DOI : 10.35124/bca.2019.19.2.4435

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 4435-4439, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

INVOLVEMENT OF CYP17 GENE POLYMORPHISM WITH BENIGN PROSTATIC HYPERPLASIA IN BABYLON PROVINCE

Nibras Yahya Hussein, Mufeed J. Ewadh and Abdulrazzaq Salman

Department of Biochemistry, College of Medicine, Babylon University, Iraq.
e-mail: ghufranasal@yahoo.com

(Received 21 February 2019, Revised 12 May 2019, Accepted 29 May 2019)

ABSTRACT : The present study was designed to evaluate the frequency and association of CYP17 gene (-34 T↔C) SNP with benign prostatic hyperplasia (BPH) in Babylon province. To achieve this aim, 146 patients with BPH and 102 apparently healthy control group were subjected to the study. DNA was extracted from whole blood for all samples. Genotyping of CYP17 gene (-34 T↔C) SNP were carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results indicated that the homozygous genotype (CC) of CYP17 gene (-34 T↔C) SNP was found to be significantly increase the risk of BPH by two folds with respect to those of the wild genotype (TT) of CYP17 gene (-34 T↔C) SNP. The heterozygous genotype (TC) of CYP17 gene (-34 T↔C) SNP was found to be none significantly increase the risk of BPH with respect to those of the wild genotype (CC) of CYP17 gene (-34 T↔C) SNP. The minor allele (C) of CYP17 gene (-34 T↔C) SNP was significantly higher in BPH patients when compared with that of the control group. In conclusion, the CYP17 gene (-34 T↔C) SNP is involved in the pathogenesis of BPH. The homozygous genotype (CC) was found to be significantly increase the risk of BPH by two folds with respect to those of the wild genotype (TT). The minor allele (C) of CYP17 gene (-34 T↔C) SNP was significantly higher in BPH patients when compared with that of the control group.

Key words : Benign prostatic hyperplasia, cytochrome 17, polymorphism.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is the nonmalignant enlargement of the prostate gland. It refers to hyperplasia of stromal and glandular epithelial that arises in the periurethral transition zone of the prostate that surrounds the urethra (Miller *et al*, 2009; Strandberg, 2000). The etiology of BPH is multifactorial and under endocrine control. Laboratory and clinical studies have identified two factors necessary for the BPH development, these factors are dihydrotestosterone and aging. Dihydrotestosterone is very essential for prostate growth. The serum testosterone levels decrease as a result of aging mainly due to decreased stimulation of Leydig cells and increased conversion of testosterone in the peripheral tissues to estrogens which induce androgen receptor. The aging prostate becomes more sensitive to androgens (Gann *et al*, 1996; Wilding, 1992).

Steroidal hormones, essentially androgen and estrogens, play an important role in the physiological growth and development of prostate gland, hence any genetic and environmental factors may play a role in regulating blood levels of circulating steroidal hormones. Thus, genes involved in the metabolic pathways of these hormones, if expressed differentially may alter the risk of development of BPH (Konwar *et al*, 2008; Habuchi *et al*, 2000). There is an evidence to support the proposition of hormonal etiology of BPH involving androgen action. Androgen is required for differentiation and growth of the prostate in utero and at puberty. Testosterone, the most abundant circulating androgen is synthesized from cholesterol by a series of enzymatic reactions involving several of the cytochrome P450 enzymes such as CYP17 (Neuhausen *et al*, 1990; Picado-Leonard *et al*, 1987).

The cytochrome 17 (CYP17) enzyme functions at key branch points in steroid hormone biosynthesis in the adrenal gland, ovary and gonads. The human CYP17 gene is located in chromosome 10 at position 10q24.3 and contains eight exons. It spans a length of 1527 bp. Transcription initiation site is mapped approximately 180 bp upstream of the initiation codon in exon 1 and ~1.7 kb mRNA is produced (Ranbir *et al*, 2009; Ying *et al*, 2005).

