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Invited Review

Genetics of primary ovarian insufficiency

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Primary ovarian insufficiency (POI) is characterized by a loss of ovarian function before the age of 40 and account for one major cause of female infertility. POI relevance is continuously growing because of the increasing number of women desiring conception beyond 30 years of age, when POI prevalence is >1%. POI is highly heterogeneous and can present with ovarian dysgenesis and primary amenorrhea, or with secondary amenorrhea, and it can be associated with other congenital or acquired abnormalities. In most cases POI remains classified as idiopathic. However, the age of menopause is an inheritable trait and POI has a strong genetic component. This is confirmed by the existence of several candidate genes, experimental and natural models. The variable expressivity of POI defect may indicate that, this disease may frequently be considered as a multifactorial or oligogenic defect. The most common genetic contributors to POI are the X chromosome-linked defects. Here, we review the principal X-linked and autosomal genes involved in syndromic and non-syndromic forms of POI with the expectation that this list will soon be upgraded, thus allowing the possibility to predict the risk of an early age at menopause in families with POI.

Conflict of interest

Authors have no conflicts to declare.

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In most of the women from industrialized countries, the median age at menopause occurs between 50 and 52 years of age (1), but early menopause (EM) occurs in about 10% of the women before 45 years of age, and in 1–2% before 40 years (2, 3). As the fertility impairment starts around 20 years before menopause (4), premature ovarian aging or primary ovarian insufficiency (POI) did not represent a relevant disease when the expected lifespan was <65 years and women usually conceived in their twenties. But, due to the cultural and socioeconomical changes of the last decades, nowadays a high number of women program their first pregnancy beyond 30 years of age (5) and POI has therefore acquired a particular importance because this disease is currently diagnosed when fertility is irreversibly affected (6) (Table 1). In most cases, POI becomes clinically [primary amenorrhea (PA) or secondary amenorrhea (SA) for >4 months] and biochemically manifest [high follicle-stimulating hormone (FSH), low estradiol and anti-Mullerian hormone (AMH)] when the ovarian follicular reserve is already severely depleted, thus justifying studies aiming to define tests able to predict the risk of an early age at menopause. A reliable test would allow young women recognized to be at risk of POI to program their fertility by anticipating the age of their spontaneous pregnancy or cryopreserving their oocytes.

The heterogeneous manifestations and multifactorial origin of POI

The clinical presentation of POI is highly heterogeneous as it can be associated with ovarian dysgenesis (OD) and PA or with anticipated depletion of the ovarian reserve and SA before 40 years of age. Such heterogeneity is reflected also by the different mechanisms potentially accounting for POI. During human embryogenesis, developing ovaries house around 7 millions of primordial follicles. Most of these follicles will be rapidly lost by apoptosis, while some germ cells will continue to divide, a few will enter meiosis to become primordial

Table 1. Relevant impact of POI on woman's and social health

Condition	Consequences	Comments
Infertility	Psychological	Severe impact for affected woman and family
	Interventions to rescue fertility after POI onset	Frequently ineffective
	Oocyte cryopreservation before follicle depletion	These interventions are rarely supported by NHS
	Oocyte donation	
Hormonal defects	Flushes, sweating, irritability/anxiety, sleep disorders	Premature aging at several tissue levels has increasing impact due to the prolongation of life expectancy, but may be reduced by appropriate HRT.
	Impaired performance (physical and mental)	
	Bone disease (osteoporosis)	
	Metabolic and cardiovascular diseases	
	Neurodegenerative diseases	Costs for examinations and therapeutics.

HRT, hormone replacement therapy; NHS, National Health System; POI, primary ovarian insufficiency.

oocytes. Half a million oocytes will not be ovulated and only approximately 400 are ovulated before physiological menopause occurs. Multiple mechanisms have been described to be causatives of POI onset, including: (i) presence of a smaller pool of primordial follicles, (ii) increased follicular atresia, or (iii) an altered maturation and/or recruitment of primordial follicles (7). Nevertheless, in the majority of cases, including a subset associated with PA and OD (8, 9), POI occurs because of premature exhaustion of the primordial follicular pool. A wide range of etiological causes may activate such mechanisms including genetic, autoimmune, metabolic, toxic, infectious and iatrogenic factors (10). At present, about 25% of all forms of infertility at early age can be classified as iatrogenic and related to cancer treatment. The benefits of fertility preservation in these situations are well established (11, 12), but freezing of oocytes or ovarian fragments can be advised also in women at high-risk of POI. However, fertility preservation for these women should be supported by an efficient test able to predict POI when the ovarian reserve is still intact.

Multiple evidences support a strong genetic component underlying the pathogenesis of idiopathic POI (13). One important clue is the role of familiarity in the determination of menopausal age in mothers and daughters (13–15) and a recent genome wide study identified loci that are significantly associated with age at natural menopause (16). Surveys focused on large series of women with POI found that 4–31% had one or more affected family members, depending on the population studied, but this incidence further increase in presence of a familial history of EM (17). Different modes of inheritance can be found in POI families, but the maternal transmission is by far the most frequent (10, 18, 19). The concomitant presence of POI and EM in the same pedigree suggests that POI may have genetic underlying causes with a highly variable expressivity (20), thus supporting the view of a complex multifactorial diseases characterized by a great genetic heterogeneity probably involving the contribution of stochastic events, several alleles and/or epigenetics.

Over the past several years, the candidate gene approach contributed to find a correlation between the ovarian phenotype and several genetic variations in different genes. Some of those candidates arose from experimental or natural animal models showing ovarian failure, however, in many cases no variants in the corresponding human orthologues have been found, but this might be due to the small size of the cohorts or the ethnicity group investigated. Among the genes associated with POI, only a few (such as *FMR1* premutation, *BMP15*, *GDF9*, and *FSHR*) have been incorporated as diagnostic biomarkers (21), and scientific community and patients ask for more research before using other genes as a routine tool (22). Additionally, submicroscopic copy number variations (CNVs), both rare and common, have recently emerged as an important genetic risk category for POI, both in cases of PA and SA. Nevertheless, a large number of genes or genomic loci, affected by common CNVs or single nucleotide polymorphisms (SNPs) and identified by genome-wide association studied (GWA), may have a role in the disease susceptibility but can explain only a small proportion of the total heritability of POI (23). The analysis of a few cohorts of 46,XX POI patients by means of high throughput techniques, such as comparative genomic hybridization array (array-CGH) and SNP array, has led to the identification of CNVs affecting several X-linked and autosomal loci with a possible role in female fertility (24–34). Similarly, the recent application of whole-exome sequencing (WES) to a few POI multigenerational familial cases has succeeded in revealing rare single nucleotide variants affecting genes implicated in ovarian function (35–48). As already reported in other complex diseases characterized by a great genetic heterogeneity, it is likely that patients with POI may harbor multiple genetic variants. For that reason, henceforth the investigations on POI candidate genes could be rapidly performed by using genetic panels based on the next-generation sequencing (NGS), which enable the study of multiple candidate genes in the same patient for a dozen of patients simultaneously (49, 50). The regulatory function of microRNAs (miRNAs) in oocyte maturation and ovarian follicular development might be implicated in the development of POI by affecting different signaling pathways (51–53).

