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REVIEW

Genetic associations with diminished ovarian reserve: a systematic review of the literature

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Abstract
Purpose Diminished ovarian reserve (DOR) affects 10 % of women seeking fertility treatment. Although it is much more prevalent than premature ovarian failure, less is known about its etiology. The purpose of this article is to review the possible genetic causes of, and associations with, pathologic DOR.
Methods A systematic review was conducted using PubMed from 1966 through November 2013.
Results Twenty-one articles identified genes associated with DOR: one gene mutation (*FMR1*), three polymorphisms (*GDF9*, *FSHR*, and *ESR1*), and seven genes differentially expressed between women with DOR and controls (*AMH*, *LHCGR*, *IGF1, IGF2, IGF1R, IGF2R* and *GREM1*). Six candidate genes were discovered in mice, including *Foxl2*, *Gdf9*, *Bmp15*, *Aire*, *Wnt4*, and *Gpr3*. Two case reports of chromosomal translocations were also identified.
Conclusions While the etiology of pathologic DOR is likely multifactorial, it is possible that many cases attributed to an idiopathic cause may have a genetic component. Larger studies are needed to expose the impact gene mutations, polymorphisms, and epigenetics have on pathologic DOR.

Keywords DOR · Poor ovarian reserve · Genetic causes · Genes · Premature ovarian aging · POR

Capsule Diminished ovarian reserve (DOR) can be a normal process of aging. In other cases DOR occurs at earlier ages, pathologic DOR, a multifactorial condition which may be caused by genetic or epigenetic anomalies. We review what is currently known about the contribution of genetic causes to the clinical condition of DOR.

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Introduction

Approximately 10 % of women seeking fertility treatment have diminished ovarian reserve (DOR), defined as a decreased number or quality of oocytes [1–4]. While the definition is imprecise, DOR is associated with infertility and poor ovarian response to controlled ovarian hyperstimulation (COH). The Bologna Criteria published in 2011 defined poor ovarian response as having two of three of the following criteria: older than 39 years of age, prior poor ovarian response to conventional stimulation protocol (less than three oocytes retrieved), and abnormal ovarian reserve testing [5]. Of note, we draw a distinction between the age-related *physiologic* DOR and the *pathologic* DOR, as explained below. Abnormal ovarian reserve testing may be based on decreased antral follicle count (AFC <5) on ultrasound, decreased anti-müllerian hormone (AMH <0.5–1 ng/mL), or elevated levels of follicle stimulating hormone (FSH >10 IU/L on cycle day 2 to 4) [6, 5, 7, 8]. Inconsistency within the literature also exists regarding the definition of many terms associated with DOR. For the purpose of this review, primary ovarian insufficiency (POI) is equivalent to premature ovarian failure (POF) [9–11]. POI is defined as women under age 40 with amenorrhea for at least 6 months and a day 3 FSH level in the menopausal range (>40 IU/L). It is possible in some patients that DOR and POI occur within a spectrum, as they share many etiologies and features. Furthermore, studies of poor ovarian response and abnormal ovarian reserve testing in in vitro fertilization (IVF) cycles suggest an earlier age of menopause in some patients with DOR [12–14]. Despite an earlier age of menopause, most affected patients will not meet the definition for POI, yet some may become menopausal before the age of 45 years old. POI affects 1 % of women, whereas DOR affects a much larger portion of the infertile population [9–11, 15–18].

DOR can have multiple etiologies including genetic, autoimmune, idiopathic, and iatrogenic [19]. Ovarian surgery,

chemotherapy, and radiation are iatrogenic causes of DOR resulting from direct destruction of tissue or the toxic effects of therapy on the follicular pool. These risk factors for DOR are easily detected through a careful history. Age is another risk factor for DOR. At some point in a woman's reproductive life span, she will meet the criteria for DOR as she approaches menopause. It is expected that her risk of DOR varies directly with increasing age. Therefore, we regard the age-related type of DOR as *physiologic* DOR [20]. Other causes of DOR, such as autoimmune and idiopathic, will be superimposed on this physiologic DOR and may thus be considered *pathologic* DOR, because they reflect abnormal ovarian aging [20]. Stated differently, pathologic DOR reflects a process that accelerates the normal physiologic decline in ovarian function with age. It is important to differentiate between the two processes in the clinic, as they represent different groups of patients that require different counseling clinically. Identifying pathologic DOR prior to undergoing an IVF cycle with ovarian stimulation would allow for proper counseling and may help to set appropriate expectations for the patient.

The cause for the prematurely lower number of follicles can occur at many different levels of follicular and oocyte development. There could be a smaller pool of primordial follicles due to a decreased production of oocytes embryologically, abnormal follicular development, increased follicular atresia, or defects in the communication between oocytes and granulosa cells. Multiple genes play a role in folliculogenesis. The impact of mutations and polymorphic variant genes involved in folliculogenesis will be reviewed since they may play a role in pathologic DOR.

It is possible that an undetermined percentage of pathologic DOR has an underlying genetic etiology, as in those women with a family history of premature and early menopause who are at increased risk for early menopause, DOR, and/or POI [2, 10, 21]. For example, a study examining 71 chromosomally normal patients characterized as idiopathic POI found 31 % of cases to be familial, exemplifying the large genetic basis of POI [22]. The purpose of this review is to identify the genes that may be associated with pathologic DOR, including those found in humans, as well as candidate genes found in mice, to achieve the goal of ultimately developing a genetic screening panel for pathologic DOR prior to initiating an IVF cycle. The murine model of DOR approximates but does not mirror the human DOR phenotype; however, with decreased number of follicles, increased atretic follicles, elevated FSH levels, decreased litter size, and earlier diestrus or menopause, we believe it best mimics DOR.

