The prevalence of cerebral aneurysms (CAs) in the world’s population ranges from 1% to 6%. The rupture of a CA can cause aneurysmal subarachnoid hemorrhage (SAH), which results in a high fatality rate of 65% and disables 50% of the patients who survive it. Current therapeutics for treating CAs include mainly microsurgical clipping and endovascular treatment. However, both of these treatments are invasive and confer a nonnegligible risk of procedural morbidity.

Great efforts have been made to investigate the molecular mechanisms of CA formation and development. It has been suggested that extracellular matrix disruption, inflammatory reactions, phenotypic vascular smooth muscle cell (SMC) modulation, dysfunction of endothelial cells, and lncRNA expression profile of long noncoding RNAs in human cerebral aneurysms: a microarray analysis

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OBJECTIVE The pathogenesis of cerebral aneurysms (CAs) remains largely unknown. Long noncoding RNAs (lncRNAs) were reported recently to play crucial roles in many physiological and biological processes. Here, the authors compared the gene-expression profiles of CAs and their control arteries to investigate the potential functions of lncRNAs in the formation of CAs.

METHODS A prospective case-control study was designed to identify the changes in expression of lncRNAs and mRNAs between 12 saccular CA samples (case group) and 12 paired superficial temporal artery samples (control group). Microarray analysis was performed to investigate the expression of lncRNAs and messenger RNAs (mRNAs), and reverse-transcription quantitative polymerase chain reaction was used to validate the microarray analysis findings. Then, an lncRNA target-prediction program and gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were applied to explore potential lncRNA functions.

RESULTS A comparison between the case and control groups revealed that 1518 lncRNAs and 2545 mRNAs were expressed differentially. By using target-prediction program analysis, the authors constructed a complex network consisting of 2786 matched lncRNA-mRNA pairs, in which ine1 mRNA was potentially targeted by one to tens of lncRNAs, and vice versa. The results of further gene ontology and KEGG pathway analyses indicated that lncRNAs were involved mainly in regulating immune/inflammatory processes/pathways and vascular smooth muscle contraction, both of which are known to have crucial pathobiological relevance in terms of CA formation.

CONCLUSIONS By comparing CAs with their control arteries, the authors created an expression profile of lncRNAs in CAs and propose here their possible roles in the pathogenesis of CAs. The results of this study provide novel insight into the mechanisms of CA pathogenesis and shed light on developing new therapeutic intervention for CAs in the future.
and overproduction of reactive oxygen species play crucial roles in the pathogenesis of CAs. However, the molecular mechanisms involved in the formation of CAs are so complex that the current knowledge remains insufficient.

Long noncoding RNAs (lncRNAs) are >200 nucleotides in length and are a class of single-stranded RNAs that lack protein-coding ability. IncRNAs can promote and repress neighboring and distant target genes by connecting to microRNA and acting as tethers, guides, decoys, and scaffolds for interactions with proteins. Recent studies found that IncRNAs can promote, fine-tune, and restrain inflammatory processes, reduce apoptosis of vascular smooth muscle cells (SMCs), and protect the endothelium against dysfunction. All of these biological processes are pathologically relevant to CA formation. Hence, it is postulated that lncRNAs might have important functions in the pathogenesis of CAs.

Taking advantage of microarray technology, we conducted a prospective case-control study to investigate the expression profile of lncRNAs in CAs in comparison with that of their control arteries, and we further propose their putative roles in CA formation.

**Methods**

**Study Design and Samples**

This prospective case-control study was approved by the Beijing Tiantan Hospital Ethics Committee, and informed consent was obtained from each of the patients. Between September 2014 and July 2015, patients who were diagnosed with saccular CA and who underwent microsurgical clipping in our department were enrolled. Aneurysmal tissue was obtained during surgery without endangering the patients. We collected control superficial temporal arteries (STAs) if they were injured during the pterional or lateral frontal craniotomy. CA samples and paired STAs from the same individuals were used in this study. Overall 12 CAs and 12 paired STAs were obtained. After collection, the samples were transferred immediately into liquid nitrogen and stored in a freezer at −80°C until RNA extraction.

**RNA Extraction and Microarray Hybridization**

According to manufacturer instructions, total RNA was extracted from the samples by using Trizol reagent (Sigma). Then, RNA purity and concentration were determined by using a spectrophotometer (NanoDrop ND-1000). Furthermore, RNA integrity was determined by capillary electrophoresis with an RNA 6000 Nano LabChip kit and the Bioanalyzer 2100 (Agilent Technologies). If the integrity number value of an RNA was >6, its extracts were used for further analysis.