In the next section, we elucidate the main genetic mechanisms involved in the pathogenesis of POI (Table 2).

Table 2. List of genetic defects associated with POI and their estimated frequencies

	Estimated frequency in POI	References
X chromosome defects		
Turner's syndrome and related defects	4–5%	(9, 55, 59, 60)
Triple X syndrome	1–4%	(62)
Fragile X syndrome (*FMR1* premutation)	3–15%	(119, 120, 122, 132)
DIAPH2 disruption (translocation)	Unknown	(18, 19)
BMP15 variants	1.5–12%	(143–149)
PGRMC1 variants	1.5%	(155)
Autosomal defects		
Complex diseases	Rare	
Galactosemia (*GALT*)		(94, 95, 98)
BPES (*FOXL2*)		(79, 88)
APECED (*AIRE*)		(65, 70, 71)
Mitochondrial diseases (*POLG*)		(102, 103)
Demirhan syndrome (*BMPR1B*)		(109)
PHP1a (*GNAS*)		(101)
Ovarioleucodystrophy (*EIF2B*)		(106)
Ataxia telangiectasia (*ATM*)		(108)
Perrault syndrome (*HSD17B4*, *HARS2*, *CLPP*, *LARS2*, *C10ORF2*)		[a]
Premature aging syndromes:		
Bloom syndrome (*BLM*)		(113, 114)
Werner syndrome (*WRN*)		(117)
GAPO disease (*ANTXR1*)		(118)
Isolated disease		
FSH/LH resistance (*FSHR* and *LHCGR*)	0–1%	(42, 47, 134, 135)
INHA variants	0–11%	(138, 139)
GDF9 variants	1.4%	(32, 146, 150)
FOXL2 variants	Rare	(50, 92, 93)
FOXO3 variants	2.2%	(168, 169)
NOBOX variants	0–6%	(50, 175, 177–179, 182)
FIGLA variants	1–2%[b]	(183, 184)
NR5A1 variants	1.6%	(164, 165)
LHX8 variants	Rare	(50, 185, 186)
DNA replication/meiosis and DNA repair genes variants (*DMC1*, *MSH4*, *MSH5*, *SPO11*, *STAG3*, *SMC1*β, *REC8*, *POF1B*, *HFM1*, *MCM8*, *MCM9*, *SYCE1*, *PSMC3IP*, *NUP107*, *FANCA*, *FANCC*,*FANCG*)	Unknown	(35, 38–41, 45, 156–162)

FSH, follicle-stimulating hormone; LH, luteinizing hormone; POI, primary ovarian insufficiency.
[a]Refer to specific article on this same issue.
[b]1% in Indian and 2% in Chinese women, respectively.

Syndromic POI

Turner syndrome and X chromosome defects

Turner syndrome (TS) is the consequence of complete or partial loss of one X chromosome. In almost all patients, the resulting phenotype is that of a female with infertility due to POI and short stature, variably associated with other extra-gonadal abnormalities. The 45,X has been recognized as the characteristic karyotype associated with TS with an incidence of about 1:2500 live female births (54). However, a recent revision of previous data argues that the surviving individuals are most likely 45,X/46,XX mosaicisms or structural abnormalities of the X chromosome (55). In women with TS, oocyte-loss occurs in the early stages of meiotic prophase and ovarian development, resulting in OD and PA with elevated FSH levels since infancy (9). Nevertheless, spontaneous menarche and pregnancies have been reported (56, 57). The TS phenotype may be explained by several mechanisms, including the lack of a homologous partner for the X chromosome at meiosis (58), but the most substantiated one is the lack of required dosage of particular X-linked gene products (like *SHOX*) that physiologically escape X inactivation (59). The requirement for a double dosage of X-linked genes is supported by the complete spontaneous puberty reached in about one third of patients with high level mosaicisms (56, 60). Recently, we documented the spontaneous puberty in one TS patient with short stature and a full duplication of the *BMP15* gene on the short arm of X chromosome in presence of low level mosaicism (<10%), suggesting a relevant role for a double dose of this gene in ovarian development (60, 61).

X chromosome abnormalities have long been recognized as a frequent cause of many forms of familial as well as sporadic POI. They include triple X syndrome

(62), turner mosaics, deletions, isochromosomes and translocations between X chromosome and autosomes (18). Critical regions for normal ovarian development have been proposed on the long arm of the X chromosome: Xq13-21, which is interested by most of the breakpoints of balanced translocations, and Xq23-27, which is associated with interstitial deletions (18). The size of the critical Xq region might alternatively explain the ovarian defect (18). Other possible deleterious consequences of balanced translocations include the direct disruption of relevant loci or a 'position effect' caused by the rearrangements on contiguous genes, which might cause changes in gene transcription. Transcriptional characterization of breakpoint regions led to the identification of five genes interrupted by translocations: the *XPNPEP2* (MIM *300145) gene in Xq25, the *POF1B* (MIM *300603) gene in Xq21.2, and the *DACH2* (MIM *300608) gene in Xq21.3, the *CHM* (MIM *300390) gene in Xq21.2 and the *DIAPH2* (MIM *300108) gene in Xq22 (18, 19). However, breakpoints described in women with POI were frequently mapped outside of genic regions in Xq21, consistent with models for POI involving extra-X chromosome effects caused by X to autosome translocations. In addition, some translocations may adversely affect X chromosome structure and, consequently, meiotic pairing, therefore increasing apoptosis of germ cells at meiotic checkpoints (63) and ultimately exacerbate POI.

Moreover, X-linked POI pathogenesis may also have an epigenetic component, as suggested by the fact that a down-regulation of genes specifically expressed in oocyte during follicle maturation were reported upon heterochromatin rearrangements of the Xq13-q21 region (64).

Autoimmune polyendocrinopathy syndrome type I

Autoimmune polyendocrinopathy syndrome type I (APS1, MIM #240300) is caused by mutations in the autoimmune regulator gene (*AIRE*, MIM *607358). The syndrome is characterized by having two out of three major clinical findings: Addison's disease (AD), and/or hypoparathyroidism, and/or chronic mucocutaneous candidiasis. Generally in this syndrome, the AD has its onset in childhood or early adulthood. APS1 is frequently associated with chronic active hepatitis, malabsorption, juvenile-onset pernicious anemia, alopecia, and primary hypogonadism. On the other hand, diabetes mellitus and autoimmune thyroid disease are infrequent in APS1. Other forms of autoimmune polyendocrinopathy syndromes are APS2 or APS3 (65). APS2 (MIM #269200) includes AD in association with autoimmune thyroid disease and/or diabetes mellitus type 1 and its genetics remain to be well defined. APS3 includes patients with autoimmune thyroid disease and another autoimmune disorder not to include AD.

