Drug addiction is associated with leukocyte telomere length

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Telomeres are protective chromosomal structures that play a key role in preserving genomic stability. Telomere length is known to be associated with ageing and age-related diseases. To study the impairment of telomeres induced by drug abuse, we conducted an association study in the Chinese Han population. Multivariate linear regression analyses were performed to evaluate the correlation of leukocyte telomere length (LTL) with addiction control status adjusted for age and gender. The results showed that drug abusers exhibited significantly shorter LTLs than controls (P = 1.32e−06). The time before relapse also presented an inverse correlation with LTL (P = 0.02). Drug abusers who had used heroin and diazepam displayed a shorter LTL than those taking other drugs (P = 0.018 and P = 0.009, respectively). Drug abusers who had ingested drugs via snuff exhibited longer LTLs than those using other methods (P = 0.02). These observations may offer a partial explanation for the effects of drug addiction on health.

T elomeres, the repetitive sequences at the ends of chromosomes, are protective chromosomal structures that appear to play a key role in preserving genomic stability[1,2]. Telomere dysregulation can lead to cell death, cell senescence, or abnormal cell proliferation[3], and studies have demonstrated that telomere length is associated with longevity and age-related diseases, such as Alzheimer's disease[4] and vascular dementia[5].

Drug addiction is characterised by compulsive drug-taking behaviour and high rates of relapse. Drug addiction not only causes medical, social, and economic problems but also severely harms a drug abuser's health[6−8]. It has beeen shown that older heroin abusers have significantly more chronic health problems and social functioning problems than the population norm[9]. Additionally, tobacco, cannabis, heroin, and methadone use are significantly related to particular physiological deficiencies, and opiate dependence is associated with age-related trajectories of dysfunction in several organ systems[10]. In the present study, we investigated the correlation between telomere length and drug addiction, both of which are closely associated with age-related physiological problems.

Results

Table 2 provides the characteristics of the participants, including their age, gender, BMI, and biochemical characteristics, for both the addiction and control cohorts. Continuous variables are shown as the mean ± SD.

The LTL was measured successfully in 916 of the 923 individuals examined (success rate > 99%). The telomere length data were natural log transformed to achieve a normal distribution. The relationship of the T/S ratio with age is shown in Fig. 1. The T/S ratio was observed to be significantly correlated with age (P = 0.046). From this population, we derived a declining age/telomere formula: LTL (T/S ratio) = −0.0016*YEAR + 0.8679; R^2 = 0.0042, which indicates that the LTL declined by 0.0016 T/S per year on average between the ages of 20 and 70 (Fig. 1). There was no significant association between the LTL and clinical characteristics (Table 3). Additionally, no significant correlation was observed between the LTL and demographic data (Table 4).

