学 位 論 文 の 要 旨

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学 位 論 文 名　Two Genomic Regions of Chromosome 1 and 18 Explain Most of the Stroke Susceptibility Under Salt Loading in SHRSP/Izm

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論 文 内 容 の 要 旨

INTRODUCTION

The stroke-prone spontaneously hypertensive rat (SHRSP) genetically suffers from severe hypertension and cerebral stroke. Information about the genetic mechanisms underlying cerebral stroke in SHRSP may provide important clues to understanding the pathogenesis of cerebrovascular diseases based on severe hypertension. To clarify the genetic mechanisms of stroke-susceptibility in SHRSP, a quantitative trait locus (QTL) analysis was performed. As a quantitative trait to evaluate stroke susceptibility, we employed stroke-latency under salt-loading because the incidence of stroke differs greatly between SHRSP and SHR under salt-loading, which implies that salt-sensitive mechanisms are the key to understanding the susceptibility to stroke in this model.

MATERIALS AND METHODS

Data on the stroke latency and blood pressure were obtained from 295 F2 rats of a cross

between SHRSP/Izm and SHR/Izm. Salt-loading was performed by feeding the rats with 1 % salt water. DNA was extracted from liver and seventy-four simple sequence repeat markers (SSRs) distributed throughout the whole genome were genotyped. QTL analysis was performed with MapManagerQTX version 20. Based on the results of the QTL analysis, four reciprocal congenic strains targeting two QTLs on chromosome (chr) 1 and 18 were constructed through backcrossing SHRSP and SHR, and established congenic strains were mated with each other to construct two reciprocal double congenic strains. The stroke latency was measured in these congenic strains under salt loading. Blood pressure was measured either by the tail-cuff method or by the radio-telemetry. Four hundred and thirty SSRs in the two QTL regions were genotyped in 11 substrains of SHRSP and SHR. Two additional subcongenic strains were constructed by backcrossing one of the congenic strains with SHRSP. The whole-genome of SHRSP and SHR was sequenced using the next-generation sequencing strategy covering 20 times the rat genome. The sequence reads were mapped on the Rattus norvegicus genome assembly (rn4) with a computer software. Quantitative real-time PCR (RT-PCR) was performed on mRNA extracted from the kidney of SHRSP and SHR using SYBR Green as an indicator. All the animal procedures were approved by the local committee for animal research of Shimane University.

RESULTS AND DISCUSSION

Two major QTLs for stroke-latency were identified on chr 1 and 18. Interestingly, the QTL on chr 1 overlapped with the QTL for blood pressure, while the QTL on chr 18 had no effects on blood pressure. Evaluation of 6 reciprocal single and double congenic rats for these QTLs showed that substitution of the SHRSP- for the SHR-fragment at the chr-1 and -18 QTLs increased the relative risk for stroke by 8.4 and 5.0, respectively. The combined effect of the two QTLs was 10 times greater than that of the background genome (by Cox hazard model), indicating that the two genomic regions that were 4 % of the genome explained the most of the stroke susceptibility in SHRSP. Blood pressure monitoring by the radio-telemetry indicated that the combination of the two QTLs had a clear effect on the salt-dependent blood pressure increase, suggesting an important role for the salt-sensitive blood pressure increase in the susceptibility of SHRSP to stroke. Although the initial QTL identified on chr 1 was as wide

as 62 Mbp, a haplotype analysis of 11 substrains of SHRSP and SHR using 340 SSR markers in the chr-1 QTL suggested that a 7-Mbp fragment was most likely to harbor the responsible gene(s). Indeed, this was confirmed by a study of additional subcongenic strains targeting this 7-Mbp region. In the QTL on chr 18, a potential candidate gene, *Nedd4l*, was identified. This gene is known to interact with the epithelial sodium channels to promote its degradation. The whole-genome sequence analysis revealed, however, that no differences in the coding sequence of *Nedd4l* were observed between SHRSP and SHR. Further, quantitative RT-PCR showed that the expression of *Nedd4l* in the kidney under salt-loading was paradoxically greater in the salt-sensitive congenic strain. These results did not support the pathological role of this gene in SHRSP.

CONCLUSION

The present study indicated a major role for two QTLs on chr 1 and 18 in stroke susceptibility in SHRSP. The salt-sensitive blood pressure increase was implied to play a key role in the stroke susceptibility. Further narrow-down of the regions will be necessary to identify the genes responsible for the stroke-susceptibility in SHRSP.

論文審査及び最終試験又は学力確認の結果の要旨

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論文審査の結果の要旨

日本では、脳卒中による死亡率は減少している一方で、高齢化に伴って患者数は漸増していること、要介護となる原因の第1位を占めていることが知られている。このことは、脳卒中の発症要因解明とそれに基づく予防の重要性が減っていないことを示している。申請者らは、高血圧および脳卒中の遺伝的モデルである stroke-prone spontaneously hypertensive rat (SHRSP)に着目し、このモデルラットで脳卒中感受性遺伝子を同定することがヒトにおける脳卒中の遺伝的危険因子の解明につながると考えて、本研究を実施した。SHRSP と、高血圧は発症するが脳卒中を起こしにくいモデルである SHR とを交配して得た孫世代 295 匹に食塩負荷を行い、脳卒中発症までの潜伏期間（stroke latency）を測定した。全ゲノム上に分布する 128 個の遺伝子マーカーを用いて遺伝的に解析したところ、第 1, 18 染色体上に強い関与を示す領域を発見した。そこで、この領域が実際に stroke latency に影響するかどうかを調べるために、SHR と SHRSP の間で当該領域を入れ換えたコンジェニック系統 6 種類を作成して検討したところ、2 つの領域が stroke latency に対して相加的な影響を示すことを実証できた。申請者らは脳卒中感受性遺伝子同定を目指して更にこの領域を狭めることを試み、11 系統の SHR, SHRSP 亜系で、第 1, 18 染色体の脳卒中関連領域から選んだ、それぞれ 340 個と 90 個の遺伝子マーカーを調べた。その結果、第 1 染色体では 7 Mbp まで領域を狭められることを明らかにし、新たにこの 7 Mbp の領域をターゲットとするコンジェニック系統を作成してこの領域が脳卒中発症にかかわることを実証した。本研究は脳卒中の新たな遺伝的危険因子同定に向けて大きな前進が得られた学術的に価値の高い研究である。

最終試験又は学力の確認の結果の要旨

申請者は SHRSP と SHR を用いた遺伝解析で脳卒中感受性遺伝子領域を明らかにした。候補遺伝子の探索により、高血圧や脳卒中と直接関連する遺伝子同定に向けて意義のある研究である。周辺の知識も豊富であり、学位授与に値すると判断した。　（主査　田邊一明）

申請者はSHRSPとSHRを用いて、交配、コンジェニック系統の作成、１１系統の亜系の遺伝子解析など、様々な手法を用いた長期間におよぶ検討により、脳卒中感受性遺伝子の同定を行った。得られた結果は科学的意義が高く、臨床的にも重要な知見である。公開審査時の質疑応答も適切で、関連する知識も十分であり、学位授与に値すると判定した。　（副査　紫藤治）

申請者は、SHRとSHRSPを交配して得られた孫世代を対象に遺伝的解析を実施した。その結果、第1、第18染色体に脳卒中発症に関与する領域を発見した。本研究は脳卒中発症の発生機序の解明に寄与すると考え、学位授与に値すると判定した。　（副査：藤田委由）

（備考）要旨は、それぞれ 400 字程度とする。