Letter to the Editor

Association between Polymorphisms in Telomere-Associated Protein Genes and the Cholinesterase Activity of Omethoate-Exposed Workers*

FAN Ya Hui[1], LI Xin Ling[1], LIU Xiao Hua[1], GUO Zhi Feng[1], YAN Meng Qing[1], DUAN Xiao Ran[1,2], MIAO Wen Bin[3,#], and WANG Wei[1,#]

Organophosphorus pesticides (OPs) are extensively used for their high efficiency, broad spectrum, and low residue. However, the health hazards caused by long-term, low-dose exposure to OPs are easily ignored. Omethoate is a large class of OPs that is widely used in China. The inhibition of the cholinesterase (ChE) activity is the main toxicity mechanism of OPs, and such an activity is used as a biomarker of exposure to OPs[1].

Telomeres are composed of noncoding DNA repeats, telomere-binding proteins (TBPs), and telomerase, which gradually shorten during cell division. POT1-TIN2 Organizing Protein (TPP1), a kind of TBP, actively recruits telomerase to telomeres, protects chromosome ends, and regulates telomere length together with POT1[2]. TGF β-regulated and epithelial cell-enriched phosphatase 1 (TEP1), a mammalian telomerase-associated protein, is associated with telomerase activity and the telomerase reverse transcriptase, and it specifically interacts with telomerase RNA[3]. TEP1 and TPP1 are telomere-associated protein genes that participate in telomere length regulation and terminal protection to affect chromosome stability[4,5]. In addition, studies have shown that telomere- associated proteins first affect chromosome stability and then change the expression level of ChE-related genes, thus affecting ChE production and degradation[6].

Single nucleotide polymorphism (SNP) is a common form of single-base mutation and can affect the mRNA expression levels of their genes or protein functions. So far, the correlations among the polymorphisms of TPP1, TEP1 genes, and the ChE activity are unclear. Therefore, this study explored the relationship between TPP1, TEP1 gene polymorphisms, and ChE activity.

A total of 180 workers exposed to omethoate for more than eight years were included in the exposure group. In addition, 115 healthy persons from a company in the same area without a history of exposure to omethoate or other toxicants comprised the control group. Individuals with histories of chronic diseases or other acute and chronic infections were excluded. People who smoked at least one cigarette a day for more than half a year were defined as smoking; people who drank alcohol more than twice a week in the last six months were defined as drinking. Demographic characteristics, occupational histories, and biological samples were collected by trained professionals. All subjects signed informed consent, and the study was approved by the Ethics Committee of Zhengzhou University.

In this study, whole blood, red blood cell, and plasma ChE activities were measured, and the damage induced by omethoate was represented by the red blood cell ChE activity. The detailed determination method could be seen in our previous study[7].

Eleven polymorphic loci of TEP1 rs1713449, TEP1 rs1760897, TEP1 rs1760903, TEP1 rs938886, TEP1 rs1760904, TEP1 rs4246977, TPP1 rs1800752, TPP1 rs3758978, TPP1 rs7488, TPP1 rs1128396, and TPP1 rs2555173 were screened through the HapMap, NCBI-SNP, and 1,000 Genomes databases or published works. PCR and single-base extension primers were designed by the Assay Designer 3.1 software and were synthesized by Thermo Fisher Scientific Co., Ltd, 2020 (Supplementary Table S1 available in www.besjournal.com). The SNPs were genotyped

doi: 10.3967/bes2022.060

*The work was supported by Programs for Science and Technology Development of Zhengzhou [131PPTGG376] and the Outstanding Youth Grant of Zhengzhou University [1521329035].

1. Department of Occupational Health and Occupational Diseases, College of Public Health, Zhengzhou University, Zhengzhou 450001, Henan, China; 2. Biotherapy Center, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, Zhengzhou 450052, Henan, China; 3. Director of Efficacy and Safety Evaluation Laboratory, Pony Testing Group Shanghai Co., Ltd., Shanghai 201600, China

with a MassARRAY® matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry platform (Agena, Inc., 4.0 San Diego, CA, USA).

All statistical analyses were conducted using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). The independent samples *t*-test was performed to compare the ChE activity between the exposure and control groups. Covariance was used to analyze the effects of genetic polymorphisms on the ChE activity, and the Dunnett method was employed to perform the comparisons between the two groups. Generalized linear models (GLMs) were used to determine the influencing factors of the ChE activity. All statistical tests were two-sided, and the statistical significance level was set at $\alpha = 0.05$.

