Short Report

Novel ZFPM2/FOG2 variants in patients with double outlet right ventricle


Congenital heart defects (CHDs) occur in about 0.5–1% of all newborns and are the most common birth defects. Double outlet right ventricle (DORV) accounts for approximately 1–3% of all CHDs. Similar to Tetralogy of Fallot (TOF), DORV is a subtype of contruncal heart defects (CTDs) and is anatomically characterized by a malposition of the great arteries. We described a boy with chromosomal translocation: 46, XY t (8; 18) (q22; q21) that may disrupts the ZFPM2/FOG2 locus. The coding sequences of ZFPM2/FOG2 were determined in 38 patients with sporadic DORV, 95 patients with TOF, and 12 patients with transposition of the great arteries. Five DNA sequence variants affecting variably conserved residues of ZFPM2/FOG2 were identified in patients with TOF type or ventricular septal defect type of DORV. Three novel mutations (p.V339I, p.K737E, and p.A611T) were reported for the first time. The other two mutations (p.M703L and p.Q889E) were reported in patients with congenital diaphragmatic hernia but not in patients with CHD. Our finding suggests that variants of the ZFPM2/FOG2 gene might be a common cause of DORV.

Conflict of interest
The authors declare no conflict of interest.

Double outlet right ventricle (DORV) accounts for approximately 1–3% of all congenital heart defects (CHDs). Similar to Tetralogy of Fallot (TOF), DORV is a subtype of contruncal heart defects (CTDs) and is anatomically characterized by a malposition of the great arteries (1). DORV is highly etiologically heterogeneous, and the pathogenesis of DORV is still largely unknown. Because anatomic abnormalities such as overriding aorta, ventricular septal defect (VSD), pulmonary stenosis and right ventricular hypertrophy can present in both TOF and DORV patients, the distinction between DORV and TOF is not always clear, especially between Fallot-type DORV or DORV-type TOF (2).

The transcription factor ZFPM2/FOG2 is located at chromosomal region 8q23.1, which is found to be duplicated in DORV patients (3), or translocated (with disruption of the ZFPM2/FOG2 locus) in a patient with atrial septal defect and gonadal dysgenesis (4). ZFPM2/FOG2 is a zinc-finger transcription factor that interacts with GATA4 (5). Similar to GATA4, ZFPM2/FOG2 plays a critical role in heart development (6, 7), gonadal differentiation (8), coronary vascular development (9), and congenital diaphragmatic hernia (10, 11). Mice lacking ZFPM2/FOG2 died at embryonic days 13.5 with multiple cardiac defects associated with TOF in humans (6). In one study, ZFPM2/FOG2 missense mutations were found in 4% (2/47) of patients with TOF (12). A recent study of a larger population of patients with CHD found only 1 out of 178 (0.5%) patients with TOF carried a ZFPM2/FOG2 mutation (7). In the same population, 2 out of 13 patients with DORV (15.3%) carried mutations in ZFPM2/FOG2 (13). These results imply that ZFPM2/FOG2 mutations might be a major cause of DORV.
Novel ZFPM2/FOG2 variants

We describe a 10-year-old boy with Langer-Giedion syndrome and DORV who carries a chromosomal translocation that may have disrupted the ZFPM2/FOG2 locus. We also performed a survey of DORV patients for ZFPM2/FOG2 mutations. Our study suggests that ZFPM2/FOG2 variants are probably a common cause of DORV in the Chinese population.

Materials and methods

Patients

From 1st March 2009 to 1st October 2010, 2523 patients under 14 years old with CHD attended the Department of Pediatric Cardiology at the Second Xiangya Hospital. Among them, 412 patients with TOF, 62 patients with DORV and 27 patients with transposition of the great arteries (TGA) underwent surgery. The parents of 95 patients with TOF, 38 patients with DORV and 12 patients with TGA provided informed consent and genomic DNA was prepared from peripheral blood of these patients. The patients were ascertained by echocardiography and cardiac catheterizations, and the cardiac anatomy were confirmed at the time of surgery in the Department of Cardiothoracic Surgery of the Second Xiangya Hospital. Two hundred and fifty ethnically matched control subjects underwent echocardiography to exclude CHD. Genomic DNA was prepared using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, Santa Clarita, CA). This study was approved by the Review Board of the Second Xiangya Hospital of the Central South University of China.

