Association of *DNMT1* Gene Polymorphisms with Systemic Lupus Erythematosus in a Han Chinese population

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Abstract

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the production of autoantibodies directed against nuclear self-antigens and the damage of multiple organs. The abnormal expression of *DNMT1* plays an important role in the pathogenesis of SLE. The aim of this study was to examine whether polymorphisms in the *DNMT1* gene are associated with the risk of SLE in Han Chinese. A total of eight SNPs within *DNMT1* were genotyped in 1068 Han Chinese including 493 patients with SLE and 575 healthy control subjects. SNP rs2162560 showed a significant association with decreased susceptibility to SLE under best fits recessive genetic model after Bonferroni correction (OR=0.503, 95% CI=0.320-0.789, *P*=0.002). Further functional analysis based on bioinformatics revealed that rs2162560 was in regulatory elements for binding by the transcriptional factors and a *DNMT1* cis expression quantitative trait locus (eQTL) that the variant allele A of rs2162560 increased *DNMT1* expression compared to the G allele. Our results suggest that genetic polymorphisms in *DNMT1* may involve in the development of SLE in Chinese Han population.

Introduction

Systemic lupus erythematosus (SLE) is considered as a prototypic autoimmune disease that is characterized by a diverse array of antinuclear autoantibody production, complement activation, immune complex deposition, and subsequent multiple organ damage. Although the etiology of SLE is still obscure, the pathogenic mechanisms of SLE are associated with the interaction between environmental exposure and genetic variations, which could result in systemic epigenetic control defects[1].

Epigenetic modifications involve heritable changes in gene expression patterns without alternations in the underlying DNA sequence such as DNA methylation. In eukaryotic cells, DNA methylation patterns are catalyzed by three main types of DNA methyltransferases (DNMTs), including DNMT1, DNMT3A and DNMT3B. DNMT3A and DNMT3B are responsible for establishing new DNA methylation, whereas DNMT1 preferentially replicates existing methylation patterns and maintains DNA methylation. DNMT1 is a major enzyme that determines genomic methylation patterns and functions, and maintains methyltranferase. DNA methylation plays important roles in the modulation chromatin structure, transcriptional regulation of gene expression, genomic stability and embryonic development[2]. Aberrant DNA methylation has been implicated in the development of many malignant and autoimmune diseases[3]. In fact, numbers of studies have addressed that global DNA hypomethylation can be observed in T cells extracted from patients with SLE compared with those from normal controls, suggesting that DNA hypomethylation contributes to the pathogenesis of SLE[4]. Although the underlying mechanism by which SLE patients show hypomethylated DNA remains unclear, a growing body of evidence indicates that down-regulation of *DNMT1* contributes to the global DNA hypomethylation in $CD4^+$ T cells in SLE. Early studies found that global hypomethylation of genomic DNA observed in $CD4^+$ T cells of lupus patients was associated with the decreased DNMT1 levels and activity[5]. Subsequent investigations further demonstrated global hypomethylation secondary to decreased DNMT1 levels[6-9]. In addition, some medicines can cause DNA hypometylation and induce

autoimmunity of SLE by repressing expression of *DNMT1* such as procainamide and hydralazine as a competitive antagonist of DNMT1 and an ERK inhibitor, respectively[10,11]. In accord with the above findings, several reports also revealed that a defect in DNMT1 is the primary reason for DNA hypomethylation in SLE[12-15]. Intriguingly, more recent works have demonstrated that some miRNAs contributed to DNA hypomethylation in lupus $CD4^+$ T cells by directly and indirectly targeting DNMT1[16-18]. Altogether, all these studies highlight the importance of DNMT1 in the pathogenesis of SLE.

The *DNMT1* gene is located on chromosome 19p13.3-p13.2 with a total size of about 62kb. Mutations in coding regions of the *DNMT1* gene have been reported to result in abnormality of the whole catalytic domain in colorectal cancer, further causing alternation of genomic DNA methylation status[19]. Numerous studies have demonstrated unambiguously that genetic variants in *DNMT1* are associated with some disease susceptibilities[20-26]. Furthermore, polymorphism rs75616428, a non-synonymous SNP in exon 4 of *DNMT1* was demonstrated to be weakly associated with an increasing production of anti-SSB (La) antibody in SLE patients in the Korean population[27]. Given the critical role of *DNMT1* in the pathogenesis of SLE, the present investigation was undertaken to evaluate possible associations between polymorphisms of the *DNMT1* gene with SLE susceptibility and clinical outcomes of SLE within a Chinese Han population.

