論文の内容の要旨

論文題目 Behavioral function of Hesr1 and Hesr2 through dopamine transporter expression.
（ドーパミントランスポーター発現制御を介したHesr1およびHesr2の行動への影響）

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The dopamine system is involved in motor function, mood, motivation, reward and so on. Synaptic dopamine level is tuned by dopamine transporter (DAT) which is one of the main targets of several psychoactive drugs. A functional genetic polymorphism has been described in the 3'-untranslated region (UTR) of exon 15 in DAT (Figure). This 3'-UTR contains a 40-bp long variable number of tandem repeats (VNTR) domain. The polymorphism within this region is known to be associated with neuropsychiatric disorders such as ADHD, PD, alcoholism and drug abuse, and with modified gene expression depending on the genotype *in vivo* and in mammalian cell lines. In my Master thesis and our previous study, we demonstrated that HESR1 and HESR2 reduced the DAT reporter gene expression.

In this study, I tried to clarify following two topics, (1) Discovery of the behavioral function of the Hesr1 and Hesr2, (2) Molecular characterization of HESR1 and HESR2 focusing on its *cis*-element and co-factor. For (1), first, I investigated localization of the Hesrs in mice brain and next, screened altered behavior in the knockout (KO) mice comparing with the wild type mice using series of behavioral test battery. For (2), I conducted luciferase reporter assay with DAT reporter gene and its deletion constructs to identify HESR-responsive elements in the neuroblastoma. At the same time, I tried to detect HESRs-androgen receptor (AR) interaction affecting DAT gene expression because HESR1 was reported to be a co-repressor for the AR in a study of prostate cancer. In addition, sexual dimorphisms in dopamine-related function and expression of the DAT are reported.

As a result, I found the following: (1) Localization of Hesr1 and Hesr2 in dopaminergic neurons was detected in mice midbrain by immunohistochemistry and enhanced prepulse inhibition (PPI) was observed in the Hesr1 KO mice. In the presence of prepulse, it is widely known that startle response to strong sensory stimulus is inhibited comparing with the case without prepulse. In other words, prepulse makes organisms less startle. It is also reported that

schizophrenia patients exhibit lower PPI, suggesting that they have deficits in an operational measure of sensorimotor gating. This lower PPI in the patients can be rescued by dopamine receptor antagonists. So, I conducted PPI test again after injection of dopamine agonist to the mice. Then, the injection of agonist reduced the enhanced PPI in the KO mice and the dose dependent sensitivity to the agonist differed between wild type and KO mice. We also determined the DAT expression level of the KO mice by Western blotting after behavioral tests. There was no difference in expression level between the wild type and KO mice though the mRNA of DAT in the KO mice postnatal day 0 was up-regulated in our previous study. These things suggest that Hesr1 modulates DAT expression in developmental stage and it affects sensitivity to dopamine and sensorimotor gating in adults. (2) The deletion of the VNTR domain increased reporter gene expression, suggesting that the VNTR has an inhibitory role for DAT expression and is responsive to HESRs. The activities of luciferase reporter with 10 repeats allele which is most common in population was decreased in the presence of the HESR1 or HESR2 expressed transiently while slightly increased in the presence of the AR. Furthermore, when the HESR1 or 2 and AR were co-expressed, the inhibitory effect of HESRs became larger. Some functional modification is thought to occur when the HESRs and AR are coupled.

Thus, My data suggest suggests that expression of Hesr1 could have influence to the dopaminergic system including sensorimotor gating in physiological level. However, knockout methods are useful but sometimes give rise to artificial condition. We should search for the events where expression or function of HESRs can be modified depending on some behavioral context such as hormonal change. Relation of the VNTR of DAT and HESRs need to be investigated by context dependent manner.

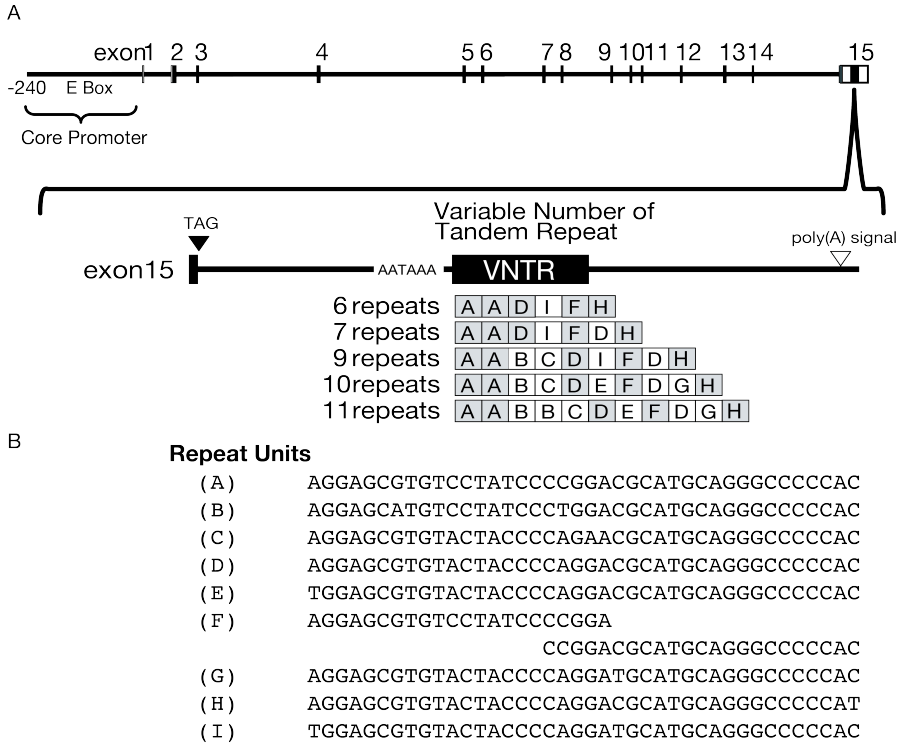

Figure. Structure of 3'-UTR in *DAT1* gene. (A) The genomic structure of human dopamine transporter (*DAT1*) gene. The coding region(closed box), non-coding region (open box), VNTR domain, and constant parts of the repeat units (gray box) are shown. Exon 15 of DAT1 contains a stop codon (arrowhead) and polyadenylation signal (open arrowhead). Upstream of the VNTR domain are six nucleotides (AATAAA) that resemble a polyadenylation signal. The allelic variants of the VNTR indicate the repeat unit type (A)-(I) for each allele. (B) Nucleotide sequence of each unit of the VNTR polymorphism in the 3'-UTR of *DAT1*.