A Novel Nonsense MMP21 Variant Causes Dextrocardia and Congenital Heart Disease in a Han Chinese Patient

Zhuang-Zhuang Yuan1,2,3, Liang-Liang Fan2,3, Zi-Chen Jiang4, Yi-Feng Yang1 and Zhi-Ping Tan1*

1 Clinical Center for Gene Diagnosis and Therapy, Department of Cardiovascular Surgery, The Second Xiangya Hospital of Central South University, Changsha, China, 2 Department of Cell Biology, School of Life Sciences, Central South University, Changsha, China, 3 Hunan Key Laboratory of Animal Models for Human Diseases, School of Life Sciences, Central South University, Changsha, China, 4 University of California, San Diego, San Diego, CA, United States

The position and morphology of human internal organs are asymmetrically distributed along the left–right axis. Aberrant left–right patterning in the developing embryo can lead to a series of congenital laterality defects, such as dextrocardia and heterotaxy syndrome. Laterality defects are a genetic condition; however, pathogenic genetic lesions are found in only one-fifth of patients. In this study, whole-exome sequencing was conducted for 78 patients with laterality defects. We identified a novel stopgain variant in MMP21 (c.G496T; p.G166*) in a Chinese patient with mirror-image dextrocardia. This variant caused a truncated MMP21 mRNA containing only the signal peptide and propeptide, while the coding sequence of matrix metalloproteinase-21 was almost entirely absent. To the best of our knowledge, this novel variant is the first homozygous stopgain variant identified in dextrocardia patients, and the first MMP21 variant found in East Asia. Our findings expand the spectrum of MMP21 variants and provide support for the critical role of MMP21 during left–right patterning in the Han Chinese population.

Keywords: congenital heart defect (CHD), whole exome sequencing, stopgain variant, dextrocardia, MMP21

INTRODUCTION

In mammals, the proper left–right (L–R) patterning of internal organs is extremely complex and highly precise. Disordered L–R patterning can lead to a broad spectrum of laterality defects, including situs inversus totalis (SIT), a mirror image of situs solitus (SS), and heterotaxy (HTX), in which at least one organ is discordant along the left–right axis (1, 2). Dextrocardia, a rare congenital heart anomaly and HTX, is caused by the failure of normal L–R asymmetry patterning during heart development. As one of the first congenital heart malformations to be recognized, dextrocardia was mentioned in the early 17th century (3). SIT is rarely associated with congenital malformations, while HTX is highly associated with a series of congenital malformations. Most dextrocardia patients have other defects of the heart and abdominal organs (4).

Matrix metallopeptidase 21 (MMP21) encodes a member of the matrix metalloproteinase (Mmps) family that is known to hydrolyze extracellular matrix (ECM) components and is crucial for morphogenesis (5, 6). Vertebrates Mmps and their inhibitors are expressed in cardiomyocytes during the early stages of cardiac development, which are required for early heart tube assembly and
modulate cardiac morphogenesis events, such as heart tube formation, heart directional looping, and differentiation of ostial cells (5).

Using N-ethyl-N-nitrosourea (ENU)-induced mutagenesis and whole-exome sequencing (WES), Li et al. (7) and Akawi et al. (8) identified Mmp21 mutations in mice with congenital heart disease (CHD) and laterality defects, respectively (7). Additionally, MMP21 mutations in several HTX families and sporadic cases were identified by WES (7–11). These mutations and laterality phenotypes in humans and mice indicated that MMP21 plays a critical role in the L–R patterning of visceral organs.

Here, we identified a novel MMP21 variant in one Han individual from among 78 patients with dextrocardia by WES.
CASE PRESENTATION

Seventy-eight patients were recruited in this study from Second Xiangya Hospital after providing written informed consent, and family history was extensively investigated. After strict clinical and radiographic examination, all patients were diagnosed with dextrocardia with or without primary ciliary dyskinesia (PCD) and/or CHD. This study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University.

The patient with MMP21 variation, a 7-year-old boy, was born at full term and liable to catch colds but developed normally. He was diagnosed with complex CHD, mirror-image dextrocardia (Figure 1A), single-ventricle, pulmonary stenosis, and transposition of the great arteries, without PCD. According to the parents of the proband, there are no other people with cardiovascular diseases in his family. A total cavopulmonary connection operation was conducted for the patient and achieved a good outcome. Written informed consent was obtained from the legal guardians of the patient for the publication of any potentially identifiable images or data included in this article.

