Original Paper

Mutant MAPK7-Induced Idiopathic Scoliosis is Linked to Impaired Osteogenesis

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Key Words

Mapk7 • Idiopathic scoliosis • Zebrafish • HMSC • Osteogenesis • RPS6KA3

Abstract

Background/Aims: Three rare *MAPK7* variants that predispose individuals to adolescent idiopathic scoliosis have previously been identified. However, the mechanism underlying the effects of the mutations remain unknown. **Methods:** Human mesenchymal stem cells (hMSCs) were isolated from both patients and healthy volunteer donors, and *MAPK7* expression was detected by western blotting and real-time quantitative PCR (RT-qPCR). Zebrafish embryos were injected with *mapk7* morpholinos or co-injected with morpholinos and wild-type (WT) *MAPK7* messenger RNA (mRNA) at the one-cell stage, followed by calcein staining to evaluate bone formation. hMSCs were transfected with *MAPK7* small interfering RNAs and osteogenesis was induced for 14 days. Alizarin red staining was performed and osteoblast markers were detected by western blotting and RT-qPCR. Since *RPS6KA3* is a downstream target of *MAPK7* and plays an important role in the osteogenesis, zebrafish embryos were then injected with *rps6ka3* morpholinos, or co-injected with *rps6ka3* or *mapk7* morpholinos and WT *RPS6KA3* mRNA at the one-cell stage. **Results:** *MAPK7* expression in the patient group was much lower than in the control group. Morpholino-induced *mapk7* knockdown in zebrafish embryos led to body curvature, which was significantly reversed by WT *MAPK7* mRNA. Calcein staining revealed that *mapk7*-knockdown delayed the ossification of the vertebrae. *MAPK7* silencing in hMSCs impaired osteogenesis and downregulated osteoblast marker expression. Morpholino-induced *rps6ka3*-knockdown in zebrafish embryos led to body curvature, which was reversed by WT *RPS6KA3* mRNA. Interestingly, *RPS6KA3* mRNA also partially reversed the phenotype

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induced by *mapk7* morpholinos. **Conclusion:** Impaired osteogenesis is linked to mutant *MAPK7*-induced idiopathic scoliosis, and *RPS6KA3* may play an important role in this process.

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Introduction

Adolescent idiopathic scoliosis (AIS) is an intricate genetic disorder characterised by a three-dimensional structural deformity as well as lateral curvature of the spine. AIS afflicts 2-3% of school-age children and accounts for about 85% of all idiopathic patients [1]. Previous studies have revealed a strong genetic etiology, and recent genome-wide association studies and whole exome sequencing (WES) studies have identified several variants in genes associated with AIS, such as *GPR126* [2], *BNC2* [3], *PAX1* [4], *LBX1AS1* [5], *POC5* [6], and *AKAP2* [7]. However, the precise biological pathways underlying the predisposition of these variants to AIS are unknown. Prior evaluations have shown that AIS patients have atypical skeletal growth [8] and consistently lower bone mineral densities [9] than those of sex- and age-matched controls. Furthermore, relative anterior overgrowth with disproportionate endochondral-membranous bone growth contributes to the development of AIS [10]. Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate to bone, cartilage and adipose cells. Intramembranous and endochondral ossification, two important components of bone formation, begin with MSC proliferation and condensation [11]. A previous study has reported that MSCs from patients with AIS undergo abnormal osteogenic differentiation [12].

Recently, we determined rare AIS-predisposing variants in the *MAPK7* gene (which encodes mitogen-activated protein kinase 7) by WES along with a linkage screen, followed by targeted sequencing in the Han Chinese population [13]. *MAPK7*, also termed extracellular signal-regulated kinase 5 (ERK5), is part of the MAP kinase family. Numerous evaluations have demonstrated that classical RAS/MAPK signaling, including RAS, RAF, MEK1/2, and ERK1/2, is required for typical skeletal growth, and variation in this pathway can lead to several skeletal abnormalities in humans, including scoliosis [14-16].