We used a multivariate linear regression model to analyse the correlation of the T/S ratio with addiction status, adjusted for age and gender. Overall, the individuals in the addiction cohort exhibited significantly
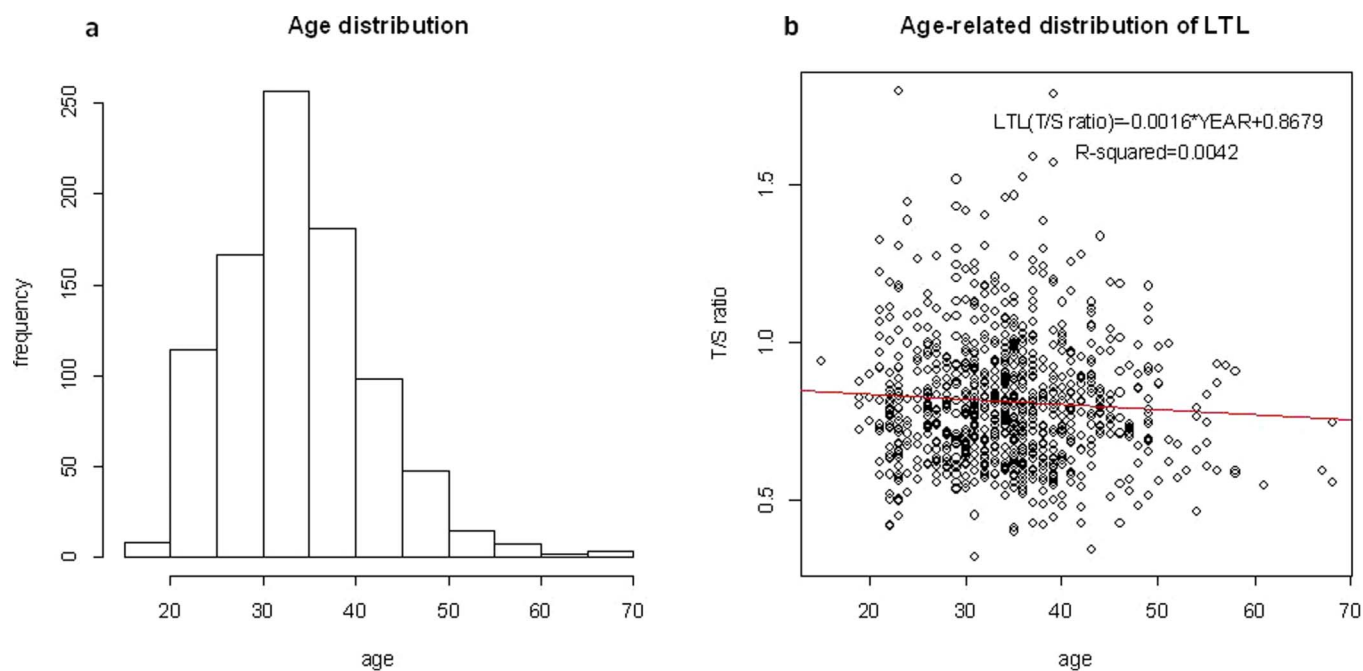
Figure 1 | (a) The age distribution of all participants in the present study. (b) The distribution of the T/S ratio according to age.

shorter LTLs than the controls after adjusting for age and gender (0.778[0.761–0.795] vs. 0.839[0.821–0.857], P = 1.32e–06, Table 5).

Drug addiction can cause drug abusers to suffer intense stress. To investigate the influence of stress in the addiction cohort, we investigated the effect of stress on two factors: libido and attitude towards life. Neither of these factors appeared to be significantly correlated with the LTL (Table 1).

To widen the scope of the study, a multivariate linear regression was performed to analyse the association of the LTL with aspects of a drug abuser's drug consumption, adjusted for age and gender (Table 1). We discovered a significant correlation between the LTL and the time before relapse (between quitting drug use and relapsing) (P = 0.02), whereas the frequency, quantity, and period of drug use were not significantly associated with the LTL. We analysed the correlation of the LTL with different methods of drug use (Table 6). We discovered that those drug abusers who ingested drugs via snuff exhibited longer LTLs (P = 0.019). However, only 26 of the drug abusers took drugs using this method. Therefore, the significant positive correlation of the LTL with snuff found in this study only serves as a reference for future studies.

We classified the drugs taken by the participants into three categories according to their effects on the central nervous system. We utilised a multivariate linear regression model to analyse the association of the LTL with each of these three categories in combination with the different categories of drug users, adjusted for age and gender (Table 7). Depressant drugs were associated with a shorter LTL (P = 0.038), whereas there was no significant association of the LTL with stimulant drugs (P = 0.525). Furthermore, we discovered negative correlations of the LTL with heroin (P = 0.018) and diazepam (P = 0.009) in the addiction cohort, while tramadol (P = 0.051) and triazolam (P = 0.086) users tended to display a shorter LTL, while drug abusers taking ketamine (P = 0.094) tended to exhibit a longer LTL.

We did not find a significant association between the LTL and the effects of polysubstance use. The P values obtained for the number of drug types and whether both depressant and stimulant drugs were taken by the same drug user were 0.881 and 0.829, respectively.