Genetic Analysis

Whole peripheral blood samples of the patients were obtained and stored in EDTA tubes. Genomic DNA was extracted by QiAamp DNA Blood Mini Kit (250) (Qiagen, Valencia, CA, USA).

The WES was mainly performed in the Novogene Bioinformatics Institute (Beijing, China). The exomes were captured by means of Agilent SureSelect Human All Exon V6 kits and sequenced on Illumina NovaSeq6000 (Illumina Inc, San Diego, USA). The WES data was filtered using three criteria: (1) Variations outside the coding regions (e.g., intergenic, intronic, and untranslated regions) and synonymous variants were excluded; (2) high allele frequency (>0.01%) compared to population-based databases (e.g., 1,000 Genomes Project, ESP, ExAC, and gnomAD) were excluded; and (3) prediction of a deleterious functional effect using bioinformatics programs (e.g., MutationTaster, CADD, SIFT, and Polyphen2), loss of function, and damaging variations were reserved.

Sanger sequencing was performed to validate the identified variant, and the primers were designed by PrimerQuest Tool (F: 5′-TTTCATCCGTGTCCTGCACTCTCTCCTTACCTCCTCCAAAG-3′; R: 5′-GTCGCTTTAC-3′). PCR conditions consisted of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, for a total of 35 cycles using 2 × Power Taq PCR MasterMix (BioTeke, Beijing, China). PCR products were electrophoresed on 1% agarose gels. The PCR fragment was subsequently cut, and purified fragments were sequenced on 3730XL sequencer (Applied Biosystems).

Identification of MMP21 Variation

WES was conducted and generated 12 Gb data with 99% coverage and a depth of >100×. After filtration of the WES data (Table 1), we finally identified a biallelic stopgain variant (c.G496T; p.G166∗) in MMP21 and further confirmed it by Sanger sequencing (Figure 1B). This variant caused a truncated MMP21 mRNA containing only the signal peptide and propeptide, while the coding sequence of matrix metalloproteinase-21 was almost entirely absent (Figure 1C). This variant was predicted to be “disease causing” by MutationTaster and not found in the 1,000 Genome Browser, the ExAC Browser, the Exome Variant Server, or 200 unrelated ethnically matched healthy controls. The controls were individuals presenting for routine health checkups or volunteers without similar symptoms or any positive family history of cardiovascular disorders (male/female: 100/100, mean age 36.1 ± 4.3 years). In addition, the variant had the highest CADD score of 35 suggesting that it is deleterious. Multiple alignment of mmp21 orthologs in other animal species showed that the amino acid sequence after position 166 is highly conserved. According to ACMG standards and guidelines, this variant was categorized as pathogenic (PV1, PM2, PM4, PM6, and PP3) and identified as the genetic lesion of the patient.

DISCUSSION

Our group aimed to identify laterality defect genes in a cohort of 78 patients with dextrocardia and CHD. Approximately 10% had variations in genes, i.e., NODAL, ZIC3, NKX2-5, and CCDC151. WES was conducted to identify the causative genes in these patients. A novel biallelic stopgain MMP21 variant (c.G496T; p.G166∗) was identified and predicted to be a disease-causing variant by MutationTaster and Combined Annotation Dependent Depletion (CADD). This variant was also not present in the current 1,000 Genome Browser, the ExAC Browser, or the Exome Variant Server. This variant caused a truncated MMP21 mRNA containing only a signal peptide and propeptide, while two functional domains (ZnMc superfamily; HX superfamily) were entirely absent, which may induce the decay of MMP21 mRNA in the patient according to nonsense-mediated mRNA decay theory (12, 13). MMP21 variants that caused laterality defects and complex CHD in humans are summarized in Table 2. To our knowledge, this novel variant (c.G496T; p.G166∗) was the first homozygous stopgain variant identified in dextrocardia patients and the first MMP21 variant found in mainland China, suggesting the critical role of MMP21 during left–right patterning in the Han Chinese population.