Results

Characteristics of participants. The demographic characteristics of participants studied are summarized in Table 1. Among the patients with SLE, there were 445 women and 48 men with a mean ± SD age of 38.50 ± 13.08 years and a mean disease duration of 6.3 years. The ethnically matched healthy controls comprised 516 women and 59 men with a mean ± SD age of 39.98 ± 13.23 years. There was no significant difference between the two groups in terms of the distribution of age and percentage of female subjects.

Table 1. Clinical characteristics of the present study

Clinical profiles	SLE(n=493)	controls(n=575)
Age, mean (years)	38.50±13.08	39.98±13.23
sex ratio(female/total)	90.3%	89.7%
Disease duaration, mean(years)	6.3	
ACR criteria (%)		
Malar rash	70.6(348)	
Discoid rash	18.3(90)	
Photosensitivity	19.1(94)	
Oral ulcer	17.1(84)	
Arthritis	73.9(364)	
Serositis	31.4(155)	
Renal disorder	59.8(295)	
Neurologic disorder	11.7(58)	
Hematologic disorder	84.6(417)	
Immunologic disorder	77.3(381)	
Antinuclear antibody	98.4(485)	
Antibodies (%)		
Anti-Sm antibody	37.6(185)	
Anti-RNP antibody	41.3(204)	
Anti-Ro antibody	63.5(313)	
Anti-La antibody	27.3(135)	
Anti-dsDNA antibody	59.9(295)	

Association of *DNMT1* SNPs and susceptibility to SLE. A total of eight SNPs within *DNMT1* were successfully genotyped in 1068 Han Chinese including 493 patients with SLE and 575 healthy control subjects. The minor allele frequencies (MAFs) of the all tested SNPs in our samples were similar to the CHD dataset of the HapMap. No SNPs showed deviation from Hardy-Weinberg equilibrium in control group.

Table 2. Genotype and allele distribution of *DNMT1* SNPs in SLE patients and healthy controls

SNP/location	samples	Genotypes [n (%)]			*P*	Alleles [n (%)]		*P*
rs2336691		GG	AG	AA		G	A	
Intron 1	cases	340(69.0)	122(24.7)	31(6.3)	0.028	802(81.3)	184(18.7)	0.032
	controls	354(61.6)	184(32.0)	37(6.4)		892(77.6)	258(22.4)	
rs16999593		TT	CT	CC		T	C	
Exon 4	cases	363(73.6)	115(23.3)	15(3.1)	0.996	841(85.3)	145(14.7)	0.95
	controls	424(73.7)	134(23.3)	17(3.0)		982(85.4)	168(14.6)	
rs75616428		CC	GC	GG		C	G	
Exon 4	cases	437(88.6)	55(11.2)	1(0.2)	0.907	929(94.2)	57(5.8)	0.703
	controls	505(87.8)	69(12.0)	1(0.2)		1079(93.8)	71(6.2)	
rs2162560		GG	AG	AA		G	A	
Intron 8	cases	246(49.9)	217(44.0)	30(6.1)	**0.005**	709(71.9)	277(28.1)	0.189
	controls	288(50.1)	221(38.4)	66(11.5)		797(69.3)	353(30.7)	
rs16999358		GG	AG	AA		G	A	
Exon 12	cases	425(86.2)	66(13.4)	2(0.4))	0.019	916(92.9)	70(7.1)	0.017
	controls	461(80.2)	113(19.7)	1(0.2)		1035(90.0)	115(10.0)	
rs2241531		CC	CG	GG		C	G	
Intron 13	cases	135(27.4)	262(53.1)	96(19.5)	0.743	532(54)	454(46)	0.951
	controls	165(28.7)	292(50.8)	118(20.5)		622(54.1)	528(45.9)	
rs1863771		CC	CT	TT		C	T	
Intron 21	cases	310(62.9))	152(30.8)	31(6.3)	0.314	772(78.3)	214(21.7)	0.638
	controls	360(62.6)	190(33.0)	25(4.3)		910(79.1)	240(20.9)	
rs11880388		GG	AG	AA		G	A	
Intron 28	cases	237(48.1)	204(41.4)	52(10.5))	0.303	678(68.8)	308(31.2)	0.27
	controls	286(49.7)	244(42.5)	45(7.8)		816(71.0)	334(29.0)	

Bold means P< 0.006 (0.05/8) being considered significant after Bonferroni correction.

