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Tfap2b mutation in mouse results in patent ductus arteriosus and renal malformation

Jing Wang, MS,[a] *Wei Ji, MD,*[b] *Diqi Zhu, PhD,*[b] *Wenfeng Wang, MS,*[c] *Yiwei Chen, MS,*[b] *Zhen Zhang, PhD,*[c] *and Fen Li, MD*[b,*]

[a] *Department of Infectious Disease, Shanghai Children's Medical Center Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China*

[b] *Department of Pediatric Cardiology, Shanghai Children's Medical Center Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China*

[c] *Department of Translational Research Institute, Shanghai Children's Medical Center Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China*

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ABSTRACT

Background: Transcription factor *TFAP2B* is associated with Char syndrome in humans and is characterized by patent ductus arteriosus (PDA) and facial and finger abnormalities. In a previous study, we detected a *c.435_438delCCGG TFAP2B* mutation in a family with PDA, and no facial dysmorphism or finger abnormalities were observed. This 4-base pair (bp) deletion in exon 2 resulted in a truncated protein of about 21 kDa in cultured cells *in vitro*. However, it is not clear why *c.435_438delCCGG* mutation carriers are present with isolated PDA instead of Char syndrome.

Materials and methods: We successfully established a mouse model bearing *Tfap2b c.435_438delCCGG* mutation using CRISPR/Cas9 technology. The mutant mice were phenotyped using histological analysis, and the development of ductus smooth muscles in mutant mice was examined by immunohistochemistry.

Results: The *c.435_438delCCGG* homozygous mutant mice were characterized by delayed closure of the ductus arteriosus (DA) and renal malformation. Furthermore, the *c.435_438delCCGG* mutation might result in PDA by affecting the development of ductus arterious smooth muscle cells.

Conclusions: Using the *c.435_438delCCGG* homozygous mice, we verified the nature of the *c.435_438delCCGG* mutation and established a new and useful animal model to explore the function of *Tfap2b* and the mechanisms of PDA and renal formation. These findings may be useful for the development of therapies for those rare disorder.

Introduction

The transcription factor *TFAP-2 beta* is a member of the *TFAP2* family and is involved in embryonic development and cell cycle regulation; it is enriched in the neural crest and exhibits dynamic expression related to the migration of neural crest cells.[1,2] Similar to other members of the *TFAP2* family, the TFAP2 protein consists of a variable transactivation domain at the N-terminus and a highly conserved helix-span-helix dimerization domain for DNA binding at the C-terminus.[3-5]

* *Corresponding author*. Department of Pediatric Cardiology, Shanghai Children's Medical Center Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China. Tel.: +86 189 3083 0811; fax: +86 216 408 5875.
E-mail address: lifen@scmc.com.cn (F. Li).

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TFAP2B mutations are associated with Char syndrome, a disorder characterized by patent ductus arteriosus (PDA), an unusual facial appearance, and abnormalities of the fingers in humans.[6-8] Khetyar *et al*.[9] reported that a single-base substitution in exon 3 of *TFAP2B* causes familial isolated PDA, without craniofacial or finger abnormalities. *TFAP2B* point mutations may be risk factors for nonsyndromic PDA. In mice, *Tfap2b* is not only expressed in distal tubular epithelial cells in late embryonic stages but is also specifically expressed in the ductus smooth muscle tissues. Moreover, *Tfap2b* is involved in the maintenance of the highly differentiated state of ductus smooth muscle cells and plays an important role in the survival and maintenance of the collecting duct and tubular epithelial cells.[10-12] *Tfap2b*-deficient mice die shortly after birth due to congenital polycystic kidney disease or PDA. Moser *et al*.[11] confirmed that polycystic kidney disease from ED16.5 to birth results from excessive apoptosis of renal epithelial cells. The complete loss of *Tfap2b* also causes heart-limb defects, including PDA and an accessory postaxial digit of the forelimb.[12] However, the pathogenesis of these three abnormalities and the mechanism through which *Tfap2b* interacts with other genes are not clear.