In 1990, Ahonen et al. reported candidiasis as the presenting symptom in the majority of the patients with APS1 (66) and POI was present in 60% of the women over the age of 13. Two laboratories concurred in the isolation of the causal gene for APS1 in 1997 and designated it *AIRE* (67, 68). The gene is located at 21q22.3 The AIRE protein contains two zinc finger motifs consistent with a role as transcription factor. AIRE is involved in the induction of tolerance to self-antigens by inducing the expression of peripheral tissue self-antigens in thymic stromal cells. Normally, this promotes the clonal deletion of differentiating T cells that recognize these self-antigens. In the absence of AIRE protein, many tissue-specific self-antigens fail to be expressed in the thymus, thus leading to multi-organ autoimmunity due to the failure in the negative selection of auto-reactive T cells (69). Negative selection normally causes death of T cells which have receptors that are highly specific for self-peptides. If these auto-reactive T cells are left unchecked autoimmunity may result. Around 50 mutations in *AIRE* have been identified and are inherited in a recessive manner.

Soderbergh et al. in their study of a cohort of 90 patients with APS1 found an association between AD and the presence of antibodies against 21-hydroxylase (P450 c21) and side-chain cleavage enzyme (P450scc) (70). In the same report, hypogonadism was exclusively associated with antibodies against P450scc with an odds ratio of 12.5. Jasti et al. examined the fertility and ovarian function of Aire-deficient mice and found that only 16% were able to produce two litters (71). By 20 weeks of age approximately half of these mice exhibited ovarian follicle depletion. This was associated with ovarian infiltration of proliferating CD3+ T lymphocytes and the presence of serum antibodies against oocytes, as well as stromal and luteal cells. Taken together the findings are consistent with the idea that ovarian dysfunction and eventual follicular depletion are mechanisms of infertility in Aire-deficient mice.

Women with POI related to steroidogenic cell autoimmunity have lymphocytic autoimmune oophoritis as the mechanism of their POI (72). Reato et al. investigated the prevalence of POI in women with autoimmune AD (73). In this specific clinical setting they found POI in 41% of women with APS1 and 16% of women with APS2. Falorni et al. examined the prevalence of steroidogenic cell autoantibodies in women with POI who also had adrenal autoimmunity (74), and found that these women were frequently positive for 17-hydroxylase and/or P450scc autoantibodies. Recent studies show that POI women with positive anti-steroidogenic cell autoantibodies have frequently conserved AMH levels (75, 76), a finding that may indicate a prevalent theca cell involvement, at least in the initial phases of the ovarian failure. Recently, mono-allelic dominant mutations in the first plant homeodomain (PHD1) zinc finger of *AIRE*, which exert a dominant negative effect, have been identified at relatively high frequencies in different populations in presence of late onset and milder manifestations compared with classical APS1, such as only hypothyroidism and POI, and follow incomplete inheritance (77). In this context, POI might represent the first or even the only manifestation of a non-classical form of organ-specific autoimmunity.

Ovarian antibodies detected by indirect immunofluorescence lack specificity and testing for them is therefore

not warranted (78). Ovarian biopsy does not provide information that helps management and is not indicated outside of an investigational protocol.

Blepharophimosis, ptosis, epicanthus inversus syndrome

Blepharophimosis, ptosis, epicanthus inversus syndrome (BPES, MIM #110100) is an autosomal dominant eyelid malformation characterized by BPES and telecanthus. When the condition is associated with POI it is considered type I BPES. When not associated with POI it is considered type II BPES. Forkhead transcription factor L2 (*FOXL2*, MIM *605597) mutations are known to be associated with BPES (79). Animal models of human BPES, including the goat with polled/intersex syndrome (PIS) and the Foxl2 knock-out mice, were shown to replicate the findings in humans (80, 81). FOXL2 role in granulosa cells (GC) physiology includes the promotion of GC differentiation and postnatal maintenance of ovaries. Briefly, FOXL2 regulates: (i) AMH expression through the interaction with steroidogenic factor 1 (SF-1) (82, 83), (ii) follistatin gene transcription by cooperating with SMAD3 (84), (iii) estradiol signaling by triggering the activin-dependent expression of ESR2 (85) and (iv) maintenance of GC identity through the indirect repression of SOX9 (85).

More than 260 *FOXL2* variants have been reported in individuals with BPES types I and II, demonstrating that phenotypic features are caused by the pleiotropic effect of a single gene (FOXL2 Mutation Database at http://medgen.ugent.be/LOVD2/home.php) (86). Intragenic mutations of all types represent about 80% of the genetic defects found in BPES cohorts. Genomic rearrangements, comprising deletions encompassing *FOXL2* entire gene or located outside its transcription unit, represent 12% and 5% of all genetic defects, respectively (87).

FOXL2 intragenic mutations resulting in truncated proteins before the poly-Ala tract are typically associated with BPES type I, whereas poly-Ala expansions would rather lead to BPES type II (88). However, in most cases *FOXL2* mutations are not in accordance with the ovarian phenotype, especially the missense mutations located within the forkhead domain (FHD). Therefore, some authors proposed that mutants could be sorted into two classes: those that potentially alter protein–protein interactions and those that might disrupt the interactions with DNA (89). The *in silico* tool based on a crystallographic-derived molecular model for testing variants affecting the FHD looks at the localization of the side chain of each amino acid of the FHD helices, which strongly correlates with their impact on FOXL2 transactivation capacity (89). Finally, two different reporter promoters are used to test the *FOXL2* variants' transcriptional activity in order to assess the associated risk of POI (90). However, recently reported cases emphasize the importance of long-term clinical follow-up of ovarian function also in patients with a poly-Ala expansion (7).

FOXL2 sequence variants in women with POI but without palpebral abnormalities appear to be a rare event (91). To date, only three variants have been documented (92, 93). Very recently, another *FOXL2* variant has been identified by NGS and described as loss-of-function (50).

Galactosemia and carbohydrate-deficient glycoprotein syndromes

Proper galactose metabolism is required for normal ovarian function. Galactosemia (MIM #230400) is a hereditary disorder of galactose metabolism caused by deficiency of galactose-1-phosphatase uridyltransferase (GALT, MIM *606999) enzyme, with an incidence in Europe and North America of about 1:30,000–1:50,000) (94). Galactosemia presents with the worst complications in organs with high GALT expression (liver, kidney, ovary and heart). Up to 80–90% female patients with *GALT* homozygous mutations that partially or completely abolish GALT activity show a severe phenotype and exhibit POI (94, 95). More than 150 causative mutations have been described in *GALT* gene (96), however, more than 70% of cases associated with impaired GALT function are caused by two common mutations (p.Q188R and p.K285N) (97). Lacking the proper metabolism of galactose, patients with *GALT* mutations accumulate galactose in the ovary to toxic levels and follicles undergo accelerated atresia (98). FSH levels can be increased since birth to puberty and the timing of the damage to the ovary can vary, but is frequently associated with PA (94). Spontaneous pregnancies have been reported in a few women with galactosemia, even when biochemical markers (undetectable AMH and estradiol and high gonadotrophins) were indicative of ovarian failure (99).

