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Analysis of the AMH rs10407022 Polymorphism Reveals Monomorphism in an Iranian IVF Cohort Consistent with POSEIDON Stratification Criteria: A Case-Control Study

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ABSTRACT

Background: Poor ovarian response (POR) during in vitro fertilization (IVF) remains a major challenge, adversely affecting both the emotional wellbeing of patients and clinical outcomes. Genetic polymorphisms, particularly in the anti Müllerian hormone (AMH) gene, such as rs10407022, have been suggested to contribute to POR. This study aimed to investigate the association between the rs10407022 polymorphism and POR, as defined by the POSEIDON criteria, among Iranian women.

Methods: In this analytical case-control study, 232 women under 45 years with poor ovarian response according to the POSEIDON criteria and 56 women with normal ovarian response (controls) were included. Demographic, hormonal, and ovarian reserve parameters, including age, BMI, FSH, AMH, and AFC, were recorded. Genotyping of the AMH rs10407022 (G/T) polymorphism was performed using ARMS-PCR, and associations were assessed using logistic regression adjusted for age and BMI.

Results: Cases exhibited significantly lower AMH levels (0.9 ± 0.4 ng/mL vs. 2.6 ± 0.8 ng/mL, $p < 0.001$), lower AFC, and higher day 3 FSH compared to controls. Notably, all participants in both groups had only the GT genotype for rs10407022, with no GG or TT genotypes observed. This finding indicates that the rs10407022 locus was monomorphic in this Iranian cohort, precluding any significant association with POR.

Conclusion: The AMH rs10407022 polymorphism was monomorphic in this Iranian population, exhibiting only the GT genotype. While significant differences in hormonal markers were observed between POR and normal responders, this single nucleotide polymorphism (SNP) did not account for the variability in ovarian response. Further large-scale, multiethnic studies are warranted to elucidate the genetic underpinnings of POR.

Introduction

Poor ovarian response (POR) is one of the most challenging complications in assisted reproductive technology (ART) cycles, affecting approximately 9-24% of women undergoing ovarian stimulation [1]. Despite receiving adequate or high doses of gonadotropins, these patients yield a low number of oocytes, leading to increased cycle cancellation rates and reduced pregnancy chances, thereby imposing significant emotional and financial burdens [2].

To address heterogeneity in POR diagnosis, the POSEIDON criteria were introduced to to better classify patients with a "low prognosis [3]. This stratification integrates key parameters such as female age, ovarian reserve markers (AMH and AFC), and prior ovarian response, distinguishing four distinct groups. Specifically, the POSEIDON classification defines four groups based on age, ovarian reserve, and previous ovarian response:

Group 1: women <35 years with normal ovarian reserve (AMH ≥1.2 ng/mL or AFC ≥5) but an unexpected poor or suboptimal response in previous cycles.

Group 2: women ≥35 years with normal ovarian reserve but poor/suboptimal response.

Group 3: women <35 years with diminished ovarian reserve (AMH <1.2 ng/mL or AFC <5).

Group 4: women ≥35 years with diminished ovarian reserve.

This detailed classification allows differentiation between "expected" poor responders (Groups 3 and 4) and "unexpected" poor responders (Groups 1 and 2), providing a clear framework for analyzing ovarian response in distinct patient subgroups [3,4].

While biomarkers like anti-Müllerian hormone (AMH) and antral follicle count (AFC) are well-established predictors of ovarian reserve and response [5], inter-individual variability suggests that genetic factors may also play a crucial role. The AMH gene, encoding a key regulator of folliculogenesis, is a prime candidate. A specific single nucleotide polymorphism (SNP), rs10407022 (G>T), results in an amino acid change from isoleucine to serine at position 49 (p.Ser49Ile) in the AMH protein precursor [6]. This polymorphism is hypothesized to affect AMH bioactivity, potentially influencing follicular development and sensitivity to FSH [7].

