CYP17 MspA1 Gene Polymorphism and Breast Cancer Patients According to Age of Onset in Cancer Institute of Iran

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Abstract

Background: Exposure to endogenous hormones such as estrogen is known as a lifetime Breast Cancer (BC) risk factor. Polymorphisms in genes that are involved in the steroidogenic process, such as Cytochrome P450c17alpha (*CYP17*), affect individuals' susceptibility to BC. In Iran, the highest incident of BC is among young women. This study aimed to find prevalence of Single Nucleotide Polymorphisms (SNPs) in genes such as *CYP17* and significant correlation with age-oriented group of breast cancer.

Methods: In 2016, a case series study was conducted on a total population of 205 patients suffering from breast cancer referred to Cancer Institute, Imam Khomeini Hospital Complex, Tehran, Iran. This population consisted of 104 cases less than 40 yr old and 101 cases over 40. The genotype variants of *CYP17 MspA1* were determined using PCR, followed by RFLP. The association of *CYP17 MspA1* polymorphisms with the risk of BC in two different age groups was evaluated by calculating odds ratio and 95% confidence intervals using unconditional logistic regression.

Results: Carriers of at least one A2 allele may have higher risk of developing breast cancer at younger age compared to patients with A1/A1 genotype (Odds Ratio: 1.99, 95% Confidence Interval: 1.11-3.57, *P*=0.02).

Conclusion: *CYP17* gene polymorphisms may have influence on the early onset of breast cancer.

Keywords: Breast cancer, *CYP17* gene, Early onset breast cancer, Estrogen, Late-onset breast cancer

Introduction

Breast Cancer (BC) is the most prevalent malignancy and is known as the second leading cause of cancer death among women all around the world (1). In Iran, it has the highest cancer rate among women and accounts for 24.4% of all cancers affecting women (2, 3).

Mixture of genetic susceptibility and environmental exposure are widely accepted as causes of breast cancer. Over the last decades, several different factors such as early menarche, late menopause, post menopause obesity, hormone replacement therapy (HRT), and drinking alcohol

have been identified and are strongly linked to increased risk of breast cancer (1, 4, 5).

As an environmental risk factor, exposure to endogenous hormones such as estrogen is known as the lifetime BC risk factor. Although the exact mechanism is not well understood, however, there are two proposed hypotheses. The first one is based on the intrinsic characteristic of the estrogen, as it is a cell proliferation driver. Increase in the number of cells can lead to increase in the number of errors during DNA replication and ultimately increase in the incident of random mu-

tations. The second hypothesis explains that estrogen can be metabolized into quinone derivatives that are involved in DNA depurination which can make DNA prone to more errors and generate carcinogenic mutations (6, 7).

In addition, factors which have influence on the activity and/or metabolism of steroid hormones are of high importance since they potentially affect the incidence of breast cancer. A good example of this is the cytochrome P450c17alpha (*CYP17*) gene. The location of this gene is on chromosome 10 at q24.3 (8). It encodes steroid 17-alpha-hydroxylase, also known as steroid 17-alpha-monooxygenase. It performs both 17-alpha-hydroxylase and 17, 20-lyase activity. Functions of this enzyme allow the adrenal glands and gonads to synthesize both 17-alpha-hydroxylated glucocorticoids (via 17-alpha-hydroxylase activity) and sex steroids (via 17, 20-lyase activity) (9-12).

The level of endogenous estrogen varies in different people, which is the result of polymorphism in the steroidogenic genes that *CYP17* is one of them (13, 14). Currently, three important polymorphisms have been identified in *CYP17* gene. One of which is a single base polymorphism (SNP) T-> C (rs743572) in the promoter region of *CYP17* gene. This specific SNP can create an additional SP1 binding site (CCACC box) which in turn may enhance the gene expression that ultimately may lead to elevated level of estrogen (15, 16).

In Iran, highest incident of BC is among women aged between 40 to 49 yr old. The early age breast cancer is associated with worse prognosis, rapid disease progression and poorer response to treatment necessitates early screening tests and treatments base on the age of the patients (2).

Altogether, an unopposed prolonged lifetime exposure of estrogen enhances the risk of breast cancer (17, 18). Studying the prevalence of SNPs in genes such as *CYP17* shows a significant correlation with age oriented group of breast cancer patients that suggests a screening marker for risk group can be developed.

This study aimed to find prevalence of Single Nucleotide Polymorphisms (SNPs) in genes such as *CYP17* and significant correlation with age-oriented group of breast cancer.