In addition, functional cross talk of *MAPK7* with the RAS-RAF-MEK1/2-ERK1/2 signaling pathway has been documented. The activation of the *MAPK7* catalytic domain by RAS and the binding of *MAPK7* and RAF-1 have also been detected [17]. In addition, SHP2, a positive regulator of RAS-MAPK signaling, leads to *MAPK7* initiation via its role in SRC kinase activation [18]. The chondrocyte-specific deletion of *shp2* can cause the disorganization of the vertebral growth plate and lead to AIS-like deformities in mice [19]. Mutations in *RPS6KA3*, encoding a downstream effector of *MAPK7* [20], contribute to Coffin-Lowry syndrome, which causes kyphosis and/or scoliosis in 80% of patients [21]. Importantly, *Nkx3.1:Cre*-driven *mapk7* deletion in mice result in a severely curved spine and substantially reduced bone mass/osteopenia [22].

Therefore, to investigate the mechanisms underlying the role of *MAPK7* in idiopathic scoliosis, *mapk7* and *rps6ka3* were knocked down in zebrafish embryos using morpholinos, and *MAPK7* was silenced in hMSCs using siRNAs.

Materials and Methods

Participants

This study was approved by the Ethical Committee of the First Affiliated Hospital of Sun Yat-sen University, and written informed consent was obtained from Patient 1 (carrying the *MAPK7* c.886G>A variant), Patient 2 (carrying the *MAPK7* c.1943C>T variant), and healthy subjects. Subjects were evaluated by three surgeons from the First Affiliated Hospital of Sun Yat-sen University and met the current clinical criteria for AIS [23].

Isolation and culture of bone marrow-derived hMSCs

hMSCs were separated from the bone marrow of patients and healthy donors and cultured as detailed previously [24]. In brief, the bone marrow samples were diluted with phosphate-buffered saline (PBS), and the cells were then fractionated using Lymphoprep (MP Biomedicals, LLC, Santa Ana, CA, USA) density gradient medium. Cells were then resuspended in low-glucose Dulbecco's modified Eagle medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) and supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific). They were then seeded and incubated at 37°C with 5% CO₂. After 48 h, non-adherent cells were eliminated by changing the medium. Then, the medium was changed after every 3 days. When the cells reached 80-90% confluence, they were trypsinized, counted, and re-plated. The cells from passage days 3-6 were utilized in the experiments.

Immunoblotting

Cells were washed twice with ice cold PBS, harvested, and resuspended in RIPA lysis buffer (Beyotime, Shanghai, China) along with the protease inhibitor cocktail (Biotool, Houston, TX, USA). Cell lysates were acquired by centrifugation at 14,000 × g for 15 minutes at 4°C. Identical portions of every sample were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then moved to Polyvinylidene fluoride transfer membranes (Millipore, Boston, MA, USA). Membranes were blocked with 5% nonfat dry milk for 1 h at room temperature and then incubated with the specified antibodies. Antibody-specific labeling was observed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature, and visualization was performed using an ECL kit (Millipore). The band strength was determined with ImageJ software (National Institutes of Health, Bethesda, MD, USA) and normalized to GAPDH as the loading control.

Primary antibodies against MAPK7, RPS6KA3 (RSK2), p-RPS6KA3 (p-RSK2), RUNX2, and α-Tubulin were acquired from Cell Signaling Technology (Danvers, MA, USA). Antibodies against Osteopontin (OPN) and GAPDH were obtained from Abcam (Cambridge, UK). Goat anti-rabbit IgG H&L (HRP) and goat anti-mouse IgG H&L (HRP) secondary antibodies were obtained from Thermo Fisher Scientific.

