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Evaluation of Glutathione Peroxidase 4 role in Preeclampsia

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Preeclampsia (PE) is a pregnancy-specific syndrome that may be lifethreatening to pregnancies and fetus. Glutathione Peroxidase 4 (GPx4) is a powerful antioxidant enzyme that can provide protection from oxidative stress damage which plays a pivotal role in the pathology of PE. Therefore, this study aims to investigate the association between *Gpx4* polymorphisms and the susceptibility to PE in Chinese Han women. TaqMan allelic discrimination real-time PCR was used to perform the genotyping of rs713041 and rs4807542 in 1008 PE patients and 1386 normotensive pregnancies. Obviously statistical difference of genotypic and allelic frequencies were found of rs713041 in *GPx4* between PE patients and controls and the C allele has the higher risk for pathogenesis of PE ($\chi^2 = 12.292$, $P = 0.002$ by genotype; $\chi^2 = 11.035$, $P = 0.001$, OR = 1.216, 95% CI 1.084–1.365 by allele). Additionally, when subdividing these samples into CC + CT and TT groups, we found a significant difference between the two groups ($\chi^2 = 11.241$, $P = 0.001$, OR = 1.417, 95% CI 1.155–1.738). Furthermore, the genotype of rs713041 was found to be associated with the mild, severe and early-onset PE. Our results suggest that rs713041 in *GPx4* may play a key role in the pathogenesis of PE.

Preeclampsia (PE) is an unpredictable and potentially dangerous clinical complication that occurs during the second half of pregnancy, characterized by the new onset hypertension and proteinuria after 20th week of gestation¹. It is a leading cause of perinatal morbidity and mortality worldwide for which is often accompanied with multi-organ disorders such as liver rupture, stroke, pulmonary oedema, or kidney failure, which can all be lethal without effective treatment except for terminating pregnancy^{2,3}. Although extensive researches have been made for many years, the etiology and pathophysiology of this disorder remains elusive. Several factors may be associated with the development of PE, such as oxidative stress, inflammation and hereditary variants^{4,5}.

Strong evidence exists that oxidative stress plays a pivotal role in the pathology of PE^{6–8}. Oxidative stress is a state in which the balance between reactive oxygen species (ROS) and the antioxidant host defenses is disturbed and ROS prevails over the antioxidant host defenses^{9–11}. ROS include free radical species which borne unpaired electrons and their non-radical intermediates, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$). The anti-oxidants, e.g. glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase, vitamins C and E, and b-carotene played a significant role in scavenging free radicals¹². During normal gestation, ROS and the anti-oxidants are in the balanced status. However, when ROS increase or the anti-oxidants decrease, thnificante pregnancies will be in an oxidative state which may involve in the etiopathogenesis of PE¹³.

GPx4 is a powerful antioxidant enzyme that is capable of metabolizing H_2O_2 and lipid hydroperoxides to water or the corresponding unreactive alcohols which can provide protection from oxidative damage¹⁴. A cohort study revealed that *GPx4* mRNA expression decreased in PE patients compared to their respective control group. Mistry and his colleagues reported that GPx4 was in a lower level in PE than controls^{15,16}. Therefore, decreased expression of GPx4 may be associated with the development of PE. In addition, GPx4 is also reported to be involved in other functions such as male fertility and apoptosis¹⁷. Moreover, epidemiological research revealed that genetic factors appeared to have an important role in the pathology of PE and significant associations between PE and candidate genes variants that is involved in oxidative stress have been identified^{4,5,18}. Additionally, two tag SNPs

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Characteristics	PE	Control	t	p-value
Maternal age (years)	30.45 ± 5.06	30.25 ± 3.91	1.033	0.302
Age of menarche (years)	14.01 ± 1.18	14.00 ± 1.25	0.21	0.833
Gestational age at admission (weeks)	35.24 ± 3.53	39.20 ± 1.38	-33.691	<0.001
Gestational age at delivery (weeks)	35.80 ± 3.15	39.43 ± 1.15	-33.676	<0.001
Times of gravidity	2.25 ± 1.27	2.24 ± 1.16	0.369	0.712
Number of abortion	1.48 ± 0.67	1.42 ± 0.72	1.344	0.179
Systolic blood pressure (mmHg)	161.10 ± 18.68	114.71 ± 9.88	71.758	<0.001
Diastolic blood pressure (mmHg)	104.69 ± 13.69	73.44 ± 7.83	65.083	<0.001
Birth weight of offspring (g)	2505.91 ± 937.29	3405.94 ± 357.36	-27.698	<0.001

Table 1. The demographic and clinical characteristics of PE and control groups.