Materials and Methods

Study population

In this case series project conducted in 2016, 205 individuals suffering from breast cancer referred to Imam Khomeini Hospital (Tehran, Iran) were studied. All the subjects included in this study gave their informed consent.

The Ethical Committee of the Tehran University of Medical Sciences approved this study.

Patients were categorized according to the age of onset into two groups of younger onset (<40 yr) and elder onset (≥40 yr). All the patients' breast cancers were confirmed based on their positive histopathology results. A validating questionnaire was used to collect demographic information including age at time of diagnosis, marital and occupational status, educational level, hometown and residence and clinical information such as tumor characteristics, tumor location, type of breast cancer, age at menarche (under 12, 12 yr old or more) and family history of cancer (first to third degree relatives with history of cancer).

Blood Collection and DNA Extraction

Five milliliters of peripheral blood were collected from each patient and later used for DNA isolation. Genomic DNA extraction was performed using Gentra Puregene Blood Kit (Qiagen).

Genotyping

Genotyping of patients was determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis (RFLP). Briefly, 50 ng of genomic DNA was used in PCR reaction containing 50 pmol of forwarding primer as 5`-GGCTCCAGGAGAATCTTTC-*3*` and reverse primer as *5*`-GGGCCAAAACAAATAAGCTA-*3*` (19), 100umol of dNTP, 1× Taq buffer and 1 unit of Taq polymerase in total volume of $25 \mu l$. The PCR procedure was performed with initiation temperature of 94 °C for 5 min followed by

30 cycles of 94 °C for 1 min for denaturing, 57 °C for 1 min for annealing and 72 °C for 1 min for elongation time. The final cycle was followed by 72 °C for 5 min for extension. PCR products were digested with MspAI restriction enzyme (CutSmart, New England BioLab, USA) at 37 °C for 15 min for investigating the presence of A1 (not digested) and A2 (T->C with MspAI digestion recognition site) genotypes. Digested fragments were stained with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology) and further analyzed on 3% agarose gel. Digestion of the mutant genotype creates 86 and 123 bp prod-

ucts, while an uncut product indicate the wild type genotype of 209 bp.

Statistical Analysis

Data were registered into excel file and transferred to SPSS Ver. 16.0 (Chicago, IL, USA) for analysis. Student *t*-test was used for statistical analysis (*P*-value). The risk of breast cancer in either age subgroups of population was estimated by calculating Odds Ratio (*OR*). The X^2 test was done to evaluate the consistency of the frequency of *CYP17* genotypes variants with Hardy-Weinberg equilibrium.

Table 1: Demographic and disease condition data of 2 age subgroups of breast cancer patients

Variables	Under 40 Total=104 n (%)	Over 40 Total=101 n (%)
Gender		
Female	104 (100)	100 (99)
Male	0 (0)	1(1)
Marital status		
Single	15 (14)	4 (4)
Married	88 (85)	94 (93)
Missing	1 (1)	3 (3)
Occupational level		
Housewife	75 (72)	76 (75)
Working	24 (23)	24 (24)
Missing	5 (5)	1 (1)
Educational status		
Educated	91 (87)	78 (77)
Uneducated	5 (5)	17 (17)
Missing	8 (8)	6 (6)
Hometown		
Tehran	32 (31)	47 (46%)
Other cities	72 (69)	54 (53%)
Residence		
Tehran	51(49)	77 (76)
Other cities	53(51)	24 (24)
Type of BC		
Invasive ductal carcinoma	59 (57)	59 (58)
Other types	19 (18)	23 (23)
Missing	26 (25)	19 (19)
Breast Involvement		
Unilateral	100 (96)	91 (90)
Bilateral	4 (4)	9 (9)
Missing	0 (0)	1 (1)
Tumor Location (Laterality)		
Left side	48 (46)	53 (52)
Right side	48(46)	35 (35)
Both	4 (4)	11 (11)
Missing	4 (4)	2 (2)
Age at Menarche		
12 yr old or under	25 (24)	18 (18)
Over 12	51 (49)	53 (52)
Missing	28 (27)	30 (30)

Results

Out of 205 patients participated in this study, 104 cases (50.7%) were younger than 40 yr old and 101 cases (49.3%) were older than 40.

Age range in young onset group was between 19 to 39 yr old and the average was 33.7 (±4.6 standard deviation). In late onset group, the age range was between 40 and 82 and was average of 51.4 (±9 standard deviation). Only 1 case (1%) was male and belonged to late onset group.

