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RESEARCH ARTICLE

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Bone morphogenetic proteins, DNA methylation, and gut microbiota interaction in lumbar disc degeneration: A multi-omics Mendelian randomization study

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Abstract

Background: Lumbar disc degeneration (LDD) is a ubiquitous finding in low back pain. Many different etiology factors may explain the LDD process, such as bone morphogenetic proteins (BMPs), DNA methylation, and gut microbiota. Until recently the mechanisms underlying the LDD process have been elusive.

Methods: BMP-related genes were extracted from the GeneCards database. The LDD transcriptome dataset was obtained from the Gene Expression Omnibus. We used linear regression and meta-analysis to screen and integrate the differentially expressed genes associated with BMPs in LDD. Genome-wide association studies (GWASs) of LDD were from FinnGen and UKBB. The expression quantitative trait loci (eQTLs) and DNA methylation quantitative trait loci from the blood were identified via the summary data-based Mendelian randomization (SMR) method, and the possible blood BMP genes and their regulatory elements associated with the risk of LDD were prioritized. Intestinal eQTLs and fecal microbial QTLs (mbQTLs) were integrated, and the potential interactions between BMP gene expression in host intestinal tissue and the gut microbiota were revealed through SMR and colocalization analysis. The GWAS catalog (GCST90246169) was used to validate SMR results.

Results: A meta-analysis of five datasets revealed that 113 BMP genes were differentially expressed between LDD and control tissues. Seven genes were selected as candidate pathogenic genes of LDD via the three-step SMR method: *CREB1*, *BMP6*, *PTCH1*, *GLI1*, *MEG3*, *GALNS*, and *NF1*. SMR analysis also revealed five possible gut genes: *HFE*, *MET*, *MAPK3*, *NPC1*, and *GDF5*. The correlation between the gut

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microbiota and BMP gene expression in intestinal tissues was verified by eQTL-mbQTL colocalization.

Conclusion: This multi-omics study revealed that the BMP genes associated with LDD are regulated by DNA methylation. There are genetic differences between gut gene expression and the gut microbiota. These findings provide evidence for new therapeutic targets in the future.

KEYWORDS

bone morphogenetic proteins, gut microbiota, integrative omics, lumbar disc degeneration, Mendelian randomization

1 | INTRODUCTION

The radiological feature of lumbar disc degeneration (LDD) is the presence of osteophytes, endplate sclerosis, and disc space stenosis, which may be possible risk factors for back pain in adults.[1] Although the cause of LDD remains unclear, the complex interactions among aging, the mechanical environment, genetics, and insufficient metabolite transport are considered the basis of disease pathogenesis.[2] In recent years, bone morphogenetic proteins (BMPs), DNA methylation, and the gut-spine axis have been investigated. It has been shown to be related to LDD.[3–5] Elucidating the complexity behind this interaction may provide important insights into the pathogenesis of LDD and reveal potential targets for therapeutic intervention and disease prevention.

As growth factors that bind to receptor smad, BMPs are extensively involved in systemic development and regulate the growth, differentiation, and apoptosis of various cell types, including osteoblasts, chondroblasts, neurons, and epithelial cells.[4] Studies have focused on the relationship between BMPs and LDD. Both animal experiments and human studies have confirmed the protective effect of BMP-7 on intervertebral discs.[6–8] The expression level of BMP-2 is correlated with the degree of intervertebral disc degeneration (IDD).[9]

Gut microbiota metabolites are critical for the maturation and protection of the immune system. Changes in the gut microbiota may be associated with various chronic diseases, such as osteoporosis,[10] and the gut microbiota may also serve as a mediator of spinal degenerative diseases.[3] One study confirmed that the inhibition of intestinal flora translocation could improve vascular calcification through the inhibition of toll-like receptor 9-mediated BMP-2 expression.[11]

Genome-wide association study (GWAS) has been widely used in research as a way to study the entire genome of a large group of people to look for single nucleotide polymorphisms (SNPs) associated with diseases or traits. Mendelian randomization is a research method that uses genetic variation to examine the causal relationship that can alter exposure to an outcome, such as a disease state. Summary data-based Mendelian randomization (SMR) integrates LDD GWASs data and reveals a causal relationship between the gut microbiota and IDD.[12,13] This finding also indirectly proves that the intestinal flora may also play an important role in the development of LDD.

Although a growing number of studies has shown a correlation between BMPs and LDD, no study has comprehensively determined its potential causal relationship with LDD. GWASs have been used to identify genomic loci containing BMP genes associated with degenerative spine diseases.[14] However, owing to the complex linkage disequilibrium (LD) structure of the genome, the loci associated with multiple variants may not be causal.[15] Furthermore, these genetic variations may regulate DNA methylation (DNAm), gene expression, and the abundance of the gut microbiota. Integrating multiomics is an emerging method in the post-GWAS era that is used to identify key regulatory factors and explore therapeutic targets of LDD.[16]

2 | METHODS

2.1 | Research design and data resources

Figure 1 shows the design process of this study. BMP-related genes were extracted from the GeneCards database (v5.10, https://www.genecards.org), the keyword "bone morphogenetic proteins" with a correlation score ≥10 was used according to previous methods.[17,18] LDD-related datasets GSE124272, GSE150408, GSE56081, GSE34095, and GSE70362, which contained LDD patients and healthy controls (HCs), were obtained from the Gene Expression Omnibus (GEO) database, and a meta-analysis was performed to identify the differentially expressed genes (DEGs) associated with BMPs in LDD patients. The GWAS data for LDD were from the FinnGen GWAS and UKBB GWAS meta-analyses. The former had a sample size of 37 636 LDD patients and 270 964 HCs, and the latter had a sample size of 4690 LDD patients and 356 504 HCs.[19]

