The COMT Rs737865 Mediates Chemo-Brain In Breast Cancer Patients With Various Index Of Ki-67

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Research Article

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Abstract

Background: Anterior findings denoted that catechol-O-methyltransferase (COMT) have been considered as genetic risk for chemo-brain. However, the mediation of COMT polymorphism for chemo-brain in breast cancer patients with various index of ki-67 was still unknown.

Objective: The current research intended to assess the genetic risk by COMT genotype for chemo-brain in breast cancer patients with various index of ki-67.

Methods: Breast cancer patients (65 with ki-67<14%, 75 with ki-67>14%) fulfilled cognitive tests pre and post adjuvant chemotherapy, three single-nucleotide polymorphisms (SNPs) of COMT (rs165599, rs4680, rs737865) were genotyped by furnishing peripheral blood.

Results: The lower cognitive of breast cancer patients were displayed in compared with those before chemotherapy. Further, the event-based prospective memory (EBPM) scores of ki-67>14% group patients were worse than those of ki-67<14% group patients after chemotherapy (z=-7.51, p<0.01), but the time-based prospective memory (TBPM) scores of two groups were no significant difference. The COMT rs737865 A/G genotype was associated with memory protective susceptibility (co-dominant model: adjusted, OR=0.135, CI95%=0.026-0.706, p =0.018), A/G genotype carriers exhibited more well on EBPM test relative to A/A genotype.

Conclusion: The index of ki-67 was likely to be associated with EBPM decline in breast cancer patients. Moreover, the COMT rs737865 polymorphism was potential genetic risk of chemo-brain in breast cancer patients with various index of ki-67.

Introduction

Breast cancer was one of the most acquainted cancer in women, the percent of the new cases for breast cancer was 30%, publishing by American Cancer Society in year 2021[1]. Equally, breast cancer was the primary death reasons from global cancer in women, the estimated date rate of breast cancer was 6.9% in year 2020[2].It was estimated that occurrence about new cases and deaths were 303,600 and 70,400 for breast cancer in china in the year 2015[3]. The incidence rate of breast cancer was significantly on rising in the United States from 2009 to 2018, while death rates had not increased at the same speed, indicating improvements of clinical treatment in breast cancer patients[4]. The chemotherapy was one of the main systemic therapeutics, the survival rate of breast patients had greatly ameliorated in the past few decades [5]. The treatment of chemotherapy not only prolong the survival of breast patients, but also produce a series of side effects, including cognitive impairment[6]. Breast cancer patients who received chemotherapy often complained of memory loss, poor concentration, slower processing speed, decreased word-finding skills and other cognitive impairment, these cognitive changes were collectively called chemo-brain[7, 8]. The mild to severe cognitive deficit were noticed in breast cancer while enduring chemotherapy [9, 10]. Approximately 75% of cancer patients during the period of chemotherapy and 35% of cancer patients undergo chemo-brain for several months at the end of therapy [11].

Prospective memory was a complex cognitive involving future plans or intentions, which could be separated into event based prospective memory (EBPM) and time-based prospective memory (TBPM) on account of the clues[12]. The results was published in the journal of Psychooncology indicated that the EBPM impairment was found in breast cancer patients undergoing chemotherapy[13].

Chemo-brain was clinical presentation with high heterogeneity, which some of the feature of cognitive impairment were mild, some were heavy, or even some none[14, 15]. Breast cancer is a highly specific female tumor that needs to be diagnosed by immunohistochemistry, it is highly heterogeneous at molecular level on the account of molecular genetic changes, which leads to great differences in prognosis and treatment response[16]. Ki-67 is an important indicator of molecular typing, which could be divided into luminal A and luminal B subtype on account of the expression level of ki-67 in luminal breast cancer[17]. The index of ki-67 was correlated to differentiation, proliferation and invasion metastasis and prognosis of tumor cells[18]. It was found that the learning and memory function of mice was closely related to the cell proliferation of dentate gyrus by detecting the index of Ki-67[19]. The expression of ki-67 in primary pediatric brain tumors was related to poor prognosis and tumor grade[20]. The index of ki-67 was positively related to brain metastasis in lung cancer patients, which high expression indicated poor prognosis[21]. Similarly, the risk factor for brain metastasis was significantly correlated to the high expression level of ki-67 in breast cancer[22]. Our research group found that the heterogeneity of chemo-brain is closely related to its molecular typing in breast cancer patients, in other words, estrogen/progesterone receptor (ER/PR) negative breast cancer was more prone to the decline of EBPM after chemotherapy[23]. However, the chemo-brain for breast cancer patients with various index of ki-67 was still uncertain.

The SNP loci of catechol-O-methyl transferase(COMT:rs4680, rs165599, and rs737865) was relevant to cognitive competence[24]. The translational product of COMT gene plays a key role in clearing catecholamines (such as dopamine, epinephrine and norepinephrine) in human brain[25]. Small et al. [26]found that COMT val carrier was more likely to have poorer performance on tests of attention, verbal fluency among breast cancer patients treated with chemotherapy. Our team found that genetic polymorphism of COMT (rs165599) was connected with retrospective memory impairment[27].Further, The EBPM impairment was detected to be related with COMT rs737865 in breast cancer with different hormonal receptor[28]. Yet, the relationship between various index of ki-67 and COMT polymorphism for chemo-brain needs to be explored in breast cancer patients.