Majority of patients were married, homemaker and educated in both young and late onset groups. Demographic data of 2 age subgroups of breast cancer patients has shown in Table 1.

In regards to menarche age, recorded data was available for 147 patients. The rest of the 58 patients were unable to recall their exact age at menarche (Table 1).

Concerning the family history of cancer, majority of patients in both subsets of population had no family history of cancer (Table 2).

Table 2: Family history of cancer of 205 patients included in this study

Family History of Cancer	Under 40 n (%)	Over 40 n (%)
First Degree relative		
1 person	16 (15)	22 (22)
2 persons	5 (5)	7 (7)
3 persons or more	0 (0)	4 (4)
No History	83 (80)	68 (67)
Missing	0 (0)	0 (0)
Second Degree relative		
1 person	19 (18)	17 (17)
2 persons	6 (6)	12 (12)
3 persons or more	3 (3)	1 (1)
No History	75 (72)	71 (70)
Missing	1 (1)	0 (0)
Third Degree relative		
1 person	18 (17)	18 (18)
2 persons	4 (4)	2 (2)
3 persons or more	1 (1)	1 (1)
No History	81 (78)	80 (79)
Missing	0 (0)	0 (0)

In case of genotype variation in the whole population, 72 cases (35.1%) were A1/A1 (209 bp), 104 cases (50.7%) were A1/A2 (209, 123 and 86 bp) and 29 cases (14.1%) were A2/A2 (123 and 86 bp) (Fig. 1). The frequency of A1 and A2 alleles in the total population was 60.48% and 39.51%, respectively. The frequency of *CYP17* alleles in the total population did not deviate from Hardy-Weinberg equilibrium (X^2=0.77, P=0.37).
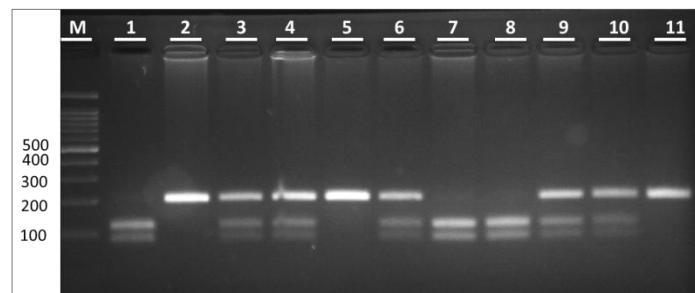

Fig. 1: *CYP17 MspA1* digested products separated on 3% agarose gel electrophoresis
Lane1 100bp ladder; Lane 2, 5 & 11 Homozygous A1 genotype (A1/A1); Lane 1, 3, 4, 6, 9 & 10 Heterozygous genotype (A1/A2); Lane 7 & 8 Homozygous A2 genotype (A2/A2).

Among the population of patients younger than 40 yr 27.8% had A1/A1, 55.7% had A1/A2, and 16.3% had A2/A2 genotype respectively (X^2= 1.76, P=0.18) while it was 42.5%, 45.4% and 11.8% (X^2=0.003, P=0.95) for elder group. No significant association was found between *CYP17* genotype variants and age of the patients. However, a significant correlation was found between the presence of at least one A2 allele and increased risk of breast cancer in patients age under 40 (P=0.02) (Table 3). In younger subset, the fre-quency of A1 and A2 allele was 55.76% and 44.23%, respectively while this was 65.34% and 34.65% for elder subset in that order.

Comparing two subsets of groups in regards to laterality, type of BC and family history of cancer no significant association was found (Table 4).

Regarding the menarche age, majority of young patients with at least one A2 allele had their first menstrual cycle at age 12 and this was statistically significant (P=0.04, OR: 0.35, 95% Confidence Interval: 0.12-0.98) (Table 4).

Table 3: Distribution of *CYP17 MSPA1* polymorphism in 2 subgroups of population

Variables	Under 40 n (%)	Over 40 n (%)	OR	95%CI	*P*
Genotypes					
Homozygous A1/A1	29 (28)	43 (43)			0.88
Heterozygous A1/A2	58 (56)	46 (46)	2.101	0.875-5.046	0.97
Homozygous A2/A2	17 (16)	12 (12)	1.124	0.488-2.587	0.784
Combined polymorph A1/A2 + A2/A2	**75(72)**	**58 (57)**	**1.996**	**1.116-3.572**	**0.02**

Table 4: Association between *CYP17 MspA1* polymorphism and potential BC risk factors such as laterality, type of breast cancer, family history of cancer and menarche age