Karyotype and FISH

Two milliliters of peripheral blood was collected from the proband and his parents. All samples were subjected to lymphocyte culture according to standard cytogenetic protocols. Fifty Giemsa–trypsin–Giemsa-banded metaphase karyotypes were analyzed for all the individuals of the family.

Fluorescence in situ hybridization (FISH) was performed using the lymphocyte culture. Hybridizations were performed with three pairs of BAC probe. Probes for chromosome 8: RP11-91G3(8q21.3), RP11-701A5 (8q22.1), and RP11-13A18 (8q24.3) were conjugated with red fluorescence. Probes for chromosome 18: RP11-260O16 (18q12.3), RP11-47F13 (18q21.1), and RP11-196B3 (18q23) were conjugated with green fluorescence.

Sequence analysis

To explore the molecular lesions that caused the diseases, the entire coding regions, including the flanking intronic sequences of ZFPM2/FOG2 (Refseq: NM_012082), TRPS1 (NM_014112), and EXT1 (NM_000127), were amplified with polymerase chain reaction (PCR) (primer sequences will be provided upon requests). Sequences of the PCR products were determined using the ABI 3100 Genetic Analyzer (ABI, Foster City, CA) as previously reported (14). The DNASTar MegAlign tool (DNASTAR, Madison, WI; http://www.dnastar.com/) was used to analyze the DNA sequence variants and the evolutionary conservation of the mutated residues in the ZFPM2/FOG2 protein.
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<tr>
<th>Patient ID</th>
<th>Types of disease</th>
<th>Exon</th>
<th>Nucleotide alteration</th>
<th>Amino acid substitution</th>
<th>POLYPHEN prediction</th>
<th>MUTATION TASTER a,b</th>
<th>POLYPHEN, polymorphism phenotyping; TOF, Tetralogy of Fallot; VSD, ventricular septal defect.</th>
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<td>c.A2107C</td>
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<td>Disease causing</td>
<td>VSD-100, De novo, Reported (11)</td>
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<td>8</td>
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<td>741729</td>
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<td>Polymorphism</td>
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Reported patients in the literature

<table>
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<th>Exon</th>
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CDH, congenital diaphragmatic hernia; DAA, double aortic arch; DORV, double outlet right ventricle; N/A, not available; PDA, patent ductus arteriosus; PH, pulmonary hypertension; POLYPHEN, polymorphism phenotyping; TOF, Tetralogy of Fallot; VSD, ventricular septal defect.

ahttp://www.mutationtaster.org/.
bRecurrence variants (characters in bold).

The POLYPHEN (polymorphism phenotyping) (15) and MUTATION TASTER (16) programs were used to predict the effects of these sequence variants on the function of the protein.

Results

In 2009, a 10-year-old boy with healthy parents and a history of CHD underwent surgery treatment for CHD. Besides cardiac features, such as DORV, he developed a mild right ventricle hypertrophy and an atrial septal defect. The patient also had typical symptoms of the Langer-Giedion syndrome and a deletion of 8q23.2 to q24.1, which includes severe development of the fetal diaphragm and multiple anomalies of the heart, including a DORV (14). The polyphen (polymorphism phenotyping) (15) and mutation taster (16) programs were used to predict the effects of these sequence variants on the function of the protein.
Novel ZFPM2/FOG2 variants

Fig. 2. Heterozygous missense variants and schematic representation of genomic organization of the human ZFPM2/FOG2 gene and functional domains of the ZFPM2/FOG2 protein. (a) Affected subjects with missense DNA sequence variants and evolutionary conservation of the mutated residue in ZFPM2/FOG2 protein. All mutated nucleotide residues are indicated by a red triangle. Mutated residues are located in the highly conserved region shown by comparison to the six species. Homo, Homo sapiens; Pan, Pan troglodytes; Felis, Felis catus; Mus, Mus musculus; Rattus, Rattus norvegicus; Canis, Canis lupus familiaris; Gallus, Gallus gallus. (b) The ZFPM2/FOG2 gene (NM_012082) with mutations found in the individuals and diagram of ZFPM2/FOG2 protein with indicated amino acid changes. The eight zinc-finger motifs are indicated by green boxes, the nuclear localization signal (NLS) is represented by a red box. V339I and M703L are located in the third and sixth zinc-finger motif respectively; K737E is located in the NLS domain; A611T and Q889E are located at the N-terminus of the fifth and seventh zinc-finger motif with unknown function.
All five patients with identified ZFPM2/FOG2 variants were examined for extracardiac anomalies and reviewed for family history. Four of the five patients were non-syndromic and had de novo mutation, whose parents did not carry any ZFPM2/FOG2 mutations. The fifth patient, No. 691152, died after surgery for complex cardiac malformations (DORV and pulmonary hypertension) with hypogenetic left ventricle and low cardiac output syndrome. His parents were non-symptomatic and refused to be tested.