Significant differences were observed in demographic characteristics, including gender, age, smoking, and drinking ($P < 0.001$), comparing the exposure and control groups (Supplementary Table S2 available in www.besjournal.com), which were described in detail in our previous study[8]. The red blood cell ChE activity in the exposure group was lower than that in the control group (2.09 ± 0.52 *vs.* 3.06 ± 0.65, $P < 0.001$) (Supplementary Table S3 available in www.besjournal.com). As illustrated in Figure 1, gender, age, smoking, and drinking had an effect on the red blood cell ChE activity ($P < 0.05$); in the exposure group, the ChE activity was associated with gender, smoking, and drinking ($P < 0.05$). However, age and working duration had no effect on the ChE activity in the exposure group ($P > 0.05$) (Supplementary Table S4 available in www.besjournal.com).

The genotypic distribution of each polymorphic locus accorded with Hardy-Weinberg balance ($P > 0.05$) indicated that the selected samples were representative. We analyzed the differences in the ChE activity between different genotypes of TPP1 and TEP1 polymorphisms (Table 1). The CT and CC genotypes of TPP1 rs1800752 had a similar ChE activity, so they were combined. TPP1 rs3758978, TPP1 rs7488, and TPP1 rs1128396 were the same. The ChE activity of the TPP1 rs1800752 CT + CC genotype was lower than that of the TT genotype (2.02 ± 0.54 *vs.* 2.18 ± 0.46, $P = 0.006$). The ChE activity of the TPP1 rs3758978 CG + GG genotype was lower than that of the CC genotype (2.01 ± 0.54 *vs.* 2.19 ± 0.46, $P = 0.003$). The ChE activity of the TPP1 rs1128396 AT + TT genotype was lower than that of the AA genotype (2.00 ± 0.53 *vs.* 2.17 ± 0.50, $P = 0.002$). Moreover, the ChE activity of the TPP1 rs2555173 AC + AA genotype was lower than that of the CC genotype (2.03 ± 0.55 *vs.* 2.13 ± 0.49, $P = 0.037$). No significant difference in genotypes was found in other loci ($P > 0.05$). C Gu et al.[5] identified that TEP1 rs1760904 AG/AA genotypes were significantly associated with a decreased risk of prostate cancer compared with the GG genotype. However, we found no statistically significant

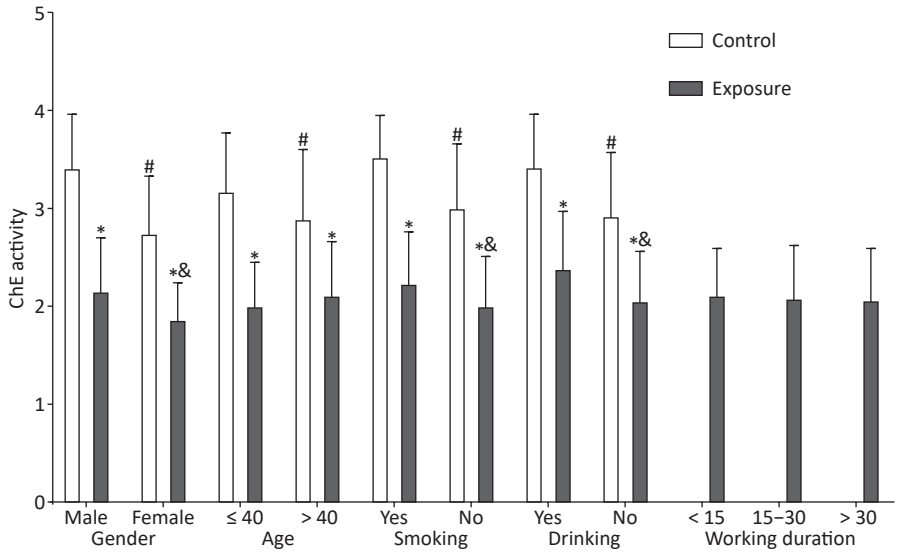

Figure 1. Effect of demographic characteristics on cholinesterase activity. The independent samples *t*-test was utilized to compare the ChE activity between the exposure and control groups. *The difference in the ChE activity between both groups after stratification was statistically significant. #The difference in the control group was statistically significant. &The difference in the exposure group was statistically significant.

difference among the different genotypes of TEP1 rs1760904.

The factors affecting the ChE activity of workers exposed to omethoate were analyzed using GLMs. The adjusted age, smoking, working duration, drinking, omethoate exposure, gender, and interaction between the TPP1 rs3758978 CC genotype and omethoate exposure might be the

