High genetic heterogeneity of Premature Ovarian Insufficiency

From DNA replication and repair to hormonal regulation
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Disclosure Statement:
Gouri Mukerjee and Ruslan Dorfman are employed by Geneyouin, a company that offers genetic screening testing.

Abstract
Primary ovarian insufficiency greatly influences a woman’s fertility potential and her overall health. The condition affects about 1–2 % of women and in most cases, the cause is undefined. Primary ovarian insufficiency (POI) may be caused by any process that results in dysfunction or depletion of ovarian follicles, reducing the number of oocytes within the ovary. The tendency for POI to run in families implies a strong genetic component underlying the condition. The most common single gene mutation to cause POI is the premutation of the fragile-X mental retardation gene 1 (FMR1), located on Xq27.3. Many other candidate genes have been suggested to play a role in the POI etiology, with mutations identified in genes involving follicle function and oogenesis, such as FOXL2, BMP15, NRSF1, Inhibin A, LHR, FSHR and the phenotype. In addition, variations in genes involved in meiosis and DNA repair have been hypothesized to affect the normal process of follicle formation and diminish ovarian reserve resulting in infertility. An alternative approach to identify novel POI candidate genes is the genome-wide analysis and we also report on a few studies that might have identified novel susceptibility genes for POI.

Introduction
A woman’s ovary has several million potential oocytes at around five months of gestational age. These are held in a quiescent state until required for ovulation, years later. Most of these potential oocytes are destroyed by the body before birth presumably as part of a quality control mechanism. Between infancy and the age of 40 years, the potential oocytes are gradually reduced from approximately one million to 10,000 in each ovary and around the age of 40, the process of egg destruction accelerates with other investigators. Unfortunately, some women can experience irregular menstrual cycles and stop producing oocytes in their early 30’s leading to the condition called Premature Ovarian Insufficiency (POI). Genetic analysis has identified aberrations in several biological pathways that can result in this condition. This review summarizes the evidence for involvement of multiple developmental genes, as well as highlights the role of known oncogenes in POI.

Disease Definition
A woman, through her reproductive life, uses fewer than 500 eggs, a tiny proportion of the original millions (Hueh 1994, Tilley 2001). Primary Ovarian Insufficiency (POI) or Premature Ovarian Failure (POF) may be caused by any process that results in dysfunction or depletion of ovarian follicles, reducing the number of oocytes within the ovary. It is defined as early menopause with elevated levels of serum gonadotrophins before the age of 40 (Coady 1982). Various terms have been used to describe this deviation from healthy ovarian function, including ‘premature menopause’ (Albright 1942) coined the term ‘primary ovarian insufficiency’ to emphasise that the primary defect was within, rather than outside the ovary. Other conditions involving endocrine disturbances outside the ovary can also result in abnormal ovulation including pituitary disorders, adrenal dysfunction, or polycystic ovary syndrome. The term ‘ovarian insufficiency’ is regarded as more scientifically accurate than ‘ovarian failure’ (De Vos 2010) as insufficiency indicates impaired ovarian function suggesting that follicular activity of the ovary might intermittently recover, years after diagnosis leading to pregnancy in some women (Nelson 2005).

Disease Diagnosis
Primary ovarian insufficiency (POI) affects about 1–2 % of women (Vegetti 2000) and in most cases, the cause is undefined. Destruction of primordial follicles by toxic agents, autoimmune response, activation of proapoptotic pathways, or accelerated follicular recruitment might result in premature depletion of the pool of primordial follicles. Accurate and timely diagnosis of POI poses challenges as hormonal and biochemical tests do not show the monthly follicle loss and thus do not indicate the true biological age of ovaries. Direct evidence of depletion of the resting pool of follicles can be reliably provided only through assessment of the total number of follicles in whole ovaries. Testing of biopsy samples of ovaries has been suggested as a diagnostic method to measure ovarian follicular reserve (Massin 2008), with other investigators concluding that analysis of laparoscopic biopsy samples cannot be used to predict follicular distribution in ovarian cortex (Lambalk 2004).

Genetics of POI
Chromosomal abnormalities account for 12% of cases (Jiao 2012), and the familial aggregation often associated with POI indicating a significant genetic contribution. Incidence of familial cases among women with POI has been reported to be as low as 4% (Conway, 1996), but it might be an underestimation and epidemiological studies have indicated incidence of familial POI as high as 30% (Cramer 1995, Torgeron 1997). In a large Italian study, Vegetti et al. (1998) found that the condition was inherited in one-third of the idiopathic POI patients. Pedigree studies on affected families showed a mode of inheritance suggestive of autosomal dominant sex-limited transmission or X-linked inheritance with incomplete penetrance (van Kasteren 1999). Using family history can help to distinguish between familial or sporadic primary ovarian insufficiency as the risk of female relatives developing this condition may be as high as 100% in familial primary ovarian insufficiency, or as low as 1% in sporadic cases (van Kasteren 1999).

