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Association of LHCGR rs2293275 genotype with ovarian aging in Chinese women: a multicenter population-based study

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Abstract

Objective To evaluate the association between the *LHCGR* rs2293275 (N312S) genotype and ovarian aging phenotypes in Han Chinese women, focusing on diminished ovarian reserve (DOR) and primary ovarian insufficiency (POI).

Study design This multicenter population-based study included 1,240 women aged 18–40 years diagnosed with DOR ($n=711$) or POI ($n=529$), alongside 72,846 ethnically and regionally matched controls from the Han Chinese Genomes Database (PGG.Han). Genotyping of rs2293275 was performed, and clinical data (menstrual history, hormonal profiles, maternal menopause age, and ART outcomes) were analyzed.

Main results The AA genotype frequency in the ovarian aging cohort (1.85%) was significantly higher than in the general Han population (0.62%, OR 3.04, 95% CI 1.99–4.64, $p<0.001$). AA carriers exhibited earlier POI diagnosis (25.5 ± 6.4 vs. 32.0 ± 5.1 years in GG carriers, $p<0.001$) and maternal menopause (41.6 ± 3.3 vs. 47.8 ± 4.1 years, $p<0.001$). In controlled ovarian stimulation cycles, AA carriers demonstrated reduced ovarian sensitivity (OSI: 3.59 vs. 1.21 in GG, $p=0.019$) despite comparable gonadotropin doses.

Conclusions The LHCGR rs2293275 AA genotype is strongly associated with accelerated ovarian aging in Han Chinese women, highlighting its potential as a biomarker for early identification of high-risk individuals. While these findings underscore genetic contributions to ovarian dysfunction, further mechanistic studies are needed to establish causality and optimize clinical translation.

Trial registration number ClinicalTrials.gov NCT05665010, registered on 2022-11-30.

Keywords LHCGR, Ovarian aging, rs2293275, Diminished ovarian reserve, Assisted reproductive technology

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Introduction

Ovarian aging (OA), characterized by the progressive decline in ovarian follicular quantity and quality, is a critical determinant of female reproductive health and overall well-being. Beyond its direct impact on fertility, OA is associated with long-term health risks, including cardiovascular disease, osteoporosis, and all-cause mortality [1, 2]. Early molecular diagnosis of OA may facilitate timely improvements in pregnancy planning, provide precise genetic counseling for families, and enhance health management during the ovarian aging process and the postmenopausal stage [3]. Notably, significant individual differences exist in these measures among women of the same age, highlighting the unpredictable nature of the reproductive aging process. Clinically, OA manifests as diminished ovarian reserve (DOR) and premature ovarian insufficiency (POI), affecting over 20% and 3.7% of women globally, respectively [3, 4]. The etiology of OA is highly heterogeneous. While genetic factors account for ~20–25% of POI cases, the molecular basis of DOR remains poorly defined [5, 6] [7]. However, the genetic basis of DOR remains underexplored, and the interrelationships and determinants of these clinical types are not well understood. This knowledge gap hinders the development of biomarkers for early diagnosis and personalized interventions, underscoring the urgency of identifying genetic contributors to OA [8].

The luteinizing hormone/choriogonadotropin receptor (LHCGR), a G protein-coupled receptor essential for follicular maturation and steroidogenesis, has emerged as a potential modulator of ovarian function [9]. Binding of luteinizing hormone (LH) to LHCGR in theca cells stimulates androgen synthesis, a precursor for estradiol production, and regulates ovulation. Exon 10 of the gene is pivotal for receptor activation by LH, as it induces extracellular conformational changes. A polymorphism, rs2293275, in exon 10 leads to the substitution of asparagine with serine (N312S) in the LHCGR protein, occurring near a glycosylation site, which may influence receptor sensitivity both in vitro and in vivo [10, 11]. While this variant has been linked to polycystic ovary syndrome (PCOS) and divergent outcomes in assisted reproductive technology (ART), findings remain contradictory. For instance, Lindgren et al. reported higher pregnancy rates in carriers of the 312 S allele during IVF, whereas Pirtea et al. found no association between LHCGR polymorphisms and ART success [12–16]. These findings suggest that genetic variations in gonadotropin receptors may serve as valuable biomarkers for predicting ART outcomes and for designing more effective treatment strategies aimed at improving live birth rates. These discrepancies likely stem from population heterogeneity, highlighting the need for population-specific investigations [13]. In the Han Chinese population, the role of LHCGR rs2293275 in OA is unexplored, despite marked ethnic differences in allele frequencies. The minor allele (A) frequency of rs2293275 is 6.87% in East Asians compared to 67.93% in Africans, suggesting that prior findings from European or African cohorts may not generalize to Asian populations. Furthermore, existing studies predominantly focus on ART outcomes and PCOS, neglecting the variant's association with ovarian aging phenotypes such as POI and DOR.

This multicenter population-based study addresses these gaps by analyzing LHCGR rs2293275 in 1,240 Han Chinese women with DOR or POI, compared to 72,846 ethnically matched controls. We aim to: (1) determine the genotype distribution of rs2293275 in OA subgroups, (2) evaluate its association with clinical parameters (e.g., age at POI diagnosis, maternal menopause age), and (3) explore its impact on ovarian response to controlled stimulation. Our findings reveal that the AA genotype is associated with accelerated ovarian aging, providing a foundation for risk stratification. However, mechanistic insights into how rs2293275 influences LHCGR function require future validation through in vitro or animal models.

