Insight into the Genomics of Premature Ovarian Failure

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Abstract

Primary Ovarian Failure (POF) is an ovarian defect characterized by the premature depletion of ovarian follicles. It causes infertility in ~1% of women <40 years of age and it has important health consequences for affected patients. POF is a heterogeneous disease, which can develop as a result of a broad variety of pathogenic mechanisms including genetic, autoimmune and iatrogenic causes. However, the mechanisms that cause ovarian dysfunction are poorly understood. Focus on genetic component of the disease has revealed the existence of several causal genetic defects, thus indicating that in addition to some monogenic forms, POF may frequently be a multifactorial disease involving several gene abnormalities and chromosome aberrations. Moreover, most recent studies have highlighted that epigenetic mechanisms may give an additional contribution to POF pathogenesis. This review gives a picture of the state of the art of the complex genetic and epigenetic defects associated with POF, being it clear that a deep comprehension of the molecular etiology of POF may in future early identify those women with higher risk of POF.

Keywords: Ovarian aging; Premature menopause; Premature ovarian failure; Premature ovarian insufficiency

Introduction

Ovarian aging process is characterized by a gradual decrease in the quantity and the quality of the oocytes [1] due to the depletion of the endowment of primordial, intermediate and primary follicles, starting in utero and extending through the menopause. During fetal life, germ cells proliferate by mitosis to reach approximately 6-7 million oogonia by the fourth month of pregnancy. From that point forward, the atresia of primordial follicle pool begins via gene-regulated apoptosis. It has been estimated that the number of germ cells falls to 1-2 million at birth and to 300,000-400,000 by the onset of puberty. During the reproductive life, through a combination of atresia and ovulation, around ~1000 follicles per month are depleted [2].

Employing mathematical models, it has been theorized that the primordial follicle pool decay rate is exponential and biphasic with a significant acceleration in depletion when the number of follicles is below 25,000 (physiologically at the age of 37-38 years). Approximately 13 years later, when the primordial follicles number drops below a critical threshold (estimated to be 1000), ovulation ceases and the menopause ensues [3-5].

More recently, a new model has been proposed combining data from fractionator and optical dissector techniques, ultrasound Antral Follicle Count (AFC) and serum anti-Mullerian hormone (AMH) measurement. This new model predicts that the decay of follicle pool is constantly accelerating rather than suddenly increasing at ~38 years [6-8]. Although this theory is more biologically plausible, inter-individual variation in primordial follicle number cannot be explained by the age alone. In Caucasian population the median age of natural menopause occurrence is 50 ± 1 years, but about 10% of women become menopausal by the age of 45 years, thus showing a low ovarian reserve considerably before the age of 37-38 years [9].

This review summarizes the state of the knowledge of Premature Ovarian Failure (POF), with the main purpose to illustrate the genetic and epigenetic mechanisms associated with its pathogenesis.

Premature Ovarian Failure: The Terminology

Premature ovarian failure is classically defined as the development of amenorrhea in women under the age of 40 years associated with follicle stimulating hormone (FSH) levels exceeding 40 mIU/ml [10,11]. The incidence of POF is 1% in women under the age of 40 years and 0.1% under the age of 30 years [12-14]. Depending on the age of onset, the disorder occurs as primary amenorrhea, without menarche, or secondary amenorrhea after the puberty [15]. Since POF has a variable clinical course, it has been recently proposed the term of primary ovarian insufficiency (POI), as a more scientifically accurate definition of the progression toward the cessation of ovarian function [16].

In the last decades, the spread of assisted reproduction technologies has given a significant contribution to the understanding of the mechanisms of ovarian aging [17]. About 5% of patients who go through standard in vitro fertilization treatments show a poor ovarian response (POR) to gonadotropin stimulation [5]. This subset of patients, who are young women (<35 years old) within explained infertility, highlights a premature declining ovarian function (PDOF) and they probably represent the “tip of the iceberg” of those women with a premature ovarian aging (POA) who are not identified as being asymptomatic and not desirous to their conception. Moreover, although robust epidemiological data defining a relationship between PDOF-POA and POF are missing, current understanding of premature ovarian senescence suggests that POA may be a milder precursor stage to POF [18-20]. Table 1 summarizes the different acronyms referring to the pathology discussed in the present review.

Etiopathogenesis of POF

The possible mechanisms leading to a premature impairment of...
the ovarian reserve are: i) decreased pool of primordial follicles, due to alteration in mechanism regulating germ cell proliferation, prolongation of oogonia and meiosis occurring before birth; ii) accelerated rate of follicle atresia by deregulation of those factors that control the rate of apoptosis (i.e., gonadotropins, estrogens, androgens, growth factors, cytokines, reorganisation of the actin cytoskeleton, nitric oxide, tumour necrosis factor-a, Fas ligand and activated peripheral T cells); iii) dysfunction in follicular recruitment or maturation [21].

