

# Both AA genotype of rs165599 and Val/Val genotype of rs4680 in *COMT* are associated with an increased risk of recurrence of prostate cancer

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## Abstract

**Introduction:** The catechol-O-methyltransferase (*COMT*) gene expression was reported to be associated with the recurrence of prostate cancer. Furthermore, it has been shown that rs165599 SNP in the 3'UTR of *COMT* affects the interaction between miR-138 and *COMT*, while another polymorphism (rs4680) located in the gene of *COMT* also affects its expression. Here, we explored the combined effect of rs165599 and rs4680 on the recurrence of prostate cancer.

**Material and methods:** Kaplan-Meier analysis was performed to analyse the PSA-progression-free survival of prostate cancer patients carrying different genotypes of rs165599 and rs4680 SNPs. IHC and Western blot were used to evaluate the expression of *COMT*. Real-time PCR was carried out to examine the expression of miR-138 and *COMT* mRNA. Luciferase assay was performed to explore the inhibitory role of miR-138 in the expression of *COMT* carrying different genotypes.

**Results:** The PSA-progression-free survival was significantly increased in prostate cancer patients carrying the rs165599-AA + rs4680-Val/Val genotype. In concordance with this, the expression of *COMT* was remarkably elevated in the tissue samples collected from prostate cancer patients carrying the rs165599-AA + rs4680-Val/Val genotype. Luciferase assay demonstrated that *COMT-G* at rs165599 was effectively suppressed by miR-138 through binding to the 3' UTR of *COMT-G*. Furthermore, *COMT* expression was notably suppressed by miR-138 precursors in primary prostate cancer cells carrying GG genotype at rs165599.

**Conclusions:** The findings of this study demonstrated that the carriers of AA genotype of rs165599 and Val/Val genotype of rs4680 showed an increased risk of prostate cancer recurrence, and these 2 polymorphisms may jointly function as a novel biomarker for the prognosis of prostate cancer recurrence.

**Key words:** prostate cancer, *COMT*, miRNA, rs165599, rs4680, recurrence in prostate cancer.

## Introduction

Pancreatic cancer (PC) is the 7<sup>th</sup> leading contributor for cancer fatalities in China and the 4<sup>th</sup> leading contributor for cancer fatalities in the

USA [1, 2]. In the USA alone, more than 250,000 PC patients die annually. As a result of the fact that little improvement has actually been made in the diagnosis as well as surgical procedures in the treatment of PC in recent years, the prognosis of PC patients is the worst among all kinds of cancers [3, 4]. It is vital to find unique approaches to address the conditions of individual PC patients.

The catechol-O-methyltransferase (COMT) gene is found on chromosome 22q11.2 [5]. There are actually 2 forms of COMT proteins translated from *COMT*: one is soluble and the other is bound on cell membrane [6]. COMT is an enzyme associated with the inactivation of catecholamines as well as catechol oestrogens, which have been demonstrated to possess carcinogenic properties along with the capacity for DNA damage and induction of PC in rats [7]. Meanwhile, COMT can catalyse the O-methylation of catechol oestrogen 2-hydroxyestradiol to produce 2-methoxyestradiol, which has anti-angiogenic and anti-proliferative properties [8]. A typical polymorphism in *COMT* is a G to A mutation that takes place at codon 158, which is also called Val158 Met or rs4680. The replacement of G for A causes the substitute of valine by methionine as well as 3- to 4-fold reduction in *COMT* activity. The Val/Val phenotype (or G/G genotype) of *COMT* has been shown to be more active and stable as compared with the Met/Met phenotype (or A/A genotype). Many studies have performed genome wide screening without pinpointing the genes of *COMT* with considerable prospect for suicidal behaviours, with a few exceptions [9–12].

MicroRNA (miRNA) makes up a family of non-coding RNAs that are less than 23 nucleotides in size and can control the endogenous expression of genes through target mRNA degradation and translational inhibition [13]. In the last few years, miRNAs have been demonstrated to regulate several biological activities, such as cell growth, neuron distinction, and apoptosis [14–16]. Significantly, growing evidence indicates that miRNA expression might be related to cancer cell growth in PC patients [14, 17, 18]. Amongst many miRNAs, miR-138 has recently been shown as a significant tumour suppressor in various cancers, including osteosarcoma, cervical cancer, ovarian cancer, non-small cell lung cancer, and gallbladder cancer [19–21]. Primarily, miR-138 prevents the spread of cancer cells and causes their apoptosis while boosting their chemo sensitivity [22–24]. It was discovered that down-regulation of miR-138-5p was linked to autophagy in PC cells. Nevertheless, elevated miR-138-5p expression prevented the activation of autophagy dependent on the SIRT1/FoxO1/Rab7 signalling. Also, SIRT1 might repress the growth of PC cells, making miR-138-5p a possible target in PC treatment [25].

It has been shown that *COMT* expression is associated with prostate cancer recurrence [26]. Furthermore, it has been shown that rs165599 in the 3' untranslated region (3'UTR) of *COMT* affects the interaction between miR-138 and *COMT*, and another polymorphism (rs4680) located in the gene of *COMT* also affects the expression level of *COMT* [27–30]. Moreover, polymorphisms located in oestrogen metabolism-related genes, such as the AA genotype and the A allele of rs4680 in *COMT*, have been proven to be inversely associated with the risk of prostate cancer in Afro-Caribbean and native African men [31]. In this study, we hypothesized that the genotype difference of rs165599 and rs4680 is associated with the risk of prostate cancer recurrence and the PSA-progression-free survival period. To study the effect of genotype difference on prostate cancer recurrence, we studied the combined effect of both rs165599 and rs4680 single nucleotide polymorphisms (SNPs) on the recurrence of prostate cancer.

