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Original Article

The COMT (rs165599) gene polymorphism contributes to chemotherapy-induced cognitive impairment in breast cancer patients

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Abstract: The present study aimed to investigate the effect of genetic polymorphisms of catechol-O-methyl transferase (COMT), apolipoprotein E (APOE), and brain derived neurotrophic factor (BDNF) on the modulation of the chemotherapy-induced cognitive impairment (CICI) in breast cancer patients. Eighty triple negative breast cancer (TNBC) and 165 non-triple negative breast cancer (NTNBC) patients were selected, and subjected to a number of neuropsychological tests, including memory questionnaires, before and after chemotherapy. Six single-nucleotide polymorphisms (SNPs), including COMT (rs165599, rs4680, rs737865), APOE (rs429358, rs7412), and BDNF (rs6265), were evaluated. The scores of breast cancer patients after chemotherapy were poorer in comparison to those before chemotherapy (t = -5.317, z = -3.372, respectively, $P < 0.01$), and the scores of TNBC patients were poorer than those of NTNBC patients were after chemotherapy (t = -5.997, z = -5.284, respectively, $P < 0.01$). Patients with the COMT (rs165599) genotype had a significantly lower chance of developing cognitive decline than the patients with the G/G genotype, and this was linear with the retrospective memory (RM) questionnaires (β = -1.441, CI (95%) = -2.781~-0.101). However, there was no significant difference between the memory scores of APOE (rs429358, rs7412) and BDNF (rs6265) carriers before or after chemotherapy. This study suggests that CICI in TNBC patients was more prominent than that in NTNBC patients after chemotherapy, and the COMT (rs165599) polymorphism was linear to the retrospective memory (RM) questionnaires, and may be a potential genetic marker for increased vulnerability to CICI in TNBC patients.

Keywords: Catechol-O-methyl transferase, polymorphisms, chemotherapy-induced cognitive impairment, breast cancer

Introduction

Breast cancer (BC) is the most frequently diagnosed cancer for women in the United States, with approximately 231,840 new cases of invasive breast cancer among US women in 2015 [1]. Although systemic treatment such as chemotherapy improves the clinical outcome of patients with BC, it is also known to have severe side effects such as cognitive impairment [2]. Chemotherapy-induced cognitive impairment (CICI) is defined as the impairment of memory, learning, attention, reasoning, executive function, visual-spatial functions, and speed of information processing during or after discontinuation of chemotherapy for cancer patients [3, 4]. The issues regarding CICI became an important topic of the National Comprehensive Cancer Network (NCCN) in 2014, but the specific mechanism is not clear. Because of early detection and advancements in treatment, the prevalence of BC survivors has increased. Most BC patients have a long-term survival rate, which provides an important time window for research on CICI in BC survivors. Longitudinal prospective trials incorporating the assessment of cognitive function have generally reported that between 20% and 61% of women with BC demonstrate alterations in cognitive function after receiving standard dose chemotherapy [5].

Memory impairment was the most prominent presentation in BC patients with CICI [6]. Neuropsychological research evidence suggests that memory can be divided into retrospective memory (RM) and prospective memory (PM) based on what is remembered [7]. In contrast to the RM that involves memory of past events, PM is defined as the ability to carry out a delayed intended action [8]. The PM is closely related to daily human life, particularly the aspect of remembering to do important things regularly. Bedard *et al.* determined the cognitive operations involved in PM deficits exhibited by chemotherapy-exposed BC survivors [9].

