（第３号様式）

学　位　論　文　要　旨

氏　　名　　文明

論　文　名　　円錐動脈幹部心奇形発生における DiGeorge 責任領域近位端に位置する *DGCR6* の関与

学位論文要旨

< Background>

DiGeorge syndrome (DGS) is one of the most common microdeldetion syndromes, with a frequency of around 1 in 4000 to 6000 live births. It is considered to be caused by dysfunction of the neural crest cells and anterior heart field during the fetal development, resulting in several symptoms including cardiac defects. Cardiac defects observed in DGS include tetralogyof Fallot (TOF), pulmonary atresia (PA), truncus arteriosus, interrupted aortic arch, type B (IAA-B), and ventricular septal defects (VSD), refered to as conotruncal heart defects.

Approximately 90% of patients with DGS have a hemizygous 3-Mb deletion at the 22q11.2 region, so called DiGeorge critical region. A hemizygous 1.5-Mb deletion was observed in 7% of the patients and the remaining 3% show less common genomic alterations such as the deletion of a shorter region. Located within the commonly deleted region on chromosome 22q11.2, haploinsufficiency of two genes, *TBX1* and *CRKL*, have been identified as causing the dysfunction of the neural crest cells in mouse model during the morphogenesis of affected regions in the syndrome.

In many cases showing only the conotruncal heart defect, deletion of 22q11.2 region cannot be detected by fluorescent in situ hybridization (FISH). We aimed to assess the possibility that gene(s) other than *TBX1* and *CRKL* could be responsible for this characteristic cardiac anomaly.

< Material and methods >

Six patients with conotruncal cardiovascular phenotype in whom 22q11.2 deletion was not detected by standard FISH analysis were included in this study. Written informed consent was obtained from the parents, and all research was approved by the institutional review board. Abnormalities in *TBX1* gene were analyzed with direct sequencing analysis of all exons, copy number assays with quantitative PCR (TaqMan copy number assays covering exon 2, 7, 12) and MLPA analysis (Congenital Heart Disease premix which include three genes, *CDC45L*, *GP1BB* and *DGCR8*, located within DiGeorge critical region). In addition, detailed copy number analysis was performed using a genome-wide high-resolution Affymetrix

氏名　文明

Cytoscan HD array containing >2.6 million copy number makers. Finally quantitative PCR was performed to confirm the results obtained by array analysis and determine detailed proximal breakpoints.

< Result >

Among the genes within the DiGeorge critical region, *TBX1* is the most probable candidate for causing the phenotype observed in DGS. In all the six patients enrolled in this study, no mutation was detected in the coding region of the *TBX1* gene. However, when copy number Copy number alteration was analyzed by high-resolution array analysis, a small deletion or duplication in the proximal end of DiGeorge critical region was detected in two patients. One patient showed a monoallelic deletion spanning approximately 108.0-375.6Kb, and the other showed duplication spanning 87.9-375.6 Kb around this region. Detailed copy number analysis by quantitative real-time PCR suggested that this small deletion/duplication region involves 4 genes at the proximal end of the DiGeorge critical region: *DGCR6, PRODH, DGCR5*, and *DGCR9*.

< Discussion >

Two out of six cases with conotruncal heart defect had atypical small deletion/duplication at the proximal end of the DiGeorge critical region containing only 4 genes. Among these genes, *DGCR5* and *DGCR9* are non-coding RNA with unknown significance, whereas the other two genes *DGCR6* and *PRODH*, encode proteins with several functions. *PRODH* encodes for proline dehydrogenase, a mitochondrial rate-limiting enzyme in the proline degradation process. Although multiple studies indicate that abnormalities of *PRODH* are associated with the risk of psychiatric disorders, no positive relationship between congenital heart defects and aberrant *PRODH* gene copy number was reported prior to this study. The *DGCR6* protein is a nuclear phosphoprotein highly expressed in heart, liver, and skeletal muscle. In a chicken model, *DGCR6* suppression in neural crest cells by retrovirus-mediated antisense transduction resulted in a high incidence of severe cardiovascular anomalies reminiscent of those found in DGS, possibly through the modulation of the expression level of *TBX1*.

Other several reported cases having small deletion/duplication in the proximal part of the DiGeorge critical region show heart defect that is observed in DGS. Our result and the others suggest that altered dosage of gene(s) other than *TBX1*, possibly *DGCR6*, may also be responsible for the development of conotruncal heart defects that are observed in patients with DGS and, in particular, in those with stand-alone conotruncal heart defects.

<Conclusion>

The findings of our study further support the pathological role of *DGCR6* in conotruncal heart defects. Although one presented deletion while the other showed duplication, *DGCR6* may be responsible in the pathogenesis of conotruncal heart defects in these patients by some common mechanism. Further analysis of more cases and a possible functional relationship between *DGCR6* and *TBX1* during cardiogenesis is necessary to clarify the role of *DGCR6* in conotruncal heart defects.

キーワード（３～５）	22q11.2 deletion, Array CGH, *DGCR6*, *TBX1*, Conotruncal heart defects