The Influence of AMHR II Polymorphisms on Infertility: A Comprehensive Study of A10G/rs11170555 and G4952A/rs3741664 in South Indian Women

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Abstract

Infertility, affecting up to 15% of couples globally, often remains unexplained. AMH (Anti-Müllerian Hormone) along with its corresponding receptor AMHR 2 play crucial roles in ovarian follicular development. Genetic polymorphisms in the AMHR II gene, specifically A10G (rs11170555) and G4952A (rs3741664), may influence fertility. This study investigates the association between AMHR II polymorphisms and infertility in South Indian women. Non experimental study involving 120 women with unexplained infertility was conducted. Genomic DNA was extracted from the peripheral blood samples obtained from all participants. Genotyping of rs11170555 and rs3741664 was performed using Real-Time PCR.

The study identified specific genotypes as more prevalent in the population, with some variations in allele distribution compared to previous studies. Recognizing the distribution of these genotypes can help to identify individuals who might be at risk of infertility linked to these polymorphisms. AMHR II polymorphisms show significant variability, influencing reproductive outcomes. Understanding these differences could enhance personalized infertility treatments. Additional studies are needed to investigatethe effects on the functionality of these polymorphisms.

Keywords: Infertility, Anti-Mullerian hormone, Ovarian Follicular development, Polymorphism.

Introduction

Infertility is a growing health issue globally, impacting one in six couples in developed countries and a significant number in developing countries[2]. The World Health Organization (WHO) reports that ranging from 10% to 15% of infertility events are unexplained[7,16]. Infertility is defined as the failure to achieve pregnancy after a year of regular, unprotected intercourse[20]. Anti-Müllerian Hormone (AMH), belonging to the family transforming growth factor-beta, has a significant role in regulating the growth of ovarian follicles[3]. AMH is secreted via the granulosa cells within the preliminary-stage ovarian follicles. Its expression persists in developing follicles until they attain a sufficient size and differentiation. At this stage, follicle-stimulating hormone (FSH) promotes the selection of follicles for dominance[16,19].

Several investigations have shown that blood AMH concentrations are indicative of the reserve of primordial follicles. They are particularly well-correlated alongside the amount of antral follicles and decrease progressively in every part of a woman's generative lifespan[14,15]. AMH influences biological processes by interacting with the receptor of AMHR 2, found on theca cells and granulosa cells[17].

Given the anticipated impact of AMH on ovarian stimulation response, it is suggested the genetic variations within the AMH signaling cascade could affect the ovarian reaction throughout COS (Controlled Ovarian Stimulation). The AMHR2 variant, which engages in this pathway, is situated on chromosome 12 and contains 11 exons[18]. Variations in the AMHR2 gene seem to influence the biological activities of hormones, which in turn may impact follicle recruitment and development, potentially contributing to infertility[10]. Investigating gene polymorphisms that influence female reproductive function, including those related to AMH along with its receptor AMHRII, can provide insights into the mechanisms of gonadal function and human fertility.

Material and Methods

Study structure and sample population: This non-experimental study was carried out between 2022 and 2023 at the Obstetrics and Gynaecology Department of Mamata Academy of Medical Sciences and General Hospital and Krishna Leela Fertility Centre, Madhapur, Hyderabad. Participants included couples seeking infertility treatment. Couples with normal diagnostic results following a comprehensive infertility investigation were classified as having Idiopathic infertility and were recruited for the study.

- **Inclusion Criteria**: Couples presented with normal diagnostic results for infertility and classified as having unexplained infertility.
- **Exclusion Criteria**: Couples in which either partner had any abnormalities detected in the hormonal, semen, or chromosomal analysis were excluded from the study.

Specimen Collection and DNA Extraction: Blood samples from peripheral veins (2 ml) were drawn from the study participants in EDTA-containing vacutainer vials. Genetic

material was isolated out of lymphocytes using the salting-out method, which is efficient, rapid and non-toxic. This method yielded approximately 6-10 µg of DNA per sample.

Genotyping of AMHR II Polymorphisms: The study focused on two polymorphisms of the AMHR II gene: A10G (rs11170555) and G4952A (rs3741664). Genetic profiling was carried out by means of RTPCR (Real-Time Polymerase Chain Reaction) followed by sequencing.

Results

In this study to find the role of AMHR2 polymorphisms in infertile women, the polymorphisms A10G/rs11170555 and G4952A/rs3741664 were genotyped.