Table 3. Different genetic models analysis of 8 SNPs in *DNMT1* gene between SLE patients and healthy controls

		Codominant		Dominant		Recessive		Additive	
		OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
	GG	1							
rs2336691	AG	0.690(0.525-0.907)	0.029	0.721(0.559-0.929)	0.012	0.976(0.596-1.598)	0.922	0.574(0.454-0.724)	0.283
	AA	0.872(0.529-1.438)							
	TT	1							
rs16999593	CT	1.002(0.753-1.334)	0.996	1.006(0.765-1.322)	0.968	1.03(0.509-2.085)	0.934	1.008(0.775-1.311)	0.952
	CC	1.031(0.508-2.093)							
	CC	1							
rs75616428	GC	0.921 (0.632-1.343)	0.907	0.924(0.636-1.344)	0.681	1.167(0.073-18.71)	0.913	0.928(0.64-1.345)	0.692
	GG	1.156(0.072-18.53)							
	GG	1							
rs2162560	AG	1.150 (0.893-1.481)	0.006	1.042(0.812-1.337)	0.745	0.503(0.320-0.789)	**0.002**	0.919(0.729-1.158)	0.473
	AA	0.532(0.335-0.846)							
	GG	1							
rs16999358	AG	0.634(0.455-0.882)	0.019	0.647(0.466-0.898)	0.009	2.338(0.211-25.863)	0.489	0.66(0.477-0.914)	0.012
	AA	2.169(0.196-24.011)							
	CC	1							
rs2241531	CG	1.097 (0.827-1.454)	0.743	1.067(0.816-1.395)	0.634	0.937(0.693-1.266)	0.669	1.051(0.811-1.363)	0.708
	GG	0.994(0.699-1.415)							
	CC	1							
rs1863771	TC	0.929 (0.715-1.207)	0.318	0.988(0.771-1.268)	0.927	1.476(0.859-2.536)	0.158	1.035(0.816-1.315)	0.775
	TT	1.440(0.832-2.492)							
	GG	1							
rs11880388	AG	1.009(0.783-1.3)	0.306	1.069(0.84-1.36)	0.587	1.389(0.914-2.111)	0.124	1.113(0.883-1.403)	0.366
	AA	1.394(0.903-2.154)							

Note: OR, odds ratio; CI, confidence interval; OR (95%CI) and *P* were adjusted by sex and age; Bold means P< 0.006 (0.05/8) being considered significant after Bonferroni correction.

We first sought to determine whether there was an association between *DNMT1* polymorphisms and SLE susceptibility. The genotype and allele frequencies of eight SNPs within *DNMT1* in SLE patients and controls were listed in Table 2. Three SNPs, rs2336691, rs2162560 and rs16999358 were found to present significant difference at genotype and or allele levels in the SLE patients as compared with the controls, but the significant difference of rs2162560 was only retained after Bonferroni correction (P<0.006, 0.05/8). Four genetic models (codominant, dominant, recessive, and additive) were chosen to further evaluate association between the eight SNPs of *DNMT1* gene and SLE risk. Statistical analyses demonstrated similar patterns as shown in Table 3. Only rs2162560 homozygosity AA significantly decreased the susceptibility to SLE by 0.32-0.789 fold as compared with carriers of G allele (GG+AG) under best fits recessive genetic model after Bonferroni correction (P<0.006, 0.05/8).

Association of haplotypes of the *DNMT1* gene with SLE. To evaluate the correlations of SNPs in the *DNMT1* gene, we further performed linkage disequilibrium (LD) and haplotype analyses. The LD structure of *DNMT1* was shown in Supplementary Figure S1. Haplotypes with frequencies ≥ 5% in the SLE patients and the control subjects were shown in Table 4. Five major haplotypes accounted for over 68% in the distribution. The most common haplotype was H1 (GCGGGCTG) appearing in patients and controls with frequencies of 19.6% and 18.9%, respectively. Similarly, haplotype H6 (ACCGGCTG) containing the risk G allele of rs2162560 showed marginally significant association with the increased risk of SLE (P=0.016, OR=1.50, 95%CI=1.08-2.09).

