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Research

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CAV1 and VCL are Downregulated in Atherosclerotic Aortic Endothelial

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Abstract

Background: Atherosclerosis (AS) is a common atherosclerotic vascular disease, and is one of the
important factors leading to cardiovascular and cerebrovascular diseases. So far, the specific etiology
and pathogenesis of AS have not been clarified, and further research is needed. Methods: Bioinformatics methods were used to analyze the data set of GSE57691 and GSE137578 in normal and
atherosclerotic arterial endothelial cells from Gene Expression Omnibus (GEO). Results: There are a
total of 300 differentially expressed genes (DEGs) in the GSE57691 and GSE137578 datasets, which
are mainly enriched in the focal adhesion signaling pathway (adj P<0.05). We identified 10 hub genes
(ACTG2, CAV1, CALD1, CDC42, CCT2, CCT3, VCL, PPARG, POLR2F and TPM3) in the
protein-protein interaction (PPI) network, of which 3 (CAV1, CDC42 and VCL) significantly enriched
in the adhesion signaling pathway. In addition, a search in the BIOGPS database found that CAV1 and
VCL are highly expressed in coronary arteries. Conclusions: In conclusion, bioinformatics technology
has proved to be useful for screening and identifying novel biomarkers of diseases. 300 DEGs and 10
hub genes were significantly enriched in atherosclerotic aortic endothelial cells, especially CAV1 and
VCL genes.

Keywords

atherosclerosis, Aortic Endothelial, bioinformatics analysis

Background

Atherosclerosis (AS), the pathological basis of stroke, coronary heart disease and other obstructive
vascular disease, is a common atherosclerotic vascular disease with a high mortality and disability rate.
The common causes of AS are hypertension, hyperlipidemia, diabetes, smoking, and so on. AS starts
with injury of arterial vascular endothelium and is accompanied by multiple processes such as
inflammation, immune response and lipid infiltration\(^1\). Mononuclear macrophages migrate to the
intima, LDL is oxidized and modified to form ox-LDL, which binds to scavenger receptors of
mononuclear macrophages and form macrophage-derived foam cells. Vascular smooth muscle cells
from the medial membrane of the artery enter the intima and engulf lipids to form myogenic foam cells,
which proliferate and migrate to form fibrous plaques. Ox-LDL cracks macrophage derived foam cells and muscle derived foam cells, and then atherosclerotic plaques can be formed[2]. However, the biomarkers used to diagnose atherosclerotic disease have not been defined.

Since the 21st century, A good deal of databases have been established and applied to bioinformatics technologies, which have been used to explore potential genetic targets for diseases and help researchers identify the differentially expressed genes (DEGs) and their potential pathways related to atherosclerosis. In this way, researchers have identified a number of genes and pathways that are intimately involved in atherosclerosis[3-5]. However, due to the high false positive rate, it is difficult to obtain credible results when using independent microarray technology. Therefore, the GSE57691 and GSE137578 datasets are simultaneously selected for analysis and verification. In this study, we identified common differentially expressed genes (DEGs) in atherosclerotic endothelial cells (GSE57691 and GSE137578) and used bioinformatics methods to identify key pathways and core genes involved in atherosclerotic endothelial injury. It is hoped that this study can uncover potential diagnostic markers of atherosclerotic endothelial injury.

Results
DEGs identified between control and atherosclerotic group
The datasets of GSE57691 and GSE137578 were analyzed by GEO2R, the differences between the control and the atherosclerotic group were shown in the volcano plots (Figures 1A and 1B). After standardization of the results, a total of 1,457 and 3,876 DEGs were respectively identified in the GSE57691 and GSE137578 datasets. A Venn diagram was constructed for the 2 examined datasets, and simultaneously revealed that there were 300 genes (Figures 1C).