Real-time quantitative PCR assay

Total RNA was extracted with an e.Z.N.A® Total RNA Kit I (Omega Bio-Tek, Norcross, GA, USA) from hMSCs and then reverse transcribed to cDNA with Superscript IV VILO Mastermix (Thermo Fisher Scientific) according to the manufacturer's instructions. Real-time PCR was conducted on a Light Cycler 480 Real-Time PCR Detection System (Roche, Basel, Switzerland) utilizing SYBR Green I Master Mix (Roche). The expression levels of MAPK7, RUNX2, ALP, and SSP1 were determined. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression level was used as the reference. Every PCR was processed in triplicate. The relative expression levels for each gene were established utilizing the 2^{-ΔΔCt} method. The primer sequences utilized in this evaluation are included in Table 1.

Plasmid construction and MAPK7 siRNA

WT versions of human MAPK7 mRNA and RPS6KA3 mRNA were produced from a flag-tagged ORF clone of human MAPK7 (Origene, Beijing, China). mRNAs were obtained from linearized constructs as previously reported [25]. The negative control and MAPK7 siRNAs (MAPK7 siRNA-1: GCTCAAGATTGGTGACTTT, and MAPK7 siRNA-2: GCTCAAGATTGGTGACTTT) were obtained from Ribobio (Guangzhou, China). hMSCs were transfected with siRNA using RNAiMAX Transfection Reagent (Thermo Fisher Scientific) according to the manufacturer's protocol.

Table 1. Primers used in this study

Gene	Primer sequence
MAPK7	Sense: 5'-GACCTGATGGAAAGCGACCTG-3'
	Antisense: 5'-TGGATGACCTGAGCCGAGTG-3'
RUNX2	Sense: 5'-ACCAGCAGCAGCTCCATATCTCTAC-3'
	Antisense: 5'-TTCCATCAGCGTCAACACCATC-3'
ALP	Sense: 5'-AATGCTGTCTCTCTGAAGAAACC-3'
	Antisense: 5'-AGTCAATGGAGTCTGGCTGTC-3'
SSP1	Sense: 5'-GCGAGGAGTTGAATGGTG-3'
	Antisense: 5'-CTTGTGGCTGTGGTTTC-3'
GAPDH	Sense: 5'-AGAAACCTGCCAATATGATGAC-3'
	Antisense: 5'-TGGGTGTCTGCTGTGAAGTC-3'
mapk7	Sense: 5'-ACCCAGCAATGATACGTCCA-3'
	Antisense: 5'-GCTTCTCTTTTCGCTCCATG-3'
rps6ka3	Sense: 5'-CGGCATTGGTGCTTATTC-3'
	Antisense: 5'-GGTTTGGAGCGTCTTTCGTT-3'
actb	Sense: 5'-GCAGAAGGAGATCAGTCCCTGGC-3'
	Antisense: 5'-CATTGCCGTACCTTCACCGTTC-3'

Zebrafish embryo injection

Embryos of the AB strain of zebrafish (*Danio rerio*) were raised and maintained as described previously [26] after obtaining permission from the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University. Embryos were staged based on the number of hours post-fertilization (hpf) or days post-fertilization (dpf) at 28.5°C. A splice-blocking morpholino (MO), 5'-TCATCCATCTTATTGGATGCCATCC-3', was designed to target the acceptor splice site of intron 4 of the *mapk7* gene (Gene Tools LLC, Philomath, OR, USA). A splice-blocking MO, 5'-AGAATGTGGCAGCCATCGCCCCCTA-3', was designed to target the splice acceptor site of intron 16 of the *rps6ka3* gene. The sequence of the standard control MO was 5'-CCTCTTACCTCAGTTACAATTATA-3'. Then, 4 ng of MO and/or 100 pg of mRNA was injected into WT zebrafish embryos (N≥100 for each group) at the one-cell stage. Whole embryo images were obtained using a Leica M205FA stereomicroscope (Leica Microsystems, Wetzlar, Germany), and a morphological analysis on the physical changes in the injected embryos, including body curvature and other skeletal malformations, was performed at 4 dpf.

