学 位 論 文 の 要 旨

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学 位 論 文 名　Identification of *Stim1* as a candidate gene for exaggerated sympathetic response to stress in the stroke-prone spontaneously hypertensive rat.

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論 文 内 容 の 要 旨

INTRODUCTION

The sympathetic nervous system (SNS) has been implied to play a key role in the pathogenesis of hypertension. The stroke-prone spontaneously hypertensive rats (SHRSP) are known to be vulnerable to various types of stress, which might contribute to the pathogenesis of severe hypertension and stroke observed in this strain, and thus the genetic factors underlying this may provide us important clues to understand the pathogenesis of hypertension in humans. Previously, by using a congenic strain (called SPwch1.72) constructed between SHRSP/Izm and the normotensive Wistar-Kyoto rat/Izm (WKY/Izm), we showed that a 1.8-Mbp fragment on chromosome 1 (Chr1) of SHRSP harbored a responsible gene (or genes) for the exaggerated sympathetic response to stress. To further narrow down the candidate region, in this study, another congenic strain (SPwch1.71) harboring a smaller fragment on Chr1 including two functional candidate genes, *Phox2a* and *Ship2*, was generated.

Physiological evaluation of the stress responses in this new congenic strain (SPwch1.71) indicated that the region harboring *Phox2a* and *Ship2* was not likely to have causative roles in the exaggerated stress response in SHRSP/Izm. In spite of that, further attempt to explore causative genes in the remaining candidate region succeeded to identify another strong candidate gene, the stromal interaction molecule 1 (*Stim1*).

MATERIALS AND METHODS

All the animal procedures were approved by the ethics committee for animal research in Shimane University. Sympathetic response to cold and restraint stress was compared among SHRSP/Izm, SPwch1.71, SPwch1.72 and WKY/Izm by three different methods [urinary norepinephrine (NE) excretion, blood pressure (BP) measurement by the telemetry system and the power spectral analysis on heart rate (HR) variability]. Briefly, restraint stress was imposed by placing rats for 3 h in a stainless-steel holder adjusted to the rat's body size. As for cold stress, a rat was placed in a cage kept at 4°C for 3 h (in the telemetry experiments) or for 6 h (in the collection of urine samples). Urinary NE was measured by HPLC. The power spectral analysis was done on HR variability under restraint stress using the telemetry for ECG. The ratio between the low frequency (LF; 0.04–1.0Hz) and the high frequency component (HF; 1.0–3.0Hz) was used as an indicator of the relative sympathetic activity. The LF/HF ratio was recorded for 30 s in every 10 min throughout the experiment, and the change in LF/HF (ΔLF/HF) was calculated as the difference between the averaged LF/HF during the periods with and without the stresses. BP and HR changes under restraint and cold stress were monitored with the telemetry system for BP (Data Science Inc, St. Paul, MN). BP and HR were monitored for 10 s in every 10 min during the experiment. The change in BP (ΔBP) and in HR (ΔHR) was calculated as the difference between the averaged BPs during the periods wth and without the stresses.

After the whole genome sequencing, sequence variations between SHRSP/Izm and WKY/Izm in the target chromosomal fragments were explored using SAMtools.

Gene and protein expression analyses were performed by quantitative reverse transcription PCR (RT-PCR) and Western blot, respectively, using the tissue of the brainstem.

Inter-strain differences were tested either by Student's t-test or by ANOVA with Dunnett's post-hoc test. $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

BP of SPwch1.71 and 1.72 did not differ significantly from that of SHRSP/Izm when measured by the telemetry. When BP change under the cold and restraint stress was monitored by the telemetry, the response was similar between SHRSP/Izm and SPwch1.71, while SPwch1.72 showed a blunted response. In accordance with this observation, increase in urinary NE excretion under the cold stress did not differ significantly between SPwch1.71 and SHRSP/Izm, while it significantly reduced in SPwch1.72 when compared with SHRSP/Izm. Further, a power spectral

analysis on HR variation indicated that ⊿LF/HF under the restraint stress was comparable between SHRSP/Izm and SPwch1.71, whereas a significant reduction was observed in SPwch1.72.

