学 位 論 文 の 要 旨

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学 位 論 文 名　Further Dissection of QTLs for Salt-Induced Stroke and Identification of Candidate Genes in the Stroke-Prone Spontaneously Hypertensive Rat

発 表 雑 誌 名（巻，初頁～終頁，年）　*Scientific Reports* (8, 9403, 2018)

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論 文 内 容 の 要 旨

INTRODUCTION

Cerebral stroke is a major health problem in Japan. As the stroke-prone spontaneously hypertensive rat (SHRSP) is thought to be a good model for cerebral stroke, genetic analysis of this model may provide new insights on the genetic risks of stroke, and may be useful for the prevention of the disease in humans.

In this context, we performed a quantitative trait locus (QTL) analysis on stroke susceptibility using F2 progenies between SHR, the stroke-resistant counterpart of SHRSP, and SHRSP and identified two major QTLs for stroke latency under salt-loading on chromosome (Chr) 1 and 18, which was then confirmed in double congenic strains.

Because the QTL regions identified were too large for further analysis, we narrowed down the QTL regions using multiple subcongenic strains in this study, and tried to identify candidate genes.

MATERIALS AND METHODS

Seven subcongenic strains for each of Chr1 and Chr18 QTLs were constructed through marker-assisted selection of pups with recombinant genotypes to cover various fragments of the QTLs. Salt loading was started by feeding 1% salt water to rats at 12

weeks of age. The rats were checked for stroke symptoms every day, and stroke latency was calculated as days between the start of salt loading and the first day that stroke symptoms were observed. Blood pressure (BP) was monitored in a newly constructed double subcongenic rat using the telemetry system.

Comprehensive analysis of expressed genes was done using a microarray system in the kidneys collected from SHR, SHRSP and two reciprocal congenic strains with and without 1-week of salt loading, and the results were confirmed by RT-PCR. The coding sequence of candidate genes were examined on whole genome sequence data of SHR and SHRSP that were obtained in a previous study. The sequence variations were confirmed by manual sequencing.

Three-dimensional protein structure was built by I-TASSER based on the sequence variations obtained through the genetic sequence analysis described above.

All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSION

In the previous study, we showed that a 18 Mbp fragment on Chr1 and a 29 Mbp fragment on Chr18 harbored major genes responsible for the stroke susceptibility in SHRSP. In the present study, we first constructed a new double subcongenic strain, SHRSPrch1.1_18.0, which covered the both fragments on Chr1 and 18 described above, to confirm the existence of genes for stroke susceptibility in these regions. The stroke latency in SHRSPrch1.1_18.0 did not significantly differ from that in the original double congenic strain SHRSPrch1_18. Further, the telemetry analysis indicated that no significant BP increase was observed under salt-loading in this strain, which was similar to SHRSPrch1_18. These observations strongly suggested that the regions covered by SHRSPrch1.1_18.0 included the gene(s) for stroke latency as well as for salt-induced BP increase that the original double congenic strains harbored. Accordingly, we focused on these regions for further dissection using subcongenic strains.

As for the QTL on Chr1, the analysis on the subcongenic strains indicated that the latency in three of the seven subcongenic strains did not significantly differ from that in the original congenic strain with the widest QTL fragment. This indicated that the common region shared with the three subcongenic strains above included the responsible genes for stroke. In addition, two subcongenic strains showed stroke latency that was not different from that in SHRSP, indicating that the region harbored by these two subcongenic strains could be excluded from the target region. Based on the compiled data on the subcongenic strains, the most promising target region was narrowed down to a 2.1

Mbp-fragment on Chr1. On the other hand, it was not successful to reduce the region on Chr18 probably because multiple genes in the region influenced the phenotype.

A microarray analysis identified three genes, *the* zinc finger protein 45-like (*Zfp45L*), the *ethylmalonic encephalopathy 1* (*Ethe1*) and the *CXC motif chemochine ligand 17* (*Cxcl17*), of which expression was affected by the allele of the 2.1 Mbp fragment. We confirmed this expression pattern by quantitative RT-PCR.

Screening for variations in coding sequnece of the genes was done on whole-genome sequence data of SHRSP and SHR. We identified five missense variations in four genes located in the 2.1 Mbp region, which were confirmed by direct sequencing. A conformation analysis of the protein products of these genes using I-TASSER implied that variations in the CBL-protooncogene C (CBL-C) and CXCL17 might cause significant changes in the protein structure.

CONCLUSION

In this study, we attempted to narrow down the QTL regions for stroke latency in SHRSP both on Chr1 and 18, and successfully reduced a target region on Chr 1 to a 2.1Mbp-fragment. We identified six candidate genes in this region, which would need further analyses to be established as the genes responsible for stroke.