Ann. For. Res. 66(1): 2518-2525, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

MOLECULAR ANALYSIS OF *CYP17* GENE PROMOTER IN IRAQI INFERTILE WOMEN

Raghad J. Abdul-Sahib and Salah H. Faraj*
Department of Biology, College of Science, University of Misan, Iraq
**Email: salah81ss@uomisan.edu.iq*

Abstract
Background and Aims: Female infertility is a complex multifactorial, and polygenic disease associated with genetic factors plays an essential role in its formation and follicle development, oocyte maturation, and steroidogenesis regulation in the ovary. The objective of this work was to pinpoint the polymorphism in the *CYP17* gene promoter. The *CYP17* gene polymorphism was discovered using DNA sequencing techniques. Results: We found that the presence of the rare allele ‘‘T’’ and heterozygous and common homozygous genotypes significantly increased the risk of infertility in women. The results detected single-nucleotide polymorphism (SNP) in the -34T>C site in the promoter of the CYP17 gene. The CYP17 gene has three genotypes according to sequence alignment, which was utilized to detect them using Geneious software V. 2020.0.4. The genotypic frequencies of TT, TC, and CC were 0.50, 0.375, and 0.125, respectively. Frequencies of T and C alleles were 0.687 and 0.313, respectively in infertility women.
Keywords: CYP17 gene, Infertility, genome

Introduction

Infertility is a global barrier affecting people all over the world and its causes and importance may vary according to geographical location and socio-economic conditions. Awareness of infertility is the first step in maintaining the power of pregnancy in lifestyle modification (**1**). Infertility is a condition when Couples who are not pregnant with more than 12 months of regular intercourse and provided that the male partner has normal sperm parameters and the woman has no known risk factors (**2**). The human genome contains a variety of genetic variations and single-nucleotide changes that alter amino acids in protein coding regions are one of the main causes of human phenotypic variation and disease incidence (**3**). CYP17A1 is a 57.4 kDa protein belonging to the cytochrome P450 family (**4**). The protein encoded by its cDNA consists of 508 amino acid residues (**5**). The cytochrome P-450c17α (CYP17) gene is located on chromosome 10 on arm q24.3 (**6**). CYP17 family (CYP17 P-450 C 17α) is associated with hyperandrogenism in women and the relationship between CYP17 gene polymorphisms and risk of PCOS (**7**). As PCOS is a complex reproductive disorder due to endocrine disruption. It is characterized by ovulatory dysfunction and androgen excess and infertile polycystic ovary syndrome due to excess androgens produced as a result of a defect in the steroid biosynthesis pathway in which cytochrome P450, hydroxylase-17 (CYP17) plays an imperative role in it (**8**). A great deal of interest has emerged in single nucleotide polymorphisms (SNPs) in the CYP17 gene, a substitution of thymine (T) with cytosine (C) at the 34 bp of the translation initiation point in the promoter region of CYP17 (rs743572 14), which has been postulated that the Sp-1 extra transcriptional binding site was produced and genotyped. Next,

the expression of the CYP17 gene was enhanced (**9**). A polymorphism in the CYP17 50-UTRMspA1 gene (rs743572)) at position 34 results in two different genotypes, TC and CC. Ovaries (**10**). The results of one study conducted on infertile women in Pakistan indicate the genotype distribution of CYP17 50-UTR MspA1 (TT, TC, CC) and that the CYP17 gene polymorphism (TC genotype) is the highest (54.9%). It is significantly associated with susceptibility to PCOS in infertile women (**11**). Also, a single nucleotide polymorphism in the 50-UTR, -34 bp results upstream of the T-to-C translation initiation point. It is believed to lead to the creation of an additional Sp1 promoter located at the CCACC box (**8,12).**

2. Materials and methods

A case-control study with 60 infertile women and 30 controls between the ages of 15 and 45 years was done. Blood was drawn from participants in the Maysan Governorate throughout the months of October and December of 2021. Each participant was a native of Maysan. The required administrative and ethical approvals for conducting this study were provided by the authorized officials of the College of Science, University of Misan. The Maysan Governorate's Al-Sadr Hospital staff was in charge of obtaining the blood samples.

