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Characterization of transcription factor AP-2 beta mutations involved in familial isolated patent ductus arteriosus suggests haploinsufficiency

Wei Ji, MD,[a,1] Matthew A. Benson, PhD,[b,1] Shoumo Bhattacharya, MD,[b] Yiwei Chen, MS,[a] Jingjing Hu, MD,[a] and Fen Li, MD[a,*]

[a] Department of Cardiology, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China

[b] Department of Cardiovascular Medicine, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

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ABSTRACT

Background: Patent ductus arteriosus (PDA) is one of the most common congenital heart defects. Transcription factor AP-2 beta (TFAP2B) mutations are associated with the Char syndrome, a disorder associated with PDA, and with facial and fingers abnormalities. Recently, we identified two TFAP2B mutations in two families without Char syndrome phenotype, c.601+5G>A and c.435_438delCCGG, and these TFAP2B mutations were associated with familial isolated PDA. The aim of this study was to identify the effects of these mutations on TFAP2B function.

Methods: Plasmids containing the wild-type or mutated TFAP2B were constructed and transfected in cells. Plasmids containing the TFAP2B coactivator, Cpb/p300-interacting transactivator 2 (CITED2), was also transfected. TFAP2B expression was detected by luciferase expression and by Western blot analysis.

Results: These mutations resulted in loss of transactivation function, which could not be improved by Cpb/p300-interacting transactivator 2. The c.601+5G>A mutated gene did not express any protein, whereas the c.435_438delCCGG mutation did not impact the transactivation function activated by the wild-type TFAP2B.

Conclusions: These results suggest that a haploinsufficiency effect of TFAP2B could be involved in familial isolated PDA.

1. Introduction

The ductus arteriosus (DA) is a specific vascular structure between the aorta and the pulmonary artery diverting the blood from the pulmonary artery into the aorta during fetal development. After birth, DA undergoes functional and anatomic closure. In some people, the DA does not close after birth, if present after the age of 3 mo, this condition is known as patent DA (PDA). Untreated PDA usually results in life-threatening conditions, such as congestive heart failure, pulmonary artery hypertension, and neonatal necrotizing enterocolitis [1]. On the other hand, DA patency is essential in

* *Corresponding author*. Department of Cardiology, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, P. R. China. Tel.: +86 150 2165 5580; fax +86 216 408 5875.
E-mail address: jiweimedsci@163.com (F. Li).

[1] These authors contributed equally to this work.

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some special pathologic conditions. Some DA-dependent congenital heart diseases, such as severe tetralogy of Fallot and single ventricle with severe pulmonary artery stenosis, require palliative systemic pulmonary shunt or stent implantation into the DA to ensure an adequate pulmonary blood flow.

The DA originates from the left dorsum part of the sixth pharyngeal arch and is histologically derived from cardiac neural crest cells (NCCs) [2,3]. Transcription factor AP-2 beta (TFAP2B), is a transcription factor that is enriched in the neural crest and could play an important role in regulating DA closure [4]. Cpb/p300-interacting transactivator 2 (CITED2) acts as a TFAP2B coactivator and enhances its transcriptional efficiency [5]. To date, most TFAP2B mutations [6–9] have been identified in Char syndrome patients, a birth defect characterized by PDA and by deformities in the face and fingers [10]. However, no mutation causing isolated PDA has been identified for a long time. Recently we reported two TFAP2B heterozygous mutations, c.601+5G>A and c.435_438delCCGG, in two unrelated families with PDA, but without features of the Char syndrome [11] (Figs. 1 and 2). In both families, the mutated allele was only observed in affected individuals. This previous study demonstrated that TFAP2B variations are not exclusively associated with the Char syndrome, but may also be associated with isolated PDA. However, although we identified the c.601+5G>A mutation in isolated PDA patients, it was also reported in Char syndrome patients [7], stressing the need for a better understanding of this pathology.

To examine the pathogenic mechanism underlying isolated PDA, we conducted a functional study of the TFAP2B c.601+5G>A and c.435_438delCCGG mutations using a transactivation assay. We hypothesized that the disruption of TFAP2B transcription by these mutations plays a major role in the etiology of isolated PDA.

