Letter to the Editor

Relationship between *TERT* Polymorphism and Telomere Length in Workers Exposed to Omethoate*

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Omethoate is a highly toxic organophosphorus pesticide that is widely used in agricultural production because of its high efficiency, broad spectrum, and low residue. Organophosphorus pesticides, such as omethoate, can inhibit acetylcholinesterase activity, leading to the accumulation of the neurotransmitter acetylcholine at cholinergic synapses. Accumulation of acetylcholine continues to stimulate cholinergic receptors, causing central nervous effects. Studies have shown that organophosphorus pesticides can cause genotoxicity in a variety of organisms, resulting in chromosomal DNA damage[1].

Telomeres are DNA-protein structures consisting of tandem hexamer repeats (TTAGGGn) at the end of the chromosome. They play important roles in chromosomal location, replication, protection, and control of cell growth. Telomerase is a DNA polymerase that synthesizes the TTAGG sequence and uses internal RNA molecules as templates to lengthen the pre-existing 3'terminal telomeres in vertebrates. It is a complex system composed of telomerase reverse transcriptase (*TERT*), telomerase RNA component and telomerase-associated protein 1 (*TEP1*). *TERT* is an important factor in maintaining telomere DNA length and chromosome stability. *TERT* is silently expressed in normal somatic and non-proliferative cells. However, in many human cancers, the *TERT* promoter mutates, resulting in abnormal expression. *TEP1*, another component of the telomerase nucleoprotein complex, catalyzes the addition of new telomeres to chromosomes. *TEP1* immunoprecipitation has revealed its telomerase activity and its relatedness to *TERT* and telomerase RNA components[2].

A study of American adults found that the environmental exposure levels to organophosphate pesticides is related to alteration in telomere length in the population[3]. We have previously studied the relationship between tankyrase (*TNKS*) gene polymorphism and telomere length in peripheral blood leukocytes. The results showed that the CG+CC genotypes in rs1055328 may affect omethoate-induced telomere length increase[4]. Additionally, in a study on the relationship between metabolizing enzyme gene polymorphisms and telomere length in omethoate-exposed workers, the extension of telomere length was related to glutathione S-transferase M1 (*GSTM1*) deletion, GG+AG genotypes, and interactions between smoking and GG+AG genotypes[5]. However, it is not clear whether the effect of omethoate on telomere length is related to the polymorphism of telomerase genes. Therefore, we investigated the effect of polymorphisms in *TERT* and *TEP1* on the telomere length of workers exposed to omethoate.

A total of 180 workers exposed to omethoate for more than 8 years were selected as the exposure group and 115 healthy people who were not exposed to toxic substances were selected as the control group. Smokers were defined as those smoking more than one cigarette a day for over half a year; alcohol drinkers were defined as those drinking more than twice a week in the past half year. This study was approved by the Life Science and Ethics Review Committee. Ten polymorphic loci associated with these genes (*TERT*: rs2736109, rs2735940, rs3215401, and rs2736100; *TEP1*:

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rs1713449, rs1760897, rs1760903, rs938886, rs1760904, and rs4246977) were studied by NCBI-SNP or Hapmap databases. The AssayDesigner3.1 software was used to design PCRs and single-base extension primers. The primer sequences of each of the polymorphic loci are listed in Supplementary Table S1, available in www.besjournal.com. Real-time fluorescence quantitative PCR assay was used to detect the DNA telomere length in peripheral blood leukocytes, and each sample was tested twice. Telomere length was determined using reference and telomere primers. The reverse and forward primers for the reference gene were *hbgd*, 5'-GCCCGGCCCGCCGCGCCCGTCCCGCCGGAGGAG-AAGTCTGCCGTT-3' and *hbgu*, 5'-CGGCGGCGGG-CGGCGCGGGCTGGGCGGCTTCATCCACGTTCACCTTG-3'. The reverse and forward primers for the telomere were 5'-TGTTAGGTATCCCTATCCCTATCCCTATCCCT-ATCCCTAACA-3' and 5'-ACACTAAGGTTTGGGTTT-GGGTTTGGGTTTGGGTTAGTGT-3'.

Statistical software (SPSS 21.0) was used for data analysis. In this study, data on telomere length were non-normally distributed. The telomere length data of the exposed and control groups were converted to normal distribution data using the Ln(X)+3 logarithmic transformation method. The *t*-test was used to compare the differences in telomere length between the two groups. The covariance method was used to analyze the relationship between variables and telomere length. The factors influencing telomere length in omethoate workers were analyzed using generalized linear models. All statistical tests were two-sided, with a statistical significance level of $\alpha = 0.05$.

