Premature Ovarian Insufficiency: Phenotypic Characterization Within Different Etiologies

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Context: Premature ovarian insufficiency (POI) is highly heterogeneous, both in phenotype and etiology. They are not yet clearly stated and correlated.

Objective: To characterize clinical presentations of a large, well-phenotyped cohort of women with POI, and correlate phenotypes with etiologies to draw a comprehensive clinical picture of POI.

Design, Patients, Interventions, and Main Outcome Measures: In this retrospective study, a total of 955 Chinese women with overt POI between 2006 and 2015 were systemically evaluated and analyzed. The phenotypic features, including menstrual characteristics, hormone profiles, ovarian ultrasonography/biopsy, pregnancy/family history, and genetic/autoimmune/iatrogenic etiologies were assessed and further compared within different subgroups.

Results: Among 955 women with POI, 85.97% presented with secondary amenorrhea (SA) and 14.03% with primary amenorrhea (PA). PA represented the most severe ovarian dysfunction and more chromosomal aberrations than SA. The decline of ovarian function in patients with SA progressed quickly. They had shortened reproductive periods (approximately 10 years) and developed amenorrhea within 1 to 2 years after menstrual irregularity. The ovaries were invisible or small, and the presence of follicles (28.43%) was correlated with other good reproductive indicators. Familial patients (12.25%) manifested better ovarian status and fewer chromosomal aberrations than sporadic patients. The etiologies consisted of genetic (13.15%), autoimmune (12.04%), and iatrogenic (7.29%), approximately 68% remaining idiopathic. There were significant differences among different etiologies, with the genetic group representing the most severe phenotype.

Conclusion: Our results regarding distinct phenotypic characteristics and association with different etiologies further confirmed the high heterogeneity of POI. Additional longitudinal clinical studies and pathogenesis research are warranted. (*J Clin Endocrinol Metab* 102: 2281–2290, 2017)

Premature ovarian insufficiency (POI), previously termed premature ovarian failure, is characterized by cessation of ovarian function before the age of 40. Although no accurate epidemiological data exist, it is estimated to affect 1% of women by age of 40 (1). Over the past few decades, ovarian insufficiency has become more common and drawn

more concerns, particularly given the increase of environment pollutants, social stress, childbearing postponement, and prolonged survival after gonadotoxic treatments (2–4).

POI is highly heterogeneous, both in phenotype and etiology (5, 6). It is not merely early menopause, nor is it permanent. In fact, the ovarian function is in a continuum of ovarian impairment in POI. Approximately 50% of women with POI experience intermittent and unpredictable resumption of ovarian activity; ovulation or spontaneous pregnancy, even years after diagnosis, occasionally occurs (7). POI encompasses a wide spectrum of causes, including genetic, autoimmune, infectious, or iatrogenic, whereas the majority remains unknown (6). Furthermore, no comprehensive understanding of the clinical spectrum in terms of different etiologies is currently available.

In the current study, clinical characteristics of a large, well-phenotyped cohort of women with POI were defined and further stratified for different etiologies to provide clues for optimal fertility consulting.

Materials and Methods

Patients

A total of 1227 women with POI were recruited and systemically evaluated at the Center for Reproductive Medicine, Shandong University, China. The study was approved by the Ethical Committee of Reproductive Medicine of Shandong University. Written informed consents were obtained from all participants. Inclusion criteria included primary amenorrhea (PA) or secondary amenorrhea (SA) for at least 4 months, and serum follicle-stimulating hormone (FSH) >40 IU/L (on two occasions >1 month apart) before age 40. The standardized evaluation consisted of clinical history, physical examination, reproductive characteristics, genetic, autoimmune, and iatrogenic factors, and ovarian biopsy in a subset of patients. A detailed questionnaire covering demographic characteristics, gynecological and obstetric data, and family/surgery/disease history was recorded. Family history was considered "positive" if another first- or second-degree relative had either POI or early menopause (menopause ≤45 years). According to the duration of reproductive age (years between menarche and amenorrhea), patients with SA were further divided into four subgroups: ≤5 years (n = 182); 6 to 10 years (n = 243); 11 to 15 years (n = 220); and >15 years (n = 176).

Hormone measurement

Blood was sampled on days 2 to 4 of menstrual cycle or randomly (for women not menstruating frequently). FSH, LH, estradiol, prolactin, and total testosterone were measured by chemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Anti-Müllerian hormone (AMH) and Inhibin-B were detected by enzyme-linked immunosorbent assay (Kangrun Biotech, Guangzhou, China). The intra-assay and interassay coefficients of variation were <10%.

Pelvic ultrasonography

Gynecological examination and transvaginal ultrasonography were routinely conducted. The ovarian surface area

(S = length × width × 0.8) and antral follicle counts were recorded. Antral follicle count was defined as the number of follicles 2 to 10 mm in early follicular phase.

Ovarian histology

Ovarian biopsy was performed with laparoscope. For each patient, bilateral biopsies (from January 2013 to November 2014) or left biopsy (after November 2014) were sampled and fixed in 4% paraformaldehyde. After embedded and serially sectioned (5 mm), three to four (one out of every five) sections were stained with hematoxylin/eosin and examined.

Genetic evaluation

Genetic evaluation included karyotype analysis, CGG repeats in FMR1 gene, and mutation screening in candidate genes. Karyotype was analyzed on GTG-banded metaphase chromosomes (400- to 450-band resolutions) (8). The FMR1 gene was amplified with fluorescence-labeled primers, and the CGG repeats of two alleles were counted by capillary electrophoresis (9). Mutations of candidate genes (e.g., FIGLA, NOBOX, GDF9, SOHLH1, SOHLH2, WT1, NR5A1, PGRMC1, FSHR, DMC1, MCM8, MSH5, and CSB-PGBD3) were screened by Sanger sequencing using specific primers (10, 11).

