The study of copy number variations in the regions of PRKAB2 and PPM1K among congenital heart defects patients

Han-Quan Dong1
Yue-Xin Du2

1. Department of Pneumology, Tianjin Children’s Hospital, Tianjin, 300074–China
2. Department of Child Healthcare, Tianjin Municipal Women and Children health care center, Tianjin, 300070, China

http://dx.doi.org/10.1590/1806-9282.65.6.786

INTRODUCTION

There are many forms of variation included in the human genome. In 2006, copy number variations were defined for a series of discoveries previously made as a DNA segment of 1 kb or larger, which presented variable copy numbers, in comparison with a reference genome.1 Copy number variations have been reported to influence gene expression by altering gene dosage and through the effect of its position.2 Several diseases have been shown to be caused by the duplication of specific genes. Hence, in this study, we analyzed the copy number variations of genes PRKAB2 and PPM1K within ventricular septal defect and tetralogy of Fallot patients.

Ventricular septal defect is the 2nd most common type of congenital heart defect (32%) and characterized as a defect in the septum between the right and left ventricle; while the tetralogy of Fallot is relatively rare, and is defined as the combination of a mispositioned aorta that overrides both ventricles, ventricular septal defect, pulmonary stenosis, and right ven-
tricular hypertrophy. Both congenital heart defects, ventricular septal defect and tetralogy of Fallot have a prevalence of 0.16% and 0.05% in births, respectively. The AMP-activated protein kinase, a central cellular energy regulator, has recently emerged as a primary candidate for its role in metabolic dysfunction. The amp-activated protein kinase is highly conserved and consists of a α-catalytic subunit and β and γ regulatory subunits. PRKAB2 (AMPKB2), the gene that encodes the β2 regulatory subunit, is located in 1q21.2.

METHODS
Study subjects

A total of 200 nonsyndromic congenital heart defect patients (100 tetralogy of Fallot patients and 100 ventricular septal defect patients) and 100 congenital heart defect-free controls were recruited from Tianjin Children’s hospital into this study. Informed consent was obtained from their parents or guardians. This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

The clinical assessment performed in patients included anthropometric measurement, physical examination for dysmorphism and malformation, and radiological evaluation. These patients also underwent chest X-ray examination, electrocardiogram, and ultrasonic echocardiogram.

Quantitative Real-Time PCR Analysis

Blood samples from congenital heart defect patients and controls were collected and stored at -20°C. Genomic DNA was extracted from peripheral blood leukocytes using standard methods (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany).

The exon regions of the PRKAB2 and PPM1K genes were used for real-time primer design. The primer sequences of these candidate genes were designed on the basis of the sequence data obtained from the NCBI database (http://www.ncbi.nlm.nih.org), using Primer Express software 3.0 (Applied Biosystems) (Table 1).

Those regions were amplified in all 200 patients and 100 congenital heart defect-free control individuals using real-time quantitative polymerase chain reaction (qPCR) with a set of gene-specific primers. The gDNA was used as a template in real-time qPCR reactions with SYBR® Green PCR Master Mix (Takara Biotechnology [Dalian] Co. Ltd.), which was performed using a 7000 real-time PCR system (Applied Biosystems, USA).

The quantitative of these target sequences was normalized to an assay from chromosome 21, C2, and the relative copy number (RCN) was determined on the basis of the comparative ΔΔCt method, with a normal control DNA as the calibrator. These experiments were repeated three times. An approximately 0.5-fold RCN and approximately 1.5-fold RCN were used for deletion and duplication, respectively.

TABLE 1. LIST OF Q-REAL-TIME-PCR PRIMERS

<table>
<thead>
<tr>
<th>Candidate Gene-Primer ID</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRKAB2-F</td>
<td>5'-GCCAAAGCTCAGTGTTGTGGTTA-3'</td>
</tr>
<tr>
<td>PRKAB2-R</td>
<td>5'-GACAGACAGACAGACAGACCTGACATCATT-3'</td>
</tr>
<tr>
<td>PPM1K-F</td>
<td>5'-CAGGAACCCAAAGGGAGGCAACT-3'</td>
</tr>
<tr>
<td>PPM1K-R</td>
<td>5'-CCTTTGCTGTGGTTTGGAG-3'</td>
</tr>
<tr>
<td>C2-F</td>
<td>5'-CAATTTCAGGTCAGGGTGAACCTCAGTA-3'</td>
</tr>
<tr>
<td>C2-R</td>
<td>5'-GCCAGGTATGATGTTTGGCTAAGTC-3'</td>
</tr>
</tbody>
</table>
RESULTS
Real-Time quantitative PCR analysis

The present study included 100 ventricular septal defects and 100 tetralogy of Fallot patients. In order to aim directly at each gene region, specific primers were designed in the exon regions of those genes, in which the amplification segment of the PRKAB2 gene was at the 8th exon, while that of the PPM1K gene was at the 7th exon. Both segments were in the 3’UTR regions.