Cytochrome P-450 C17 mediates both steroid 17 - hydroxylase activity, which converts pregnenolone to dehydroepiandrosterone and 17,20-lyase activity, which generates androstenedione from progesterone, precursors of testosterone and estrogen (Ilic *et al*, 1996; Daviglus *et al*, 1996). These androgens may then be converted to

estrone, testosterone and estradiol. Testosterone is converted to dihydrotestosterone in the prostate by the enzyme 5 Q-reductase. Dihydrotestosterone binds to androgene receptor and the dihydrotestosterone-androgene receptor complex transstimulates a number of genes with androgene receptor responsive elements. These occasionseventuallyoutcome in cell division in the prostate (Galbraith *et al*, 1997; Brentano *et al*, 1990).

Aims of the study

To estimate the frequency and association of CYP17 gene (-34 T↔C) SNP with benign prostatic hyperplasia.

MATERIALS AND METHODS

Materials

Subjects

The study included two groups, the first group comprises patients with benign prostatic hyperplasia while the second group comprises control subjects. All subjects were collected from February 2018 till July 2018. The practical part of this study was done in the laboratory of Biochemistry Department in College of Medicine, Babylon University. The study was performed on 146 patients with BPH and 102 apparently healthy control group.Any subject suffered from problems such as, renal dysfunction, liver dysfunction, diabetes mellitus, malignancies, urinary tract infection, drug dependency such as glucocorticoid, alcohol drinking were excluded from the current study.

Blood sampling

Two milliliter of blood collected in EDTA (ethylene diamine tetra acetic acid) containing tube and the samples were kept frozen at -70^{0}C until analysis of CYP17 gene polymorphism.

Methods

Genotyping of CYP17 gene -34 T↔C SNP was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The DNA extracted from frozen blood by genomic DNA mini kit (Favorgen) (Vogelstein *et al*, 1979). The DNA was amplified by PCR. A 421bp DNA fragment containing the polymorphic site-34 T↔C SNP of CYP17 gene was amplified by using specific primers (forward primer 5'-CCA TTC GCA CTC TGG AGT CAT-3' and reverse primer 5'-GAC AGG AGG CTC TTG GGG TA-3') (Carey *et al*, 1994). The PCR products were digested with MspA1I restriction enzyme (Biolabs, New England). The wild genotype (TT) remains uncut (421bp) whereas the homozygous genotype (CC) is digested into 291 and 130bp fragments. The heterozygous genotype (TC) contained three bands sized 421, 291 and 130bp. The restriction digestion products were analyzed on 2% agarose gel electrophoresis.

Statistical analysis

The results of phenotypes data were expressed as mean ± SD. Student's t-test was used for the evaluation of data. Genotype data expressed as odds ratio (OR), confidence interval (CI) 95%. Statistical analyses were performed with SPSS (version 20). P-value less than 0.05 was considered to be statistically significant.

RESULTS

The demographic characteristics of patients with BPH and controls groups were shown in Table 1.

The genotypes distribution and frequency of CYP17 gene (-34 T↔C) SNP as shown in Table 2 and Fig. 1, respectively. The analysis of results indicated that the CYP17 gene (-34 T↔C) SNP genotype frequencies of wild genotype (TT), heterozygous (TC) and homozygous genotype (CC) were 36.3%, 37.7% and 26% in BPH patients and 45.1% 42.2% and 12.7% in controls group, respectively.

The homozygous genotype (CC) of CYP17 gene (-34 T↔C) SNP was found to be significantly increase (OR = 2.537, CI 95% 1.206-5.335, P = 0.013) the risk of BPH by two folds with respect to those of the wild genotype (TT) of CYP17 gene (-34 T↔C) SNP. The heterozygous genotype (TC) of CYP17 gene (-34 T↔C) SNP was found to be none significantly increase (OR = 1.110, CI 95% 0.633-1.946, P=0.715) the risk of BPH with respect to those of the wild genotype (CC) of CYP17 gene (-34 T↔C) SNP.