It indicated that CICI is widespread and heterogeneity in BC survivors from Journal of Clinical Oncology, but the specific mechanism is not clear [10]. Previous studies have documented that single-nucleotide polymorphisms in catechol-O-methyl transferase (COMT, rs4680, rs165599, rs737865), brain-derived neurotrophic factor (BDNF, rs6265), and apolipoprotein E (APOE, rs429358 and rs7412) are implicated in aging and neuro-cognitive functioning [11]. COMT is an enzyme that catalyzes the O-methylation of catecholamine neurotransmitters such as dopamine, adrenaline, and noradrenaline [12]. Polymorphisms in this gene result from a valine (Val or G) or methionine (Met or A) substitution, with the latter reducing dopamine degradation. Several studies have reported that COMT Val carriers (Val/Val and Val/Met) are considered relatively at risk for specific cognitive deficits [13-15]. BC survivors treated with chemotherapy who also possess the COMT Val genotype are susceptible to negative effects on their cognitive health [16]. APOE is involved in lipoprotein metabolism. The well-known ε4 variant is the largest known genetic risk factor of Alzheimer disease (AD) and has been implicated in normal cognitive aging decline [17]. BC and lymphoma survivors who were treated with chemotherapy have poor performance in visual memory, spatial ability, and psychomotor functioning tests if they are carriers of the ε4 allele of the APOE gene [18]. Moreover, cognitive function in postmenopausal women with BC is modified by APOE genotypes and the combination of APOE genotype and treatment [19]. BDNF is the most widely distributed neurotrophin in the central nervous system, and is mainly expressed in the hippocampus and cortex, but also exists in the striatum, prefrontal cortex, basal forebrain, hypothalamus, brain stem, and cerebellum [20]. BDNF plays an important role in neuronal repair and survival, dendritic and axonal growth, and long-term potentiation [21]. Dooley *et al.* found the BDNF Met allele may be a risk factor for inflammation-associated cognitive depressive symptoms among BC survivors [22].

Triple-negative breast cancer (TNBC) is defined by the absence of detectable estrogen receptor (ER) and progesterone receptor (PR) expression and the lack of human epidermal growth factor 2 (HER2/neu) gene amplification [23]. And, the relationship between the COMT, APOE, and BDNF polymorphisms and the occurrence of CICI in TNBC survivors has not yet been elucidated.

At present study, we try to find whether there is difference on cognitive impairment in 80 TNBC and 165 non-triple negative breast cancer (NTNBC) patients before and after chemotherapy. Further we retrieve the genetic contribution of COMT APOE, and BDNF polymorphisms in these patients, and aim to investigate whether the three gene polymorphisms modulate CICI in TNBC survivors.

Materials and methods

Participants

A total of 245 breast cancer patients, including 80 cases of triple negative breast cancer (TNBC) and 165 cases of non-triple negative breast cancer (NTNBC), who were hospitalized from January 2013 to September 2015 in the Department of Oncology at the Second Affiliated Hospital of Anhui Medical University were recruited. Information regarding the patient's age, education, Karnofsky Performance Status (KPS), and pathological patterns was gathered and statistically analyzed.

All subjects were right-handed, had an education of more than 5 years, and were selected according to the following criteria: 1) de-novo BC confirmed by postoperative pathology; 2) standard-dose chemotherapy treatment with doxorubicin, paclitaxel, cyclophosphamide, and fluorouracil, but no hormonal therapy; 3) no restrictions on age or pathological types; 4) a score of ≥ 24 in the mini-mental state examination (MMSE); 5) normal daily life activities as

measured by the Karnofsky Performance Status (KPS, score ≥ 80); and 6) no impairment of vision, hearing, or language. BC patients with the following conditions were excluded: 1) a history of a variety of chemotherapy, radiotherapy, hormonal therapy, and other treatments; 2) cachexia or distant metastasis; 3) psychiatric symptoms such as anxiety, depression, and paranoia; 4) a history of alcohol/drug dependence and cognitive therapy; 5) clinically diagnosed dementia; 6) intracranial metastases and other abnormalities; and 7) cognitive impairment patients. This study was approved by the Research Ethics Committee of the Affiliated Second Hospital of Anhui Medical University, and all subjects provided informed consent prior to beginning the study.

Neuropsychological background tests

According to the above grouping of BC patients, a series of neuropsychological background tests was administered within four weeks of beginning chemotherapy, followed by six cycles of postoperative adjuvant, to assess general cognitive and memory functions. MMSE was administered to assess cognitive function, including time and spatial orientation, short-term memory, calculation, language, and visuo spatial skills. A verbal fluency test (VFT) was administered, where subjects were asked to name as many animals as they could in one minute. A digit span test (DST) was used to measure short-term memory in which subjects were asked to recall a series of numbers after hearing them in a randomized order. The total score was determined by the number of digits recalled in correct serial order.