PRKAB2, located at 1q21.1, which has been previously implicated in congenital heart defects, has been found to have copy number variations in the same region in the present study. According to the Greenway et al. study, there were four duplications and one deletion in five subjects with tetralogy of Fallot, and the shared segment spans a small interval on chromosome 1q21.1. The present study found a deletion at the 8th exon of the PRKAB2 gene in one tetralogy of Fallot patient, while no duplication or deletion was found in all ventricular septal defect patients (Figure 1a).

Copy number variations at 4q22.1 in tetralogy of Fallot patients have been reported in a previous study, and the region is where the PPM1K gene located. Combined with the results of our experiment, a correlation between tetralogy of Fallot and the PPM1K gene is strongly suggested. Meanwhile, all patients with ventricular septal defects were duplicated in the same region of this gene (Figure 1b).

In order to confirm the copy number variations found in the present study, real-time qPCR was performed at least three times to get rid of human-made handling errors.

As expected, there were no deletions or duplications detected in all 100 congenital heart defect-free controls according to the qPCR result.

DISCUSSION

At the 1q21.1 region, according to real-time qPCR results, deletions were found at the PRKAB2 gene region in one tetralogy of Fallot patient. Since more recent copy number variations at this locus were identified in subjects with neurocognitive, psychiatric, and developmental phenotypes, we confirm that tetralogy of Fallot subjects with 1q21.1 copy number variation identified in the present study had normal cognition, social behavior, and neurologic function.

AMPKbeta2 in the N-terminal region may play a role in isoform-specific AMP-activated protein kinase activity, and it was proven that AMPKbeta2 was highly expressed in the skeletal muscle, with low expression in the liver. AMP-activated protein kinase beta subunit contains a functional glycogen binding domain, and the mutation of glycogen binding residues may completely abolish beta-GBD binding to glycogen. This suggests that this gene is associated with diabetes. AMPKbeta2 was also expressed in the heart, along with its other subunits. However, no disease-causing mutations were found in PRKAB2 in inherited cardiomyopathies. In another study, AMPK-beta2 was shown to be regulated by the p53 protein, which may be signaled to reduce the fidelity of cell growth and division, and responds by initiating cell cycle arrest, senescence, or apoptosis.

Several SNPs at the 4q22.1 region have been reported and associated with bone mineral density (hip mineral density).
and spine) (MEPE), serum uric acid, serum urate (ABCG2) and conduct disorder (interaction) (PPM1K). However, there were no other previously reported studies on disease-candidate copy number variations in this region, except for the study of Greenway et al. 9.

PPM1K is a mitochondrial protein phosphatase that plays an important role in normal development and cell survival and was discovered to specifically bind the branched-chain-alpha-ketoacid dehydrogenase (BCKD) complex and induce the dephosphorylation of Ser293 in the presence of BCKD substrates. PPM1K inactivation in developing zebrafish embryos caused abnormal cardiac, neural development, as well as heart failure. 8 PPM1K-deficient mice exhibited catabolic defects similar to human maple syrup urine disease. 26

Previous studies have discovered that copy number variation research has focused on neurological diseases such as Parkinson’s and Alzheimer. 27, 28 In recent years, copy number variations were found to be more related with congenital disabilities. 29, 30 As one of the most common types of congenital disability, the pathological mechanism of congenital heart defects has also been said to be associated with copy number variations.

The less severe subtype ventricular septal defect is mainly due to the defect in the ventricular septum, which consists of the inferior muscular and superior membranous portion, while the ventricular septum is extensively innervated with conducting cardiomyocytes. In contrast, the tetralogy of Fallot involves four heart malformations presented together, which are pulmonary stenosis, overriding aorta, ventricular septal defect, and right ventricular hypertrophy. The first condition includes the narrowing of the right ventricular outflow tract, in which the PRKAB2 gene was found to be significantly expressed. This may be the explanation for why copy number variations that overlap the PRKAB2 region were not found in the ventricular septal defect, but only found in tetralogy of Fallot patients.

In conclusion, we replicated the disease-associated genes of PRKAB2 and PPM1K with tetralogy of Fallot in a Chinese Han population and verified that there was no correlation between those two genes and ventricular septal defect. These results indicate the same molecular population genetics in these two genes of different ethnicity. This shows that these two genes may be specific of tetralogy of Fallot candidates.

**Competing interests:**
The authors declare that they have no competing interests. There are no financial or other relationships to be declared.

---

**REFERENCES**