Variables	Patients Under 40					Patients over 40				
	Combined genotype A1/A2 & A2/A2 n (%)	Genotype A1/A1 n (%)	OR	95%CI	*P* value	Combined genotype A1/A2 & A2/A2 n (%)	Genotype A1/A1 n (%)	OR	95%CI	*P* value
Unilateral	71 (71)	29 (29)	0	0	0.998	51 (56)	40 (44)	0.348	0.069-	0.203
Bilateral	4 (100)	0 (0)				7 (77.8)	2 (22.2)		1.769	
Invasive ductal carcinoma	47 (79.7)	12 (20.3)	2.848	0.939-	0.064	30 (50.8)	29 (49.2)	0.796	0.302-	0.644
Other types	8 (42.1)	11 (57.9)		8.640		14 (60.9)	9 (39.1)		2.098	
Family history of cancer	37 (69.8)	16 (30.2)	0.791	0.335-	0.594	38 (63.3)	22 (36.7)	2	0.892-	0.093
No family history of cancer	38 (74.5)	13 (25.5)		1.871		20 (48.8)	21 (51.2)		4.486	
Menarche age under 12	14 (56)	11 (44)	0.35	0.124-	**0.047**	9 (50)	9 (50)	0.963	0.330-	0.945
Menarche age 12 or over	40 (78.4)	11 (21.6)		0.984		28 (52.8)	25 (47.2)		2.806	

Discussion

Endogenous exposure to circulating steroid hormones is known as a risk factor in developing breast cancer. There are different factors that have influence on the exposure to endogenous hormones and may affect susceptibility of the individuals to breast cancer. Polymorphisms in genes that are involved in the steroidogenic process, such as *CYP17*, are thought to be as one of these factors. Up to now, several studies have investigated the correlation of *CYP17 MspA1* polymorphism and risk of breast cancer but there is still a controversy in the results (20). In this case-series study, we demonstrated that carriers of at least one A2 allele might have higher risk of developing breast cancer at younger age compared to patients with A1/A1 genotype.

The frequencies of 34%, 52%, and 14% were reported for A1/A1, A1/A2, and A2/A2, respectively, in patients with polish origin (21). A prevalence of 41.5% for A1/A1, 47.4% was reported for A1/A2 and 11.1% for A2/A2 genotypes in Finnish population which were in accordance with our findings (22). Similar frequencies were also reported for different variants of *CYP17 MspA1* polymorphism (23).

Genotypes other than A1/A1 which may have more activity for *CYP17* enzyme for estrogen metabolism (A1/A2, A2/A2) were more frequent among younger breast cancer patients compared to older ones and this was statistically significant (*P*=0.02). Carriers of at least one A2 allele may have higher risk of developing breast cancer at younger age compared to patients with A1/A1 genotype (*OR:* 1.99, *95% CI:* 1.11-3.57). In 1996, the first research group brought up the association of A2 allele and increased risk of breast cancer (18). Higher risk of breast cancer was reported among younger patients who were Hetero- and Homozygote for A2 allele and our results are in line with their findings (19). Statistically significant association between A2 allele and early age breast cancer were reported (24). On the other hand, two meta-analyses, showed lack of correlation between *CYP17* polymorphisms and overall risk of breast cancer (20, 25-28). A reason that can be brought for the existing controversy is that different studies used different subgroups of populations defined by ethnicity, age (24, 29, 30), tumor stage/aggressiveness and menopausal status which may vary from one study to the other (31, 32). A second reason could be due to this fact that early onset of breast cancer is different with late onset in terms of pathological characteristics, diagnosis, and biological origin. This could explain the difference between 2 subgroups of population and controversy in different studies (19).

The relationship between estrogen exposure and risk of breast cancer were evaluated. Early menarche and late menopause, which means longer exposure to estrogen, increase the risk of breast cancer; however, in the present study, a significant relationship was found between young patients harboring A2 allele and late menarche. Several other studies including a meta-analysis also failed to show any association between *CYP17 MspA1* polymorphism and early menarche (33, 34). Twin and Family studies have shown that genetic factors account for 53-72% of menarche age variation (34). Thus, *CYP17* polymorphism may not be the only effect modifier in this regards and other genes may contribute to this trait variation.

Analyzing the *CYP17 MspA1* polymorphism in two subsets of population did not show any association with family history of cancer. Other genes are participating in steroidogenic pathways that may have greater roles in familial cases.