Collectively, the results of these physiological experiments indicated that the region covered by SPwch1.71 did not contribute to the difference in the sympathetic stress response between SHRSP/Izm and WKY/Izm, and could be excluded from the candidate region. We thus narrowed down the candidate region between *Trpc2* and *Olr111* as the maximal estimation, which harbored 12 candidate genes.

Sequence analysis of the 12 potential candidate genes in this region identified a nonsense mutation in the stromal interaction molecule 1 (*Stim1*) gene of SHRSP/Izm, which was shared among 4 substrains of SHRSP. A western blot analysis confirmed a truncated form of STIM1 in SHRSP/Izm. In addition, the analysis revealed that the protein level of STIM1 in the brainstem was significantly lower in SHRSP/Izm when compared with WKY/Izm. STIM1 plays a key role in the cellular Ca^{2+} dynamics through interacting with ORAI1 and/or the transient receptor potential cation channel 1 (TRPC1). Of note, the C-terminus lysine residues (K684, K685), which were lost in the truncated form in SHRSP/Izm, were reported to be essential in the interaction of STIM1 with the TRPC1. The truncatied STIM1 may be causally related to the exaggerated response of SNS in SHRSP through abnormal regulation of TRPC1.

CONCLUSION

In conclusion, we found that *Stim1* is the best candidate in terms of the gene function as well as of the potential significance of the sequence variations identified in it. To obtain conclusive evidence for pathological roles of the truncated STIM1, it is essential to clarify effects of the truncation on the cellular calcium dynamics. Further studies on the role of STIM1 in the regulation of SNS are warranted.

論文審査及び最終試験又は学力の確認の結果の要旨

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学位論文名	Identification of *Stim1* as a Candidate Gene for Exaggerated Sympathetic Response to Stress in the Stroke-Prone Spontaneously Hypertensive Rats		
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論文審査の結果の要旨

交感神経系の過度の活動亢進は、高血圧の発症に寄与すると考えられる。申請者らは、本態性高血圧症の遺伝的モデルである脳卒中易発症高血圧自然発症ラット（SHRSP）は各種ストレスに対する交感神経系の反応性亢進があること、さらに、SHRSPを背景とし第１染色体領域のみを正常血圧対照ラット(WKY)と交換したコンジェニックラットを用い、それに関与する遺伝子が同染色体上の1.8Mbpの領域に存在することを明らかにしてきた。本研究は、同領域を更に狭め、交感神経反応性亢進の原因となる遺伝子を同定することを目的とした。上記1.8Mbpの領域より小さな領域を持つコンジェニックラットを作成した。次いで、ストレスに対する交感神経活動の応答を元のコンジェニックラットと比較し、原因遺伝子の含まれる領域を1.2Mbpに狭めた。同領域内の71個の遺伝子のうち、嗅覚受容体遺伝子を除く12個の遺伝子を候補とし、変異の有無や遺伝子発現レベルを検討した。その結果、Stromal interaction molecule 1（*Stim1*）をコードする遺伝子のC末端にナンセンス変異が見つかり、STIM1蛋白のC末端に46アミノ酸の欠失があることを確認した。本研究は、高血圧発症に関与する交感神経の反応性亢進の原因遺伝子の候補を同定したもので、高血圧発症機序の解明に寄与する学術的価値の高い研究と認められたため、学位授与に値すると判断した。

最終試験又は学力の確認の結果の要旨

申請者は、本態性高血圧の発症に関与する遺伝子を同定するため、独自に作成したコンジェニックラットを用いて、外乱に対する交感神経活動の過剰な亢進とその原因遺伝子を解析した。得られた結果は基礎医学的も臨床的にも極めて意義が高い。基本的および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。（主査：紫藤　治）

申請者は、本態性高血圧症遺伝的モデル由来のコンジェニックラットによる遺伝子解析などから、交感神経反応性亢進の原因となる遺伝子の可能性として*Stim1*を導き出し得た。質疑応答も的確で、関連分野の知識も豊富であり、学位授与に値する。（副査：竹下治男）

申請者は寒冷ストレスによる過剰な交感神経緊張をもたらす遺伝子として*Stim1*の可能性をつきとめた。交感神経緊張に伴う高血圧のメカニズム解明に寄与する研究であり、また周辺の知識も豊富で学位授与に値すると判断した。（副査：田邊一明）

（備考）要旨は、それぞれ４００字程度とする。