Discussion

In the present study, it was found that drug abusers exhibited a significantly shorter LTL than the population norm, after adjustment for age and gender. This result is in accord with a study by Imam[12], which, prior to the present study, had provided the main source of evidence that drug use is associated with telomere shortening. Our results suggested that drug abusers exhibit a shorter mean telomere length (0.061 T/S) than the population norm and that this shortening is equivalent to approximately 38 years of average age-related telomere attrition. The occurrence of type 2 diabetes is inversely associated with the LTL, and type 2 diabetes is equivalent to approximately 5 years of average age-related telomere attrition[13]. Compared with the effect of diabetes on the LTL in the Chinese Han population, the severe harm to telomeres caused by drug abuse is clear. In general, telomere shortening is caused by disturbances during cell division, oxidative stress, impaired antioxidant function, or interference with telomerase activity[14–17]. The most prevalent mechanism of telomere shortening is a drug-induced increase in oxidative stress[18–20]. Heroin, amphetamine, cocaine, and marijuana exposure significantly enhance the levels of oxidants, such as reactive oxygen species (ROS) and lipoperoxides, and decrease the levels of antioxidants, such as vitamin C and beta-carotene[19–22]. The balance between oxidation and antioxidation in drug abusers is seriously disturbed. Cumulative oxidative stress may cause oxidative damage to telomeric DNA[23] as well as to antioxidant defences. This damage may accelerate the rate of telomere shortening per cell division[24] and decrease the expression of telomerase reverse transcriptase, which plays a critical role in telomerase activity and is regulated by redox-sensitive transcription factors[25–27]. Furthermore, oxidative stress is not only related to telomere shortening but also correlated with drug withdrawal syndromes[28]. Determining whether telomere shortening presents a potential relationship with drug withdrawal syndromes requires further study.

Many previous studies have found that stress, such as adverse experiences during childhood[29,30], psychological stress in rape victims[31], and even prenatal stress exposure[32], can induce an increase in oxidative stress, which is the most plausible mechanism underlying telomere shortening, as mentioned above. Family conflict and

Table 1 | Association of drug use effects and LTL

	Number of drug abusers	P value*
Libido		0.27
No change	150	
Hyposexuality	103	
Serious hyposexuality	89	
Loss of sexuality	43	
Attitude towards life (frequency of losing the desire for life)		0.18
None	82	
Rarely	89	
Occasionally	113	
Frequently	84	
Frequency of drug use		0.98
Once a week	110	
Twice to five times a week	66	
Once to twice a day	89	
Over twice a day	85	
Quantity of drugs taken on each occasion		0.64
Less than 0.1 g	79	
0.1–0.3 g	75	
0.3–1 g	73	
Over 1 g	45	
Time between first drug use and waking		0.97
Less than 10 minutes	52	
10–30 minutes	48	
30–60 minutes	68	
Over 1 hour	130	
Incidence of drug treatment		0.31
Once	154	
Twice to thrice	170	
Four to five times	27	
Over six times	12	
Time before relapse		0.02
Less than 1 month	68	
1–3 months	42	
3–6 months	39	
6–12 months	39	
1–2 years	34	
Over 2 years	66	
Years of drug abuse**	7 (3–11)	0.33
Addiction intensity (0–10)**	3.75 (1–7)	0.12

*P values were calculated using a multivariate regression model adjusted for age and gender.
**Data are shown as the median (25% quartile −75% quartile).

economic pressure can be caused by compulsive drug-taking behaviour and can lead to further stress. In the present study, we investigated the degree of stress related to the subjects' libido and attitude towards life. We also gathered data on the addiction intensity exhibited by the drug abusers based on self-reported information. None of the variables showed a significant association with the LTL. This result implies that the attrition of telomeres is directly associated with the effect of drugs, rather than with the stress caused by drug taking.

Furthermore, we discovered a significant negative correlation between the LTL and the length of time before relapse (P = 0.02). We speculate that a long interval prior to relapse could establish a homeostasis that is different from that during addiction. When the new homeostasis is broken by relapse, metabolic disorders may result in an increase in oxidative stress[33] and telomere damage, but this hypothesis requires further study.

Prior to the present study, our view was that drug abusers who took drugs through intravenous injection might display shorter LTLs because the drugs would act directly on blood cells. However, our results did not support this notion. No significant difference in telomere length was discovered between drug abusers using intravenous injection and those using other methods. To our surprise, we discovered that drug abusers who used snuff to ingest drugs exhibited longer LTLs (P = 0.019). To the best of our knowledge, no previous study had investigated differences in toxicology related to different methods of drug use. In tobacco–related studies, some results have shown that the harm attributable to the use of snuff is much lower than that for tobacco smoking, although snuff is not risk free[34,35]. Similarly, the use of snuff may minimise the damage induced by drug use, thus providing a possible reason for the longer telomeres associated with this method of ingestion.