Group	N	rs713041						rs4807542					
		CC	CT	TT	CC/CT+TT	CC+CT/TT	C	T	AA	AG	GG	A	G
PE	1008	333	497	178			1163	853	19	188	801	226	1790
Control	1386	402	661	323			1465	1307	20	286	1080	326	2446
χ^2		12.292			4.458	11.241	11.035		2.037			0.346	
p-value		0.002			0.035	0.001	0.001		0.361			0.556	
OR					1.208	1.417	1.216					0.947	
95% CI					1.014–1.439	1.155–1.738	1.084–1.365					0.791–1.134	

Table 2. The comparison of genotypic and allelic frequencies of both SNPs between PE and controls.

(rs713041 and rs4807542) in *GPx4* whose product belonged to antioxidant enzymes were reported to have an influence on its expression possibly¹⁹. Therefore, in this case-control study, we aim to explore the association between the polymorphisms of *GPx4* and the susceptibility to PE in Chinese Han women.

Results

Demographic and clinical characteristics. Table 1 shows the comparison in demographic and clinical characteristics between cases and controls. The age of cases and controls was 30.45 ± 5.06 and 30.25 ± 3.91 years old respectively, which was matched between both groups ($P = 0.302$). We have not observed significant differences in times of gravidity ($P = 0.712$), number of abortions ($P = 0.179$) and age of menarche ($P = 0.833$) between cases and controls. However, compared to controls, PE patients had earlier admitted and delivered weeks, higher blood pressure and lower birth weight of offspring ($P < 0.001$).

Genetic analysis. The participants of control group in our study were in accordance with HWE for both SNPs and had a group representative (for rs713041, $\chi^2 = 2.571$, $P = 0.109$; for rs4807542, $\chi^2 = 0.046$, $P = 0.830$).

The genotypic and allelic frequencies of rs713041 and rs4807542 in cases and controls were summarized in Table 2. For rs713041, the genotypic and allelic distributions between two groups were statistically different ($\chi^2 = 12.292$, $P = 0.002$ by genotype; $\chi^2 = 11.035$, $P = 0.001$, OR = 1.216, 95% CI 1.084–1.365 by allele). When subdividing these samples into CC and CT + TT groups, no obvious difference between cases and controls was found ($\chi^2 = 4.458$, $P = 0.035$, OR = 1.208, 95% CI 1.014–1.439). Then, when subdividing these samples into CC + CT and TT groups, we found a significant difference between cases and controls ($\chi^2 = 11.241$, $P = 0.001$, OR = 1.417, 95% CI 1.155–1.738). The C allele of rs713041 was related to the development of PE ($\chi^2 = 11.035$, $P = 0.001$, OR = 1.216, 95% CI 1.084–1.365). Nevertheless, no statistical differences were observed in rs4807542 between the two groups in terms of genotypic frequencies ($\chi^2 = 2.037$, $P = 0.361$), nor for allelic frequencies ($\chi^2 = 0.346$, $P = 0.556$, OR = 0.947, 95% CI 0.791–1.134).

We divided PE patients into mild and severe PE groups according to guidelines from the American College of Obstetricians and Gynecologists in order to further explore the association between the variants of both SNPs and PE²⁰. The genotypic and allelic frequencies of rs713041 and rs4807542 in mild or severe PE were compared with those in normotensive pregnancies respectively. In Table 3 statistical differences between mild/severe PE patients and normotensive controls in both genotypic distributions and allelic frequencies of rs713041 were observed (mild PE vs. control: $\chi^2 = 10.716$, $P = 0.005$ by genotype; $\chi^2 = 10.981$, $P = 0.001$, OR = 1.497, 95% CI 1.178–1.903 by allele; severe PE vs. control: $\chi^2 = 8.572$, $P = 0.014$ by genotype; $\chi^2 = 6.509$, $P = 0.011$, OR = 1.171, 95% CI 1.037–1.323 by allele). However, the mild/severe PE patients did not differ significantly from the controls in either genotypic distributions or allelic frequencies of rs4807542 (mild PE vs. control: $\chi^2 = 0.275$, $P = 0.872$ by genotype; $\chi^2 = 0.001$, $P = 0.978$, OR = 0.995, 95% CI 0.693–1.429 by allele; severe PE vs. control: $\chi^2 = 2.077$, $P = 0.354$ by genotype; $\chi^2 = 0.427$, $P = 0.513$, OR = 0.939, 95% CI 0.776–1.135 by allele).

