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学位（専攻分野）	博士（理学）
学位記番号	総研大甲第959号
学位授与の日付	平成１８年３月２４日
学位授与の要件	生命科学研究科　遺伝学専攻 学位規則第６条第１項該当
学位論文題目	A systematic analysis of genetic factors associated with behavioral traits by use of consomic mouse strains established from C57BL/6J and MSM/Ms
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論文内容の要旨

In our laboratory, we have focused on the behavioral diversity of wild derived mouse strains. A wild derived mouse strain, MSM indicates prominent behavioral differences from C57BL/6J. These two strains belong to different subspecies. MSM and C57BL/6J belong to musculus subspecies group and domesticus subspecies group, respectively. There is a large amount of nucleotide differences between the two strains as it nearly reaches 1%. MSM is one of the most remote strains from C57BL/6J among inbred strains of mouse. Therefore, it is expected to obtain the new knowledge for behavioral mechanisms by comparing the two strains. To study the genetic factors associated with the behavioral diversity between the two strains, we used the consomic strains, otherwise known as chromosome substituted strains (CSS), derived from these two strains. The consomic strains were established by substitution of each one chromosome of C57BL/6J by the corresponding chromosome of MSM. These strains are described as B6-[chromosome number]MSM. When only the centromere side or telomere side of a chromosome derived from MSM is kept separately as two consomic strains, they are described as B6-[chromosome number]CMSM or B6-[chromosome number]TMSM, respectively. By using a series of consomic strains covering the whole genome, we are able to detect the quantitative trait loci (QTL) on each chromosome efficiently. Furthermore, consomic strains reduce the time and cost in establishing congenic strains that include the candidate QTL.

In this study, I focused on two behavioral traits, general activity and emotionality. These two traits are the most basic behavioral traits across the animal species and important for animal to survive. It seemed that the basic mechanisms for both general activity and emotionality have been conserved in mammals. In human society, these traits deeply associate with personal quality of life (QOL). Abnormalities of these traits cause several serious psychiatric disorders. Because methods of studies for psychiatric disorders in human have limitation, approaches using rodent models have been applied for elucidating mechanisms for behavioral traits and psychiatric disorders. One of the most common approaches is analysis of knockout mouse, which has aberration on the gene associated with the focused mechanism (reverse genetics approach). By using this approach, roles of

genes on the pathway related to the focused mechanism have been revealed and important knowledge for genetic factors associated with behavioral pathway has been accumulated. However, the mechanisms of the behavioral traits are complex and that many genetic and environmental factors are associated with the traits. Therefore, forward genetic approach, which focuses on the phenotype first and followed by analysis of the gene associated with the phenotype, has also been needed for understanding the genetic mechanism and genetic factors associated with behavioral traits.

To evaluate the behavioral traits of each consomic strain cyclopaedically, first, we established the behavioral test battery. In this study, I performed the novel cage test and the light dark box test to evaluate the emotionality and the home cage test to evaluate general activity for eighteen consomic strains, which cover about two-third of the entire chromosomes. In the result of behavioral tests, many strains showed behavioral differences from C57BL/6J. Interestingly, most of the consomic strains have own distinctive behavioral traits. When I sorted the behavioral traits of each consomic strains into categories of emotionality and general activity; for example, B6-7TMSM, B6-8MSM and B6-16MSM differed from C57BL/6J for general activity but not for emotionality. On the other hand, B6-1MSM, B6-2CMSM and B6-17MSM mainly increased anxiety related behavior than C57BL/6J. B6-6CMSM showed significantly great behavioral differences from C57BL/6J for both general activity and emotionality related behavior. In total, seventeen out of eighteen tested strains showed some sort of behavioral differences from C57BL/6J. Through these behavioral tests, I successfully characterized complex behavioral traits by use of each consomic strain.

Then, I characterized the behavioral phenotype of B6-6CMSM using multiple behavioral tests because this strain showed the biggest behavioral deviation from C57BL/6J among the tested consomic strains. I evaluated the motor function, the emotionality related behavior and psychiatric disorders like phenotype using such as a rotarod test, a cued fear conditioning test and prepulse inhibition (PPI) test. In the result of these tests, B6-6CMSM did not have severe deficit for motor function in the rotarod test with constant slow speed while this strain showed the lower performance

than C57BL/6J in the rotarod test with accelerated speed. B6-6CMSM had increased fear response in comparison with C57BL/6J in the fear conditioning test. There is no significant difference for psychiatric disorders like phenotype in the tail suspension test and the PPI test. Additionally, B6-6CMSM had higher dopamine level in striatum, which relates with behavioral activity, than C57BL/6J. On the other hand, the behavioral changes following injection of the dopamine receptor agonist and dopamine transporter blocker were not different from those observed in C57BL/6J.