Autoimmune antibody detection

Adrenal cortex autoantibody (AAA) titers were detected by indirect immunofluorescence assay on monkey adrenal gland sections (Euroimmun, Lübeck, Germany). Non–organ-specific antibodies, anti-nuclear antibody, anti-cardiolipin antibody, and anti–double-stranded DNA antibody were determined by enzyme-linked immunosorbent assay (Orgentec, Mainz, Germany).

Statistical analysis

SPSS 23.0 was used for statistical analysis (SPSS Inc.). Continuous data in normality distribution were expressed as mean ± standard deviation and compared by Student t test or one-way analysis of variance; otherwise, data were presented as median (quartile interval) and compared with Mann-Whitney U test. The difference of categorical data in percentage was compared by χ^2 test or Fisher's exact test. Correlation analysis was performed for variables among different reproductive periods. $P < 0.05$ was considered statistically significant. P value was corrected by Bonferroni adjustment within multiple comparisons.

Results

Baseline characteristics

After excluding 272 women with incomplete data in the database, the current study comprised 955 women with POI, and average age at recruitment was 29.28 ± 4.75 years. Somatic anomalies (webbed neck and cubitus valgus) were found in five cases and mental retardation in three cases. Characteristics of our POI cohort are summarized in Table 1 and Fig. 1.

Reproductive characteristics and endocrine profiles

Among 955 women with POI, 821 (85.97%) presented with SA and 134 (14.03%) with PA. Women with PA did not have spontaneous menarche instead of medically induced menstruation at around age 19

Table 1.   Characteristics of 955 Women With POI

Characteristics	POI (N = 955)	SA (n = 821)	PA (n = 134)	P
Age (y)	29.28 ± 4.75	29.58 ± 4.74	27.42 ± 4.39	<0.001[a]
BMI (kg/m^2)	22.72 ± 3.23	22.75 ± 3.14	22.50 ± 3.74	0.401[a]
Age at menarche (y)	—	14.26 ± 1.59	—	—
Age at irregularity (y)	—	22.90 ± 6.34	—	—
Age at amenorrhea (y)	—	24.83 ± 5.85	—	—
Duration of amenorrhea (y)	—	4.75 ± 3.93	—	—
FSH (IU/L)	75.77 ± 26.53	76.84 ± 26.84	69.18 ± 23.59	0.001[a]
LH (IU/L)	34.94 ± 16.30	36.40 ± 16.40	26.04 ± 12.48	<0.001[a]
E2 (pg/mL)	13.00 (5.00–27.53)	14.00 (5.37–29.00)	10.00 (5.00–17.63)	<0.001[b]
T (ng/dL)	20.46 (10.65–32.64)	20.59 (10.87–32.34)	19.47 (9.08–33.67)	0.854[b]
AMH (ng/mL)	0.26 (0.18–0.80)[d]	—	—	—
Inhibin-B (pg/mL)	28.66 (16.37–37.36)[e]	—	—	—
Ovarian surface area (cm^2)	1.23 ± 0.69	1.26 ± 0.69	0.92 ± 0.59	<0.001[a]
Frequency of invisible ovary [%(n/N)]	26.18 (233/890)	23.05 (177/768)	45.90 (56/122)	<0.001[c]
Frequency of follicles [%(n/N)]	38.36 (252/657)	39.59 (234/591)	25.76 (17/66)	0.028[c]
Frequency of family history [%(n/N)]	12.25 (117/955)	12.42 (102/821)	11.19 (15/134)	0.687[c]
Frequency of chromosomal abnormality [%(n/N)]	14.71 (103/700)	11.60 (68/586)	30.70 (35/114)	<0.001[c]

Abbreviations: BMI, body mass index; E2, estradiol; LH, luteinizing hormone; T, testosterone.

[a]Student t test.

[b]Mann-Whitney U test.

[c]χ^2 test.

[d]Data of 50 patients with AMH above detection limit is shown.

[e]Data of 31 patients with Inhibin-B above detection limit is shown.

(19.26 ± 0.22 years). For patients with SA, they had a shortened reproductive period of 10.6 years [0 to 26 years; 95% confidence interval (CI), 10.15 to 10.98 years]. Most women experienced delayed menarche (14.26 ± 1.59 years) and thereafter established normal periods. No obvious signs or symptoms preceding period cessation were reported. The age at onset of irregular menstruation was 22.90 ± 6.34 years, and amenorrhea occurred 2 years later. Surprisingly, more than 50% developed amenorrhea within 1 year after irregularity occurred (69.18%, 568/821). No significant association was found between age at menarche, irregularity, or amenorrhea ($P > 0.05$). It took approximately 5 years (0 to 21 years; 95% CI, 4.48 to 5.02 years) for the confirmed diagnosis of POI after amenorrhea occurred.

Patients with POI are biochemically characterized by elevated gonadotropins and decreased estradiol. A total of 48.17% (409/849) patients showed a decline in testosterone levels (<20 ng/dL). AMH value was available for 358 patients, of which 86.03% were undetectable, and the remaining patients showed a low value of 0.26 (0.18 to 0.80) ng/mL. Inhibin-B was measured in 175 patients, of which only 17.71% had detectable value of 28.66 (16.37 to 37.36) pg/mL.

Ovarian sonography and biopsy

Of the 890 patients (93.19%) with ultrasound results, the ovaries were either undetectable (26.18%) or very small (1.22 ± 0.68 cm^2), especially for patients with PA

($P < 0.001$). Presence of follicles was found in approximately 38.36% of patients with visible ovaries, with the prevalence much higher in patients with SA than PA (39.59% vs 25.76%; $P < 0.05$) (Table 1). There were 31 cases (4.72%) with SA who had follicles 10 mm or larger. Compared with SA patients without follicles, those with follicles were older at recruitment and experienced later onset of POI ($P \leq 0.001$). The presence of follicles was also associated with lower FSH, increased estradiol, and ovarian surface area ($P < 0.05$) (Supplemental Table 1).