## Material and methods

### Human subjects and sample collection

In this work, to study the combined effect of rs165599 and rs4680 SNPs on prostate cancer, we recruited 224 prostate cancer patients and then partitioned them into 4 groups according to their genotypes of rs165599 and rs4680 SNPs: (1) rs165599-AA + rs4680-Val/Val group ( $N = 62$ ); (2) rs165599-AA + rs4680- (Val/Met + Met/Met) group ( $N = 55$ ); (3) rs165599- (GG+GA) + rs4680-Val/Val group ( $N = 58$ ); and (4) rs165599- (GG+GA) + rs4680- (Val/Met + Met/Met) group ( $N = 49$ ). The information of all participants, including their age, tumour status, and Gleason score, was collected and compared among the 4 groups. Then, one-way analysis of variance (ANOVA) was utilized to perform the statistical comparison among the above 4 groups. In the next step, tissue and peripheral blood samples were collected from all subjects for the analyses described below. The institutional ethical committee approved the protocol of this study.

### Genotyping by direct sequencing

Collected tissue and peripheral blood samples were treated with a GoldMag Mini Purification assay kit (GoldMag Co. Ltd., Xi'an, China), in accordance with the specific assay protocol provided by the assay kit manufacturer, to extract Genomic DNA content. Then, the quality and the concentration of each isolated DNA sample were assessed by using a Nanodrop 3000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) in accordance with the specific operating protocol provided by the instrument manufacturer. In the next step, the genotypes of rs165599 and rs4680 SNPs

were measured by using a multiplexed SNP Mass EXTEND assay designed with the Agena Mass ARRAY version 3.0 software (Agena Bioscience, San Diego, CA) in accordance with the specific instructions provided by the software vendor. The multiplexed SNP Mass EXTEND assay was carried out using an Agena Mass ARRAY RS1000 system in accordance with the specific operating protocol provided by the instrument manufacturer.

#### RNA isolation and real-time PCR

In order to explore the effect of rs165599 and rs4680 polymorphisms on the expression of their host gene *COMT*, we carried out qPCR and ELISA to analyse the expression of *COMT* in tissue samples of prostate cancer as well as peripheral blood samples collected from all prostate cancer patients carrying different genotypes. We also performed qPCR to examine the expression of miR-138 in these tissue and peripheral blood samples. To carry out the measurements, the total RNA content was first isolated from each sample by utilizing an RNAiso Plus RNA extraction reagent (Takara, Tokyo, Japan) in accordance with the specific protocol provided by the reagent manufacturer. Then, the isolated RNA from each sample was reverse transcribed into cDNA templates using a Taqman Advanced cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA) and a Reverse Transcription kit (Applied Biosystems, Foster City, CA) in accordance with the specific assay protocols provided by the assay kit manufacturers. Then, quantitative real-time PCR was performed by making use of a Fast-Start Universal SYBR Green Master Mix assay kit (Roche, Basel, Switzerland) in accordance with the specific assay protocol provided by the assay kit manufacturer. The real-time PCR reaction was done on an ABI Prism 7900HT real-time PCR machine (Applied Biosystems, Foster City, CA) using U6 and GAPDH as the internal controls for the normalization of measured relative expression of target genes, i.e. *COMT* mRNA and miR-138.

#### Cell culture and transfection

Primary prostate cancer cells were bought from the Shanghai Cell Bank at the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in a RPMI 1640 medium added with 10% of foetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA) and suitable antibiotics. The culture conditions were saturated humidity at 37°C in 5% carbon dioxide. To further explore the regulatory network of rs165599 polymorphisms, miR-138 and *COMT*, we cultured primary prostate cancer cells carrying the GG and AA genotypes, and then transfected them with *COMT* siRNA and miR-138 precursors, respectively. In brief,

the primary prostate cancer cells carrying the GG genotype of the rs165599 SNP were divided into 3 groups: (1) Scramble control group (primary prostate cancer cells treated with 50 nM of a scramble control siRNA); (2) *COMT* siRNA group (primary prostate cancer cells treated with 50 nM of *COMT* siRNA); and (3) miR-138 precursor group (primary prostate cancer cells treated with 50 nM of miR-138 precursors). Similarly, the primary prostate cancer cells carrying the AA genotype of the rs165599 SNP were also divided into 3 groups, i.e. (1) Scramble control group (primary prostate cancer cells treated with 50 nM of a scramble control siRNA); (2) *COMT* siRNA group (primary prostate cancer cells treated with 50 nM of *COMT* siRNA); and (3) miR-138 precursor group (primary prostate cancer cells treated with 50 nM of miR-138 precursors). In the next step, the cells were transfected with corresponding siRNA/miRNA by using Lipofectamine 3000 (Invitrogen, Carlsbad, CA) in accordance with the specific transfection protocol provided by the manufacturer of the transfection reagent. At 48 hours post transfection, the cells were harvested for the analysis of target gene expression.

#### Vector construction, mutagenesis, and luciferase assay

Our sequence analysis showed that miR-138 could potentially bind to the 3' UTR of *COMT*. In order to examine whether rs165599 polymorphisms affect the expression of *COMT*, the 3' UTR of *COMT* carrying the A and G allele of the rs165599 polymorphism was cloned into pcDNA luciferase vectors (Promega, Madison, WI), respectively, to establish *COMT-G* and *COMT-A* vectors, which were then transfected into PC3 cells along with miR-138 mimics by using Lipofectamine 3000 in accordance with the specific transfection protocol provided by the manufacturer of the transfection reagent. At 48 hours post transfection, the luciferase activity of transfected cells was assayed by using a Dual Luciferase reporter gene assay kit (Promega, Madison, WI) in accordance with the specific assay protocol provided by the manufacturer of the assay kit.