Table 1
Primer Sequences and PCR Conditions for AMHR II Gene Polymorphisms

Gene	Base Pair Change	Primer	Base Pair	Tm
AMHR II	A10G	F: 5' GGTAGGCACCCCTAGGACTAA 3' R: 5' ACTGTATTAGGAGAGCAGATGGG 3'	551	59
AMHR II	G4952A	F: 5' AAGTGCCCAGTACCCTTGATCT 3' R: 5' TCCTCCCTCTTTTGACCCAATC 3'	425	60

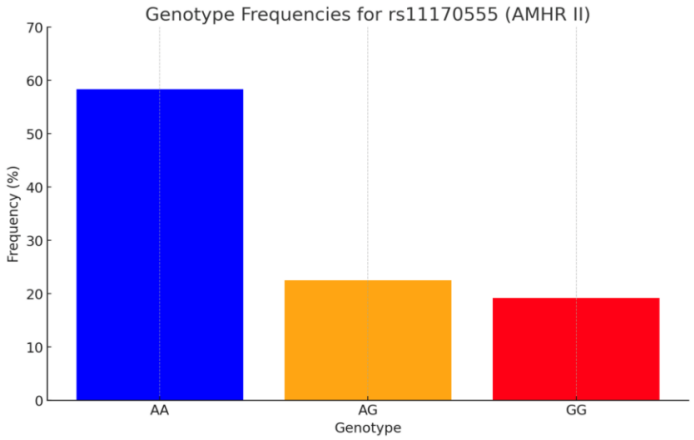

Figure 1: Genotype Frequencies for rs11170555

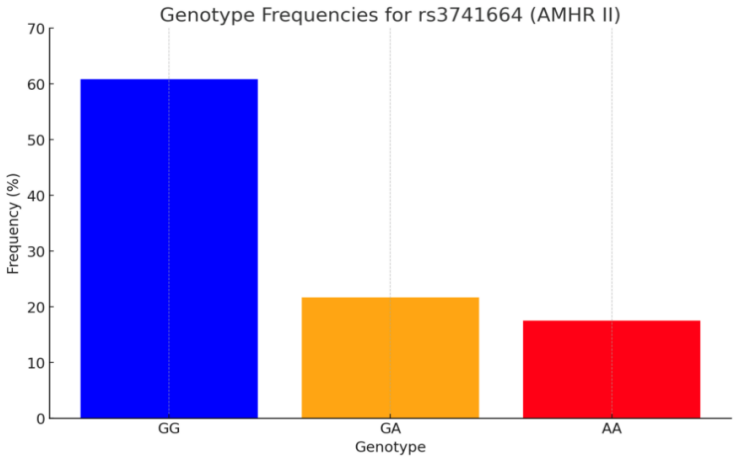

Figure 2: Genotype Frequencies for rs3741664

Table 2
Distribution of Alleles in Genotypes A10G and G4952A

AMHR II	A10G/rs11170555	G4952A/rs3741664
Homozygous AA	70 (58.33 %)	21 (17.5 %)
Heterozygous AG	27 (22.5 %)	26 (21.67 %)
Homozygous GG	23 (19.17 %)	73 (60.83 %)

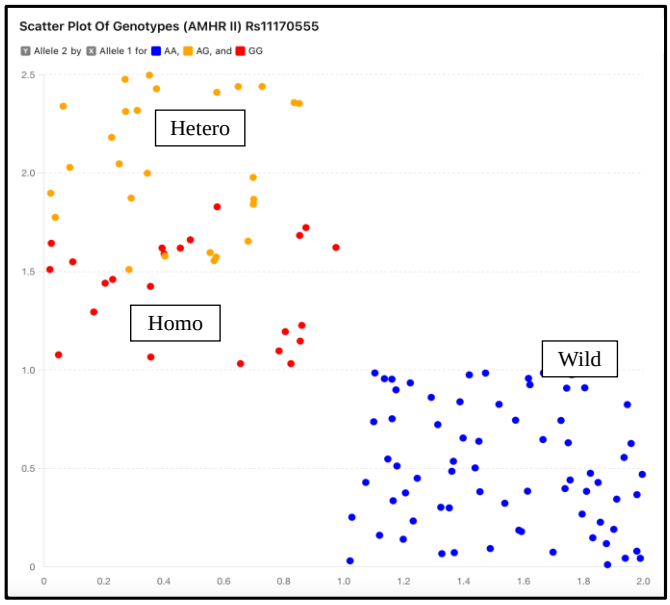

Figure 3: Scatter Plot for rs11170555

Table 3
Comparison of Genotype Frequencies for AMHR2 rs11170555 Between Our Study and Peluso et al