The current study concentrate on the chemo-brain in breast cancer survivors with various index of ki-67, and expound the genetic risk of COMT polymorphism on chemo-brain in breast cancer patients with different expression levels of Ki-67.

Materials And Methods

Participants

Add up to 175 women diagnosed with breast cancer were enrolled at the Department of Oncology, the Affiliated Second Hospital of Anhui Medical University. Groups were divided as those ki-67 < 14% (99 cases) and ki-67 > 14%(76 cases). The research was approved by the Research Ethics Committee of the Second Affiliated Hospital of Anhui Medical University, China. The written informed consent were obtained before the research from all patients.

All subjects was accorded with inclusion criteria as follows: 1) breast cancers were diagnosed by immunehistochemistry and postoperative pathology; 2) anthracycline and paclitaxel are the main standard chemotherapy regimens, but no hormonal therapy;3) Overall cognitive function was normal and the mini-mental state examination (MMSE) scores were greater than 24; 4) activities of daily living was normal and the Karnofsky performance scale score(KPS) ≥ 80 points; 5) no communication barriers. Exclusion criteria: 1) a history of radiotherapy and endocrine therapy;2) terminal stage of tumor;3) brain metastases;4) mental illness, such as dementia ;5) A history of antipsychotics and psychotherapy; and 6) severe anxiety and depression.

Neuropsychological background tests

A succession of neuropsychological background tests was performed before and after chemotherapy. MMSE was to assess general cognitive, including the following seven aspects: orientation in time and place, immediate memory, attention and computational power, delayed memory, language and visual space. For a total of 30 questions, 1 point will be given if each answer was correct, and 0 point will be given if the answer was wrong or the answer was unknown. The score was 26 or less on MMSE indicated cognitive impairment.Language competence, semantic memory and executive function were evaluated by verbal fluency test (VFT). The test requires participants to name as many words of a certain type as possible in a given time (usually one minute). One point will be given for each answer.Digit span test (DST) The number span test is mainly applied to assess the attention and immediate memory of the subjects, and record the highest score corresponding to the forward and reverse retelling numbers.

EBPM task

The subjects were told to tap the table whenever they saw two animal words (target words) in subsequent task. Further, the subjects were asked to remember to state their contact number (no more any prompt) at the end of the experiment. There were 32 Chinese cards, each of which contains 12 Chinese words, two of 12 words belong to subcategory and the other 10 words belong to major category. The patient's task was to select the two words belonging to the subcategory. When the selected words belong to target words (animal category), the subjects was required to knock on the table. Add up to 6 target words were interspersed among the task. If the subjects could remember to complete them correctly, one score was recorded, a total of 6 points. At the end of the task, the subject were demanded to say his/her contact number, and can correctly remember (record as 2 points). The total score of EBPM were 8 points.

TBPM task

Subjects were performed to finish a target behavior every once in a while, and the number of times that the target behavior which they could execute correctly were regarded as the score of TBPM task. There were 100 cards in total, of which with 12 two-digit numbers. The subject's task was to select the maximum and minimum numbers on each card, and were asked to knock on the table every five minutes after the starting of selection task. The time could be monitored through the clock placed at the right shoulder of subjects, and the total test time was 17 min. If the subjects could remember to tap the table from 10s before and 10s after the target time, 2 points will be obtained, and 1 point will be obtained if the taping the table reacted from 30 s before and 30s after the target time. The total score of TBPM were 6 points.

Genotyping

The 3 ~ 5 ml peripheral venous blood of participant was collected by vacuum blood collection tube and stored in a refrigerator at -20℃. Genomic DNA was extracted by blood genomic DNA QIAGENE Kit (Shanghai Genesky Bio-Tech Co, Ltd(http://biotech.geneskies.com). Genotyping was accomplished by Shanghai Genesky Biotechnology Co., LTD, adopting the technology of the improved multiplex ligase detection reaction (iMLDR). The three SNP loci of 175 samples were genotyped in this study. The region of the target SNP loci was amplified in one system using multiplex PCR. The amplified products were purified by exonuclease and used as templates for subsequent ligase reactions. The 5 '-end allele specific probes and a fluorescent labeled specific probe at the 3'-end were contained in each site for linkage reaction. The ligase products were amplified by PCR using fluorescent labeled universal primers. The amplified products were distinguished by fluorescence capillary electrophoresis. Finally, genotypes of each SNP loci were acquired by electrophoresis analysis. The success rate of classification was 99% and accuracy was 99.6%.