Retrospective memory (RM) and prospective memory (PM) questionnaires

All subjects were tested by PM/RM questionnaires (PRMQ). The memory questionnaires consisted of 16 items divided equally between a PM and a RM subscale. For the 8 PM questions, participants were asked to rate how often each item happened to them on a 4-point Likert scale according to memory impairment degree (4: heaviest impairment, 1: lightest impairment). For the 8 RM questions, participants were asked to rate the statements on a 4-point Likert scale (4: very often, 3: sometimes, 2: rarely, 1: never). According to the standards, the minimum total score was 16, and the maximum total score was 64. All tests were administered in a quiet environment without interference and completed within 10 minutes.

Genotyping

Peripheral venous blood was sampled into sterile anticoagulation blood tubes, and stored at -80°C until use. Genomic DNA was extracted from peripheral blood usinga blood genomic QIAGENE kit (Shanghai Genesky Bio-Tech Co, Ltd (http://biotech.geneskies.com)). DNA samples were stored at -20°C. Genotyping was carried out using the improved multiplex ligase detection reaction (iMLDR) technique, with technical support from Shanghai Genesky Biotechnologies Inc (Shanghai, China). For each SNP, the alleles were distinguished using different fluorescently labeled allele-specific oligonucleotide probe pairs. Distinct SNPs were further distinguished by different extended lengths at the 3'-end. Two negative controls were set: one with double-distilled water as the template and the other with a DNA sample without primers, while keeping all other conditions the same in one plate. Duplicate tests were designed, and the results were consistent. A random sample accounting for 5% of the total DNA samples was directly sequenced using Big Dye-terminator version 3.1 and an ABI3730XL automated sequencer (Applied Biosystems) to confirm the results of iMLDR.

Statistical analysis

All data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed with a one-way ANOVA using SPSS software (version 22.0, http://spss.en.softonic.com/; Chicago, IL, USA), and Student's tests were performed; odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression. A Mann-Whitney U-test was performed for normally and non-normally distributed data. In addition, the frequencies of the alleles, genotypes, and other categorical variables in the 2 groups were assessed by the chi-square (χ^2) test. For cognitive impairment susceptibility analyses, we used logistic regression, calculating odds ratios (ORs) and 95% confidence intervals (CIs) to assess genetic effects, assuming a general genetic model (dominant, recessive, additive models) for individual SNP analyses, and adjusting for age (continuous), KPS, years of education, and pa-

Table 1. Clinical data on breast cancer patients with TNBC and NTNBC

Parameters		Groups (Mean ± SD)	
		TNBC (n = 80)	NTNBC (n = 165)
Age (year)		48.48 ± 10.57	49.39 ± 10.61
Education (year)		10.09 ± 3.37	10.08 ± 3.63
KPS		82.5 ± 7.88	84.12 ± 7.65
Pathological pattern	Non-special type invasive carcinoma of breast	74	156
	Special type invasive carcinoma of breast	3	0
	Carcinoma in situ	3	8
	Microinvasive carcinoma	0	1

Note: Triple-negative breast cancer (TNBC); Non-triple negative breast cancer (NTNBC); Karnofsky Performance Status (KPS).

Table 2. Comparison of neuropsychological background testing between the 2 groups before and after chemotherapy

Group	N	MMSE	DST	VFT
Before chemotherapy	245	27.26 ± 1.58	6.19 ± 0.73	11.46 ± 1.52
After chemotherapy	245	26.76 ± 1.62*	5.78 ± 0.97*	9.99 ± 2.11*

Note: *: $P < 0.01$; Mini-mental state (MMSE); Digit span test (DST); Verbal fluency test (VFT).

Table 3. Comparison of RM and PM questionnaire scores between the 2 groups before and after chemotherapy

Group	N	RM	PM
Before chemotherapy	245	16.23 ± 4.03	16.01 ± 4.64
After chemotherapy	245	17.21 ± 4.59*	18.20 ± 4.51*

Note: *: $P < 0.01$; Retrospective memory (RM); Prospective memory (PM).