Materials and methods

Ethical statement

The study was approved by the Medical Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China (TJ-IRB20211016). Written informed consent was obtained from all participants, and all methods were carried out in accordance with the approved guidelines.

Study participants

The cohort comprised 529 patients diagnosed with primary ovarian insufficiency (POI) and 711 patients with diminished ovarian reserve (DOR), all of whom were Han Chinese, as illustrated in the flowchart in Fig. 1. These patients were referred to gynecology for abnormal menstruation or reproductive medicine for fertility issues between September 2021 and June 2022. This multicenter study was conducted in collaboration with several hospitals including Tongji Hospital of Huazhong University of Science and Technology, Zhongnan Hospital of Wuhan University, Renmin Hospital of Wuhan University, Hubei Maternal and Child Health Hospital, Xiangyang Central Hospital, Jiangxi Maternal and Child Health Hospital, Fujian Maternal and Child Health Hospital, the Women's Hospital affiliated with Zhejiang University School of Medicine, Changsha Maternal and Child Health Hospital, the First Affiliated Hospital of Xi'an Jiaotong University, the Fourth Hospital of Shijiazhuang, the Third Affiliated Hospital of Zhengzhou University, Guangdong

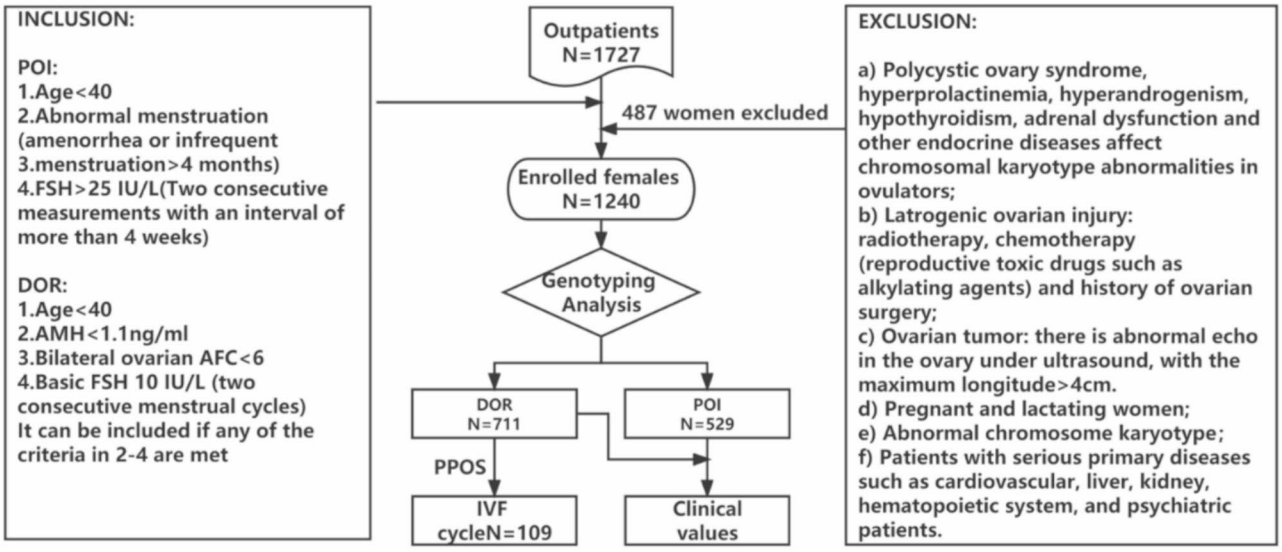

Fig. 1 Flowchart of Inclusion and Exclusion. DOR, diminished ovarian reserve; POI, primary ovarian insufficiency; PPOS, progestin-primed ovarian stimulation; IVF, in vitro fertilization

Provincial People's Hospital and the First Affiliated Hospital of Soochow University.

All patients diagnosed with POI exhibited either amenorrhea or oligomenorrhea for a minimum of four consecutive months. Additionally, they had a basal follicle stimulating hormone (bFSH) level exceeding 25 IU/L on two separate occasions within a 4-week timeframe before the age of 40. The inclusion criteria for DOR consisted of being under the age of 40 and meeting at least one of the following conditions: an anti-Müllerian hormone (AMH) level below 1.1 ng/ml, a bFSH level between 10 and 25 IU/L, or an antral follicle count (AFC) below 6 in both ovaries. Patients with a history of abnormal karyotypes, FMR1 premutation, previous ovarian surgery, chemotherapy, radiotherapy, polycystic ovarian syndrome, hyperprolactinemia, hyperandrogenemia, thyroid dysfunction, endocrine disorder, or autoimmune disease were excluded from the study. For the control group, a sample of 72,846 individuals with matching ethnic and regional backgrounds was obtained from the Han Chinese Genome Database, established by the Population Genomics Group (PGG.Han). The database can be accessible at https://www.biosino.org/HCGD/index [17, 18].

