Mapping of Four Simple Sequence Repeat (SSR) Markers on Rat Chromosome 4

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We previously reported that several markers on rat chromosome (Chr) 4 cosegregated with the occurrence of cerebral stroke and brain edema in stroke-prone spontaneously hypertensive rats (SHRSP). To obtain insights into the positional candidate genes for stroke susceptibility in this region, we mapped four genes, Taurine transporter (Taut), tumor necrosis factor receptor (Tnfr), GABA transporter (Gati) and glucose transporter-3 (GIut3) genes, using newly developed simple sequence repeat (SSR) markers on rat Chr 4. We isolated the SSRs for the genes either by screening a rat genomic library or by searching the GenBank database. By linkage analysis using two sets of backcrosses, Gati and Tnfr were mapped in the region associated with stroke, while Taut was located distant from the region. The GIut3 locus was also assigned to rat Chr 4 using a rat x mouse hybrid clone panel. These results indicated that the Tnfr, Gati and GIut3 genes were good positional candidates for the stroke susceptibility in SHRSP, suggesting that further evaluation of these genes by functional studies could prove useful. (Hypertens Res 2000; 23: 47-50)

Key Words: stroke susceptibility, stroke-prone spontaneously hypertensive rat, candidate genes, simple sequence repeat markers, comparative map

Introduction

Although hypertension is a major risk factor for cerebral stroke, other genetic and environmental factors, such as abnormalities in lipid metabolisms, diabetes mellitus, dietary habits and cigarette smoking, are also stroke risk factors. Recently, genes related to stroke susceptibility have been sought by linkage analysis in humans (1, 2) and in experimental animal models (3-6). Based on our study of stroke-prone spontaneously hypertensive rats (SHRSP), we previously reported that several markers on rat chromosome (Chr) 4 cosegregated with the occurrence of cerebral stroke and brain edema (4). These markers did not cosegregate with blood pressure, suggesting that the putative stroke susceptibility gene in this region was present independent of hypertension. In this study, we mapped 4 genes on rat Chr 4 to make a comparative map between the rat and the mouse to obtain positional candidate genes for stroke susceptibility in this region.

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**Methods**

**Library Screening**

Genomic library screening was carried out as described previously (7). Briefly, a rat genomic library constructed in the cosmid vector pH79 (8) was screened with cDNA fragments amplified by reverse transcription-polymerase chain reaction (RT-PCR) used as probes. Total RNA from the rat brain was used in the RT-PCR. Positive cosmid clones were digested with Sau 3A and subcloned into the pBluescript (Stratagene, La Jolla, CA). These plasmid libraries were then screened with 32P-endlabeled (CA)15 or (GA)15 oligomers. Positive clones were picked up, miniprepped and sequenced to identify simple sequence repeats (SSRs) and their flanking sequences.

**Genotyping and Linkage Mapping**

Polymorphisms of SSR markers were examined using 8 inbred strains of rats, SHRS/Imz, Wistar Kyoto (WKY/Imz), SHR/kyo, Donryu (DRY/Sankyo), Otsuka Long-Evans Tokushima Fatty (OLETF), Long-Evans Tokushima Otsuka (LETO), Wistar-derived tremor control (WTC/Kyo) and Brown-Norway (BN/N). SHRSP rats were maintained in our institute as described previously (9). OLETF and LETO rats were maintained in the Tokushima Research Institute (10), and other strains except for DRY/Sankyo rats were maintained in the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University. The animals were treated with appropriate care, in accordance with the guideline for animal experimentation issued by the Japan Association for Laboratory Animal Science (11).

Three sets of intercrossed or backcrossed animals, 125 progenies of F2 (SHRSP×WKY), 48 of (WTC×BN)×BN and 87 of (SHR×DRY)×SHR, were genotyped with SSR markers on Chr 4. The PCR conditions are summarized in Table 1. D4Smu4 were amplified by touchdown PCR (12). The annealing temperature was decreased by 0.5 degree/cycle from 63°C to 58°C and then fixed at 58°C for 25 cycles (Table 1). PCR products were electrophoresed on either 7.5% polyacrylamide or 4% agarose gels and visualized with ethidium bromide staining. Genotype data were analyzed with the MAPMAKER/EXP 3.0 program to make linkage maps (13).