Table 4. Comparison of neuropsychological background testing between the TNBC group and the NTNBC group after chemotherapy

Group	N	MMSE	DST	VFT
TNBC	80	26.20 ± 1.67*	5.29 ± 1.01*	8.40 ± 1.65*
NTNBC	165	27.03 ± 1.52	6.02 ± 0.86	10.76 ± 1.87

Note: *: $P < 0.01$; Mini-mental state (MMSE); Digit span test (DST); Verbal fluency test (VFT); Triple negative breast cancer (TNBC); Non-triple negative breast cancer (NTNBC).

Table 5. Comparison of RM and PM questionnaire scores between TNBC group and NTNBC group after chemotherapy

Group	N	RM	PM
TNBC	80	19.10 ± 2.36*	20.44 ± 3.41*
NTNBC	165	16.29 ± 5.10	17.12 ± 4.58

Note: *: $P < 0.01$; Retrospective memory (RM); Prospective memory (PM); Triple negative breast cancer (TNBC); Non-triple negative breast cancer (NTNBC).

thological pattern. Linear regression was performed to delineate the associations between CICI and the COMT (rs165599) polymorphism. All statistically matched two-tailed probability test, statistically meaningful standards were defined at $P < 0.01$.

Results

Analysis of clinical parameters of patients

A total of 245 patients met the inclusion criteria, of which, 80 were TNBC and 165 were NTNBC. As **Table 1** shows, there was no significant difference in age (48.48 ± 10.57 vs. 49.20 ± 10.63), years of education (10.09 ± 3.35 vs. 10.11 ± 3.87), or KPS (82.5 ± 7.88 vs. 84.09 ± 7.66). According to the pathological pattern of BC, among the TNBC group, 74 patients were identified as non-special type invasive carcinoma of the breast, 3 patients were identified as special type invasive carcinoma of the breast, and 3 patients were identified as carcinoma *in situ*. Similarly, among the NTNBC group, 156 patients were identified as non-special type invasive carcinoma of the breast, 8 patients was identified as carcinoma *in situ*, 1 patient was identified as microinvasive carcinoma.

Comparison of neuropsychological tasks, PM, and RM

As shown in **Table 2**, the MMSE was significantly decreased to 26.76 ± 1.62 after chemother-

Table 6. Identified SNPs by sequencing of COMT (rs4680, rs165599, rs737865), APOE (rs429358, rs7412) and BDNF (rs6265) in TNBC group and NTNBC group

SNP	COMT			APOE		BDNF
	rs4680	rs165599	rs737865	rs429358	rs7412	rs6265
CHR	22	22	22	19	19	11
Allele Position	19951271	19956781	19930121	45411941	45412079	27679916
Ref allele	G	G	A	T	C	C
Alt allele	A	A	G	C	T	T
MAF in TNBCs	0.213	0.394	0.241	0.075	0.100	0.463
MAF in NTNBCs	0.262	0.470	0.317	0.094	0.089	0.473
MAF in Database	0.369	0.481	0.227	0.151	0.072	0.201
Call Rate%	100%	100%	99.2%	100%	100%	100%
P for HWE	0.303	0.251	0.360	0.704	0.705	1
*P**	0.218	0.005	0.051	0.487	0.746	0.831

Note: Single nucleotide polymorphism (SNP); Chromosome (CHR); Loci alleles on the reference sequence (Ref allele); The other allele on the loci (Alt allele); Triple negative breast cancer (TNBC); Non-triple negative breast cancer (NTNBC); Minor allele frequency (MAF, data from 1000 Genomes); Hardy-Weinberg equilibrium (HWE), *p*-value for HWE in TNBC groups; **p*-value for alleles frequency differences between two groups.

apy as compared to that of before chemotherapy (27.26 ± 1.58, *: $P < 0.01$), and similarly DST and VFT scores were also significantly decreased to 5.78 ± 0.97 and 11.46 ± 1.52, respectively, after chemotherapy when compared to that before chemotherapy (6.19 ± 0.73, *: $P < 0.01$; and 11.46 ± 1.52, *: $P < 0.01$, respectively). As **Table 3** shows, the average RM score was significantly increased to 17.21 ± 4.59 after chemotherapy as compared to that before chemotherapy (16.23 ± 4.03, *: $P < 0.01$), and similarly, the average PM score was also significantly increased to 18.20 ± 4.51 after chemotherapy as compared to that before chemotherapy (16.01 ± 4.64, *: $P < 0.01$).

