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Emotionality, such as fear and anxiety, is an evolutionally conserved trait in many animals to prepare for and react against danger. However, excess level of emotionality interrupts their normal life, and it will be diagnosed as psychological disorder in human (e.g. anxiety disorder). It is known that emotionality has genetic bases as well as environmental effect, and recently a number of genes contributing to anxiety have been progressively found. In the animal model, several behavioral tests and indices are developed and used to measure emotionality of animals. However, it has been noticed that all of those emotionality-related indices do not have consistent correlation within individuals, even between measurements in the same test. That is, those indices are measuring several different aspects. In psychological studies, emotionality has been considered as a "complex of factors" rather than a single alternative construct. However, not many studies that aim to identify genes associated with emotionality have concerned this multifactorial architecture of emotionality. There are some attempts by combining genetic analysis and multivariate analysis of behavior to identify genetic loci related to the "complex of factors", but those are just a beginning. In this study, I examined the structure of those multiple factors of emotionality, validated those factors genetically, and tried to identify genetic loci related to those factors. I focused on the open-field test, which is the first model for measuring emotionality, and still common today.

At the start point of this study, I examined genetic contribution to the open-field behavior by using conventional measurements, ambulation and defecation, and some ethological measurements in a variety of wild-derived mouse strains. By describing open-field behavior in detail and examining temporal changes, I was able to identify the prominent behavioral features of each strain of mice. Conventional simple measurements lose substantial information, such as the variety of behaviors that can be displayed, and the use of too few indices might easily lead to confusion in interpreting the genetic mechanisms underlying open-field behavior or "emotionality". Principal component analysis showed that the open-field behavior consisted of three dimensions of psychological trait: "locomotor activity", "thigmotaxis", and "anxious tension state".

In order to perform genetic mapping of open-field behavior, I used consomic strains of mouse established from C57BL/6J and MSM/Ms (B6-Chr^{MSM}CSSs) in which one of each chromosome of C57BL/6J was substituted by a corresponding chromosome of MSM. By analyzing a series of CSSs, I was able to map the chromosomes associated with a certain phenotype. In addition to open-field test, two kinds of other emotionality-related tests, elevated plus-maze and social interaction test, were examined. By analyzing a panel of CSSs, I identified multiple chromosomes that have a QTL or QTLs related to conventional and ethological measurements of open-field

behaviors, elevated-plus maze, and social interaction test. Many CSSs had substantially large effect QTLs due to the non-additive effect, and thus they were expected to be superior tool for the next step of QTL analysis: identifying the quantitative trait gene. By analyzing both males and females of CSSs, I found that there were many sex-dependent QTLs. Principal component analysis of a series of CSSs validated the three factors underlying open-field behavior as in wild-derived mouse strains. Because behaviors loaded on "anxious tension state" factor have rarely been analyzed in most behavior genetic analysis, I focused on this factor for the further analysis.

One CSS, B6-17MSM, that has substituted chromosome 17 from MSM, showed increase of the "anxious tension state" factor. They also exhibited reduced novelty-induced activity and highly increased social interaction behavior, but no differences in their home-cage activity. Thus, it was expected that there is a genetic locus/loci related to some aspect of "emotionality" on the chromosome 17. For characterizing B6-17MSM in more detail, I conducted several behavioral tests and brain morphological analysis. Fear conditioning tests revealed B6-17MSM had an increased fear memory in the cue-fear conditioning but not in the context-fear conditioning. Thus, it was expected there is a genetic locus/loci related to cue-specific fear learning on the chromosome 17. On the other hand, this strain had increased incidence of hydrocephalus. Histological analysis revealed that externally-normal individuals of B6-17MSM had enlarged brain ventricle size than C57BL/6J. Despite the hydrocephalus phenotype, B6-17MSM showed normal sensorimotor gating and motor coordination as C57BL/6J.

The analysis of reciprocal F1 intercross of B6-17MSM and C57BL/6J revealed that there are prominent maternal effects on their behavior. To identify genetic loci related to those behaviors and the hydrocephalus-like phenotype, I established a series of congenic mouse strains of B6-17MSM. By analyzing those congenic strains, I successfully revealed novel genetic loci associated with the brain ventricle size on the chromosome 17. Behavioral analysis also identified several genetic loci related to each behavior. Although social interaction behavior was prominently high in B6-17MSM, any congenic strains showed increased duration of social contact. It was supposed that there are interacting epistatic genes for inducing social interaction on this chromosome.

So far, I conducted the factor analyses of open-field measurements in the wild-derived strains and consomic mouse strains, and confirmed that there are "locomotor activity", "thigmotaxis", and "anxious tension state" factors related to their behaviors. Behavioral analysis of congenic strains also revealed the existence and independence of those factors. The analysis of open-field behavior revealed two interesting congenic strains, C10 and C15; C10 has relation to "locomotor activity" factor, and C15 is associated with both "locomotor activity" and "anxious tension state" factors. Further behavioral characterization of these congenic strains showed differences of home-cage

activity and fear conditioning between C10 and C15. This result suggested that the "locomotor activity" factor and "anxious tension state" factor are independent traits and have relation to different genetic and biological pathways.