Statistical analysis

SPSS software package (version 22.0, http://spss.en.softonic.com/; Chicago, IL, USA) was applied to conduct the statistical analysis. Forest plots and histogram was drew with GraphPad Prism5 (Graph Pad Software Inc., San Diego,CA). The clinical baseline characteristics and cognitive tests were divided into ki-67 < 14% group and ki-67 > 14% group adopting independent-sample t-test or Mann-Whitney U-test for normally distributed data and non-normally distributed data. Genetic balance of populations were detected passing hardy weinberg equilibrium (HEW).The discrepancy of allelic gene, genotype frequency, pathological type and neoplasm staging were analyzed by the chi-square ($\chi 2$) test in the two groups. Binary logistic regression was devoted to assess the genetic susceptibility risk of chemo-brain, displaying as odds ratio (OR) and 95% confidence interval (CI).

The genetic models were founded to further evaluate the susceptibility factors causing cognitive dysfunction, such as co-dominant, dominant, recessive, HOM and HET models. The P value of logistic regression were adjusted for age, KPS, education, neoplasm staging, and pathological pattern. A one-way ANOVA was applied to analyze the cognitive impairment possessing different genotypes among breast

cancer patients with ki-67 > 14%. All statistical tests were two-tailed with statistical significance criterion defined at $p < 0.05$.

Results

The clinical characteristics

As shown in Table 1, ki < 14% group included 99 patients and ki-67 > 14% group included 76 patients. There were statistical difference in karnofsky performance status scale (KPS) (85.25 ± 6.90 vs. 82.37 ± 7.81, z=-2.53, $p < 0.05$).But, age (49.04 ± 9.97 vs. 48.13 ± 10.29), years of education (10.15 ± 3.85vs. 10.05 ± 3.30) were proved no significant difference. Similarly, there was statistical difference in tumor stages between the two groups ($\chi^2 = 13.84$, $p < 0.01$). Pathological patterns were proved no significant difference. In ki-67 < 14% group, 89 breast cancer patients were confirmed as non-special type invasive ductal carcinoma (IDO-NOS), 1 patients were confirmed as special type invasive ductal carcinoma (IDO-S), and 9 patients were confirmed as carcinoma in situ(CIS). Similarly, in ki-67 > 14% group, 71 breast cancer patients were confirmed as IDO-NOS, 3 patients was identified as IDO-S, 2 patient was confirmed as CIS.

Table 1
The basic clinical characteristics of breast cancer patients with various index of Ki-67

Contents		Groups	
		Ki-67 < 14% (n = 99)	Ki-67 > 14% (n = 76)
Age (Mean ± SD, year)		49.04 ± 9.97	48.13 ± 10.29
Education (Mean ± SD, year)		10.15 ± 3.85	10.05 ± 3.30
KPS (Mean ± SD, year)		85.25 ± 6.90	82.37 ± 7.81*
Pathological patterns (%)	IDC-NOS	89(89.9%)	71(93.4%)
	IDC-S	1(1.0%)	3(4.0%)
	CIS	9(9.1%)	2(2.6%)
Stages (%)	I	8(8.1%)	2(2.6%)**
	II	41(41.4%)	52(68.4%)
	III	16(16.2%)	9(11.8%)
	IV	33(34.3%)	13(17.1%)

Note: *$P < 0.05$ **: $p < 0.01$. KPS, karnofsky performance status scale; IDC-NOS, non-special type invasive ductal carcinoma of breast; IDC-S, special type invasive ductal carcinoma of breast; CIS, carcinoma in situ; MIC, microinvasive carcinoma.

Table 1

General cognitive tests: before and after chemotherapy

Table 2, before and after chemotherapy, the MMSE (27.27 ± 1.54 vs. 26.64 ± 1.70), DST (6.18 ± 0.69 vs. 5.78 ± 0.98), VFT (11.35 ± 1.54 vs. 9.81 ± 2.08), EBPM (2.67 ± 1.01 vs. 1.82 ± 1.18) and TBPM (4.99 ± 0.98 vs. 4.71 ± 0.92) scores were significantly decreased, had a significant difference (z=-3.07,z=-3.58,z=-6.95,z=-6.72,z=-2.77, respectively, $p < 0.01$).

Table 2
General cognitive test before and after chemotherapy

Task	Mean ± SD	
	Before chemotherapy (n = 175)	After chemotherapy (n = 175)
MMSE	27.27 ± 1.54	26.64 ± 1.70**
DST	6.18 ± 0.69	5.78 ± 0.98**
VFT	11.35 ± 1.54	9.81 ± 2.08**
EBPM	2.67 ± 1.01	1.82 ± 1.18**
TBPM	4.99 ± 0.98	4.71 ± 0.92**

Note: **: $p < 0.01$. MMSE indicates the mini-mental state; DST indicates the digit span test; VFT indicates the verbal fluency test. EBPM indicates the event-based prospective memory; TBPM indicates the time-based prospective memory; RM retrospective memory; PM prospective memory.