Table 4. Haplotype frequencies of *DNMT1* gene in SLE cases and healthy controls

	Haplotypes	Case No (%)	Control No (%)	OR	95%CI	*P*
H1	GCGGGCTG	194(19.6)	217(18.9)	1.05	0.85-1.31	0.638
H2	ATCGGCTG	147(14.9)	165(14.4)	1.05	0.82-1.33	0.714
H3	ACCGACTG	116(11.7)	136(11.8)	0.99	0.76-1.29	0.965
H4	GCGGGCCG	114(11.6)	119(10.4)	1.13	0.86-1.49	0.37
H5	GCCGACTA	107(10.8)	137(11.9)	0.90	0.69-1.18	0.442
H6	ACCGGCTG	85(8.7)	68(5.9)	1.50	1.08-2.09	0.016

Haplotypes were constructed from the 8 SNPs of *DNMT1* gene in the order rs11880388, rs1863771, rs2241531, rs16999358, rs2162560, rs75616428, rs16999593 and rs2336691. Only haplotypes with frequencies ≥ 5% in the SLE patients and the control subjects were presented. OR, odds ratio; CI, confidence interval; OR (95%CI) and *P* were adjusted by sex and age; P<0.008 (0.05/6) was considered significant after Bonferroni correction.

Correlation between *DNMT1* SNPs and subphenotypes of SLE. We also assessed the possibility whether *DNMT1* polymorphisms could predispose to any particular clinical/serologic manifestations of SLE. Subphenotype stratification analysis was performed by comparing allele distribution between patients with and without the subphenotype (Supplementary Table S1). Several SNPs (rs2162560, rs16999358 and rs2241531) showed association with certain clinical subphenotypes of SLE, but neither of them survived after Bonferroni correction (P >0.0004, 0.05/128).

Bioinformatic analysis. Using HaploReg database, we predicted that rs2162560 is in regulatory elements for binding by the transcriptional factors AP1 (activator protein 1), LXR1 (liver X-activated receptor 1) and RORα1 (retinoid related orphan receptor alpha1) (Supplementary Table S2). The LOD scores for the G/A alleles of rs2162560 were +12.0, +11.9, and +12.0 for AP1, LXR1 and RORα1, respectively. This showed higher affinity to regulatory motifs for the variant A allele relative to the G allele. We further found that among the set of 14SNPs being in strong linkage disequilibrium ($r^2 \geq 0.8$) with rs2162560 in the Asian population, 9 variants were within regions of enhancer histone marks, 4 were in regions of DNAase I hypersensitivity, 5 were in regions of eQTL, and 12 were in regulatory motif regions from multiple cells types, suggesting that rs2162560 was located in a regulatory region (Supplementary Table

S3). In addition, rs2162560 was observed being in absolute LD (r^2 =1) with a potential functional SNP rs6511677, a cis eQTL for *DNMT1*. Furthermore, analysis of *DNMT1* expression levels in the Blood eQTL dataset has demonstrated that rs2162560 was a *DNMT1* cis expression quantitative trait locus that the variant allele A of rs2162560 increased *DNMT1* expression compared to the G allele (P=3.4×10^{-29}, Z=11.22) (Supplementary Table S4).

Discussion

SLE is an autoimmune disorder characterized by T lymphocyte autoreactivity and the presence of autoantibodies against self antigens leading to immune complex deposition and multiple tissue damage. T cell DNA demethylation plays an important role in the pathogenesis of SLE. As a matter of fact, several studies have demonstrated that DNA extracted from T cells of SLE patients is globally hypomethylated when compared to DNA from normal T cells, causing autoimmune-related gene overexpression and autoreactive monocyte/macrophage killing and excessive B cell stimulation[12-15]. Accumulating evidence of the role of *DNMT1* in the genomic methylation showed the importance of *DNMT1* in the pathogenesis of SLE. The *DNMT1* genetic polymorphisms not only exert a modifying effect on LINE-1methylation, but also are associated with some diseases including autoimmune disorders. These suggest the hypothesis that genetic polymorphisms of *DNMT1* may involve in genetic susceptibility to SLE development. So, we want to know whether *DNMT1* polymorphisms were associated with SLE susceptibility and its clinical/serologic manifestations.