Materials and methods

A systematic review of the literature was conducted from 1966 through November 2013 using PubMed to find relevant studies on genetic causes of or associations with DOR. The combination of keywords used for the searches were "diminished ovarian reserve and genes", "diminished ovarian reserve and genetic causes", "premature ovarian aging and genes", and "premature ovarian aging and genetic causes." Eighty-nine articles were found with nine duplicates for a total of 80 articles. All abstracts were read and the articles chosen included original research examining specific genes associated with DOR. Exclusion criteria were those written on POI and review articles or editorials. We supplemented our search with a review of the references of the included articles. This resulted in identifying 21 total studies describing eight genes in humans and six candidate genes in mice. There were two case reports on chromosomal translocations that were also relevant to this review.

Results

Whereas many genetic causes of POI are well established, little is known about definitive gene mutations associated with DOR. One of the known genetic causes of POI, a mutation in the *FMR1* gene, has been implicated as a potential cause of DOR. Additionally, many known genetic causes of POI [10] have been implicated as being associated with DOR in mice, including autoimmune regulator gene (*Aire*) [23], forkhead transcription factor forkhead protein (*Foxl2*) [24], growth differentiation factor 9 (*Gdf9*) [25], and bone morphogenetic protein 15 (*Bmp15*) [26, 27]. Here, we focused on DOR to examine the strength of evidence for the association. The following sections summarize current information organized into gene mutations and polymorphisms, differential gene expression, candidate genes, as well as epigenetics associated with DOR.

Gene mutations associated with DOR

Fragile X mental retardation 1 (FMR1)

The strongest genetic association with DOR is the *FMR1* gene (Table 1). The *FMR1* gene is responsible for Fragile X Syndrome, a form of X-linked mental retardation, which occurs when >200 trinucleotide CGG (Cytosine Guanine Guanine) repeats are located at the 5′ untranslated region of the gene. This mutation causes hypermethylation of the promoter, resulting in complete absence of the FMR protein [10, 28, 29]. Although women with Fragile X Syndrome with over 200 CGG repeats do not have any form of ovarian dysfunction, those with polymorphic triplet repeats above normal (35–54 repeats) have been associated with POI [2]. *FMR1* has been shown to be the most significant single gene associated with POI [30]. Women with premutation alleles, or 55–200 CGG repeats, have a higher incidence of POI, while also carrying

Table 1 Gene mutations and polymorphisms associated with diminished ovarian reserve

Gene	Mutation	Mechanism	Frequency	Ref
GENE MUTATIONS				
FMR1	35–54 CGG repeats = intermediate allele >55 CGG repeats = premutation	Full mutation >200 repeats induces hypermethylation of *FMR1* promotor gene, silencing gene → Fragile X Syndrome (MR); carriers known to have DOR or POI	13.63 % DOR had >35 CGG repeats vs 4.17 % controls	[29]
			14.5 % DOR had intermediate allele vs 3.9 % controls	[2]
			Premutation carriers have significantly elevated FSH, decreased inhibin B/A, decreased progesterone, shorter follicular phase	[28]
			Increasing FSH and decreasing AMH associated with increasing CGG repeats	[32]
GENE POLYMORPHISMS				
GDF9	Mutation in proregion of *GDF9* (T to C)	Oocyte-derived factor essential for folliculogenesis, proliferation of GCs, cumulus expansion	2.2 % DOR had SNP vs 0 % controls; DOR pts had poor ovarian response and pregnancy failure	[34]
	Single nucleotide polypmorphism (SNP): G546A		32.1 % DOR had variant vs 19.5 % controls; Among DOR pts, 6.7 % of patients with SNP became pregnant vs 33.3 % without SNP	[7]
FSHR	Polymorphism at position 680 (Asparagine → Serine)	G-protein coupled receptor associated with intracellular phosphorylation; FSH is essential for dominant follicle recruitment	68.2 % poor responders (PR) Asn/Ser genotype, PR 31.8 % Ser/Ser vs 0 % NOR; PR 0 % Asn/Asn vs 32.4 % NOR Polymorphism correlated with ovarian response	[35]
	Polymorphism at position 680 (Arginine→ Serine) and 307 (Threonine→ Alanine)		Ala307-Ser680/Ala307-Ser680 genotype more prevalent in ovarian dysfunction (26 %) and poor responders (33.3 %) than good responders (12.5 %), young control group (7.7 %), older control group (17.5 %)	[36]
ESR1 (alpha receptor)	PvuII polymorphism in intron 1	Downregulate *ESR1* gene expression	DOR: TT allele 34.5 % vs 24.5 % young controls, 17 % older controls	[39]
			DOR 1.75 OR CC allele vs control	[38]

the risk of passing on the Fragile X Syndrome to future generations. Premutation carriers have been found to have shorter menstrual cycles secondary to a decreased follicular phase length, as well as hormonal reflections of premature ovarian aging displayed by an increased FSH and decreased inhibin A and B levels [28].