The allele distribution and frequency of CYP17 gene (-34 T↔C) SNP as shown in Table 3 and Fig. 2, respectively. The allele frequencies of T and C of CYP17 gene (-34 T↔C) SNP were found to be 55.1% and 44.9% in benign prostatic hyperplasia patients respectivelyand 66.2% and 33.8% in the control group, respectively. The minor allele frequencies (C) of CYP17 gene (-34 T↔C) SNP in BPH patients and control groups were found to be 44.9% and 33.8%, respectively. It was significantly higher (P<0.05) in BPH patients when compared with that of the control group.

DISCUSSION

Several genetic polymorphisms of CYP17 gene have been identified. One of the most common polymorphism of the CYP17 gene is (-34 T↔C) SNP. For CYP17 gene (-34 T↔C) SNP, the 5'-untranslated promoter region of CYP17 gene contains a single base pair thymidine (T) to cytosine (C) polymorphism that may create a new splitting site (CCACC box) at 34 bp upstream from the initiation

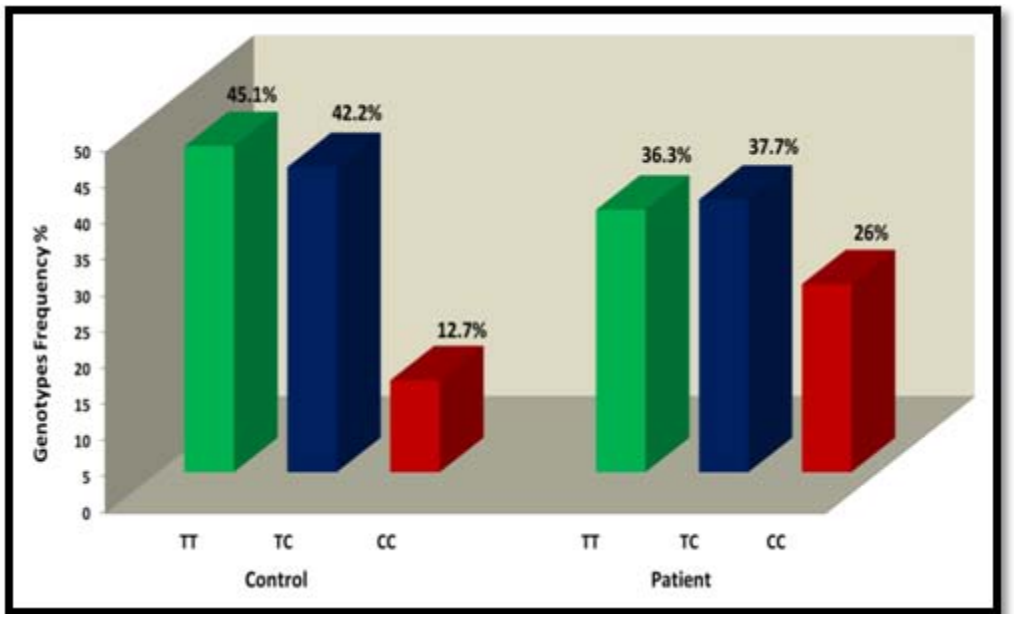

Fig. 1 : Genotypes frequency of CYP17 gene (-34 T↔C)SNP in patients with Benign Prostatic Hyperplasia and the controls groups. TT: Wild genotype, CC: Heterozygous and CC: Homozygous genotype.

