

Association of Catechol-O-Methyltransferase Gene Polymorphism with Benign Prostatic Hyperplasia in Babylon Province

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Abstract

Background: Benign prostatic hyperplasia (BPH) is a common nonmalignant disorder in elderly men. **Objectives:** The objective of the present study was planned to evaluate the frequency and association of catechol-O-methyltransferase (COMT) gene G↔A (Val 158 Met) single-nucleotide polymorphism (SNP) with BPH in Babylon Province. **Materials and Methods:** To accomplish this purpose, 146 patients with BPH and 102 apparently healthy controls were subjected to the study. DNA was extracted from whole blood for all samples. Genotyping of COMT gene G↔A (Val 158 Met) SNP was carried out by allele-specific oligonucleotides-polymerase chain reaction. **Results:** Results indicated that the homozygous genotype (Met158Met) (AA) of COMT gene G↔A (Val 158 Met) SNP was found to be significantly increase the risk of BPH by three folds with respect to those of the wild genotype (Val158Val) (GG) of COMT gene G↔A (Val 158 Met) SNP. The heterozygous genotype (Val158Met) (GA) of COMT gene G↔A (Val 158 Met) SNP was found to be none significantly increase the risk of BPH with respect to those of the wild genotype (Val158Val) (GG) of COMT gene G↔A (Val 158 Met) SNP. The minor allele frequencies (A) of COMT gene G↔A (Val 158 Met) SNP were significantly higher in BPH patients when compared with that of the control group. **Conclusions:** The COMT gene G↔A (Val 158 Met) SNP is involved in the pathogenesis of BPH.

Keywords: Benign prostatic hyperplasia, catechol-O-methyltransferase, polymorphism

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common nonmalignant disorder in elderly men.^[1,2] The pathogenesis of BPH is theorized that steroid hormone-induced cell proliferation, inflammation, and inefficiency of the apoptotic cell death as well as environmental and genetic factors may contribute to the disease.^[3]

Androgens might not be the only significant hormone in the development of BPH, and estrogen shows an essential role during the development of prostate, and studies have shown that excessive estrogenization during prostatic development may contribute to the high incidence of BPH currently observed in the aging male population. Serum and intraprostatic estradiol levels tend to increase in men with age.^[4-7]

Several studies have demonstrated that the aging prostate becomes more sensitive to androgens. Further investigations have demonstrated a positive correlation between levels of free

testosterone and estrogen and the volume of the gland. The latter may suggest that the association between aging and BPH might reflect increasing estrogen levels of aging, resulting in induction of the androgen receptor and thus sensitizing the prostate to free testosterone.^[8,9] Estrogen effects on the prostate gland may also be indirectly mediated through alterations in other serum hormones. Estrogens stimulate the pituitary release of prolactin, and prolactin induces prostate enlargement and decreased apoptosis.^[7]

Steroidal hormones, mainly androgen and estrogens, play an important role in the physiological growth and development

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Submission: 21-10-2019 **Accepted:** 18-11-2019 **Published Online:** 17-03-2020

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How to cite this article: Hussein NY, Ewadh MJ, Al-Salman AR. Association of catechol-O-methyltransferase gene polymorphism with benign prostatic hyperplasia in Babylon Province. *Med J Babylon* 2020;17:54-7.

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10.4103/MJBL.MJBL_83_19

of the 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 androgen and estrogens, if expressed differentially, may alter the risk of development of BPH.^[3,10]

The catechol-O-methyltransferase (COMT) is coded as the COMT gene and is one of the enzymes essential for metabolizing circulating serum catecholamine's, which catalyzes the O-methylation of various compounds, such as catechol estrogens. The general function of COMT is the elimination of biologically active or toxic catechols and some other hydroxylated metabolites.^[11,12] The human COMT gene is located in chromosome 22 at position 22q11.1. It has six exons.^[13,14]

The estrogens are metabolized by cytochrome 1A1 and cytochrome 1B1 to produce 2-hydroxyestradiol (2-OHE) and 4-OHE, respectively. These metabolites may mediate their effect by activation of the classical estrogen receptor, interaction with other specific receptors or effectors, direct binding to DNA, or by other mechanisms to produce biological effects. The 4-OHE binds to and activates the estrogen receptor with approximately the same affinity as estradiol. However, the interaction with the hormone receptor is markedly reduced for the 2-OHE, which, therefore, may possess a weaker hormonal potency as compared with the parent hormone, estradiol.^[15-18]

The aims of this study were to evaluate the frequency and association of COMT gene G↔A (Val 158 Met) single-nucleotide polymorphism (SNP) with BPH.

MATERIALS AND METHODS

Subjects and study design

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

Blood sampling

Two milliliters of blood collected in ethylenediaminetetraacetic acid containing tube, and the samples were kept frozen at -70°C until analysis of COMT gene polymorphism.