Semi-quantitative reverse transcription (RT-PCR) was conducted to determine the mRNA expression of *mapk7*. In brief, total RNA was isolated at 48 hpf with RNAiso Plus (Clontech Laboratories, Inc., Fremont, CA, USA) following the manufacturer's instructions. cDNA was synthesized from 0.3–1 µg of total RNA utilizing a Thermo Fisher Scientific Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The gene-specific primer sequences are included in Table 1. The samples were resolved by electrophoresis on 2.0% agarose gel.

Calcein staining

Calcein (C₃₀H₂₆N₂O₁₃; Sigma Aldrich, St. Louis, MO, USA) was used to stain calcified bone. Immersion solutions (0.2%) were set up by dissolving 2 g of calcein powder in 1 L of deionized water. Owing to the intense acidifying impact of calcein, a suitable portion of NaOH (0.5 N) was added to the solution to adjust the pH to its initial value. Zebrafish embryos were netted and immersed in the solution for 20 min in Petri dishes. After immersion, the embryos were rinsed several times in fresh water and then immersed in fresh water for 10 min to let the extra unbound calcein spread out of tissues. The embryos were then killed in tricaine-methanesulfonate (Sigma Aldrich) and mounted on glass slides with 3% methyl-cellulose (Sigma Aldrich). Whole embryo images were obtained using a Leica M205FA stereomicroscope.

Osteogenic differentiation

Prior to osteogenic differentiation, MSCs were plated and grown in complete medium until they reached 70%–80% confluence. They were then cultured in osteogenic medium for 14 days. The osteogenic medium consisted of low-glucose DMEM, 10% FBS, 0.1 µmol/L dexamethasone, 10 mmol/L β-glycerol phosphate, and 50 µg/mL ascorbic acid, and the medium was changed every 3 days.

Alizarin Red S staining

Following the induction of osteogenic differentiation for 14 days, the cells were rinsed twice with PBS and fixed in 4% formaldehyde for 30 min. Then, the cells were stained with 0.2% Alizarin Red S solution (Sigma-Aldrich) for 5 min at 37°C. The cells were then cleansed thrice with PBS and images were obtained. The red color indicated calcium deposits. Stains were eluted with 100% dimethyl sulfoxide (Sigma-Aldrich) to establish the amount of Alizarin red staining and absorbance was measured at 405 nm. Every experiment was conducted in triplicate.

Statistical analysis

All quantitative results are presented as means ± standard deviation (SD). Statistical analyses were performed utilizing two-tailed independent Student's *t* test for comparisons between two groups and one-way analysis of variance and Dunnett's post-hoc tests for multiple comparisons. All statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, IL, USA). The level of statistical significance was established at *P* < 0.05.

Results

Low expression of MAPK7 in patient-derived MSCs

hMSCs were exacted from two patients who received surgery and exhibited the *MAPK7* c.886G>A and *MAPK7* c.1943C>T variants (Fig. 1A). RT-qPCR revealed that the *MAPK7* mRNA level in patients was much lower than in the control group (Fig. 1B). *MAPK7* protein expression levels were also quantified by western blotting. *MAPK7* protein levels in the patient group was much lower than those in the control group (Fig. 1C-D).

Zebrafish embryos with *mapk7*-morpholino knockdown exhibited body curvature

Since vertebrate spines have astonishing similarities between species, and *MAPK7* is highly conserved among humans and zebrafish (amino acid identity, 73%), we evaluated the role of *MAPK7* in skeletal development in the zebrafish model. We injected zebrafish embryos with an MO designed to target the splice acceptor site of intron 4 of the *mapk7* gene (Fig. 2A). An RT-PCR analysis of the *mapk7* MO-injected embryos showed that exon 5 of the *mapk7* gene was skipped (Fig. 2A-B). Since exon 5 encodes the majority of the *mapk7* kinase domain, its skipping is assumed to result in a nonfunctional product. Three days post-fertilization, body curvature was observed in the morphants, and co-injection of human WT *MAPK7* mRNA significantly reversed the phenotype (Fig. 2 C-D).