A total of 86 patients with SA and visible ovaries in sonography underwent ovarian biopsies. The majority showed bilateral small or steak ovaries with hard texture. Most ovaries only contained fibrous connective tissue covered by cuboidal epithelium, with atretic follicles or corpora albicantia occasionally (Supplemental Fig. 1). The presence of follicles, either quiescent or growing, was detected in 13 (15.12%) cases. A follicular reserve with primordial follicles was found in 8 cases, primary follicles in 11, secondary follicles in 9, and early antral follicles in 4 (Supplemental Fig. 1). No differences of phenotypic parameters were observed in patients with or without follicles ($P > 0.05$) (Supplemental Table 2). No significant concordance in the frequency of visible follicles between ultrasound and biopsy data was found (concordance coefficient κ = 0.038; $P > 0.05$).

Pregnancy history before POI diagnosis

Among patients with pregnancy history before POI diagnosis, 188 (22.9%) women with SA had a total of
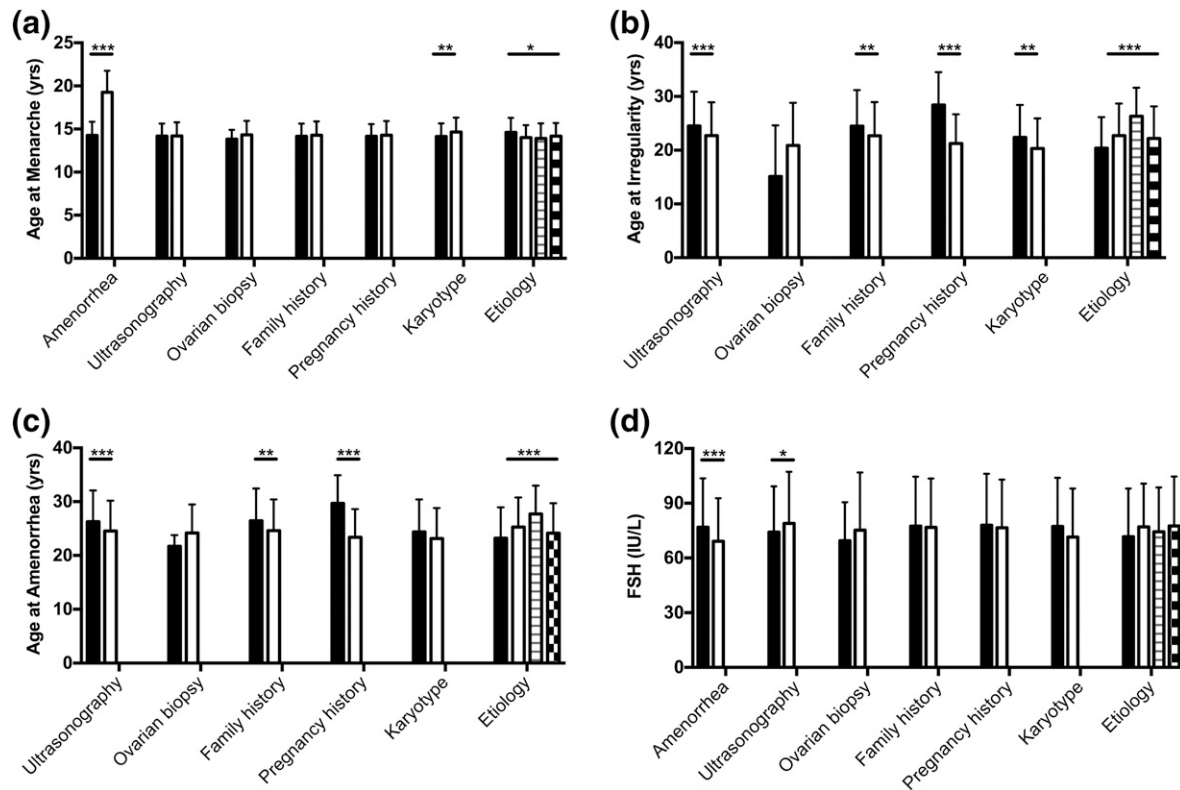
Figure 1. Clinical characteristics in women with POI by different grouping. (a) Age at menarche. (b) Age at irregularity. (c) Age at amenorrhea. (d) FSH levels of women. Amenorrhea: SA (black bar) or PA (white bar); ultrasonography: SA patients with (black bar) or without (white bar) visible follicles; ovarian biopsy: SA patients with (black bar) or without (white bar) follicles; family history: SA patients with (black bar) or without (white bar) family history; pregnancy history: SA patients with (black bar) or without (white bar) pregnancy history before amenorrhea; karyotype: SA patients with (black bar) or without (white bar) chromosomal abnormality; etiology: genetic (black bar), autoimmune (white bar), iatrogenic (striped bar), or idiopathic (boxed bar). Data were expressed as mean ± standard deviation. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

377 pregnancies. No pregnancies occurred in any of the PA patients. 32.64% (110/337) resulted in term delivery, and 10.98% (37/337) experienced spontaneous miscarriage. Later onset of menstrual irregularity and amenorrhea was observed in patients who had been pregnant in the past compared to those who had not ($P < 0.001$) (Supplemental Table 3; Fig. 1). As expected, the longer duration of reproductive age, the higher prevalence of pregnancy, and parity were observed ($P < 0.001$) (Supplemental Table 6).

Family history

Family history, either POI or early menopause, was reported in 12.25% (117/955) of patients, including 103 mothers, 4 maternal aunts, and 10 siblings. No difference in the rate of positive family history was found between patients with PA and SA ($P = 0.687$) (Table 1). Among patients with SA, although no difference of age at menarche was found between patients with and without family history, patients with family history experienced irregular menses and amenorrhea much later than those sporadic ($P < 0.01$) (Supplemental Table 4; Fig. 1). The later the amenorrhea occurred, the higher the frequency of positive family

history was observed ($P < 0.05$) (Supplemental Table 6). Thirty-three individuals with pregnancy (32.35%) had family history of POI compared with 155 individuals (21.56%) in sporadic cases ($P < 0.05$) (Supplemental Table 4).