Table 2

General cognitive tests: after chemotherapy

Table 3, the TBPM scores of breast cancer patients in ki-67 < 14% group after chemotherapy was mildly elevated than in ki-67 > 14% group (4.68 ± 0.99 vs. 4.75 ± 0.80), had no statistical difference ($P > 0.05$). On the contrary, the MMSE scores was elevated in ki-67 < 14% group after chemotherapy than in ki-67 > 14% group (26.93 ± 1.49 vs.26.26 ± 1.88,$P < 0.05$). Similarly, the DST, VFT, and EBPM scores were significant difference in ki-67 < 14% and ki-67 > 14% group after chemotherapy (DST: 6.02 ± 0.85 vs. 5.48 ± 1.07; VFT: 10.38 ± 1.99 vs.9.05 ± 1.96; EBPM: 2.37 ± 1.08 vs. 1.09 ± 0.85, respectively, $P < 0.01$)

Table 3
Cognitive test with various index Ki-67 groups after chemotherapy

Task	Groups (Mean ± SD)	
	Ki-67 < 14%(n = 99)	Ki-67 > 14% (n = 76)
MMSE	26.93 ± 1.49	26.26 ± 1.88*
DST	6.02 ± 0.85	5.48 ± 1.07**
VFT	10.38 ± 1.99	9.05 ± 1.96**
EBPM	2.37 ± 1.08	1.09 ± 0.85**
TBPM	4.68 ± 0.99	4.75 ± 0.80#

Note: #: $p > 0.05$,*: $P < 0.05$ **: $p < 0.01$. MMSE: mini-mental state; DST: digit span test; VFT: verbal fluency test; EBPM: event-based prospective memory; TBPM: time-based prospective memory.

Table 3

Sequencing analysis

Table 4 indicated that the allelic distribution of COMT rs737865 was statistically different between ki-67 < 14% and ki-67 > 14% groups ($P = 0.037$). The SNPs (rs4680, rs165599, rs737865) of COMT were all to be conformed to Hardy-Weinberg equilibrium (HWE) in 2 groups ($p > 0.05$). This revealed that the three SNP loci we chose in our study were in genetic balance and group representation was discovered in ki-67 < 14% and ki-67 > 14% groups.

Table 4
Information for 3 genotyped SNPs of COMT in various index ki-67 groups

SNP	COMT		
	rs4680	rs165599	rs737865
CHR	22	22	22
Allele Position	19951271	19956781	19930121
Allele type	G/A	G/A	A/G
MAF	0.255	0.500	0.307
P for HWE	0.318	1	1
P*	0.931	0.197	0.037*

Note: *: $P < 0.05$,Single nucleotide polymorphism(SNP); Chromosome (CHR); Minor allele frequency (MAF, data from 1000 Genomes); Hardy-Weinberg equilibrium(HWE), *p*-value for HWE in 2 groups; $^{*}p$-value for allelic frequency differences between two groups.

Table 5, there was a statistically different in rs737865 (recessive model: $\chi^2 = 5.156$, $p = 0.025$) genotypic frequency distribution. Furthermore, as shown in Table 6 and Fig. 1, binary logistic regression analysis results revealed that the patients with the A/G (adjusted, OR = 0.135, CI (95%) = 0.026–0.706, $p = 0.018$) genotypes of COMT rs737865 had significantly lower the risk of occurring cognitive impairment than the patients with the A/A genotype. For genetic model, the recessive model and HOM model of rs737865 with G/G genotype (adjusted, OR = 0.162, CI (95%) = 0.032–0.818, $p = 0.028$; OR = 0.123, CI (95%) = 0.022–0.680, $p = 0.016$, respectively) could decrease the risk of chemo-brain. There was no statistically significant difference in the locus of COMT rs4680 and rs165599 between the ki-67 < 14% and ki-67 > 14% groups.

Table5. Genotype frequencies of COMT (rs4680, rs165599, rs737865) in various index ki-67 groups

SNP	Model	Genotype	Ki-67 <14%	Ki-67 >14%	χ^2	P
rs4680	Co-dominant	G/G	56	44		
		G/A	36	25	0.408	0.819
		A/A	7	7		
	Dominant	G/A+A/A	43	32	0.031	0.879
		G/G	56	44		
	Recessive	A/A	7	7	0.267	0.780
		G/G+G/A	92	69		
rs165599	Co-dominant	G/G	24	23	1.765	
		G/A	49	39		0.400
		A/A	26	14		
	Dominant	G/A+A/A	75	53	0.793	0.394
		G/G	24	23		
	Recessive	A/A	26	14	1.499	0.276
		G/G+G/A	73	62		
rs737865	Co-dominant	A/A	46	43	5.747	
		A/G	41	30		0.052
		G/G	12	2		
	Dominant	A/G+G/G	53	32	2.017	0.171
		A/A	46	43		
	Recessive	G/G	12	2	5.156	0.025*
		A/A+G/A	87	73		

Note: *: *P*<0.05, The χ^2 test of P values for SNP polymorphisms distribution differences between ki-67<14% and ki-67>14% group; Models: Various genetic models that were defined as 1 (MM + Mm) versus 0 (mm) for dominant;1 (mm) versus 0 (MM +Mm) for recessive; and 0 (mm) versus 1 (Mm) versus 2 (MM) for co-dominant (M and m represent major and minor alleles, respectively).