The causes that may activate such mechanisms are highly heterogeneous. About 25% of POF cases are iatrogenic, being related to postsurgical ovarian failure and cancer treatments (radiotherapy and chemotherapy) [21-23]. It has been also hypothesized that POF may be induced by environmental factors, such as cigarette smoking, heavy metals, solvents, pesticides, plastics, industrial chemicals [21]. Moreover, there is evidence that POF can be secondary to infections (mumps, herpes zoster, cytomegalovirus), autoimmune (i.e., systemic lupus erythematosus, Hashimoto’s thyroiditis, Addison’s disease, rheumatoid arthritis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) and metabolic diseases (galactosemia) [21,24,25]. Some cases of POF are syndromic, i.e., in Turner syndrome, carbohydrate-deficient glycoprotein syndromes, pseudohyoparathyroidism type 1a, progressive external ophthalmoplegia, autoimmune polyendoligandular syndrome type I, and ovarian leukoostrophy [21]. In more than 50% of cases the etiology is idiopathic and probably genetic. The POF can result from different genetic mechanisms. To date, mutations associated with POF have been identified in a small number of genes and none of the mutations are associated with >10% of cases. Therefore, it supports the view of POF as a complex multifactorial disease probably involving many different loci.

### Chromosome X Defects in POF

About 9% of POF are related to X chromosome aberrations that comprise both numerical (monosomy and trisomy) and segmental (deletions, isochromosomes and balanced X: autosomal translocations) abnormalities [26-28]. The complete or partial absence of one X chromosome is associated with Turner syndrome characterized by defective ovarian function and gonadal dysgenesis [29]. The clinical features in 45,XX females may primarily be determined by the impairment of X chromosomes at meiosis [30] and the haploinsufficiency of X-linked genes (such as SHOX/PHOG) that physiologically escape from X-inactivation as their diploid dosage is required for oogenesis [31]. X trisomy is the most frequent aneuploidy which affects 1 in 900 women in general population and association between the 47,XXX genotype and hypergonadotrophic POF has been reported.

As regarding segmental defects, deletions in POF patients are commonly at Xq21.3–q27 (POF1 region), whereas breakpoints of balanced X:autosomal translocations are preferentially localized to Xq13.3–q21.1 (POF2 region) [32,33]. To date, no single gene on the X chromosome in these regions has consistently been found to be involved in POF. Therefore, it may be that any structural defect involving the X chromosome may alter normal chromosome pairing during meiosis, leading to accelerated follicular atresia.

Recently, Quitter et al. and Jin and Warren [34,35] performed a large analysis using a complete X tiling path array to detect cryptic copy number variations (CNV) in idiopathic cytogenetically normal POF patients. The new data reported in this study revealed unknown polymorphic CNV not previously associated with the disease. These chromosome amplifications and deletions are likely to alter the expression of novel clusters of POF-candidate X-linked sequences, including genes involved in chromosome pairing and segregation (POF1, CENP1, USP9X), hormone-dependent oocytes development and maturation (ST3), and apoptotic responses (AIFM1, BCORL1).

As a consequence, there are multiple discrete intervals on the human X chromosome that may impact on those cellular processes that are important for normal ovarian function.

### Candidate Genes on the X Chromosome

Identification of deletions and translocations in POF1 and POF2 regions has suggested several POF-candidate genes, such as CHM (Xq21.2), POFIB (Xq21.2), DACH2 (Xq21.3), DIAPH2 (Xq22), XPNPEP2 (Xq25) [36-38], although very few mutations have actually been detected in these loci [39,40].

Another “POF critical region”, where several Turner syndrome traits are located, maps to the short arm of X chromosome and includes zinc finger protein, X-linked (ZFX, Xp22.1–21.3) and bone morphogenic protein 15 (BMP15, Xp11.2) [26]. The BMP15, as one of the member of TGFβ superfamily, encodes a growth and differentiation factor, which regulates follicle maturation, follicular germ cells sensitivity to FSH action, germ cells apoptosis, oocyte developmental competence, and ovulation [41-44]. In humans, several missense variations in BMP15 gene have been found in association with POF with a frequency between 1.5 and 12% [45-47]. A reduced production of bioactive BMP15 protein, probably due to a mechanism of haploinsufficiency, may lead to ovarian dysgenesis through: i) an impairment of the anti-apoptotic effects on germinal cells, then favoring follicle atresia; ii) an altered recruitment of pre-antral follicles by gonadotropins [35].