Congenital disorders of glycosylation [carbohydrate-deficient glycoprotein (CDG) syndromes] are rare and complex diseases caused, among others, by mutations in *PMM2* (*CDG1*, MIM *601785) gene, which encodes a phosphomannomutase enzyme required for the conversion of mannose-6-phosphate into mannose-1-phosphate. Genetic defects of such enzymes generally determine severe systemic disorders, and ovarian defects may be seen indicating that a defective glycosylation of ovarian glycoproteins is critical for ovarian function (100).

Pseudo-hypoparathyroidism type 1a

Pseudo-hypoparathyroidism type 1a (PHP1a, MIM #103580) is a generalized form of hormone resistance characterized by renal resistance to parathyroid hormone (PTH), resulting in hypocalcemia and hyperphosphatemia. Moreover, it is characterized by resistance to other hormones including thyroid-stimulating hormone (TSH), gonadotropins and growth-hormone-releasing hormone (GHRH) and a variety of clinical features known as Albright hereditary osteodystrophy. Gonadal dysfunction with delayed or incomplete sexual maturation, amenorrhea or oligomenorrhea and/or infertility is very frequent. In about 70–80% of cases, PHP1a is caused by maternally inherited heterozygous loss-of-function variants in the *GNAS* gene (MIM

*139320). Methylation defects at the same locus can also be disease-causing. The *GNAS* gene encodes for the protein Gsα, which is the first intracellular element downstream of gonadotropin receptors and whose activation couples the stimulation of FSH and luteinizing hormone (LH) receptors (FSHR and LHCGR) to their enzymatic effector, adenylyl cyclase. The presence of gonadotropin resistance and POI in these patients is justified by the preferential expression of a mutant maternal allele in gonads as in other target tissues of peptide hormones acting through the same GPCR-Gsα-cAMP pathway (101).

Progressive external ophthalmoplegia

The *POLG* gene (MIM *174763) encodes for the enzyme that synthesizes new mitochondrial DNA and corrects mitochondrial DNA errors. Patients with *POLG* mutations present with autosomal dominant (MIM #157640) or recessive (MIM #258450) progressive external ophthalmoplegia (PEO), a disease characterized by weakness of the ocular muscles and myopathy secondary to the depletion of mitochondria. *POLG* mutations which cluster in the polymerase (*pol*) domain undergo a typical dominant inheritance pattern, while those affecting the proofreading (exonuclease, *exo*) domain follow recessive inheritance. POLG *pol*-domain mutations have been consistently reported in several large families in co-segregation with POI and parkinsonism (102, 103).

Ovarioleucodystrophy

Ovarioleukodystrophies are by definition genetic neurological disorders characterized by the involvement of the white matter of the central nervous system associated with POI (104). Some of the patients have unusual association of POI with 'vanishing white matter disease' (VWM, MIM #603896) observed on cerebral magnetic resonance imaging, with variations in any of the five subunits of eukaryotic initiation factor 2B (*EIF2B*). This factor acts in response to cellular stress preventing the accumulation of denatured proteins. The age at onset of neurologic degeneration correlates positively with the severity of ovarian dysfunction. Moreover, in some cases, POI manifests before the neurological symptoms or occurs when neurological abnormalities are subclinical (105). Therefore, the involvement of *EIF2B* mutations should be considered even in patients with apparent isolated POI. The screening of a large panel of patients without leukodystrophy or neurological symptoms showed no mutations in *EIF2B* genes (106), thus indicating that *EIF2B* mutations are not responsible for non-syndromic forms of POI.

Ataxia telangiectasia

Ataxia telangiectasia mutated gene (*ATM*, MIM *607585) encodes a cell-cycle checkpoint kinase which is involved in the cellular response to DNA damage, in the processing of the DNA strand breaks that occur during meiosis, during immune system maturation and for telomere maintenance (107). *ATM* mutations are the underlying causes of ATM (MIM #208900), an autosomal-recessive disorder which includes cerebellar degeneration, oculomotor dysfunction, immunodeficiency, predisposition for cancer, radiosensitivity and chromosome instability as well as gonadal abnormalities and reduced germ cell pool. Mutations in the *ATM* gene generally result in the total loss of the protein. A loss-of-function mutation of the *ATM* gene has been associated with OD and defects in primordial germ cells development (108).

Demirhan syndrome

Mutations in *BMPR1B*, the gene coding for bone morphogenetic protein receptor 1B (MIM *603248), have been found to cause a subtype of acromesomelic chondrodysplasia with genital anomalies, amenorrhea and hypergonadotrophic hypogonadism, defined Demirhan syndrome (109). Acrosomelic chondrodysplasias are hereditary skeletal disorders characterized by short stature, very short limbs and hand/foot malformations. BMPR1B is a receptor for member of the transforming growth factor-beta (TGF-β) family and is fundamental for gonadal and skeletal development, as confirmed by the existence of naturally occurring *BMPR1B* variants found in association with the hyperproliphic Booroola phenotype in sheep and the female knock-out mice presenting with brachydactyly and infertility (110–112).

Premature aging syndromes

Several syndromes characterized by symptoms of 'premature aging' are associated with POI (or azoospermia in males). Bloom syndrome (MIM #21090) is a rare autosomal recessive disorder caused by mutations in the gene coding for the DNA helicase BLM (MIM #604610), which result in genomic instability. The main symptoms of Bloom syndrome include short stature, distinctive skin rashes on sun-exposed areas, moderate immunodeficiency, increased cancer risk and hypogonadism in both sexes (113). Nevertheless, successful pregnancies in women with Bloom syndrome have been reported, although rarely, in literature (114, 115). Recessive mutations in the *WRN* gene (MIM #604611), which encodes another DNA helicase, are the causatives of Werner syndrome (MIM #604611), a form of adult progeria characterized by sklerodermic-like skin, cataract, premature arteriosclerosis, increased cancer risk and atrophic gonads (116). Also in these patients, successful pregnancies have been reported, although rarely (117). Another form of syndromic premature aging associated with POI is represented by growth retardation, alopecia, pseudoanodontotia, and optic atrophy (GAPO) syndrome (MIM #230740), which is caused by recessive mutations in a gene involved in cell adhesion and migration, *ANTXR1* (MIM *606410). GAPO syndrome is characterized by severe growth retardation, alopecia, optic atrophy and distinctive facial features. Ovaries of women affected

by GAPO syndrome display extensive deposition of hyaline extracellular material and premature follicular depletion (118).