A quantitative trait locus (QTL) is a region of DNA associated with a specific phenotype or trait that varies within a population. An expression quantitative trait locus (eQTL) is a locus that explains a fraction of the genetic variance of a gene expression phenotype. A methylation quantitative trait locus (mQTL) is a genetic variant that affects DNAm levels at CpG sites. A microbial quantitative trait locus (mbQTL) is a genetic variant that influences the abundance of microbial populations within the host. The blood eQTL summary statistics of the BMP genes were obtained via eQTLGen, which included the genetic data of blood gene expression for 31 684 individuals from 37 datasets.[20] The pooled blood mQTL data were from a meta-analysis of two European cohorts: the Brisbane Systematic Genetics Study ($n = 614$) and the Midorthian Birth Cohort ($n = 1366$).[21,22]

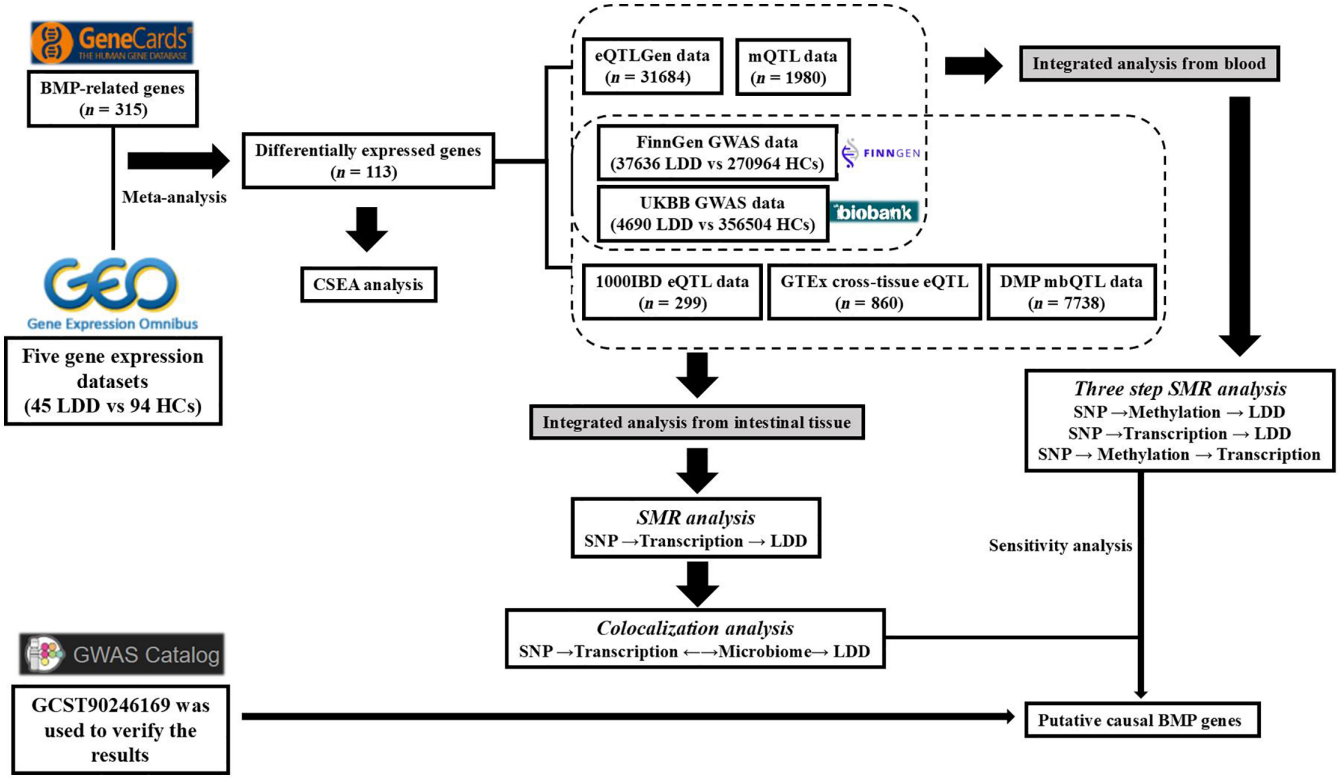

FIGURE 1 Workflow of this study. A series of analyses were performed to identify candidate bone morphogenetic protein (BMP) genes associated with the pathogenesis of lumbar disc degeneration (LDD). BMP-related genes were extracted from the GeneCards database. Five transcriptome datasets, including LDD patients and healthy controls (HCs), were obtained from the GEO database, and a meta-analysis was conducted to ascertain the differential expression of LDD-associated BMP genes, followed by cell type-specific expression analysis (CSEA). A three-step summary data-based Mendelian randomization (SMR) method was used to integrate the genome-wide association study (GWAS) summaries and *cis*-eQTL (expression quantitative trait locus)/*cis*-mQTL (methylation quantitative trait locus) data from blood, and the putative blood BMP genes and their regulatory elements associated with LDD risk were prioritized (p-SMR-multi <0.01; HEIDI $p > 0.05$). Sensitivity analysis was performed after the initial SMR to test for heterogeneity (HEIDI method, $p > 0.05$ indicating the absence of heterogeneity). Furthermore, we meta-analyzed intestinal *cis*-eQTLs from two public summaries (GTEx and 1000IBD) and further integrated intestinal *cis*-eQTLs with fecal microbial quantitative trait loci from the Netherlands Microbiology Project (DMP) to obtain results via SMR, heterogeneity, and colocalization analysis (p-SMR-multi <0.05; HEIDI test $p > 0.05$; colocalization PPH4 >0.5). The above SMR results were validated with GWAS catalog data (GCST90246169).

Intestinal eQTL data were obtained from the Genotype-Tissue Expression (GTEx) project ($n = 860$)[23] and the 1000IBD cohort ($n = 299$).[24] The current study focused only on *cis*-eQTLs and *cis*-mQTLs, which constitute SNPs within a 1 Mb distance from the start and end of genes. The fecal mbQTL data were from the Dutch Microbiome Project (DMP) study, which included data from 7738 individuals to assess the genetic influence of the host on the gut microbiota.[25] External validation was performed using the GWAS catalog data, accession number for GCST90246169, to validate the SMR result.