2-1: Genomic DNA extraction and genotyping

Genomic DNA was extracted from whole blood using the gSYNC™ DNA Extraction Kit manufactured by the Taiwanese Geneaid Company. A fragment (459bp) of the *CYP17* gene in the women was amplified by using the primer F:5`- CATTCGCACTTCTGGAGTC -3 and R: 5`-GGCTCTTGGGGTACTTG -3` (**13**). The PCR amplifications were conducted in a 50 µl volume containing 5 µl genomic DNA, 25 µl of Master Mix, 4 µl each primer, 11 µl free water. The amplification conditions were as follows: initial denaturation at 95˚C for 5 min followed by 35 cycles of denaturation at 95˚C for1 min, annealing at 56˚C for 0.75 min, and extension at 72˚C for 0.75 min, and then the final extension at 72 C for 7 min. The PCR results were using electrophoresis at 2% agarose-gel with the visualized by contact with ultraviolet light as in the Figure (1).

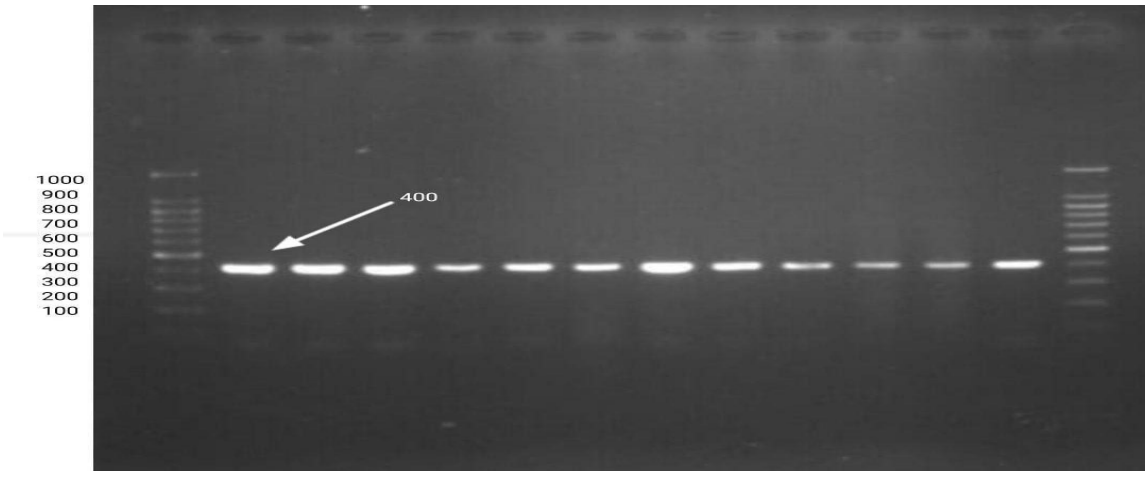

Figure (1) The amplification product of CYP17 gene on a 1% agarose gel

Results and Discussion

The results of the analysis of nitrogenous base sequences using the Multiple 2 Alignment (MSA) method (Clustal omega 1.2.3., 2022) revealed the detection of a single genetic change of the CYP17 gene in sterile women compared with the control group and with the sequences mentioned in the gene bank for the same gene under accession number EU322845.1. A single polymorphism was detected in the promoter region of CYP17 gene, specifically at site 725, and it was a significant genetic mutation for both primary and secondary sterility samples, in which the nitrogenous base of thymine (T) was replaced by the nitrogenous base of cytosine (C) at a 34 basis point from the start of translation in the promoter region. (T>C) at site 725 (T725C) of the total nucleotide sequence of the promoter region as in Figure (2).Figure (2) shows that the non-conservative point mutation (T725C) led to a change in the genetic code from CTG to CCG and thus led to the replacement of amino acids at the level of the resulting protein, as the amino acid asparagine (Asn) was changed to the amino acid serine (Ser) at the site 680 (Asn680Ser)The result was identical to the results of the researcher (**7**), which These indicated that the T/C gene polymorphism played a role in increasing the susceptibility to PCOS when carrying the C allele, suggesting that the *CYP17* gene polymorphism is an important factor in the metabolic and hormonal disruption associated with the infertility of PCOS. And also the result of the researcher (**14**) on women in North India who suffer from menstrual disorders due to anovulation, infertility and hyperandrogenism, as the overproduction of androgens in the ovaries is the main pathological feature of PCOS, which is the main cause of female infertility, due to genetic polymorphism (-34T>C) susceptibility to developing PCOS in North India. The results were also identical to the results of the researcher (**8**) when studying the association of T/C polymorphisms of *CYP17* gene with polycystic ovaries and hyperandrogenism that causes infertility in Kashmiri women. Replacing the nitrogenous base thymine (T) with the nitrogenous base cytosine (C) led to a genetic formation that causes infertility and this is similar to the results of (**11**) in one of the studies conducted