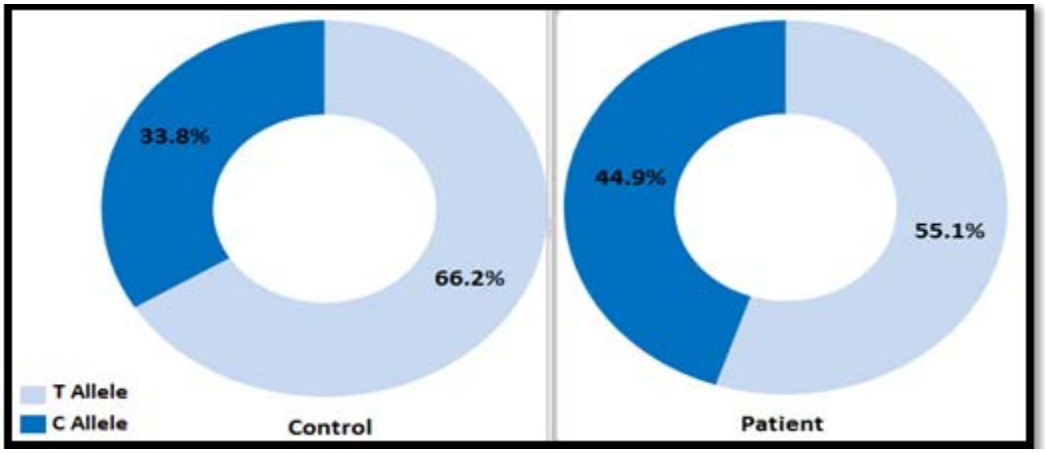

Fig. 2 : Alleles Frequency of CYP17 Gene (-34 T↔C)SNP in Patients with Benign Prostatic Hyperplasia and the Controls Groups.

of translation and downstream from the putative transcription start site, therefore providing an additional promoter activity with an increased rate of transcription of CYP17 mRNA, *i.e.* altering the expression level of CYP17 (Sharp *et al*, 2004; Tomonori *et al*, 2000; Ranbir *et al*, 2009).

The CYP17 gene is highly expressed in the human prostate gland and plays an important role in metabolism of steroidal hormones; hence CYP17 polymorphisms which cause changes in enzymatic activity may alter the metabolism of steroidal hormones, possibly altering BPH risk (Ellem *et al*, 2004). A study by Zmuda *et al* (2001) reported that men with the homozygous genotype (CC) of CYP17 gene (-34 T↔C) SNP had a higher bioavailable testosterone level than men with the wild genotype (TT) of CYP17 gene (-34 T↔C) SNP. Heterozygotes genotype (TC) of CYP17 gene (-34 T↔C) SNP had intermediate testosterone values. This connection is in line with the observation that a subset of BPH has a genetic transmission (Guoqi *et al*, 2013; Sanda *et al*, 1994).

It has been reported that the volume of BPH is positively correlated with serum testosterone and estradiol levels, therefore a distinct sex-steroid hormone caused by the CYP17 genotype will presumably contribute to the development of BPH (Partin *et al*, 1991; Greenwald *et al*, 1974). In this study, the homozygous genotype (CC) of CYP17 gene (-34 T↔C) SNP was found to be significantly increase the risk of BPH by two folds with respect to those of the wild genotype (TT) of CYP17 gene (-34 T↔C) SNP. The presence of (C) allele of CYP17 gene (-34 T↔C) SNP may create an additional promoter activity for CYP17 gene expression than (T) allele of CYP17 gene (-34 T↔C) SNP that lead to

Table 1 : Demographic characteristics of patients with benign prostatic hyperplasia and the controls groups.

Characteristics	Groups	Mean ± SD	Range	P value
No.	Control		102	
	Patient		146	
Age (y)	Control	54.93 ± 8.68	38-65	
	Patient	57.76 ± 6.83	40-69	> 0.05
Weight (kg)	Control	76.07 ± 13.53	49-104	
	Patient	86.78 ± 14.4	55-120	< 0.001
Height (m)	Control	1.68 ± 0.09	1.49-1.85	
	Patient	1.69 ± 0.08	1.51-1.83	> 0.05
BMI (kg/m²)	Control	26.79 ± 4.09	19.77-34.72	
	Patient	30.25 ± 3.42	19.66-38.07	< 0.001
Duration of disease	Patient	5.05 ± 2.43 year	1 month-12 year	-

Table 2 : Genotypes distribution of CYP17 gene (-34 T↔C)SNP in patients with Benign Prostatic Hyperplasia and the controls groups.