We defined early-onset PE patients as those diagnosed before the 34th week of gestation, known to be more severely affected than those with later-onset PE²¹. As shown in Table 4, we identified significant differences in both genotype and allelic frequencies of rs713041 between early-onset PE patients and controls while no statistical

Group	N	rs713041					rs4807542				
		CC	CT	TT	C	T	AA	AG	GG	A	G
Mild PE	158	64	70	24	198	118	3	31	124	37	279
Control	1386	402	661	323	1465	1307	20	286	1080	326	2446
χ^2		10.716			10.981		0.275			0.001	
p-value		0.005			0.001		0.872			0.978	
OR					1.497					0.995	
95% CI					1.178–1.903					0.693–1.429	
Severe PE	850	269	427	154			16	157	677	189	1511
Control	1386	402	661	323			20	286	1080	326	2446
χ^2		8.572			6.509		2.077			0.427	
p-value		0.014			0.011		0.354			0.513	
OR					1.171					0.939	
95% CI					1.037–1.323					0.776–1.135	

Table 3. The comparison of genotypic and allelic frequencies of both SNPs between mild/severe PE and control groups.

Group	N	rs713041					rs4807542				
		CC	CT	TT	C	T	AA	AG	GG	A	G
Early-onset PE	523	181	253	89	615	431	12	105	406	129	917
Control	1386	402	661	323	1465	1307	20	286	1080	326	2446
χ^2		10.896			10.825		1.704			0.237	
p-value		0.004			0.001		0.427			0.615	
OR					1.273					1.056	
95% CI					1.102–1.470					0.849–1.312	
Late-onset PE	485	152	244	89	548	422	7	83	395	97	873
Control	1386	402	661	323	1465	1307	20	286	1080	326	2446
χ^2		5.175			3.841		2.824			2.221	
p-value		0.075			0.052		0.244			0.136	
OR					1.159					0.834	
95% CI					1.000–1.342					0.656–1.059	

Table 4. The comparison of genotypic and allelic frequencies of both SNPs between early/late-onset PE and control groups.

significances were found between patients with later-onset PE and normotensive pregnancies (early-onset PE vs. control: $\chi^2 = 10.896$, $P = 0.004$ by genotype; $\chi^2 = 10.825$, $P = 0.001$, OR = 1.273, 95% CI 1.102–1.470 by allele; later-onset PE vs. control: $\chi^2 = 5.175$, $P = 0.075$ by genotype; $\chi^2 = 3.841$, $P = 0.052$, OR = 1.159, 95% CI 1.000–1.342 by allele). For rs4807542, there were no statistical differences in genotypic distributions and allelic frequencies between early/late-onset PE and control groups (early-onset PE vs. control: $\chi^2 = 2.678$, $P = 0.262$ by genotype; $\chi^2 = 1.126$, $P = 0.289$, OR = 1.146, 95% CI 0.891–1.475 by allele; later-onset PE vs. control: $\chi^2 = 2.917$, $P = 0.233$ by genotype; $\chi^2 = 2.196$, $P = 0.138$, OR = 0.853, 95% CI 0.691–1.053 by allele).

Analysis of genotype-phenotype relationship. Table 5 demonstrated the results of a detailed genotype-phenotype analysis of rs713041 which involved clinical characteristics and laboratory examination. When PE patients were divided into CC, CT and TT groups, no significant differences in the parameters between the three groups were found, including systolic pressure, diastolic pressure, birth weight of offspring, white blood cell count (WBC), neutrophil count, red blood cell count (RBC), haemoglobin (Hb), platelet (PLT), prothrombin time (PT), activated partial thromboplastin time (APTT), alanine transaminase (ALT), aspartate transaminase (AST), urea nitrogen, creatinine ($P > 0.05$). Moreover, there were no statistical significances either when PE patients were fractionized into CC/CT + TT groups and CC + CT/ TT groups ($P > 0.05$).