Genotyping of catechol-O-methyltransferase

Genotyping of COMT gene G↔A (Val 158 Met) SNP was carried out by allele-specific oligonucleotides-polymerase chain reaction (ASO-PCR). The DNA extracted from frozen blood by genomic DNA mini kit (Favorgen).^[19] The SNP of COMT gene, i.e., valine to methionine substitution at codon

158 (G↔A) was predicted by ASO-PCR, and for each sample, two reactions of PCR were set up. The first reaction includes amplification of gene by PCR with forward primer (common) and reverse primer (wild), i.e., G allele for valine, whereas the second reaction includes amplification of gene by PCR with forward primer (common) and reverse primer (mutant), i.e., A allele for methionine.^[20,21]

The DNA was amplified by PCR. A 169 bp DNA fragment containing the polymorphic site G↔A (Val 158 Met) SNP of COMT gene was amplified using specific primers (forward primer [common] 5'-ACTGTGGCTACTCAGCTGTG-3' and reverse primer 5'-GCATGCACACCTTGTCTTCAC-3' [G allele for Val] and reverse primer 5'-GCATGCACACCTTGTCTTCAT-3' [A allele for Met]).^[21] The PCR products were analyzed on 2% agarose gel electrophoresis.

Statistical analysis

The results of phenotypes data were expressed as mean ± standard deviation. Student's *t*-test was used for the evaluation of data. Genotype data expressed as odds ratio (OR) and confidence interval (CI) 95%. Statistical analyses were performed with SPSS version 20 (SPSS, IBM Company, Chicago, USA). *P* < 0.05 was considered to be statistically significant.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patient's verbal and analytical approval before sample was taken. The study protocol and the participant information and consent form were reviewed and approved by a Local Ethics Committee.

RESULTS

The demographic characteristics of patients with BPH and control groups are shown in Table 1.

The genotype distributions and frequency of COMT gene G↔A (Val 158 Met) SNP are shown in Table 2 and Figure 1, respectively. The analysis of results indicated that the COMT gene G↔A (Val 158 Met) SNP genotype

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

Characteristics	Groups	Mean ± SD	Range	P
<i>n</i>	Control	102		
	Patient	146		
Age (year)	Control	54.93±8.68	38-65	>0.05
	Patient	57.76±6.83	40-69	
BMI (kg/m ²)	Control	26.79±4.09	19.77-34.72	<0.001
	Patient	30.25±3.42	19.66-38.07	
Prostate volume (mL)	Control	28.59±12.52	11.84-52.7	<0.001
	Patient	56.73±27.69	17.97-157	
Duration of disease	Patient	5.05±2.43 year	1 month-12 year	-

BMI: Body mass index, SD: Standard deviation

frequencies of wild genotype (Val158Val) (GG), heterozygous genotype (Val158Met) (GA), and homozygous genotype (Met158Met) (AA) were 15.8%, 67.8%, and 16.4% in BPH patients and 26.5%, 64.7%, and 8.8% in controls, respectively.

The homozygous genotype (Met158Met) (AA) of COMT gene G↔A (Val 158 Met) SNP was found to be significantly increase (OR = 3.130, CI 95% = 1.214–8.067, $P = 0.018$) the risk of BPH by three folds with respect to those of the wild genotype (Val158Val) (GG) of COMT gene G↔A (Val 158 Met) SNP.

The heterozygous genotype (Val158Met) (GA) of COMT gene G↔A (Val 158 Met) SNP was found to be none significantly increase (OR = 1.760, CI 95% = 0.930–3.330, $P = 0.081$) the risk of BPH with respect to those of the wild genotype (Val158Val) (GG) of COMT gene G↔A (Val 158 Met) SNP.

The allele distribution and frequency of COMT gene G↔A (Val 158 Met) are shown in Table 3 and Figure 2, respectively. The allele frequencies of G and A of COMT gene G↔A (Val 158 Met) SNP were found to be 49.7% and 50.3% in BPH patients, respectively, and 58.8% and 41.2% in the control group, respectively. The minor allele frequencies (A) of COMT gene G↔A (Val 158 Met) SNP in BPH patients and control groups were found to be 50.3% and 41.2%, respectively. It was significantly higher ($P < 0.05$) in BPH patients when compared with that of the control group.

DISCUSSION

Several genetic polymorphisms of COMT gene have been identified. One of the most common polymorphism of the COMT gene is G↔A (Val 158 Met) SNP. The COMT gene G↔A (Val 158 Met) SNP is located in coding region in codon 158, in which guanine (G) to adenine (A) that lead to valine (Val) is substituted by methionine (Met).