on women who had infertility cases in Pakistan, where genetic polymorphism (-34T>C) in the 5'-UTR promoter region of the CYP17 gene was significantly associated with susceptibility to PCOS in infertile women. It is also identical to what the researcher (**9**) identified 17 articles related between genetic polymorphisms. T/C of the CYP17 gene and PCOS, and it was considered that carrying the C allele increases the risk of PCOS and thus infertility, but the results did not match a study he conducted (**15**) for the CYP17A1 gene in Iraqi women, where the researcher concluded No association between CYP17 and PCOS.

```
EU332845.1      GGTGATCAACTGACCTCCCTTACCTAGCTCCTCCTCCGGAGGTTTGCCCTGGAGTTGAGC    540
Control         ---------CTGACCTCCCTTACCTAGCTCCTCCTCCGGAGGTTTGCCCTGGAGTTGAGC    51
Primary         ---------CTGACCTCCCTTACCTAGCTCCTCCTCCGGAGGTTTGCCCTGGAGTTGAGC    51
Secondary       ---------CTGACCTCCCTTACCTAGCTCCTCCTCCGGAGGTTTGCCCTGGAGTTGAGC    51
                         **************************************************

EU332845.1      CAGCCCTTGAGGAGGCCTTCACTCCCACCGCCTCTCTCCCTTCTGGATATGAGCTCAGGC    600
Control         CAGCCCTTGAGGAGGCCTTCACTCCCACCGCCTCTCTCCCTTCTGGATATGAGCTCAGGC    111
Primary         CAGCCCTTGAGGAGGCCTTCACTCCCACCGCCTCTCTCCCTTCTGGATATGAGCTCAGGC    111
Secondary       CAGCCCTTGAGGAGGCCTTCACTCCCACCGCCTCTCTCCCTTCTGGATATGAGCTCAGGC    111
                ************************************************************

EU332845.1      CTGGCTGGGCTCCAGGAGAATCTTTCCACAAGGCAAGAGATAACACAAAGTCAAGGTGAA    660
Control         CTGGCTGGGCTCCAGGAGAATCTTTCCACAAGGCAAGAGATAACACAAAGTCAAGGTGAA    171
Primary         CTGGCTGGGCTCCAGGAGAATCTTTCCACAAGGCAAGAGATAACACAAAGTCAAGGTGAA    171
Secondary       CTGGCTGGGCTCCAGGAGAATCTTTCCACAAGGCAAGAGATAACACAAAGTCAAGGTGAA    171
                ************************************************************

EU332845.1      GATCAGGGTAGCCCTTTAAAAGGCCTCCTTGTGCCCTAGAGTTGCCACAGCTCTTCTACT    720
Control         GATCAGGGTAGCCCTTTAAAAGGCCTCCTTGTGCCCTAGAGTTGCCACAGCTCTTCTACT    231
Primary         GATCAGGGTAGCCCTTTAAAAGGCCTCCTTGTGCCCTAGAGTTGCCACAGCTCTTCTACT    231
Secondary       GATCAGGGTAGCCCTTTAAAAGGCCTCCTTGTGCCCTAGAGTTGCCACAGCTCTTCTACT    231
                ************************************************************

EU332845.1      CCACTGCTGTCTATCTTGCCTGCCGGCACCCAGCCACCATGTGGGAGCTCGTGGCTCTCT    780
Control         CCACTGCTGTCTATCTTGCCTGCCGGCACCCAGCCACCATGTGGGAGCTCGTGGCTCTCT    291
Primary         CCACCGCTGTCTATCTTGCCTGCCGGCACCCAGCCACCATGTGGGAGCTCGTGGCTCTCT    291
Secondary       CCACCGCTGTCTATCTTGCCTGCCGGCACCCAGCCACCATGTGGGAGCTCGTGGCTCTCT    291
                ************************************************************

EU332845.1      TGCTGCTTACCCTAGCTTATTTGTTTTGGCCCAAGAGAAGGTGCCCTGGTGCCAAGT       837
Control         TGCTGCTTACCCTAGCTTATTTGTTTTGGCCCAAGAGAAGGTGCCCTGGTGCCAAGT       348
Primary         TGCTGCTTACCCTAGCTTATTTGTTTTGGCCCAAGAGAAGGTGCCCTGGTGCCAAGT       348
Secondary       TGCTGCTTACCCTAGCTTATTTGTTTTGGCCCAAGAGAAGGTGCCCTGGTGCCAAGT       348
                ********************************************************
```

Figure (2) Comparison of CYP17 gene base sequences in control and patients and accession number (EU332845.1)

Distribution of the genotypes of the *CYP17* gene (-34 T>C) in patient and control samples.