2.2 | Statistical analysis

2.2.1 | Meta-analysis of DEGs

DEGs related to BMP in LDD patients and HCs were analyzed via a linear regression model adjusted for age, sex, tissue, and medication use (if metadata were available). To increase statistical power, we pooled blood, nucleus, and annulus biopsies and added tissue location as a covariate in linear models. DEGs were analyzed separately in the five gene expression datasets, and then fixed effects meta-analysis was performed via the R package *metafor*.

2.2.2 | Intestinal *cis*-eQTLs meta-analysis

To include as many intestinal *cis*-eQTLs as possible, we first performed a meta-analysis on the *cis*-eQTLs of the BMP gene GTEx of the transverse colon, sigmoid colon, and small intestine via the related samples (MeCS) method, with consideration of sample overlap. The intestinal *cis*-eQTLs from the 1000 IBD cohort were highly comparable (consistency rate >97%) to those detected in the nondisease GTEx dataset.[24] Therefore, we used traditional inverse variance weighting meta-analysis on two independent datasets in SMR (v1.3.1).

2.2.3 | SMR and colocalization analysis

SMR tools have been established to examine whether the effects of SNPs on a phenotype are mediated by molecular features such as gene expression, DNAm, and the gut microbiota. Colocalization analysis aimed to study overlapping variants that may cause different traits. The integration of GWAS data with other molecular QTL data via SMR or colocalization improved the detection of candidate causal SNPs.

For blood tissue analysis, the SMR multitool was used to determine the causal inferences of the BMP genes, and the LD was calculated via the European Reference 1000 Genomes. Three-step SMR analysis was performed: (1) SNPs were the tools, blood gene expressions were exposed, and BMP was the outcome; (2) SNPs were the tools, blood DNAms were exposed, and BMP was the outcome; and (3) SNPs were the tools, blood DNAms were exposed, and blood gene expressions were the outcome. The third step included only significant signals from steps 1 and 2. The final candidate signals were defined as those that (1) passed all three-step SMR false discovery rates (*p*-SMR-multi) <0.01; (2) were suggestively significant genome-wide ($p < 1 \times 10^{-5}$) in all eQTLs and mQTLs; and (3) excluded heterogeneity in the dependent instrument (HEIDI) test results with $p > 0.05$.

Intestinal tissue analysis was performed via the SMR tool to determine the cause-and-effect relationships between GWASs and *cis*-eQTLs. This included SNPs as instruments, intestinal gene expression as an exposure, and LDD as an outcome (*p*-SMR-multi <0.05, HEIDI $p > 0.05$, *cis*-eqtl $p < 1 \times 10^{-5}$).

Sensitivity analysis was performed after primary SMR analysis was performed via the other two MR methods. We tested the heterogeneity of individual causal effects via the HEIDI test. A HEIDI <0.05 indicated the presence of heterogeneity. Owing to the limited ability to assess the causal relationship between the gut microbiota and disease, colocalization was chosen to assess the potential interactions between gut gene expression and the microbiota.[26,27] Colocalization is a method used to assess whether two traits have common causal variation in a region. Analysis was performed via the *coloc* R package, and PPH4 >0.5 was used as the threshold for a shared genetic effect between two traits.[28,29]

3 | RESULTS

3.1 | Meta-analysis of the differential expression of BMP genes in LDD patients and HCs

To understand the function of the BMP genes in LDD, we included five gene expression datasets and used linear regression and meta-analysis to compare the RNA expression in the disc tissues of LDD patients ($n = 45$) and HCs ($n = 94$) (Table S1). A total of 315 BMP-related genes with correlation scores ≥10 were downloaded from GeneCards (Table S2). Meta-analysis revealed that a total of 113 BMP genes were differentially expressed between LDD and control tissues ($p < 0.05$) (Table S3). Furthermore, we performed cell type-specific expression analysis on these DEGs. Among the 23 general cell classifications, BMP-related DEGs were significantly enriched in the bone marrow ($p < 0.05$) (Table S4).

3.2 | Integration of GWAS and BMP-related eQTL/mQTL data

There is a relationship between BMPs and LDD. DNAm located in promoters or enhancers commonly influences the regulation of disease-associated target genes. Therefore, we aimed to identify candidate causal genes for LDD and explore their possible underlying epigenetic mechanism of gene regulation in the blood. A three-step SMR method was used, and only the significant results in all three SMR analyses that passed sensitivity checks were interpreted as suggestive causal genes (see Section 2). In this study, 113 BMP-related DEG *cis*-eQTLs and their *cis*-mQTLs were integrated with the largest available GWAS summary statistics for LDD.

Specifically, the integration of eQTL results from the eQTLGen Consortium and LDD GWAS summary statistics resulted in nine BMP-related genes (*p*-SMR-multi <0.01, HEIDI $p > 0.05$) (Table S5). Moreover, we identified 1096 DNAm probes (near genes within 1 Mb) by integrating the same LDD GWAS results and mQTL summary statistics from the meta-analysis of the Brisbane Systems Genetic Study and Lothian Birth Cohorts (Table S6). Further integration analysis of putative LDD-causal *cis*-eQTL and *cis*-mQTL data prioritized 21 DNAm probes potentially regulating 7 neighboring genes: CREB1, BMP6, PTCH1, GLI1, MEG3, GALNS, and NF1 (*p*-SMR-multi <0.01, HEIDI $p > 0.05$) (Table S7). These CpG sites were significantly enriched in fibroblast primary cells, muscle cells, and primary T cells, which are of mesoderm origin and related to the skeletal motor system and immunity (Table S8).

3.3 | Putative LDD-causal genes mediated by blood methylation regulation of gene expression

Our three-step SMR analysis prioritized GLI1, which is a gene that encodes a protein that functions as a transcription factor protein. This study revealed that the SNP signals associated with GLI1 were significant across the data from LDD GWAS, eQTL, and mQTL studies. The DNAm probe cg09253762 was found to be located in the enhancer region. The methylation level of this site had a positive effect on GLI1 expression (betaSMR = 0.115) and LDD onset (betaSMR = 0.143), whereas the GLI1 expression level was positively associated with disease (betaSMR = 0.337). Together, our results suggest a putative mechanism whereby a lower DNAm level at the enhancer region of GLI1 upregulates the expression of GLI1 and subsequently increases LDD risk (Figure 2).