Table 6
Genetic susceptibility of COMT (rs4680, rs165599, rs737865) gene in various index ki-67 groups

SNP	Model	Genotype	Ki-67	Ki-67	Binary logistic regression	
			< 14%	> 14%	OR(95%CI)	P
rs4680	Co-dominant	G/G	56	44	-	-
		G/A	36	25	1.318(0.415–4.186)	0.639
		A/A	7	7	1.477(0.445–4.905)	0.524
	Dominant	G/A + A/A	43	32	0.964(0.508–1.831)	0.912
		G/G	56	44		
	Recessive	A/A	7	7	1.378(0.448–4.242)	0.572
		G/G + G/A	92	69		
	HOM	-	-	-	1.106(0.347–3.523)	0.865
	HET	-	-	-	0.912(0.459–1.815)	0.794
rs165599	Co-dominant	G/G	24	23	-	-
		G/A	49	39	0.553(0.217–1.408)	0.214
		A/A	26	14	0.604(0.265–1.376)	0.230
	Dominant	G/A + A/A	75	53	0.788(0.381–1.630)	0.520
		G/G	24	23		
	Recessive	A/A	26	14	0.586(0.268–1.283)	0.181
		G/G + G/A	73	62		
	HOM	-	-	-	1.014(0.969–1.061)	0.553
	HET				1.010(0.974–1.046)	0.595
rs737865	Co-dominant	A/A	46	43		
		A/G	41	30	0.135(0.026–0.706)	0.018*
		G/G	12	2	0.198(0.038–1.036)	0.055
	Dominant	A/G + G/G	53	32	0.562(0.294–1.074)	0.081

Note: *: *P* < 0.05, P value for binary logistic regression analysis;odds ratio (the OR); 95% confidence interval(95%CI)； co-dominant model were defined as 0 (mm) versus 1 (Mm) versus 2 (MM); Dominant models were defined as 1 (MM + Mm) versus 0 (mm); recessive models were defined as 1 (mm) versus 0 (MM + Mm); Homozygote (HOM) were defined as 1 (MM) versus 0 (mm); and Heterozygote (HET) were defined as 1 (Mm) versus 0 (mm), (M and m represent major and minor alleles, respectively).

SNP	Model	Genotype	Ki-67	Ki-67	Binary logistic regression	
			< 14%	> 14%	OR(95%CI)	P
		A/A	46	43		
	Recessive	G/G	12	2	0.162(0.032–0.818)	0.028*
		A/A + G/A	87	73		
	HOM	-	-	-	0.123(0.022–0.680)	0.016*
	HET				0.681(0.348–1.335)	0.264

Note: *: *P* < 0.05, P value for binary logistic regression analysis;odds ratio (the OR); 95% confidence interval(95%CI)□ co-dominant model were defined as 0 (mm) versus 1 (Mm) versus 2 (MM); Dominant models were defined as 1 (MM + Mm) versus 0 (mm); recessive models were defined as 1 (mm) versus 0 (MM + Mm); Homozygote (HOM) were defined as 1 (MM) versus 0 (mm); and Heterozygote (HET) were defined as 1 (Mm) versus 0 (mm), (M and m represent major and minor alleles, respectively).

Table 4

Table 5

Table 6

Figure 1

The correlation analysis between COMT rs737865 polymorphism and chemo-brain

Means and standard deviations for neuropsychological test were revealed in Table 7 and Fig. 2. The A/G and G/G genotype carriers of COMT rs737865 revealed taller scores on EBPM (1.31 ± 0.82 vs. 0.91 ± 0.84, *P* < 0.05) tests than A/A carriers. Similarly, the A/G genotype carriers of COMT rs737865 presented statistically raised scores on EBPM (1.33 ± 0.80 vs. 0.91 ± 0.84,*P* < 0.05) than A/A carriers in ki-67 > 14% group for breast cancer patients.

Table 7
Comparison for cognitive test with different COMT (rs737865) genotypes.

rs737865	A/G + G/G VS A/A		G/G VS A/A + A/G		G/G VS A/A		A/G VS A/A	
MMSE	26.66 ± 1.45	25.91 ± 2.08	27.00 ± 1.41	26.72 ± 1.88	27.00 ± 1.41	25.91 ± 2.08	26.63 ± 1.47	25.91 ± 2.08
DST	5.56 ± 1.10	5.42 ± 1.06	5.50 ± 1.41	5.48 ± 1.08	5.50 ± 1.41	5.42 ± 1.06	5.57 ± 1.10	5.42 ± 1.06
VFT	9.00 ± 1.88	9.07 ± 2.05	8.00 ± 2.83	9.07 ± 1.96	8.00 ± 2.83	9.07 ± 2.05	9.07 ± 1.85	9.07 ± 2.05
EBPM	1.31 ± 0.82	0.91 ± 0.84*	1.00 ± 1.41	1.08 ± 0.85	1.00 ± 1.41	0.91 ± 0.84	1.33 ± 0.80	0.91 ± 0.84*
TBPM	4.78 ± 0.79	4.72 ± 0.83	5.00 ± 1.41	4.74 ± 0.80	5.00 ± 1.41	4.72 ± 0.83	4.77 ± 0.77	4.72 ± 0.83

Note: *P< 0.05.MMSE: mini-mental state; DST: digit span test; VFT: verbal fluency test; EBPM: event-based prospective memory; TBPM: time-based prospective memory.