Comparison of neuropsychological background tasks, RM, and PM after chemotherapy

As shown in **Table 4**, MMSE score after chemotherapy in the TNBC group (26.20 ± 1.67) was significantly lowerthan that in the NTNBC group (27.03 ± 1.52, *: $P < 0.01$), and similarly, the DST and VFT values after chemotherapy (5.29 ± 1.01 and 8.40 ± 1.65, respectively) in the TNBC group were also significantly lower than in the NTNBC group (6.02 ± 0.86, *: $P < 0.01$, and 10.76 ± 1.87, *: $P < 0.01$, respectively).

As shown in **Table 5**, the average RM score after chemotherapy in the TNBC group (19.10 ± 2.36) was significantly higher than that in the NTNBC group (16.29 ± 5.10, *: $P < 0.01$), and similarly, the average PM score after chemotherapy in the TNBC group (20.44 ± 3.41) was significantly higher than that in the NTNBC group (17.12 ± 4.58, *: $P < 0.01$).

The unit SNP loci analysis

Sequencing analysis (**Table 6**) revealed that the allelic distribution of COMT (rs165599) was significantly different between TNBC and NTNBC survivors (*: $P < 0.01$). As **Table 7** shows, there was a significant difference in COMT rs165599 (co-dominant model: $\chi^2 = 8.922$, $P = 0.012$; dominant model: $\chi^2 = 7.096$, $P = 0.008$; recessive model $\chi^2 = 4.354$, $P = 0.037$), and rs737865 (co-dominant model: $\chi^2 = 7.565$, $P = 0.022$; recessive model: $\chi^2 = 7.56$, $P = 0.006$) genotypic frequency distribution. Furthermore, logistic regression analysis results showed that the patients with the GA (adjusted, OR = 0.515, CI (95%) = 0.272-0.977, $P = 0.048$) and AA (adjusted, OR = 0.318, CI (95%) = 0.136-0.742, $P = 0.048$) genotypes of COMT rs165599 had significantly lower odds of developing cognitive decline than the patients with the G/G genotype. The rs165599 was found to significantly increase the risk of CICI in addictive models (OR = 0.556, CI (95%) = 0.365-0.847, $P = 0.037$), but not in dominant or recessive models. Similarly, the A/G (OR = 0.995, CI (95%) = 0.562-1.762, $P = 0.049$) and the G/G (OR = 0.157, CI (95%) = 0.035-0.711, $P = 0.049$) genotypes of the COMT rs737865 were also significantly different from the G/G genotype. When comparing the cognitive outcomes of the reces-

Table 7. Genetic associations between COMT (rs4680, rs165599, rs737865), APOE (rs429358, rs7412), BDNF (rs6265) and cognitive impairment susceptibility