Karyotype analysis

Karyotype was analyzed in 700 POI cases, and 14.71% presented abnormal. Of these anomalies, 94 (91.26%) involved the X chromosome; 3 (2.91%) involved only autosomes; and 6 (5.83%) were nonmosaic 46,XY and 45,X/46,XY mosaicism. The frequency of karyotypic aberrations was significantly higher in patients with PA than SA (30.70%, 35/114 vs 11.60%, 68/586; $P < 0.001$) and higher in sporadic cases than those with family histories (15.80%, 97/614 vs 7.23%, 6/83; $P < 0.05$). SA patients with abnormal karyotype experienced menarche and irregularity later than those with normal karyotype ($P < 0.01$), whereas no difference was observed with age of amenorrhea ($P = 0.091$) (Supplemental Table 5; Fig. 1). Interestingly, patients with a longer reproductive period tended to have decreased chromosomal abnormality ($P = 0.072$) (Supplemental Table 6). The prevalence of pregnancy was also

significantly lower in patients with abnormal karyotype ($P < 0.05$) (Supplemental Table 5).

Gene mutations

Candidate genes associated with folliculogenesis/oogenesis (*e.g.*, *FIGLA*, *NOBOX*, *GDF9*, *SOHLH1*, *SOHLH2*, *WT1*, and *NR5A1*), endocrine hormone/receptor (*PGRMC1* and *FSHR*), DNA damage repair/meiosis (*e.g.*, *DMC1*, *MCM8*, *MSH5*, and *CSB-PGBD3*), and premutation of *FMR1* were screened in different subsets of the idiopathic POI cohort with normal karyotype (Table 2). *FMR1* premutation (CGG repeats 55 to 199) was observed in only two patients (0.53%, 2/379) and none of the matched controls. Both alleles in POI cases demonstrated a small but significant shift toward a single additional CGG repeat in the *FMR1* gene ($P < 0.001$). Among other candidate genes, *FIGLA*, *NOBOX*, *WT1*, *NR5A1*, *FSHR*, *MCM8*, *MSH5*, and *CSB-PGBD3* were proven causatively by functional validation experiments.

Causative genes *MSH5* and *CSB-PGBD3* were first identified by whole-exome sequencing in two POI pedigrees, and their deficiencies were related to impaired DNA repair (Table 1; Supplemental Fig. 2). Given the novel nonsynonymous variants, if any were identified, each gene deficiency generally explains at most 2%. All variants were heterozygous except the *MSH5* mutation in two POI siblings. None carried two different or compound mutations. Therefore, in total, known gene mutations could account for less than 3.29% (23 cases) of patients with POI.

Autoimmune factors

A total of 38 patients with SA (6.02%, 38/631) presented with autoimmune disorders, including hypothyroidism or Hashimoto thyroiditis (n = 17), Graves disease (n = 8), psoriasis (n = 5), rheumatoid arthritis (n = 4), systemic lupus erythematosus (n = 2), takayasuarteritis (n = 1), and allergic purpura (n = 1). Autoantibody data were available for 250 idiopathic cases with SA (data

Table 2. Candidate Gene Mutations Found in Women With POI

Gene	Location	Case (n)	Control (n)	Mutation Rate (%)	Variants	Mechanism
FMR1	Xq27.3	379	402	0.5	Premutation	—
GDF9	5q31.1	100	96	1	p.T238A	—
NOBOX	7q35	200	—	—	—	—
FIGLA	2q13.3	100	—	2	p.G6fsX66	Truncated protein
					p.140delN	Impaired interaction with TCF3
NANOS3	19p13.13	80	—	—	—	—
SOHLH1	9q34.2	364	400	0.55	p.S317F	—
					p.E376K	
SOHLH2	13q13.3	364	400	1.1	p.E79K	—
					p.E105G	
					p.T321P	
NR5A1	9q33	384	400	0.26	p.Y5D	Impaired transactivation of Amh, Inha, Cyp11a1, and Cyp19a1
PTEN	10q23.3	161	—	—	—	—
CDKN1B	12p13.1-p12	200	200	—	—	—
SKP2		200	200	—	—	—
WT1	11p13	384	384	0.52	p.P126S	Impaired transactivation of Amh, Fshr, Cyp19a1, and Cdh1
					p.R370H	
PGRMC1	Xq22-24	196	200	0.5	p.P186S	—
FSHR	2p16	200	200	1	p.M265V	—
					p.R59X	Truncated protein
DMC1	22q13.1	192	—	—	—	—
EXO1	1q43	186	—	—	—	—
HELQ	4q21.23	192	—	—	—	—
PRIM1	12q13.3	192	—	—	—	—
MCM8	20p12.3	192	192	1.04	p.H317L	Impaired DNA repair
					p.H601R	
CSB-PGBD3[a]	10q11.23	Pedigree POI-1[b]			p.G746D	Impaired DNA repair
		432	400	0.46	p.V1056I	
MSH5[a]	6p21.33	Pedigree POI-2[b]			p.E215X	Impaired DNA repair
		432	400	—	p.D487Y	
					—	

[a]Mutations in causative genes *CSB-PGBD3* and *MSH5* were first identified by whole-exome sequencing in POI pedigrees.

[b]Detailed pedigree information is shown in Supplemental Fig. 2.

under review). The positive prevalence of AAA was much higher in POI than the control women (19.20% vs 5.86%; $P < 0.01$), whereas no difference of nonspecific antibodies (anti-cardiolipin antibody, anti-nuclear antibody, and anti–double-stranded DNA antibody) was found. No significant difference of clinical phenotypes existed between the patients with positive and negative AAA. Fifteen AAA-positive POI patients were followed up to assess the association of AAA presence with later onset of Addison disease. None but one had symptom of Addison disease after 3 years of POI diagnosis. Thirteen POI patients (six AAA positive and seven negative) underwent ovarian biopsy, and all presented with small and hard-textured ovaries. The histology showed atrophy and fibrosis, completely devoid of follicles.