Table 7

Figure 2

Discussion

Through the detection of COMT gene polymorphism in peripheral blood and the evaluation of MMSE, DST, VFT, EBPM and TBPM, the results declared that 1) The EBPM and TBPM deficits were presented in breast cancer following chemotherapy; 2) breast cancer patients with ki-67 > 14% revealed more poorly than patients with ki-67 < 14% on the task of MMSE,DST, VFT and EBPM after chemotherapy; 3) There were genotypic differences about COMT rs737865 between ki-67 < 14% and ki-67 > 14% groups, A/G genotype was associated with memory protective susceptibility, A/G genotype carriers exhibited more better on EBPM test relative to A/A genotype, and the COMT rs737865 polymorphism could be a potential genetic factor for chemo-brain in breast cancer patients with various index of ki-67.

The cognitive function of animals could be affected by single or combined chemotherapy, and the learning and memory functions related to hippocampus are impaired after chemotherapy[29]. Doxorubicin, as a commonly used chemotherapeutic drug for breast cancer, patients treated with doxorubicin had poor scores on cognitive scales and visuospatial skills tests[30]. Koppelmans et al.[31] conducted cognitive tests for breast cancer patients and found that the rapid onset and delayed verbal memory, processing speed, executive function and psychomotor speed were significantly lower than those in the control group, even after the end of treatment. Janelsins et al.[32] found that the cognitive impairment could partially recover after 6 months of chemotherapy in breast cancer, but it did not return to the level before chemotherapy, originating from a longitudinal study. The different degrees of EBPM damage was existed in breast cancer patients following chemotherapy, and hormone receptors were

related to chemo-brain, specifically, ER-/PR- breast cancer had worse cognitive function[23]. In this study, the finding indicated that patients with ki-67 > 14% breast cancer suffered worse chemotherapy-related EBPM deficits than those with ki < 14% breast cancer.

Ki-67, a large molecule antigen located in the nucleus, which was expressed in all cell cycles except G0 phase and had been identified as a highly efficient molecular marker for cell proliferation[33]. The marker of ki67 was closely related to the degree of malignancy, which possessed important reference value for predicting the prognosis of tumor, and had become a routine item in the pathological examination of breast cancer[34].The higher levels of ki-67 are associated with pathological diagnosis for primary central system tumor, with nerve numb, cognitive deficit, and disturbance of consciousness and other neuropsychiatric symptoms[35]. Minisini et al.[36] found that the risk of developing brain metastases was higher in breast cancer patients with high ki-67, which are associated with a worse prognosis and cognitive decline. Moderate ki-67 level was found to be significantly related with positive concentration performance before adjuvant treatment for cognitive task[37]. The study found that breast cancer patients with high level expression of ki67 were more sensitive to chemotherapy[38, 39]. Chemotherapy could lead to a series of cognitive dysfunction, which was consistent with the poor cognitive function of breast cancer patients with high index of ki-67 after chemotherapy in this study.

COMT gene polymorphism can affect dopamine concentration in the brain, and it has been found that Met carriers were more susceptible to decrease COMT enzyme activity relative to Val carriers[40]. The three SNPs of COMT (rs 4680, rs737865 and rs165599) were the common sites in the study of psychiatric disorders[41, 42]. The cognitive functions such as memory, attention and executive control for Val carriers of COMT were significantly lower than those of Met carriers in schizophrenia patients[43]. As well, Juarez-Cedillo et al. [44]found that COMT polymorphisms were associated with dementia susceptibility and cognitive impairment when investigating the elderly in the community. COMT was widely distributed in the hippocampus, which could directly regulate the level of dopamine in the hippocampus to affect the hippocampal structure[45, 46]. The structure and function of hippocampus were closely related to working memory, episodic memory and spatial memory[47, 48]. McDermott et al. [49]indicated that the expression of COMT was significantly decreased by high level estrogen, which adjusted the concentration of catecholamine in hippocampus, and enhanced the formation of fear memory. Our past research declared that COMT rs165599 was genetic factors influencing chemo-brain in triple negative breast cancer patients[27]. Furthermore study, we found the influence of COMT rs737865 in EBPM deficits on chemo-brain in ER-/ER- or ER+/PR + breast cancer patients[28]. What we found in this study, consistent with our previous research, was that the A/G genotype of COMT rs737865 patients projected better on TBPM task following chemotherapy, which was genetic factors for chemo-brain in breast cancer with various index of ki-67.