SNP	Model	Genotype	TNBC	NTNBC	P[a] (χ^2)	Logistic regression		FDR-BH adjusted[c]
						OR (95% CI)	P[b]	
rs4680	Co-dominant	G/G	51	91		-	-	-
		G/A	24	61	0.441	0.747 (0.411-1.358)	0.339	1
		A/A	5	13		0.725 (0.243-2.166)	0.564	1
	Dominant	G/G	51	91	0.201	0.743 (0.424-1.303)	0.300	0.659
		G/A+A/A	29	74				
	Recessive	G/G+G/A	75	152	0.647	0.804 (0.274-2.358)	0.691	1
		A/A	5	13				
	Addictive	-	-	-	-	0.802 (0.514-1.251)	0.331	0.662
rs6265	Co-dominant	T/T	16	38				
		T/C	42	80	0.812	1.159 (0.613-2.195)	0.650	1
		C/C	22	47		0.931 (0.426-2.034)	0.858	1
	Dominant	T/T	16	38	0.872	1.085 (0.593-1.984)	0.791	0.791
		T/C+C/C	64	127				
	Recessive	T/T+T/C	58	118	0.592	0.847 (0.436-1.647)	0.625	1
		C/C	22	47				
	Addictive	-	-	-	-	0.977 (0.667-1.433)	0.907	0.907
rs165599	Co-dominant	G/G	28	32		-	-	-
		G/A	41	91	0.012	0.515 (0.272-0.977)	0.042	0.048
		A/A	11	42		0.318 (0.136-0.742)	0.008	0.048
	Dominant	G/G	28	32	0.008	0.455 (0.246-0.838)	0.012	0.069
		G/A+A/A	52	133				
	Recessive	G/G+G/A	69	123	0.037	0.495 (0.237-1.034)	0.061	0.184
		A/A	11	42				
	Addictive	-	-	-	-	0.556 (0.365-0.847)	0.006	0.037
rs737865	Co-dominant	A/A	43	80		-	-	-
		A/G	34	62	0.022	0.995 (0.562-1.762)	0.986	0.049
		G/G	2	23		0.157 (0.035-0.711)	0.016	0.049
	Dominant	A/A	43	80	0.385	0.771 (0.445-1.336)	0.354	0.658
		A/G+G/G	36	85				
	Recessive	A/A+A/G	77	142	0.006	0.158 (0.036-0.697)	0.015	0.089
		G/G	2	23				
	Addictive	-	-	-	-	0.652 (0.422-1.009)	0.055	0.164
rs429358	Co-dominant	T/T	68	135		-	-	-
		T/C	12	29	0.682	NA	NA	1
		C/C	0	1		NA	NA	1
	Dominant	T/T	68	135	0.536	0.812 (0.386-1.709)	0.583	0.700
		T/C+C/C	12	30				
	Recessive	T/T+T/C	80	164	0.485	NA	NA	1
		C/C	0	1				
	Addictive	-	-	-	-	0.812 (0.386-1.709)	0.583	0.700
rs7412	Co-dominant	C/C	64	136		-	-	-
		C/T	16	28	0.485	1.364 (0.673-2.764)	0.389	1
		T/T	0	1		1.31E-09 (0-inf)	0.993	1
	Dominant	C/C	64	136	0.646	1.32 (0.653-2.67)	0.439	0.659
		C/T+T/T	16	29				
	Recessive	C/C+C/T	80	164	0.485	1.2E-09 (0-inf)	0.999	1
		T/T	0	1				
	Addictive	-	-	-	-	1.25 (0.631-2.478)	0.522	0.700

Note: [a]The χ^2 test of *p* values for SNP polymorphisms distribution differences between 2 groups; [b]*p* value for logistic regression analysis after adjusting for age, years of education, KPS and pathological type; [c]By controlling the False Discovery Rate (FDR) to determine the threshold of *p* value; 95% confidence interval (95% CI); The False Discovery Rate (FDR); Triple negative breast cancer (TNBC); Non-triple negative breast cancer (NTNBC).