The telomere length in the exposed group (3.52 ± 0.62) was longer than that in the controls (3.00 ± 0.36) ($t = 9.108$, $P < 0.001$). Additionally, we analyzed the effects of sex, age, smoking, alcohol consumption, and omethoate exposure on telomere length. The results showed that, except in smokers, the telomere length in the exposed group was significantly longer than that in the control group ($P < 0.05$) (Supplementary Table S2, available in www.besjournal.com). After the Hardy-Weinberg equilibrium test, the genotype distribution of each genetic polymorphism did not deviate ($P > 0.05$), indicating that the control group was representative. Covariance analysis was used to analyze differences in telomere length between different genotypes of the *TERT* and *TEP1* polymorphisms (Table 1). At the *TERT* rs2736109 polymorphism, the telomere length of the GG genotype was close to that of the AG genotype, thus leading to their fusion. The results showed that the telomere length of the AG+GG genotype was significantly longer than that of the AA genotype in the exposed group ($P = 0.029$). In the control group, the telomere length of the TT genotype of the *TERT* rs2736100 polymorphism was shorter than that of the GT genotype ($P = 0.037$). There were no significant differences in genotypes between the other loci. The rs2736100 genetic variation is associated with a range of cancers and related disorders. A case-control study of 828 people suggested that individuals with TG or GG had a higher risk of non-small-cell lung cancer compared to individuals with TT in the rs2736100 genotype[6]. Gu et al.[7] reported that the G allele of rs2736100 is significantly associated with a decrease in telomere length in Caucasians. However, we found that the telomere length of the *TERT* rs2736100 TT genotype was significantly lower than that of the GT genotype in the normal control population ($P = 0.037$), this could be due to ethnic differences.

In the generalized linear model, telomere length was used as the dependent variable; exposure, *TERT* rs2736109, and *TERT* rs2736100 were used as independent variables; and sex, age, smoking, drinking, and working period were used as covariates to enter the model. Generalized linear model analysis showed that exposure (b = 0.568, $P < 0.001$) and *TERT* rs2736109 (GG+AG) (b = 0.240, $P = 0.045$) affected telomere length, and no other factors were found to affect telomere length (Table 2). The polymorphism rs2736109 is located in the specific promoter region of *TERT*. The specific binding of the *TERT* promoter and the transcription factor GATA-2 can initiate the *TERT* transcription process. All members of the GATA transcription factor family bind to a specific nucleotide sequence (T/A (GATA) A/G). Instead of the G-allele in *TERT* promoter, the mutant A allele in rs2736109 generates a new GATA-1 binding locus[8], which decreases the transcription efficiency of *TERT* by competitive inhibition. GATA-1 encodes two zinc finger structure motifs, c-terminal zinc finger (c-znf) and N-terminal zinc finger (n-znf). N-znf, interacts with the nuclear protein transcription factor *FOG1*[9]. Studies have shown that *FOG1* can inhibit the activity of *GATA-1*[10]. This may result in lower transcriptional activity of *GATA-1* than for *GATA-2*. Therefore, the combination of the *TERT* promoter and *GATA-1* can reduce the expression of the *TERT* mRNA. Finally, the mutant A allele of rs2736109 may lead to shorter telomere lengths. The telomere length in the AA genotype was significantly lesser than that in the GG+AG genotype, which is