Clinical and biochemical measurements

Body mass index (BMI) was calculated using height and weight measurements. The serum concentration of anti-Müllerian hormone (AMH) was measured at recruitment utilizing a chemiluminescence immunoassay diagnostic kit from Kangrun Biotech in Guangzhou, China. All AMH measurements were performed in the same laboratory at Tongji Hospital. Serum follicle stimulating hormone (FSH) levels were assessed on menstrual cycle day 2–5 or any day of amenorrhea without dominant follicles, employing a chemiluminescence-based immunometric assay at the respective study site. An experienced ultrasonologist evaluated the antral follicle count (AFC) via transvaginal ultrasound on menstrual cycle day 2–5 or on any day of amenorrhea without dominant follicles. Follicles with a diameter of 2–9 mm were counted.

In this study, we evaluated the ovarian response to stimulation using three indicators: the follicular output rate (FORT, defined as the ratio of preovulatory follicle count to antral follicle count (AFC)) [19]; the follicle-to-oocyte index (FOI, calculated as the number of oocytes divided by AFC) [20]; and the ovarian sensitivity index (OSI, which is the ratio of the number of retrieved oocytes to the total dose of FSH administered) [21]. Clinical confirmation of pregnancy was established through a blood test and ultrasound examination to detect the presence of a gestational sac and embryonic heart activity.

Statistical analysis

All analyses were conducted using SPSS version 26 (IBM, Armonk, NY, USA). The clinical characteristics of patients were described using medians and interquartile ranges. Comparisons between the POI and DOR groups were performed using nonparametric Mann-Whitney tests. The Hardy-Weinberg equilibrium (HWE) was assessed using Fisher's exact probability test. Chi-squared analysis or Fisher's exact probability test was employed to compare the LHCGR rs2293275 genotypes and allele frequencies between the groups. Age, BMI, baseline hormones, AFC, total gonadotrophin dose, stimulation duration, oocyte count, and embryo development were compared between groups using Student's

Table 1 Baseline characteristics

	Group median(P25, P75)		MannWhitney U	MannWhitney z	*p*
	DOR($n=711$)	POI($n=529$)			
Age	33.000(29.0,36.0)	33.000(26.0,37.0)	180951.000	-1.100	0.271
BMI	21.644(20.0,23.9)	21.094(19.5,22.9)	161204.000	-4.306	0.000**
Menarche age	13.000(12.0,14.0)	13.000(12.0,14.0)	166159.500	-1.216	0.224
No. Of pregnancies	1.000(0.0,2.0)	1.000(0.0,2.0)	168790.500	-0.909	0.364
AMH	0.590(0.3,0.9)	0.030(0.0,0.1)	23601.500	-26.393	0.000**
bFSH	8.480(5.6,12.9)	49.900(29.0,87.3)	25634.000	-26.043	0.000**
Menopausal age of mother	49.000(45.0,51.0)	49.000(45.0,50.0)	36902.000	-1.214	0.225

* $p<0.05$ ** $p<0.01$

BMI, body mass index; AMH, anti-Müllerian hormone; bFSH, basal follicle-stimulating hormone; LH, luteinizing hormone

Table 2 Genotype distribution and genetic model analysis of rs2293275 in ovarian aging(OA) cohort and PGG.HAN Chinese population

rs2293275		Group(%)		OA(%)	PGG.HAN(%)	$P_{POIvs.DOR}$	$P_{DORvs.PGG}$	$P_{POIvs.PGG}$	$P_{OAvs.PGG}$
		DOR	POI						
Genotype	GG	572(80.45)	421(79.58)	993(80.08)	59,216(85.49)	0.643	0.003*	0.000**	< 0.001**
	GA	128(18.00)	96(18.15)	224(18.06)	9621(13.89)				
	AA	11(1.55)	12(2.27)	23(1.85)	428(0.618)				
Sum		711	529	1240	69,265				
Allele	G	1272(89.45)	938(88.66)	2210(89.11)	128,053(92.44)	0.530	0.000**	0.000**	0.000**
	A	150(10.55)	120(11.34)	270(10.89)	10,477(7.56)		OR 1.441 (1.216–1.709)	OR 1.564 (1.292–1.893)	OR 1.493 (1.314–1.697)
Sum		1422	1058	2480	138,530				
Dominant	GG	572(80.45)	421(79.58)	993(80.08)	59,216(85.49)	0.706	0.000**	0.000**	0.000**
	GA/AA	139(19.55)	108(20.42)	247(19.92)	10,049(14.51)		OR 1.432 (1.188–1.726)	OR 1.512 (1.222–1.870)	OR 1.466 (1.273–1.688)
Sum		711	529	1240	69,265				
Recessive	GG/GA	700(98.45)	517(97.73)	1217(98.15)	68,837(99.38)	0.352	0.003*	0.000**	0.000**
	AA	11(1.55)	12(2.27)	23(1.85)	428(0.62)		OR 2.527 (1.383–4.620)	OR 3.733 (2.090–6.669)	OR 3.04 (1.991–4.642)
Sum		711	529	1240	69,265				

* $p<0.05$ ** $p<0.01$

t-test or analysis of variance. Binary logistic regression analysis was conducted to evaluate the impact of LHCGR c.935 A > G on OA, adjusting for potential confounding factors. A *p*-value of less than 0.05 was deemed statistically significant.