Another key example is CREB1, which has a coordinating effect with BMPs and participates in the development of IDD. We found that the DNAm probe cg25923788, located in the promoter region, was causally negatively associated with CREB1 expression (betaSMR = −0.119). Consistently, LDD was related to higher CREB1 gene expression

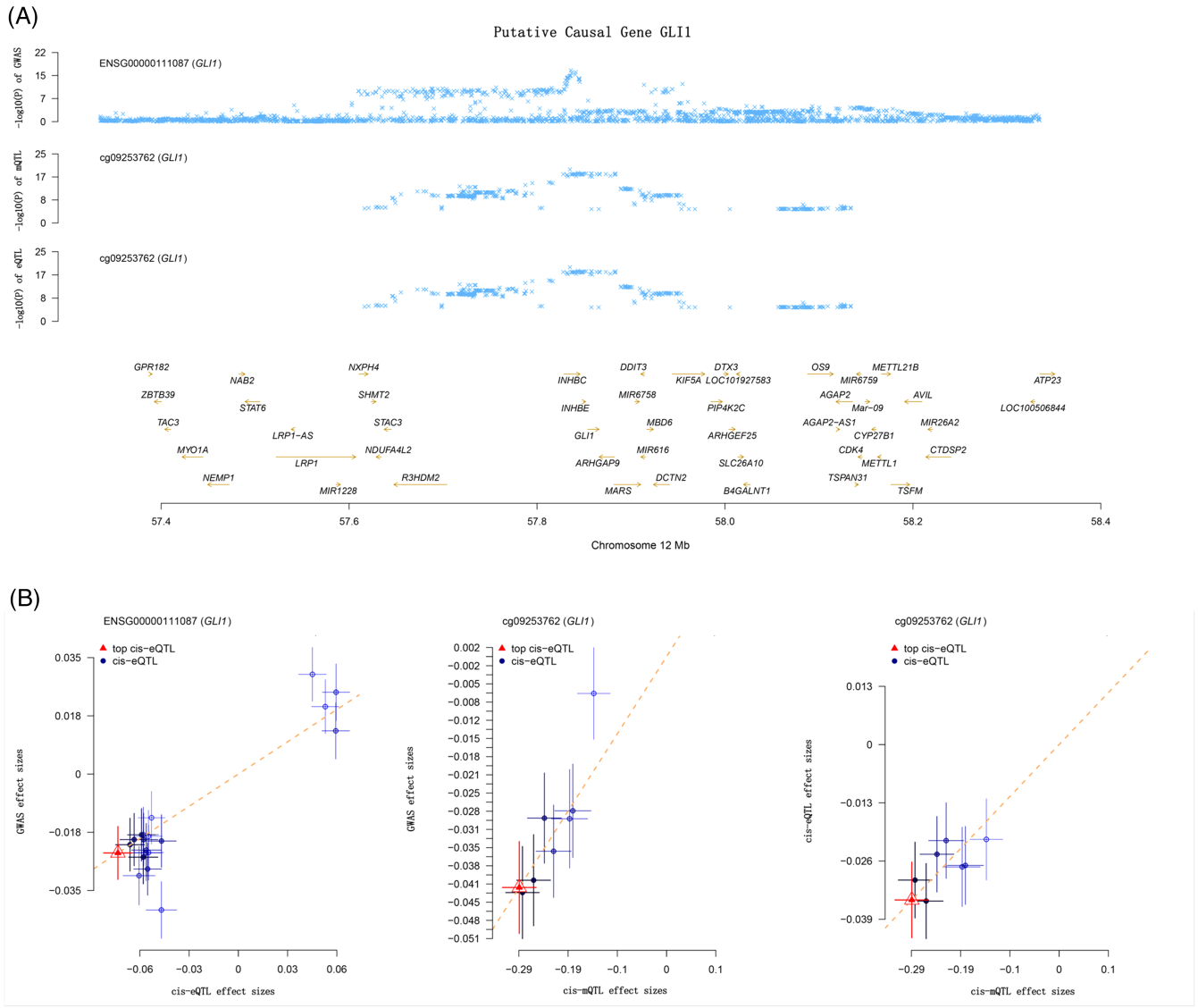

FIGURE 2 Three-step summary data-based Mendelian randomization (SMR) analysis putative causal gene GLI1 and mechanisms in lumbar disc degeneration (LDD) using blood tissue. (A) Locus zoom plots showing the consistent genetic effects from LDD genome-wide association study (GWAS), *cis*-mQTL (methylation quantitative trait locus), and *cis*-eQTL (expression quantitative trait locus) nearby GLI1 (from upper to lower panels, all minimum $p < 1 \times 10^{-5}$). (B) Three-step SMR indicating significant causal relationships between gene expressions and LDD onset mediated by methylation (all three-step *p*-SMR-multi <0.01, HEIDI test $p > 0.05$). From left to right: SMR between gene expression and LDD GWAS, SMR between gene methylation and LDD GWAS, and SMR between gene methylation and expression.

(betaSMR = 0.136) and lower methylation levels (betaSMR = −0.098). Thus, the putative mechanism could be that the genetic variants upregulate CREB1 expression by influencing the promoter DNAm status, showing a protective effect on LDD onset (Figure 3).

3.4 | Integration of GWAS and BMP-eQTL/mbQTL data from intestinal tissue

Genetic effects on gene expression vary across blood and intestine tissues, which could reflect different LDD-causal genes. Moreover, host genetics and the gut microbiota are known to play critical roles in LDD. Intestinal tissues are in direct contact with gut microbes and sense local changes in BMP levels; therefore, we hypothesized that integrating *cis*-eQTLs and mbQTLs from intestinal tissue would provide novel candidate targets with putative host–microbiota interactions. We performed a meta-analysis of *cis*-eQTL data from three intestinal tissues (sigmoid colon, transverse colon, and small intestine) obtained from the GTEx project ($n = 860$), adjusting for sample overlap. SMR analysis demonstrated the potential causal role of five intestinally expressed genes in LDD (*p*-SMR-multi <0.05, HEIDI $p > 0.05$): HFE, MET, MAPK3, NPC1, and GDF5 (Figure 2; Table S9).