In this study, we discovered that drug abusers who had taken heroin and diazepam displayed shorter LTLs than those taking other drugs (P = 0.018 and P = 0.009, respectively, adjusted for age and gender). We conclude that heroin and diazepam may cause an increase in oxidative stress. Notably, the two drugs found to be significantly associated with the LTL are both depressant drugs that act on the central nervous system. Table 7 provides the mean T/S ratio for each type of drug. Those drug abusers who used depressant drugs exhibited shorter LTLs than those who used stimulant drugs, implying that the negative significant association of telomere length with depressant drugs (P = 0.038) was not simply related to heroin and diazepam. Whether the depressant effect plays a role in telomere shortening is an interesting subject for future research. The effects

Table 2 | Participant characteristics

	Drug abusers	Controls	All
Age (year)	33.79 ± 7.60	34.46 ± 8.16	34.16 ± 7.92
Sex			
Male	199	210	409
Female	216	289	505
Unknown	0	9	9
BMI (kg/m²)	22.03 ± 2.31	22.27 ± 3.79	22.14 ± 3.03
GPT (U/L)	56.78 ± 88.31	31.34 ± 72.15	42.41 ± 80.50
GOT (U/L)	37.79 ± 42.90	22.53 ± 11.61	29.17 ± 30.53
GGT (U/L)	29.25 ± 29.63	25.42 ± 24.96	26.57 ± 26.49
Triglyceride (mmol/L)	1.15 ± 0.66	1.31 ± 1.01	1.26 ± 0.92
Cholesterol (mmol/L)	4.49 ± 0.90	4.64 ± 0.93	4.59 ± 0.92
HDLC (mmol/L)	1.31 ± 0.27	1.22 ± 0.29	1.25 ± 0.29
LDLC (mmol/L)	2.96 ± 0.76	3.18 ± 0.93	3.11 ± 0.89
BUN (mmol/L)	3.53 ± 0.82	4.58 ± 1.08	4.26 ± 1.12
CREA (μmol/L)	58.00 ± 11.10	68.00 ± 15.36	64.90 ± 14.91
Uric acid (μmol/L)	304.8 ± 86.8	357.1 ± 91.6	341.0 ± 93.3
GLU (mmol/L)	4.49 ± 0.49	5.11 ± 0.98	4.92 ± 0.90

The values are presented as the mean ± SD where applicable. BMI, body mass index; GPT, glutamic-pyruvic transaminase; GOT, glutamic-oxaloacetic transaminase; GGT, gamma-glutamyl transferase; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; CREA, creatinine; GLU, glucose.

Table 3 | Partial Pearson's correlation coefficients of telomere length (T/S ratio) and participant characteristics

Participant characteristics	r	P value
Age (yrs)	−0.067	0.046
BMI (kg/m²)	0.060	0.269
GPT (U/L)	−0.069	0.134
GOT (U/L)	−0.061	0.185
GOT/GPT	0.017	0.655
GGT (U/L)	−0.060	0.116
Triglyceride (mmol/L)	0.010	0.806
Cholesterol (mmol/L)	−0.005	0.907
HDLC (mmol/L)	−0.030	0.446
LDLC (mmol/L)	−0.020	0.603
BUN (mmol/L)	0.031	0.417
CREA (μmol/L)	−0.061	0.113
BUN/CREA	0.060	0.122
Uric acid (μmol/L)	−0.005	0.902
GLU (mmol/L)	0.059	0.130

The partial Pearson's correlation coefficient, r, and the P value were adjusted for age. BMI, body mass index; GPT, glutamic-pyruvic transaminase; GOT, glutamic-oxaloacetic transaminase; GGT, gamma-glutamyltransferase; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; CREA, creatinine; GLU, glucose.

of polysubstance use were not significantly associated with the LTL, suggesting that different drug types might function together to shorten telomere length. Additionally, no additive effect was observed when multiple drugs were used. In this study, tramadol (P = 0.051) and triazolam (P = 0.086) were shown to confer a risk of shorter LTLs, whereas drug abusers who took ketamine (P = 0.094) tended to display longer LTLs. Determining whether tramadol and triazolam are associated with telomere shortening will require further study.