Results

Cohort characteristics

A total of 1,240 women diagnosed with ovarian aging (DOR: $n=711$; POI: $n=529$) were enrolled from more than 10 centers across six genetic regions of China (southwest: 33.31%; southeast: 29.11%; south coast: 14.68%; central: 14.11%; northwest: 8.06%; northeast: 0.73%). Table 1 summarized the key clinical characteristics. Patients with POI exhibited significantly higher basal FSH levels (median 49.9 vs. 8.5 IU/L, $p<0.01$) and lower AMH levels (median 0.03 vs. 0.59 ng/mL, $p<0.01$) compared to patients with DOR. No statistically significant differences were observed in age, menarche age, or maternal menopause age between the DOR and POI groups. The majority of participants were non-smokers and non-drinkers.

Genotyping distribution and genetic risk of rs2293275 in patients with OA

The allele frequency of rs2293275 was found to be in Hardy-Weinberg equilibrium ($p>0.05$). Table 2 provides the distribution of rs2293275 across groups (the DOR and POI subsamples, the overall OA sample, and the PGG.HAN reference population). The rs2293275 genotype frequencies in the overall ovarian aging cohort (AA: 1.85%, GA: 18.06%, GG: 80.08%) deviated significantly from the Han reference population (AA: 0.62%, GA: 13.89%, GG: 85.49%; $p<0.001$). The minor allele A was found to be associated with an increased risk of OA (OR 1.47, 95% [13] 1.27–1.69; $p<0.001$). Subgroup analysis revealed elevated risks for both DOR (OR 2.53, 95% CI 1.38–4.62, $p=0.003$) and POI (OR 3.73, 95% CI 2.09–6.67, $p<0.001$) under the recessive model (Table 2). The AA genotype frequency was higher in POI (2.27%) than DOR (1.55%), though inter-subgroup differences were nonsignificant ($p=0.35$).

Geographic and ethnic variation in rs2293275 allele frequency

The global allele distribution was obtained from the single nucleotide polymorphism database of the National Center for Biotechnology Information (Fig. 2A). Globally, the A allele frequency varied markedly across ethnicities: highest in Africans (67.93%), intermediate in Europeans (22.15%), and lowest in East Asians (6.87%). Notably, genotype frequencies vary significantly among different continental ethnicities, with the East Asian group demonstrating a distinct distribution compared to the others.

The rs2293275 allele frequencies of genetic regions corresponding to the cohort were evaluated (Fig. 2B and C). Supplementary Table 1 provides detailed information on population sizes and allele frequencies for each region, as derived from the PGG.SNV database. Within China, regional differences were observed, with the A allele frequency in ovarian aging cohorts exceeding local Han populations in the southeast (10.53% vs. 7.02%, $p < 0.001$), central (11.71% vs. 8.44%, $p = 0.013$), and southwest (10.77% vs. 6.98%, $p < 0.001$) (Fig. 2; Table 3).

Associationof rs2293275 with clinical phenotypes

Violin charts and nonparametric tests were employed to evaluate the performance of six clinical indicators associated with the LHCGR genotype: AMH, bFSH, age at initial diagnosis, maternal age at natural menopause (ANM), number of pregnancies, and BMI.AA carriers experienced earlier POI diagnosis (25.5 ± 6.4 vs. 32.0 ± 5.1 years in GG carriers, $p < 0.001$) and maternal menopause (41.6 ± 3.3 vs. 47.8 ± 4.1 years, $p < 0.001$) (Table 4; Fig. 3A–F). Multivariable regression confirmed the AA genotype as an independent predictor of earlier POI onset (β = −6.31, $p = 0.001$), accounting for 18.9% of variance in diagnosis age (Table 5). Kaplan-Meier analysis further