The advantage of this study is to integrate cognitive neuropsychology, genetics and oncology to explore the mechanism of chemotherapy brain, including memory scale tests, genetic dection, and chemotherapy regimen selection. However, the limitation and challenge of this study should also be stated. First, this study only had a before-and-after comparison, missing a healthy control group, the expression of COMT

and cognitive test in healthy women could not be known. Second, this study pay attention to early cognitive impairments that occurred one month after chemotherapy. It is unknown whether results are suitable to follow up cognitive impairment research. Third, the sample size was insufficient, the quantity of breast cancer and healthy controls need to be supplemented in subsequent study. Four, the task scale of EBPM and TBPM were made by referring to Mcdougal's research methods, the task were subjective memory representation, the objective cognitive scales for tumor patients may be better.

Conclusion

In a word, our study initially found the relationship between chemotherapy related EBPM impairment and genetic polymorphism in breast cancer patients with various index of ki-67. The heterogeneity of chemo-brain possible mediated through COMT rs737865 polymorphism, and this mediation may express an opinion that COMT polymorphism was potential genetic risk of chemo-brain in breast cancer patients with various index of ki-67.

Abbreviations

COMT, catechol-O-methyltransferase

EBPM, event based prospective memory

TBPM, time-based prospective memory

ER/PR, estrogen/progesterone receptor

MMSE, mini-mental state examination

KPS, Karnofsky performance scale score

VFT, verbal fluency test

DST, digit span test

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval and Consent to participate

Research Ethics Committee of the Second Affiliated Hospital of AnHui Medical University, China issued the approval of this study. The manuscript complies with the Ethical Rules applicable for this journal. The

manuscript complies with the current laws of the country in which they were performed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study

Consent for publication

The Author agrees to publication in the Journal of breast cancer research

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Statement of conflict of interest

All authors declare that they have no conflicts of interest for this study.

Authors' contributions

Wen Li performed data collection, cognitive tests, and wrote the manuscript; Sheng Yu performed data analysis; Senbang Yao and Lingxue Tang performed Clinical data acquisition and peripheral venous collection; Huaidong Cheng designed the project. All authors contributed to manuscript editing.