Patients carrying the intermediate allele, which includes 35 to 54 CGG triplet repeats, are considered to be in a "grey zone." Although those with this allele were previously considered to have no specific phenotype, Bretherik et al. [30] found an increased intermediate carrier prevalence of 16 % among POI patients compared to 4.8–7.1 % of controls. Contradictory to this, a study by Bennett et al. [31] reported that although the presence of a premutation allele was statistically significant in POI patients compared to controls, intermediate alleles were not found to be increased among POI women.

The *FMR1* gene is also implicated in DOR patients. Several studies suggested an increased incidence of grey zone and premutation carriers among DOR patients [2, 29]. In a study in the Basque region by Barasoain et al. [29], 13.65 % of DOR patients had alleles with 35 or greater repeats compared to only 4.17 % of controls. Similarly, a multicenter prospective cohort study examined intermediate and premutation alleles in women with DOR and found 14.5 % of DOR patients had intermediate alleles compared to 3.9 % of controls [2].

A pilot study found a positive correlation between rising FSH levels and the number of CGG repeats, as well as AMH levels <1.0 ng/mL being significantly associated with CGG repeats above 32 [32]. However, these results differ from previously reported literature and await validation with a larger sample size. Given current data, the majority of data support an association of DOR with intermediate alleles.

Gene polymorphisms associated with DOR

Polymorphisms exist within genes and are a source of variation between individuals. Particularly, the promoter region of genes contains polymorphisms and such polymorphisms have been shown to contain a large amount of genetic variation [33]. Gene polymorphisms associated with DOR are discussed in Table 1.

Growth differentiation factor 9 (*GDF9*)

GDF9 is an oocyte-derived factor that is essential for folliculogenesis, granulosa cell proliferation, and cumulus expansion [25, 34, 7]. It is a member of the transforming growth factor-beta superfamily. Knockout mice unable to express the *Gdf9* gene were infertile due to the inability to form follicles progressing beyond having a single layer of granulosa cells and were unresponsive to added FSH stimulation [25].

In humans, polymorphisms of *GDF9* have been associated with DOR [7, 34]. One hundred three Chinese women with DOR were genetically analyzed for three single nucleotide polymorphisms (SNPs) of *GDF9* and compared to 123 age-matched women with infertility and normal ovarian reserve (NOR) [7]. NOR was defined as a day 3 FSH below 10 and/or estradiol level less than 80 pg/mL. Approximately 32 % of women with DOR had the GA/AA genotype compared with 19.5 % of those with NOR ($p<0.05$). A higher prevalence of the GA/AA genotype was found in those with poor ovarian response during IVF cycles.

Another study of Chinese women also analyzed the genotype of the *GDF9* gene [34]. Three out of 139 women (2.2 %) with DOR had a specific mutation (p.R146C) substituting arginine for cystine, whereas this mutation was absent in the 159 women in the control group. The women with this mutation also had poor quality oocytes and complete pregnancy failure. Although a second mutation was found with different frequencies between the groups, the mutation was present in both the DOR and control group. There was no difference in pregnancy outcomes, so it was thought to be a common variant in some Chinese women.

Follicle-stimulating hormone receptor (*FSHR*)

FSH is a glycoprotein produced by the anterior pituitary largely responsible for folliculogenesis and recruitment of the dominant follicle prior to ovulation. FSHR is G-protein coupled receptor, which is primarily located on granulosa cells in the ovary. Polymorphisms have been identified within the *FSHR* gene that differed in frequency among poor ovarian responders [35, 36].

In a study of patients referred to an infertility center undergoing COH for an IVF cycle, 108 subjects were genotyped for polymorphisms in the *FSHR* gene [35]. This study group was divided into 3 groups: NOR, poor ovarian responders, and hyper-ovarian responders. Poor ovarian responders were defined as women with less than four mature follicles after COH. Hyper-responders at risk for ovarian hyperstimulation syndrome were defined as those with more than 14 mature follicles. The polymorphism in the *FSHR* gene the investigators genotyped was at amino acid position 680. The SNP changed the amino acid from asparagine (Asn) to serine (Ser). Of the 22 poor responders, 15/22 (68.2 %) were heterozygotes for the SNP (Asn/Ser), while the remaining 31.8 % were homozygotes for the SNP (Ser/Ser). In the normal responders group, a similar percentage 46/68 (67.6 %) were heterozygotes for the SNP (Asn/Ser), however the remaining 32.4 % did not have the SNP (Asn/Asn). As mentioned previously, a poor response to ovarian stimulation is a hallmark of DOR. Therefore, based on this study, it can be extrapolated that patients with the polymorphism at position 680 that substitutes a serine amino acid for asparagine are at higher risk of DOR, although the sample size is considered small for a genotyping study.

A similar study performed by Livshyts et al. [36] examined two polymorphisms within the *FSHR* gene. The SNP at position 680 was the same serine substitution for arginine as in the study by Sheikhha et al. [35]. Additionally, a second SNP at position 307 was also found, which changed threonine (Thr) to alanine (Ala) [36]. The investigators compared poor responders ($n=39$) together with the ovarian dysfunction group ($n=102$) to good responders ($n=40$), and two control groups, each normo-ovulatory and with children but stratified by age (control group I <35 years old, $n=40$; control group II >35 years old, $n=130$). The Ala307-Ser680/Ala307-Ser680 genotype was more prevalent in those with ovarian dysfunction (26 %) and poor responders (33.3 %) compared to good responders, control group I, and II (12.5 %, 7.7 %, 17.5 %, respectively). Although a small percentage of the controls and good responders also have the genotype homozygous Ala307-Ser680, a higher prevalence is seen among ovarian dysfunction ($p<0.05$) and poor responders ($p<0.05$) using pairwise comparisons. In summary, the results of both of these studies showed that these polymorphisms in *FSHR* were found in different frequencies in patients with poor ovarian response and suggest patients with the genotype have a higher prevalence of DOR.