Discussion

PE is a pregnancy-specific syndrome and may be accompanied with severe complications when left untreated, which will threaten the health of mothers and fetus²². Although the etiology of PE is unclear, some causes have been reported that may have a possible role in the development of the disorder. A causal relationship between oxidative stress and the pathophysiology of PE was demonstrated to exist^{23,24}. Oxidative stress arises from an imbalance between the generation of ROS and the antioxidants that prevent harmful effects of ROS. Various molecules, including lipids, proteins, and DNA, can be affected and damaged by this disparity⁹.

	①CC	②CT	③TT	①vs②vs③	①vs②	①vs③	②vs③	①vs②+③	①+②vs③
	Mean ± SD	Mean ± SD	Mean ± SD	p-value	p-value	p-value	p-value	p-value	p-value
Systolic blood pressure (mmHg)	162.06 ± 20.09	160.37 ± 17.91	161.33 ± 18.04	0.438	0.203	0.672	0.56	0.252	0.644
Diastolic blood pressure (mmHg)	105.05 ± 14.51	104.06 ± 13.46	105.81 ± 12.69	0.289	0.308	0.545	0.142	0.568	0.244
Birth weight of offspring (g)	2498.36 ± 944.44	2481.73 ± 937.38	2587.12 ± 924.74	0.465	0.811	0.33	0.22	0.863	0.348
RBC ($\times 10^{12}/L$)	4.00 ± 0.65	4.05 ± 0.58	4.03 ± 0.52	0.549	0.275	0.559	0.792	0.287	0.83
Hb (g/L)	113.12 ± 17.76	115.67 ± 20.11	114.60 ± 17.54	0.164	0.057	0.399	0.518	0.074	0.624
WBC ($\times 10^9/L$)	9.60 ± 3.02	9.65 ± 2.85	9.49 ± 2.70	0.811	0.789	0.687	0.519	0.951	0.611
neutrophil ($\times 10^9/L$)	7.21 ± 2.76	7.18 ± 2.68	7.07 ± 2.44	0.845	0.87	0.569	0.636	0.737	0.709
PLT ($\times 10^9/L$)	203.22 ± 69.88	207.16 ± 69.83	196.85 ± 72.23	0.237	0.429	0.329	0.094	0.797	0.205
PT (s)	10.07 ± 1.66	10.29 ± 1.71	10.05 ± 1.62	0.124	0.084	0.861	0.111	0.202	0.225
APTT (s)	31.02 ± 3.86	30.83 ± 3.79	30.70 ± 4.03	0.65	0.501	0.381	0.7	0.537	0.546
ALT (IU/L)	26.09 ± 43.32	26.30 ± 48.20	22.98 ± 32.65	0.68	0.945	0.454	0.395	0.825	0.499
AST (IU/L)	29.28 ± 35.96	30.55 ± 39.32	29.32 ± 36.70	0.87	0.635	0.991	0.711	0.708	0.779
urea nitrogen (mmol/L)	4.86 ± 2.15	4.90 ± 3.83	4.74 ± 1.87	0.846	0.848	0.689	0.563	0.451	0.96
creatinine (umol/L)	68.05 ± 33.21	66.59 ± 33.51	67.20 ± 23.46	0.814	0.521	0.774	0.83	0.545	0.861

Table 5. Associations between genotypes of rs713041 and characteristics among PE patients.

GPx4 is an intracellular antioxidant selenoprotein which can provide protection from oxidative damage. It is a monomer that consists of a cytosolic (cGPx4), a mitochondrial (mGPx4) and sperm nuclear GPx4 (snGPx4) isoforms. cGPx4 exists in cells ubiquitously; mGPx4, as well as snGPx4, mainly exists in testis while only trace amounts are expressed in other tissues^{17,25}. It plays a pivotal role in prohibiting the development of oxidative stress by reducing H₂O₂ and lipid hydroperoxides to water or the corresponding alcohols. Furthermore, it can directly reduce bound phospholipid hydroperoxides within membranes and lipoproteins, which is the only enzymatic antioxidant known to have this unique function of all the GPxs²⁶. Because of the significant function of GPx4 to counteract oxidative stress, decrease in GPx4 level may be associated with the pathogenesis of PE, in which oxidative stress plays a key role. Evidences also indicated that the GPx4 mRNA and protein level were lower in PE patients than those in their normotensive counterparts^{15,16}. In addition, GPx4 is also reported to be involved in other cell functions such as male fertility, apoptosis and modulating inflammation through regulating leukotriene biosynthesis and cytokine signaling pathways^{17,27}.

