Original Article

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 (*Tau*), tumor necrosis factor receptor (*Tnfr*), GABA transporter (*Gat1*) and glucose transporter-3 (*Glut3*) 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, *Gat1* and *Tnfr* were mapped in the region associated with stroke, while *Taut* was located distant from the region. The *Glut3* locus was also assigned to rat Chr 4 using a rat x mouse hybrid clone panel. These results indicated that the *Tnfr*, *Gat1* and *Glut3* 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 or the human to obtain positional candidate genes for stroke susceptibility in this region.

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Table 1. A List of Newly Developed Simple Sequence Repeat (SSR) Markers on Rat Chromosome 4.

Loci (gene symbols) : Gene names	Accession No.	Primers	Product sizes (SSRs) ^a	PCR conditions	
				Ann. temp./MgCl ₂	Alleles ^b
D4Smu1 (<i>Taut</i>)	M96601	5' CGCTTTCCTGCCCCTTTACTA 3' 5' GCCCATGGGTTTCTTTTG 3'	223bp(CA) ₂₂	60°C/1.5 mM	P=W=S=D=O=WT>F=B
: Taurine transporter					
D4Smu2 (<i>Tnfr</i>)	AF128408	5' TTCGGGGAAGGAACACACTT 3' 5' CGGAGACCTGAGAAGCCACAC 3'	206bp(CA) ₂₉	65°C/1.5 mM	P=W=S=D=F=O=WT>B
: Tumor necrosis factor receptor					
D4Smu3 (<i>Gat1</i>)	AF128409	5' GGCATGGTATTGGCATGGTAGA 3' 5' AGGTTTTTCACAAGGGGAGGG 3'	147bp(CA) ₂₄	65°C/1.5 mM	P=W=S=F=O=WT>D=B
: GABA transporter					
D4Smu4 (<i>Glut3</i>)	AF128410	5' TGCCCTGGTTTCTTCATCATC 3' 5' AACCCAGGGCCTTGGCTTCTT 3'	156bp(GA) ₂₃	TD63-58°C ^c 1.5 mM	P=W=S=D=F=O=WT=B
: Glucose transporter-3					

^aProduct sizes and numbers of repeats are based on sequenced plasmid clones or on the reference. ^bGenotyped rat strains are P, SHRSP/Izm; W, WKY/Izm; S, SHR/kyo; D, DRY/Sankyo; F, OLETF; O, LETO; WT, WTC/Kyo; BN, BN/N. ^cTouchdown PCR: Annealing temperature was decreased from 63°C to 58°C by 0.5 degree at every cycle and then fixed at 58°C for 25 cycles.

Methods

Library Screening

Genomic library screening was carried out as described previously (7). Briefly, a rat genomic library constructed in the cosmid vector pHCT9 (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, minipreped 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, SHRSP/Izm, Wistar Kyoto (WKY/Izm), 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 and WKY 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

Fig. 1. Linkage maps of rat chromosome 4. The left panel is the map for an F2 (SHRSP×WKY) cross of 125 progenies, the center panel is for a backcross (WTC×BN)×BN of 48, and the right panel is for a backcross (SHR×DRY)×SHR of 87. Map distance is calculated by MAPMAKER/EXP 3.0 program and represented in Kosambi's unit. Human and mouse homologous locations are indicated on the right. Markers in bold letters were newly developed in this study. Underlined markers are mapped by more than two crosses. *Markers previously showed a significant linkage with stroke in SHRSP (4).

human chromosomal regions that were expected to be homologous to rat Chr 4. A (CA)₂₂ 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×BN)×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×BN)×BN or a (SHR×DRY)×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×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.

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.

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