Estrogen alpha receptor (*ESR1*)

Estrogen binds to estrogen receptors, and plays an important role in granulosa cell proliferation, folliculogenesis, and fertility. Two types of nuclear estrogen receptors are found in humans: alpha and beta. Within the ovary, the alpha-receptors are typically expressed more in the theca cells, whereas the beta-receptors are more highly expressed in granulosa cells [37].

Eleven estrogen receptor polymorphisms have been implicated in regulation of the menstrual cycle, low fecundity, and polycystic ovarian disease. M'Rabet et al. [38] examined these polymorphisms in a large group of predominantly white American infertile women ($n=348$), and compared them to those with POI ($n=48$), while using fertile women ($n=200$) as controls. The SNPs were identified and amplified by PCR. The homozygous CC (Cytosine Cytosine) allele of the PvuII

polymorphic variant of the *ESR1* gene was more prevalent in infertile and POI women compared to fertile controls. Eighty-three women in the infertile group had elevated levels of FSH above 9 IU/L and would meet criteria for DOR. These women also had a higher prevalence of this allele with an odds ratio of 1.750 (95 % confidence interval=1.030–2.973).

Another study examined the same polymorphism in a group of women in the Ukraine [39]. Women with ovarian dysfunction were compared to two control groups. The groups were stratified by age (control group I <35 years old, control group II >35 years old) with both normo-ovulatory and having a history of prior conception. Ovarian dysfunction was defined as women under age 40 years old with amenorrhea for more than 6 months and FSH levels above 25 IU/L. Thirty-nine out of 113 (34.5 %) of those with ovarian dysfunction had the homozygous TT (Thymine Thymine) allele, whereas 25/102 (24.5 %) of control group I and 9/53 (17 %) of control group II had the TT allele. These results were similar to a Korean study, which found a higher prevalence of the TT allele in women with POI [40].

Examination of IVF outcomes has shown that women with the CC allele of the PvuII variant of *ESR1* develop more follicles (p=0.033) with COH [37]. However, no difference in clinical pregnancy outcome was observed. Although this study supported the polymorphism as a risk factor for DOR from the perspective of ovarian stimulation response, ultimately it did not alter pregnancy outcomes in this study. The lack of difference in pregnancy outcome may have been because of insufficient statistical power.

The four studies [37–40] were conducted in different ethnic populations, which may explain the discrepancy in results. Ethnic differences in this allele frequency, or other polymorphisms dependent on ethnicity that interact with the PvuII variant of *ESR1*, might explain the different effects of the variant on the diagnosis of DOR. The different definitions used may have also affected the results. Therefore, it is important to note that the first study [38] used the standard definition of DOR, whereas the second study [39] used an ovarian dysfunction group that was more similar to the POI phenotype. It appears that, at least within certain populations, the PvuII variant of *ESR1* is associated with DOR.

Gene expression associated with DOR

Gene expression profiling with cDNA microarrays allows for differentiation between a diseased and normal state in large groups. This technique permits identification of candidate genes potentially involved in the pathogenesis of disease. Chin et al. [41] performed a cDNA microarray analysis of pooled follicular granulosa cells of 9 women with DOR and 9 women with NOR. The investigators identified many genes that were differentially expressed between the DOR and NOR groups, including forkhead-like transcription factor 13, Pumilio 2, Notch 3, and STAT-induced STAT inhibitor 3. Although the results were not confirmed by reverse transcriptase polymerase chain reaction (RT-PCR), it still suggested that differential gene expression might play a part in distinguishing those susceptible to DOR. May-Panloup et al. [3] performed a similar study examining genes differentially expressed within the corona radiata cells between four patients with DOR and four patients with NOR first using microarray analysis. The investigation found 16 candidate genes for validation based on the microarray analysis. The investigators then validated the differential expression of the identified genes using quantitative RT-PCR in a larger group of 20 patients with DOR and 20 patients with NOR. Six genes were ultimately validated as differentially expressed including connective tissue growth factor (*CTGF*), CXXC finger protein 5 (*CXXC5*), forkhead box C1 (*FOXC1*), follistatin-like 3 (*FSTL3*), prostaglandin–endoperoxide synthase 2 (*PTGS2*), and suppressor of cytokine signaling 2 (*SOCS2*) [3]. However, each differentially expressed gene only showed a <2-fold difference in expression between the two groups. Other gene expression studies identified four genes with at least a 2-fold

Table 2 Differential gene expression associated with DOR

Gene	Gene Expression	Mechanism	Frequency	Ref
AMH	Differential gene expression in granulosa cells (GCs)	Higher levels associated with normal ovarian reserve, produced by small follicles	2.02-fold higher in control vs DOR	[42]
LHCGR	Differential gene expression in GCs	G-protein coupled receptor, known to be induced by FSH on GCs (luteinization)	2.19-fold higher in DOR vs control	[42]
IGF1, *IGF2*, *IGF1R*, *IGF2R*	Differential gene expression in cumulus and mural GCs	Stimulate GC proliferation and differentiation, enhance estradiol production	*IGF1* and *IGF2* downregulated 4.33- and 4.18- fold in DOR vs control pts in cumulus GCs; 4.35 and 3.89 fold in mural GCs *IGF1R* and *IGF2R* downregulated 5.32- and 2.13-fold in cumulus GCs	[43]
GREM1	Differential gene expression in GCs	Member of family of genes that regulates folliculogenesis, downstream effector of *GDF9*	Downregulated 4.02-fold in DOR vs control pts	[45]

differential gene expression between those with DOR and NOR (see Table 2). These genes with differential expression between women with DOR and NOR represent candidates for further research.