The genotypes were detected by using Geneious Software (version 10.1.3). The sequences of standard and infertile samples of the first and second types were compared with the genotypes of women present in The World Gene Bank (Accession Number: EU322845), and the alignment of the sequences of women among them showed that three genotypes were found in the studied locus -34T> C of the (*CYP17*) gene, where the presence of one blue curve indicates the wild genotype (Wild). (CC) the absence of a mutation in both alleles, but the presence of two blue and red curves indicates the heterozygous genotype (TC) a mutation in one of the alleles, whether the base above the curve changes to C or not, and the presence of one black curve indicates a change The base above the curve to C indicates the mutated (homozygous) genotype, that is, a change in both alleles (TT) Fig. (3).

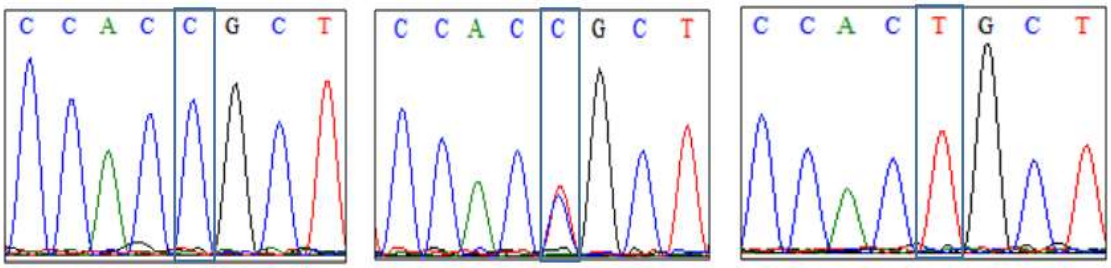

Figure (3) Genotypes of the C-34 T>C sit of the *CYP17* gene

Table (1) shows the numbers and percentages of genotypes in samples of patients and control, as it was noted that the percentage of homozygous genotype (wild) TT in patients and control is unequal, reaching (50%) in patients and (100%) in control, and for the heterozygous genotype TC, it was found that its percentage was (37.5%) in patients, while it was observed that it was completely absent in the control samples, while the homozygous (mutant) CC genotype was (12.5%) In patients, in the control samples, its complete absence was observed. Regarding the frequency of the T allele in patients and control samples, it was (68.7%) and (1.0), respectively. As for the frequency of the C allele, it was (31.3%) in patients and there was no repeat in the control (Fig 4).

Table (1): Genotypes percentage of the CYP17 gene polymorphism among patients and control group.

Genotype	Patients (%)	Control (%)	OR	(95%CI)	*P* value
TT	4 (50)	4 (100)	0.11	0.005-2.727	0.18 NS
P.F	0.89				
TC	3 (37.5)	-	5.73	0.230-142.550	0.29 NS
E.F	0.375				
CC	1 (12.5)	-	1.80	0.059-54.333	0.34 NS
E.F	0.125				

OR= Odds ratio ،CI = Confidence Intervals, P.F = Preventive fraction, E. F= Etiological fraction