In rare cases, sufficient ovarian follicles are present but they do not function i.e. oocytes do not mature in regular cycles. However, in a large proportion of cases no cause is found and they are classified as idiopathic or karyotypically normal spontaneous ovarian failure (Lam 2000).

Unraveling the genetic causes of POI
Several methods have been used to elucidate the role of genetic contributors in the pathogenesis of POI — transgenic ‘knockout’ animals, mutation screening in affected women, analysing pedigree data in linkage analysis. Genetic association studies aim to identify candidate genes or genome regions that contribute to a specific trait or disease by identifying a correlation between disease status and genetic variation (Cordell 2005) and we report on several candidate genes that are believed to contribute to POI.
POI genes on the X-chromosome

Premature ovarian senescence is many times associated with abnormalities in the X chromosome. Women with structural and numerical abnormalities in the X chromosome make up the largest subgroup with POI. During early embryonic development, one of the X chromosomes is randomly inactivated by methylation in female somatic cells (Sato 2004). In some women, X chromosome structural abnormalities, such as large deletions and unbalanced translocations, skewed patterns of X chromosome inactivation (SXXCI) may result with the abnormal inactivation pattern being transmitted to the germ cells. Other women may inherit only one X chromosome (45,X) leading to congenital Turner’s syndrome (Sybert 2004). Although one X chromosome is sufficient to allow the normal development of most organs and initial differentiation of ovaries, oocytes need two active X chromosomes. Defective X chromosome leads to insufficient gene dosage of many genes, and haploinsufficiency of the X chromosome results in depletion of the oocyte pool in the first 10 years of life.

Link between Fragile X and POI

Mutations in the FRM1 gene can also lead to the expansion of a CGG trinucleotide repeat located at the 5’UTR region of the gene. Long repeats of CGG trinucleotides lead to reduced gene expression and Fragile X mental retardation syndrome. Repeat lengths between 59 and 199 of the CGG repeat confer an unstable premutation state. Women with the premutation state have a substantially increased risk of POI. Besides Turner’s syndrome, premutation in the FRM1 gene is the most common known congenital cause of POI. Cryptic deletions in FMR2 gene, located near the FRM1, have also been suggested as an X chromosome-linked cause of primary ovarian insufficiency (Murray 1999).

Multiple rare mutations in oocyte development and a hypothalamic-pituitary regulation genes contribute to the risk of POI

FOLLX2 is a member of the forkhead/hepatocyte nuclear factor 3 gene family of transcription factors that plays a role in sex determination. Mutations in FOLLX2 cause congenital hypogonadotropic hypogonadism (CHH), an X-linked syndrome (BPD1) that is characterized by premature death of egg cells (Crinòpin 2001). FoxL2 knockout mice were shown to replicate the findings in humans (Schmidt 2004). Reduced FoxL2 expression resulted in the characteristic cranio-facial alterations and infertility with folliculogenesis being blocked at the early stages. A functional study supporting the role of FoxL2 mutations in nonsyndromic POI was reported by LAUSSON et al. (2009). A novel FoxL2 missense mutation p.G138N in a case of POI without BPD1 was identified. The subcellular localization of the mutant protein was normal, but its functional activity was significantly lower than that of normal FoxL2 protein.

NRSAn1

NRSAn1 gene, also called steroidogenic factor 1, plays a key role in ovarian development and function. Mutations in the gene were detected in members of four families with a history of POI but not in the 700 control alleles (Lorenzoni 2009). Mutations were associated with a range of ovarian anormalities, with functional analysis revealing that mutant proteins had altered transcriptional activity that is important for follicle growth and maturation.

Members of the TGF superfamily

Heterozygous mutations in BMP15 (Bone Morphogenetic Protein 15), an oocyte-specific growth/differentiation factor that stimulates follicle growth and ovarian development during early folliculogenesis, has been implicated in POI (Di Pasquale 2009). It is presumed to be expressed both from X chromosomes in oocytes, and could potentially show a gene dosage effect. BMP15 maps to a locus on the short arm of X chromosome (Xp11.2), within a ‘POI critical region’ (Persani 2009). In humans, mutations in BMP15 gene have been found in POI cohorts. Rossetti et al. (2009) demonstrated that BMP15 protein coding variations resulted in reduced production of bioactive BMP15 proteins in comparison with wild type, thus functional effects of these mutations is consistent with a mechanism of haploinsufficiency. Mature BMP15 proteins with missense variations also showed significant reduction in their biological effects in human cell-lines (Rossetti 2009).

Inhibit A, NOBOX

Inhibit A plays an important role in regulating ovarian function either as a negative modulator of pituitary FSH synthesis. Inhibit A (INHA) gene knockout mice have raised FSH levels, are sterile, and have mixed gonadal tissue in the adult with nearly 100% penetrance (Matzuk 1992). Therefore, Inhibit A was regarded as a candidate gene for mutational studies. One missense variation of INHA gene (p.A257T) was found to be associated with POI in several populations: the INHA variant was identified in Indian, New Zealand and Slovenian patients (Shellogg 2008, Duit 2004). An Italian study also reported a significant association between the A257T allele in INHA and sporadic (4.5%) and familial (7.7%) POI cases (Manzoni 2002).