Anti-müllerian hormone (*AMH*)

AMH is a hormone produced by pre-antral follicles, and is a biomarker of granulosa cell mass, and thus indirectly, ovarian reserve. Typically, serum AMH values below 0.8 ng/mL have been found to be consistent with elevated FSH levels and severely diminished ovarian reserve [6]. Skiadas et al. [42] examined membrana granulosa cells from women with DOR. The membrana granulosa cells were obtained from 13 women under the age of 35 years old with DOR and 13 women with NOR. NOR patients were oocyte donors with day 3 FSH level <8 mIU/mL, day 3 estradiol level ≤50 pg/mL, and had ≥10 follicles of ≥12 mm diameter at the time of ovulation trigger. The cells were screened for differential gene expression using microarray and underwent confirmatory studies using quantitative RT-PCR. One of the genes identified was *AMH*, which showed a 2.02-fold increased expression in NOR patients over DOR patients. While this may have been expected given that elevated serum AMH levels are correlated with NOR, it was the first study to show increased gene expression at the tissue level.

Luteinizing hormone/choriogonadotropin receptor (*LHCGR*)

Luteinizing hormone receptors (LHCGR) are found on the theca cells, as well as on granulosa cells after induction by FSH. These receptors are important in ovarian steroidogenesis, luteinization, and therefore, progesterone synthesis and fertility. Skiadas et al. [42] found a 2.19-fold increased expression of the *LHCGR* gene in DOR patients over NOR patients. The increased gene expression of *LHCGR* in DOR patients could be explained by premature luteinization and upregulation of luteinizing hormone receptors on follicular cells, which might occur with DOR patients given the increased FSH in the follicular phase. It is also possible that the increased *LHCGR* gene expression could have been a result of the higher levels of FSH administrated during COH, which is often required for DOR patients. Further studies are needed to identify the reason for increased expression of the *LHCGR* gene in patients with DOR.

Insulin-like growth factor 1 and 2 (*IGF1/IGF2*) and receptors (*IGF1R/IGF2R*)

IGF-1 and IGF-2 are hormones that have an autocrine and paracrine effect at the level of the granulosa and theca cells. These growth factors promote steroidogenesis and granulosa cell proliferation. Greenseid et al. [43] examined granulosa cell expression of the genes *IGF1*, *IGF2*, and their receptors, *IGF1R* and *IGF2R*. A prospective study of four patients with DOR and four patients with NOR was performed using microarray and confirmatory quantitative RT-PCR of pooled granulosa cells. Over one thousand statistically significant differentially expressed genes were identified between the DOR and NOR groups. The genes of the IGF family ligands within cumulus granulosa cells, *IGF1* and *IGF2*, were downregulated 4.33- and 4.18-fold, respectively, whereas the genes for the corresponding receptors were downregulated 5.32- and 2.13-fold, respectively. In mural granulosa cells, only *IGF1* and *IGF2* genes showed a statistically significant downregulation, 4.35- and 3.89- fold, respectively. This differential gene expression could be related to the diminished reproductive capacity among DOR patients given the biologically plausible role the IGF family of genes play in ovarian folliculogenesis.

Gremlin 1 (*GREM1*)

GREM1 is a member of a family of genes that are highly regulated in folliculogenesis and is a down-stream effector of the *GDF9* gene, which is essential in folliculogenesis and fertility as mentioned previously. A 15-fold increased expression of the *GREM1* gene was seen in cumulus cells stripped from oocytes during IVF with intracytoplasmic sperm injection (ICSI) that resulted in higher quality embryos when compared to lower quality embryos [44]. Jindal et al. [45] performed a prospective study examining granulosa cell expression of the *GREM1* gene based on these findings using microarray and confirmatory quantitative RT-PCR. They showed a 4.02-fold decreased expression of the *GREM1* gene in DOR patients compared to NOR patients. Despite the small sample size of only four patients per group of this study, the finding suggested that decreased expression of the *GREM1* gene was found in the DOR group. Furthermore, it provides a potential gene linking DOR with embryo quality. Studies with larger sample sizes are needed to confirm these associations.

Candidate genes in mice associated with DOR

Many genes that are first identified in animal models are then used to guide identification of similar genes in humans. Six candidate genes have been identified in mice that caused a DOR phenotype characterized by decreased number of follicles, increased atretic follicles, elevated FSH levels, decreased litter size and frequency, and earlier diestrus onset or menopause (Table 3). A few of these candidate genes have also been implicated in humans.