In addition to the above study, I conducted genetic analysis of other important emotion, aggression. Aggression has considerable importance for animal's living and is evolutionally ancient behavior. Because the wild-derived strain MSM/Ms still retains considerable aggression, it was expected that B6-Chr^{MSM}CSSs would have advantages to identify genetic loci associated with the aggressive behavior. In this study, I focused on one CSS, B6-15MSM, which has substituted chromosome 15 from MSM, and examined their aggression in the resident-intruder paradigm. Resident-intruder test revealed that B6-15MSM shows elevated aggressive behavior toward the same genotype intruder compared to C57BL/6J. By analyzing both homogenous pairs and reciprocal, heterogenous pairs in the resident-intruder test, I found prominent effect of the opponent (intruder) in their aggressive behavior: aggressive behavior was increased when the intruder was B6-15MSM but not C57BL/6J. The analysis of reciprocal F1 progeny indicated there are dominance effect on the tail-rattling and submission behavior, and also maternal effect on attack behavior. Preliminary analysis of congenic strains showed the possibility to identify the genetic loci associated with the aggressive behavior of B6-15MSM, and suggested there are multiple genetic loci related to the aggressive behavior on chromosome 15.

論文の審査結果の要旨

高等動物の複雑な行動、特に「情動性（emotionality)」について遺伝学的な解析を体系的に適用した例は非常に少ない。例えばマウスの異なる系統に見られる特徴的な行動（臆病さ、攻撃性など）は量的形質として遺伝的に複雑な制御を受けていると考えられるが、実際にゲノム中にどれくらい、どのような遺伝子が関わっているかという遺伝的基盤の全体的な知見は得られていない。高橋さんはこの問題にアプローチするため、染色体置換（コンソミック）系統、染色体部分置換（コンジェニック）系統などを用いて、詳細な行動観察をおこなった。

まず、それぞれ非常に異なった行動を示す野生由来マウス 10 系統を対象に、情動性検定としては最も普遍的な open-field 行動を 12 の行動項目にわたって観察し、因子分析をおこなった。結果、このような多種類のマウスでもその open-field 行動は「移動活動量（locomotor activity)」「走触性（thigmotaxis)」「不安緊張性（anxious tension state）という 3 つの心理特性（因子）により制御される、という知見を得た。

これらの心理特性をさらに遺伝学的に解析するため、野生由来系統 MSM の染色体 1 本ずつを実験用マウスである B6 系統に導入した（置き変えた）コンソミック系統、21 系統に対して同じ検定をおこなった。さらに高架式十字迷路と social interaction test の両検定もおこなった。これらの結果より、それぞれの行動項目に対して複数の染色体上に量的形質遺伝子座（QTL）が存在することが判明した。雌雄別々に検定することにより、性依存的な QTL も同定された。これらのデータの因子分析からも、open-field 行動を制御する 3 因子の存在が確認された。すなわち、心理特性に遺伝学的にアプローチする上でこれらのコンソミック系統の有用性が示された。それぞれの染色体には比較的強い効果を示す QTL が観察されたので、単なる QTL 解析にとどまらず当該遺伝子を同定するためのツールとしても有用であることが明らかである。

次に高橋さんは、情動行動において B6 との顕著な差がみとめられたコンソミック系統（B6-17MSM）を取り上げてさらに解析を進めた。B6-17MSM は情動行動の中でもこれまで行動遺伝学的解析例がほとんど無い不安緊張性に関連した行動が特に昂進している。この B6-17MSM 系統を恐怖条件付け（fear-conditioning）など 4 種類の行動検定を行ない、cue-fear conditioning における恐怖学習のみ昂進していることを明らかにした。正逆交配実験より、これら行動への母性効果も観察された。さらに詳細な解析と将来の遺伝子クローニングを目指して、17 番染色体の一部のみをそれぞれ置換した 13 種類のコンジェニック系統を作出し、行動解析をおこなったところ、幾つかの行動項目に対応する QTL を染色体上にマップすることができた。一方、social interaction 行動に関する QTL は染色体全体に分散し、複数の遺伝子座に制御された行動であることが明らかとなった。また、副産物として、脳水腫様症状の原因遺伝子座も単一領域にマップすることができた。また同時に進めた B6-15MSM の解析からは、攻撃行動に関わる複数の QTL が同定できた。

高橋さんはユニークなアプローチを着実に、体系的かつ詳細に進め、膨大なデータを得た。その成果は複雑な行動の遺伝的基盤を明らかにする上で大きな進展であり、非常に興味深い。審査員全員一致で博士論文として十分な成果であることを確認した。