DATE: 10____May____2024

SUMMARY of
FY2023 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Determining the protein structure of TBX1 variants by ultra-high magnetic field NMR spectroscopy
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Summary

Hemizygous deletion at human chromosome 22q11.2 is associated with elevated rates of schizophrenia and diverse neurodevelopmental disorders, including ASD, intellectual disability, and attention-deficit hyperactivity disorder. Ultra-rare variants of some 22q11.2 CNV have been identified in samples of ASD, schizophrenia, and neurodevelopmental disorders. In particular, a few cases of ultra-rare TBX1 varaints (e.g., frameshift deletions) are associated with ASD and schizophrenia. Genetic mouse models for dose alterations of small segments and single genes within 22q11.2 CNV have pinpointed some segments and driver genes for distinct dimensional aspects of mental illnesses. Dose alterations of a ~200kb segment, including Tbx1, impacts social and sensorimotor gating dimensions in mice. Constitutive heterozygosity of Tbx1 in a homogeneous genetic background impairs social communication and interaction in mice. To further examine the precise impact of Tbx1 variants, we have recently developed a mouse model of Tbx1 frameshift deletion. This variant is located in an exon that encodes a protein sequence needed for the translocation of this transcription factor into the cell nucleus. Given its functional relevance to many aspects of neurodevelopmental disorders, it is imperative to achieve a better understanding of the structural basis of TBX1 variants on the function of a transcription factor. However, the 3D structure of the TBX1 protein, as well as how this structure is altered by protein-modifying variants, remains unknown. Additionally, details about the interacting factors of TBX1 are not yet understood.

In this collaborative research, we aim to elucidate the function of TBX1 by analyzing its 3D structure and interaction modes using solution NMR methid. So far, we have successfully prepared a stable isotope-labeled human TBX1 T-box domain and measured its NMR spectrum. The molecular weight of the T-box domain is approximately 20 kDa, but by preparing the ^{2}H,^{15}N-labeled T-box and utilizing a 950 MHz ultra-high-field NMR, we have successfully observed nearly all amide signals on the ^{1}H-^{15}N TROSY HSQC spectrum. Additionally, the spectral pattern confirmed that the T-box retains its 3D structure. Furthermore, titration experiments with the target DNA successfully captured changes in chemical shifts and signal sensitivities of specific amide signals. This indicates that the T-box domain is involved in interactions with the target DNA.

***Deadline: May 10, 2024**
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