The epidemiological observations that POI is often associated with syndromes characterized by premature aging further support the growing idea that even non-syndromic POI could be considered as a form of ovary-specific accelerated aging.

Non-syndromic POI

Fragile X mental retardation 1

The fragile X mental retardation 1 (*FMR1*) gene is located on the X chromosome and includes a trinucleotide repeat sequence, (CGG)n, in its 5′ untranslated region. Common alleles include 6-44 CGG repeats, typically with AGG interspersions every 9 or 10 repeats. When expanded to 55–200 repeats, this 'premutation' becomes unstable when transmitted and has the potential to expand beyond 200 repeats in the next generation. The resulting 'full' mutation then leads to the full silencing of the *FMR1* gene due to hypermethylation of the repeat and regulatory regions and causes fragile X syndrome, the most common inherited form of intellectual and developmental disabilities in males. The premutation is carried by about 1 in 250 women. Among women who carry the premutation, approximately 15–24% have POI (119), the disorder referred to as fragile X-associated premature ovarian insufficiency (FXPOI). About 11.5% [95% confidence interval (CI): 5.4–20.8%] of women with familial POI and 3.2% (95% CI: 1.4–6.2%) of those with sporadic POI carry the premutation (119). Thus, the *FMR1* premutation has emerged as the leading known heritable cause of both sporadic and familial POI (19).

Studies conducted on women carrying the premutation allele that are still having regular menstrual cycles revealed that their hormonal hypothalamic-pituitary-gonadal axis profile is strikingly similar to that of aging ovaries: increased gonadotropins and decreased inhibin B in the follicular phase and decreased inhibin A (INHA) and progesterone in the luteal phase (120). More recent studies have shown reduced levels of AMH among premutation carriers compared with non-carriers, again, consistent with an aging ovary (121).

Two risk factors have surfaced as potential predictors of risk and severity of FXPOI, namely repeat size (122) and mean age at menopause of first degree relatives (123). With respect to repeat size, a non-linear relationship with severity of FXPOI has been established: premutation carriers with the highest risk for FXPOI turn out to be those with about 80–100 repeats, not those with >100 repeats. Consistently, among those with 80–100 repeats, the onset of FXPOI was earliest, rarely before 20 years. Several studies aimed at the investigation of the role of intermediate CGG repeat size (45–54 repeats) in POI have been performed but produced varying results. Indeed, some studies highlighted an increased frequency of intermediate alleles in 'occult' POI (124–126), while, more recently, other authors did not show any positive association, despite a significantly larger sample size, thus limiting the consideration of normal- and intermediate *FMR1* repeat size in the diagnostic evaluation of women with POI, or in order to predict POI onset in women at risk (127, 128). Additional research is needed to better define the repeat size alleles which assesses the highest risk and the underlying mechanisms. The second established risk factor is mean age at menopause of first degree relatives. Accordingly, modifier genes could play a substantial role in the variability of age at menopause among premutation carriers.

The mechanism leading to FXPOI is still unexplained. As the premutation repeat size increases, the level of *FMR1* transcripts abnormally increases and the level of FMRP, the resulting protein, decreases (129). The toxic effect of the premutation could have its influence at several levels. FMR1 mRNA studies in the mouse (130) and FMRP expression studies in fetal ovaries (131) indicate that FMRP is highly expressed in the germ cells of the fetal ovary. Thus, FMRP may play a role in oogonia proliferation and the determination of the initial size of the ovarian reserve. Interestingly, several studies have indicated that FMRP is also expressed in GC of maturing follicles, but not in primordial/primary stages (130, 131). Schuettler et al. (132) suggested that this cellular shift of FMRP expression to GC during follicle maturation after birth indicates a role for FMRP in the maturation of an oocyte.

Based on the knowledge that FMRP is involved in the suppression of transcripts' translation, it may be possible that increased levels of FMRP in specific moments during development could lead to the insufficiency of proteins necessary in oocyte development, or for follicle development and survival. Alternatively, the large CGG repeat track in the premutation allele mRNA may determine a cumulative toxic effect in GC, leading to an increased rate of follicular atresia later in a woman's reproductive life.

Gonadotropin receptors

FSH and LH receptors are glycoprotein hormone receptors belonging to the G-protein-coupled receptors (GPCRs) family. Together with their binding hormones, LH and FSH, these receptors are involved in regulating reproductive hormonal signaling in both males and females. Rare loss-of-function mutations affecting these receptors cause gonadotropin resistance with hypergonadotrophic hypogonadism (133). For example, the homozygous missense mutation that determines the p.A189V substitution in the extracellular domain of the *FSHR* gene (MIM *136435) causes PA, hypergonadotropic hypogonadism and hypoplastic ovaries with impaired follicular growth. However, this mutation appears to be particularly frequent only in the Finnish population, as result of a founder effect. From *in vitro* studies, the mutant receptor is retained inside the cells thus causing a complete FSH resistance (134). Other mutations in different regions of the *FSHR* gene have been reported in women with the classic biochemical phenotype of premature ovarian insufficiency (FSH higher than LH levels). Very recently, thanks to the

WES approach, two novel causative *FSHR* missense variants (p. I418S and p.D408Y) have been identified in two distinct families, each one composed of two sisters diagnosed with PA and hypergonadotropic gonadal failure (42, 47). Complete FSH resistance is associated with absent pubertal development and PA and partial forms are characterized by post-pubertal POI and SA. However, both the complete and partial forms undergo a typical recessive inheritance (133).

Homozygous inactivating variants of the *LHCGR* gene (MIM *152790) are a rare cause of POI in women with 46,XX karyotype. They represent a particular form of hypergonadotropic hypogonadism characterized by LH levels higher than those of FSH. Studies in males affected with Leydig cell hypoplasia evidenced the particular phenotype of ovarian insufficiency in women with LH resistance (135). A severe LH resistance cause the POI phenotype characterized by oligoamenorrhea or SA with evidence of multiple follicles at the antral stage at ultrasound. Although different mature follicles are present, as evidenced by ovarian biopsies, ovulation does not occur.

TGF-β family

Both the oocyte and GC within the ovarian follicle express several TGF-β-like factors (136), which promote proliferation and differentiation in the tissues where they are expressed. These factors include: growth and differentiation factors (GDFs), bone morphogenetic proteins (BMPs), as well as inhibins, activins or AMH. TGF-β-like factors are commonly expressed as pre-pro-proteins, which undergo proteolytic cleavage during the secretory pathway. The precursors are specifically cleaved to generate the 'mature' ligand, which alone or in combination with other secreted factors promote the cell signaling cascade. The pro-region is important for the processing of the pro-protein by driving the dimerization and secretion of the mature peptides. Several of these factors acting within the ovarian follicles are required for maintaining the follicle homeostasis and for proper folliculogenesis. Therefore, the related encoding genes are considered as candidates to be investigated in women with POI (7, 112).