Table 1. Telomere length for polymorphisms in the genes *TERT* and *TEP1*

SNPs	Exposure			Control		
	n^a	$\bar{x} \pm s$	P^b	n^a	$\bar{x} \pm s$	P^b
TERT rs2736109						
AA	13	3.17 ± 0.42	Ref	12	2.95 ± 0.43	Ref
GG+AG	161	3.55 ± 0.63	0.029	101	3.00 ± 0.35	0.602
TERT rs2735940						
TT	35	3.48 ± 0.55	Ref	25	2.97 ± 0.37	Ref
CT	90	3.57 ± 0.67	0.397	59	2.98 ± 0.35	0.790
CC	54	3.47 ± 0.57	0.964	30	3.03 ± 0.37	0.934
TERT rs3215401						
-/-	76	3.51 ± 0.55	Ref	46	3.00 ± 0.38	Ref
-/C	82	3.56 ± 0.71	0.711	51	2.99 ± 0.33	0.676
CC	20	3.37 ± 0.44	0.299	16	2.98 ± 0.37	0.885
TERT rs2736100						
TT	52	3.45 ± 0.60	Ref	35	2.92 ± 0.31	Ref
GT	85	3.56 ± 0.66	0.315	62	3.05 ± 0.38	0.037
GG	25	3.47 ± 0.55	0.976	12	2.95 ± 0.32	0.688
TEP1 rs1713449						
TT	27	3.60 ± 0.65	Ref	16	2.90 ± 0.31	Ref
CT	59	3.53 ± 0.57	0.732	48	2.98 ± 0.35	0.539
CC	92	3.48 ± 0.65	0.483	49	3.04 ± 0.38	0.214
TEP1 rs1760897						
CC	10	3.84 ± 0.54	Ref	6	3.16 ± 0.24	Ref
CT	62	3.49 ± 0.61	0.180	38	2.93 ± 0.37	0.247
TT	106	3.50 ± 0.63	0.114	67	3.02 ± 0.35	0.554
TEP1 rs1760903						
TT	64	3.51 ± 0.59	Ref	48	2.96 ± 0.37	Ref
CT	79	3.50 ± 0.68	0.920	44	3.00 ± 0.34	0.402
CC	36	3.60 ± 0.54	0.414	21	3.09 ± 0.35	0.130
TEP1 rs938886						
CC	24	3.50 ± 0.59	Ref	12	2.98 ± 0.26	Ref
CG	60	3.56 ± 0.60	0.617	48	2.97 ± 0.36	0.814
GG	90	3.53 ± 0.59	0.790	50	3.04 ± 0.38	0.613
TEP1 rs1760904						
CC	64	3.50 ± 0.58	Ref	46	2.95 ± 0.37	Ref
CT	76	3.51 ± 0.70	0.920	46	3.02 ± 0.36	0.305
TT	37	3.58 ± 0.54	0.451	21	3.02 ± 0.33	0.331
TEP1 rs4246977						
TT	75	3.49 ± 0.59	Ref	59	2.95 ± 0.37	Ref
CT	81	3.59 ± 0.59	0.309	50	3.05 ± 0.33	0.177
CC	20	3.37 ± 0.86	0.521	5	3.02 ± 0.38	0.383

***Note*.** [a]Some samples were missing due to limitations of detection methods. [b]Covariance analysis compares differences in telomere length between genotypes, adjusted for sex, age, smoking, drinking, and working period. Ref: The reference group of the two comparisons, using the LSD method.

Table 2. Factors influencing telomere length

Parameter	β (95% *CI*)	Standard Error	χ^2	P^a
Intercept	2.836 (2.435, 3.238)	0.2047	191.997	< 0.001
Exposure	0.568 (0.415, 0.721)	0.0781	52.868	< 0.001
*TERT*rs2736109 (GG+AG)	0.240 (0.005, 0.476)	0.1200	4.014	0.045

***Note*.** [a]The generalized linear model was used to analyze telomere length, and adjusted for sex, age, smoking and drinking.

consistent with the results of this study.

To our knowledge, this is the first study to investigate the relationship between *TERT* and *TEP1* polymorphisms and telomere length in workers exposed to omethoate. Our study has several limitations. First, it is a cross-sectional design and therefore does not address the temporal or causal relationship between omethoate exposure and telomere length decrease. Further follow-up is required to confirm these relationships. Second, the molecular mechanisms of the selected SNPs in telomere length shortening remain unclear, and cell-base experiments are required to elucidate these mechanisms. Finally, factors that might affect telomere length, such as chronic diseases and inflammatory conditions, were not considered due to limited information.

In summary, we explored the relationship between telomerase gene (*TERT* and *TEP1*) polymorphisms and telomere length in long-term low-level omethoate-exposed workers. The *TERT* rs2736109 polymorphism is the main factor affecting telomere length. Through this study, the molecular mechanism of organophosphorus pesticides that cause telomere prolongation was further explored. Our study provides a basis to screen for workers susceptible to occupational exposure. This is conducive to improving workers' health protection and reducing occupational contact damage.

Conflicts of Interest None.

Author Contributions CHENG Shuai and LIU Bin wrote the manuscript; YANG Yong Li and GUO Zhi Feng analyzed the data; DUAN Xiao Ran performed the experiments; LIU Su Xiang and LI Lei contributed to specimen collection; YAO Wu and WANG Wei contributed constructs; WANG Wei designed the experiments. All authors commented on the article before submission.

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Ethical Conduct of Research The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent was obtained from the participants involved.

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