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学 位 論 文 題 目　Effect of *Esr1* gene polymorphisms on parental behavior in female mice

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(Form 3)

Summary of Doctoral Thesis

Name in full- Mallarapu, Lalithadevi

Title- Effect of *Esr1* gene polymorphisms on parental behavior in female mice

Parental care is critical for offspring's survival. Across mammalian species, females exhibit extremely diverse parental care (maternal behaviors), generating questions about how animals recognize their offspring and how brain circuits drive and control maternal display of affection. Estrogen plays an essential role in onset of parental behavior in females and exerts its actions through receptors. Estrogen receptor 1 (ESR1) is one of the estrogen receptors (ERs) which is encoded by *Esr1* gene. The expression of ESR1 in hypothalamic medial preoptic area (MPOA) determines the female parental behavior. ESR1-KO female mice showed reduced estrogen sensitivity and impairment in parental behavior, and optogenetic inactivation of MPOA-ESR1 expressing cells impaired pup approach and retrieval behavior in female. Previous studies implicated that *Esr1* gene polymorphisms results in several behavioral changes like aggression, episodic memory, anxiety, depression, obsessive-compulsive disorder and harsh maternal parenting in humans. Even though, mice are widely used model species for studying molecular and neural mechanism underlying parental behavior, none of the studies were focused on effect of polymorphisms. In this study, I addressed how polymorphisms in *Esr1* gene can affect parental behavior in female mice.

In the present study, *Esr1* gene sequence of 18 mice strains (downloaded from Mouse Genome Informatics database) was compared to check whether there is any variation in the coding region. Initially, using multiple sequence alignment tool, I identified a difference in length of GCC repeat region of exon 1 of *Esr1* gene between wild and laboratory strains. This GCC repeat region was considered for further identification of polymorphisms. In the next stage

of my study, I have chosen one of the wild derived mice strains MSM/Ms (MSM; found in Mishima, Japan) and commonly used laboratory strain C57BL/6J (B6) for checking the variation in *Esr1* GCC repeat region. These mice strains are already reported to have a difference in their behavior, where MSM shows higher aggression and social behavior when compared to B6. Specific primers were designed and coding exon 1 sequence of *Esr1* gene of MSM was compared with B6 by performing the sequence analysis. A GCC repeat length polymorphism (GCC; codes for alanine; AAA) which results in difference of polyalanine repeat numbers was identified in exonic region of *Esr1* gene in MSM when compared to B6.

To analyze the role of this "GCC repeat length", we developed an $Esr1^{\Delta 9/\Delta 9}$ mice in which the "9bp" sequence was deleted in B6 mice through CRISPR/Cas9-mediated genome editing. Interestingly, females with heterozygous deletion of "9bp" ($Esr1^{\Delta 9/+}$) showed more severe phenotype than homozygous deletion ($Esr1^{\Delta 9/\Delta 9}$). The low pup (offspring) survival and abnormal maternal behavior (reluctant to retrieve pups) was observed in $Esr1^{\Delta 9/+}$ females. Other pup-directed behaviors like nursing were not significantly changed between the genotypes. However, the other measurements like crouching over and pup licking which involves active females' involvement with pups was observed to be lower in $Esr1^{\Delta 9/+}$ females. Immuno-histochemical analysis of MPOA region, showed lower number of ESR1 positive cells in $Esr1^{\Delta 9/+}$ females when compared with $Esr1^{+/+}$ and $Esr1^{\Delta 9/\Delta 9}$ females. As MPOA ESR1 positive cells are the key mediators of pup approach and retrieval, abnormal maternal behavior of $Esr1^{\Delta 9/+}$ females can be correlated to lower number of MPOA ESR1 positive cells. Upon pup exposure to females, the cFOS, a marker of neural activity, was less expressed in MPOA of $Esr1^{\Delta 9/+}$ females than the $Esr1^{+/+}$ and $Esr1^{\Delta 9/\Delta 9}$ females. As $Esr1^{\Delta 9/+}$ females showed less interaction with pups during the

pup exposure, cFOS expression was not increased in these females. Next, I also checked the double-positive neurons with ESR1 and cFOS. I observed that the percentage of double-positive neurons (ESR1 and cFOS) was lower in *Esr1*$^{\Delta 9/+}$ females. As lower ESR1 positive neurons were present in these females, the excitation also lower compared to control group. *Esr1* $^{\Delta 9/+}$ females showed higher *Esr1* mRNA expression and lower protein levels when compared to wild type control. These results suggest that heterozygous deletion of 9bp in B6 genetic background results in reduced pup retrieval in females. Altogether, this is the first study to reports how *Esr1* genetic polymorphism affects maternal behavior in mice.

Form8 : Separate Sheet
（様式８・別紙１）

Results of the doctoral thesis defense

博士論文審査結果

Name in Full
氏 名　Mallarapu, Lalithadevi

Title
論文題目　Effect of *Esr1* gene polymorphisms on parental behavior in female mice

Estrogen シグナルは動物の生殖行動や社会性行動に重要である。Mallarapu さんは、野生由来マウス系統 MSM と実験用マウス系統 C57BL/6 (B6)との間で、Estrogen 受容体 α 遺伝子（*Esr1*）に９bp の挿入欠失多型が存在することを見つけた。この遺伝子多型の結果、B6 の Estrogen 受容体 αN 末の AF1 ドメインには、MSM やヒトには含まれない３アミノ酸（AAA）の挿入が存在することになる。

Mallarapu さんは、CRISPR/Cas9 法を用いて、B6 の *Esr1* 遺伝子から９bp を欠失させて MSM 型にした変異アレル $Esr1^{\Delta 9bp}$ を作成した。この変異をヘテロ接合で持つ $Esr1^{\Delta 9bp/+}$母マウスは、野生型やホモ接合型 $Esr1^{\Delta 9bp/\Delta 9bp}$ 母マウスと比較して、保育する仔マウスの生存率が低いことがわかった。そこで未交尾雌マウスを用いて母性行動テストを行い、*Esr1* 遺伝子型の母性行動への影響を調べた。その結果、$Esr1^{\Delta 9bp/+}$マウスは新生仔回収などの母性行動の出現が弱いことを確認した。脳の視床下部 MPOA 領域で発現する ESR1 が、母マウスの母性行動に関与することが報告されている。Mallarapu さんは、免疫組織化学染色により、$Esr1^{\Delta 9bp/+}$マウスでは、野生型やホモ接合型 $Esr1^{\Delta 9bp/\Delta 9bp}$ マウスに比べて、MPOA 領域の ESR1-陽性細胞が少ないことを見つけた。さらに刺激に応答して発現する最初期遺伝子 cFOS の発現を指標にすることで、$Esr1^{\Delta 9bp/+}$マウスではこれらの ESR1-陽性細胞の神経活動が低いことを示した。さらに、ウエスタンブロットによる定量を行い、$Esr1^{\Delta 9bp/+}$マウスの視床下部では、９bp の多型領域を含む 65kD の ESR1 アイソフォームの量が減少していることを示した。一方、９bp の多型領域を含まない 46kD の ESR１アイソフォームの量には遺伝子型による違いは認められなかった。

以上の結果は、単一遺伝子における多型が動物の行動に影響する可能性を示しており興味深い。ヘテロ接合時にタンパク質の量が減少する例は珍しく、今後新規機構の解明のためのモデルケースとなることが期待できる。確実な結果を基に慎重な議論を行う学位論文は、学位基準を満たすと委員会で判断した。