Microarray hybridization was then performed on an lncRNA + mRNA Human Gene Expression Microarray V4.0 4 × 180K chip (CapitalBio Corp). In brief, 1 μg of total RNA extracted from the samples was amplified and transcribed into double-stranded cDNA using CbcScript reverse transcriptase according to the manufacturer’s protocol (CapitalBio). Double-stranded cDNA products were purified using a PCR NucleoSpin Extract II kit, eluted with 30 μl of elution buffer, and then mixed with 40 μl of in vitro transcription reaction mixes at 37°C for 14 hours with a T7 enzyme mix for amplifying cRNA. After reverse transcription with CbcScript II reverse transcriptase, a Klenow enzyme-labeling strategy was used.

**Data Analysis and Bioinformatics**

The GeneSpring v13.0 software package (Agilent Technologies) was used for data normalization, quality control, and calculation of the differences in gene expression. Genes were considered differentially expressed if their fold-change value was ≥2 or ≤−2 between the two groups and their p value was <0.05 (t-test for case/paired-control ratio; the p value was corrected by the falsecovery rate). Hierarchical clustering with average linkage was used to calculate the distinguishable IncRNA and mRNA expression patterns. With the help of gene annotations from the University of California, Santa Cruz (http://genome.ucsc.edu/), we defined cis target genes as those with differentially expressed mRNA transcribed within a 10-kb window upstream or downstream of the differentially expressed IncRNA genomic location. trans target-gene predictions were made by comparing the full sequence of a differentially expressed IncRNA with the three untranslated region of differentially expressed mRNAs using BLAT tools (stand-alone BLAT v35x1fast sequence search command line tool, download from: http://hgdownload.cse.ucsc.edu/admin/exe/) with the default parameter setting. Gene ontology (GO) analysis was based on information in the GO database (http://geneontology.org/). On the website, “GO biological process complete” was selected at the “annotation data set” selection, and the other parameters were set with their default value. The selection criteria for GO-term enrichment were fold enrichment >2 and p < 0.05 (the p value was corrected by Bonferroni correction for multiple testing). In addition, pathway analysis, which was used to identify significant pathways for the differentially expressed genes, was based on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (https://david.ncifcrf.gov/) using Fisher’s exact test, and p < 0.05 was a selection criterion for enriched pathways.

**Validation With Reverse-Transcription Quantitative Polymerase Chain Reaction**

Eight randomly selected lncRNA and mRNA transcripts (p29111, p4995, p15422, p1608, A_33_P3511265, A_24_P77968, A_21_P0014047, and A_24_P272310) were validated by reverse-transcription quantitative polymerase chain reaction (RT-qPCR). The RNA left after microarray hybridization was reverse transcribed to complementary DNAs (cDNAs) with PrimeScript RT reagent kit (TaKaRa Bio, Inc.). RT-qPCR was performed according to the SYBR Premix Ex Taq II (Tli RNaseH Plus) instructions. Then, the reactions were processed with a 7500 real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Inc.). A standard dilution curve was used to determine amplification efficiency. Glyceraldehyde 3-phosphate dehydrogenase was used as the control gene to normalize gene expression in each sample.

**Results**

CA and STA samples were obtained from 12 patients...
Expression profile of lncRNAs in human cerebral aneurysms

Expression Profiles of lncRNAs and mRNAs

Hierarchical clustering revealed systematic variations between the case and control groups in the expression of lncRNAs (Fig. 1). Compared with the lncRNAs in the STAs, 1518 lncRNAs were differentially expressed in CAs; 413 were upregulated, and 1105 were downregulated. According to the genomic locations that implied that the lncRNAs have potential functions in regulating their neighboring protein-coding genes, each of the differential-

ly expressed lncRNAs was classified into 1 of 5 categories (antisense, intergenic, intronic, divergent, and other) (Fig. 2). More than one-third of these lncRNAs belong to the intergenic IncRNA category, of which many are conserved across mammalian species and are functional.10

In addition, the protein-coding mRNA profiles between the CAs and STAs were also compared. In comparison with the mRNAs in the STAs, 2545 mRNAs were differentially expressed in CAs; 1150 were upregulated and 1395 were downregulated.

Validation of the Microarray Results by RT-qPCR

To evaluate the consistency of the microarray, 4 lncRNAs and 4 mRNAs were randomly selected and analyzed further with RT-qPCR in 12 CAs and 12 STAs. As expected, the expression trends of these transcripts were similar to those from the microarray results (Fig. 3).