Forkhead transcription factor forkhead protein L2 (*Foxl2*)

The *FOXL2* gene is expressed in the eyelid, the ovary (granulosa cells), and in the anterior pituitary (gonadotropes and

Table 3 Candidate genes identified in mice associated with diminished ovarian reserve

Gene	Mutation	Mechanism	Phenotype	Ref
Foxl2	Conditional Knockout (anterior pituitary gonadotrope cells)	Part of family of forkhead transcription factors	Subfertility (decreased litter frequency and size), decreased ovarian weight, decreased FSH beta mRNA expression	[24]
	Knockout		Infertile, perinatal mortality, hypoplastic pituitaries	
Gdf9	Knockout	Oocyte-secreting factor essential in early ovarian folliculogenesis and fertility	Infertile (no litters produced), lack follicles beyond primary single layer GC, lack corpus lutea, FSH elevated to levels of ovarian failure	[25]
Bmp15	Over-expression in oocytes (transgenic mice)	Oocyte-secreted factor, works with GDF9 in folliculogenesis, important for ovulation	Decreased primary follicles, increased secondary and atretic follicles, early diestrus (menopause) = ovarian reserve exhausted earlier	[26]
	Knockout		Subfertile (decreased litter size and litters/month), decreased ovulation and fertilization (50 % embryos fertilized vs 93 % in controls)	[27]
Aire	Knockout	Autoimmune transcriptional regulator promotes recognition of self antigens in thymus	Delayed puberty, 50 % infertile, depleted follicles faster, decreased litter number and size, 83 %+anti-ovarian antibodies	[23]
Wnt4	Conditional knockout (granulosa cells)	Required for ovarian follicular development; regulates genes required for steroidogenesis	Decreased litter size 3.86 vs 7.19 ($p<0.05$), decreased ovarian weight 2.56 mg vs 5.25 mg ($p<0.05$) At 42 days old, decreased healthy antral follicles	[51]
Gpr3	Knockout	G-protein coupled receptor with constitutive G_{stim} signaling activity to maintain oocytes in prophase I	Premature resumption of meiosis, decrease litter size, decrease embryo blastocyst transformation, poor ovarian response (increase abnormal oocytes)	[53]

thyrotropes) [24]. In humans, mutations of this gene before the polyalanine tract lead to truncated proteins causing Blepharophimosis Syndrome (BPES) type 1, an autosomal dominant disorder featuring short palpebral fissures, epicanthus inversus, ptosis of the eyelids, other ocular findings, and POI [46, 47]. Although this gene is known to cause POI in humans, it has not yet been reported to cause DOR. A DOR phenotype, however, was identified in conditional knockout mice (CKO) of *Foxl2* [24].

Foxl2 knockout mice have been shown to develop POI by premature activation of primordial follicles and consequent follicular depletion [24, 47]. CKO mice that had *Foxl2* only disrupted in the gonadotrope cells appeared to develop overtly normal and had subfertility similar to DOR [24]. These CKO mice produced fewer total litters, decreased litter size, and decreased litter frequency ($p<0.001$). These mice were also gonadotropin deficient with significantly reduced serum FSH levels, confirmed by reduced FSH beta protein and mRNA expression. Decreased levels of LH receptors were also seen in the ovaries. The decreased levels of LH receptors was likely related to the decreased FSH expression and diminished ability for luteinization of granulosa cells. Altogether, this phenotype featured subfertility and FSH deficiency.

Growth differentiation factor 9 (*Gdf9*)

Although the *GDF9* gene has been suggested to be associated with DOR in humans, simply identifying a polymorphism does not provide information about the gene's role in pathogenesis. In this case, an animal model can reveal the specific role of the *GDF9* gene in ovarian function. Therefore, a brief review of the role of the *GDF9* gene in animal models is warranted. In a study by Dong et al. [25], the ovaries of *Gdf9* knockout mice were examined histologically. The ovaries of the *Gdf9* knockout mice were significantly smaller than wild type ovaries and failed to show any normal follicles beyond the primary follicle with a single layer of granulosa cells. No theca cells were found. The chromatin from these follicles exhibited impaired meiotic competence. When cultured, the oocytes did not proceed with normal meiosis and failed to form meiotic spindles. Follicular cysts were common in the knockout mice. The serum collected from these mice showed elevated FSH and LH consistent with hypergonadotropic hypogonadism. The knockout mice were also unresponsive to FSH and superovulation. *Gdf9* knockout mice were completely infertile, illustrating that this gene is essential for folliculogenesis.

Bone morphogenetic protein 15 (*Bmp15*)

BMP15 is an oocyte-secreted factor that works together with GDF9 to play a key role in folliculogenesis and fertility. In contrast to *Gdf9* knockout mice which have been found to be completely infertile because of a block in primary folliculogenesis, *Bmp15* knockout mice have shown subfertility with defects in ovulation and early embryonic development [26]. McMahon et al. [26] studied transgenic mice with an overexpression of the human gene *BMP15* proregion instead of knockout mice. There was no difference in the number of primordial follicles between wild type and

transgenic mice; however, a significant decrease in primary follicles and increase in secondary follicles in transgenic mice was observed in 25-day old mice. Additionally, an increased number of atretic follicles were also found in adult mice. These findings were consistent with accelerated follicular development in the transgenic mice. Bmp15 also decreased the FSH receptor mRNA expression in the transgenic mice. Litter frequency was the same in mice 1.5-5 months of age; however, older transgenic mice aged 6–11 months showed a decreased litter frequency and increased estrous cycle length. Litter size was the same between transgenic and wild type mice regardless of age. Transgenic mice began to show increased diestrus length earlier than wild type mice. They found 87 % entered constant diestrus or menopause by 20 months, whereas 94 % of wild type mice were still cycling normally at this age. In summary, overexpression of the *Bmp15* gene in mice led to accelerated follicle development, increased atresia, and decreased FSH receptor mRNA. This ultimately led to premature ovarian aging and earlier decline in the follicular pool.