Fig. 1. (A) Low expression of *MAPK7* in patient MSCs. hMSCs were exacted from the proband of sporadic patients carrying two of the three previously reported *MAPK7* variants. (B) RT-qPCR revealed that the *MAPK7* mRNA levels in patients were much lower than those in the control group. (C and D) *MAPK7* protein levels in the patient group were much lower than those in the control group. Band strength was quantified using ImageJ and normalized to the levels of *GAPDH* as the loading control.

Fig. 2. *Mapk7*-morpholino knockdown zebrafish embryos exhibited body curvature. (A) Schematic diagram of the targeting site of *mapk7*-MO. (B) RT-PCR analysis of the *mapk7* MO-injected embryos showed that exon 5 of *mapk7* was skipped. (C and D) Body curvature was observed in the morphants, and co-injection of human WT *MAPK7* mRNA significantly reversed the phenotype. Scale Bar: 500 μ m. N $\geq$ 100. (E and F) The number of ossified vertebrae in the morphants decreased significantly in response to the *mapk7*-morpholinos. ***P<0.01.

To examine whether there are any defects in osteogenesis in the *mapk7* knockout zebrafish model, ossification of the vertebrae in the morphants was examined at 14 dpf by calcein staining. The numbers of ossified vertebrae in the morphants decreased significantly in response to *mapk7* MOs (Fig. 2E-F).

Knockdown of MAPK7 impaired osteogenesis in hMSCs

To further investigate the role of MAPK7 in the process of osteogenesis, the MAPK7 gene was silenced using siRNA in hMSCs from healthy donors. The siRNA efficiency was examined by RT-qPCR and western blotting (Fig. 3A-B). hMSC differentiation into osteoblasts was then induced and Alizarin Red staining was performed and quantified at day 14. MAPK7 silencing led to an impaired osteogenic capacity in the hMSCs (Fig. 3C-D). RT-qPCR revealed that the

Fig. 3. Knockdown of MAPK7 with siRNA impaired osteogenesis in hMSCs. (A) Quantitative PCR and (B) immunoblotting were conducted to ensure silencing efficiency. The osteogenic differentiation of cells was then induced for 14 days. The osteogenic differentiation capacity was visualized using Alizarin Red S (ARS). (C) Staining for ARS was observed by microscopy in representative samples. (D) ARS staining was quantified based on absorbance at 405 nm. (E) Expression of RUNX2, ALP, and SSP1 was detected by quantitative PCR. (F) Expression of RUNX2, OPN, RPS6KA3, and p-RPS6KA3 was detected by immunoblotting. Values in A, D, and E represent the mean $\pm$ SD of three independent experiments. *** $P < 0.001$.

Fig. 4. Overexpression of *rps6ka3b* mRNA reversed the body curvature of *mapk7* morphants. (A) Diagram demonstrating the targeting sites of *rps6ka3*-morpholinos. (B) RT-PCR analysis of the *rps6ka3* MO-injected embryos was conducted to ensure knockdown efficiency. (C) Body curvature was observed in the morphants. (D) Percent distribution of normal embryos vs. embryos with axial deformity, or dead embryos. $N \geq 100$.

osteoblast marker genes *RUNX2*, *ALP*, and *SSP1* were downregulated by treatment with *MAPK7* siRNA (Fig. 3E). Osteogenic marker proteins and their downstream molecules were then detected by western blotting. *MAPK7* siRNA downregulated both *RUNX2* and *OPN* during the osteogenic process (Fig. 3F). However, *MAPK7* siRNA led to reduced phosphorylation of *RPS6KA3* (*RSK2*), instead of affecting the protein expression of total *RPS6KA3* (Fig. 3F).