Discussion

In this study, we examined the frequency of ZFPM2/FOG2 mutations in Chinese patients with sporadic DORV diseases. From 38 such individuals, we identified five heterozygous ZFPM2/FOG2 variants, while no TRPS1 and EXT1 variants were found in this cohort. Three of the five ZFPM2/FOG2 variants were not reported previously in any human diseases. The other two variants were previously reported in patients with congenital diaphragmatic hernia, a disease often associated with cardiac defect (10, 11, 17). Consistent with the role of ZFPM2/FOG2 in diaphragmatic hernia and cardiac development, haploinsufficiency of GATA4, an interacting transcription factor of ZFPM2/FOG2, can cause complex CHD and diaphragmatic hernia (18). All five variants altered evolutionarily conserved amino acids. Two programs for analyzing protein functions, POLYPHEN and MUTATION TASTER, predicted that the p.M703L and p.K737E variants are detrimental to the function of ZFPM2/FOG2 while the p.A611T and p.Q889E variants are probably benign polymorphisms (Table 1). POLYPHEN predicted that the p.V339I variant to be a benign polymorphism while the MUTATION TASTER suggested that this variant to be disease-causing. This discordance in protein function prediction might be caused by the different algorithms used by these two programs (15, 16). We also used these programs to examine sequence variants identified previously in other studies and found that different predictions were generated for three out of seven variants (Table 1). Our findings of de novo variants of ZFPM2/FOG2 in DORV patients confirmed previous studies by De Luca et al. (13) and extended the importance of ZFPM2/FOG2 in the development of complex CHD with an outflow tract defect (such as TOF and DORV).

Clinically the distinction between TOF and DORV with subaortic VSD has been controversial. Although 50% override rule is well accepted in diagnosing DORV, some researchers use a more restrictive criterion (90% overriding). In addition, other researchers propose the absence of fibrous continuity between the mitral and aortic valves as a definition of DORV (19, 20). We follow the STS-EACTS International Nomenclature classification and a 70% override rule, and classify DORV into four subtypes: VSD type, Fallot type, TGA type and non-committed VSD type (21). In our study, two patients with ZFPM2/FOG2 mutations exhibit VSD-type DORV, while three patients exhibited TOF-type DORV. This result suggests that ZFPM2/FOG2 mutations are more closely related to the TOF and VSD types of DORV and may serve as a molecular classification of these types of DORV. However, we cannot rule out the possibility that ZFPM2/FOG2 mutations are also related to other types of DORV or CHDs due to the limited number of patients of this study.

We determined the entire coding sequences of ZFPM2/FOG2, TRPS1 and EXT1 in the proband and found no sequence variants. The result implies that haploinsufficiency of ZFPM2/FOG2 could cause DORV, which is consistent with recent studies that suggest copy number variations are associated with CHD (22–24). No mutations of TRPS1 and EXT1 were identified in any of the 145 patients, suggesting that TRPS1 or EXT1 mutations are not involved in DORV. We determined the entire coding sequences of NKX2.5, GATA4, and TBX5 from the same cohort of 145 patients and identified no sequence variants. This result suggests that mutations of these genes are not commonly involved in DORV as well.

In conclusion, we mapped the chromosomal deletion in a DORV patient with multiple developmental defects and identified five (three novel) ZFPM2/FOG2 mutations in 38 patients (a 13% frequency) with sporadic DORV. These DNA sequence variants altered conserved amino acids of the ZFPM2/FOG2 protein, which may not or may have deleterious effects on the protein function based on POLYPHEN and MUTATION TASTER prediction. We did not identify ZFPM2/FOG2 sequence variants in 95 TOF and 12 TGA patients, suggesting a close association of ZFPM2/FOG2 mutations with DORV but not other types of CHD. Our study also suggests that mutations of the transcription factor genes TRPS1 and EXT1 are not commonly involved in the development of CHD. Our result, together with the finding of an association between ZFPM2/FOG2 gene and TOF/DORV by De Luca et al. (13), suggests that mutations of the ZFPM2/FOG2 gene might be a common cause of DORV.

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References

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