To further explore the role of intestinal BMP genes from the perspective of host–microbiota interactions, we integrated mbQTL summary statistics with putative LDD-causal *cis*-eQTLs via colocalization analysis. This analysis was assumed to determine the probability that

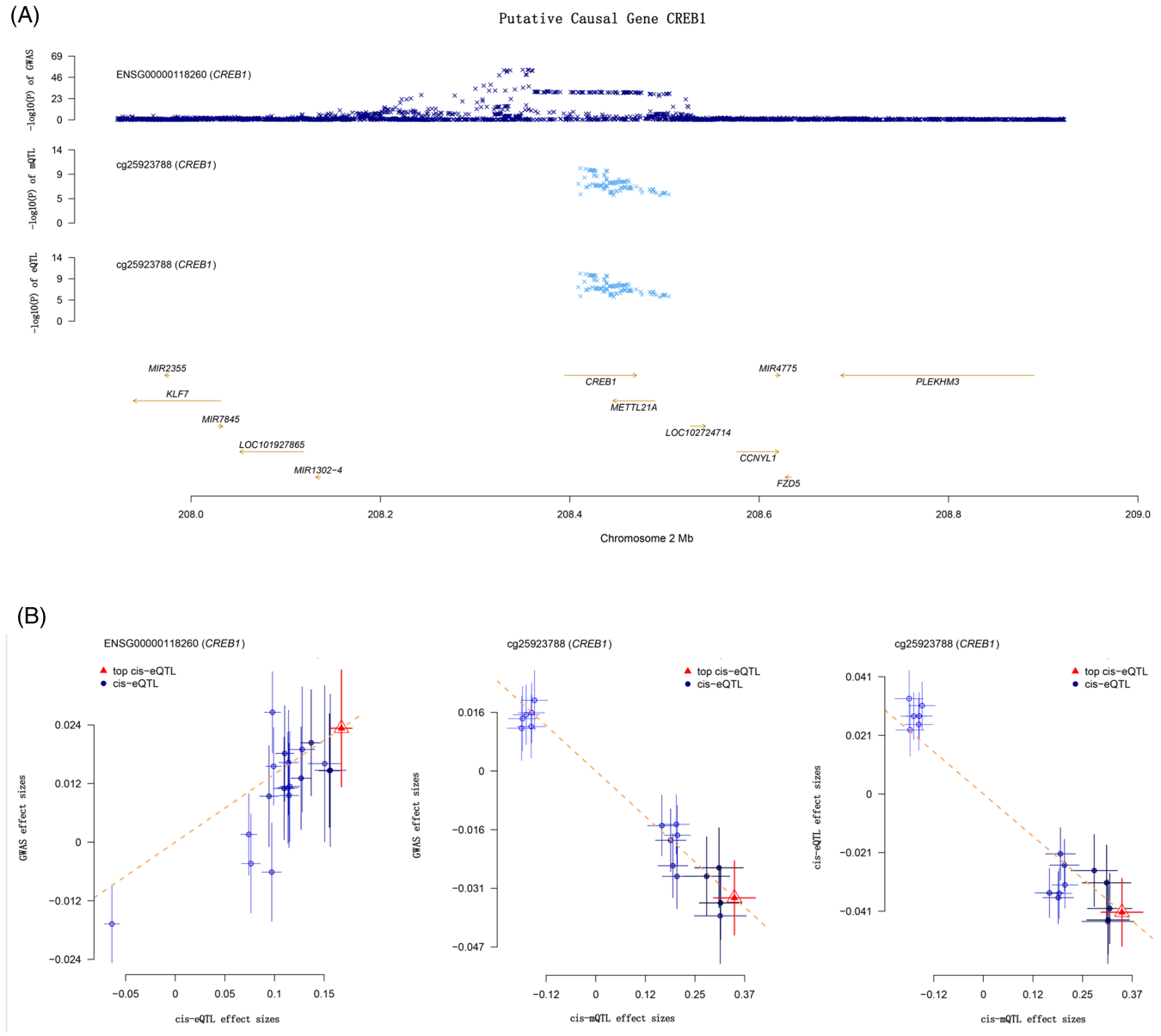

FIGURE 3 Three-step summary data-based Mendelian randomization (SMR) analysis putative causal gene CREB1 and mechanisms in lumbar disc degeneration (LDD) using blood tissue. (A) Locus zoom plots showing the consistent genetic effects from LDD genome-wide association study (GWAS), *cis*-mQTL (methylation quantitative trait locus), and *cis*-eQTL (expression quantitative trait locus) nearby CREB1 (from upper to lower panels, all minimum $p < 1 \times 10^{-5}$). (B) Three-step SMR indicating significant causal relationships between gene expressions and LDD onset mediated by methylation (all three-step *p*-SMR-multi <0.01, HEIDI test $p > 0.05$). From left to right: SMR between gene expression and LDD GWAS, SMR between gene methylation and LDD GWAS, and SMR between gene methylation and expression.

the genetic determinants of mucosal gene expression were shared with the gut microbiota. GWAS data for gut microbiota was from DMP data (Table S10). SMR was not used because of power issues resulting from the moderate effects of host genetics on the gut microbiota. Six gene expression-microbial pathway pairs were detected at the threshold of PPH4 >0.5, including MAPK3—*Lactobacillus delbrueckii*, NPC1—*Parabacteroides merdae*, GDF5—*Bacteroides finegoldii*, HFE—*Dorea*, NPC1—*Bifidobacterium catenulatum*, and GDF5—*Bacteroides massiliensis* (PPH4 = 0.528, 0.972, 0.871, 0.550, 0.56, 0.950, respectively) (Figure 5; Table S11).

The above SMR results were verified with GWAS Catalog under accession number GCST90246169 data (Table S12).