In the present study, we investigated the association of *DNMT1* polymorphisms with the risk of developing SLE in a Han Chinese population from Southwest China. We observed for the first time that a tag SNP rs2162560 located in intron 8 of *DNMT1* was associated with decreased susceptibility to SLE with statistical power of 82%.

According to HaploReg database, our in silico functional analysis showed that

rs2162560 was located in regulatory region, where the variant A allele of rs2162560 presented higher affinity to the transcription factor regulatory motifs compared to the G allele. Furthermore, analysis of *DNMT1* expression levels in the Blood eQTL dataset has demonstrated that rs2162560 was a *DNMT1* cis expression quantitative trait locus that the variant allele A of rs2162560 increased *DNMT1* expression compared to the G allele. These bioinformatic results of rs2162560 were concordant with the observation that global DNA hypomethylation and the decreased DNMT1 levels appeared in T cells extracted from patients with SLE compared with those from normal controls, which provides a functional interpretation for the genetic association of rs2162560 variant A of *DNMT1* with decreased susceptibility to SLE. Of special note, our in silico functional analysis showed that rs2162560 affected the binding of transcription factor RORα1. RORα has been proved to be implicated in the regulation T helper (Th) 17 cell differentiation[28]. The interleukin-17 (IL-17)-producing Th 17 cell subset exerts pro-inflammatory activity in the pathogenesis of several autoimmune diseases including SLE[29,30]. Altogether, these bioinformatics analyses may shed more light on the role of *DNMT1* in the pathogenesis of SLE.

However, few association studies concerning *DNMT1* polymorphisms with SLE have been reported. Until now, only one study has assessed the associations between *DNMT1* polymorphisms and SLE[27]. In that study of SLE patients from a Korean population, Park *et al.* analyzed 8 *DNMT1* polymorphisms, among which four SNPs (rs2241531, rs16999593, rs1863771 and rs7516428) also appeared in our research. Their results suggested that none of the eight SNPs studied was associated with the risk of SLE. Nevertheless, further analyses of association with autoantibody production in SLE patients showed that rs75616428, a nonsynonymous SNP (V120L) in exon 4, was associated with anti-La antibody production (P=0.04), although this association did not survive correction for multiple testing. Nevertheless, *DNMT1* has not been reported to be associated with SLE at the genome-wide significance level in genome-wide association studies (GWAS)[31,32]. Whether our *DNMT1* SNPs were sufficiently represented in the platform used in these GWAS is unclear. The potential cause for this discrepancy of association of *DNMT1* with SLE between the GWAS

and our study also includes heterogeneity in clinical subphenotypes among different populations. In addition, ethnic differences in the genes or environmental factors that interact with *DNMT1* might also explain the disparity. In fact, population difference in the genetic background was observed even between the closely related northern Chinese and southern Chinese populations. Of interest, in earlier whole-genome scanning study of SLE families, Namjou *et al.* investigated 71 multiplex SLE American pedigrees and identified potential susceptibility loci at chromosome 19p13.2, where *DNMT1* coincidently exists[33]. This possibly indicated that the SLE susceptibility of the genetic region 19p13.2 could be implemented by *DNMT1*. It is therefore important to evaluate these results further by systematically attempting to replicate them in further studies.

There are some limitations in this study that should be noted. The sample size in the present study was still relatively small although our study has a statistical power of greater than 80% to detect the significant effect between the SNPs analyzed and SLE, except for rs75616428 (64%). In particular, no any significant association was detected in our subphenotype stratification analysis. However, this finding should be treated with caution as the statistical power was found to be low given the limited sample sizes of our stratified subphenotype analysis performed among paired patient groups. In addition, our study was performed only in Chinese Han population.

In summary, our study for Chinese population provides the first report that genetic variant rs2162560 in *DNMT1* is associated with the susceptibility to SLE. Considering the biological function of *DNMT1*, we suggest that *DNMT1* is a potentially novel candidate gene for SLE. Nevertheless, further studies with a larger sample size in different populations are needed to validate our results.

