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.