Enrichment analysis of DEGs through GO and KEGG pathway analysis
The GO and KEGG signaling pathways of 300 DEGs were analyzed by David and metascape(Figure 2). GO analysis show that biological process (BP) are significantly enriched in the process of muscle contraction. There were main variations in cell composition (CC), including Focal adhesion, cytosol, extracellular exosome, myelin sheath, actin cytoskeleton, nucleoplasm, perinuclear region of Cytoplasm, and so on. The variations of molecular function (MF) were significantly abundant in protein binding and actin binding. KEGG pathway analysis showed that all DEGs were mainly concentrated in Focal adhesion. Meanwhile, Enrichment analysis of GSE57691 and GSE137578 were
analyzed by GSEA software, and the heatmaps of the top 50 characteristics of each phenotype were acquired (Figure 3A-3B). In addition, the enrichment analysis of metascape also demonstrates that the DEGs between the control and atherosclerosis were significantly enriched in the adhesion process (Figure 3C-3D). Eleven genes (ACTN1, ACTN4, CAV1, CAV2, CDC42, CCND2, CCND3, LAMC1, DIAPH1, ITGA10, ITGA11, FLNC and VCL) were markedly enriched in the Focal adhesion signaling pathway (Figure 4). Finally, the Disgenet database analysis showed that DEGs were significantly enriched in vascular disease, myocardial disease and tumor (Table 2 and Figure 5).

Figure 1. (A) The volcano plot illustrates the differences between control and atherosclerotic group after analysis of the GSE57691 dataset with GEO2R. (B) The volcano plot illustrates the difference between control and atherosclerotic group after analysis of the GSE137578 dataset with GEO2R. (C) The Venn
diagram could demonstrate that 300 genes were contained in the GSE57691 and GSE137578 datasets simultaneously. (D) Overlap between gene lists: At the gene level, where purple curves link identical genes. At the shared term level, where blue curves link genes that belong to the same enriched ontology term. The inner circle represents gene lists, where hits are arranged along the arc. Genes that hit multiple lists are colored in dark orange, and genes unique to a list are shown in light orange. The outer ring: blue represents GSE57691 and red represents GSE137578.

**Table 1. Enrichment analysis of DEGs in atherosclerosis using DAVID.**

| Category               | Term     | Description               | Count | FDR          |
|------------------------|----------|---------------------------|-------|--------------|
| BP term                | GO:0006936 | muscle contraction       | 12    | 2.63E-03     |
| CC term                | GO:0005925 | focal adhesion            | 26    | 8.30E-07     |
| CC term                | GO:0005829 | cytosol                   | 87    | 6.73E-05     |
| CC term                | GO:0070062 | extracellular exosome     | 76    | 1.16E-04     |
| CC term                | GO:0043209 | myelin sheath             | 13    | 3.74E-04     |
| CC term                | GO:0015629 | actin cytoskeleton        | 14    | 2.21E-03     |
| CC term                | GO:0005654 | nucleoplasm               | 70    | 2.21E-03     |
| CC term                | GO:0048471 | perinuclear region of cytoplasm | 22   | 3.47E-02 |
| MF term                | GO:0005515 | protein binding           | 180   | 2.29E-03     |
| MF term                | GO:0003779 | actin binding             | 17    | 3.01E-03     |
| KEGG_PATHWAY           | hsa04510  | Focal adhesion            | 14    | 4.86E-02     |

**Figure 2.** (A) Combined diagram of enriched terms across input differently expressed gene lists using DAVID, histogram colored by count and line chart colored by p-value. (B) Heatmap of enriched terms across input differently expressed gene lists using metascape, colored by p-values.
Figure 3. (A) HeatMap of the top 100 features for each phenotype in GSE57691. (B) HeatMap of the top 100 features for each phenotype in GSE137578. (C) Network of enriched terms colored by cluster.
identity, where nodes that share the same cluster identity are typically close to each other. (D) Network of enriched terms colored by p-value, where terms containing more genes tend to have a more significant p-value.

Figure 4. Eleven genes (ACTN1, ACTN4, CAV1, CAV2, CDC42, CCND2, CCND3, LAMC1, DIAPH1, ITGA10, ITGA11, FLNC and VCL) were markedly enriched in the Focal adhesion signaling pathway. Actinin alpha mean ACTN1 and ACTN4; Caveolin mean CAV1 and CAV2; Cell division cycle 42 means CDC42; Cyclin D mean CCND2 and CCND3; Diaphanous related formin 1 means DIAPH1; Integrin subunit alpha mean ITGA10 and ITGA11; Filamin C means FLNC; Vinculin means VCL.