Inhibin A

Inhibin is a candidate gene involved in regulating ovarian function either as negative modulator of pituitary FSH synthesis or as a paracrine factor within the ovarian follicles. Based on studies of transgenic mice with *INHA* gene deletion which early develop stromal/granulosa cell tumors with raised FSH levels and infertility with nearly 100% penetrance, it has been postulated that inhibin functions *in vivo* as a tumor suppressor in the gonads of mice (137). The association between inhibin and POI was first suggested by the identification in a woman with POI of a 46,XX,t(2;15)(q32.3;q13.3) translocation causing a breakpoint in the α subunit of inhibin (*INHA*; MIM *147380, locus 2q33-36), therefore prompting the mutational screening of this gene (138). One recurrent variation of *INHA* (p.A257T) has been consistently found in women affected by POI of different ethnicities, with a prevalence of 0–11% depending on the population studied. A large-scale association study in Italian and German POI cohorts, however, evidenced no significant differences in variant frequency between patients and controls (139). Nevertheless, a further meta-analysis of the random effects on the risk of POI in carriers of the *INHA* variant from the most relevant studies revealed a combined risk difference of 0.04 (138). Based on these results, *INHA* gene might be considered as a susceptibility locus for POI.

Bone morphogenetic protein 15

BMP15 (MIM *300247) is an oocyte-specific growth/differentiation factor which is involved in follicular development and in the regulation of many GC processes (112, 136). The main BMP15 actions include: (i) the promotion of follicle growth and maturation, (ii) regulation of follicular GC sensitivity to FSH, (iii) prevention of GC apoptosis, (iv) promotion of oocyte developmental competence and (v) determination of ovulation quota (61, 136). Consistent with a role for this gene in folliculogenesis and ovulation, ewes with heterozygous naturally occurring mutations have an increased ovulation rate, while homozygous carriers show infertility with complete block of folliculogenesis. Unlike mutated Bmp15 homozygous ewes, female knock-out mice show only subfertility (140). All together, these data indicate that the role of BMP15 appears to differ between species and seems more critical in mono-ovulating species (such as sheep and human) than in the poly-ovulating ones (mice). Accordingly, mouse seems to lack a biologically active Bmp15 molecule (141), but the over-expression of a biologically active Bmp15 in mice leads to accelerate folliculogenesis and causes an early onset of ovarian failure (142). *BMP15* maps to a locus on Xp critical to ovarian reserve determination where several of the TS traits are located including ovarian failure (19, 59). In women, mutations in *BMP15* gene have been associated with both PA and SA in several POI cohorts with a prevalence ranging from 1.5% to 15% (7). BMP15 p.Y235C was the first mutation reported in association with hypergonadotropic ovarian insufficiency in two Italian sisters with PA and OD (143). The Y235 residue is highly conserved among species and corresponds to a site of positive selection in the hominidae clade during evolution. When functionally tested, this alteration enhances the BMP15-induced transcriptional activity and causes increased GC steroidogenesis (144). In contrast, BMP15 p.Y235C was unable to increase GC proliferation, as previously shown (143). Several other variants have been further identified with variable frequency in worldwide POI cohorts (145–151). Almost all of the identified *BMP15* variants are missense substitutions found in the heterozygous state and located in the gene sequence encoding the pro-region of the protein which lead to hampered processing, together with an important reduction in their biological functions (149). Although, some of these variants have also been found in low percentage in

the control populations, a finding that may question their pathogenic role, a recent revision of the frequency of *BMP15* variants in POI and control populations revealed a 10-fold higher frequency of heterozygous *BMP15* variations in cases (61). In a recent study on X chromosome mosaicism, we identified a tandem duplication of the single *BMP15* gene in a patient with 45,X karyotype, who experienced a spontaneous menarche followed by regular menses for 4 years. This *BMP15* duplication would have enabled a small amount of functional follicles to survive atresia and reach pubertal age by means of a partial compensation to the haploinsufficiency for the other X-linked genes. Consistent with this, fluorescent in-situ hybridization (FISH) and array-CGH experiments demonstrated that the presence of a mosaicism with the euploid cell line >10% would be sufficient for spontaneous menarche to happen (60). Taken together, these data highlight how *BMP15* gene dosage contributes to the ovarian phenotype of patients with TS and further support the hypothesis that *BMP15* is an ovary-determining X-linked gene (61, 152). This finally brings additional support to the idea that inactivating mutations in this gene can represent a predisposing event for POI.

Growth differentiation factor 9

Growth differentiation factor 9 (*GDF9*, MIM *601918), also named *GDF9b*, is homologous to the gene encoding for *BMP15.* GDF9 expression is high in the oocytes, where its products can form non-covalent heterodimers with BMP15, which are active in surrounding follicular GC. Evidences from experimental animals have strongly suggested that GDF9 activity is crucial in poly-ovulating species: in mice, for instance, GDF9 is fundamental for folliculogenesis (112, 136). Concerning mono-ovulating species, natural *GDF9* mutations have been described both in Cambridge and Belclare sheeps, where the ovarian phenotype was analogous to that seen in *BMP15* mutants (140). In humans, all the *GDF9* variations described so far in different cohorts are all missense and in the heterozygous state, affecting exclusively the pro-region with a prevalence of 1.4%. None were detected in the control populations (112). A duplication affecting the regulatory region of GDF9 was detected by array-CGH in a patient with early onset of SA. The duplicated region contains three newborn ovary homeobox (NOBOX)-binding elements and an E-box, important for *GDF9* gene regulation, and it is likely causative of POI (32). Rarely, some insertion/deletion or missense variants in GDF9 have been reported in mothers of dizygotic twins (153, 154), with an incidence around 4%, thus supporting GDF9 as a determinant of the ovulation quota also in humans.

Progesterone receptor membrane component 1

Progesterone receptor membrane component 1 (*PGRMC1*, MIM *300435) gene has been described as candidate for POI following the identification of an X/autosome translocation in Xq13-26, within the X 'critical region' in association with POI. Further, a missense substitution located in the intracellular C-terminus of PGRMC1, which impair the anti-apoptotic action of progesterone in the developing ovary leading to premature ovarian follicles depletion, has been found at the heterozygous state by the genetic screening of 67 women with POI (155).

Genes affecting DNA replication, meiosis and DNA repair

Variations in genes involved in creation and repair of DNA double-strand breaks for recombination, DNA damage checkpoint control, cell cycle progression or formation of the synaptonemal complex may be associated with POI, as strongly suggested by mouse models showing a POI-like phenotype.

Among those genes known to be potentially associated with POI, which include *DMC1* or *LIM15* (MIM *602721), *MSH4* (MIM *602105), *MSH5* (MIM *603382), and *SPO11* (MIM *605114), variations in *MSH5* and *DMC1* have been identified by the genetic screening of women with POI (156). However, this study has not been confirmed yet in larger populations and further studies are needed to evaluate the functional consequences of the identified variants in order to corroborate the link with POI onset.