However, evidence regarding the association between rs10407022 and ovarian stimulation outcomes has been inconsistent. A 2020 meta-analysis suggested that T-allele carriers might yield a higher number of mature oocytes [8], while other studies reported no significant association with oocyte yield or presented conflicting results across different populations [9,10]. These discrepancies underscore the need for further research in specific ethnic groups. This case-control study aimed to investigate the association between the AMH rs10407022 polymorphism and the risk of poor ovarian response among Iranian women undergoing IVF, classified according to the POSEIDON criteria.

Materials and Methods

Study design and participants

This analytical case-control study was conducted at the Yazd Reproductive Sciences Institute, Yazd, Iran. The present study was part of an MSc. thesis and was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd (IR.SSU.RSI.REC.1398.008). Informed and written consent was obtained from all participants, and sampling was carried out according to the approved guidelines. A total of 232 women younger than 45 years of age, classified as poor ovarian responders based on the POSEIDON criteria and candidates for ART, were included in the study. Participant eligibility was determined by a gynecologist according to predefined inclusion and exclusion criteria. Exclusion criteria included a history of ovarian radiotherapy, severe endometriosis, prior pelvic surgery, smoking, and any condition that could interfere with the evaluation of ovarian reserve. Eligible patients were categorized into the four POSEIDON

groups following clinical assessment. After obtaining written informed consent, peripheral blood samples were collected from all participants. As a comparison, 56 women with normal ovarian response were enrolled as the control group, and blood samples were obtained to assess the frequency of the targeted polymorphism.

Data collection

Demographic and clinical parameters, including age, BMI, and hormonal profiles (AMH, FSH, luteinizing hormone [LH], thyroid-stimulating hormone [TSH], prolactin, and AFC), were collected. Ovarian stimulation protocols followed standard clinical practice.

Genotyping

Genotyping of the AMH rs10407022 (G>T) polymorphism was performed using the tetra-primer ARMS-PCR method. Genomic DNA was extracted from peripheral blood samples and stored at –20 °C until analysis. The ARMS-PCR assay used specific primers, including outer primers (OF: ATAGGGGTCTGTCCTGCAC / OR: CTCCAGGTGTAGGACCACC) and inner primers (IF: GGACTGGCCTCCAGGTAT / IR: CACAGAGGCTCTTGTGAGC).

This primer set amplified allele-specific fragments of 265 bp for the T allele, 378 bp for the G allele, and a 498 bp outer control fragment. PCR amplification consisted of an initial denaturation at 95 °C for 5 minutes, followed by 34 cycles of denaturation at 95 °C for 40 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 40 seconds, with a final extension at 72 °C for 5 minutes, followed by a hold at 4 °C. PCR products were separated on a 1.5% agarose gel, stained with Safe Stain, and visualized under UV light to determine genotypes based on the observed banding patterns.

Statistical analysis

Continuous variables were compared using t-tests or Mann-Whitney U tests, as appropriate, while categorical variables were analyzed using chi-square tests. Logistic regression, adjusted for age and BMI, was used to assess the association between genotypes and POR. All analyses were performed using SPSS version 25, with a significance level set at $P < 0.05$.

Results

Analysis of patients stratified into the four POSEIDON groups revealed expected patterns in age distribution and key hormonal markers. The cohort's age structure aligned with the POSEIDON framework, with younger poor responders (Group 1: mean age 27.9 years; Group 3: 32.0 years) distinct from their older counterparts (Group 2: 36.5 years; Group 4: 39.6 years). The control group of normal responders had a mean age of 26.9 years. As anticipated, AMH levels effectively distinguished the subgroups based on ovarian reserve. Groups 1 and 2 exhibited AMH levels above the 1.2 ng/mL threshold, while levels in Groups 3 and 4 fell below this cutoff (see Table 1 for complete hormonal data). Statistical comparisons among groups are presented in Table 1. Significant differences ($P < 0.05$) were observed for age, AMH, FSH, LH, TSH, and prolactin, whereas TPO did not show significant differences. Analysis of the AMH rs10407022 (G/T) polymorphism revealed that all participants in both the case (poor ovarian response) and control (normal ovarian response) groups exhibited only the GT genotype. No GG or TT genotypes were observed. This finding indicates that the rs10407022 locus was monomorphic in this Iranian cohort. Consequently, due to the absence of genetic variation, it was not possible to assess any association between this specific polymorphism and ovarian response outcomes. Further analysis highlighted subgroup-specific endocrine patterns: TSH differed significantly only in Groups 1 and 2, prolactin levels were significantly higher in Groups 1–3, and no significant differences were observed for TPO between any groups.