3.5 | Putative LDD-causal genes involved in intestinal gene–microbiota interactions

MAP K3 acts in a signaling cascade that regulates various cellular processes, such as proliferation, differentiation, and cell cycle progression, in response to a variety of extracellular signals. We prioritized

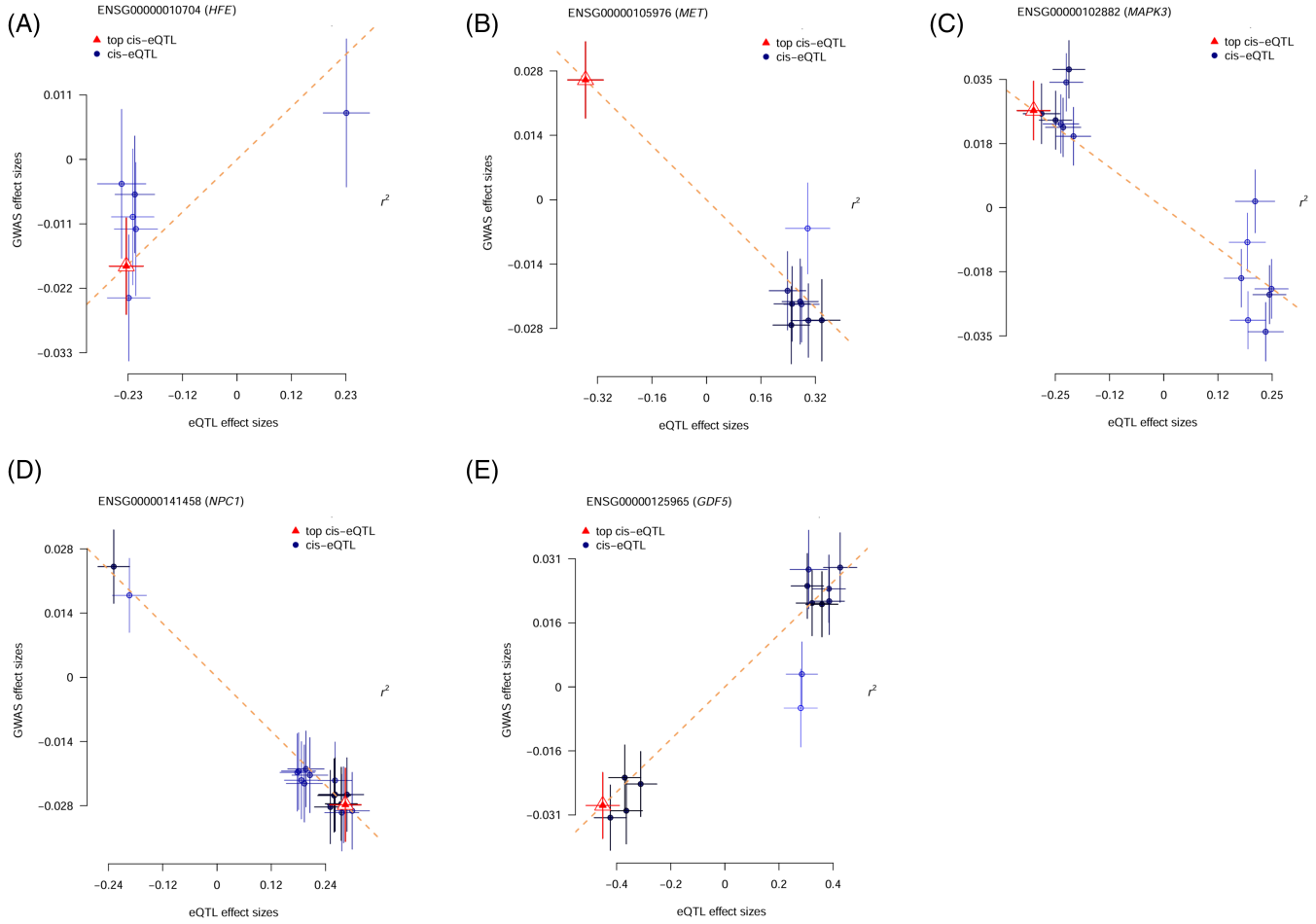

FIGURE 4 Summary data-based Mendelian randomization (SMR) analysis demonstrated the potential causal role of five intestinally expressed genes in lumbar disc degeneration (LDD) (all *p*-SMR-multi <0.05; HEIDI test $p > 0.05$). (A–E) The genes of *HFE*, *MET*, *MAPK3*, *NPC1*, and *GDF5*, respectively.

MAPK3 as a candidate BMP causal gene in LDD intestinal tissues associated with the gut microbiota on the basis of SMR and colocalization analysis (Figure 4A). Our study revealed that elevated MAPK3 expression likely plays a causal role in LDD onset (betaSMR = −0.144). Furthermore, SNPs regulating MAPK3 expression might also affect microbial metabolic functions according to the results of the colocalization analysis. Specifically, *L. delbrueckii* is correlated with MAPK3 (PPH4 = 0.528) and plays a role in the pathogenesis of LDD. Our study also revealed that elevated NPC1, GDF5, and HFE expression likely played causal roles in LDD onset (betaSMR = −0.096, 0.065, and 0.077, respectively).

4 | DISCUSSION

To the best of our knowledge, this study is the first to leverage a multiomics integration method to detect putative causal BMP genes and the underlying mechanisms in LDD using blood, disc, and intestinal tissues. We identified 113 BMP-related DEGs out of 315 potential genes associated with LDD via a sizable meta-analysis of disc tissue transcriptome data and successfully validated these DEGs in our cohort. Integration of GWAS with the eQTLs and mQTLs of these DEGs from the peripheral blood prioritized seven putative BMP genes and their regulatory elements associated with LDD onset: *CREB1*, *BMP6*, *PTCH1*, *GLI1*, *MEG3*, *GALNS*, and *NF1*. Moreover, the integration of intestinal eQTL data revealed five candidate causal genes, of which *MAPK3*, *NPC1*, *GDF5*, and *HFE* were involved in intestinal gene–microbiota interactions, through further colocalization analysis. In LDD patients, the intestinal microbiota may affect BMP gene expression, or there may be a correlation. Our study is therefore fundamental, as we seek to fill in the gaps in our understanding of distinguishing causal or remotely related BMP genes and identifying the correlated interactions of LDD in a genomic context.