Iatrogenic factors

Acquired iatrogenic factors accounted for 7.29% (46/631) of cases with SA. Before the onset of ovarian insufficiency, 12 patients (1.90%) experienced pelvic radiation or chemotherapy exposure, whereas 34 (5.39%) underwent ovarian surgery, including 4 unilateral oophorectomy, 25 ovarian cystectomy (20 unilateral, 5 bilateral), and 5 unilateral oophorectomy with contralateral cystectomy. The pathological type of ovarian cysts included endometriosis cysts, teratomas, and serous or mucinous cystadenomas. Menstrual cycles were recalled in 20 patients (mean age, 25.45 ± 5.65 years) with regular periods before ovarian surgeries (9 bilateral and 11 unilateral). It took approximately 3 years (0 to 10 years; 95% CI, 1.49 to 4.61 years) to develop amenorrhea after

surgeries, and almost one-half (45%, 9/20) displayed ovarian insufficiency within 1 year. No significant differences, in terms of duration from surgery to amenorrhea and the percentage of patients developing POI within 1 year after surgeries, were observed between patients with bilateral and unilateral surgery. The age receiving surgery was positively correlated with the age of amenorrhea ($r = 0.817$, $P < 0.001$).

The known plausible etiologies of POI with SA were assessed and consisted of genetic (13.15%), autoimmune (12.04%), and iatrogenic (7.29%), with approximately 68% remaining idiopathic. The detailed distribution is outlined in Table 3. There were significant differences of age at menarche/amenorrhea/recruitment and prevalence of previous pregnancy among those four categories (all $P < 0.05$; Fig. 1). Patients with known genetic anomalies experienced a trend toward later menarche, earlier amenorrhea, and consequently earlier diagnosis and lower prevalence of pregnancy compared with those with autoimmune or iatrogenic etiologies. No significant difference existed between patients with autoimmune and iatrogenic etiology.

Discussion

POI imposes a great challenge on women's fertility and lifelong health. It is of increasing clinical importance given its rising prevalence. However, it remains poorly understood and under-researched. Controversy persists regarding its nomenclature and recruitment criteria; high heterogeneity in etiology and phenotype as well as absence of early diagnosis and optimal treatment regimens

Table 3.   Characteristics of Patients With SA Within Different Etiologies

Characteristics	Genetic (n = 83)	Autoimmune (n = 76)	Iatrogenic (n = 46)	Idiopathic (n = 426)	P
Age (y)	28.22 ± 4.68	29.79 ± 4.62	31.22 ± 4.79[d,e]	29.09 ± 4.51[d]	0.008[a]
BMI (kg/m^2)	22.96 ± 3.50	22.94 ± 3.35	22.63 ± 3.12[e]	22.72 ± 3.09	0.866[a]
Age at menarche (y)	14.62 ± 1.69	14.01 ± 1.44[d]	13.91 ± 1.75[e]	14.17 ± 1.52	0.042[a]
Age at irregularity (y)	20.38 ± 5.76	22.71 ± 5.96	26.33 ± 5.30[d]	22.17 ± 5.98	<0.001[a]
Age at amenorrhea (y)	23.23 ± 5.71	25.27 ± 5.52	27.70 ± 5.41[d,e]	24.16 ± 5.57	<0.001[a]
Duration of amenorrhea (y)	4.99 ± 4.35	4.52 ± 3.63	3.52 ± 3.07[e]	4.83 ± 3.97	0.149[a]
FSH (IU/L)	71.67 ± 26.41	77.02 ± 23.75	74.32 ± 24.36[e]	77.50 ± 27.17	0.332[a]
LH (IU/L)	30.33 ± 14.98	36.82 ± 12.33	37.94 ± 15.83[e]	36.37 ± 16.35[d]	0.011[a]
E2 (pg/mL)	18.35 (5.91–34.40)	11.85 (5–29.25)	14.63 (5.15–24.25)[e]	13.96 (6–28.95)	0.392[b]
T (ng/dL)	18.91 (8.44–28.69)	21.73 (13.07–36.13)	21.51 (13.43–30.37)[e]	20.55 (10.87–32.64)	0.437[b]
Prevalence of pregnancy [%(n/N)]	7.2 (6/83)	21.1 (16/76)	34.8 (16/46)[d,e]	19.5 (83/426)	0.004[c]
Prevalence of parity [%(n/N)]	16.7 (1/6)	62.5 (10/16)	43.8 (7/16)[e]	50.6 (42/83)	0.280[c]

Abbreviations: BMI, body mass index; E2, estradiol; LH, luteinizing hormone; T, testosterone.

[a]One-way analysis of variance.

[b]Mann-Whitney U test.

[c]χ^2 test.

[d]Significant difference was found compared with genetic group. Bonferroni-corrected P value of 0.0083 (six tests in total) was set as the threshold.

[e]No significant difference was found when comparing autoimmune group and iatrogenic group. Bonferroni-corrected P value of 0.0083 (six tests in total) was set as the threshold.

still exist. Here, we focus on the overt stage of POI, define and characterize various presentations, and correlate phenotypes with etiologies to draw a comprehensive clinical picture of POI.

Clinical phenotypes

POI is variable in its clinical presentation. PA may represent the most severe defect in ovarian function, characterized by early-onset, specific phenotype and high prevalence of chromosomal aberration. Women with SA had considerably shortened reproductive window (approximately 10 years). They experienced menarche 2 years later than that of general Chinese girls (approximately 12 years) (12). And their menses ceased around age 25, within 2 years, and surprisingly more than 50% within 1 year, after irregularity occurred. This time frame is significantly shorter than the physiological interval of 5 to 10 years between onset of cycle irregularity and menopause (13). This highlights the rapid decline of ovarian function in POI. It has been reported that earlier age at menarche was associated with earlier age at menopause because of increased frequency of ovulation (14). However, our data, consistent with others (15, 16), did not show this relationship. Another concern was the delayed diagnosis. It took approximately 5 years for a confirmed diagnosis of POI after amenorrhea. Panay et al. (17) reported that 50% of women with amenorrhea had to consult three physicians before any ovarian reserve evaluation was performed. In another study, in 25% of women, the diagnosis of POI took more than 5 years (18). Therefore, it is critical to evaluate the ovarian function in adolescents with late menarche or young women with irregular periods.