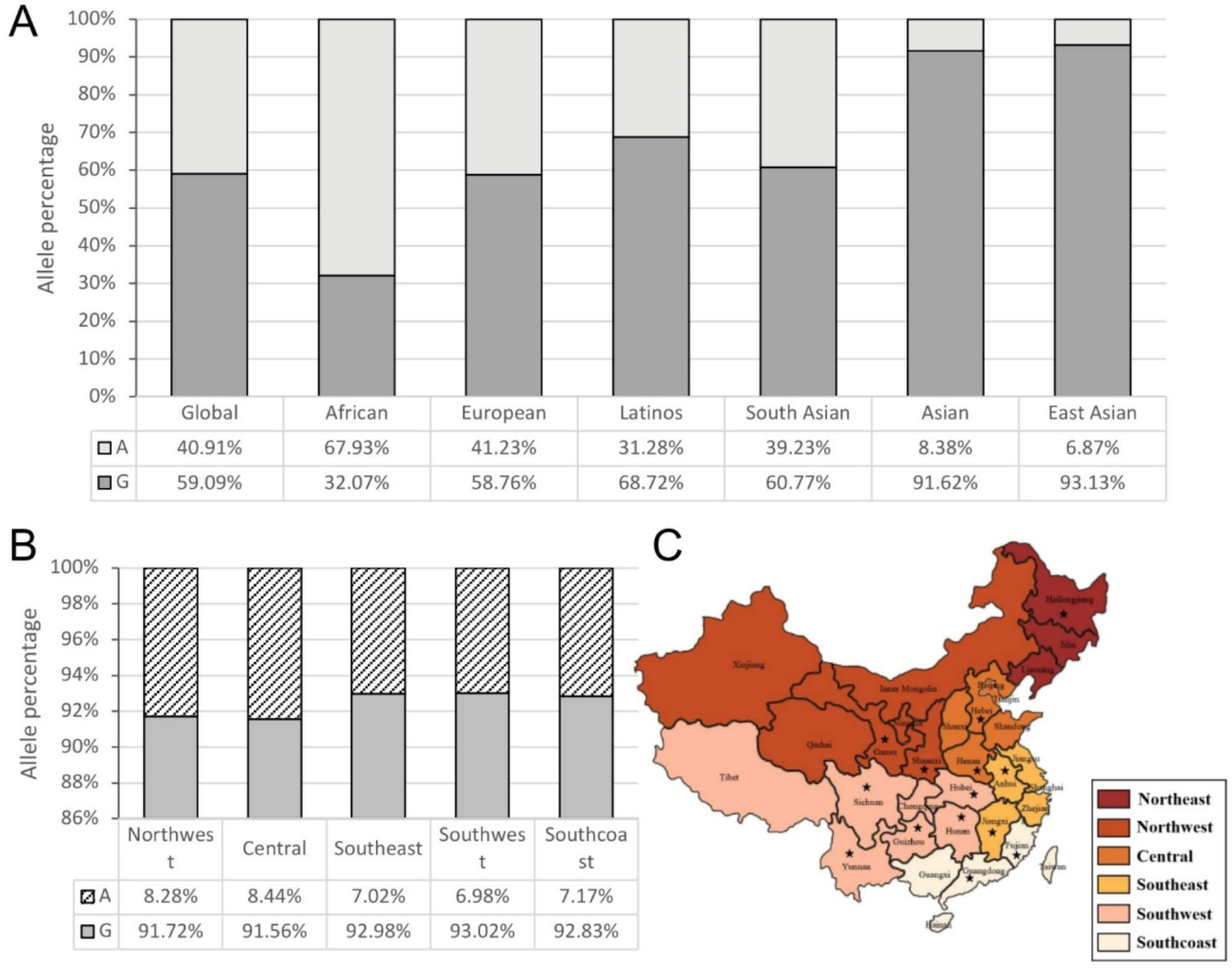

Fig. 2 Geographic and allele distribution of LHCGR genotypes worldwide and in China. (**A**) Global Allele distribution of LHCGR rs2293275, cited from https://www.ncbi.nlm.nih.Gov/snp/. (**B**) Allele Distribution of LHCGR rs2293275 in PGG.HAN. (**C**) Genetic geographical division and distribution of sub centers in China

Table 3 Allele frequency analysis of rs2293275 in ovarian aging patients and the corresponding subgrous of the Han Chinese population based on different genetic regions

Genetic region		DOR	POI	OA(%)	PGG.HAN(%)	$P_{DORvs.HAN}$ OR (95%CI)	$P_{POIvs.HAN}$ OR (95%CI)	$P_{OAvs.HAN}$ OR (95%CI)
Southeast	**G**	361	285	646 (89.47)	49,496 (92.98)	0.002* 1.651 (1.209–2.254)	0.055 1.440 (0.993 ~ 2.090)	0.000** 1.558 (1.225 ~ 1.980)
	A	45	31	76 (10.53)	3738 (7.02)			
Central	**G**	182	127	309 (88.29)	36,713 (91.56)	0.458 1.192 (0.750 ~ 1.894)	0.013* 1.793 (1.129 ~ 2.850)	0.029* 1.439 (1.037 ~ 1.997)
	A	20	21	41 (11.71)	3385 (8.44)			
Southwest	**G**	414	323	737 (89.23)	7942 (93.02)	0.015* 1.481 (1.080 ~ 2.030)	0.001* 1.774 (1.277 ~ 2.465)	0.000** 1.609 (1.272 ~ 2.036)
	A	46	43	89 (10.77)	596 (6.98)			
Northwest	**G**	79	97	176 (88)	10,601 (91.72)	0.046* 1.823 (1.010 ~ 3.290)	0.476 1.256 (0.671 ~ 2.352)	0.061 1.511 (0.981 ~ 2.326)
	A	13	11	24 (12)	957 (8.28)			
Southcoast	**G**	229	98	327 (89.84)	23,301 (92.83)	0.234 1.299 (0.844 ~ 2.000)	0.032* 1.848 (1.054 ~ 3.242)	0.030* 1.464 (1.039 ~ 2.064)
	A	23	14	37 (10.16)	1801 (7.17)			

* $p < 0.05$ ** $p < 0.01$

Table 4 Clinical parameters based on LHCGR rs2293275 genotype (Association study)