Genotype	Control	Patient	OR	95% CI	P-value
TT	46	53	Reference	Reference	Reference
TC	43	55	1.110	0.633-1.946	0.715
CC	13	38	2.537	1.206-5.335	0.014
Total	102	146			

TT : Wild genotype, CC: Heterozygous and CC: Homozygous genotype.

Table 3 : Alleles Distribution of CYP17 Gene (-34 T↔C) SNP in Patients with Benign Prostatic Hyperplasia and the Controls Groups.

Allele	Control	Patient	OR	95% CI	P-value
T	135	161	Reference	Reference	Reference
C	69	131	1.592	1.099-2.306	0.013
Total	204	292			

increase the rate of CYP17 gene transcription (mRNA). The increase of CYP17 mRNA cause increase in CYP17 enzyme synthesis (activity), which lead to increasing in androgen production and cell division in the prostate, thereby may increases the risk of BPH.

The current results are in consistence with the results of Lin-Lin *et al* (2017), Ananthan *et al* (2016) and Vivek *et al* (2014) studies in which the homozygous genotype (CC) of CYP17 gene (-34 T↔C) SNP was found to increase the risk of BPH with respect to those of the wild genotype (TT) of CYP17 gene (-34 T↔C) SNP. Also the minor allele (C) of CYP17 gene (-34 T↔C) SNP was significantly higher in BPH patients when compared with that of the control group. Conversely this results differed from those described by Asmahan *et al* (2014), who did not find an association between CYP17 gene (-34 T↔C) SNP and BPH.

CONCLUSION

The CYP17 gene (-34 T↔C) SNP is involved in the pathogenesis of BPH. The homozygous genotype (CC) was found to be significantly increase the risk of BPH by two folds with respect to those of the wild genotype (TT). The minor allele (C) of CYP17 gene (-34 T↔C) SNP was significantly higher in BPH patients when compared with that of the control group.

REFERENCES

Ananthan V, Sudha B and Pragna B (2016) Association of CYP17 Gene Polymorphism with Benign Prostatic Hyperplasia. *J. App. Med. Sci.* **4**(7), 2630-2635.

Asmahan A, Wissam R and Mohammed A (2014) Association of Benign Prostate Hyperplasia with Polymorphisms in VDR, CYP17 and SRD5A2 Genes among Lebanese Men. *Asian Pacific Journal of Cancer Prevention* **15** (3), 1255-1262.

Brentano S, Picado-Leonard J and Mellon S (1990) Tissue- specific, cyclic adenosine 3',5'-monophosphate-induced and phorbolester repressed transcription from the human P450c17 promoter in mouse cells. *Mol Endocrinol*. **4** (12), 1972-1979.

Carey A, Waterworth D and Patel K (1994) Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17. *Hum. Mol. Genet.* **3**, 1873-1876.

Daviglus M, Dyer A and Persky V (1996) Dietary beta-carotene, vitamin C, and risk of prostate cancer: results from the Western Electric Study. *Epidemiology* **7**, 472-477.

Ellem S, Schmitt J and Pedersen J (2004) Local aromatase expression in human prostate is altered in malignancy. *J. Clin. Endocrinol. Metab*. **89**, 2434-2441.

Galbraith S and Duchesne G (1997) Androgens and prostate cancer: biology, pathology and hormonal therapy. *Eur. J. Cancer* **33**, 545-554.

Gann P, Hennekens C and Ma J (1996) Prospective study of sex hormone levels and risk of prostate cancer. *J. Natl Cancer Inst.* **88**, 1118-1126.

Greenwald P, Kirmiss V and Polan A (1974) Cancer of the prostate among men with benign prostatic hyperplasia. *J. Natl. Cancer*

Inst. **53**, 335-340.

Guoqi S, Ling G and Fuliang T (2013) Lack of Association Between CYP17 Mspa1 Polymorphism and Prostate Cancer Risk: A Meta-Analysis of 14 494 Cases and 15 971 Controls. *Medicina (Kaunas)* **49**(2), 51-55.