Table 8. Correlation analysis between COMT (rs165599) and CICI

	Model	Genotype	B (95% CI)	*P* value
MMSE	Dominant	G/G G/A+A/A	0.049 (-0.426~0.524)	0.840
	Recessive	G/G+G/A A/A	0.075 (-0.425~0.571)	0.768
	Addictive	-	-0.029 (-0.254~0.349)	0.848
	HOM	G/G	0.096 (-0.509~0.701)	0.757
	HET	G/A	0.301 (-0.467~0.528)	0.904
DST	Dominant	G/G G/A+A/A	-0.689 (-1.16~-0.218)	0.005
	Recessive	G/G+G/A A/A	-0.358 (-0.856~-0.141)	0.161
	Addictive	-	-0.410 (-0.709~-0.110)	0.008
	HOM	G/G	-0.803 (-1.402~-0.203)	0.009
	HET	G/A	-0.644 (-1.138~-0.151)	0.011
VFT	Dominant	G/G G/A+A/A	0.914 (0.025~1.802)	0.045
	Recessive	G/G+G/A A/A	0.657 (-0.276~1.59)	0.169
	Addictive	-	0.611 (0.047~1.175)	0.035
	HOM	G/G	1.208 (0.078~2.339)	0.037
	HET	G/A	0.798 (-0.133~1.729)	0.094
PM	Dominant	G/G G/A+A/A	1.166 (-0.1532~2.486)	0.084
	Recessive	G/G+G/A A/A	0.353 (-1.034~1.741)	0.618
	Addictive	-	0.601 (-0.239~1.44)	0.162
	HOM	G/G	1.161 (-0.520~2.842)	0.177
	HET	G/A	1.169 (-0.216~2.553)	0.099
RM	Dominant	G/G G/A+A/A	-1.441 (-2.781~-0.101)	0.036
	Recessive	G/G+G/A A/A	-0.639 (-2.05~0.772)	0.376
	Addictive	-	-0.817 (-1.669~0.035)	0.062
	HOM	G/G	-1.594 (-3.3~0.113)	0.068
	HET	G/A	-1.381 (-2.786~0.024)	0.055

Note: Homozygote (HOM); Heterozygote (HET); Beta, Regression coefficient; 95% confidence interval (CI (95%)).

sive (OR = 0.158, CI (95%) = 0.036-0.697, *P* = 0.089), dominant (OR = 0.771, CI (95%) = 0.445-1.336, *P* = 0.658), and addictive models (OR = 0.652, CI (95%) = 0.422-1.009, *P* = 0.164), no significant association was established for COMT (rs7378-65). Neither APOE (rs429358, rs74-12) nor BDNF (rs6265) showed any statistically significant differences between the two groups.

The correlation analysis between COMT, APOE, and BDNF gene polymorphisms and CICI

As **Table 8** shows, the correlation analysis between CMOT (rs165599) and CICI (MMSE, DST, VFT, RM, and PM) demonstrated that the G/G genotype (β = -0.803; CI (95%) = -1.402~-0.203; *P* = 0.009), G/A genotype (β = -0.644; CI (95%) = -1.138~-0.151; *P* = 0.011), and dominant model (β = -0.689, CI (95%) = -1.16~-0.218, *P* = 0.005) were significantly associated with DST. Specifically, the dominant model (β = -1.441, CI (95%) = -2.781~-0.101) was found to be significantly associated with respective memory questionnaires.

Discussion

This study demonstrated that the results of the neuropsychological assessment and PRMQ were significantly different in BC patients before and after chemotherapy, and significantly different in TNBC patients compared to NTNBC patients, with lower MMSE, lower DST, lower VFT, higher RM and higher PM scores. In addition, the distribution of alleles and genotypes of COMT (rs165599) between the TNBC and NTNBC groups was significantly different, and had an obvious linearity (β = -1.441, CI (95%) = -2.781~-0.101) with the RM questionnaires.

CICI of BC patients occurred with varying degrees of severity and was affected by many factors, the pathogenesis of which is complex; however the exact mechanism is not clear yet

[24, 25]. A large number of studies have documented that cognitive impairment was a commonly occurring problem in BC patients after chemotherapy, involving several cognitive areas such as memory, attention, and executive functions, of which the decline or loss of memory was the most prominent feature [26-28], Cheng *et al.* found that BC patients, after adjuvant chemotherapy, showed deficits in PM questionnaires [29]. More importantly, the consequence of CICI was even more worrying than recurrence and metastasis of the tumor in the long-term survival patients with BC [30]. Sahin *et al.* found that the cognitive dysfunction seen in BC patients after chemotherapy severely affected the quality of daily life [31]. In our study, the MMSE, DST, and VFT scores were significantly decreased after chemotherapy compared to before chemotherapy. Meanwhile, RM and PM scores were significantly increased after chemotherapy. Our data analysis verified the conclusion that BC patients have considerable memory decline after chemotherapy.