2. Materials and methods

2.1. Plasmids

The pcDNA3-TFAP2B expression plasmid (containing the wild-type TFAP2B [NM_003221]), the p3*AP2-Bluc plasmid (containing the luciferase reporter gene with three copies of the TFAP2B recognition sequence), the pcDNA3-lacZ plasmid, the pcDNA3-CITED2 plasmid (containing the TFAP2B coactivator, CITED2), and the empty vector pcDNA3 were all provided by S.B. (Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA). The c.435_438delCCGG expression plasmid, CMV-CCGGdel, was developed by site-directed mutagenesis polymerase chain reaction using the primers presented in Table 1. The CMV-Δexon3 vector expressing TFAP2B without exon 3 was made using the primers presented in Table 1; these primers matched the 3′ terminal part of exon 2 and the 5′ terminal part of exon 4. Both mutated TFAP2B vectors were confirmed by sequencing.

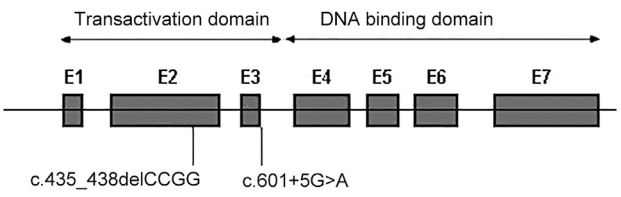

Fig. 1 – Schematic representation of the TFAP2B gene and position of the two mutations. The c.601+5G>A mutation is located in the junction area of exon 3 and intron 3. The c.435_438delCCGG is located in the C-terminal part of exon 2. Boxes represent the exons.

2.2. Cell transfection and transactivation assay

U2-OS cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma, St Louis, MO) supplemented with 10% fetal bovine serum (Sigma), penicillin (100 U/mL, Sigma), streptomycin (10 μg/mL, Sigma) and l-glutamine (4 mM, Sigma). Cells were seeded into a 96-well plate, 5000 cells/well. For transactivation assays, cells were transfected with 20 ng of CITED2 or pcDNA3 vector, 100 ng of p3*AP2-Bluc plasmid, 20 ng of CMV-lacZ plasmid, and 20 ng of wild-type or mutant plasmids using Fugene HD (Promega, Madison, WI). To evaluate the activation of the luciferase reporter gene (p3*AP2-Bluc) by increased doses of CITED2, we transfected 10, 20, or 40 ng of CITED2 together with 20 ng of wild-type or TFAP2B mutant plasmids, and 20 ng of the CMV-lacZ plasmid for normalization. To detect the effects of the CMV-CCGGdel plasmid (30 ng) on cotransfected wild-type TFAP2B (30 ng), cells cotransfected with pcDNA3 (30 ng) and CMV-CCGGdel (30 ng) were compared, and cells transfected with pcDNA3 (60 ng) were used as control. Twenty-four hours after transfection, luciferase expression was measured using the luciferase assay system (Promega, E1500), according to the manufacturer's instructions. LacZ levels were assessed as previously described [12], and luciferase expression levels were normalized to LacZ levels. Results are presented as relative luciferase value from three independent transfection experiments performed in triplicate.

2.3. Western blotting

U2-OS cells were seeded in a 12-well plate (80,000 cells/well) and transfected with 800 ng of pcDNA3, wild-type TFAP2B, c.435_438delCCGG, or c.601+5G>A plasmids using Fugene HD (Promega). Western blotting was performed after 24 or 48 h. We used the TFAP2B N-terminal specific polyclonal antibody (Sigma, AV38282) as the primary antibody (1 μg/mL) and horseradish peroxidase-labeled goat anti-rabbit as the secondary antibody. Proteins were detected using the enhanced chemiluminescence (ECL) advanced Western blotting detection kit (GE Healthcare, Waukesha, WI).

3. Results

3.1. TFAP2B mutations disrupt transactivation

As expected, wild-type TFAP2B-induced luciferase expression, which was increased by CITED2 cotransfection. However,