In our previous study, we found a heterozygous *c.435_438delCCGG* mutation in *TFAP2B* in all affected individuals of an isolated PDA family lacking facial and finger abnormalities. This mutant gene had a 4-base pair (bp) CCGG deletion in exon 2 and encoded a predicted abnormal 189 amino acid protein owing to a premature stop codon, p.Arg145Argfsx45.[13] We also found that the *TFAP2B c.435_438delCCGG* mutation altered the transcription of the target sequences and only produced a residual protein of about 21 kDa, which lacked a DNA-binding domain, in cultured cells *in vitro*.[14]

Based on these previous results, we hypothesize that the *c.435_438delCCGG* mutation in *TFAP2B* may be a key mechanism underlying isolated PDA. Accordingly, in this study, we established a mouse model bearing the *c.435_438delCCGG* mutation (*Tfap2b*$^{Mu/Mu}$) using CRISPR/Cas9 technology to determine the effects of this mutation at the molecular level. Interestingly, we found that *Tfap2b*$^{Mu/Mu}$ mice showed partially delayed closure of the ductus arteriosus (DA) and renal malformation but did not show limb abnormalities, unlike mice bearing the *Tfap2b*-null mutation. The effects of this *c.435_438delCCGG* mutation in our mouse model may be associated with the truncated N-terminal protein, which could disrupt transcription and translation. We think that the residual protein may include an important motif for interactions with target genes involved in the regulation of limb and DA formation.

Materials and methods

Tfap2b$^{Mu/Mu}$ *mouse generation and genotyping*

Heterozygous mutant mice with the *Tfap2b c.435_438delCCGG* mutation (*Tfap2b*$^{Mu/Wt}$) in exon 2 were produced using CRISPR/Cas9 technology and maintained by Shanghai Biomodel Organism Science and Technology Development (Shanghai, China). The specific guide-RNA (gRNA) and oligo donor DNA were designed as follows: gRNA1, 5′-CTACCACTCTGTCCGCC GGC-3′; gRNA2, 5′-GCAGCAGCACGTCCGGCCGG-3′; and oligo donor DNA sequence, 5′-CCGGGCAGCCTTGCCCCAGCTCTCCG GCCTTGATCCCCGAAGGGACTACCACTCTGTCCGCCGGCCGG ACGTGCTGCTGCATTCCGCACATCACGGCCTGGACGCCGGCA TGGGCGACAGCCTCT-3′. Mutant mice were crossed with C57BL/6 wild-type mice and genotyped to select *Tfap2b*$^{Mu/Wt}$ mice. *Tfap2b*$^{Mu/Mu}$ mice were obtained by mating *Tfap2b*$^{Mu/Wt}$ mice. After mice were sacrificed, tissue samples from neonatal mice were collected for genomic DNA extraction and polymerase chain reaction (PCR). The following primers were used for PCR: forward, 5′-CACGATGGCGTCCCAAGC-3′ and reverse, 5′-AAACCGCCACCACCTTGT-3′. PCR products were 647 bps for the wild-type allele and 643 bps for the mutant allele. It was very difficult to distinguish the 4-bp difference on an ordinary agarose gel. Accordingly, we genotyped all samples by DNA sequencing using the forward primer. This study was performed with the approval of the local ethics committee, and all experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Western blot analyses

Total proteins isolated from midbrain tissues were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Equal amounts of protein were then transferred to a nitrocellulose filter membrane and incubated with primary antibodies overnight at 4°C. Anti-Tfap2b N-terminal-specific polyclonal antibodies (AV38282, 1:100; Sigma, St. Louis, MO) was used as the primary antibody, and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibodies (1:2000; Boster, Pleasanton, CA) were used as reference antibodies. After washing with Tris-buffered Saline with Tween 20, the membrane was incubated for 30 min with horseradish peroxidase-labeled goat anti-rabbit IgG (1:2000; Jackson, Bar Harbor, ME) at room temperature.

Histopathology of the hearts and kidneys of mice

The hearts and kidneys of mutant and control mice were dissected. The organs were fixed in 10% formaldehyde solution and embedded in paraffin. All tissue sections were sliced, mounted on glass slides, and stained with hematoxylin and eosin (H&E) or Nuclear Fast Red for microscopic examinations.

Immunochemistry

Heart tissues from mutant and wild-type mice were cut into 8-μm-thick sections, and tissue sections of DA were incubated with primary antibodies overnight at 4°C. Primary antibodies included anti-myosin light-chain kinase (MLCK, EP1458Y [ab76092]; Abcam, Cambridge, UK), anti-SM22α (ab10135; Abcam), and anti-Fibronectin antibodies (sc-6952; Santa Cruz Biotechnology, Santa Cruz, CA). Sections were then incubated with biotinylated secondary antibodies (SA1021, SA1022) at 37°C for 30 min. The hematoxylin staining was performed for 60 s (SABC Immunochemical Staining Kit; Boster).