Conclusion

Polymorphism in *CYP17* is possibly age specific and in addition to other risk factors may have influence on increased risk of breast cancer in early age. *CYP17 MspA1* polymorphism can be used as a potential genetic marker for identification of high-risk groups. However, a larger population is needed to confirm the major influence of *CYP17 MspA1* variants on early onset breast cancer among Iranian women.

Ethical Consideration

Ethical issues (including plagiarism, obtaining informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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References

1. Antognelli C, Del Buono C, Ludovini V, Gori S, Talesa VN, Crinò L, Barberini F, Rulli A (2009). CYP17, GSTP1, PON1 and GLO1

gene polymorphisms as risk factors for breast cancer: an Italian case-control study. *BMC Cancer,* 20;9:115.

2. Babu GR, Samari G, Cohen SP, Mahapatra T, Wahbe RM, Mermash S, Galal OM (2011). Breast cancer screening among females in Iran and recommendations for improved practice: a review. *Asian Pac J Cancer Prev,* 12:1647-1655.

3. Harirchi I, Kolahdoozan S, Karbakhsh M, Chegini N, Mohseni S, Montazeri A, Momtahen A, Kashefi A, Ebrahimi M (2011). Twenty years of breast cancer in Iran: downstaging without a formal screening program. *Ann Oncol,* 22:93-97.

4. Yager JD, Davidson NE (2006). Estrogen carcinogenesis in breast cancer. *N Engl J Med,* 354:270-282.

5. Dumitrescu R, Cotarla I (2005). Understanding breast cancer risk-where do we stand in 2005? *J Cell Mol Med,* 9:208-221.

6. Yue W, Santen R, Wang J-P, Li Y, Verderame M, Bocchinfuso W, Korach K, Devanesan P, Todorovic R, Rogan E (2003). Genotoxic metabolites of estradiol in breast: potential mechanism of estradiol induced carcinogenesis. *J Steroid Biochem Mol Biol,* 86:477-486.

7. De Bont R, Van Larebeke N (2004). Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis,* 19:169-185.

8. Yoshimoto FK, Auchus RJ (2015). The diverse chemistry of cytochrome P450 17A1 (P450c17, CYP17A1). *J Steroid Biochem Mol Biol,* 151:52-65.

9. Reid AH, Attard G, Barrie E, de Bono JS (2008). CYP17 inhibition as a hormonal strategy for prostate cancer. *Nat Clin Pract Urol,* 5:610-620.

10. van den Akker EL, Koper JW, Boehmer AL, Themmen AP, Verhoef-Post M, Timmerman MA, Otten BJ, Drop SL, De Jong FH (2002). Differential inhibition of 17α-hydroxylase and 17, 20-lyase activities by three novel missense CYP17 mutations identified in patients with P450c17 deficiency. *J Clin Endocrinol Metab,* 87:5714-5721.

11. Blackburn HL, Ellsworth DL, Shriver CD, Ellsworth RE (2015). Role of cytochrome P450 genes in breast cancer etiology and treatment: effects on estrogen biosynthesis, metabolism, and response to endocrine therapy. *Cancer Causes Control,* 26:319-332.

12. Setiawan VW, Schumacher FR, Haiman CA et al (2007). CYP17 genetic variation and risk of breast and prostate cancer from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3). *Cancer Epidemiol Biomarkers Prev,* 16:2237-2246.

13. Rannala B (2001). Finding genes influencing susceptibility to complex diseases in the post-genome era. *Am J Pharmacogenomics,* 1:203-221.

14. Sharp L, Cardy AH, Cotton SC, Little J (2004). CYP17 gene polymorphisms: prevalence and associations with hormone levels and related factors. a HuGE review. *Am J Epidemiol,* 160:729-740.

15. Ye Z, Parry JM (2002). The CYP17 MspA1 polymorphism and breast cancer risk: a meta-analysis. *Mutagenesis,* 17:119-126.

16. Rai R, Sharma KL, Misra S, Kumar A, Mittal B (2014). CYP17 polymorphism (rs743572) is associated with increased risk of gallbladder cancer in tobacco users. *Tumour Biol,* 35:6531-6537.

17. Mense SM, Remotti F, Bhan A, Singh B, El-Tamer M, Hei TK, Bhat HK (2008). Estrogen-induced breast cancer: alterations in breast morphology and oxidative stress as a function of estrogen exposure. *Toxicol Appl Pharmacol,* 232:78-85.

18. Feigelson HS, Coetzee GA, Kolonel LN, Ross RK, Henderson BE (1997). A polymorphism in the CYP17 gene increases the risk of breast cancer. *Cancer Res,* 57:1063-1065.