NS= Not significant at the P<0.05 probability level

Allelic frequency of *CYP17* gene in control

Allelic frequency of *CYP17* gene in Patients

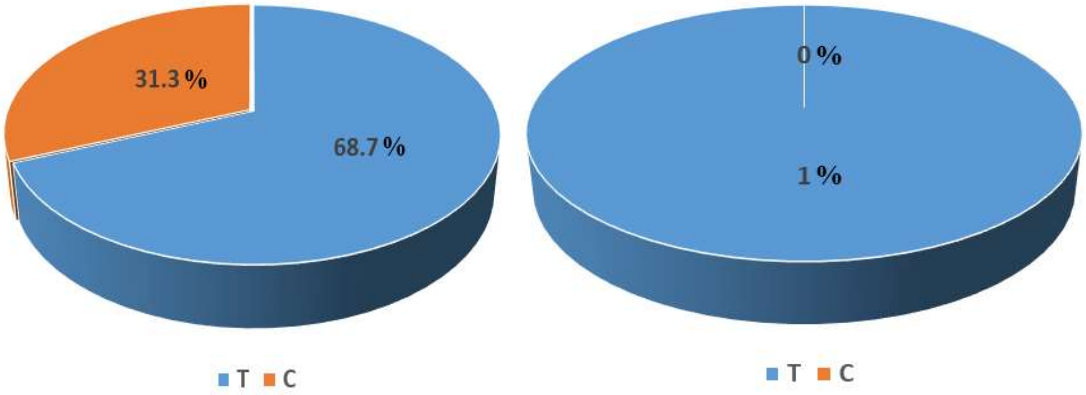

Figure (4) Repeats of the T and C alleles of the *CYP17* gene in a sample of patients and control Women

The results of the *CYP17* gene show a non-significantly difference between the expected and observed for the control group, with a *P*=0.7189 value at its P<0.05 probability level, according to the Hardy-Weinberg equilibrium law. As shown in Table 2, a Hardy-Weinberg distribution applies to the control group in this situation.

Table (2): CYP17 gene structures, their observed and expected numbers, and the Hardy-Weinberg equilibrium value in patients and control women**.**

Gene	Genotype	Observed number	Expected number	Hardy-Weinberg equilibrium (%)	*P* value
CYP17	**TT**	4	3.78	47.27	0.7189
	TC	3	3.44	42.97	
	CC	1	0.78	9.77	

Consanguineous marriage is common in these countries, which accounts for the deviation from the Hardy-Weinberg equilibrium in the control sample. This possibility exists in our society very probable. 7 The small size of the control sample is another factor that could cause a departure from the Hardy-Weinberg equilibrium because this equilibrium is extremely sensitive to the low frequencies of alleles in homozygous individuals. Studies have found a substantial link between the SNP -34T/C in CYP17A1 and PCOS, that has been corroborated by researchers working with people of different racial and ethnic backgrounds, including Indians (14). Additionally, both males and females have reported using the *CYP17A1* allele -34 as a useful biomarker to diagnose breast cancer [16, 17, 18]. Additionally, it is most likely a biomarker linked to male prostate cancer [19]. The abnormal activity of the *CYP17A1* gene may have an impact on the sequential reactions of the steroidogenesis pathways, which, along with other defective genes in the pathway, may contribute to infertility in PCOS women, despite the fact that these studies seem to indicate that *CYP17A1* is

not the susceptibility gene that directly causes infertility. The several genetic variations connected to infertility in women.

Funding

No specific grant was given to this article by funding organizations in the public, private, or nonprofit sectors.

Authors' declaration:

The authors declared have no conflicts of interest to disclose.

ORCID:

Salah H. Faraj: https://orcid.org/0000-0001-9794-7396

Ethical approval

The ethical guidelines of the institutional and national research committees, the 1964 Helsinki Declaration, and its later amendments, or comparable ethical norms, were followed in all procedures carried out in studies involving human participants. All patients signed a consent form prior to the study.

References

1.Deyhoul, N., Mohamaddoost, T., & Hosseini, M. (2017). Infertility-related risk factors: a systematic review. Int J Womens Health Reprod Sci, 5(1), 24-29.

2.Garolla, A., Pizzol, D., Carosso, A. R., Borini, A., Ubaldi, F. M., Calogero, A. E., ... & Foresta, C. (2021). Practical clinical and diagnostic pathway for the investigation of the infertile couple. Frontiers in Endocrinology, 1032.

3.Pei, J., Kinch, L. N., Otwinowski, Z., & Grishin, N. V. (2020). Mutation severity spectrum of rare alleles in the human genome is predictive of disease type. PLoS computational biology, 16(5), e1007775.

4.Estrada, D. F., Laurence, J. S., & Scott, E. E. (2016). Cytochrome P450 17A1 interactions with the FMN domain of its reductase as characterized by NMR. Journal of Biological Chemistry, 291(8), 3990-4003.