A X-linked mutation leading to an increased risk for POF is the fragile X premutation [48]. It is characterized by a large CCG repeat track (55-199 repeats) in the 5' untranslated region of the fragile X mental retardation 1 (FMR1) gene located at Xq27.3. Premutation carriers have been identified in 0.8% to 7.5% of women with sporadic POF and in up to 13% of women with familial forms [49]. The small expansions (55-79 repeats) are unmethylated and a FMR1 mRNA gain-of-function toxicity may underlie the altered ovarian function occurring in premutation carriers [50], since the accumulation of the RNA-binding FMR protein may impair the expression of genes required for oocyte development in fetal ovary [35,51]. On the other hand, longer repeats are hypermethylated and the expression of the FMR1 gene is repressed, thus favoring follicle atresia [52].

### Autosomal Candidate Genes of POF

In the last decades, researchers focused on classic gene-specific candidate-driven studies, mostly based on genetically modified mouse
GWAS have evolved as an alternative approach for finding novel effects (FSH, FSHR, LH, LHR, CYP17 and CYP19); ii) genes that affect the rate of initial recruitment from the primordial follicle pool into growing follicles (BMP15, GDF9, FOXL2 and GPR3); iii) DNA binding proteins, transcription factors like NOBOX and LHX8, and RNA binding proteins like NANOS.

Hormones such as FSH, LH and their receptors, play an important role in follicular development. Although follicle stimulating hormone receptor (FSHR) is the only well characterized autosomal recessive gene that causes non-syndromic POF [58], heterozygous polymorphic variants involving the gonadotropin receptors have been detected in both patients and control individuals, thus they do not appear to be the cause of POF [59-61]. Similarly, polymorphisms in Estrogen Receptor (ER) gene have been associated with POF, but further validation studies in larger groups of patients are needed [62].

Meiotic events play an essential role in establishment of the primordial pool of follicles. Interestingly, mutations of DMCI (a DNA strand exchange protein that acts on double-strand breaks in meiosis [63]), MSH5 (a meiosis-specific protein [64]), and ATM (involved in DNA damage checkpoint control and activated in response to double-strand breaks [65]) have been associated with POF [66,67].

The long survival of primordial quiescent follicles is necessary for preservation of the length of reproductive life. It has been thus supposed that dysfunction of molecules that maintain the primordial follicles may cause POF by damaging the primordial pool or over-activating primordial follicles. An oocyte-specific gene, essential for primordial follicle formation, is factor in germline alpha (FIGLA) that has been found mutated in POF women [68]. Other oocyte-specific factors -FOXO2 (forkhead box protein L2) and NOBOX (newborn ovary homeobox gene) - are important for transition of primordial follicles to primary follicles. Interestingly, mutations in FOXO2 and in the homeobox domain of the NOBOX gene are associated with POF in humans [69-71], although conflicting results have been reported by other studies [72,73].

Recently, several genes have been identified as negative regulators of follicular activation. In their absence-as in mice lacking PTEN, FOXO3a, or P27KIP1- the pool of prematurely activated, primordial follicles undergo atresia [74-76]. These studies support the idea that deregulated activation of primordial follicles may be a cause for POF. Consistently, mutation screenings in POF patients revealed two potentially pathogenic variations in FOXO3a and FOXO1a forkhead transcription factors [77,78].

Defects in follicular development lead to lack of functional ovarian follicles and anovulation. In this context, mutations in BMP15 have been associated with POF, as mentioned above. Besides BMP15, other TGFβ family members have arelevant role in the progression of folliculogenesis. Among them, there are GDF9, which is expressed in the oocyte and forms BMP15/GDF9 heterodimers [79], and inhibit A (INHA), that acts as a negative modulator of pituitary FSH synthesis or as a paracrine factor [80]. Rare insertion/deletion and missense variations in GDF9 [81-84] and INHA [85-89] have been observed in POF patients.

Genome-Wide association Studies of POF

Over the last ten years Genome-Wide Association Studies (GWAS) have evolved as an alternative approach for finding novel candidate genes and chromosomal loci of human diseases. In contrast to methods which specifically test one or a few genetic regions, the GWAS investigate the entire genome. The approach is therefore said to be non-candidate-driven. GWAS typically focus on associations between single-nucleotide polymorphisms (SNPs) and complex traits, by comparing DNA of cases and controls groups.