Table 1. Baseline Characteristics and Hormonal Profiles of the Study Participants According to POSEIDON groups and normal responders

Variable	POSEIDON Group 1	POSEIDON Group 2	POSEIDON Group 3	POSEIDON Group 4	Normal Responders	P-value
Age (years)	27.98 ± 3.94	36.56 ± 3.23	32.03 ± 4.64	39.69 ± 3.09	26.95 ± 3.08	< 0.001
AMH (ng/mL)	5.19 ± 4.19	2.82 ± 1.49	0.77 ± 0.36	0.67 ± 0.30	0.60 ± 0.42	< 0.001
FSH (IU/L)	8.83 ± 2.98	7.94 ± 1.82	7.41 ± 3.46	6.19 ± 3.74	5.42 ± 2.52	< 0.001
LH (IU/L)	27.51 ± 6.90	22.76 ± 4.99	20.52 ± 9.57	14.64 ± 7.45	8.55 ± 3.79	< 0.001
TSH (mIU/L)	2.81 ± 1.34	4.03 ± 2.44	2.89 ± 2.44	2.61 ± 1.09	2.41 ± 1.18	0.04
Prolactin (ng/mL)	48.52 ± 5.42	47.41 ± 3.11	26.00 ± 4.11	24.42 ± 5.08	16.48 ± 4.03	< 0.001
TPO (IU/mL)	20.35 ± 16.30	9.49 ± 2.57	9.20 ± 12.11	7.91 ± 1.42	6.46 ± 1.18	0.28

Data are presented as mean ± standard deviation (SD). Comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparisons. A p-value < 0.05 was considered statistically significant.
Abbreviations: AMH, Anti-Müllerian Hormone; FSH, Follicle-Stimulating Hormone; LH, Luteinizing Hormone; TSH, Thyroid-Stimulating Hormone; TPO, Thyroid Peroxidase Antibodies.

Discussion

The findings of this study provide a detailed endocrine profile of patients classified according to the POSEIDON criteria for POR, reinforcing the clinical utility of this stratification system while highlighting distinct hormonal patterns that may underlie different etiologies of POR.

The clear stratification in AMH levels across the POSEIDON groups aligns with the framework's foundation, which uses ovarian reserve markers for categorization. Groups 1 and 2 (<35 years) exhibited AMH levels >1.2 ng/mL, consistent with normal ovarian reserve but suboptimal response, potentially implicating functional or iatrogenic factors [11]. In contrast, Groups 3 and 4 (≥35 years) had AMH levels <1.2 ng/mL, confirming the expected diminished ovarian reserve associated with advanced age [12]. This distinction is critical for tailoring therapeutic strategies, such as protocol optimization for younger patients versus addressing reduced follicular pool in older patients [13]. The significant alterations in gonadotropin levels further elucidate the underlying pathophysiology. The dysregulation of FSH and LH across all POSEIDON groups indicates pervasive disruption of the hypothalamic-pituitary-ovarian (HPO) axis in POR [14]. Elevated FSH in older groups (2 and 4) reflects diminished ovarian feedback, while aberrant levels in younger groups (1 and 3) may indicate ovarian resistance or inefficiency [15].