As a distinct type of BC, TNBC has unique clinical features and biological behavior. Clinically, chemotherapy is commonly used in the treatment of TNBC because of its high sensitivity; however, the recurrence and metastasis rate are still higher than in NTNBC patients. Liedtke *et al.* found that TNBC patients have a higher recurrence with the risk of death post-operatively in the first 3 years compared with that of NTNBC patients [32]. In this study, we focused on a series of CICIs between TNBC and NTNBC patients, including cognitive neuropsychological investigation of MMSE, DST, VFT, and PRMQ. Our data analysis demonstrated that the MMSE, DST, and VFT scores in TNBC patients after chemotherapy were significantly higher compared to those in NTNBC patients. Meanwhile, the RM and PM scores in TNBC patients were significantly lower than in NTNBC patients. This provides evidence of CICI in TNBC survivors.

The mechanism of CICI is complicated in BC, and the integrity of the human genome plays a key role in the biological systems (including brain tissue) normal functions [33]. Newhouse *et al.* found that estrogen and progesterone affect cognitive function by influencing the brain cholinergic system [34]. A large number of studies have found that hormones not only provide neurotrophic and protective effects, but also enhance and maintain speech and memory function [35]. The prefrontal lobe and hippocampus memory area in the brain are the main target organs of estrogen and progesterone. Yuen *et al.* found that stress-induced glutamatergic deficits and memory impairment are influenced by the blocking of estrogen receptors or aromatase in females [36]. Masuda *et al.* found that the prognosis of BC was related to the expression of estrogen, progesterone, and human epidermal growth factor-2 [37]. CICI has significant heterogeneity for BC survivors. The relationship between genetic risk factors and chemotherapy-induced memory impairment in TNBC survivors will be elaborated on in this study.

The mechanism of CICI is complicated in BC. A previous study demonstrated that COMT, BDNF, and APOE play key roles in aging and neurocognitive functioning [38]. COMT gene polymorphism is a common genetic variant affecting cognition and prefrontal dopamine levels [39, 40]. Our study observed that TNBC patients performed more poorly than NTNBC patients on tests of cognitive neuropsychology including on PRMQs, further supporting that certain COMT (rs165599) genotypes could modify the presence of cognitive differences as a function of BC treatment, especially on the RM questionnaires. The potential mechanism for the interaction between chemotherapy and COMT polymorphisms in TNBC survivors is not clear. As an enzyme that can methylate estrogen metabolites resulting in their inactivation, past research found that the COMT gene possesses a G→A structural polymorphism at codon 158, resulting in a methionine (Met/A) instead of valine (Val/G), and the enzyme activity of the A/A genotype was 2-4 fold lower than that of G/G carriers [41]. The observation that PM and RM performance among TNBC survivors after chemotherapy is poor, is consistent with predictions regarding the effect of chemotherapy on the frontal lobes of cancer survivors, as well as the role of dopamine in the functioning of these brain structures [42]. We speculated that TNBC patients who were A allele carriers of COMT (rs165599) may exhibit poorer memory performance.

There are several limitations worthy of discussion. First, this study was entirely composed of the Han population; whether the observed results can be applied to other diverse popula-

tions is not clear. The second is the lack of sufficient numbers of TNBC and NTNBC patients. Future studies should include a large sample of BC patients who have undergone chemotherapy to examine the reliability and validity of neuropsychological tests, and to validate whether memory impairment is a feature of BC patients after completion of chemotherapy. The third limitation is that this is a cross-sectional study of small sample size, and it failed to observe any dominant pattern of deficits that could be associated with a particular brain region. Neuroimaging evidence can be applied to similar future studies.

In conclusion, our study found some differences in PM and RM impairment and genetic polymorphisms in TNBC patients; however, additional evidence is required through further large follow-up studies to confirm our observations. These results indicate that CICI may be correlated to differential expression of the COMT (rs165599) polymorphism, and this correlation may play an important role in the understanding of risk factors for poorer cognitive health among TNBC patients after chemotherapy, and not only provides a data analysis between TNBC and NTNBC patients, but also provides molecular biological mechanisms of CICI for BC patients.

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Disclosure of conflict of interest

None.

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