Habuchi T, Liqing Z and Suzuki T (2000) Increased risk of prostate cancer and benign prostatic hyperplasia associated with a CYP17 gene polymorphism with a gene dosage effect. *Cancer Res*. **60**, 5710-5713.

Ilic M, Vlajinac H and Marinkovic J (1996) Case-control study of risk factors for prostate cancer. *Br. J. Cancer* **74**, 1682-1686.

Konwar R, Chattopadhyay N and Bid H (2008) Genetic polymorphism and pathogenesis of benign prostatic hyperplasia. *BJU Int*. **102**, 536-544.

Lin-Lin Z, Yu S and Ling-Ling H (2017) Associations of SRD5A2/CYP17/CYP19/VDR gene polymorphisms with the development and clinical progression of benign prostatic hyperplasia: a case-control study in northern Chinese population. *Int J Clin Exp Pathol*. **10** (8), 8660-8676.

Miller J and Tarter T (2009) Combination therapy with dutasteride and tamsulosin for treatment of symptomatic enlarged prostate. *CliInterv Aging* **4**, 251-258.

Neuhausen S, Skolnick M and Cannon-Albright L (1990) Familial prostate cancer studies in Utah. *Br. J. Urol*. **79**, 15-20.

Partin A, Oesterling J and Epstein J (1991) Influence of age and endocrine factors on the volume of benign prostatic hyperplasia. *J. Urol*. **145**, 405-409.

Picado-Leonard J and Miller W (1987) Cloning and sequence of the human gene for P450c17 (steroid 17 alpha-hydroxylase/17,20lyase): similarity with the gene for P450c21. *DNA* **6**, 439-448.

Ranbir C, Lipsy G and Hitender T (2009) CYP17 Gene Polymorphism and its Association in North Indian Prostate Cancer Patients. *Anticacer Research* **29**, 1659-1664.

Ranbir C, Lipsy G and Hitender T (2009) *CYP17* Gene Polymorphism and its Association in North Indian Prostate Cancer Patients. *Anticancer Research* **29**, 1659-1664.

Sanda M, Beaty T and Stutzman R (1994) Genetic susceptibility of benign prostatic hyperplasia. *J. Urol.* **152**, 115-119.

Sharp L, Cardy A and Cotton S (2004) CYP17 gene polymorphisms: prevalence and associations with hormone levels and related factors. A HuGE Review. *Am. J. Epidemiol*. **160**, 729-740.

Strandberg J (2000) Comparative pathology of benign Prostatic hyperplasia. In: Leper Hgedd. *Prostatic Disease*. Philadelphia, W.B Sanders **37**(6), 329-343.

Tomonori H, Zhang L and Takehiro S (2000) Increased Risk of Prostate Cancer and Benign Prostatic Hyperplasia Associated with a *CYP17* Gene Polymorphism with a Gene Dosage Effect. *Cancer Research* **60**, 5710-5713.

Vivek K, Basu D and Sudip K (2014) Association of CYP1A1, CYP1B1 and CYP17 gene polymorphisms and organochlorine pesticides with benign prostatic hyperplasia. *Chemosphere* **108**, 40-45.

Vogelstein B and Gillepie D (1979) Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA* **76** (2), 615-619.

Wilding G (1992) The importance of steroid hormones in prostate cancer. *Cancer Surv.* **14**, 113-130.

Ying L, Zhi-Xing Y and Vassilios P (2005) Cytochrome P450 17α-Hydroxylase/17, 20Lyase (CYP17) Function in Cholesterol Biosynthesis: Identification of Squalene Monooxygenase) Epoxidase) Activity Associated with CYP17 in Leydig Cells. *Mol Endocrinol*. **19** (7), 1918-1931.

Zmuda J, Cauley J and Kuller L (2001) A common promotor variant in the cytochrome P450c17alpha (CYP17) gene is associated with bioavailability testosterone levels and bone size in men. *J Bone Miner Res*. **16**, 911-917.