#### Western blot analysis

The total protein content was extracted from each sample by making use of a radio immunoprecipitation assay kit (Solarbio Science & Technology Co. Ltd., Beijing, China) in accordance with the specific assay protocol provided by the assay kit manufacturer. Then, 50 µg of each protein sample was resolved by using SDS-PAGE and blotted onto polyvinylidene fluoride (PVDF) membranes, which was then blocked by utilizing 5% skimmed milk at ambient temperature for 1 hour before they were incubated for 24 hours at 4°C

with anti-COMT primary antibody (Abcam, Cambridge, MA) followed by incubation with suitable HRP-conjugated secondary antibodies for 1 hour at room temperature in accordance with the specific instructions provided by the antibody manufacturer. After colour development by utilizing an enhanced chemiluminescence (ECL) assay kit (Amersham Pharmacia, Piscataway, NJ) in accordance with the specific assay protocol provided by the assay kit manufacturer, the relative protein expression of *COMT* in each sample was determined by means of a Bio-Rad imaging system (Bio-Rad Laboratories, Hercules, CA) in accordance with the instructions provided by the machine manufacturer.

### Immunohistochemistry (IHC)

In order to explore the effect of rs165599 and rs4680 polymorphisms on the expression of their host gene *COMT*, we collected prostate cancer tissue samples from all patients and performed immunohistochemistry to evaluate *COMT* expression in each sample. In brief, the collected prostate cancer tissue samples were embedded in paraffin, fixed, deparaffinized, made transparent, and blocked with 5% goat serum before they were treated with anti-COMT primary antibodies and matching Alexa Fluor 488-labelled secondary antibodies in sequence (Santa Cruz Biotechnology, Santa Cruz, CA) in accordance with the instructions provided by the antibody manufacturer. After counterstaining with DAPI, the positive expression of *COMT* in each sample was evaluated by using a confocal microscope.

### ELISA

The *COMT* activity in the collected peripheral blood samples was measured using a commercial ELISA assay kit (Thermo Fisher Scientific, Waltham,

MA) in accordance with the specific assay protocol provided by the assay kit manufacturer.

### Statistical analysis

Data analysis was carried out using SPSS 19.0 software (SPSS, Chicago, IL). The Kaplan-Meier method was used to compare the prostate-specific antigen (PSA)-progression-free survival of patients in the 4 groups. Comparisons between 2 groups was done by using Student's *t* tests. The results are presented as mean  $\pm$  standard deviation. One-way ANOVA was used to compare multiple groups. A *p* value of  $< 0.05$  was deemed statistically significant.

### Results

#### Patients with rs165599-AA + rs4680-Val/Val genotypes had the highest PSA-progression-free survival

In this work, we recruited 224 prostate cancer patients and partitioned them into 4 groups according to their genotypes at rs165599 and rs4680 SNP. The information of participants including their age, tumour status, and Gleason score was collected and is shown in Table I. ANOVA was utilized to perform the statistical comparison among the above 4 groups, and the results revealed that there is no obvious difference in all characters among the groups. The Kaplan-Meier method was used to compare the PSA-progression-free survival of patients in the 4 groups. The PSA-progression-free survival was highest in patients carrying the rs165599-AA + rs4680-Val/Val genotype, and it was lowest in patients carrying the rs165599-(GG+GA) + rs4680-(Val/Met + Met/Met) genotype. The PSA-progression-free survival of patients carrying the rs165599-AA + rs4680-(Val/Met + Met/Met) and rs165599-(GG+GA) + rs4680-Val/Val genotypes was moderate (Figure 1).

**Table I.** Basic information of participants grouped according to their genotypes

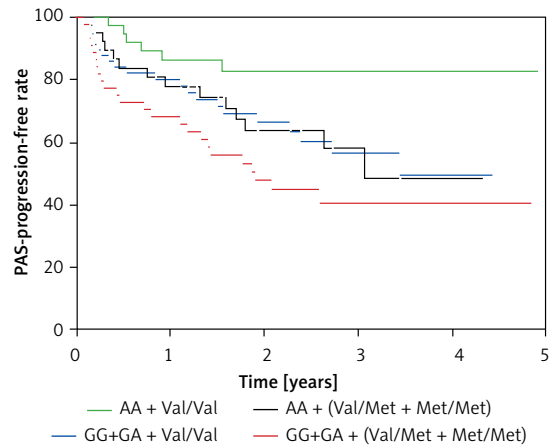
Characteristics	Rs165599-AA + Rs4680-Val/Val (N = 62)	Rs165599-AA + Rs4680- (Val/Met + Met/Met) (N = 55)	Rs165599- (GG+GA) + Rs4680-Val/Val (N = 58)	Rs165599-(GG+GA) + Rs4680- (Val/Met + Met/Met) (N = 49)	P value
Age [years]	73.4 $\pm$ 5.4	70.6 $\pm$ 5.6	74.0 $\pm$ 5.8	71.2 $\pm$ 5.1	0.772
Tumour status					0.632
T1	1 (1.6)	1 (1.8)	2 (3.4)	1 (2.0)	
T2	10 (16.1)	11 (20.0)	8 (13.8)	8 (16.3)	
T3	33 (53.2)	28 (50.9)	30 (51.7)	28 (57.1)	
T4	18 (29.1)	15 (27.3)	18 (31.1)	12 (24.6)	
Gleason score					0.717
2-7	32 (51.6)	30 (54.5)	30 (51.7)	22 (44.9)	
8-10	30 (48.4)	25 (45.5)	28 (48.3)	27 (55.1)	