In conclusion, our results indicate that drug addiction is significantly associated with shorter telomeres. These findings may help to explain some of the effects of drug addiction on health.

Table 4 | Association of demographic data with LTL

Demographics	Number of participants	P value
Occupation		
Businessman	3	
Farmer	28	0.562
Officer	2	
Unemployed	135	0.510
Service	41	0.463
Worker	12	0.699
Skilled worker	9	
Soldier	4	
Student	1	
Other	74	0.337
Marriage Status		
Unmarried	216	0.624
Married	116	0.921
Remarried after divorce	5	
Unmarried after divorce	51	0.899
Unmarried after widowed	2	
Education		
Below primary school	59	0.589
Primary school	85	
Junior middle school	59	
Senior high school	28	
College degree	1	

P values were calculated using a multivariate regression model adjusted for age and gender. Education was included as an ordered categorical variable, while marriage and occupation were unordered categorical variables. The variables were transformed into dummy variables before analysis.

Table 5 | Association of addiction status with LTL

	Drug abusers (n = 413)	Controls (n = 503)	All (n = 916)	P value
T/S ratio	0.778 ± 0.179	0.839 ± 0.208	0.812 ± 0.199	1.32e−06

The values are presented as the mean ± SD. P values were calculated using a multivariate regression model adjusted for age and gender.

Methods

Participants. All of the study participants were Han Chinese individuals with a history of smoking. A cohort of 415 drug abusers (199 females and 216 males) between the ages of 15 and 61 (33.79 ± 7.60) was recruited from drug rehabilitation centres under the jurisdiction of the Department of Justice of Fujian Province. A control cohort consisting of 508 healthy subjects (210 females, 289 males, and 9 samples without gender information) between the ages of 20 and 68 (34.46 ± 8.16) was recruited from the Physical Examination Centre of the Second People's Hospital of Fujian Province. The control cohort specifically excluded subjects who had any history of opiate drug consumption prior to the study. Participant self-assessment indicated that all of the participants were free of serious illness, including infectious diseases, cardiovascular diseases, mental disorders, and cancer, at the time of participation. Details regarding the participants' lifestyle, level of stress (based on their libido and attitude towards life), drug consumption (e.g., the category, quantity, and frequency), demographic data (including education, marriage, occupation, and personal medical history) and physiological characteristics (e.g., height, weight, GPT (glutamic-pyruvic transaminase), and GOT (glutamic-oxaloacetic transaminase)) were obtained using standardised questionnaires and protocols. The body mass index (BMI) was calculated as weight/height². Parameters related to stress and drug consumption were considered categorical variables (Table 1). The study protocol was approved by the ethics committee of the Fujian University of Traditional Chinese Medicine, and all subjects gave informed consent.

Procedure. All subjects participated in a 12-hour overnight rapid blood draw to allow LTL and physiological measurements to be conducted. Trained research assistants then completed a survey of physiological characteristics for all participants, and the participants completed questionnaires immediately after breakfast.

Measurement of leukocyte telomere length (LTL). Leukocyte DNA was extracted using the QIAamp blood mini kit (QIAGEN, Valencia, CA). Telomere length was measured using an established and validated qPCR-based technique[11]. The relative telomere length was calculated as the T/S (telomere/single copy) ratio using RNase P as a reference (ABI) for each sample. The quantities of telomere repeats and of RNase P as a reference were also determined for each sample in duplicate in 10 μl reactions within the same plate in an ABI Applied Biosystems 7900 HT Thermal Cycler (Applied Biosystems).

The telomere reaction contained 1 × SYBR green TaqMan Gene Expression master mix (Applied Biosystems, Foster City, California), 300 nM Tel-F primers, 300 nM Tel-R primers, and 1 ng of template DNA (primers: Tel-F: 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′; Tel-R: 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′). A commercial kit was used according to the manufacturer's instructions to estimate the level of RNase P gene expression as an internal standard (TaqMan RNase P Detection Reagents Kit, Applied Biosystems) using 1× primers and the TaqMan® probe reagent, 1× TaqMan® Genotyping Master mix, and 3 ng of template DNA. The cycling conditions for the telomere and RNase P assays were as follows: 95°C incubation for 10 min, followed by either 50 cycles of 95°C for 15 sec and 60°C for 1 min.