As the understanding of the pathogenesis of LDD has increased, an increasing number of studies have demonstrated the role of genetic factors in LDD.[30–32] As growth factors, BMPs are widely involved in the development process of the whole body and regulate the growth, differentiation, and apoptosis of various cell types, including osteoblasts, chondroblasts, neurons, and epithelial cells.[4] Moreover, the roles of BMPs in inflammation and immunomodulation have

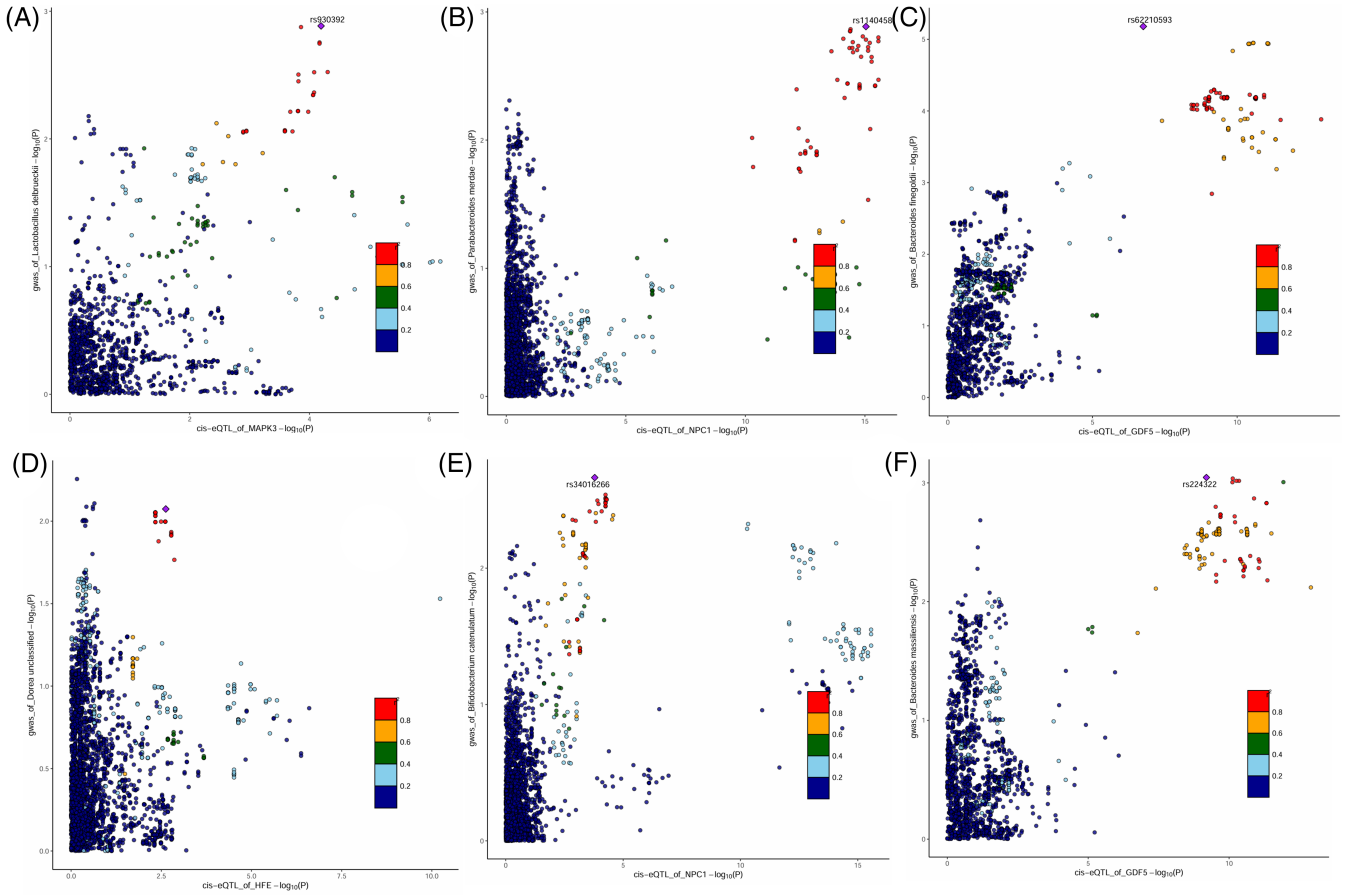

FIGURE 5 The locus comparisons between *cis*-eQTLs (expression quantitative trait loci) and mbQTLs (methylation quantitative trait loci) by colocalization analysis (all PPH4 >0.5) were shown. Six gene expression-microbial pathway pairs were detected. (A–F) The expression-microbial pathway pairs of MAPK3—*Lactobacillus delbrueckii*, NPC1—*Parabacteroides merdae*, GDF5—*Bacteroides finegoldii*, HFE—*Dorea*, NPC1—*Bifidobacterium catenulatum*, and GDF5—*Bacteroides massiliensis*, respectively.