The expression of *COMT* in tissue samples and peripheral blood was remarkably elevated in patients carrying the rs165599-AA + rs4680-Val/Val genotype

We performed immunohistochemistry to evaluate *COMT* expression in various samples. The expression of *COMT* was significantly higher in tissue samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype, and it was remarkably lower in tissue samples collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype (Figure 2). Moreover, we carried out qPCR and ELISA to analyse the expression of *COMT* in tissue samples and peripheral blood. The expression of *COMT* mRNA in tissue samples (Figure 3 A) and peripheral blood (Figure 3 B) was in good agreement with that revealed by IHC analysis.

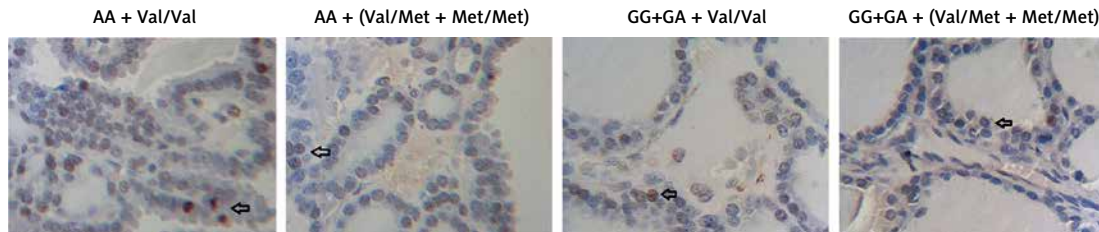
MiR-138 inhibited the expression of *COMT* carrying the rs165599-G genotype by binding to the 3' UTR of *COMT*

No obvious difference was observed for the expression of miR-138 in either tissue samples (Fig-

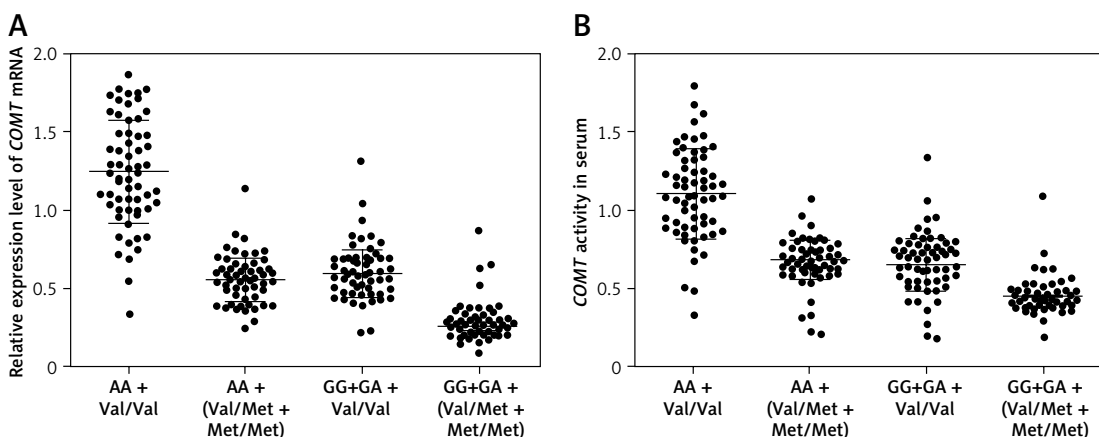


**Figure 1.** The PSA-progression-free survival was significantly higher in patients carrying the rs165599-AA + rs4680-Val/Val genotype and lower in patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype

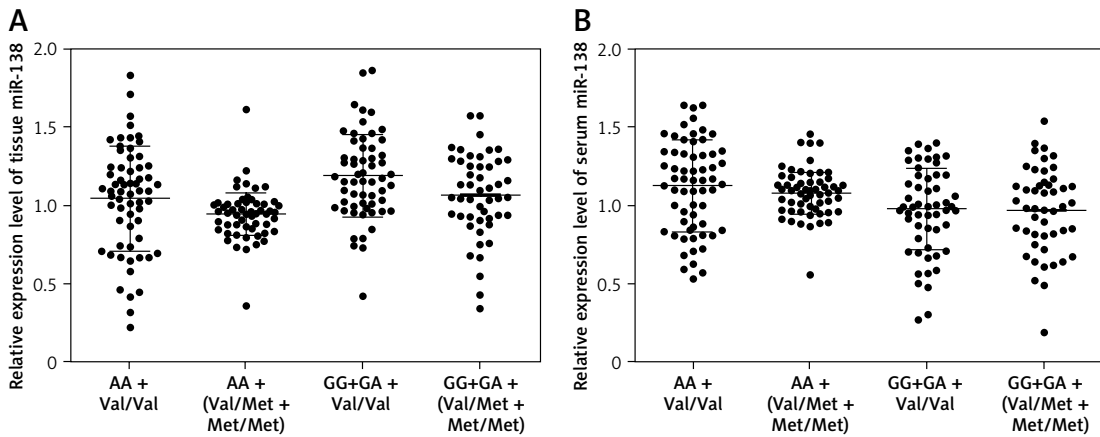
ure 4 A) or peripheral blood (Figure 4 B). Sequence analysis showed that miR-138 could potentially bind to the 3' UTR of *COMT* (Figure 5 A). In order to examine whether rs165599 polymorphisms affect the expression of *COMT*, luciferase vectors