In order to reveal the genetic factor associated with the behavioral phenotypes, I analyzed the QTLs on chromosome 6. I especially focused on genetic factors associated with the general activity and emotionality. In the result of QTL analysis for the novel cage activity, activity in the light dark box and the home cage activity, I obtained mainly two QTLs. One QTL located in the centromere side (QTL-C) associated with the novel cage activity and activity in the light dark box. Furthermore, the QTL-C was also associated with general activity for females but not for males. The other QTL located in telomere side (QTL-T) was associated with only the home cage activity for both females and males. Therefore, it is suggested that different genes regulate emotionality and general activity of B6-6CMSM. The QTL-C mainly associates with emotionality of both sexes, and sex specific general activity. The QTL-T regulates the general activity of both sexes. There are complex mechanisms associated with behavioral traits on chromosome 6. Therefore, I established a series of congenic strains for QTL by introducing shorter segment of chromosome 6 via meiotic recombination to narrow down the candidate regions.

In this study, I obtained novel information for QTLs associated with multiple behavioral traits. I indicated the candidate regions associated with emotionality related traits and general activity of B6-6CMSM, and I was able to narrow down the candidate region associated with behavioral phenotype. I am expecting to understand the gene associated with behavioral traits by combing this QTL information, accumulated data for single nucleotide polymorphism between C57BL/6 and MSM and expression data of each gene.

論文の審査結果の要旨

申請者の論文は、ラボラトリーマウスであるMus musculus domesticus 由来のC57BL/6Jと日本産野生マウスであるM. m. molossinusに由来する系統MSMとの交配により作成されたコンソミックマウス系統群を用いて、マウスの２つの行動特性に関する表現型解析を行い、これらの表現型を支配する遺伝子が染色体上のどの領域にあるかを、３段階の遺伝解析により検出したものである。

動物の行動特性を支配する遺伝的要因は多数存在すると考えられ、複雑な系を解きほぐして行く必要があるが、おとなしいラボラトリーマウスと活発な野生マウス系統の交配から作成されたコンソミック系統、つまり染色体置換系統は、この問題を解くのに適した系統群であると考えられた。また、これらの系統間に見られる行動多様性の解析は、ヒトにおける行動疾患の原因をさぐる上でも欠かせないモデル系になると考えられる。

西君はこの系統群の特性を利用し、行動特性としての自発活動量および情動性の２つの表現形質が系統間でどのように異なるかを解析する実験に取り組んだ。表現型としての行動解析には、これまで使われていた試験法を取り入れつつ、より正確な解析のため、試験区間を分割して活動回数や持続時間、平均活動時間および行動パターンの変化を抽出する方法などを新たに取り入れた行動試験バッテリーを組み、系統間の行動特性の差を検出することに成功した。この論文では、ホームケージ活動、新規ケージ活動、と明暗箱行動テストを採用した。その結果、用いた１８のコンソミック系統の中で、第６染色体のセントロメア末端領域にMSMのゲノムをもつ系統 (B6-6CMSM)が、自発活動、情動性共にC57BL/6J系統と最も異なった行動特性を示すことを明らかにした。

第６染色体の領域と行動形質との相関をより詳細に評価するため、B6-6CMSM を用いて、さらに刺激反応、学習などへの反応を５項目の試験により解析すると共に、生体内ドーパミンレベルの測定を行った。この結果、MSM の染色体６末端領域には、C57BL/6J と比べ学習性は変わらず、恐怖情動を上昇させ、音に対する驚愕反応性を低下させる要因のあることがわかった。また、線条体におけるドーパミン放出量の増加も観察された。平行して第６染色体についての QTL 解析を行い、ホームケージ活動、新奇ケージ活動と明暗箱行動に作用力を持つ QTL を検出した。この結果、いずれの行動特性についても、コンソミック系統での試験と同様に、大きな作用力をもつ座位を第６染色体のセントロメア領域近傍に特定できた。

最後に、これらの QTL を分解して、作用遺伝子を絞り込むため C57BL/6J に B6-6CMSM を戻し交雑し、標的領域を細分化したコンジェニック系統を４系統（B6-6CMS-C1, C2, C3, C4）作成し、行動表現型試験を実施した。この結果、それぞれの系統が QTL の結果と対応する位置に表現型に違いをもたらす要因を持つ可能性が高いと考えられ、候補領域の絞り込みを進めることが出来た。

この研究は、行動を規定する要因を遺伝学的に優れた材料を作りながら絞り込んで行こうとするもので、絞り込みの過程で多くの表現形質の評価を工夫しつつ、遺伝学的に数段階のステップを経て、目的の遺伝子に近づくことが出来た。これらの結果は、いまだかなりな困難を伴うこの研究分野を切り開く試みのひとつとして評価できる。よってこの論文は、遺伝学専攻の博士論文としての水準を十分に満たすものであると審査員全員が判断した。