**Results**

Taurine transporter (Taut), tumor necrosis factor receptor (Tnfr), GABA transporter (Gat1) and glucose transporter-3 (Glut3) genes were selected from the mouse and the
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human chromosomal regions that were expected to be homologous to rat Chr 4. A (CA)$_2$ repeat was found in the 3' untranslated region (3'UTR) of the reported Taut cDNA sequence (GenBank M96601). We accordingly designed a SSR marker for the Taut locus, as shown in Table 1, and mapped it on rat Chr 4 by linkage analysis using a (WTC $\times$ BN) $\times$ BN cross (Fig. 1, D4Smu1).

Three SSRs for Tnfr, Gat1 and Glut3 were newly isolated through the screening of a rat genomic library. Tnfr and Gat1 were mapped on Chr 4 by linkage using a (WTC $\times$ BN) $\times$ BN or a (SHR $\times$ DRY) $\times$ SHR cross (Fig. 1, D4Smu2 and D4Smu3, respectively). D4Smu4, a SSR for the Glut3 locus, was not polymorphic among the eight strains examined in this study, so it was assigned to rat Chr 4 using a rat X mouse hybrid clone panel (14). Somatic hybrids positive for D4Smu4 were completely concordant with the rat Chr 4 (data not shown).

Discussion

The present study showed that the Gat1 and Tnfr loci were located between D4Mgh7 and D4Mgh11, which were reported to link with stroke susceptibility (4), while Taut was located near the Npy locus, substantially distant from the region (Fig. 1). These results indicated that a wide region on rat Chr 4 was homologous to mouse Chr 6. Although Glut3 could not be mapped by linkage, we concluded after considering the homology among rat, mouse and human chromosomes that it is probably between Gat1 and Tnfr (Fig. 1).

Tumor necrosis factor (TNF) is known as a cytokine that plays an important role in putative cascade-evoking apoptosis. TNF levels were shown to increase in the presence of ischemic brain injury (15). Further, in Tnfr-knocked out mice, the brain damage caused by the middle cerebral artery occlusion (MCAO) was significantly greater than that in normal mice (16). The TNF signal, therefore, may be one of the determinants of the neuronal susceptibility to ischemia. The Tnfr locus mapped in this study indicated that Tnfr is a good candidate as a stroke susceptibility gene in SHRSP rats. Functional studies also implicated Gat1 and Glut3. Recent studies showed that an inhibitor of the GABA transporter reduced the extent of ischemic injury (17). Glut3, known as a neuron-specific glucose transporter, was also reported to be increased in the rat brain after MCAO (18). The authors argued that up-regulation of Glut3 levels in neurons might spare metabolic energy during the postischemic depression of cerebral blood flow.

Somatic hybrids positive for D4Smu4 were completely concordant with the rat Chr 4 (data not shown).

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Taurine was reported to protect neural cells from
exotoxin-induced ischemia (19). In SHRSP, dietary taurine was shown to prevent cerebral stroke (20). Although Taut was thought to be a good candidate as a stroke susceptibility gene in SHRSP rats, it was mapped distant from the stroke-linked markers on rat Chr 4. Further study is necessary to evaluate whether Taut can be a positional candidate or not.

The present study indicated that the Tnfr, Gat1 and Glut3 genes were good positional candidates for stroke susceptibility in SHRSP rats. Although we found no difference in the coding sequence of Tnfr between SHRSP/Izm and WKY/Izm rats (data not shown), future studies of these genes, including a comparison of the expression levels as well as the sequence of them between the SHRSP and the WKY rat, will allow further evaluation of them as candidates for stroke susceptibility genes.

References