Long Noncoding RNA Target-Gene Prediction

Procedures for defining the target genes of differentially expressed lncRNAs included 2 steps. The first step was predicting potential lncRNA targets via cis and trans prediction programs. The second step was integrating the predicted potential lncRNA targets with the differentially expressed mRNAs. This procedure resulted in a complex lncRNA target network that consisted of 2786 matched lncRNA-mRNA pairs for 559 differentially expressed lncRNAs (160 lncRNAs were upregulated and 399 lncRNAs were downregulated in the CAs) and 408 differentially expressed mRNAs (144 mRNAs were upregulated and 264 mRNAs were downregulated in CAs). Within the network, a single mRNA was potentially tar-

TABLE 1. Summary of clinical and aneurysm sample features

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Location</th>
<th>Size (cm)</th>
<th>Ruptured</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>51</td>
<td>MCA</td>
<td>1.6</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>63</td>
<td>MCA</td>
<td>1.6</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>47</td>
<td>ACoA</td>
<td>0.6</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>56</td>
<td>ACoA</td>
<td>0.6</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>67</td>
<td>MCA</td>
<td>0.7</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>46</td>
<td>ICA</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>65</td>
<td>ACA</td>
<td>3.5</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>59</td>
<td>ICA</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>57</td>
<td>ACoA</td>
<td>0.8</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>45</td>
<td>ICA</td>
<td>2.7</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>45</td>
<td>ACoA</td>
<td>0.8</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>58</td>
<td>ACoA</td>
<td>0.8</td>
<td>Yes</td>
</tr>
</tbody>
</table>

ACA = anterior cerebral artery; ACoA = anterior communicating artery; ICA = internal carotid artery; MCA = middle cerebral artery.

with CA (age 55 ± 8 years [mean ± SD]) (Table 1). All the patients were of Chinese Han ethnicity.
geted by one to tens of lncRNAs, and vice versa (Supple-
mentary Table 1 and Supplementary Fig. 1), and the link
numbers a node has could measure gene centrality, which
determines the relative importance.

**Gene Ontology and KEGG Pathway Analysis**

Then, functional analysis of these target genes was
performed. By using GO biological process analysis,
functional terms for upregulated (Supplementary Table 2)
and downregulated (Supplementary Table 3) target genes
in the CA groups were enriched significantly. Most of
the upregulated and downregulated functional GO terms
were related to the immune/inflammatory system and
muscle contraction, respectively. In addition, as shown in
Table 2, the top 5 upregulated biological processes were
mainly T-cell/lymphocyte chemotaxis/migration, and the
top 5 downregulated biological processes included mainly
smooth muscle contraction and muscle cell differentiation.

**FIG. 2.** Subgroups of the differentially expressed lncRNAs according to their genomic location and relationships with nearby
protein-coding genes.

**FIG. 3.** RT-qPCR validation of selected lncRNAs and mRNAs in 12 CAs and 12 STAs. The relative expression represents the rela-
tive fold-change levels of the CA group values over the STA group values. Error bars indicate the standard error within the CA and
STA groups; the horizontal dotted line indicates an expression level of 1. Figure is available in color online only.
TABLE 2. Top 5 GO biological processes of upregulated and downregulated target genes in CAs versus STAs

<table>
<thead>
<tr>
<th>GO Term</th>
<th>Fold Enrichment</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upregulated target genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO:0010818: T-cell chemotaxis</td>
<td>&gt;5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GO:0072678: T-cell migration</td>
<td>&gt;5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GO:0048247: lymphocyte chemotaxis</td>
<td>&gt;5</td>
<td>0.002</td>
</tr>
<tr>
<td>GO:0072676: lymphocyte migration</td>
<td>&gt;5</td>
<td>0.008</td>
</tr>
<tr>
<td>GO:0070228: smooth muscle contraction</td>
<td>&gt;5</td>
<td>0.017</td>
</tr>
<tr>
<td>Downregulated target genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO:0006939: smooth muscle contraction</td>
<td>&gt;5</td>
<td>0.009</td>
</tr>
<tr>
<td>GO:0006936: muscle contraction</td>
<td>&gt;5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GO:0003012: muscle system process</td>
<td>&gt;5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GO:0051146: striated muscle cell differentiation</td>
<td>&gt;5</td>
<td>0.026</td>
</tr>
<tr>
<td>GO:0042692: muscle cell differentiation</td>
<td>&gt;5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* CAs versus STAs.