Overexpression of *rps6ka3b* mRNA reversed the body curvature of *mapk7* morphants

To further investigate the role of *rps6ka3* in the body curvature caused by *mapk7* knockdown, we injected zebrafish embryos with an MO designed to target the splice acceptor sites of introns 16 and 20 of *rps6ka3* (Fig. 4A). An RT-PCR analysis of the MO-injected embryos showed that exons 17-20 of *rps6ka3* were skipped (Fig. 4 A-B). Since exon 17 and exon 21 encode the majority of the *mapk7* phosphorylation and docking sites, respectively, its skipping is expected to result in the failure of *mapk7* binding to *rps6ka3*. Body curvature was observed in both types of morphants at 4 dpf (Fig. 4C), and the co-injection of WT *rps6ka3* mRNA significantly reversed the phenotype (Fig. 3D). Importantly, the co-injection of WT *rps6ka3* mRNA with *mapk7* morpholinos partially reversed the body curvature (Fig. 4D). Therefore, the loss of function of *mapk7* may lead to idiopathic scoliosis partly by impairing the activation of the *rps6ka3* protein.

Discussion

Previously, we reported that three rare *MAPK7* variants predispose individuals to AIS. However, the underlying mechanism remains unknown. In this study, we evaluated the functional changes associated with two of these variants using hMSCs from patients. RT-qPCR and western blotting showed that the *MAPK7* c.886G>A and *MAPK7* c.1943C>T variants lead to low *MAPK7* expression. Thus, we focused on the effects of the loss of *MAPK7* on the development of the skeletal system.

MAPK7 plays a crucial role in vertebrate embryogenesis, and gene disruption is lethal during mouse development [27, 28]. Very recently, the loss of *Mapk7* as a result of *Nkx3.1-Cre* was linked to a severe spinal deformity and an osteopenic phenotype. In this model, a curled tail phenotype was noted in approximately 30% of *Erk5^{fl/fl}* mice [29]. In our study, we found that knocking down *mapk7* in zebrafish embryos results in a scoliosis-like body curvature, and this could be reversed by co-injection with human WT *MAPK7* mRNA.

AIS is the most frequently noted spinal deformity, and prior evaluations have demonstrated that the vertebrae in scoliotic spines exhibit atypical shape changes that are possibly impacted by the bone density distribution inside the vertebrae. A low bone mass and osteopenia have been reported in the axial and peripheral skeletons of patients with AIS. The z-score for BMD at the femoral neck of the subjects with AIS was significantly lower than that of the normal controls. In addition, the patients with AIS had irregular bone quality with a greater prevalence of osteopenia [30]. Biomechanical simulation indicated that a low bone mineral mass may aggravate curve progression and induce more serious lumbar compensatory scoliosis in patients with AIS [31]. At the apical vertebra, the mean bone density at the concave side of the cortical shell was 23.5% greater than that of the convex cortical shell, and the cancellous bone density along the central 60% of the lateral path from convex to concave increased by 13.8% [32]. A substantially deformed curved thoracic spine, with a linked loss of trabecular bone volume, was also noted in the above-mentioned *Erk5^{fl/fl}* mice. Importantly, we observed delayed ossification of the vertebrae in the morphants at 14 dpf, suggesting that abnormal bone formation plays an important role in the mutant *mapk7*-induced phenotype.