To analyze the function of BMP15, Yan et al. [27] created mutant *Bmp15* alleles in mice. Homozygous mutant mice were subfertile, showing a statistically significant decreased litter size and frequency. *Bmp15* knockout mouse ovaries appeared relatively normal and showed signs of normal folliculogenesis; however, when superovulated, produced a decreased number of eggs and developed denuded or trapped oocytes unable to ovulate. These findings suggested that BMP15 was needed for proper ovulation in the mice. Interestingly, *Bmp15* knockout sheep exhibited a similar phenotype to the *Gdf9* knockout mice: infertility due to a complete block in folliculogenesis [27]. It is unclear why this difference between species exists, but it underscores the need to investigate the *BMP15* gene as a pathogenic mechanism in women with DOR.

Autoimmune regulator (*Aire*)

Autoimmune causes of POI have been well documented in the literature, however, its link to DOR has been less well established [15, 17]. Several studies have suggested an autoimmune association with DOR [48, 49, 4, 50]. Unfortunately, no standard exists for defining those with autoimmune features. Most studies have used only one positive autoimmune antibody to classify a patient as having abnormal autoimmune function [48, 4, 49].

AIRE gene mutations in humans are responsible for Autoimmune Polyglandular Syndrome type 1 (APS-1), which manifests as chronic mucocutaneous candidiasis, hypoparathyroidism, adrenal insufficiency, and other autoimmune processes including infertility [23]. AIRE is a glycoprotein produced in the medullary thymus where it promotes the expression of tissue-restricted antigens to develop central tolerance, or immunologic tolerance to self. It is responsible for negative selection of self-reactive T cells. Failure to do so permits autoreactive T cells to escape into the peripheral system, which can manifest as an autoimmune disease. Jasti et al. [23] studied the reproductive lifespan of *Aire* knockout mice. Delayed onset of puberty was observed in *Aire* knockout mice when compared to wild type mice (p=0.0009). Although mating behaviors were normal, only 50 % of knockout mice produced a litter and only 17 % produced a second litter. Of the mice that did not develop a litter, 83 % showed impaired estrous cycles. These mice exhibited either complete depletion or a decreased number of follicles, which would correlate with DOR. FSH levels were also elevated in 29 % of knockout mice. In the mice that had elevated FSH, their ovaries displayed follicular depletion. When histologic sections of the ovaries were examined, 57 % of the knockout mice showed T cell infiltration of the ovary as early as 4 weeks of age. By 20 weeks of age, 96 % of AIRE-deficient mice had T cell infiltration. Although T cell infiltration likely played a factor in follicular depletion, it did not immediately cause depletion, as T cell infiltration was seen prior to follicular depletion.

Next, Jasti et al. [23] analyzed the sera of the mice for autoimmune antibodies, specifically anti-ovarian antibodies (AOA). Eighty-three percent of AIRE-deficient mice had positive a AOA, whereas none of the wild type mice displayed positive antibodies. There were no statistically significant differences in ovulation and fertilization outcomes between the knockout and wild type mice. Interestingly, wild type ovaries were reimplanted subrenally into knockout mice which resulted in 4/6 mice undergoing complete follicular depletion and lymphocytic infiltration. Jasti et al. [23] concluded that infertility and subfertility could be caused by mutations in the *Aire* gene. These results suggest that the reduced fertility of the mice was due in part to an immune-mediated follicular decline supported by the positive autoimmune antibodies and T cell infiltration in transplanted wild type ovaries into *Aire* knockout mice.

Wingless-type MMTV integration site family, member 4 (*Wnt4*)

Wnt4 is part of a family of genes that is important in many embryonic processes. *Wnt4* knockout mice have been shown to have a decreased ovarian reserve by depletion of primordial follicles and display perinatal mortality secondary to kidney defects [51]. Therefore, Boyer et al. [51] studied *Wnt4* CKO mice by deleting the gene only in granulosa cells within the ovary. Litter size was significantly decreased in CKO mice by 54 %. Ovarian weight was approximately 50 % of wild type mice. At 5 days old, there was no difference in primordial or primary follicles; however, by 42 days old, there was a statistically significant decreased number of follicles in the CKO mice. The number of healthy antral follicles was significantly

reduced ($p<0.05$). Expression of steroidogenic genes including steroidogenic acute regulatory (StAR) protein and aromatase enzyme was decreased among CKO mice; however, only serum progesterone, but not estrogen, was decreased. This was the first study to show that *Wnt4* gene expression is important in the normal adult murine ovary and that WNT4 might affect fertility by decreasing the number of healthy follicles and steroidogenic factors.

G protein-coupled receptor 3 (*Gpr3*)

Meiotic arrest of oocytes is maintained in the diplotene phase of prophase I until the LH surge of ovulation. This arrest, maintained by cyclic adenosine monophosphate (cAMP) levels within the oocyte, is derived from a constitutive stimulating G-protein, the GPR3 [52]. Ledent et al. [53] generated *Gpr3* knockout mice to assess their phenotype. A decreased litter size was seen in these knockout mice at all ages ($p<0.001$). Knockout mice displayed an increase in extrusion of the first polar body, which signified premature progression of meiosis and an inability to maintain meiotic arrest. Although the number of oocytes produced during ovulation was the same between controls and knockout mice, knockout mice showed an increased number of fragmented oocytes during superovulation. Embryos of GPR3 deficient mice were poorer quality and showed a decreased progression to the blastocyst stage ($p<0.001$). Knockout mice also had significantly higher FSH levels and shorter estrous cycles than control mice. This finding suggested premature ovarian aging and thus may be related to DOR.