TABLE 3. KEGG pathways of upregulated and downregulated target genes in CAs versus STAs

<table>
<thead>
<tr>
<th>KEGG Term</th>
<th>Fold Enrichment</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upregulated target genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa04062: chemokine signaling pathway</td>
<td>4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsa04514: cell adhesion molecules</td>
<td>4.3</td>
<td>0.003</td>
</tr>
<tr>
<td>hsa04620: Toll-like receptor signaling pathway</td>
<td>4.7</td>
<td>0.004</td>
</tr>
<tr>
<td>hsa04142: lysosome</td>
<td>4.0</td>
<td>0.008</td>
</tr>
<tr>
<td>hsa04662: B-cell receptor signaling pathway</td>
<td>5.0</td>
<td>0.008</td>
</tr>
<tr>
<td>hsa04060: cytokine–cytokine receptor interaction</td>
<td>2.5</td>
<td>0.02</td>
</tr>
<tr>
<td>hsa05322: systemic lupus erythematosus</td>
<td>3.8</td>
<td>0.02</td>
</tr>
<tr>
<td>hsa04672: intestinal immune network for IgA production</td>
<td>5.8</td>
<td>0.015</td>
</tr>
<tr>
<td>Downregulated target genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa04270: vascular smooth muscle contraction</td>
<td>8.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsa04510: focal adhesion</td>
<td>3.1</td>
<td>0.002</td>
</tr>
<tr>
<td>hsa05414: dilated cardiomyopathy</td>
<td>4.5</td>
<td>0.002</td>
</tr>
<tr>
<td>hsa04920: adipocyte signaling pathway</td>
<td>5.2</td>
<td>0.003</td>
</tr>
<tr>
<td>hsa04070: phosphatidylinositol signaling system</td>
<td>4.7</td>
<td>0.004</td>
</tr>
<tr>
<td>hsa04670: leukocyte transendothelial migration</td>
<td>3.5</td>
<td>0.007</td>
</tr>
<tr>
<td>hsa05410: hypertrophic cardiomyopathy</td>
<td>4.1</td>
<td>0.007</td>
</tr>
<tr>
<td>hsa04020: calcium signaling pathway</td>
<td>2.8</td>
<td>0.013</td>
</tr>
<tr>
<td>hsa05416: viral myocarditis</td>
<td>3.9</td>
<td>0.018</td>
</tr>
<tr>
<td>hsa05412: arrhythmogenic right ventricular cardiomyopathy</td>
<td>3.7</td>
<td>0.023</td>
</tr>
</tbody>
</table>

IgA = immunoglobulin A.

* CAs versus STAs.

Expression profile of lncRNAs in human cerebral aneurysms

As KEGG pathway analysis (Table 3) also revealed consistently that upregulated target genes participated significantly in immune/inflammatory-related pathways. Among these pathways, the chemokine signaling pathway was the most affected. Moreover, in this pathway, chemokine ligand 5 (CCL5), a well-known chemokine implicated in aneurysms, was found to be targeted by up to 17 lncRNAs, which implies its centrality (Supplementary Fig. 2). The other pathways enriched by upregulated target genes included the B-cell receptor signaling and cytokine–cytokine receptor interaction pathways, among others. The most affected pathway enriched by downregulated target genes was vascular smooth muscle contraction. As shown in Fig. 4, a number of genes involved in the vascular smooth muscle contraction pathway were targets of lncRNAs. Other pathways related to vessel integrity and mechanical strength, such as the focal adhesion and dilated cardiomyopathy pathways, were also found to be enriched by the downregulated target genes.

Discussion

This study was a purely descriptive analysis in which the comprehensive role of lncRNAs in the pathogenesis of CAs was investigated. We identified the lncRNA and mRNA expression profile in CAs in comparison with that in their paired control arteries. Afterward, by bioinformatics analysis, we found that lncRNAs were involved mainly in the regulation of biological immune/inflammatory processes/pathways and vascular smooth muscle contraction, both of which are known to have crucial pathobiologic relevance in terms of CA formation.