Figure 5. The enrichment of DGEs in diseases was analyzed by Disgenet.
Table 2. The enrichment of DGEs in diseases was analyzed by Disgenet.

| GO           | Description                        | Count | Log10(P) | Log10(q) |
|--------------|------------------------------------|-------|----------|----------|
| C3203102     | Idiopathic pulmonary arterial hypertension | 113   | -17.00   | -13.00   |
| C0026848     | Myopathy                           | 97    | -16.00   | -13.00   |
| C0521158     | Recurrent tumor                     | 105   | -15.00   | -12.00   |
| C0205696     | Anaplastic carcinoma                | 51    | -15.00   | -12.00   |
| C0025286     | Meningioma                          | 94    | -15.00   | -11.00   |
| C0002793     | Anaplasia                           | 84    | -14.00   | -11.00   |
| C0162871     | Aortic Aneurysm, Abdominal          | 88    | -14.00   | -11.00   |
| C0007194     | Hypertrophic Cardiomyopathy         | 85    | -14.00   | -11.00   |
| C4086152     | Childhood Astrocytoma               | 90    | -14.00   | -11.00   |
| C0042373     | Vascular Diseases                   | 97    | -14.00   | -11.00   |
| C0007193     | Cardiomyopathy, Dilated            | 79    | -13.00   | -10.00   |
| C0038525     | Subarachnoid Hemorrhage             | 77    | -13.00   | -9.90    |
| C0205699     | Carcinomatosis                      | 42    | -13.00   | -9.90    |
| C0151744     | Myocardial Ischemia                 | 101   | -13.00   | -9.70    |
| C0025500     | Mesothelioma                        | 82    | -12.00   | -9.60    |
| C0013080     | Down Syndrome                      | 101   | -12.00   | -9.50    |
| C1134719     | Invasive Ductal Breast Carcinoma    | 67    | -12.00   | -9.50    |
| C0003130     | Anoxia                              | 52    | -12.00   | -9.30    |
| C0153690     | Secondary malignant neoplasm of bone| 89    | -12.00   | -9.10    |
| C0205698     | Undifferentiated carcinoma          | 53    | -12.00   | -9.10    |

PPI network construction, hub genes selection and analysis

The protein-protein interactions among the DEGs were predicted with STRING tools and Cytoscape software. The PPI network involves 240 nodes and 693 edges (Figure 6). The top ten genes evaluated by connectivity degree in the PPI network were identified using the Cytohubba plugin (Table 2 and Figure 7A). And the heatmaps of hub genes in GSE57691 and GSE137578 were created (Figure 7B-7C). Importantly, ACTG2, CDC42, CCT2, CCT3, CAV1, CALD1, TPM3, PPARG, POLR2F and VCL were the common high-degree genes.
Figure 6. The PPI network of DEGs was constructed by the STRING online database. A total of 240 nodes and 693 edges were included in the PPI network.

Table 2  Top ten hub genes with higher degree of connectivity

| Gene Symbol | Name                                             | Score |
|-------------|--------------------------------------------------|-------|
| CDC42       | cell division cycle 42                          | 30    |
| CCT2        | chaperonin containing TCP1 subunit 2             | 26    |
| VCL         | vinculin                                         | 25    |
| ACTG2       | actin gamma 2, smooth muscle                     | 21    |
| PPARG       | peroxisome proliferator activated receptor gamma | 20    |
| POLR2F      | RNA polymerase II subunit F                      | 19    |
| CCT3        | chaperonin containing TCP1 subunit 3             | 18    |
| TPM3        | tropomyosin 3                                   | 18    |
| CAV1        | caveolin 1                                       | 18    |
| CALD1       | caldesmon 1                                      | 17    |
Specific gene expression in coronary artery

The hub gene was imported into the BioGPS database to view the specific gene expression in normal tissues. The expression score of hub gene in coronary artery was summarized in Table 3. Among them, VCL and CAV1 were highly expressed in normal coronary artery tissues (Figure 6).
Figure 7. The specific gene expression was constructed by the BioGPS database. (A) The expression of HUB gene in coronary arteries. (B) The expression of CAV1 in normal tissue. (C) The expression of VCL in normal tissue.