The known pathogenic genes of laterality defects account for only 15–20% of cases and are mainly related to the NODAL/TGFβs signaling pathway, SHH signaling pathway, and ciliary functions (11). Nodal cilia play a role in the origination of L–R asymmetry patterning (14–17). The observation that more than half of patients with primary ciliary dyskinesia (PCD) accompanied by SIT or HTX further reinforces the connection (2). In a prospective study involving 767 participants, Shapiro et al. (2) found that at least 12.1% of patients with classical PCD have HTX ranging from classic to subtle. In our previous studies, we reported a novel CCDC151 mutation in a patient with PCD and SIT and computed tomographic scanning of the sinus and lungs showing diffuse bronchiectasis and chronic sinusitis, respectively. His nasal nitric oxide concentrations (nNO) were far below (2 ppb) the PCD-specific nNO cutoff value (287 ppb). These results indicated that the CCDC151 mutation damaged cilia and further affected L–R patterning (18). However, we did not find PCD or other clinical phenotypes related to cilia in the
### The gene list after WES data filtration of the patient.

| CHR  | POS        | REF | ALT | GeneName | Mutation                                                                 | Genotype | OMIM                      | Database                  | MutationTaster | CADD         | ACMG                      | Significance |
|------|------------|-----|-----|----------|--------------------------------------------------------------------------|----------|---------------------------|---------------------------|----------------|-------------|---------------------------|--------------|
| 17   | 56,598,212 | AG  | A   | SEPTIN4  | NM_001198713: exon11:c.1254–10C>−                                     | Hom      | Unknown variant           | 0.99999         | D              |              | PM2,PM6                  | Uncertain    |
| 10   | 5,032,240  | G   | GGA | AKR1C2   | NM_001354: exon11:c.930–10−>TC                                          | Hom      | AR; 46XY sex reversal     | 0.99999, P –        | –              | 0.170684, 3.168 | BP4          | Uncertain    |
|      | 152,988,952| TG  | T   | BCAP31   | NM_001199457: exon1:1.c.157 +10C>−                                     | Hom      | AR; Deafness, dystonia, and cerebral hypomyelination                    | 0.99976, P –        | –              | PM2, PM6                  | Uncertain    |
|      | 135,574,521| AG  | A   | BRS3     | NM_001727: exon3:c.1188delG:p.E396fs                                  | Hom      |                          | 0.99999         | P –            | PM4          | Uncertain    |
|      | 103,267,971| C   | G   | H2BFWT   | NM_001002916: exon1:c.G26 2C:p.V88L                                   | Hom      |                          | 0.99906         | P –            | PM2, PP3                  | Uncertain    |
| 6    | 33,037,413 | C   | CA  | HLA-DPA1 | NM_001242524: exon3:c.346+5->T                                        | Hom      | Unknown variant           | 0.99999         | –              | PM2, PM6                  | Uncertain    |
| 10   | 127,462,601| C   | A   | MMP21    | NM_147191: exon2:c.G49 6Tp.Q166X                                      | Hom      | AR; Heterotaxy            | 1,D             | 9.265177, 35   | PVs1, PM2, PM4, PM6, PP3 | Pathogenic   |
| 13   | 24,798,487 | C   | T   | SPATA13  | NM_001166271: exon2:c.C142 0Tp.P474S                                   | Hom      | Unknown variant           | 0.99717, P –     | 4.289395, 24.0 | –            | Uncertain    |