	LHCGR rs2293275 Median (P_{25}, P_{75})			Kruskal-Wallis H	*p*
	0.0($n = 993$)	**1.0($n = 224$)**	**2.0($n = 23$)**		
AMH	0.230(0.0,0.7)	0.220(0.0,0.7)	0.130(0.0,0.8)	0.209	0.901
Basal FSH	14.590(7.4,44.1)	14.260(7.5,42.6)	16.730(10.4,63.9)	1.097	0.578
Age at diagnosis	33.000(29.0,37.0)	32.000(27.3,35.0)	25.000(21.0,29.0)	34.409	0.000**
Menopausal age of mother	49.000(49.0,49.0)	49.000(45.3,49.0)	45.000(40.0,49.0)	45.196	0.000**
Menarche age	13.000(12.0,14.0)	13.000(12.0,14.0)	13.000(12.0,14.0)	3.608	0.165
Number of pregnancies	1.000(0.0,2.0)	1.000(0.0,2.0)	0.000(0.0,3.0)	1.847	0.397
BMI	21.484(19.8,23.4)	21.499(19.6,23.4)	20.324(19.3,22.0)	3.176	0.204

* $p < 0.05$ ** $p < 0.01$

demonstrated a 9-year earlier median onset age in AA carriers (30 vs. 39 years, $p < 0.001$) (Fig. 3H).

Impact of rs2293275 on ovarian response to stimulation

Among the 711 patients with DOR, 235 of them sought ART for infertility. Out of these, 109 patients underwent PPOS. The primary IVF outcome parameters were analyzed based on LHCGR genotype (Table 6 and Supplementary Table 2). Interestingly, the OSI showed a significant difference based on the rs2293275 genotype ((OSI: 3.59 vs. 1.21 in GG, $p = 0.019$). Patients with the AA genotype ($n = 2$) had only one-third the number of retrieved oocytes compared to patients with the GG genotype, at the same effective FSH dose. However, no significant associations were observed between the rs2293275 genotype and AFC, basal LH value, gonadotropin, FOI, FORT, number of oocytes, MII oocyte rate, or number of high-quality embryos.

Discussion

Ovarian aging, a multifactorial process influenced by genetic and environmental determinants, poses significant challenges to female fertility and long-term health. This multicenter population-based study elucidates the role of the LHCGR rs2293275 polymorphism in accelerating ovarian aging among Han Chinese women, with three key advancements: (1)Population-specific genetic risk: The AA genotype of rs2293275, though rare (1.85%), confers a 3.7-fold increased risk of premature ovarian insufficiency (POI), highlighting its potential as a biomarker for early intervention in high-risk populations. (2)Transgenerational impact: AA carriers experience POI diagnosis and maternal menopause 7 and 6 years earlier than GG carriers, respectively, suggesting a heritable component in ovarian aging trajectories. (3)Pharmacogenetic implications: Reduced ovarian sensitivity in AA carriers during controlled stimulation ($p = 0.019$)