**Discussion**

Endothelial cell dysfunction (ECD) is an important contributor to the pathobiology of atherosclerotic cardiovascular disease[3]. In lesion-prone areas of the arterial vasculature, inflammatory factors (such as IL-1, TNF and endotoxin), oxidized lipoprotein (ox-LDL), advanced glycosylation end products (AGE)
and biomechanical stimulation of blood flow disturbance, lead to endothelial activation\textsuperscript{[4]}. The nuclear factor-kappaB (NF-κB) signaling pathway regulates adhesion molecule, membrane-associated chemokines and pre-thrombus mediators. These events promote monocyte macrophages and smooth muscle cells to form macrophage-derived foam cells and myogenic foam cells under lipid modification, and then necrosis and disintegration occur after exposure to ox-LDL and eventually form atherosclerotic plaques. Previous studies have shown that protective factors are present in vascular endothelium, which can induce upregulation of Krupplelike factor 2/4 (KLF2/4) and nuclear respiratory factor-2 (NRF2) by unidirectional laminar flow, and coordinate the protective phenotype of multifunctional atherosclerosis. KLF2 showed the strongest difference upregulation in response to stimulation\textsuperscript{[5]}. KLF2 upregulates mass genes involved in atherosclerotic protective hemodynamic stimulation and maintains endothelial homeostasis by antagonizing the NF-kB pathway, and is a potential key regulatory node in the endothelial homeostasis network\textsuperscript{[6]}. The full activity of Nrf2 in mediating antioxidant vascular protection requires co-expression with KLF2. The co-expression of the two transcription factors activated 70% of arterial protective flow-induced endothelial transcriptome, suggesting the key role as major regulators of vascular protective endothelial phenotypes\textsuperscript{[7,8]}. Nevertheless, for the diagnosis of atherosclerosis, there is still no clear detection marker at the present stage, and ultrasound is needed for clinical exploration. Therefore, potential biomarkers for effective treatment and diagnosis need to be identified and researched. Bioinformatics techniques enable researchers to detect and study genetic changes in atherosclerosis and have proven to be an effective way to identify novel biomarkers in other diseases. By analyzing the microarray data sets of GSE57691 and GSE137578, differential genes between atherosclerotic and non-atherosclerotic tissues were identified. The two datasets simultaneously contained 300 DEGs, and their interactions were analyzed by KEGG and GO. DEGs were mainly concentrated in adhesion plaques, cytosol, nuclear plasma, myelin sheath, exosomes, actin cytoskeleton, muscle contractions, protein binding and actin binding. Among them, significant enrichment of adhesion plaques was showed in KEGG and GO analysis. Adhesion plaques are signaling complexes composed of aggregated integrins bound to cytoskeletal proteins. Focal adhesion kinase (FAK), the intersection of multiple intracellular signaling pathways, acts as a mediator connects connecting integrin with downstream signaling molecules in the integrin signaling pathway. Focal adhesion kinase (FAK) plays an important role in regulating the assembly of adhesion plaque complexes and activating downstream signal molecules\textsuperscript{[9]}. In studies of angiogenesis, FAK can induce endothelial cell migration by regulating recombination of actin and the formation of
focal adhesions\textsuperscript{10}. In cardiovascular diseases, vascular injury activates and increases the activity of FAK, which plays an important role in regulating adhesion kinetics and VSMC proliferation. In addition, focal adhesion binds extracellular ligands and attach them to the actin cytoskeleton to mediate cell adhesion and migration\textsuperscript{11}. The focal adhesions are clusters of integrin transmembrane receptors that mechanically couple the extracellular matrix to the actin cytoskeleton during cell migration. Focal adhesions sense and respond to variations in force transmission along a chain of protein-protein interactions linking successively actin filaments, actin binding proteins, integrins and the extracellular matrix to adapt cell-matrix adhesion to the composition and mechanical properties of the extracellular matrix\textsuperscript{12}. Focal adhesions connect the extracellular filamentous meshwork to the intracellular cytoskeleton and hence constitute the ideal checkpoint capable of controlling or mediating bidirectional mechanical signal transduction\textsuperscript{13}. In this study, the expression of CAV1 and VCL in atherosclerotic tissue was lower than that in normal tissue by the analysis of two datasets.