CHR, Chromosome; POS, position; REF, reference sequence base; ALT, alternative base identified; Hom, homozygous; AR, autosomal recessive; P, polymorphism; D, disease causing; BP, Benign Supporting; PP, Pathogenicity Supporting; PM, Pathogenicity Moderate; PVs, Pathogenicity Very Strong. The database included 1000G, ESP and gnomAD browser.
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| Family | Cardiac anomalies | Extra-cardiac laterality defects | Mutations | Protein change | Inheritance | Reference |
|--------|-------------------|---------------------------------|-----------|----------------|-------------|-----------|
| 1      | Complex CHD, dextrocardia | Intestinal malrotation, polysplenia | c.677T>G, c.1203G>A | p.(Ile226Thr), p.(Trp401*) | Compound heterozygous | (9) |
| 2      | Complex CHD, dextrocardia | Situs ambiguus (spleen, liver) | deletion of exons 1-3, c.365delT | p.(Met122Serfs*55) | Compound heterozygous | |
| 3      | Complex CHD | Midline liver, intestinal malrotation, polysplenia | c.1A>G | p.(Met1?) | Homozygous | |
| 4      | Complex CHD | Left pulmonary isomerism, left sided liver, right-sided stomach, polysplenia | c.91C>T, c.643G>A | p.(Arg31Trp), p.(Glu215Lys) | Compound heterozygous | |
| 5      | CHD, dextrocardia | Situs ambiguus (thoracic and abdominal) | c.308_309delAG | p.(Glu103Alafs*154) | Homozygous | |
| 6      | Complex CHD | Situs ambiguus (thoracic) | c.961G>C | p.(Ala321Pro) | Homozygous | |
| 7      | Complex CHD | Situs ambiguus (thoracic and abdominal) | c.1078C>T | p.(Arg380Cys) | Homozygous | |
| 8      | Complex CHD | Situs ambiguus (abdominal) or Situs inversus totalis | c.1124G>A | p.(Arg375His) | Homozygous | |
| 9      | CHD, dextrocardia | None | c.1222C>G, c.1585_1588dup | p.(Arg408Gly), p.(Val303Glyfs*3) | Compound heterozygous | |
| 10     | Complex CHD | Thoracic situs inversus | c.101C>T, c.1372C>T | p.(Ser34Leu), p.(Arg458*) | Compound heterozygous | |
| 11     | Complex CHD, dextrocardia | None | c.163C>T, c.1372C>T | p.(Arg55Trp), p.(Arg458*) | Compound heterozygous | |
| 12     | Complex CHD, dextrocardia | None | c.1024_1025delAA | p.(Lys342Argfs*13) | Homozygous | (10) |
| 13     | Complex CHD, dextrocardia | Right-sided stomach | c.847C>T, c.947G>A | p.(His283Tyr), p.(Trp316*) | Compound heterozygous | |
| 14     | Complex CHD, dextrocardia | None | c.1380_1381delGA, c.854T>C | p.(Lys461Valfs*14), p.(Ile285Thr) | Compound heterozygous | |
| 15     | Complex CHD, dextrocardia | Situs anomaly | c.557G>T | p.(Ser186Ile) | Homozygous | (11) |
| 16     | Complex CHD, dextrocardia | Situs anomaly | c.643G>A | p.(Glu215Lys) | Homozygous | |
| 17     | Complex CHD, dextrocardia | None | c.496G>T | p.(Gly166*) | Homozygous | Present study |

**MMP21** variant patient, suggesting that **MMP21** was unrelated to cilia and involved in L–R patterning in another way. In addition, some known variants of **NODAL**, **ZIC3**, and **NKX2-5** were identified in this cohort.

Knockdown or genome editing of the **MMP21** ortholog in zebrafish resulted in heart-looping defects in a dose-dependent manner (9, 10). Mutant zebrafish embryos also showed concomitant disruption of the laterality marker southpaw in the lateral plate mesoderm and disrupted notch signaling in vitro and in vivo, suggesting that the heart-looping defect is related to abnormal L–R patterning and that **MMP21** is a negative regulator of notch signaling (10). Consistent with the findings from zebrafish, mutant mouse embryos generated by Mmp21 genome editing and N-ethyl-N-nitrosourea (ENU)-induced mice that have a homozygous missense mutation in Mmp21 both exhibit CHD and laterality defects (7). The findings suggested that **MMP21** plays an important role in the establishment of asymmetric organ development.

The Drosophila genome encodes two Mmps, Mmp1 and Mmp2. Experimental results from Drosophila demonstrated that Mmps play essential roles in promoting ECM remodeling, cell polarization, and lumen formation during Drosophila cardiogenesis. In humans, up to 25 Mmps have been identified with overlapping functions, and only **MMP21** has been confirmed to be involved in L–R patterning (5, 19). It is very significant to identify the deleterious variants affecting L–R patterning in other members of the MMP family in both human and animal models.

In conclusion, our finding expands the spectrum of **MMP21** variants and provides extra support that **MMP21** play important roles in L–R patterning.

**DATA AVAILABILITY STATEMENT**

The datasets generated for this study can be found in NCBI SRA, NCBI Accession No. PRJNA668249.
ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Second Xiangya Hospital of the Central South University. A signed written informed consent was obtained from the legal guardians of the patient for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Z-PT designed the overall study and performed data analysis. Z-ZY and L-LF processed the WES data, validated the mutation, and Equipments of Central South University, Grant/Award Number: CSUZC201940.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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