**Figure 2.** Immunohistochemistry analysis showed that the expression of *COMT* protein was increased in the tissue samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in tissue samples collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype



**Figure 3.** The expression and activity of *COMT* were increased in patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype. **A** – QPCR analysis showed that the expression of *COMT* mRNA was increased in the tissue samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in the tissue samples collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype. **B** – ELISA analysis showed that the activity of *COMT* was increased in the serum collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in the serum collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype



**Figure 4.** No obvious difference was observed for the expression of miR-138 in the tissue samples and serum collected from patients carrying different genotypes. **A** – No apparent difference was observed for the expression of miR-138 in the tissue samples collected from patients carrying different genotypes. **B** – No apparent difference was observed for the expression of miR-138 in the serum collected from patients carrying different genotypes

carrying *COMT-G* and *COMT-A* were established and transfected into PC3 cells along with miR-138 mimics. The luciferase activity of *COMT-G* vector was significantly suppressed by miR-138 mimics, whereas no apparent suppression was observed for *COMT-A* vector (Figure 5 B).

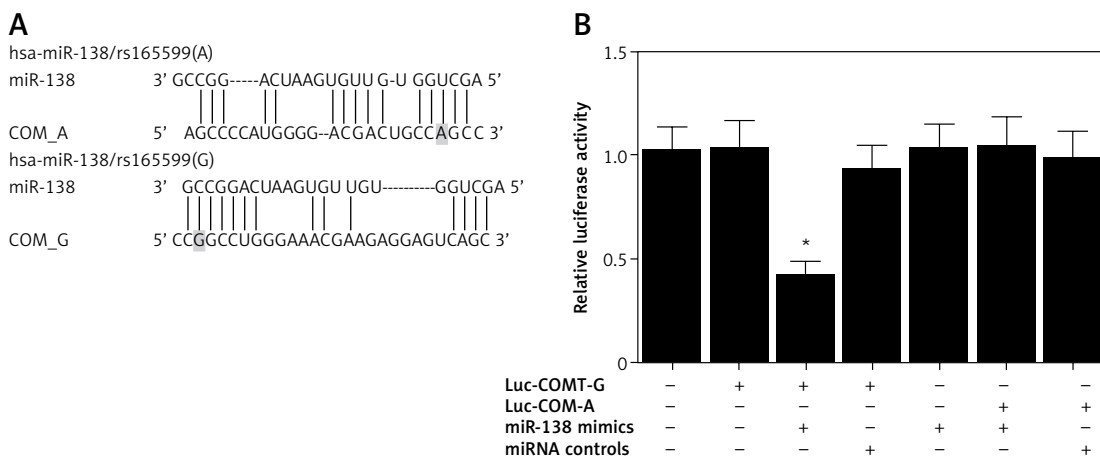
**MiR-138 precursors effectively suppressed the expression of *COMT* in primary prostate cancer cells carrying the GG genotype**

To further explore the regulatory network of rs165599 polymorphisms, miR-138 and *COMT*, we cultured primary prostate cancer cells carrying the GG and AA genotypes, and then transfected them with *COMT* siRNA and miR-138 precursors, respectively. The dramatically elevated expression of miR-138 in primary prostate cancer cells carrying the GG (Figure 6 A) and AA (Figure 7 A) genotypes

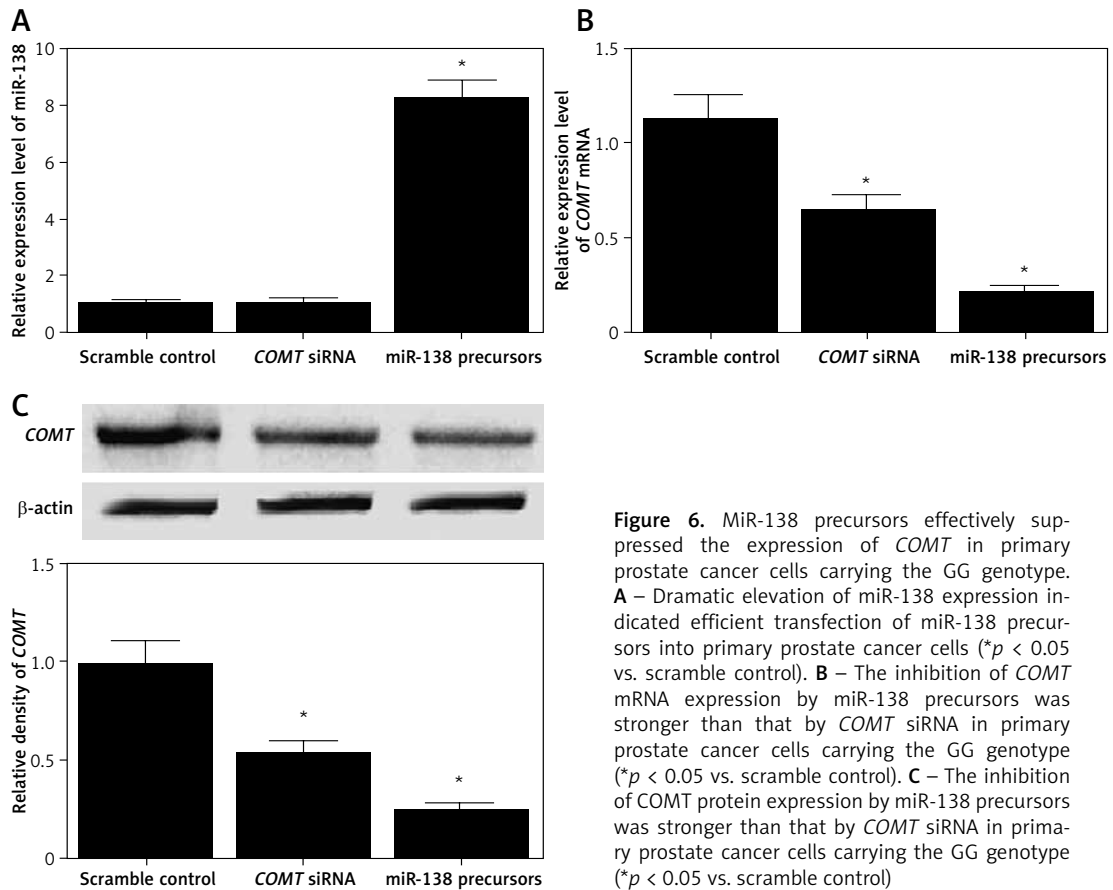
indicated successful transfection of miR-138 precursors. QPCR and Western blot analysis showed that the expression of *COMT* mRNA (Figure 6 B) and protein (Figure 6 C) was notably decreased in primary prostate cancer cells carrying the GG genotype after transfection with miR-138 precursors. The inhibitory effect of miR-138 precursors on the expression of *COMT* in primary prostate cancer cells carrying the GG genotype was even stronger than that of *COMT* siRNA. In addition, the expression of *COMT* mRNA (Figure 7 B) and protein (Figure 7 C) was decreased to a lesser extent in primary prostate cancer cells carrying the AA genotype after transfection with miR-138 precursors.