As expected, all patients exhibited typical endocrine profiles with continuously elevated gonadotropin and decreased estradiol. About 50% of patients manifested deficiency of testosterone. This is in agreement with the previous studies, which have shown that the production of ovarian testosterone was insufficient in POI (19, 20). Recently, AMH and Inhibin-B have been suggested as early markers for diminished ovarian reserve impending POI (21). However, given that our cohorts were already in the stage of ovarian failure, AMH and Inhibin-B were low or undetectable as reported previously (22, 23).

Most ovaries in all patients with POI (71.57%), particularly in patients with PA (85.25%), were either undetectable or very small, lacking growing follicles. The presence of follicles might indicate the residual or resumption of ovarian function, especially with follicles larger than 10 mm. Patients with presence of follicles tended to experience menstrual disturbance later and had better hormone parameters and larger ovaries. Histologic analysis of ovarian biopsy has also been proposed.

However, given that small biopsies are not predictive of the whole nature, it is not standardized or routinely recommended as an invasive procedure (7). In our study, ovarian biopsy was performed in a small subset of SA patients with visible ovaries under ultrasonography. The presence of growing follicles was found in only 15.12% of patients, a lower prevalence compared with previous reports (24). In addition, clinical characteristics did not differ between patients with or without follicles, nor was concordance found in follicular pattern at pelvic ultrasonography compared with ovarian biopsy. Therefore, our results reconfirm that ovarian biopsy is uninformative for evaluation and diagnosis. Nonetheless, ovarian biopsy, in the context of management, may be of therapeutic benefit given the success of in vitro culture of immature oocytes or primordial follicles (25).

Extensive evidence has indicated a strong familial, and therefore genetic, component to POI (5, 6). In our cohort, 12.3% had at least one affected relative, similar to what reported by Bachelot et al. (14%) (26). POI women with affected relatives tended to experience menstrual disturbance and amenorrhea later and had more chance to get pregnant compared with sporadic POI. These data indicate considerably better ovarian function in women with family histories. Consistent with our findings, Bidet et al. (24) reported that positive family history was a good predictor for resumption of ovarian activity. Therefore, early diagnosis of familial POI will help in prediction of early menopause or POI, which would provide other reproductive choices of childbearing planning or freezing oocytes/embryos.

Plausible etiologies

Different etiologic factors and pathogenic mechanisms have been reviewed comprehensively (6, 27); however, phenotypic characteristics with different etiologies in POI remain elusive. In this study, known plausible etiologies were divided into genetic, autoimmune, and iatrogenic factors, while one-half remained idiopathic. Our results demonstrated that POI likely comprises different subtypes with different etiopathologies. Spontaneous genetic POI represents the most unique and severe phenotype of premature aging, distinct from that of autoimmune or iatrogenic induced.

The genetic factor has long been considered as a component of POI. Here we focused on chromosomal abnormalities and gene mutations. Chromosomal aberration is a common cause of POI, although percentages vary widely among reported series. By reviewing the five largest POI studies, a prevalence of 10% to 15% seems reasonable (8, 28–30). The X chromosome defects, including numerical aberration and rearrangements, are an important factor for ovarian dysfunction (8). The frequency

of abnormality was much higher in patients with PA compared with SA, which was consistent with previous reports (30). A higher prevalence of abnormalities is correlated with shorter reproductive windows and lower prevalence for pregnancy and parity, which confirms the important role played by two intact chromosomes for fecundity. More interestingly, chromosomal abnormality is more common in sporadic POI patients compared with those with affected relatives, suggesting that the majority of these anomalies are *de novo* rather than inherited, whereas gene deficiencies may account for most POI patients with family history.

POI is highly heterogeneous with no single underlying dominant gene deficiency (6, 27). Taken together, over 80 candidate genes have been identified, and approximately 25% of genes were validated to be causative functionally (27). These identified gene deficiencies account for only a small proportion of POI patients, and most of them had notable ethnic differences in mutation frequency. Our results that no perturbations, weak associations with low incidence, or no same variants existed in different individuals further confirmed the heterogeneity of its pathogenesis. Of note, the prevalence of *FMR1* premutation in our cohort was considerably lower than previously reported (31). Thus, *FMR1* testing is not part of the workup of women with POI in our clinics as recommended in the United States and Europe (32, 33). Furthermore, defects in different candidate genes may be responsible for different phenotypes. For instance, deficiency in genes involved in reduced primordial follicle pool is considered associated with PA, whereas those related to dysregulated folliculogenesis or accelerated depletion are more common in SA. It would be yet more desirable to classify POI on the basis of aberrant ovarian differentiation and folliculogenesis or screen distinct gene panels in a large phenotypically defined POI cohort with isolated or syndromic features, sporadic or familial forms, PA or SA, and early or later onset. Given the low yields of a single-candidate gene approach, identifying genes not isolated but interrelated functionally within pathways with next-generation sequencing and implementation of a systems approach is anticipated.