Chromosomal translocations

Chromosomal translocations occur when parts of nonhomologous chromosomes are rearranged and crossed. A translocation can interrupt a gene and result in a loss of genetic information when rearrangement occurs. Two cases in the literature have identified women with diminished ovarian reserve associated with a translocation [54, 19]. The first was the case of a mosaic balanced Robertsonian translocation between the long arms of chromosomes 13 and 21 [54]. The patient was classified as DOR based on elevated FSH levels, normal menstrual cycles, and a decreased AFC. This patient also had a family history of early menopause, suggesting a genetic component of DOR. This phenotype may have been related to a disruption in chromosome 21 because it has been shown that women with trisomy 21 undergo menopause at an earlier age [55].

The second case report involved a family with an unbalanced X;18 translocation that took place in the middle of the *POF1* locus [19]. Two loci on the X chromosome have previously been described that, when interrupted, were likely to cause POI [17, 19]. The proband in this study [19] was diagnosed with DOR based on a low AFC and low ovarian volume, but with normal menses and FSH levels. As part of her workup, the patient was tested for the *FMR1* gene mutation, which showed an unexpected result. The normal *FMR1* allele showed an unmethylated band of 30 CGG repeats, but the expected methylated inactive band was not identified. This unexpected result suggested that a rearrangement or deletion near the *FMR1* gene may have occurred to cause DOR, which was ultimately mapped to the *POF1* locus. Fluorescence in situ hybridization (FISH) analysis of the chromosomes confirmed this unbalanced translocation, which was also detected in the proband's mother. However, the proband's mother had normal reproductive ability and did not show evidence of DOR. The difference between the mother's phenotype and daughter's phenotype was likely an example of incomplete penetrance or variable expressivity based on skewed X inactivation. These DOR phenotypes associated with chromosomal translocations suggest that further genomic testing may help identify new genetic factors in ovarian reserve.

Epigenetic causes of DOR

Epigenetics is a term used to describe heritable changes in gene function that do not entail a change in DNA sequence [56]. Examples include DNA methylation, histones, and post-translational modification—all of which can be affected by the environment. Vinclozolin, a commonly used fungicide, has previously been shown to affect rat and rabbit sperm production [57]. To study the effects of vinclozolin along with other common environmental toxins including insecticides, plastics, and jet fuel on ovarian disease, these substances were injected into pregnant female rats [58]. The primordial follicle pool was examined in these rats as well as in the next three generations of offspring. All generations showed a decreased number of primordial follicles ($p<0.001$). Differential DNA methylation was seen between the controls and the vinclozolin lineage F3 generation. Differential DNA methylation pattern demonstrated that the F3 generation rats had changes in the epigenome that persisted for generations following one exposure. Furthermore, over 500 genes were differentially expressed between the controls and vinclozolin lineage F3 generation, some of which were involved in lipid metabolism and steroid precursor synthesis. This animal study suggested that environmental toxins that affected the epigenome in female rats may also create a similar DOR phenotype in humans.

A review article examining all environmental endocrine-disrupting chemicals (EDCs) affecting fertility disorders in females identified similar toxins including methoxychlor (MTX, pesticide), genistein (phytoestrogen), and bisphenol A (BPA, plasticizer) [59]. While the EDCs directly affected the fertility of the female mouse exposed, they also affected future generations and caused differential methylation patterns suggesting epigenetic effects. Further studies need to be

conducted to determine the epigenetic effects these ubiquitous toxins have on human ovaries and reproduction.

Future directions

NextGen Sequencing technology and genome wide association studies (GWAS) can be used to identify genetic variants within specific patient populations. A GWAS database has already begun to emerge for POI cases [10]. POI has proven to be largely familial [21, 22]. As we have shown here, evidence supports that pathologic DOR behaves in a similar fashion. Genetic studies have revealed upregulated or downregulated gene expression within the pathologic DOR population. Although small sample sizes were often examined, these studies suggest specific genes that are associated with pathologic DOR. Therefore, we may be able to identify some women with pathologic DOR as having a genetic cause instead of classifying them as having an idiopathic cause of DOR. We hold the view that it is essential to clearly distinguish physiologic age-related DOR from pathologic, or early onset DOR. The reason is that with early onset DOR, proper counseling of patients may ultimately depend on identification of genetic causes, and thus the distinction is clinically important. Drawing such a clinical distinction has been suggested for genetic causes of other reproductive disorders where practical considerations for genetic testing are entertained [60].

In summary, pathologic DOR affects a significant number of women with infertility; however, in the majority of cases, the etiology remains obscure. As more gene polymorphisms, mutations, and epigenetic factors become associated with pathologic DOR, a panel of tests may be developed to screen for these patients prior to undergoing ovarian stimulation for IVF. At the present time, this will be especially useful in the counseling of women prior to undergoing these procedures so they can make informed decisions about infertility treatment. In the future, as our understanding of folliculogenesis improves, identifying specific genes causing pathologic DOR may allow for novel targeted therapies for these women.

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