The first GWAS in POF was reported by Kang et al. [90]. This two-stage association study in Korean women (101 cases and 87 controls) suggested that PTHB1 gene may be associated with POF. In another study (99 cases and 235 controls) a possible association with ADAMTS19, a gene expressed in female mouse gonads, has been suggested [39]. However, replication in an independent cohort of 60 POF patients and 90 controls did not confirm a clear association. Moreover, the Authors did not observe strong evidence for any of 74 selected POF candidate regions being associated with idiopathic POF in Caucasian females, although suggestive association was observed for SNPs that mapped in BDNF, CXCL12, LHR, USP9X and TAF4B, all possible candidate genes on the basis of animal models. The weakness of both these studies is the small sample size, thus replications in independent larger cohorts are warranted to obtain a significant statistical power.

CVN in POF

Recent developments and applications of genome-wide structural variation technologies, such as array comparative genomic hybridization (aCGH), have led to the identification of CVN. The CVN, defined as regions of DNA larger than 1 kb that display copy number differences in the normal population, contribute to genetic variation associated with diseases or susceptibility to diseases [40]. Indeed, CVN can influence transcriptional and translational levels of overlapping or nearby genes [91]. Thus, in addition to GWAS based on SNPs, there is increasing interest toward the association of structural variants with complex traits.

The first study aimed to assess the presence and the prevalence of CVN in sporadic and familiar POF was performed by Aboura et al. [92] by DNA microarrays comprising 4500 bacterial artificial chromosome (BAC) clones spread on the entire genome. The authors reported eight statistically significantly different CVN on the X chromosome and autosomes and, within them, they identified genes involved in reproductive disease (DNAH5 and NAIP), reproductive endocrinology (DUSP22 and NUPR1), and folliculogenes (AKT1), thus representing five potential candidate genes in POF.

Oligonucleotide-microarrays with higher resolution allowed the identification of 44 micro-deletions and microduplications potentially causative for POF [93]. Intriguingly, these aberrant chromosome regions harbor genes involved in meiosis (PLCB1, RB1CC1, MAP4K4), DNA repair (RBBP8), and folliculogenesis (IMMP2L, FER1L6, MEIG1) pathways.

Very recently, a SNP array-based study confirmed that CVN associate with POF and that the majority of candidate genes for ovarian failure are located on autosomes [94]. In fact, in addition to only one novel microdeletion located on the X chromosome, the authors discovered seven novel autosomal microdeletions and seventeen novel autosomal microduplications among 88 successfully arrayed POF women.

Future studies in larger cohorts of patients are warranted to validate whether recurring CVN are present in women with POF and to discern the clinical utility of molecular karyotyping methods, such as high-
resolution aCGH, in replacing conventional karyotyping. Moreover, animal models that target novel human deletions and/or duplications may be useful in elucidating the functional role of such genetic variants in reproductive biology.

Genetics of Familial Idiopathic POF

Although epidemiological evidence based on mother-daughter pairs supports the heritability of menopausal age [95,96] and some Authors reported cases of familial premature menopause [97,98], very little is known about the inheritance pattern of the idiopathic POF. The overall incidence of familial POF ranges between 4% and 31% depending on the inclusion criteria adopted and the availability of a detailed family history [95,96,98]. However, due to rare familial cases with full pedigrees, few genome-wide linkage analyses have been performed so far. The first two studies on relatively large pedigrees established linkage to the POF critical region Xq21.1-q21.3.3 [99] and to a 15.8 Mb region on chromosome 5 (5q14.1-q15) [100] which harbors novel candidate POF susceptibility genes. Sequencing of POF2 region identified a point mutation in the exon 10 of POFIB gene. The disruption of mutated POFIB binding to nonmuscle actin filaments may lead to up-regulation of primordial oocytes apoptosis through a loss of function of POFIB in pairing meiotic chromosomes or in cytoskeletal dynamics [99].

On one hand, the most accepted theory suggests an either maternal or paternal dominant transmission of POF [98,100]. It is often difficult to distinguish an autosomal dominant pattern of inheritance from an X-linked one since transmitting males with both affected and unaffected daughters, while expected for autosomal dominant inheritance can also occur with X-linkage if penetrance is incomplete. In families with maternal transmission the risk of recurring POF is always 50% (39.5% corrected by incomplete penetrance), where as in families with paternal transmission the risk is 100% (79.1% corrected by penetrance) when the disorder has an X-linked pattern of inheritance, and it decreases to 50% (39.5% corrected by penetrance) when the dominant pattern of inheritance is autosomal. Therefore, a specific genetic counseling is necessary to properly assess the reproductive risk [98].

