

Title	Clinical Outcomes and Genetic Analyses of Restrictive Cardiomyopathy in Children
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Supplemental material I

Supplemental Methods

Study Design and Data Collection

We retrospectively analyzed clinical records of RCM patients under 15 years old diagnosed between 1998 and 2021. Patients were followed-up at the Osaka University Hospital, Osaka, Japan. Clinical data included age at diagnosis, sex, symptoms, reasons to reach the final diagnosis of RCM, arrhythmias, genetic testing before referring to our hospital, echocardiographic data, catheterization data, use of ventricular assist devices, heart transplantation, and survival. RCM diagnosis was based on echocardiography and catheterization data showing dilated atria with preserved ventricular ejection fraction, elevated ventricular end diastolic pressures, and a “dip and plateau” pressure pattern of the left ventricle.

Whole Exome Sequencing

Genomic DNA was extracted from peripheral blood samples using DNeasy Mini Kit (Qiagen, Hilden, Germany). WES was conducted as previously described.²¹ Briefly, we prepared DNA libraries using SureSelect Human All Exon V6 kits (Agilent Technologies, Santa Clara, CA, USA) and sequenced the libraries using HiSeq 3000 systems (Illumina, San Diego, CA, USA). Candidate variants were present on the 257 genes associated with cardiomyopathy, and those with a minor allele frequency < 0.5% were extracted. To assess the potential functional impacts of variants, four bioinformatics algorithms were used: HGMD, Intervar, CADD, and Protein variation effect analyzer (Provean). Missense

variants were considered “pathogenic” if classified simultaneously as “DM” by HGMD and “Pathogenic” or “Likely pathogenic” by Intervar, CADD > 25, PROVEAN < -2.5, and previously reported as causing RCM, HCM, or noncompaction cardiomyopathy.

Statistical Analyses

Continuous clinical variables are presented as medians [interquartile range (IQR)]. Kaplan–Meier survival curves with log-rank tests were utilized to analyze overall survival and transplant-free survival based on the diagnosis of RCM. Unpaired *t*-tests were used to compare two groups when the normal distribution was confirmed by Shapiro–Wilk tests. Otherwise, Mann–Whitney U tests were used to compare groups. Fisher’s exact tests were conducted to compare groups. *P* values < 0.05 were considered statistically significant. All statistical analyses were performed using JMP pro 14 software.

Supplemental material II

Clinical summary of the RCM patient who had *TNNI3* (I195T) missense variant

The female patient who had *TNNI3* (I195T) missense variant was diagnosed by school heart screening at 6 years old without any symptom. After the final diagnosis of RCM, she had low output syndrome, and then she had heart transplantation at 7 years old without VAD implantation. She had no family history of cardiomyopathy or VT/Vf event. LVEDP and RVEDP were 23 mmHg and 13 mmHg at diagnosis, respectively. PVRI was 1.6 Wood Unit·m² and CI was 2.8 L/min/m². LVEF by echocardiography was 56%. We believe the clinical features of this patient was similar to the pathogenic variant positive group, however I195T was not previously reported as a pathogenic or likely-pathogenic variant. Therefore, we could not validate the pathogenicity of this variant, and we excluded this case from further analysis.