STAG3 encodes a meiosis-specific subunit of the cohesin ring, which ensures correct sister chromatid cohesion. Using WES analysis of a large consanguineous Palestinian family, a homozygous 1-bp deletion inducing a frameshift mutation in *STAG3* segregated with POI, a finding that is further supported by the phenotype of knock-out female mice with fetal oocytes arrested at early prophase I and oocyte depletion within 1 week of age (38). Variants in two other genes part of the cohesin complex (*SMC1*β and *REC8*) have been recently identified in association with POI onset (38). These genes regulate sister chromatid cohesion and recombination between homologous chromosomes. The pathogenic role of the identified variants in POI is supported by studies of *Smc1*$\beta^{-/-}$ and *Rec8*$^{-/-}$ female mice that show very early ovarian defects and are sterile as a result of early meiotic arrest in oocytes (157, 158).

A mutation in *POF1B* gene was discovered in a homozygous state in association with POI by sequencing a Lebanese family (159). *POF1B* is an X-linked gene located within the critical region for normal ovarian function, known to escape X inactivation, which encodes a protein that interacts with actin filaments, and the authors speculate that POF1B could have a role in the pairing of meiotic chromosomes and that alteration in its function could increase germ-cell apoptosis and lead to POI.

A shared compound heterozygous mutation in another meiotic gene, *HFM1*, which encodes a protein necessary for homologous recombination of chromosomes, resulted in autosomal recessive POI in two affected Chinese sisters. The mother and father each carried one of the mutations, and both parents were clinically normal. Sequencing of *HFM1* in an additional cohort of 69 Chinese women with sporadic POI identified another compound heterozygous mutation. All the identified variants were not found in 316 matched controls (160). Another

gene required for homologous recombination, *PSMC3IP* (MIM *608665) has been found to harbor variations causative of POI (35).

MCM8 and MCM9 are members of the highly conserved mini-chromosome maintenance proteins (MCM) complex involved in homologous recombination and repair of double-stranded DNA breaks. SNP analysis and WES revealed several autosomal-recessive variants causing genomic-instability and association with hypergonadotropic hypogonadism (40, 41, 161).

A protein-truncating homozygous mutation was identified by exome sequencing in the *SYCE1* gene, encoding an essential component of the synaptonemal complex, where paired chromosome homologs closely associate in meiosis before crossover, in an Israeli Arab family with a consanguineous pedigree (39).

Recently, mutations in *NUP107* (MIM *607617) have been described in association with POI and OD (45). NUP107 is a component of the nuclear pore complex, which mediates nucleocytoplasmic transport of macromolecules, such as transcription factors, thus promoting cell-specific gene-expression. Although the role of this protein is to be confirmed, related genes in *Drosophila melanogaster* are necessary for mitosis and meiosis progression. Similarly, NUP107 could have a role in maintaining the meiotic cycle also in humans and variations affecting this gene could lead to impaired meiosis.

Finally, another chromosomal instability disorder associated with POI is Fanconi anemia, which can be caused by alterations in *FANCA* (MIM *607139), *FANCC* (MIM *613899) and *FANCG* (MIM *602956) genes (162).

Transcription factors

SF-1 or NR5A1

The *NR5A1* (MIM +184757) gene encodes for a nuclear receptor whose expression can be detected early in embryo development in bipotential gonads, where it plays a key role as a transcriptional regulator of genes involved in the hypothalamic-pituitary-steroidogenic axis (163), such as *STAR*, *CYP11A1*, *CYP17A1*, *CYP19A1*, *LH/CGR* and *INHA*. Mutations of *NR5A1* have been reported in cases of 46,XY disorders of sex development (DSD), with or without adrenal failure. The first evidence of an association between NR5A1 function and POI came from the detection of mutations in this transcription factor in members of four families with histories of both 46,XY DSD and 46,XX POI, as well as in 2 of 25 women with isolated ovarian insufficiency, but in none of 700 control alleles (164). The association between *NR5A1* mutations and POI pathogenesis was further confirmed by Janse et al. with a mutation frequency of 1.6% (165). Patients carrying *NR5A1* mutations show a wide spectrum of ovarian anomalies, ranging from SA to PA, or even gonadal dysgenesis. *NR5A1* mutations were further tested in *in vitro* experiments, which demonstrated that each mutant protein displayed an altered transactivational activity on gonadal promoters important for follicle growth and maturation.

Forkhead transcription factors

The forkhead family of transcription factors consists of over 100 genes encoding for proteins involved in a range of developmental processes, including a role in TGF-β signaling by means of binding to members of the Smad family proteins. Similarly to *FOXL2*, few other FHD-containing transcription factors have been demonstrated to have a role in ovarian function: *FOXO3a* (MIM *602681), *FOXO1a* (MIM *136533) and *FOXO4* (MIM *300033). *Foxo3a* knock-out female mice, for instance, show a premature development of follicles, followed by oocyte death, which results in a marked age-dependent decline in their reproductive fitness and, ultimately, in infertility (166). Alike, transgenic mice with a constitutive expression of Foxo3a in the oocytes show a delay in follicular development and oocyte growth resulting in infertility. Moreover, Foxo3a seems to have a regulatory role on BMP15, as the constitutive expression of Foxo3a determines a significant reduction of BMP15 expression (167). The striking ovarian phenotype of *Foxo3a* mouse models highly resembles the human POI phenotype, suggesting that *FOXO3a* could be a candidate gene for POI in women. Accordingly, two potentially pathogenic variations (p.S421L and p.R506H) in *FOXO3a* were found in a first screening in 2 out of 90 POI cases from New Zealand and Slovenia (2.2%), but not in controls (168). A subsequent analysis conducted on a cohort of 50 French patients identified only one missense variant (p.Y593S) probably with no deleterious impact on protein function (169).

Oocyte-specific transcription factors

NOBOX (MIM *610934) and *FIGLA* (factor in germline alpha) (MIM *608697) both encode for oocyte-specific transcription factors which in turn regulate genes unique to oocytes. NOBOX is a homeodomain-containing, oocyte- and GC-specific protein (170, 171) able to directly regulate the expression of key oocyte-specific factors such as Gdf9, Oct4 and KIT-L (172–175). In mice, NOBOX expression can be detected in embryonic ovaries as early as embryonic day 15.5, and its knockout has been shown to cause female sterility due to accelerated postnatal oocyte loss and blockade in the primordial to primary follicle transition (172, 176). Follicles are then replaced by fibrous tissue in female knockout mice in a manner similar to OD in women (172). Further corroborating the importance of NOBOX activity for folliculogenesis, heterozygous NOBOX mutations have been consistently reported in women with sporadic POI of African and Caucasian origin at a prevalence of approximately 6% (POF5, OMIM #611548) (175, 177–179), suggesting to consider NOBOX as the first autosomal candidate gene involved in POI (178). Vice versa, mutations in the homeobox domain of NOBOX seem not to be common explanations for POI in Asian women (180, 181). We recently documented that several NOBOX mutants form intracellular aggregates and are unable to enter the nucleus and activate transcription (182). Interestingly such mutants conserve the ability to interact with FOXL2, thus presumably reducing also its access to the nucleus.