ERK5 (*MAPK7*) is activated downstream of MAP2K MEK5 (*MAP2K5*). ERK1/2, ERK5, p38, and JNK comprise the conventional group of MAPKs based on the conservation of the Thr-Xaa-Thr motif in the activating loop [33]. Li et al [34]. provided novel insights into the mechanisms underlying mechanosensation by highlighting the association between *MAPK7*

activation and cytoskeletal reorganization in osteoblasts experiencing fluid shear stress (FSS). MAPK7 was quickly phosphorylated in pre-osteoblastic MC3T3-E1 cells upon the occurrence of FSS. The integrity and reorganization of the cytoskeleton were pivotal in this process, in which the cytoskeleton-dependent activation of FAK (focal adhesion kinase) was involved in the FSS-induced initiation of MAPK7. The MEK5/ERK5 pathway also regulates FSS-induced Runx-2 expression in osteoblast differentiation, and the expressions of Cyclin B1 and CDK1 may also be upregulated, causing MC3T3-E1 cell proliferation [35, 36].

Mekk3 (Mek kinase 3) encodes an upstream kinase of MEK5; it has been linked to bone mineral density prompted by M-CSF in an oligonucleotide microarray examination of gene expression patterns in mouse bone marrow mononuclear cells during the process of osteoclast differentiation [37, 38]. *Mekk2*, a kinase downstream of the ERK5 pathway, is also essential in the regulation of osteoblast activity [39]. The atypical expression of Runx2 and reduced BMD in patients with AIS suggest the abnormal regulation of osteoblast differentiation. Runx2 could have a critical role in the pathogenesis of the reduced BMD in AIS [40]. In our study, the silencing of *MAPK7* by siRNA impaired osteogenesis in hMSCs, and significantly down-regulated the expression of RUNX2.

Coffin-Lowry syndrome is a unique X-linked disorder in which males present with severe mental delays and dysmorphism (approximately 80% exhibit kyphosis and/or scoliosis) [21]. It results from mutations in the *RPS6KA3* gene located at Xp22.2, which encodes RSK2, a growth-factor-regulated protein kinase. *RPS6KA3* mutations are exceedingly heterogeneous and cause the loss of phosphotransferase activity in the RSK2 kinase, most frequently due to early translation termination [41]. MAPK7 can specifically activate *RPS6KA3* *in vitro*, and the activation of MAPK7 weakens its binding to RSK2, indicating that *RPS6KA3* is liberated at activation [20]. ATF4 is a pivotal substrate of *RPS6KA3* and is needed for the timely onset of osteoblast differentiation, the terminal differentiation of osteoblasts, and osteoblast-specific gene expression. Furthermore, RSK2 and ATF4 post-transcriptionally mediate the synthesis of Type I collagen, the prominent constituent of the bone matrix. Thus, an *Atf4*-deficiency results in later bone formation during embryonic development and diminished bone mass during postnatal life [42]. The large C-terminal domain of MAPK7 is not needed for the binding or activation of *RPS6KA3* by MAPK7; however, the common docking (CD domain) of MAPK7 and the docking (D domain) of RSK are necessary for their association [20]. Prior evaluations have demonstrated that mice without *RPS6KA3* develop osteopenia due to damaged osteoblast function and normal osteoclast differentiation. This phenotype is linked to reduced expression of *Phex*, an endopeptidase that mediates bone mineralization [43]. By targeting the ERK docking and phosphorylation sites using *rps6ka3* MOs, we successfully replicated the body curvature in zebrafish embryos. Importantly, the co-injection of *mapk7* MO and WT human *RPS6KA3* mRNA significantly prevented this phenotype, suggesting that mutant MAPK7 contributes to idiopathic scoliosis partly via *RPS6KA3*.

In conclusion, the results of this study revealed the critical part of *Mapk7* in bone development and homeostasis *in vivo* and *in vitro*. The loss of *Mapk7* was linked to extreme spinal deformity and an impaired osteogenesis phenotype. In addition, our results suggest that the reduction of MAPK7 pathway activity and the loss of *MAPK7* gene expression induce damaged osteogenesis via *RPS6KA3* and/or its substrates. This model broadens our knowledge and understanding of the molecular events that mediate bone homeostasis, spinal deformities, and/or osteopenia.

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Disclosure Statement

The authors declare that they do not have any conflict of interests.

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