AMHR2	rs11170555	
	Our Study (N=120)	**Peluso C et al[12] (N=186)**
Homozygous AA	58.33 %	61.3 %
Heterozygous AG	22.5 %	27.4 %
Homozygous GG	19.17 %	11.3 %

rs11170555

1. **Homozygous AA (70 individuals, 58.33%)**:
   - This genotype is considered the wild type.
   - The majority of individuals possess this genotype, indicating that it is the most common form in the population.
   - The wild type (AA) is the most prevalent genotype, suggesting that the normal function of the AMHR II gene is predominant in this population.
2. **Heterozygous AG (27 individuals, 22.5%)**:
   - This genotype is normal type.
   - This genotype is present in a significant portion of the population, but not as common as the wild type.
3. **Homozygous GG (23 individuals, 19.17%)**:
   - This genotype is the polymorphic allele.
   - A smaller portion of the population has this genotype, which could potentially be associated with altered function of the AMHR II gene and might indicate potential reproductive health issues

rs3741664

1. **Homozygous GG (73 individuals, 60.83%)**:
   - This genotype is considered the wild type for rs3741664.
   - The majority of individuals possess this genotype, indicating that it is the most common form in the population.
2. **Heterozygous GA (26 individuals, 21.67%)**:
   - This genotype is normal type for rs3741664.
3. **Homozygous AA (21 individuals, 17.5%)**:
   - This genotype is the polymorphic allele.
   - A smaller portion of the population has this genotype, suggesting it is less common compared to the wild type GG genotype.

The genotype distribution is visualized with blue dots for wild (AA), orange dots for heterozygous (AG) and red dots for homozygous (GG) allelic types. The X-axis displays the intensity or frequency of single variation, while the Y-axis indicates the intensity or frequency of the other variant. The wild type (AA) points are concentrated in a specific area, making up 58.33% of the dataset. Heterozygous (AG) points are spread between the AA and GG regions, comprising of 22.5% of the dataset. Homozygous (GG) points are in a distinct area, representing 19.17% of the dataset.

The genotype distribution is visualized with blue dots for wild (GG), orange dots for heterozygous (GA) and red dots for homozygous (AA) genotypes. The X-axis displays the intensity or frequency of single variation, while the Y-axis indicates the intensity or frequency of the other variant.

The wild type (GG) points are concentrated in a specific area, making up 60.83% of the dataset. Heterozygous (GA) points are spread between the GG and AA regions, comprising of 21.67% of the dataset.

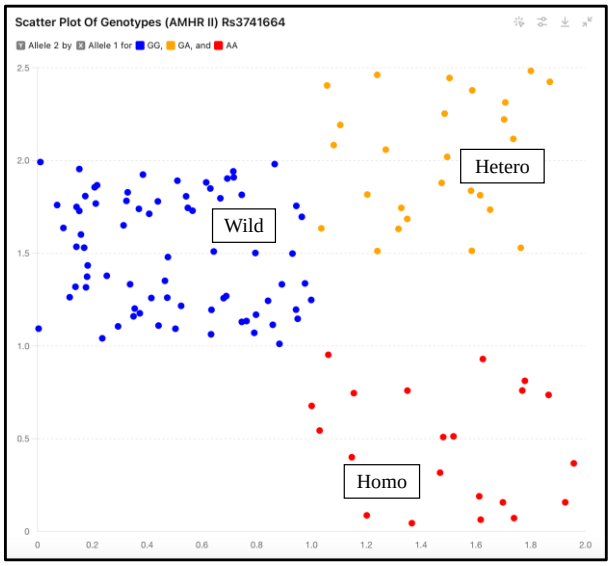

Figure 4: Scatter Plot for rs3741664

Table 4
Comparison of Genotype Frequencies for AMHR2 rs3741664 Between Our Study and Peluso et al

AMHR2	rs 3741664	
	Our Study (N=120)	Peluso C et al[12] (N=186)
Homozygous AA	17.5 %	0 %
Heterozygous AG	21.6 %	28.5 %
Homozygous GG	60.83 %	71.5 %

Homozygous (AA) points are in a distinct area, representing 17.5% of the dataset. The scatter plots visually show the distribution of different genotypes in the population for each polymorphism, with the concentration of points in specific regions indicating the prevalence of certain genotypes. The presence of heterozygous (AG/GA) genotypes in the middle regions of the plots suggests genetic variation within the population.