Autoimmune disturbances could contribute to a subset of POI (5% to 30%) (34, 35). The ovary is a susceptible target of autoimmune attacks given its dynamics of folliculogenesis, ovulation, luteum formation, and dysgenesis. Once the autoimmune destruction initiates, ovarian follicles might be attacked and depleted prematurely, and consequently POI occurs. However, reliable diagnosis of autoimmune premature ovarian insufficiency (aPOI) remains elusive. POI occurs more frequently in women affected by autoimmune diseases and *vice versa*. Consistent with previous evidence (26, 35), thyroiditis was

the most common coexistent autoimmune disorder, followed by psoriasis and rheumatoid arthritis in this study. Common antigens among different organs and overall autoimmune misbalance might exist in POI. Presence of serum autoantibodies represents potential markers to predict the risk of POI (36). The most promising indicators of aPOI are steroid cell autoantibodies and AAA, and the presence of steroid cell autoantibodies is almost exclusive in patients positive for AAA (37). We found that the positive frequency of AAA was significantly higher in patients with POI, confirming its role as a potential marker for aPOI. However, no difference of reproductive characteristics was observed between AAA-positive and -negative patients. And both groups presented with atrophic ovaries in ovarian biopsy. Ovarian inflammation might be transient and not present by the late stage in our patients. Consistent with the biphasic nature of inflammation, an atrophic ovary remained long after the inflammation rapidly disappeared. Therefore, further examination in earlier, yet progressive, POI patients is warranted to elucidate the plausible autoimmune causes. Additionally, an autoimmune screen for thyroid and adrenal autoantibodies is essentially important for future surveillance of thyroid or adrenal deficiency in patients with POI.

With the advance of "oncofertility," iatrogenic ovarian damage is increasingly common. The well-documented iatrogenic causes of POI are chemotherapy, pelvic radiotherapy, and immunosuppressive agents. These therapies significantly induce and accelerate apoptosis or atresia of ovarian follicles. Pelvic surgery is another risk factor for ovarian insufficiency (7, 38) given its deleterious effect on the follicle pool and ovarian blood supply. Our data demonstrated that POI occurred within 2 years after the surgery, which was faster than previously reported (39). The earlier ovarian surgery was performed, the earlier amenorrhea occurred. Thus, fertility counseling is suggested to inform patients of the risk of ovarian dysfunction after surgery, and follow-up is recommended to monitor ovarian reserve.

Conclusion

Our results depict a comprehensive picture of clinical phenotype with different etiologies in women with POI; however, our study only involved patients with overt POI. Future studies, which longitudinally stage reproductive aging and incorporate patients at an earlier stage, are warranted for early diagnosis or to develop strategies that improve fertility. As the cause of POI still remains undetermined in a majority of patients, the pathogenesis and association with phenotype and risk prediction still need to be explored.

Acknowledgments

References

1. Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol.* 1986;**67**(4):604–606.
2. De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. *Lancet.* 2010;**376**(9744):911–921.
3. Patel S, Zhou C, Rattan S, Flaws JA. Effects of endocrine-disrupting chemicals on the ovary. *Biol Reprod.* 2015;**93**(1):20.
4. Hart RJ. Physiological aspects of female fertility: role of the environment, modern lifestyle, and genetics. *Physiol Rev.* 2016;**96**(3):873–909.
5. Simpson JL. Genetic and phenotypic heterogeneity in ovarian failure: overview of selected candidate genes. *Ann N Y Acad Sci.* 2008;**1135**:146–154.
6. Tucker EJ, Grover SR, Bachelot A, Touraine P, Sinclair AH. Premature ovarian insufficiency: new perspectives on genetic cause and phenotypic spectrum. *Endocr Rev.* 2016;**37**(6):609–635.
7. Nelson LM. Clinical practice. Primary ovarian insufficiency. *N Engl J Med.* 2009;**360**(6):606–614.
8. Jiao X, Qin C, Li J, Qin Y, Gao X, Zhang B, Zhen X, Feng Y, Simpson JL, Chen ZJ. Cytogenetic analysis of 531 Chinese women with premature ovarian failure. *Hum Reprod.* 2012;**27**(7):2201–2207.
9. Guo T, Qin Y, Jiao X, Li G, Simpson JL, Chen ZJ. FMR1 premutation is an uncommon explanation for premature ovarian failure in Han Chinese. *PLoS One.* 2014;**9**(7):e103316.
10. Qin Y, Jiao X, Dalgleish R, Vujovic S, Li J, Simpson JL, Al-Azzawi F, Chen ZJ. Novel variants in the SOHLH2 gene are implicated in human premature ovarian failure. *Fertil Steril.* 2014;**101**(4):1104–1109.e6.
11. Jiao X, Qin Y, Li G, Zhao S, You L, Ma J, Simpson JL, Chen ZJ. Novel NR5A1 missense mutation in premature ovarian failure: detection in han chinese indicates causation in different ethnic groups. *PLoS One.* 2013;**8**(9):e74759.
12. Song Y, Ma J, Hu PJ, Zhang B. Geographic distribution and secular trend of menarche in 9-18 year-old Chinese Han girls. *Beijing Da Xue Xue Bao.* 2011;**43**(3):360–364 (Health Sciences).
13. Broekmans FJ, Knauff EA, te Velde ER, Macklon NS, Fauser BC. Female reproductive ageing: current knowledge and future trends. *Trends Endocrinol Metab.* 2007;**18**(2):58–65.
14. Ozdemir O, Cöl M. The age at menopause and associated factors at the health center area in Ankara, Turkey. *Maturitas.* 2004;**49**(3):211–219.
15. Stanford JL, Hartge P, Brinton LA, Hoover RN, Brookmeyer R. Factors influencing the age at natural menopause. *J Chronic Dis.* 1987;**40**(11):995–1002.
16. Yasui T, Hayashi K, Mizunuma H, Kubota T, Aso T, Matsumura Y, Lee JS, Suzuki S. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas.* 2012;**72**(3):249–255.
17. Panay N, Fenton A. Premature ovarian failure: a growing concern. *Climacteric.* 2008;**11**(1):1–3.
18. Alzubaidi NH, Chapin HL, Vanderhoof VH, Calis KA, Nelson LM. Meeting the needs of young women with secondary amenorrhea and spontaneous premature ovarian failure. *Obstet Gynecol.* 2002;**99**(5 Pt 1):720–725.
19. Kalantaridou SN, Calis KA, Vanderhoof VH, Bakalov VK, Corrigan EC, Troendle JF, Nelson LM. Testosterone deficiency in young women with 46,XX spontaneous premature ovarian failure. *Fertil Steril.* 2006;**86**(5):1475–1482.
20. Janse F, Knauff EA, Niermeijer MF, Eijkemans MJ, Laven JS, Lambalk CB, Fauser BC, Goverde AJ; Dutch Premature Ovarian Failure Consortium. Similar phenotype characteristics comparing familial and sporadic premature ovarian failure. *Menopause.* 2010;**17**(4):758–765.
21. Knauff EA, Eijkemans MJ, Lambalk CB, ten Kate-Booij MJ, Hoek A, Beerendonk CC, Laven JS, Goverde AJ, Broekmans FJ, Themmen AP, de Jong FH, Fauser BC; Dutch Premature Ovarian Failure Consortium. Anti-Mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. *J Clin Endocrinol Metab.* 2009;**94**(3):786–792.
22. Li HW, Anderson RA, Yeung WS, Ho PC, Ng EH. Evaluation of serum antimullerian hormone and inhibin B concentrations in the differential diagnosis of secondary oligoamenorrhea. *Fertil Steril.* 2011;**96**(3):774–779.
23. Goswami D, Arif A, Saxena A, Batra S. Idiopathic primary ovarian insufficiency: a study of serial hormonal profiles to assess ovarian follicular activity. *Hum Reprod.* 2011;**26**(8):2218–2225.
24. Bidet M, Bachelot A, Bissauge E, Golmard JL, Gricourt S, Dulon J, Coussieu C, Badachi Y, Touraine P. Resumption of ovarian function and pregnancies in 358 patients with premature ovarian failure. *J Clin Endocrinol Metab.* 2011;**96**(12):3864–3872.
25. Massin N, Czernichow C, Thibaud E, Kuttenn F, Polak M, Touraine P. Idiopathic premature ovarian failure in 63 young women. *Horm Res.* 2006;**65**(2):89–95.
26. Bachelot A, Rouxel A, Massin N, Dulon J, Courtillot C, Matuchansky C, Badachi Y, Fortin A, Paniel B, Lecuru F, Lefrère-Belda MA, Constancis E, Thibault E, Meduri G, Guiochon-Mantel A, Misrahi M, Kuttenn F, Touraine P; POF-GIS Study Group. Phenotyping and genetic studies of 357 consecutive patients presenting with premature ovarian failure. *Eur J Endocrinol.* 2009;**161**(1):179–187.
27. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. *Hum Reprod Update.* 2015;**21**(6):787–808.
28. Baronchelli S, Conconi D, Panzeri E, Bentivegna A, Redaelli S, Lissoni S, Saccheri F, Villa N, Crosti F, Sala E, Martinoli E, Volonte M, Marozzi A, Dalpra L. Cytogenetics of premature ovarian failure: an investigation on 269 affected women. *J Biomed Biotechnol.* 2011:370195.
29. Kalantari H, Madani T, Zari Moradi S, Mansouri Z, Almadani N, Gourabi H, Mohseni Meybodi A. Cytogenetic analysis of 179 Iranian women with premature ovarian failure. *Gynecol Endocrinol.* 2013;**29**(6):588–591.
30. Lakhal B, Braham R, Berguigua R, Bouali N, Zaouali M, Chaieb M, Veitia RA, Saad A, Elghezal H. Cytogenetic analyses of premature ovarian failure using karyotyping and interphase fluorescence in situ hybridization (FISH) in a group of 1000 patients. *Clin Genet.* 2010;**78**(2):181–185.
31. Wittenberger MD, Hagerman RJ, Sherman SL, McConkie-Rosell A, Welt CK, Rebar RW, Corrigan EC, Simpson JL, Nelson LM. The FMR1 premutation and reproduction. *Fertil Steril.* 2007;**87**(3):456–465.
32. American College of Obstetricians and Gynecologists Committee on Genetics. ACOG Committee Opinion No. 469: Carrier screening for fragile X syndrome. *Obstet Gynecol.* 2010;**116**(4):1008–1010.

33. Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, Cifkova R, de Muinck Keizer-Schrama S, Hogervorst E, Janse F, Liao L, Vlaisavljevic V, Zillikens C, Vermeulen N; European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI. ESHRE Guideline: management of women with premature ovarian insufficiency. *Hum Reprod*. 2016;**31**(5):926–937.
34. Silva CA, Yamakami LY, Aikawa NE, Araujo DB, Carvalho JF, Bonfá E. Autoimmune primary ovarian insufficiency. *Autoimmun Rev*. 2014;**13**(4-5):427–430.
35. Bakalov VK, Gutin L, Cheng CM, Zhou J, Sheth P, Shah K, Arepalli S, Vanderhoof V, Nelson LM, Bondy CA. Autoimmune disorders in women with turner syndrome and women with karyotypically normal primary ovarian insufficiency. *J Autoimmun*. 2012;**38**(4):315–321.
36. Falorni A, Laureti S, Candeloro P, Perrino S, Coronella C, Bizzarro A, Bellastella A, Santeusanio F, De Bellis A. Steroid-cell autoantibodies are preferentially expressed in women with premature ovarian failure who have adrenal autoimmunity. *Fertil Steril*. 2002;**78**(2):270–279.
37. La Marca A, Brozzetti A, Sighinolfi G, Marzotti S, Volpe A, Falorni A. Primary ovarian insufficiency: autoimmune causes. *Curr Opin Obstet Gynecol*. 2010;**22**(4):277–282.
38. Muzii L, Di Tucci C, Di Feliciantonio M, Marchetti C, Perniola G, Panici PB. The effect of surgery for endometrioma on ovarian reserve evaluated by antral follicle count: a systematic review and meta-analysis. *Hum Reprod*. 2014;**29**(10):2190–2198.
39. Takae S, Kawamura K, Sato Y, Nishijima C, Yoshioka N, Sugishita Y, Horage Y, Tanaka M, Ishizuka B, Suzuki N. Analysis of late-onset ovarian insufficiency after ovarian surgery: retrospective study with 75 patients of post-surgical ovarian insufficiency. *PLoS One*. 2014;**9**(5):e98174.