The results for TSH and prolactin introduce additional complexity. The significant difference in TSH levels only in Groups 1 and 2 suggests that subclinical thyroid dysfunction may be a more relevant co-factor in younger POR patients [16]. Similarly, the pattern for prolactin, with significant differences in Groups 1, 2, and 3 but not in Group 4, implies that hyperprolactinemia may impair response in younger patients or those with moderate reserve, whereas its impact is negligible in the context of severely diminished reserve [17]. The absence of significant differences in TPO antibody levels between POR groups and normal responders suggests that autoimmune thyroiditis may not be a primary driver of POR in this cohort [18]. This underscores that the POSEIDON criteria primarily capture disturbances intrinsic to the HPO axis and ovarian aging, rather than systemic autoimmune conditions.

A key finding of this study was the absence of genetic variation at the AMH rs10407022 locus in our Iranian population, with only the heterozygous GT genotype detected across all samples. This result contrasts with some previous studies in other ethnic groups, which have reported the presence of both G and T alleles and investigated their potential association with ovarian response [6, 8-10]. For instance, studies in Caucasian and Chinese

populations have documented varied genotype distributions for this SNP, suggesting ethnic heterogeneity in allele frequencies [9, 10]. The monomorphic nature of this SNP in our cohort precluded any evaluation of its role in predicting POR among Iranian women. This discrepancy highlights the influence of ethnic background on the genetic architecture of genes involved in reproduction, such as AMH. Although the rs10407022 polymorphism was found to be monomorphic in this Iranian cohort, this finding provides important insight into the population-specific genetic background influencing ovarian physiology. The absence of allelic variation suggests a potential ethnic or regional fixation of the GT genotype in Iranian women, which could partly explain differences in ovarian response profiles across populations. Such population-specific genetic homogeneity emphasizes the need for broader genome-wide approaches and cross-ethnic studies to uncover genetic determinants of poor ovarian response. It underscores the necessity for large-scale, multi-ethnic genetic studies to identify population-specific variants and to comprehensively elucidate the genetic determinants of ovarian response.

Conclusion

In conclusion, this study demonstrates that the POSEIDON classification effectively captures distinct endocrine phenotypes associated with POR. The significant variations in AMH, FSH, and LH across all groups confirm systemic disruption of the HPO axis in POR. The selective significance of TSH and prolactin in specific subgroups suggests that these hormones may modulate ovarian response, influenced by age and ovarian reserve. The lack of association with TPO antibodies refines the focus on primary ovarian and hypothalamic-pituitary factors. Despite the monomorphism at the AMH rs10407022 locus, our findings underscore the importance of evaluating genetic variation within distinct ethnic contexts. Previous studies in Asian populations reported that carriers of the TT genotype of rs10407022 had a lower number of retrieved oocytes compared to GG/GT carriers [8]. In contrast, a study in a Polish cohort found no significant association between this SNP and ovarian parameters [8]. Our study provides the first report of AMH rs10407022 genotype distribution in an Iranian IVF cohort, revealing a monomorphic GT genotype. This population-specific pattern highlights the importance of considering ethnic differences in genetic studies and may inform personalized ovarian stimulation strategies. The observed monomorphism highlights potential population-specific patterns that could shape ovarian response. Future genomic studies incorporating larger, multiethnic cohorts and additional AMH pathway variants are warranted to elucidate genetic contributions to poor ovarian response. These insights can guide personalized diagnostic and therapeutic strategies for managing poor ovarian responders. Future studies should explore other genetic and molecular factors contributing to POR in diverse populations.

Conflict of Interest

The authors declare no conflicts of interest regarding the study.

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Ethical Considerations

The study was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences (Code: IR.SSU.RSI.REC.1398.008).

Author's Contribution

Conceptualization, N.N.L and F.M; Methodology, NNL and F.M; Conducting the data collection and performing the analyses, F.D and S.M.M; Assisting in interpreting the results and reviewing the literature, F.M; Reviewing

and approving the final manuscript, M.Y and F.M.

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