Additionally, the difference in prevalence between homozygous wild type and homozygous polymorphic alleles highlights the genetic diversity present in the population. Understanding the distribution of these genotypes can aid in identifying individuals who may be at risk for infertility associated with these polymorphisms. Moreover, the data can be used to guide further research on the functional implications of these genetic variations, providing valuable insights into their impact on reproductive health.

Discussion

Only a limited number of studies have compared AMHR II polymorphisms. AMH binds specifically to the AMHR type II receptor (AMHRII), which was first cloned by two separate research groups in 1994[3,6]. The AMHRII gene is located on chromosome 12q13, spans approximately 8.7 kilobases and is composed of 11 exons. AMHRII functions as a specific type II receptor while ALK2, ALK3 and ALK6 serve as type I receptors. The cytoplasmic signaling is mediated by Smad proteins 1, 5 and 8. The roles of cofactors, coactivators and corepressors in AMH signaling, as well as their interactions with other signaling pathways, remain unclear at this time[21].

The AA genotype is the most common genotype in both studies, with similar proportions. The AG genotype is more common in the Peluso et al's[10]. study compared to our study. The GG genotype shows a notable difference, being more prevalent in our study compared to Peluso et al's[10] study. The AA genotype is present in our study but completely absent in Peluso et al's[10]. study, indicating a significant genetic variation between the two populations. The AG genotype is more common suggesting that this heterozygous state might be more frequent in their population. The GG genotype is the most common in both studies, with a higher prevalence in the Peluso et al's[10] study compared to our study. Durlinger and colleagues[4] observed that mice lacking AMH showed a more rapid recruitment of follicles compared to control mice, leading to increased sensitivity to FSH. Variations in AMH and AMHR2 genes are linked to differences in the ovarian response threshold to FSH[2]. These genetic variations seem to influence the biological activities of the hormone, which in turn impacts follicle recruitment and development[21].

In patients with normo-gonadotrophic normo-estrogenic anovulatory infertility, these polymorphisms may serve as important predictors for evaluating the unique FSH threshold level. The variations among the required dose of

administered FSH to induce an ovarian response could influence the risk of hyper-response, which may lead to complications such as ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies[5,9]. Several studies have investigated different SNPs in the AMHR II receptor gene, particularly in the promoter region, comparing these polymorphisms with AMH, FSH and estradiol levels. The majority of research has focused on the -482 A>G SNP, as examined by researchers such as Kevenaar et al[8], Schug et al[12], Yoshida et al[21] and Rigon et al[11].

The homozygous −482 A>G mutation may lead to reduced AMH signaling. Since this SNP is located in the promoter region, it could also be in linkage disequilibrium with other nearby SNPs, potentially influencing gene expression[8]. The −482 A>G SNP is situated at a site that may serve as a binding location for the c-Myb and c-Myc transcription factors, potentially altering promoter activity[12]. Yoshida et al's[21] study indicates that the homozygous −482 A>G mutation may lead to impaired follicular development. In cases of normal pregnancy, where the −482 A>G homozygous mutation was present, it was consistently associated with ISV5-6 and these cases exhibited poor obstetric outcomes.

The allele frequencies of the IVS 10+77 A>G , IVS 5–6 C>T and−482 A>G polymorphisms are substantially higher (P<.05) in women with infertility relative to control group. However, this distribution of frequency of genotypes between the two groups is similar[11]. Peluso et al[10]. observed that the A10G/11170555 polymorphism of the AMHR II gene was linked to serum levels of estradiol and AMH. However, the study found no such correlation for the A-482G, C1749G and G4952A polymorphisms of the AMHR II gene.

Conclusion

The genotype findings provide important insights into the genetic variability of the AMHR II gene and its potential impact on reproductive health. Recognizing these genetic differences is crucial for advancing research and enhancing personalized approaches to infertility treatment. Customizing treatments according to an individual's genotype, such as specific interventions for those with the GG genotype versus the AA genotype, may lead to better reproductive outcomes. Further investigation is necessary to understand the functional consequences of these polymorphisms. This genetic diversity is key in developing targeted medical treatments and interventions.

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