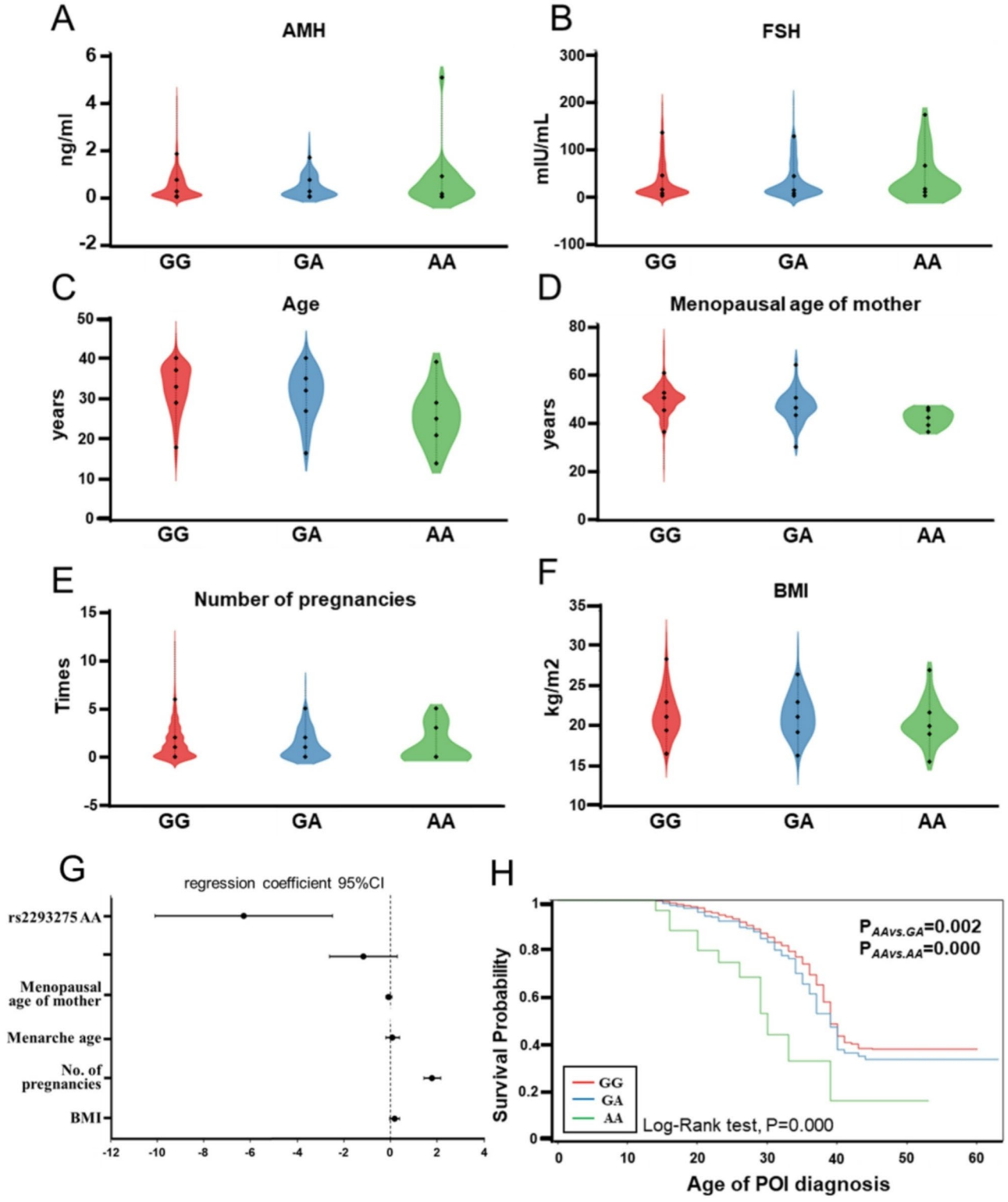

Fig. 3 Clinical significance of LHCGR rs2293275. (**A-F**) Clinical parameters (AMH, basic FSH, Age at diagnosis, menopausal age of mother, number of pregnancies and BMI) based on LHCGR rs2293275 genotype. (**G**) Impaction of rs2293275 genotype and clinical parameters on the menopausal age of POI patients. (**H**) Contribution of LHCGR rs2293275 genotype to the onset of POI

Table 5 Effects of genotype and clinical parameters on menopausal age of patients with POI

	regression coefficient (t value)	95% CI	VIF
constant	28.305** (6.291)	19.487 ~ 37.123	-
rs2293275 AA	-6.312** (-3.251)	-10.117 ~ -2.507	1.046
rs2293275 GA	-1.184 (-1.593)	-2.639 ~ 0.272	1.026
Menopausal age of mother	-0.082 (-1.178)	-0.218 ~ 0.054	1.049
Menarche age	0.089 (0.619)	-0.194 ~ 0.372	1.010
Number of pregnancies	1.791** (9.979)	1.439 ~ 2.143	1.012
BMI	0.174 (1.661)	-0.031 ~ 0.379	1.005
Sample size	529		
R^2	0.189		
Adjusted R^2	0.180		
***F*value**	$F(6,522) = 20.292, p = 0.000$		

Dependent variable: menopause age of POI patients

D-W value: 1.666

* $p < 0.05$ ** $p < 0.01$, T value in parentheses

Table 6 Assisted reproductive technology outcomes after PPOS ovarian stimulation considering LHCGR N312S genotype groups

	rs2293275 Medin M(P_{25}, P_{75})			Kruskal-Wallis H	*p*
	GG($n = 87$)	GA($n = 20$)	AA($n = 2$)		
AFC(bilateral)	3.000(2.0,5.0)	3.000(3.0,5.0)	5.000(4.0,6.0)	1.741	0.419
Gonadotropin injection days	9.000(7.0,11.0)	9.000(7.3,10.0)	8.000(7.0,9.0)	0.424	0.809
Total gonadotropin injections(IU)	2100.000(1275.0,2850.0)	2100.000(1237.5,2681.3)	1425.000(1200.0,1650.0)	1.086	0.581
FOI	0.500(0.3,1.0)	0.722(0.4,1.0)	0.625(0.5,0.8)	2.266	0.322
FORT	50.000(33.3,100.0)	59.921(40.0,91.7)	54.167(33.3,75.0)	0.205	0.902
OSI	1.212(0.7,2.2)	1.753(1.1,3.1)	3.587(3.5,3.6)	7.963	0.019*
Number of mature follicles before ovulation	2.000(1.0,3.0)	2.000(1.3,4.0)	2.500(2.0,3.0)	0.543	0.762
Number of retrieved oocytes	2.000(1.0,3.0)	2.500(1.0,5.0)	3.000(3.0,3.0)	2.514	0.285
MII stage oocyte	2.000(1.0,3.0)	2.000(1.0,4.0)	2.500(2.0,3.0)	1.812	0.404
MII rate	1.000(0.8,1.0)	1.000(0.7,1.0)	0.833(0.7,1.0)	1.968	0.374
High quality embryo	0.000(0.0,1.0)	1.000(0.0,1.0)	0.500(0.0,1.0)	1.417	0.492
Clinical pregnancy	0.000(0.0,0.0)	0.000(0.0,0.8)	0.000(0.0,0.0)	0.695	0.706

* $p < 0.05$ ** $p < 0.01$

Note: the sample size of AA group is small ($n = 2$), and the results should be interpreted with caution

underscores the need for genotype-tailored ART protocols, despite limitations in sample size.

While these findings advance our understanding of genetic contributions to ovarian dysfunction, they also reveal critical knowledge gaps—particularly the molecular mechanisms linking rs2293275 to follicular depletion and the clinical utility of genotyping in diverse ethnic cohorts.