Table 1. Relationships between genetic polymorphism and ChE activity

SNP	$n^{\#}$	Control $\bar{x} \pm s$	*P*	$n^{\#}$	Exposure $\bar{x} \pm s$	*P*
TEP1 rs1713449						
TT	16	2.77 ± 0.59	Ref	27	2.02 ± 0.53	Ref
CT	48	3.08 ± 0.62	0.136	59	2.06 ± 054	0.851
CC	49	3.13 ± 0.70	0.057	92	2.12 ± 0.50	0.669
CT+CC	113	3.10 ± 0.66	0.067	151	2.10 ± 0.52	0.724
TEP1 rs1760897						
CC	6	3.20 ± 0.61	Ref	10	2.02 ± 0.40	Ref
CT	38	3.01 ± 0.67	0.650	62	2.01 ± 0.51	0.789
TT	67	3.09 ± 0.67	0.727	106	2.14 ± 0.53	0.635
CT+TT	105	3.06 ± 0.66	0.691	168	2.09 ± 0.52	0.845
TEP1 rs1760903						
TT	48	2.97 ± 0.66	Ref	64	2.02 ± 0.46	Ref
CT	44	3.17 ± 0.68	0.311	79	2.12 ± 0.56	0.523
CC	21	3.09 ± 0.60	0.446	36	2.14 ± 0.51	0.450
CT+CC	65	3.14 ± 0.65	0.273	115	2.12 ± 0.54	0.432
TEP1 rs938886						
CC	12	2.97 ± 0.64	Ref	24	1.99 ± 0.53	Ref
CG	48	3.07 ± 0.62	0.973	60	2.06 ± 0.55	0.632
GG	50	3.12 ± 0.69	0.589	90	2.13 ± 0.50	0.492
CG+GG	98	3.10 ± 0.65	0.750	150	2.10 ± 0.52	0.521
TEP1 rs1760904						
CC	46	2.98 ± 0.66	Ref	64	2.01 ± 0.46	Ref
CT	46	3.16 ± 0.67	0.319	76	2.14 ± 0.56	0.345
TT	21	3.05 ± 0.62	0.803	37	2.12 ± 0.53	0.559
CT+TT	67	3.13 ± 0.65	0.392	113	2.13 ± 0.55	0.346
TEP1 rs4246977						
TT	59	3.01 ± 0.70	Ref	75	2.06 ± 0.52	Ref
CT	50	3.11 ± 0.60	0.507	81	2.09 ± 0.50	0.292
CC	5	3.21 ± 0.67	0.437	20	2.18 ± 0.60	0.269
CT+CC	55	3.12 ± 0.60	0.418	101	2.11 ± 0.52	0.212
TPP1 rs1800752						
TT	43	3.06 ± 0.62	Ref	70	2.18 ± 0.46	Ref
CT	56	3.10 ± 0.69	0.762	80	2.01 ± 0.56	0.007*
CC	12	3.04 ± 0.61	0.805	27	2.04 ± 0.51	0.098
CT+CC	68	3.09 ± 0.67	0.849	107	2.02 ± 0.54	0.006*

Continued

SNP	$n^{\#}$	Control $\bar{x} \pm s$	*P*	$n^{\#}$	Exposure $\bar{x} \pm s$	*P*
TPP1 rs3758978						
CC	45	3.02 ± 0.63	Ref	71	2.19 ± 0.46	Ref
CG	55	3.09 ± 0.69	0.665	80	2.01 ± 0.55	0.004*
GG	12	3.04 ± 0.61	0.916	27	2.04 ± 0.51	0.080
CG+GG	67	3.08 ± 0.68	0.730	107	2.01 ± 0.54	0.003*
TPP1 rs7488						
AA	95	3.06 ± 0.66	Ref	140	2.11 ± 0.51	Ref
AG	16	3.09 ± 0.65	0.303	35	2.00 ± 0.55	0.605
GG	0	0		3	2.04 ± 0.16	0.895
AG+GG	16	3.09 ± 0.65	0.303	38	2.00 ± 0.53	0.646
TPP1 rs1128396						
AA	59	3.04 ± 0.64	Ref	95	2.17 ± 0.50	Ref
AT	45	3.11 ± 0.69	0.676	66	2.00 ± 0.53	0.004*
TT	8	3.02 ± 0.66	0.516	14	2.02 ± 0.56	0.093
AT+TT	53	3.09 ± 0.68	0.856	80	2.00 ± 0.53	0.002*
TPP1 rs2555173						
CC	62	3.06 ± 0.66	Ref	102	2.13 ± 0.49	Ref
AC	44	3.09 ± 0.66	0.791	65	2.05 ± 0.55	0.097
AA	8	2.97 ± 0.71	0.571	11	1.87 ± 0.55	0.037*
AC+AA	52	3.07 ± 0.66	0.681	76	2.03 ± 0.55	0.037*

Note. The covariance was obtained to compare the difference in the ChE activity among the genotypes, adjusted for gender, age, smoking, drinking, and working duration. Ref: The reference group for comparing different genotypes. SNP: Single nucleotide polymorphism. #Some samples were missing due to limitations of detection methods. *The difference was statistically significant

influencing factors of the ChE activity of omethoate-exposed workers ($P < 0.05$) (Table 2). The ChE activity of females was lower than that of males, indicating that women were more susceptible to omethoate than men. Drinking might be another potential protective factor in the ChE activity, which was similar to the finding that moderate alcohol consumption could increase antioxidant activity[9], suggesting that drinking might have played a similar role in workers exposed to omethoate. Hernandez et al.[10] evaluated pesticide-induced oxidative stress and found an interaction between pesticide exposures and genes. They suggested that the interaction between these genes and the pesticides may play a key role in the development of many chronic and degenerative diseases.