GPx4, located in chr19p13.3, comprised by 7 exons and several introns in human. Regina Brigelius-Flohé found that it was lethal to the mice when *gpx4* was knocked out entirely from the whole genome¹⁷. Variants in *GPx4* could influence its antioxidant capacity and other functions²⁸. Two candidate SNPs (rs713041 and rs4807542) in *GPx4* were chosen as the tag SNPs. The polymorphism of rs713041, located in the 3'UTR which is near the Sec insertion sequence element at position 718, could modulate the synthesis of *GPx4* through changing the affinity of the Sec insertion machinery for its Sec insertion sequence element and protein that combines with the 3'UTR¹⁹. Moreover, lipid metabolism and several cancers are reported to be related to this polymorphism^{29–32}. Both SNPs are seated in the exons and might influence the gene expression¹⁹. As these two SNPs might affect the expression of *GPx4* and the antioxidant capacity of its product which is pivotal in the pathogenesis and development of PE, we performed this case–control study to explore the relationship between polymorphism of *GPx4* and PE.

In our study, we observed significant differences in genotypic and allelic frequencies of rs713041 between PE patients and their normotensive counterparts, which can demonstrate an association between *GPx4* rs713041 polymorphism and PE. Furthermore, for rs713041, the C allele was higher in PE patients than that in normal controls and may be the risk allele for the development of PE by calculating of the value of OR. When cases were divided into CC + CT/TT groups, we found significant differences with OR1.417, through which we can conclude that patients with CC or CT genotype were more inclined to develop PE than patients with TT genotype. However, we failed to identify an association between the genotype or allelic frequencies of rs4807542 and PE.

In addition, when subdividing cases into mild/severe or early-onset/late-onset groups, the genotypic distributions and allelic frequencies of rs713041 were associated with mild PE, severe PE and early-onset PE with OR1.497, OR1.171, OR1.273 by allele respectively. However, for rs4807542 no statistically significant differences were demonstrated between healthy pregnancies and patients with PE in all these subgroups. In conclusion, our results suggest that the polymorphism of rs713041 in *GPx4* may play a pivotal role in the pathogenesis and progression of PE.

To our knowledge, this was the first time to investigate the polymorphisms of *GPx4* in patients with PE. However, previous studies had explored the association between genetic polymorphisms in other oxidative stress related genes and PE. Ji Hyae Limsuch *et al.* indicated that the variant genotype of the *COMT* (MetMet) was associated with an increased risk of PE³³; Ebru *et al.* demonstrated that the –463G/A polymorphism of *MPO* could be an intriguing susceptibility factor of PE in Turkish population³⁴; and Lucia and his colleagues proposed that polymorphisms of rs4880 in *SOD2* was significantly associated with PE³⁵. In addition, it was reported that rs713041 in *GPx4* was associated with idiopathic recurrent miscarriage and it could be regarded as a predictor of cerebral stroke in Russian patients with essential hypertension, while Xiao *et al.* stated that both SNPs were related to the development of Kashin–Beck disease in the Chinese population^{19,36,37}.

A limitation of our study is that all participants were ethnic Han Chinese coming from Shandong province. As regional and racial differences are probably to influence the results, our observations could not represent other human races. Furthermore, we did not explore the relationship between PE and environmental factors such as diet, smoking and stress which may participate in the pathogenesis and development of PE. In addition, one or even several genetic variants might not influence gene expression for that PE is a multifactorial hereditary disease but we only investigated two SNPs of *GPx4*. The relationship between rs713041 and the susceptibility to PE may be direct association meaning that the site rs713041 has function such as regulating gene transcription, influencing gene expression and so on. However, the association may also be indirect meaning that the site rs713041 has linkage disequilibrium with other adjacent functional sites, which just is a linkage marker without function itself. Therefore, functional experiments measuring *GPx4* expression and activity need to be conducted to strengthen our conclusions in the future. Despite some limitations, our study suggested that *GPx4* may involve in the development of PE in Chinese Han pregnancies. Larger-scale studies involving candidate genes and variant SNPs are necessary to be conducted in different races and regions with functional and environmental analyses to validate our findings and further explore the pathogenesis of PE.