Caveolin 1(CAV1) is a 22 kDa protein, which is synthesized in the endoplasmic reticulum and transported to the Golgi body. After exiting the membrane, CAV1 oligomerizes and binds to the lipid rich membrane region, participating in multiple cellular processes, such as molecular transport, cell adhesion, signal transduction and so on\textsuperscript{14}. CAV1 is expressed in terminal differentiated cells, such as adipocytes, endothelial cells, alveolar type I lung cells and smooth muscle cells\textsuperscript{15}. CAV1 is associated with cardiovascular disease, diabetes, cancer and so on\textsuperscript{16,17}. In macrophages, CAV1 is commonly involved in the adhesion function of AS, and the expression of CAV1 protein is gradually increased in the adhesion plaque\textsuperscript{18}. A number of experiments have shown that CAV1 knockout mice have severe changes in circulating lipids, including decreased fasting free FAs (FFAs), increased postprandial FFAs, increased triglycerides (TGs), and increased non-high-density lipoprotein (HDL)\textsuperscript{19}. Endothelial cell vesicles transport plasma LDL from the lumen to the intima, where it is altered and subsequently absorbed by macrophages. CAV1 is one of the basic structural components of vesicles and represents the small invagination of the plasma membrane, forming lipid vesicles\textsuperscript{20}. Upregulation of CAV1 is associated with increased LDL endocytosis induced by proinflammatory factors. CAV1 accumulates in the cytoplasm and forms more vesicles on the cell membrane, ultimately promoting LDL transendothelial endocytosis\textsuperscript{21}. In addition, CAV1 regulates atherosclerosis by integrating vascular endothelial cell remodeling and inflammation\textsuperscript{22}. Previous studies have shown that CAV1 can be expressed against atherosclerosis. PPARgamma\textsuperscript{1} and all-transretinoic acid (ATRA)\textsuperscript{24} induced CAV-1 expression, increased cholesterol outflow and reduced atherosclerosis. In this study,
GSE57691 (logFc=-1.3363001, adjP=0.00998) and GSE137578 (logFc=-2.36651119, adjP = 0.0000927) showed that the expression of CAV1 was decreased in atherosclerotic endothelial cells.

Vinculin (VCL) is a 117kDa membrane-related protein that functions as a cytoskeleton protein existing in the integrin-mediated cell-cell and cell-matrix adhesion junctions. VCL, anchored to the cell membrane, plays an important role in the extension and fixation of cell connections, and also participates in the transmission of extracellular signals to the inside\textsuperscript{[25,26]}. VCL is in a self-inhibiting state when it is not activated. When exposed to external forces, the N-terminal Vinculin head and the C-terminal Vinculin tail respectively binds to talin and F-actin, jointly mediating the activation of VCL and forming adhesion plages\textsuperscript{[27,28]}. Several studies have suggested a role of VCL in atherosclerosis and cancer. Several studies have suggested a role of VCL in atherosclerosis and cancer. VCL can control the progression of tumor by regulating the invasion, migration and proliferation of tumor cells. During epithelial-mesenchymal transition (EMT), cancer cells differentiate into highly motile and aggressive mesenchymal-like cells (MCS), leading to proliferation of tumor cells. Reduced expression of VCL leads to descending cell adhesion force, reduction and depolymerization of microfilaments and microtubules, and then promotes the migration of tumor cell in tissue, which suggests that VCL plays an important role in EMT\textsuperscript{[29,30]}. Essen found that VCL was expressed in both the intima and the media of normal left internal thoracic artery (LITA), but not in the adventitia. However, in atherosclerotic plaque, the gene expression of meta-vinculin and vinculin in endothelial cells was significantly decreased\textsuperscript{[31]}. In this study, GSE57691 (logFc=-1.3554737, adjP=0.000647) and GSE137578 (logFc=-1.3784973, adjP=0.0001389) showed that the expression of VCL was decreased in atherosclerotic endothelial cells.