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Figures

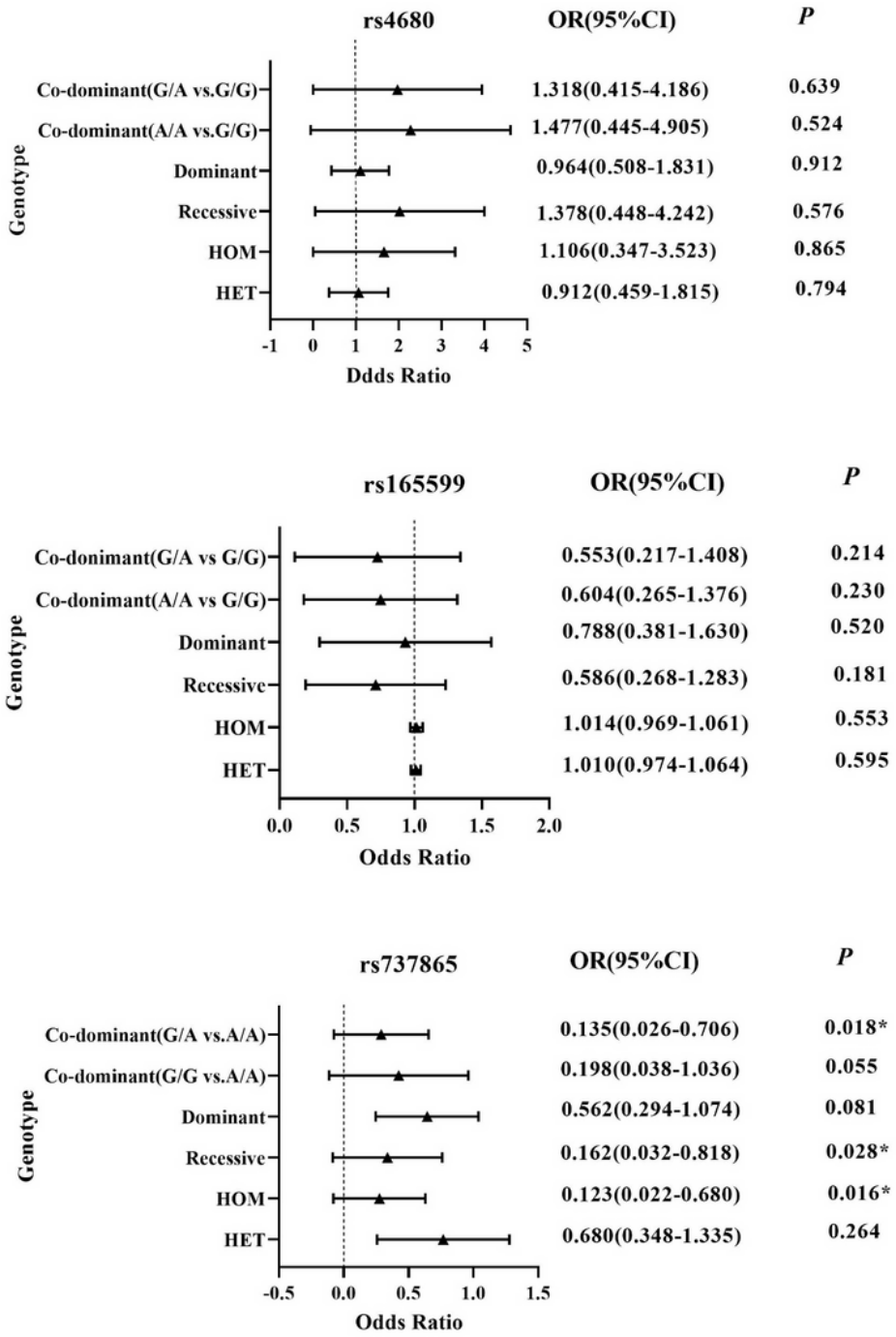

Figure 1

The forest plots of susceptibility analysis in COMT (rs4680, rs165599, rs737865) for ki-67<14% and ki-67>14% group

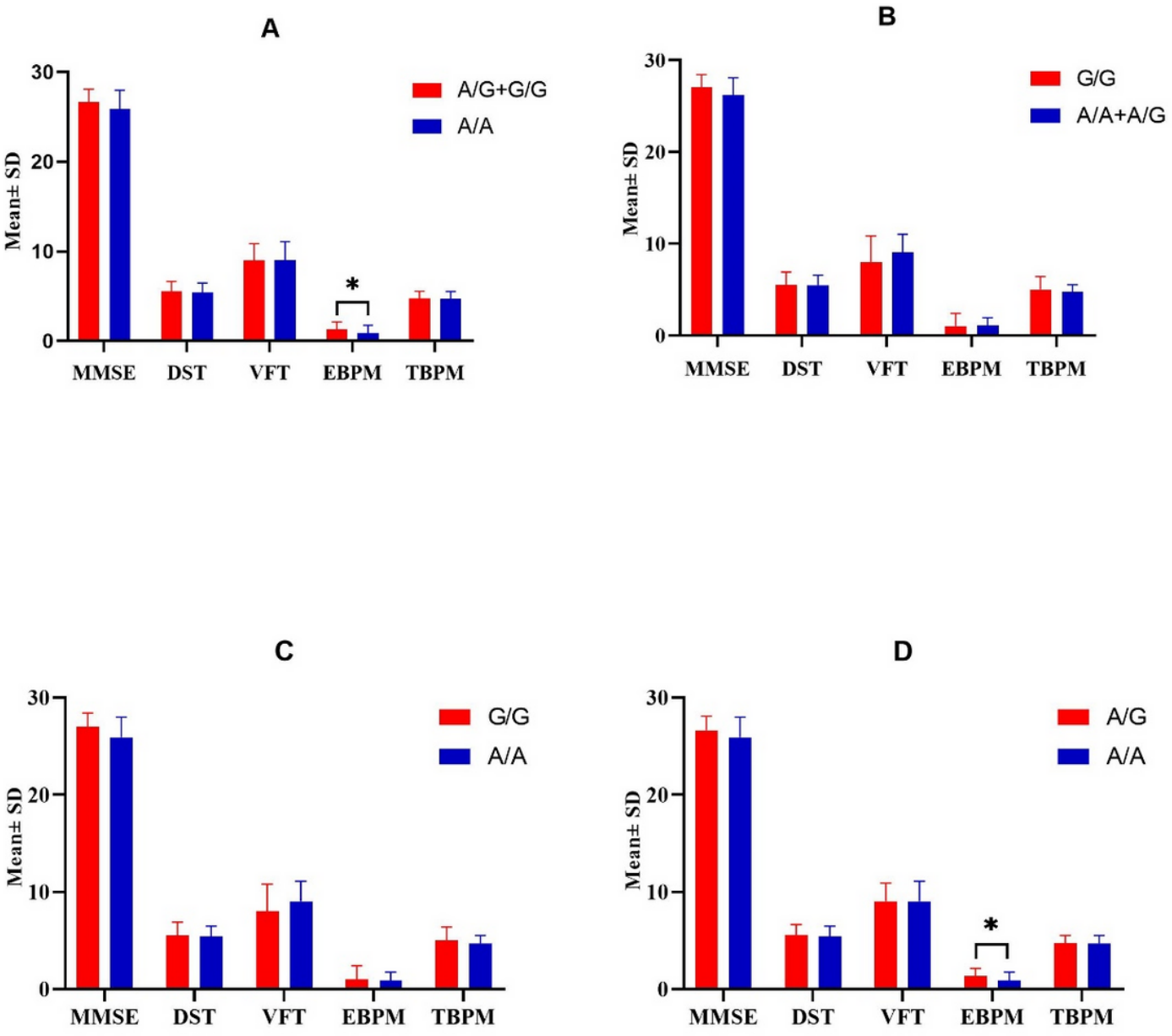

Figure 2

The histogram of cognitive test with different COMT (rs737865) genotypes