et al. Analysis of X chromosome genomic DNA sequence copy number variation associated with premature ovarian failure (POF). Hum Reprod 2010;25(8):2139–2150
15 Zhen XM, Sun YM, Qiao J, Li R, Wang LN, Liu P. [Genome-wide copy number scan in Chinese patients with premature ovarian failure]. Beijing Da Xue Xue Bao 2013;45(6):841–847
16 Laven JS. Genetics of early and normal menopause. Semin Reprod Med 2015;33(6):377–383
17 de Vries L, Behar DM, Smirin-Yosef P, Lagovsky I, Tzur S, Basel-Vanagaite L. Exome sequencing reveals SYCE1 mutation associated with autosomal recessive primary ovarian insufficiency. J Clin Endocrinol Metab 2014;99(10):E2129–E2132
18 Fonseca DJ, Patiño LC, Suárez YC, et al. Next generation sequencing in women affected by nonsyndromic premature ovarian failure displays new potential causative genes and mutations. Fertil Steril 2015;104(1):154–62.e2
19 Laissue P. Aetiological coding sequence variants in non-syndromic premature ovarian failure: from genetic linkage analysis to next generation sequencing. Mol Cell Endocrinol 2015;411:243–257