Genetic association of LHCGR rs2293275 with ovarian aging

This multicenter study provides robust evidence that the LHCGR rs2293275 AA genotype is strongly associated with accelerated ovarian aging in Han Chinese women, particularly in POI (OR 3.73, 95% CI 2.09–6.67). The AA genotype frequency in our cohort (1.85%) was threefold higher than in the general Han population (0.62%), aligning with its elevated risk profile. Notably, AA carriers experienced POI diagnosis 7 years earlier than GG carriers, with maternal menopause occurring 6 years prematurely (Fig. 3H), suggesting a transgenerational genetic influence. These findings are consistent across diverse Chinese regions (Fig. 2B–C), underscoring the variant's population-specific relevance.

While the LHCGR rs2293275 AA genotype was strongly associated with accelerated ovarian aging, its impact on classical LH-mediated steroidogenesis (e.g., AMH, FSH) remained nonsignificant. Intriguingly, a trend toward elevated basal E2 levels in GA carriers (vs. GG) suggests potential context-dependent receptor modulation, such as partial hyperactivity or selective signaling pathway activation (e.g., cAMP/PKA vs. β-arrestin) [22, 23]. The absence of broader steroidogenic disruptions may reflect the variant's preferential influence on follicular dynamics (e.g., recruitment/apoptosis) rather than global hormonal regulation [12]. However, the retrospective design and limited GA/AA subgroup sizes preclude definitive conclusions. Future studies should

assess dynamic steroidogenic responses (e.g., post-hCG stimulation) and employ in vitro/vivo models to dissect rs2293275's mechanistic role in LH signaling.

Clinical implications and population heterogeneity

The marked ethnic variation in rs2293275 allele frequency (African: 67.93% vs. East Asian: 6.87%) highlights the necessity of population-specific genetic studies. Within China, regional allele frequency differences (e.g., southwest: 10.77% vs. northwest: 8.28%) further emphasize the need for geographically tailored genetic counseling. Our findings contrast with prior reports in Sichuan Han populations [16], where A allele frequencies were lower (6.98% vs. 10.89% in our OA cohort), likely due to phenotypic differences between general infertility and ovarian aging populations.

Notably, while the AA genotype was more prevalent in POI (2.27%) than DOR (1.55%), inter-subgroup genetic differences were nonsignificant ($p = 0.35$). This parallels reports of shared genetic etiologies between DOR and POI, suggesting a continuum of ovarian dysfunction rather than distinct entities [3, 24]. Longitudinal studies are needed to determine if DOR patients with AA genotype progress to POI.

Pharmacogenetic potential of rs2293275 in ART

In ART cycles, AA carriers exhibited reduced ovarian sensitivity (OSI: 3.59 vs. 1.21, $p = 0.019$), implying that higher LH supplementation might optimize outcomes in this subgroup—a hypothesis supported by prior pharmacogenetic studies [25–27]. However, the small AA sample ($n = 2$) limits definitive conclusions. To assess the robustness of our findings, we conducted sensitivity analyses excluding the AA subgroup ($n = 2$). The results confirmed that GA carriers still exhibited significantly lower ovarian sensitivity (OSI: 1.753 vs. 1.212, $p = 0.019$) compared to GG carriers, supporting the generalizability of our conclusions (Supplementary Table 3). However, the small GA subgroup ($n = 20$) limited power to detect differences in other outcomes (e.g., oocyte yield, embryo quality). Future studies with larger cohorts are needed to validate these findings.The absence of genotype effects on oocyte yield or embryo quality may reflect the study's focus on DOR/POI populations, where baseline ovarian reserve is universally low. Future work should evaluate rs2293275 in normo-responders to assess its broader pharmacogenetic utility.

Limitations

First, the retrospective design may introduce selection bias, particularly in ART outcome analyses where treatment protocols were not standardized. Second, the low AA genotype frequency (1.85%) limited statistical power for subgroup analyses, a challenge inherent to rare variant studies. Third, while the PGG.Han database provided robust controls, its inclusion of males and non-ovarian aging populations may dilute genetic associations. Prospective cohorts with longitudinal follow-up are needed to validate these findings.

Conclusion

This study establishes LHCGR rs2293275 as a novel genetic marker associated with accelerated ovarian aging in Han Chinese women, particularly for POI risk prediction. The AA genotype's association with earlier diagnosis age and maternal menopause underscores its potential in family-based fertility counseling. While our findings illuminate population-specific genetic contributions to OA, functional studies are essential to unravel the molecular mechanisms linking rs2293275 to follicular depletion. Clinically, genotyping this variant may aid in identifying high-risk women for early intervention, though its integration into ART protocols requires further validation.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

Wenqing Ma and Shuangmei Ye contributed to study concept and design, and interpreting the data, composed the statistical dataset, performed the analyses, and wrote and revised the manuscript.Lifeng Tian, Min Liu, Rui Wang and Xuezhou Yang were responsible for follow-up of sub centers and collection of clinical data.Man Wang, Fangfang Fu, Wu Ren, Lei Dang contributed to interpreting the data and critical revision of the manuscript. Tian Wang, Wenwen Wang, Shixuan Wang, Yan Sun and Yan Li contributed to interpreting the data and critical revision of the manuscript.All authors reviewed and approved the final version and no other person made a substantial contribution to the paper.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China (TJ-IRB20211016). All participants provided written informed consent and all methods were carried out in accordance with the approved guidelines.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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