Methods

Study subjects. A total of 493 SLE patients and 575 healthy controls were recruited from Yunnan Province in Southwest China. All SLE patients fulfilled the American College of Rheumatology classification criteria for the classification of SLE[34] and

were recorded with detailed clinical data for the stratified analysis. Healthy controls had no medical history or current symptoms of rheumatism and immunology diseases, nor any abnormal clinical examination results. All subjects were genetically unrelated Han Chinese according to self-reported ancestry. Healthy controls were matched to SLE cases on the basis of gender, age, ethnicity, and local residential region. This study was reviewed and approved by the Research Ethics Committee of Yunnan University. Informed consent was obtained from all participants. All experiments were performed in accordance with relevant guidelines and regulations.

DNA preparation. Genomic DNA was extracted from peripheral blood leukocytes with a standard phenol-chloroform method, and stored at -70□ until use.

SNP selection and genotyping. SNP selection was based on the SNP information in the public database (NCBI dbSNP, http//www.ncbi.nlm.nih.gov/projects/SNP/; HapMap, http//hapmap.ncbi.nlm.nih.gov/, phase 3, CHB) and the previously published literature[27]. Haplotype tagging SNPs were selected from the Han Chinese data in the HapMap Project using SNPBrowserTM Software v4.0 to capture SNPs with a minimum minor allele frequency (MAF) of 0.05 with a pair-wise r^2 of 0.8 or greater. In the *DNMT1* gene, eight SNPs (rs2241531, rs11880388, rs2162560, rs2336691, rs16999358, rs16999593, rs75616428, rs1863771) were selected, among which the first six were Tag SNPs.

Polymerase chain reaction-fragment length polymorphism (PCR-RFLP) was performed for genotyping. AS a quality control, 10% of samples for each SNP were randomly chosen and directly sequenced using the ABI PRISM Big Dye Terminator V3.1 Sequencing Kit and ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, California, USA). There was 100% correspondence between the PCR-RFLP based genotyping and direct sequencing results.

Bioinformatic analysis. Variant effects on regulatory motifs were investigated using the HaploReg v4.1 database

(http://www.broadinstitute.org/mammals/haploreg/haploreg.php) and calculated according to the equation LOD (alt)－LOD (ref), where LOD is the logarithm of odds, alt is the alternative sequence, and ref is the reference sequence. A positive result indicated that the predicted relative affinity was higher for the alternative sequence, while a negative result indicated that the predicted relative affinity was higher for the reference sequence. To explore whether the identified SNPs had an expression quantitative trait loci (eQTL) effect, associations between sequence variation and gene expression were determined. Sequence variation and gene expression profiling data were from the blood eQTL data set of non-transformed peripheral blood samples of 5,311 individuals (http://www.genenetwork.nl/bloodeqtlbrowser).

Statistical analysis. Deviations of genotype frequencies in controls from those expected under the Hardy-Weinberg equilibrium were assessed by the Chi-square test. The linkage disequilibrium (LD) structures of the eight SNPs of the *DNMT1* gene were constructed using HaploView software 4.2 according to the genotyping data of the cases and controls. Either Chi-square test or Fisher's exact test was performed by comparing the distributions of frequencies of allele, genotype and haplotype between cases and controls. Association analysis using the odds ratio (OR), 95% confidence interval (95%CI) and corresponding *P* was carried out through unconditional logistic regression model with an adjustment of age and gender. Subphenotype stratification analysis was performed, in which association was evaluated by comparing allele distribution between patient with and without the subphenotype (paired patient groups). All statistical tests were two-tailed and *P*-values were corrected by applying the Bonferroni method for multiple testing. The adjusted *P* value for significance was set at 0.05/N, where N is the number of multiple testing. All analyses were performed using SPSS17.0 (SPSS Inc., Chicago, Illinois, USA). The power of the statistical tests was calculated using the QUANTO software program[35].

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Acknowledgements

We are grateful to all the participants in this study. This work was supported by the National Natural Science Foundation of China (31471187 & 81372360).

Author Contributions

Conceived and designed the study: C.X., W.H., S.Z. H.B.; Collected the sample: X.L., B.W.; Performed the experiments: W.H., S.Z., H.B., Y.Z., T.L.; Analyzed the data: C.X., W.H., S.Z., H.Y.; Drafted and revised the manuscript: C.X., W.H., H.Y., B.Z.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/serp

Competing financial interest: The authors declare no competing financial interest.