gradually been explored.[33,34] Many studies have shown that BMPs are important regulators of IDD.[4,7,35] Through SMR analysis, we identified seven possible causal relationships between BMP genes and LDD susceptibility through genetic regulation of the epigenome and transcriptome, suggesting that epigenetic factors and gene expression play important roles in disease onset. Among these genes (*CREB1*, *BMP6*, *PTCH1*, *GLI1*, *MEG3*, *GALNS*, and *NF1*), the causal roles of *PTCH1* and *MEG3* have been extensively characterized in LDD.[36–38] The relationship between the expression of other genes and the pathogenesis of LDD has also gradually been discovered. For example, the cAMP-response element binding protein (CREB) acts as a transcription factor that binds to the cAMP response element (CRE) of the promoters of its target genes.[39] CREB may have a coordinating effect with BMPs and participate in the development of intervertebral disc degeneration. One study confirmed that CREB activates BMP2 transcription in osteoblasts.[40] CREB1 has been reported to cooperate with BMP-stimulated SMAD signaling to increase the activation of the Smad6 promoter in chondrocytes.[41] One study confirmed that CREB1 knockdown mimicked the effects of miR-140 overexpression on extracellular matrix degradation, which was related to tumor necrosis factor-α or interleukin-1β signaling and led to IDD.[42] Another example is the glioma-associated oncogene homolog (GLI) family, which includes nuclear transcription factors of the Hedgehog signaling pathway. Gli1 plays an important role in BMP9-induced osteogenic differentiation and is completely absent in the nucleus pulposus compartment of both juvenile and adult mice.[43,44] This work revealed that DNAm sites near promoter regions were significantly associated with CREB1 and GLI, indicating a coregulatory pattern involving multiple epigenetic regulatory elements.[45] Although previous GWAS data on spondylosis have identified genetic variants located in genes such as BMP6, NIPAL1, and CNGA1,[14] it remains unclear whether these genes have a causal effect on LDD. On the basis of our SMR analysis, we hypothesize that genetic variants could regulate the expression of these genes through DNA methylation, thereby affecting LDD pathogenesis.

LDD is a degenerative disease, but there is increasing evidence that the gut microbiota is involved in its pathogenesis.[3,13] The gut microbiota may affect lumbar discs in three potential ways: (1) bacteria cross the intestinal epithelial barrier into the intervertebral disc; (2) bacteria regulate the mucosal and systemic immune system; and

(3) bacteria regulate the metabolism of intestinal epithelial substances.[46] BMP signaling functions in the inflammatory response of the intestinal epithelium.[47] Studying the genetic effects of LDD on BMP gene expression in the intestine via intestinal eQTLs (the most pertinent tissue type) may be meaningful for confirming the gut-spine axis. As the intestinal barrier directly contacts luminal microbes and short-chain fatty acids and other metabolites, inflammation in the intestine may be associated with LDD through host–microbiota interactions.[48] Our SMR-based analysis identified HFE, MET, MAPK3, NPC1, and GDF5 as putative causal genes in intestinal tissue. Four genes, MAPK3, NPC1, GDF5, and HFE, were further prioritized when the interactions between host genetics and the microbiota were considered. The gut microbiota may be involved in disc metabolism. One study confirmed that MAPK3, especially the phosphorylation of MAPK3, is a potential therapeutic target for LDD treatment.[49] Our study revealed six gene expression–microbial pathway pairs, including *MAPK3–L. delbrueckii*. One study confirmed that *Lactobacillus* enhances the immune response of bone marrow-derived macrophages by activating MAPKs.[50] It is possible that the same immune response mediated by Lactobacillus and MAPK3 also occurs in disc tissue. GDF5, a member of the BMP family, is expressed in the cartilage primordium during early limb development.[51] Some studies have suggested that GDF5 may maintain the structure and function of the intervertebral disc.[51,52] Our study revealed that *Bacteroides* may be associated with GDF5. *Bacteroides* have the ability to alter serum valeric acid and may further alter bone metabolism.[53] Perhaps the effects of *Bacteroides* on the metabolites of intestinal bacteria are related to the expression of GDF5 and affect the metabolism of disc tissue. However, further evidence on the basis of genetic background is needed to precisely explain the potential role of the gut-spine axis in LDD.

Integrating multiomics from multiple tissues enables researchers to dissect GWAS signals, such as the prioritization of genes and disease mechanisms. We prioritized a list of novel genes and DNAm sites for follow-up functional studies using the largest up-to-date LDD GWAS and BMP-targeted approach. More importantly, this is the first study providing evidence to support a causal role of BMP genes interacting with the gut microbiota in intestinal tissue. Gene therapy is changing the immune environment of the intervertebral disc and promoting disc regeneration in various ways, leading to therapeutic advancements for the treatment of LDD.[54] Techniques such as fecal microbiota transplantation are being explored in the treatment of diseases.[55] Therefore, we believe that this study provides a reference for new therapeutic targets in the future.

Some limitations of this study warrant recognition. First, the meta-analysis of disc tissue DEGs included different data resources. Second, cell type-dependent eQTLs vary with disease progression. Third, we focused only on the *cis*-regions of BMP genes in the analysis, despite the possibility that *trans*-eQTL SNPs (SNPs and the center of the gene >5 Mb) may have a widespread impact on regulatory networks. Fourth, patient cohort studies are needed to validate the results. Fifth, some database data lacks the original patient information. Finally, functional experiments are still needed to validate our findings. Moreover, as multiple factors can influence the expression of BMP genes, we believe that integrating other omics data at different molecular levels (such as those of proteins and metabolites) with large sample sizes may lead to novel discoveries and improve the characterization of putatively involved causal mechanisms of BMP in LDD.

5 | CONCLUSIONS

This study expands our knowledge of the potential causality of BMPs and the underlying biological mechanisms in LDD on the basis of a multiomics MR approach. We demonstrated that LDD onset putatively results from the expression of several candidate BMP genes through DNAm, gene expression, and interactions with the gut microbiota. Host–microbiota interactions between our newly identified causal BMP genes and microbial taxa and pathways are worth studying at the functional level to gain more in-depth insights into the underlying biological mechanisms. This study advances fundamental research into the role of BMP in LDD and pinpoints potentially novel therapeutic targets for clinical practice.

AUTHOR CONTRIBUTIONS

Xiao-Long Chen and Xiang-Yu Li mainly contributed to the conception of the study and wrote the manuscript. Peng-Yun Wang made important contributions to the revision and review of this study. Xiang-Yu Li created graphs. Qi-Jun Wang, Dong-Fan Wang, Shuai-Kang Wang, Yu Wang, Wei-Guo Zhu, Wei Wang, and Chao Kong collected database and analyzed data. Shi-Bao Lu made an important contribution to the revision of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the article content was composed in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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