However, other studies have found no differences in variants with frequent between POI cases and controls (Corre 2009).

NOBOX and FIGLA are oocyte-specific transcription factors, and deletion of either of these genes could accelerate post-natal oocyte loss. Mutations in NOBOX seem to occur more frequently in the Caucasian POI population. The NOBOX missense variant, p.R351H, first identified in 1 of 96 Caucasian POI subjects, could disrupt the binding of the NOBOX homodomain to DNA (Qin 2007). Bousley et al. (2011) subsequently demonstrated that loss of function function NOBOX mutations accounted for 6.2% of POI cases in a Caucasian cohort of 178 participants.

Gonadotropin receptors

FSH and LH are glycoprotein hormone receptors which together with their binding hormones, LH and FSH, are essential for normal reproductive function. A linkage analysis in a Finnish population revealed a significant association between a locus containing both FSHR and LHFR genes and ovarian developmental disorder. Sequencing of the entire FSHR gene revealed a homonymous missense mutation, p.A189V (Attimski 1995) that has been observed only in the Finnish population suggesting a founder effect. From in vitro studies it was observed that the p.A189V mutation had altered receptor folding and it failed to reach the plasma membrane, causing complete FSH resistance.

STAT5

Using a combination of genome wide linkage analysis and exome sequencing in a consanguineous (people descended from the same ancestor) family with POI, Cabrera et al. (2014) identified a homogenous 1-bp deletion in the gene encoding steroidogenic factor 1 (STAG3). All affected family members analyzed were homogygous for the mutation. This finding was supported by the phenotype of female mice with a homogenous disruption in Stag3. These mice were sterile and their fetal oocytes were arrested at early prophase 1, leading to oocyte depletion at one week of age.

Genes involved in meiosis and DNA repair

It has been proposed that genetic defects in meiotic genes associated with POI may be widespread among women with POI. These mice have phenotypes resembling human POI. Wang et al (2014) identified a heterozygous mutation in a meiotic gene, HMFI1, which encodes a protein necessary for homologous recombination of chromosomes, in two sisters suffering from POI. Variants in genes that affect the normal processes of primordial germ-cell proliferation, oocyte meiosis, and formation are plausible candidates in the pathogenesis of POI. Also, HMFI1-deficient mice are infertile (Guarnelli 2013).

BRCAl mutations, fertility treatments and POI

As infertility is associated with breast and ovarian cancer risks, Oktay et al (2010) hypothesized that mutations in the BRCA1 and BRCA2 genes could be involved in fertility treatments. Low response to ovariative stimulation is a strong indication of diminished ovarian reserve and infertility. As DNA repair is deficient in patients with BRCA mutations, their oocytes may be more prone to DNA damage, and when DNA damage cannot be repaired, apoptotic pathways are activated. Thus, oocytes with deficient BRCA function may be prematurely eliminated, resulting in early depletion of oocyte pool and, as a consequence, OA. Oktay et al (2010) found that in BRCA mutation-positive patients, the incidence of low ovarian response was significantly higher compared to BRCA mutation-negative patients. Of note, all BRCA mutation-positive low responders to fertility treatment had BRCA1 mutations, but not BRCA2. These findings can explain, in part, the link between infertility and breast/ovarian cancer risks.

Genome Wide Association Studies in POI

Genome Wide Association Studies (GWAS) have been conducted to scan across the genome for loci associated with POI. The drawback of this method is that it does not take into account the biological relevance of the variants discovered.

PTB1 and ADAMTS19

In a two-stage association study in a Korean population (101 cases and 107 controls). Kang et al. (2009) showed a strong association of POI with the PTB1 gene. PTB1 was first identified in osteoblast cells and then in other tissues, but not the ovary; and its physiological function remains unknown. PTB1 has been linked to both cell survival and discovery-driven, providing a comprehensive approach that is based on a case-control design. The association was confirmed in another study of 97 cases and 108 controls. The association of POI with the PTB1 gene was first identified in osteoblast cells and then in other tissues, but not the ovary; and its physiological function remains unknown. PTB1 has been linked to both cell survival and discovery-driven, providing a comprehensive approach that is based on a case-control design. The association was confirmed in another study of 97 cases and 108 controls. The association of POI with the PTB1 gene was first identified in osteoblast cells and then in other tissues, but not the ovary; and its physiological function remains unknown. PTB1 has been linked to both cell survival and discovery-driven, providing a comprehensive approach that is based on a case-control design.
A rapid decline in the cost of sequencing is enabling effective prenatal analysis and whole-genome variation analysis along with choromosomal deletions and copy number analysis. Application of genome or exome sequencing to identify variants can confirm genetic testing that provides the ethical consequences of applying genetic screening should be evaluated on a case-by-case basis. ●

References