5.Petrunak EM, DeVore NM, Porubsky PR, Scott EE (November 2014). "Structures of human steroidogenic cytochrome P450 17A1 with substrates". The Journal of Biological Chemistry. 289 (47): 32952–64. doi:10.1074/jbc.M114.610998. PMC 4239641. PMID 25301938

6.Kardelen, A. D., Toksoy, G., Baş, F., Abalı, Z. Y., Gençay, G., Poyrazoğlu, Ş., ... & Darendeliler, F. (2018). A rare cause of congenital adrenal hyperplasia: clinical and genetic findings and follow-up characteristics of six patients with 17-hydroxylase deficiency including two novel mutations. Journal of Clinical Research in Pediatric Endocrinology, 10(3), 206.

7.Liu, X., Xu, M., Qian, M., & Yang, L. (2021). CYP17 T/C (rs74357) gene polymorphism contributes to polycystic ovary syndrome susceptibility: evidence from a meta-analysis. Endocrine Connections, 10(12), R305-R316.

8.Ashraf, S., Rasool, S. U. A., Nabi, M., Ganie, M. A., Jabeen, F., Rashid, F., & Amin, S. (2021). CYP17 gene polymorphic sequence variation is associated with hyperandrogenism in

Kashmiri women with polycystic ovarian syndrome. Gynecological Endocrinology, 37(3), 230-234.

9.Ali, R. M., Shkurat, T. P., Alexandrova, A. A., Bugrimova, E. S., Lomteva, S. V., & Ammar, M. N. (2022). Association of CYP17 gene polymorphism (rs743572) with polycystic ovary syndrome. Meta Gene, 31, 100996.

10.Wiweko, B. (2011). Relation between CYP17 polymorphism and hyperandrogenemia in polycystic ovarian syndrome. Indonesian Journal of Obstetrics and Gynecology.

11.Munawar Lone, N., Babar, S., Sultan, S., Malik, S., Nazeer, K., & Riaz, S. (2021). Association of the CYP17 and CYP19 gene polymorphisms in women with polycystic ovary syndrome from Punjab, Pakistan. Gynecological Endocrinology, 37(5), 456-461.

12.Carey, A. H., Waterworth, D., Patel, K., White, D., Little, J., Novelli, P., ... & Williamson, R. (1994). Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17. Human molecular genetics, 3(10), 1873-1876.

13.Antognelli, C., Del Buono, C., Ludovini, V., Gori, S., Talesa, V. N., Crinò, L., ... & Rulli, A. (2009). CYP17, GSTP1, PON1 and GLO1gene polymorphisms as risk factors for breast cancer: An Italian case-control study. BMC cancer, 9(1), 1-14.

14.Kaur, S., Ali, A., Ahmad, U., Siahbalaei, Y., Pandey, A. K., & Singh, B. (2019). Role of single nucleotide polymorphisms (SNPs) in common migraine. The Egyptian Journal of Neurology, Psychiatry and Neurosurgery, 55(1), 1-7.

15.Mohammed MB, AL-Awadi SJ, Omran MA. Association between polycystic ovary syndrome and genetic polymorphisms of CYP 17 gene in Iraqi women. Iraqi J Biotechnol. 2015;14(2):99–110.

16. Bardia, A.; Gucalp, A.; Dacosta, N.; Gabrail, N.; Danso, M.; Ali, H.; Blackwell, K.L.; Carey, L.A.; Eisner, J.R.; Baskin-Bey, E.S.; et al. Phase 1 study of seviteronel, a selective CYP17 lyase and androgen receptor inhibitor, in women with estrogen receptor-positive or triple-negative breast cancer. Breast Cancer Res. Treat. 2018, 171, 111–120.

17. Sun, J.; Zhang, H.; Gao, M.; Tang, Z.; Guo, D.; Zhang, X.;Wang, Z.; Li, R.; Liu, Y.; Sun,W.; et al. Association between CYP17 T-34C rs743572 and breast cancer risk. Oncotarget 2017, 9, 4200–4213.

18. Ferzoco, R.M.; Ruddy, K.J. The Epidemiology of Male Breast Cancer. Curr. Oncol. Rep. 2015, 18, 1–6. [CrossRef]

19. Effah, C.Y.;Wang, L.; Agboyibor, C.; Drokow, E.K.; Yu, S.;Wang,W.; Wu, Y. Polymorphism in the Androgen Biosynthesis Gene (CYP17), a Risk for Prostate Cancer: A Meta-Analysis. Am. J. Men's Health 2020, 14, 1557988320959984.