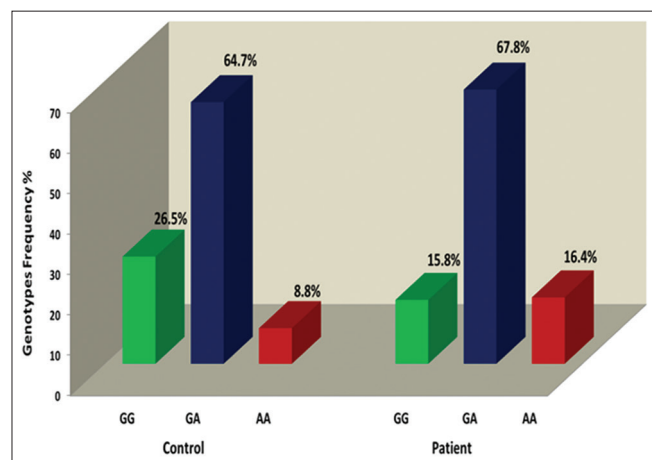


Figure 1: Genotypes frequency of catechol-O-methyltransferase gene G↔A (Val 158 Met) single-nucleotide polymorphism in patients with benign prostatic hyperplasia and the controls groups. GG: wild genotype (Val158Val), GA: heterozygous (Val158Met), AA: homozygous genotype (Met158Met)

The COMT gene G↔A (Val 158 Met) SNP produces three genotype groups: wild genotype (Val158Val) (GG), heterozygous genotype (Val158Met) (GA), and homozygous genotype (Met158Met) (AA), which subsequently leads to different enzyme activities.^[22,23]

The wild genotype (Val158Val) (GG) has a high enzymatic activity, heterozygous genotype (Val158Met) (GA) has intermediate enzymatic activity while homozygous genotype (Met158Met) (AA) has low enzymatic activity. For homozygous genotype (Met158Met) (AA), there are about a three to four-fold reduced enzymatic activity compared with the wild genotype (Val158Val) (GG).^[24,25]

In this study, the homozygous genotype (Met158Met) (AA) of COMT gene G↔A (Val 158 Met) SNP was found to be significantly increase the risk of BPH by three folds with respect to those of the wild genotype (Val158Val) (GG) of COMT gene G↔A (Val 158 Met) SNP. The presence of (A)

Table 2: Genotypes distribution of catechol-O-methyltransferase Gene G↔A (Val 158 Met) single-nucleotide polymorphism in patients with benign prostatic hyperplasia and the control groups

Genotype	Control	Patient	OR	95% CI	P
GG	27	23	Reference	Reference	Reference
GA	66	99	1.760	0.930-3.330	0.081
AA	9	24	3.130	1.214-8.067	0.018
Total	102	146	-	-	-

GG: Wild genotype (Val158Val), GA: Heterozygous (Val158Met) and AA: Homozygous genotype (Met158Met), OR: Odds ratio, CI: Confidence interval

Table 3: Alleles distribution of catechol-O-methyltransferase Gene G↔A (Val 158 Met) single-nucleotide polymorphism in patients with benign prostatic hyperplasia and the control groups

Allele	Control	Patient	OR	95% CI	P
G	120	145	Reference	Reference	Reference
A	84	147	1.448	1.009-2.078	0.044
Total	204	292	-	-	-

OR: Odds ratio, CI: Confidence interval

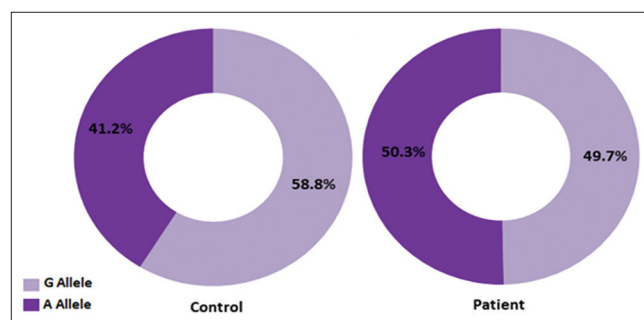


Figure 2: Alleles frequency of catechol-O-methyltransferase gene G↔A (Val 158 Met) single-nucleotide polymorphism in patients with benign prostatic hyperplasia and the controls groups

allele of COMT gene G↔A (Val 158 Met) SNP may reduce the COMT enzymatic activity three to four-fold than (G) allele of COMT gene G↔A (Val 158 Met) SNP that leads to increase the estradiol level.

The estradiol has several effects on the prostate, which include increase prostatic expression of estrogen receptors, induction of the androgen receptor and thus sensitizing the prostate to free testosterone and also estrogens stimulate the pituitary release of prolactin, and the prolactin induces prostate enlargement and decreased apoptosis. In all these mechanisms the homozygous genotype (Met158Met) (AA) may increase the risk of BPH.

The current results are in consistence with the results of Omrani *et al.*^[26] study in which the homozygous genotype (Met158Met) (AA) of COMT gene G↔A (Val 158 Met) SNP was found to increase the risk of BPH with respect to those of the wild genotype (Val158Val) (GG) of COMT gene G↔A (Val 158 Met) SNP. Furthermore, the minor allele (A) of COMT gene G↔A (Val 158 Met) SNP was significantly higher in BPH patients when compared with that of the control group. Conversely, these results differed from those described by Tanaka *et al.*^[21] who did not find an association between COMT gene G↔A (Val 158 Met) SNP and BPH.

CONCLUSIONS

The COMT gene G↔A (Val 158 Met) SNP is involved in the pathogenesis of BPH. The homozygous genotype (AA) was found to be significantly increasing the risk of BPH by three folds with respect to those of the wild genotype (GG). The minor allele (A) of COMT gene G↔A (Val 158 Met) SNP was significantly higher in BPH patients when compared with that of the control group.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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