On the other hand, very recently Caburet et al. [101] reported a recessive autosomal pattern of inheritance in one large consanguineous Middle-Eastern POF-affected family. In particular, the Authors identified two regions with a LOD ≥ 3.26 on 7p21.1-15.3 and 7q21.3-22.2, which were supported as candidate loci by homozygosity mapping. The region on 7q21.3-22.2 includes DLX5 and DLX6 genes that are involved in steroidogenesis, and SHFM1 which is required for oogenesis and normal female fertility in animal models. Although these three genes are implicated in the etiology of the Split Hand/Split Foot Malformation Type 1 syndrome, that is not associated with POF, their possible function in the ovary led the Authors to sequence them, but they did not detected any causal mutations.

Epigenetics of POF

A genetic disease is classically caused by changes in DNA sequence due to point mutations and/or chromosome aberrations. Additional molecular mechanisms involved in genetic diseases are functionally modifications of the genome-the so called "epigenetic abnormalities" – which comprise DNA methylation, histone modifications, chromatin structure and non-coding RNAs. They can all regulate proximal promoter activity as well as distal gene expression.

According to a first model, chromatin structure alterations may be responsible of an epigenetic origin of POF. Firstly, rearrangements involving X chromosome (X: autosome translocations, terminal deletions) have been suggested to adversely affect X chromosome structure leading to defective meiotic pairing, that might increase apoptosis of germ cells at meiotic checkpoints [102]. The importance of chromatin structure in the pathogenesis of X-linked POF has been then confirmed by Rizzolio et al. [103] who reported that heterochromatin rearrangements of the Xq12-q21 region may down regulate oocyte-expressed genes during oocytes and follicle maturation.

Starting from the observation that in POF patients most X-autosome balanced translocation break points map in "critical regions" that do not contain transcribed sequences (i.e.; Xq13.3-q26) [104-106], the same group postulated that key genes may be not necessarily included in regions of deletion or amplification or disrupted by translocations. Specifically, Rizzolio et al. [107] proposed that autosomal genes, expressed in the oocytes, when translocated to the active X chromosome undergo their down expression driven by the epigenetic mechanisms regulating X-linked genes. Therefore, two different mechanisms may be responsible of X-linked POF. One, acting in Turner syndrome and in POF with partial X monosomies, is dependent on haploinsufficiency of X-linked genes for ovarian function. The second one acts on autosomal ovary-expressed genes when translocated to the X chromosome critical region and may result in their down regulation by a position effect of cis regulatory sequences.

More recently, a new paradigm has been considered for the etiology of ovarian diseases such as POF: epigenetic abnormalities can be induced by exposure to a variety of environmental toxins [108]. Moreover, if the exposure occurs during fetal gonadal development, these epigenetic abnormalities can be fixed into the germ line and be passed to offspring, thus increasing susceptibility to an ovarian adult-onset disease. Molecular analyses disclosed that the effect of the environmental compound exposure on germ cells consists of differential expression of more than 500 genes and alterations in only 43 DNA methylated regions (DMR) [108]. Since this relatively low number of epigenetic DMR sites could not explain the large number of differentially expressed genes observed, the Authors stated that the epigenetic regulatory sites associated with the DMR may influence distal gene expression through non-coding RNAs. Therefore, the hypothesis developing is that also the epigenetic pathogenesis of POF is a multifactorial phenomenon.

Summary and Future Perspectives

In recent years, the candidate gene approach allowed identification of many genes and pathways involved in POF. However, known genetic alterations in POF patients are detected in only 20-25% of the cases originally classified as idiopathic. Therefore, the pathogenic mechanism of POF is still largely unknown. Certainly, high-throughput genome-wide studies are giving a large contribution for discovery novel POF genes and in the near future application of innovative next generation sequencing technology may open new prospects to decipher the multifactorial genetic etiology of POF. The final aim of this effort should be the development of a genetic test for early prediction of menopausal age, after further validation of candidate genes, linkage and association studies.

Presently, subtle changes in ovarian function with advancing age (i.e., serum concentrations of estradiol, progesterone, luteinizing hormone and activin as well as follicle dynamics) seem interesting but they are not clinically useful as predictive test. More long term prognostic ability has been attributed to family history, serum levels of AMH and FSH, and AFC [109]. To date, none of these markers is
able to early predict the evolution toward POF with a good diagnostic accuracy and only karyo type and FMRI premutation testing are routinely used in counseling of infertility for women with idiopathic POI or belonging to POF families.

Feasibility of a high quality, well powered genetic test may open the possibility of an efficient counseling service for female infertility and establish “ad hoc” interventions for the prevention of the consequences of POF. Moreover, those women being identified with a high risk of suffering from POF might make important decisions concerning their conception and eventually apply for fertility preservation techniques.

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