Methods

Subjects. We recruited 1008 diagnosed PE patients as the case group and 1386 age-matched normotensive pregnancies as the control group. All the subjects are Chinese Han women that come from Binzhou Medical University Hospital, the Affiliated Hospital of Qingdao University, Linyi People's Hospital, Liaocheng People's Hospital, the Maternal and Child Health Care of Zaozhuang, Yantai Yuhuangding Hospital and Yantai Shan Hospital between January 2013 and November 2015. The research staffs filled out the questionnaire which contains maternal age, gestational weeks of admission and delivery, blood pressure, pregnancy and delivery history, clinical symptoms, and results of laboratory examinations. All PE patients and normal controls signed the informed consent. Our study was approved by the ethics committee of the Affiliated Hospital of Qingdao University and the methods were carried out in accordance with the relevant guidelines.

PE was defined as onset of hypertension ($\geq 140/90$ mmHg) with detectable urinary protein (≥ 0.3 g/24 h, or $\geq 1+$ by dipstick) after 20 weeks of gestation which may be accompanied by headache, blurred vision and upper abdominal discomfort. Both PE and control pregnant women are age matched and have no previous history of PE and a systemic disease such as chronic hypertension, heart disease, diabetes mellitus, thyroid function disorder, kidney disorders, hepatic diseases excluding from artificial insemination, twin or multiple pregnancy, and macrosomia in the present gestation.

Genetic studies. DNA was isolated from peripheral blood lymphocytes using a Qiagen DNA extraction kit (Qiagen, Hilden, Germany). The TaqMan allelic discrimination real-time PCR was used to genotype the polymorphisms of rs713041 and rs4807542 in *GPx4*. We used the Taqman probes and primers which were designed by Applied Biosystems of Life Technologies (New York, USA). The primers of rs713041 were 5'-CCGCCCGAGCCCCTGCCACGCCCT-3' (forward) and 5'-GGAGCCTTCCACCGCACTCATGAC-3' (reverse). The primers of rs4807542 were 5'-GCCGCCTTTGCCGCTACTGAAGCC-3' (forward) and 5'-GCGCTGCTGTGGGGCTCTGGCCG-3' (reverse). The polymerase chain reaction (PCR) system which was 25 μ l totally consisted of 1.25 μ l 20 \times SNP Genotyping Assay, 12.5 μ l 2 \times PCR Master Mix, and 11.25 μ l DNA and DNase-free water. C1000TM thermal cycler system was used to conduct the Amplifications with the following conditions: 95 °C for 3 min, followed by 45 cycles at 95 °C for 15 sec and 60 °C for 1 min. The fluorescent signals from VIC/FAM-labeled probes were detected each cycle. Bio-Rad CFX manager 3.0 software was used to conduct the genotyping.

Statistical analysis. We used statistical software package SPSS 21.0 (SPSS Inc., Chicago, IL, USA) to perform all analyses. Hardy—Weinberg equilibrium (HWE) was assessed in the controls by chi-square test. Differences between cases and controls in demographic and clinical characteristics were compared using Student's t-test. The genotype-phenotype analysis was conducted by an analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$ (two-sided). We used Pearson's χ^2 test (if expected values were below 5, Fisher's exact test was used) to compare differences in allelic and genotypic distributions between two groups and a level of $P < 0.025$ (two-sided) was considered statistically significant when Bonferroni's correction was made. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to show the relative risk degree.

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Author Contributions

K.C. and S.L. conceived and designed the experiments. X.P. and Y.L. performed the experiments and analyzed the data. J. Li, M.L., J.W., X.L., J. Liu, X.J., Z.J. and Z.H. contributed the reagents, materials and helped collect the samples. X.P. and Y.L. wrote the paper. K.C. and S.L. revised the paper. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Additional Information

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