This study observed an interaction of telomere-associated protein genes and environmental factors that affects human health, thereby providing clues for the screening of susceptible workers exposed to omethoate and the mechanism of inheritance variation. However, this research has some

Table 2. Influencing factors of the ChE activity

Parameter	β (95% *CI*)	χ^2	*P*
Constant	2.808 (2.556, 3.061)	474.301	< 0.001*
Drinking	0.271 (0.078, 0.463)	7.615	0.006*
Exposure	−0.903 (−1.235, −0.571)	28.392	< 0.001*
Female	−0.408 (−0.555, −0.262)	29.918	< 0.001*
TPP1 rs3758978 CC	−0.034 (−0.229, 0.161)	0.114	0.736
Exposure × rs3758978 CC	0.250 (0.001, 0.499)	3.867	0.049*

Note. Adjusted for age, smoking, and working duration by using the GLM method. GML: Generalized linear models. *The difference was statistically significant.

limitations. First, it is a cross-sectional study, which may require further follow-up to confirm its correlation. Second, the OPs were metabolized and excreted in the urine, usually within 24−48 hours of exposure. Therefore, the relationship of urinary metabolites with the ChE activity and gene polymorphism may need to be further evaluated.

In conclusion, this study suggests that drinking, omethoate exposure, gender, and the interaction between the TPP1rs3758978 CC genotype and omethoate exposure may be the influencing factors of the ChE activity of omethoate-exposed workers.

No potential conflicts of interest were disclosed.

The authors express their gratitude to all the individuals who volunteered to participate in this study.

FAN Ya Hui, data analysis and manuscript drafting; LI Xin Ling, LIU Xiao Hua, GUO Zhi Feng, and YAN Meng Qing, project investigation and quality control; DUAN Xiao Ran, experiment; MIAO Wen Bin, manuscript revision; WANG Wei, funding acquisition and project design; all authors commented on the article before submission.

[#]Correspondence should be addressed to MIAO Wen Bin, E-mail: moumm999@sina.com,Tel: 86-371-67781466; WANG Wei, E-mail: ww375@zzu.edu.cn

Biographical note of the first author: FAN Ya Hui, female, born in 1996, MPH, majoring in occupational cancer and biomarker.

Received: November 12, 2021;

Accepted: March 29, 2022

REFERENCES

1. Chowdhary S, Bhattacharyya R, Banerjee D. Acute organophosphorus poisoning. Clin Chim Acta, 2014; 431, 66−76.
2. Yu KX, Zeng ZR, Cheng S, et al. TPP1 Enhances the Therapeutic Effects of Transplanted Aged Mesenchymal Stem Cells in Infarcted Hearts via the MRE11/AKT Pathway. Front Cell Dev Biol, 2020; 8, 588023.
3. Liu Y, Snow BE, Hande MP, et al. Telomerase-associated protein TEP1 is not essential for telomerase activity or telomere length maintenance *in vivo*. Mol Cell Biol, 2000; 20, 8178−84.
4. Xin HW, Liu D, Zhou SY. The telosome/shelterin complex and its functions. Genome Biol, 2008; 9, 232.
5. Gu C, Li Q, Zhu Y, et al. Genetic variants in the *TEP1* gene are associated with prostate cancer risk and recurrence. Prostate Cancer Prostatic Dis, 2015; 18, 310−6.
6. Ding MC, Yang YL, Duan XR, et al. Association of genetic polymorphisms of telomere binding proteins with cholinesterase activity in omethoate-exposed workers. Ecotoxicol Environ Saf, 2018; 161, 563−68.
7. Zou KL, Zhou XS, Wang W, et al. The association between polymorphisms in miRNA and the cholinesterase activity of workers in an omethoate-exposed environment. Int J Environ Health Res, 2020; 32, 1020−29.
8. Duan XR, Yang YL, Wang SH, et al. Changes in the expression of genes involved in cell cycle regulation and the relative telomere length in the process of canceration induced by omethoate. Tumour Biol, 2017; 39.
9. Prickett CD, Lister E, Collins M, et al. Alcohol: Friend or Foe? Alcoholic Beverage Hormesis for Cataract and Atherosclerosis is Related to Plasma Antioxidant Activity. Nonlinearity Biol Toxicol Med, 2004; 2, 353−70.
10. Hernández AF, Lacasaña M, Gil F, et al. Evaluation of pesticide-induced oxidative stress from a gene-environment interaction perspective. Toxicology, 2013; 307, 95−102.