19. Bergman-Jungeström M, Gentile M, Lundin AC, Wingren S (1999). Association between CYP17 gene polymorphism and risk of breast cancer in young women. *Int J Cancer,* 84:350-353.

20. Mao C, Wang X-W, He B-F, Qiu L-X, Liao R-Y, Luo R-C, Chen Q (2010). Lack of association between CYP17 MspA1 polymorphism and breast cancer risk: a meta-analysis of 22,090 cases and 28,498 controls. *Breast Cancer Res Treat,* 122:259-265.

21. Jakubowska A, Gronwald J, Menkiszak J, Górski B, Huzarski T, Byrski T, Tołoczko-Grabarek A, Gilbert M, Edler L, Zapatka M (2010). BRCA1-associated breast and ovarian cancer risks in Poland: no association with

commonly studied polymorphisms. *Breast Cancer Res Treat,* 119:201-211.

22. Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma V-M, Benhamou S, Vainio H, Uusitupa M, Hirvonen A (2000). Steroid metabolism gene CYP17 polymorphism and the development of breast cancer. *Cancer Epidemiol Biomarkers Prev,* 9:1343-1348.

23. Kristensen VN, Haraldsen EK, Anderson KB, Lønning P, Erikstein B, Kåresen R, Gabrielsen OS, Børresen-Dale A-L (1999). CYP17 and Breast Cancer Risk The Polymorphism in the 5' Flanking Area of the Gene Does Not Influence Binding to Sp-1. *Cancer Res,* 59:2825-2828.

24. Chakraborty A, Murthy N, Chintamani C, Bhatnagar D, Mohil R, Sharma P, Saxena S (2007). CYP17 gene polymorphism and its association with high-risk north Indian breast cancer patients. *J Hum Genet,* 52:159-165.

25. Cristina Tote Franco Pinotti S, da Silva IDCG, Carvalho CV, Nazário ACP (2012). MspA1 polymorphism of the CYP17 gene in breast cysts. *Gynecol Endocrinol,* 28:443-446.

26. Chang J-H, Gertig DM, Chen X, Dite GS, Jenkins MA, Milne RL, Southey MC, McCredie M, Giles GG, Chenevix-Trench G (2005). CYP17 genetic polymorphism, breast cancer, and breast cancer risk factors: Australian Breast Cancer Family Study. *Breast Cancer Res,* 7:R513-R521.

27. Santos SS, Jácome GPO, Koifman R, Koifman S (2014). CYP17, CYP19, and NQO1 Genetic Polymorphisms and Breast Cancer Susceptibility in Young Women in Brazil. *Br J Med Med Res,* 4:68.

28. Yao L, Fang F, Wu Q, Yang Z, Zhong Y, Yu L (2009). No association between CYP17 T-34C polymorphism and breast cancer risk: a meta-analysis involving 58,814 subjects. *Breast Cancer Res Treat,* 122:221-227.

29. Spurdle AB, Hopper JL, Dite GS, Chen X, Cui J, McCredie MR, Giles GG, Southey MC, Venter DJ, Easton DF (2000). CYP17 promoter polymorphism and breast cancer in Australian women under age forty years. *J Natl Cancer Inst,* 92:1674-1681.

30. Shin MH1, Lee KM, Yang JH, Nam SJ, Kim JW, Yoo KY, Park SK, Noh DY, Ahn SH, Kim B, Kang D (2005). Genetic polymorphism of CYP17 and breast cancer risk in Korean women. *Exp Mol Med,* 37:11-17.

31. TÜZÜNER BM, Öztürk T, Kisakesen HI, Ilvan Ş, Zerrin C, Öztürk O, Isbir T (2010). CYP17 (T-34C) and CYP19 (Trp39Arg) polymorphisms and their cooperative effects on breast cancer susceptibility. *In Vivo,* 24:71-74.

32. Verla-Tebit E, Wang-Gohrke S, Chang-Claude J (2005). CYP17 5'-UTR MspA1 polymorphism and the risk of premenopausal breast cancer in a German population-based case-control study. *Breast Cancer Res,* 7:R455-R464.

33. Dvornyk V1, Waqar-ul-Haq (2012). Genetics of age at menarche: a systematic review. *Hum Reprod Update,* 18:198-210.

34. Pei Y-F, Zhang L, Deng H-W, Dvornyk V (2008). CYP17 MspA1 polymorphism and age at menarche: a meta-analysis. *Dis Markers,* 25:87-95.