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# 学 位 論 文 の 要 旨
## (Summary of the Dissertation)

論文題目　Phenotype-genotype relationships in *Xenopus sox9* crispants provide insights into campomelic dysplasia and vertebrate jaw evolution
(ツメガエルの *sox9* 変異体群を用いたヒト屈曲肢異形成症と脊椎動物の顎進化の研究)

広島大学大学院統合生命科学研究科
生命医科学　プログラム
学生番号　D206250
氏　　名　Nusrat Hossain

## (Summary of the Dissertation)

Animal models have facilitated remarkable advancements in unraveling disease pathogenesis which would have been impossible to accomplish in humans considering the ethical prohibitions. In recent years, knocking out a gene with CRISPR/Cas9 has become a valuable way to investigate a variety of gene functions by directly introducing mutations into the targeted gene of a selected model organism. Therefore, CRISPR/Cas9 alongside an ideal animal model helps to create innovative paths in the drug discovery pipeline and study the function of candidate genes related to human diseases. In vertebrates, crispants (F0 generation mutants) have been generated using various animal models, including mice, zebrafish, and *Xenopus,* by introducing CRISPR reagents into fertilized eggs. The resulting mutations generally show mosaicism due to random editing of the target loci in each cell at different stages of embryonic development. Therefore, researchers must breed F1 or F2 generations to obtain uniform germline transmission and homozygous mutant animals. However, such mosaicisms are advantageous in certain cases. These include rapid functional analysis of the genes whose non-mosaic mutations result in early developmental lethality; somatic mosaicism enables the crispants to survive beyond the lethal phase and enables to examine the null phenotype in a specific group of cells. Along with other model animals, *Xenopus* has

recently emerged as a powerful time- and cost-effective disease model in which CRISPR/Cas9 system works quite efficiently. In general, *X. tropicalis* is considered a comparatively better predictive animal model as it has a diploid genome compared to the duplicated pseudotetraploid genome of *X. laevis*. Especially, the high synteny between the *X. tropicalis* and the human genome is a favorable advantage in F0 reverse genetics, as exploring the phenotypes in genetically modified embryos can be done without requiring time-consuming crosses. The recent advancement of CRISPR/Cas9 technology helped the *Xenopus* community to emphasize its essential role in observing genetic manipulations through disease modeling, such as microphthalmia, retinitis pigmentosa, kidney defect, limb malformations, ciliary dyskinesia, etc. Although most previous studies have successfully modeled various non-syndromic diseases, it remains unclear whether *Xenopus* is useful for modeling syndrome-type diseases. Thus, it remains elusive whether *X. tropicalis* crispants are indeed useful in modeling such pleiotropic defects and in elucidating phenotype-genotype relationships responsible for the variable appearance of multiple defects in human patients with syndromic diseases.

To address those questions, I have chosen the *SOX9* gene as an entry point, whose coding mutation can cause campomelic dysplasia syndrome (CD), a syndromic disease characterized by micrognathia, laryngomalacia, cleft palate, limb deformity, male-to-female sex reversal, hearing impairment, cardiac, renal, lung, and pancreas defects. Although multiple defects in CD have been independently characterized using different sets of mutant mice but due to the prenatal death of the heterozygous *Sox9* mutant mice the knowledge of phenotype-genotype relationships that explain the phenotypic variations in CD is still limited. In my study, I successfully induced partial syndromic pleiotropic phenotypes mimicking CD in *Xenopus tropicalis*, targeting a newly identified jawed vertebrate-specific amino-terminal domain (JAD) using the CRISPR/Cas9 mediated gene knockout technique. I used Inference of CRISPR Edits (ICE) software from Synthego (https://ice.synthego.com/#/) to visualize nucleotide sequences of the composite alleles, and to calculate the rate of each allele and the total indel rate for each crispant. This study is first of our knowledge to examine *sox9* knockout strategies in *X. tropicalis* and we validated that CRISPR knockout technique can be use to establish F0 *X. tropicalis* embryos while avoiding early embryonic lethality associated with altered *sox9* gene expression. Interestingly, genotyping of the crispants with a variety of

allelic series of mutations suggested that the heart and gut defects depend primarily on frame-shift mutations expected to be null, whereas the jaw, gill, and ear defects could be induced not only by such mutations but also by in-frame deletion mutations expected to delete the jawed vertebrate-specific domain from the encoded Sox9 protein. Remarkably, the jaw deficient phenotype caused by in-frame deletions of the region encoding the JAD of *Sox9*, not only recapitulated the micrognathia in human CD patients but is also reminiscent of a jawless vertebrate, lamprey, suggesting a crucial role for JAD in the evolution of jawed vertebrates. The combination of various tissue anomalies in *sox9* crispants also generated the possibility of studying a part of the mechanisms that produce the combinatorial patterns of CD-related defects, without reproducing heterozygous mutations close to those of the human patients. Furthermore, the jaw dysplasia phenotype of the *sox9* crispants, which appeared to be caused by the partial truncation of JAD, highlights the use of crispants for investigating tissue-specific roles for each functional domain included in a single protein, and provides a novel insight into the jaw evolution. I believe that the results of this project will provide valuable insights regarding the effectiveness of *Xenopus* as a disease model and pave the way for a deeper understanding regarding phenotype-genotype relationships in the phenotypic variations of CD patients.