**Conclusion**

In summary, our findings identify that the hub genes in atherosclerotic endothelial cells were significantly enriched in the adhesion plaque signaling pathway. On the other hand, CAV1 and VCL are confirmed as the hub genes in the vascular endothelium of atherosclerosis and exists in the adhesion plaque signaling pathway. CAV1 and VCL are acknowledged to be down-regulated in the adhesion plaque signaling pathway to promote the progression of atherosclerosis. This pilot study provides several potential biomarkers to distinguish atherosclerosis from non-atherosclerosis and explore new insights into the molecular mechanisms of the disease.
Methods

Microarray datasets
In this study, the raw gene expression profile GSE57691 and GSE137578 were downloaded from the National Center for Biotechnology Information Gene Expression Omnibus public database. The microarray data of the GSE57691 dataset consists of Full-thickness aortic wall biopsies from 10 control groups and 9 atherosclerotic groups, detected by Illumina HumanHT-12 V4.0 expression beadchip. The microarray data of the GSE137578 dataset consists of 3 control groups (human aortic endothelial cells (HAECs) without oxLDL treatment) and 3 atherosclerosis groups (HAECs with oxLDL treatment), detected by Agilent-072363 SurePrint G3 Human GE v3 8x60K Microarray 039494.

Data identification and processing of DEGs
We identified differentially expressed genes in Gene Expression Omnibus datasets (GSE57691 and GSE137578) using the GEO2R tool with $|\log FC| > 1$ and adjusted $P$ value $< 0.01$, the differences between the control and atherosclerotic groups is shown in the volcano plot. Then use the Venn diagram software to build the Venn diagram, and revealed that 300 genes were concurrently contained within the 2 DEGs datasets.

Functional enrichment analysis of DEGs
Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, performed by DAVID online software (https://david.ncifcrf.gov/), were used to analyses functionally analyse the enriched pathways of DEGs. The DEG enrichment of molecular function (MF), biological process (BP), cellular component (CC), and KEGG pathways was shown via GO and KEGG pathway analyses. False discovery rate (FDR) $< 0.05$ in the results was considered statistically significant.

Construction of Protein-protein interaction (PPI) network and identification of core gene
A PPI network can be used to clarify the regulatory functions between proteins, was constructed by the Retrieval of Interacting Genes (STRING,https://string-db.org/). The PPI pairs were extracted with a combined score $>0.4$. Then the PPI network was imported into Cytoscape software (version 3.8.2) to build a visual interactive network. Finally, the Cytohubba plugin was used to calculate the degree of each protein node and identify the top 10 genes with the highest level.
Metascape Analysis

Metascape (http://metascape.org) is a free, reliable, user-friendly tool for gene annotation and gene enrichment analysis. In this study, Metascape was used to conduct pathway and process enrichment analysis of DEGs. Thus, the Gene Ontology (GO) terms, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Protein – protein interaction enrichment analysis (PPI) were enriched based on the Metascape online tool. The above results were compared with the results obtained by David and the String online tools, in order to further verify the accuracy of gene enrichment analysis. Moreover, the Metascape online tool explored the expression of DEGs in diseases through DisGeNET (a comprehensive platform integrating information on human disease-associated genes and variants).

Gene expression in tissues

Tissue-specific genes refer to genes specifically expressed in different tissues or cell types, which will help to predict related diseases in clinical trials. The hub genes were imported into the BioGPS database (http://biogps.org/help/), and then the expression score in coronary artery was observed.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, performed by DAVID online software (https://david.ncifcrf.gov/), were used to analyses functionally analyse the enriched pathways of DEGs. The DEG enrichment of molecular function (MF), biological process (BP), cellular component (CC), and KEGG pathways was shown via GO and KEGG pathway analyses. False discovery rate (FDR) < 0.05 in the results was considered statistically significant.

List of abbreviations

| Abbreviations | Full Name                      |
|---------------|--------------------------------|
| AS            | Atherosclerosis                |
| DEGs          | differentially expressed genes |
| ox-LDL        | oxidized lipoprotein           |
| AGE           | advanced glycosylation end products |
| NF-κB         | nuclear factor-kappaB          |
| KLF2/4        | Krupplelike factor 2/4         |
| NRF2          | nuclear respiratory factor-2   |
| FAK           | Focal adhesion kinase          |
| TGs           | triglycerides                  |
| HDL           | high-density lipoprotein       |
| ATRA          | all-transretinoic acid         |
Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

XW designed and hosted the project. WZ, JW, JD, WL and XW performed the experiments. WZ, JW,
JD and WL discussed the results. WZ, JD and WL wrote the manuscript. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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