**Discussion**

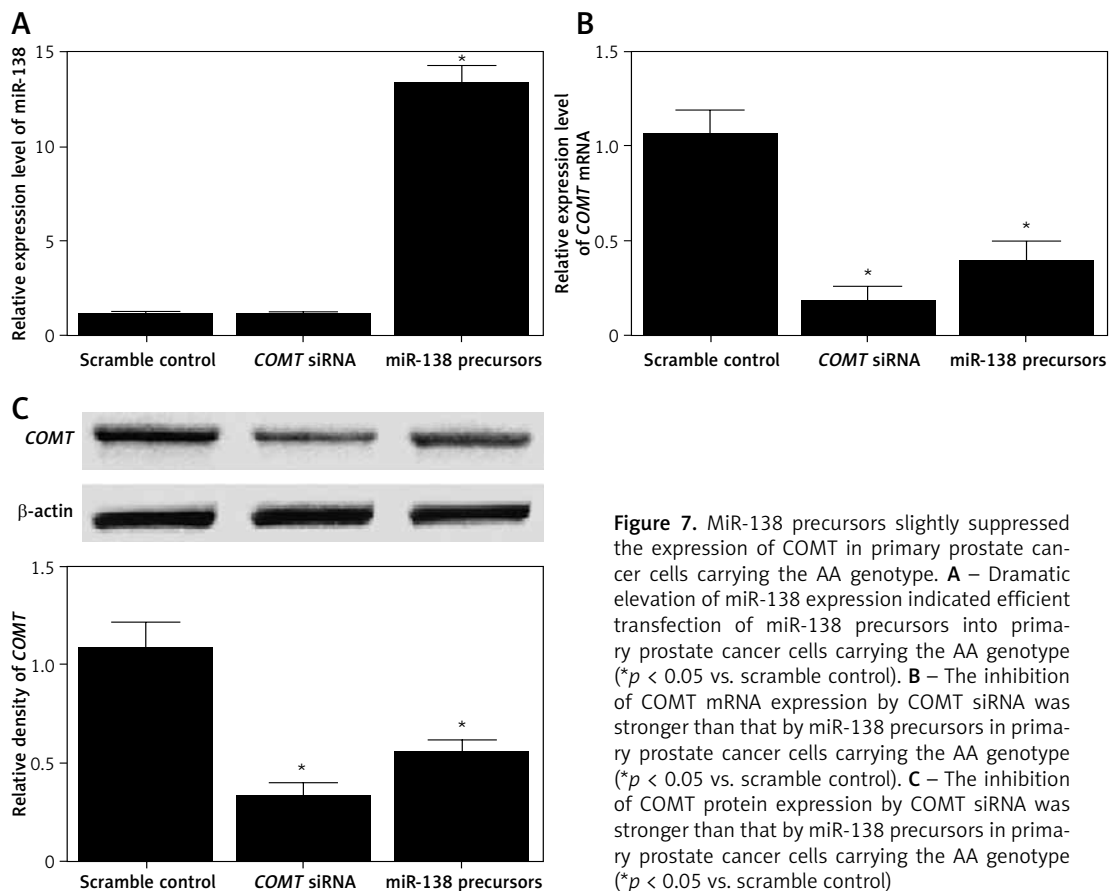
The findings of this study support our hypothesis that the genotypes of rs165599 and rs4680



**Figure 5.** The luciferase activity of *COMT-G* was inhibited by miR-138. **A** – Sequence analysis showed that miR-138 could potentially bind to *COMT* carrying the G allele of rs165599 SNP. **B** – The luciferase activity of *COMT-G* was suppressed by miR-138 in PC3 cells (\* *P* value < 0.05 vs. miRNA controls)