Table 3. Particular health conditions of POI patients associated with variations in the listed genes

Particular phenotypes	Genetic associations
Hearing defects (Perrault syndrome)	*HSD17B4*, *HARS2*, *CLPP*, *LARS2*, *C10ORF2*
Progressive external ophthalmoplegia and tremor (parkinsonism)	*POLG*
Blepharophymosis, epicanthus inversus	*FOXL2*
Resistance to multiple hormones (PTH, GHRH, LH/FSH, TSH), short stature, short IV metacarpus, overweight	*GNAS*
Candidiasis, Addison disease	*AIRE*
Hypothyroidism	*AIRE* (mutations in the PHD1 domain), *MCM8*
X-linked mental retardation or tremor-ataxia in relatives	*FMR1* premutation
LH elevation higher than FSH, large ovarian follicles present, anovulation	*LHCGR*
Variable presence of small pre-antral follicles	*FSHR*
DSD (Swyer syndrome) in male relatives	*NR5A1*
Galactosemia	*GALT*
Vanishing white matter (VWM) disease with progressive neurological deterioration	*EIF2B*
Ataxia telangiectasia	*ATM*
Dehmiran syndrome	*BMPR1*
Short stature (cardiac malformations, lymphedema)	Turner mosaicism
Recurrent spontaneous dizygotic twinning	*GDF9*
46,XX ovarian dysgenesis	*BMP15*, *MCM9*, *FSHR*, *NUP107*, *PSMC3IP*, *ATM*
Premature aging syndromes (Bloom syndrome, Werner syndrome, GAPO disease)	*BLM*, *WRN*, *ANTXR1*

DSD, disorders of sex development; FSH, follicle-stimulating hormone; GHRH, growth-hormone-releasing hormone; LH, luteinizing hormone; POI, primary ovarian insufficiency; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone.

FIGLA is a basic helix-loop-helix (bHLH) transcription factor involved in the regulation of the expression of zona pellucida genes. *Figla*$^{-/-}$ female mice display a lack of primordial follicles formation concomitantly with a rapid loss of oocytes after birth, suggesting that *FIGLA* variations might have a role in the pathogenesis of POI in humans. A mutational study conducted on 100 Chinese women affected by POI revealed two heterozygous deletions (which were not detected among 304 ethnically matched controls) in two unrelated cases. Functional *in vitro* studies confirmed that both variants might have a pathogenic role (183). Two additional variants, p.R83C (positioned within the functional domain bHLH) and p.S141T (located outside the functional domain, but possibly impairing the protein–protein interaction between FIGLA and TCF3), were further identified in a cohort of Indian women with POI. Both variants were predicted as potentially pathogenic and disease-causing by *in silico* analysis, but were not experimentally tested (184). Recently, thanks to the NGS approach a rare loss-of-function variant (p.A41V) has been identified in 1 out of 100 women with idiopathic POI (50).

LHX8 gene is a member of the LIM-homeobox transcription factor family which encodes for a transcription factor acting as a germ-cell-specific critical regulator of early oogenesis. NGS of 100 women with POI detected the first reported variant of this gene. When functionally tested, the mutant exhibits a lower transcriptional activity on the promoter of Lin28A (a protein regulating primordial germ cell development) (50). Supporting the LHX8 critical role for maintenance and differentiation of the oocyte during early oogenesis, the murine knockout model display an impaired transition from primordial to growing follicles, together with a very rapid loss of primordial follicles. The ovaries of *Lhx8*$^{-/-}$ mice show an aberrant expression of oocyte-specific genes, such as *Gdf9*, *Pou5f1*, and *Nobox* (185). Nevertheless, only one *LHX8* variant was found in 100 patients (50) while a previous study found no variants in a cohort of 95 Caucasian women with POI (186), thus suggesting that while *LHX8* mutations may be a rare finding in POI.

The relevance of genetic investigations in patients with POI

In the last years, thanks to the sustained efforts in the investigation of new genetic POI determinants the prevalence of known genetic alterations in women with an idiopathic premature ovarian insufficiency is estimated at 25–30% and the pathogenic mechanism of POI onset still remain unknown in approximately 70% of cases. Indeed, the existing literature on POI is only the beginning of a better understanding of the molecular mechanisms and regulation of ovarian aging process and POI pathogenesis. The identification of the causative genetic alteration (or alterations) in a patient already diagnosed with POI is especially useful for her female relatives, who in case of positivity for the genetic screening, could be addressed to either egg or ovarian tissue freezing with later thawing and use in assisted reproductive technology at the appropriate age, embryo cryopreservation, anticipated pregnancy planning or novel patient-oriented protocols and tailored treatments, which could be made when fertility is still present and

no physical or biochemical parameters are altered (the 'occult' phase). In this perspective this precautionary measures will finally allow the preservation of female fertility of all women, preventing emotional and psychological discomfort of the patients and worthless costs for ineffective infertility treatments. This perspective is becoming increasingly important, as a high number of women want to conceive into their thirties and forties. At present, genetic screening for female infertility risk and/or assessment should be performed for the most prevalent genetic alterations (i.e. X chromosome abnormalities by karyotype or array-CGH- and the *FMR1* premutation). The prompt identification of such abnormalities would be of extreme importance in a context of family counseling not only for female fertility preservation, but also for the risk of X-linked male mental retardation associated with *FMR1* full mutation. Moreover, the recent papers on the field agree on the possibility of extending genetic investigations to also include genes like *BMP15*, *FIGLA*, *NOBOX* and *NR5A1*. Furthermore, the presence of POI signs together with distinctive phenotypes should advise for other, more specific, genes to be investigated (Table 3). Nevertheless, clinicians should pay careful attention in counseling patients on the basis of data derived from cohorts of different ethnicity as notable differences in prevalence exist among different populations (i.e. in case of *FSHR*, *BMP15*, *GDF9*, *NOBOX*, and *FOXL2* variants) (180, 181, 187–192). Henceforth, the investigations on POI candidates should be multi-ethnic and involve larger sample sizes. Consistently, several groups around the world are working toward the amelioration of the sensitivity of genetic screening in the near future and possibly develop NGS panels with adequate performance for the prediction of the risk of EM and POI (50). Recent studies suggest that a more extensive analysis at genomic level (which goes further than the suspected candidate gene investigation) will facilitate the identification of causative gene or genes responsible for POI. This scenario may indeed broaden specialists' chances for an efficient counseling service aimed at infertile women and establish '*ad hoc*' interventions for the prevention and management of the consequences of premature ovarian insufficiency.

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