Along with the samples, each run also contained a calibrator sample (DNA from pooled samples). Dilution series (0.675-5 ng in two-fold dilutions) were run for both the telomere and RNase P assays to establish the linear range. Good linearity was observed across this range (R² > 0.97). Any samples outside this range were diluted and run again. For quality control, all samples were run in duplicate, and the duplicate values were checked for correlation. Samples showing a CV >2% were excluded and re-run. In addition, to test the reproducibility of the assay, multivariate samples were

Table 6 | Association of different methods of drug use with LTL

Methods of drug use	Number of drug abusers	P value
Intravenous	147	0.497
Hypodermic	5	—
Oral	26	0.720
Snuff	26	0.019
Opium pipe	99	0.489

P values were calculated using a multivariate regression model adjusted for age and gender.

Table 7 | Association of different drug types with LTL

Type of drug	Number of drug abusers	T/S ratio (mean ± SD)	P value
Depressant drugs			0.038
Heroin	281	0.763 ± 0.167	0.018
Opium	8	—	—
Dolantin	21	0.739 ± 0.214	0.198
Morphine	9	—	—
Methadone	83	0.771 ± 0.154	0.935
Dihydroetorphine	3	—	—
Buprenorphine	3	—	—
Diazepam	58	0.736 ± 0.195	0.009
Secobarbital	3	—	—
Tramadol	102	0.757 ± 0.196	0.051
Marijuana	37	0.816 ± 0.225	0.339
Bucinperazine	3	—	—
Stimulant drugs			0.525
Somedon	35	0.806 ± 0.236	0.601
MDMA	63	0.808 ± 0.241	0.582
Methamphetamine	168	0.789 ± 0.198	0.798
Cocaine	7	—	—
Ephedrine	4	—	—
Ketamine	105	0.819 ± 0.220	0.094
Hallucinogenic drugs			0.086
Triazolam	62	0.753 ± 0.198	0.086
CNB	1	—	—

P values were calculated using a multivariate regression model adjusted for age and gender. MDMA, methylenedioxymethamphetamine. CNB, Caffeine and sodium benzoate.

randomly chosen and run again, and a high level of agreement was observed between the T/S ratios from the 2 runs ($R^2 = 0.831$, $P < 0.0001$).

Statistical analysis. In this study, the mean telomere length was considered a quantitative trait and expressed as the T/S ratio. Because the data were not normally distributed, log-transformed data were used for the tests and determination of correlations. Partial Pearson's correlation coefficients were calculated between the LTLs and each physiological parameter (e.g., BMI, GOT, GPT) and adjusted for age. As demographic data (e.g., marriage, education) are categorical variables, we transformed these parameters into dummy variables and conducted a multivariate linear regression analysis to calculate the correlation of the LTL with each variable adjusted for age and gender. A multivariate linear regression model was also utilised to examine the association of the LTL with addiction status adjusted for age and gender as well as to analyse the association of the T/S ratio with data on the addiction intensity, frequency of drug treatment, frequency of relapse and details regarding stress and drug usage for the subjects; all data were adjusted for age. These data were provided by the participants in the addiction cohort. Factors that applied to fewer than 10 individuals were excluded from the regression model to ensure the reliability of the results. To uncover the effects of polysubstance use on the LTL, we also analysed the association of the LTL with the number of drug types that each user had taken, as well as whether the drug abusers used both depressant drugs and stimulant drugs; the data were adjusted for age and gender. All analyses were performed using R version 2.14.0. A P value < 0.05 was considered significant in these analyses.

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Author contributions

J.Y. Ye and Z.Y. Yang contributed equally to this study. L. He and Y. Liu supervised the experiment. Z.Y. Yang and C.D. Li collected the samples and participants' characteristics. Q. Shen designed the experimental protocol. J.Y. Ye carried out the experiment. Y. Liu, Z.Y. Yang, J.Y. Ye, D.Z. Zhou, L. Cao, T. Wang, J. Wu, D.X. Cui, S.G. He, and G.Y. Qi analysed and discussed the experimental results. Finally, J.Y. Ye, Y. Liu, and Z.Y. Yang wrote the paper.

Additional information