**Figure 6.** MiR-138 precursors effectively suppressed the expression of *COMT* in primary prostate cancer cells carrying the GG genotype. **A** – Dramatic elevation of miR-138 expression indicated efficient transfection of miR-138 precursors into primary prostate cancer cells ( $*p < 0.05$  vs. scramble control). **B** – The inhibition of *COMT* mRNA expression by miR-138 precursors was stronger than that by *COMT* siRNA in primary prostate cancer cells carrying the GG genotype ( $*p < 0.05$  vs. scramble control). **C** – The inhibition of *COMT* protein expression by miR-138 precursors was stronger than that by *COMT* siRNA in primary prostate cancer cells carrying the GG genotype ( $*p < 0.05$  vs. scramble control)



**Figure 7.** MiR-138 precursors slightly suppressed the expression of *COMT* in primary prostate cancer cells carrying the AA genotype. **A** – Dramatic elevation of miR-138 expression indicated efficient transfection of miR-138 precursors into primary prostate cancer cells carrying the AA genotype ( $*p < 0.05$  vs. scramble control). **B** – The inhibition of *COMT* mRNA expression by *COMT* siRNA was stronger than that by miR-138 precursors in primary prostate cancer cells carrying the AA genotype ( $*p < 0.05$  vs. scramble control). **C** – The inhibition of *COMT* protein expression by *COMT* siRNA was stronger than that by miR-138 precursors in primary prostate cancer cells carrying the AA genotype ( $*p < 0.05$  vs. scramble control)

are associated with the risk of prostate cancer recurrence and the PSA-progression-free survival period. In this study, we found that the PSA-progression-free rate was highest in patients carrying the rs165599-AA + rs4680-Val/Val genotype and lowest in patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype. In addition, the expression and activity of *COMT* were significantly higher in samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype, and they were remarkably lower in samples collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype.

Yu *et al.* found that miR-138-5p displayed a suppressive impact on the growth of PC, suppressing the formation of tumours [30]. Tian *et al.* showed that miR-138-5p inhibited autography and PC growth, suggesting that miR-138-5p could function as a vital factor in PC [25, 32]. One SNP, rs165599, demonstrated the highest correlation with mental illness [33]. While SNP rs165599 is actually located downstream of the site of *COMT* polyadenylation and hence cannot be found in a lot of EST sequences recorded in the GenBank database, this SNP could be amplified from brain cDNA free of genomic contamination, which confirms that SNP rs165599 is actually expressed in the brain [34]. As a result, the site of *COMT* with additional 3 polyadenylation should also be present in the human brain. When SNP rs165599 was utilized as an expression tag for *COMT* transcripts containing the 3' sequence, notable variations were seen in allelic expression, indicating that the SNP is actually linked to larger modifications in the transcripts containing the 3' UTR. In this study, we carried out luciferase assays to explore the effect of rs165599 polymorphisms on the expression of *COMT*. The luciferase activity of *COMT-G* vector was remarkably suppressed by miR-138 mimics. Furthermore, we cultured primary prostate cancer cells carrying GG and AA genotypes to explore the effect of miR-138 precursors on the expression of *COMT*. MiR-138 precursors showed a stronger effect on suppressing the expression of *COMT* mRNA and protein than *COMT* siRNA in primary prostate cancer cells carrying the GG genotype, but not in primary prostate cancer cells carrying the AA genotype.

However, there are limitations to our study. The number of patients included in our study for genetic analysis was too small to be convincing and supportive. Also, the genetic analysis was not comprehensive. In our future study, it will be necessary to include a more comprehensive genetic analysis.

In conclusions, the findings of this study demonstrate that the carriers of the AA genotype of rs165599 and Val/Val genotype of rs4680 SNPs

showed an increased risk of prostate cancer recurrence and shorter PSA-progression-free survival period, both of which indicated a worse prognosis. Therefore, these 2 polymorphisms may jointly function as a novel biomarker for the prognosis of prostate cancer recurrence.

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## Conflict of interest

The authors declare no conflict of interest.

## References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; 60: 277-300.
2. Rethemiotaki I, Rethemiotakis A. Link between prostate cancer diagnosis and stroke in the United States during 2007–2017. *Arch Med Sci Atheroscler Dis* 2019; 4: 94-102.
3. Hirata K, Egawa S, Kimura Y, et al. Current status of surgery for pancreatic cancer. *Dig Surg* 2007; 24: 137-47.
4. Gillen S, Schuster T, Meyer Zum Buschenfelde C, Friess H, Kleeff J. Preoperative/neoadjuvant therapy in pancreatic cancer: a systematic review and meta-analysis of response and resection percentages. *PLoS Med* 2010; 7: e1000267.
5. Bastos P, Gomes T, Ribeiro L. Catechol-O-methyltransferase (COMT): an update on its role in cancer, neurological and cardiovascular diseases. *Rev Physiol Biochem Pharmacol* 2017; 173: 1-39.
6. Parkin GM, Udawela M, Gibbons A, Scarr E, Dean B. Catechol-O-methyltransferase (COMT) genotypes are associated with varying soluble, but not membrane-bound COMT protein in the human prefrontal cortex. *J Hum Genet* 2018; 63: 1251-8.
7. Matsuzaka CT, Christofolini D, Ota VK, et al. Catechol-O-methyltransferase (COMT) polymorphisms modulate working memory in individuals with schizophrenia and healthy controls. *Braz J Psychiatry* 2017; 39: 302-8.
8. Perkovic MN, Strac DS, Tudor L, Konjevod M, Erjavec GN, Pivac N. Catechol-O-methyltransferase, cognition and Alzheimer's disease. *Curr Alzheimer Res* 2018; 15: 408-19.
9. Zubenko GS, Maher BS, Hughes HB, 3rd, Zubenko WN, Scott Stiffler J, Marazita ML. Genome-wide linkage survey for genetic loci that affect the risk of suicide attempts in families with recurrent, early-onset, major depression. *Am J Med Genet B Neuropsychiatr Genet* 2004; 129B: 47-54.
10. Perlis RH, Huang J, Purcell S, et al. Genome-wide association study of suicide attempts in mood disorder patients. *Am J Psychiatry* 2010; 167: 1499-507.
11. Nolan KA, Volavka J, Czobor P, et al. Suicidal behavior in patients with schizophrenia is related to COMT polymorphism. *Psychiatr Genet* 2000; 10: 117-24.
12. Baud P, Courtet P, Perroud N, Jollant F, Buresi C, Malfosse A. Catechol-O-methyltransferase polymorphism (COMT) in suicide attempters: a possible gender effect on anger traits. *Am J Med Genet B Neuropsychiatr Genet* 2007; 144B: 1042-7.
13. Pillai RS. MicroRNA function: multiple mechanisms for a tiny RNA? *RNA* 2005; 11: 1753-61.



14. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; 6: 857-66.
15. Feng W, Feng Y. MicroRNAs in neural cell development and brain diseases. *Sci China Life Sci* 2011; 54: 1103-12.
16. Bian S, Sun T. Functions of noncoding RNAs in neural development and neurological diseases. *Mol Neurobiol* 2011; 44: 359-73.
17. Papagiannakopoulos T, Kosik KS. MicroRNAs: regulators of oncogenesis and stemness. *BMC Med* 2008; 6: 15.
18. Blandino G, Fazi F, Donzelli S, et al. Tumor suppressor microRNAs: a novel non-coding alliance against cancer. *FEBS Lett* 2014; 588: 2639-52.
19. Zhu Z, Tang J, Wang J, Duan G, Zhou L, Zhou X. MiR-138 Acts as a Tumor Suppressor by Targeting EZH2 and Enhances Cisplatin-Induced Apoptosis in Osteosarcoma Cells. *PLoS One* 2016; 11: e0150026.
20. Jiang B, Mu W, Wang J, et al. MicroRNA-138 functions as a tumor suppressor in osteosarcoma by targeting differentiated embryonic chondrocyte gene 2. *J Exp Clin Cancer Res* 2016; 35: 69.
21. Xiao L, Zhou H, Li XP, et al. MicroRNA-138 acts as a tumor suppressor in non small cell lung cancer via targeting YAP1. *Oncotarget* 2016; 7: 40038-46.
22. Ye XW, Yu H, Jin YK, et al. miR-138 inhibits proliferation by targeting 3-phosphoinositide-dependent protein kinase-1 in non-small cell lung cancer cells. *Clin Respir J* 2015; 9: 27-33.
23. Wang Q, Tang H, Yin S, Dong C. Downregulation of microRNA-138 enhances the proliferation, migration and invasion of cholangiocarcinoma cells through the up-regulation of RhoC/p-ERK/MMP-2/MMP-9. *Oncol Rep* 2013; 29: 2046-52.
24. Song T, Zhang X, Wang C, et al. MiR-138 suppresses expression of hypoxia-inducible factor 1alpha (HIF-1alpha) in clear cell renal cell carcinoma 786-O cells. *Asian Pac J Cancer Prev* 2011; 12: 1307-11.
25. Tian S, Guo X, Yu C, Sun C, Jiang J. miR-138-5p suppresses autophagy in pancreatic cancer by targeting SIRT1. *Oncotarget* 2017; 8: 11071-82.
26. Suzuki M, Mamun MR, Hara K, et al. The Val158Met polymorphism of the catechol-O-methyltransferase gene is associated with the PSA-progression-free survival in prostate cancer patients treated with estramustine phosphate. *Eur Urol* 2005; 48: 752-9.
27. Ren X, Zhang L, Xiao Q, Huang D, Liu Q, Zhang Y. Association between COMT polymorphism, labor anxiety, and analgesia in pregnant women. *J Pain Res* 2019; 12: 779-85.
28. Lamb YN, Thompson JM, Murphy R, et al.; ABC Study group. Perceived stress during pregnancy and the catechol-O-methyltransferase (COMT) rs165599 polymorphism impacts on childhood IQ. *Cognition* 2014; 132: 461-70.
29. Szemraj-Rogucka Z, Szemraj J, Masiarek K, Majos A. Circulating microRNAs as biomarkers for myocardial fibrosis in patients with left ventricular non-compaction cardiomyopathy. *Arch Med Sci* 2019; 15: 376-84.
30. Yu C, Wang M, Li Z, et al. MicroRNA-138-5p regulates pancreatic cancer cell growth through targeting FOXC1. *Cell Oncol (Dordr)* 2015; 38: 173-81.
31. Brureau L, Moningo D, Emeville E, et al. Polymorphisms of estrogen metabolism-related genes and prostate cancer risk in two populations of African ancestry. *PLoS One* 2016; 11: e0153609.
32. Yang M, Sun S, Guo Y, Qin J, Liu G. Long non-coding RNA MCM3AP-AS1 promotes growth and migration through modulating FOXC1 by sponging miR-138-5p in pancreatic cancer. *Mol Med* 2019; 25: 55.
33. Shifman S, Bronstein M, Sternfeld M, et al. A highly significant association between a COMT haplotype and schizophrenia. *Am J Hum Genet* 2002; 71: 1296-302.
34. DeYoung CG, Getchell M, Kuposov RA, et al. Variation in the catechol-O-methyltransferase Val 158 Met polymorphism associated with conduct disorder and ADHD symptoms, among adolescent male delinquents. *Psychiatr Genet* 2010; 20: 20-4.