Increasing evidence has pointed to chronic vascular inflammation as the leading factor in the pathogenesis of CA. In particular, several lncRNAs are now emerging as important regulators in the modulation of the immune/inflammatory system. They are involved in the control of proinflammatory gene expression, immune cell susceptibility to viruses, monocyte differentiation, and several crucial regulators in the inflammatory system, including nuclear factor κB (NFκB), cyclooxygenase 2 (Cox2), and tumor necrosis factor–α (TNFα). In the context of CA, we also found that the number of biological immune/inflammatory processes and pathways were potentially targeted by lncRNAs (Table 2). Among all of these biological processes, the most affected was the regulation of T-cell/lymphocyte chemokinesis/migration. The infiltration of these inflammatory cells into the arterial wall is believed to be an early event in aneurysm formation. In addition, lymphocytes, mainly T lymphocytes, are the dominant cells producing proinflammatory cytokines in CA tissue and are associated with CA rupture. Therefore, it could be hypothesized that lncRNAs promote CA formation and development by regulating the recruitment and infiltration of T cells/lymphocytes into the CA walls. The chemokine signaling pathway was consistently found to be the most affected pathway in our pathway enrichment analysis. Chemokines are a group of structurally related cytokines that can promote cell migration, especially that of inflammatory cells. In particular, several chemokines potentially targeted by lncRNAs play important roles in aneurysm formation and development. For in-
stance, in this study, CCL5 was found to be a central gene targeted by up to 17 lncRNAs. CCL5 is a potent chemoattractant of inflammatory cells that express chemokine C-C motif receptors (CCRs), including macrophages, T cells, and dendritic cells. Within animal models of aortic aneurysm and human aortic aneurysm biopsy specimens, CCL5 is upregulated consistently, and blocking the association of CCL5 and CXC chemokine ligand 4 (CXCL4) might inhibit aortic aneurysm development and progression. SMCs are the predominant matrix-synthesizing cells of the vascular wall and are responsible for the structural integrity of the arterial wall. The phenotypic modulation of SMCs from a differentiated phenotype concerned with contraction to a dedifferentiated phenotype that promotes inflammation and matrix breakdown is believed to be an important contributor to the pathogenesis of CAs. In our study, genes involved in vascular smooth muscle contraction, such as MYOCD, ACTA2, MYH11, and MYL9, were predicted to be targeted by lncRNAs. MYOCD, targeted by 4 lncRNAs, functions as a transcriptional coactivator of serum response factor (SRF) and modulates the expression of smooth muscle–specific SRF target genes. In the establishment and maintenance of the SMC contractile phenotype, MYOCD has a dominant role by directing an SMC differentiation program. It should be noted that a previous study found that the expression level of MYOCD was reduced with attenuated lncRNA SENCR, which provided experimental evidence for the regulating roles of lncRNAs on contractile genes. However, in our study, SENCR was not differentially expressed between CAs and STAs, and its relationship with MYOCD was not included in the subsequent bioinformatics analysis. The other 3 genes encode structural muscle contraction proteins. ACTA2 encodes α-actin, which is a major constituent of the contractile apparatus of cells. Defects in this gene cause thoracic aortic aneurysms and dissections. MYH11 and MYL9 encode structural proteins that belong to the myosin heavy chain and light chain family, respectively. The protein encoded by MYH11 functions as a major contractile protein that converts chemical energy to mechanical energy through the hydrolysis of ATP. Mutations in MYH11 also cause human thoracic aortic aneurysms and dissections. The protein encoded by MYL9 regulates muscle contraction by modulating the

FIG. 4. Downregulated target genes in the vascular smooth muscle contraction pathway. Target genes are marked with a red star. (Image generated using the DAVID website [https://david.ncifcrf.gov/].)
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ATPase activity of myosin heads.\textsuperscript{23} Altogether, lncRNAs might contribute to the pathogenesis of CAs by regulating loss of the contractile SMC phenotype.

In addition, in this study, the downregulated target genes were also enriched in vessel integrity and mechanical strength–related pathways, including the focal adhesion and dilated cardiomyopathy pathways, which suggests that lncRNAs might regulate CA formation by impairing the mechanical strength of vessels.

Our study has some potential limitations. First, despite the relatively large number of CAs, the total number of samples in this study was still small because it is difficult to obtain CA tissue. Second, because of the difficulty in obtaining intracranial arteries from humans, we chose STAs as the control arteries, which is a method established in previous similar studies.\textsuperscript{18,22,24} Nonetheless, STAs are not intracranial arteries, and the morphological and phenotypic differences between STAs and intracranial arteries might have affected the final results. Third, this was a purely descriptive study, not a mechanistic one, so we cannot be sure whether the identified differentially expressed lncRNAs were the cause of CA formation or if they were just secondary to the presence of CA. Last, but not least, the functions of lncRNAs are based on bioinformatics prediction and require further experimental validation.

Conclusions

By comparing CAs and their control arteries, we identified differentially expressed lncRNAs and proposed their possible roles in the pathogenesis of CAs. The results of our study provide novel insight into the mechanisms of CA pathogenesis and shed light on developing new therapeutic interventions for CA in the future.

Acknowledgments

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References


Disclosures
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions
Conception and design: Zhao. Acquisition of data: Hao Li, Yue, Hao. Analysis and interpretation of data: Hao Li, Zhang. Drafting the article: Hao Li. Critically revising the article: Zhao. Statistical analysis: Hao Li. Administrative/technical/material support: Haowen Li. Study supervision: Wang, Yu, Zhang, Cao.

Supplemental Information
Online-Only Content
Supplemental material is available with the online version of the article.

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