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A Novel Renal Carcinoma Predisposing Gene of the Nihon Rat Maps on Chromosome 10

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A novel rat model of hereditary renal cell carcinoma (RC) was found in a rat colony of the Sprague-Dawley (SD) strain in Japan, and named the "Nihon" rat in 2000. This study was designed to map the RC susceptibility gene in the Nihon rat using 113 backcross animals. Our present data clearly show that the *Nihon* gene is genetically linked to *interleukin-3* (*IL3*) gene (χ^2=93.6, Lod score=25.16), *lethal (2) giant larvae* (*LLGL1*) locus (χ^2=109.0, Lod score=31.56) and *myosin heavy chain, embryonic skeletal muscle* (*MYHSE*) gene (χ^2=90.6, Lod score=23.87), which are located on the distal part of rat chromosome 10. The order of the genes is the *Eker* (*Tsc2*) gene (located on the proximal part of rat chromosome 10; human chromosome 16p 13.3)—21.3 cM—*IL3* gene (human 5q23-31)—4.4 cM—*Nihon* gene—0.9 cM—*LLGL1* locus (human 17p11.2)—4.4 cM—*MYHSE* gene (human 17p13.1). We also detected loss of the wild-type allele at the *MYHSE* locus, fitting Knudson's "two hit" model. Thus, the Nihon rat should have a mutation of a novel tumor suppressor gene related to renal carcinogenesis.

Key words: Nihon rat — *Tsc 2* gene mutant (Eker) rat — Renal carcinogenesis — Tumor suppressor gene — Rat chromosome 10

Hereditary cancer was first described in the rat by Eker and Mossige in 1954 in Oslo.[1)] The Eker rat model of hereditary renal carcinoma (RC) was the first example of a Mendelian dominantly inherited predisposition to a specific cancer in an experimental animal. We and others identified a germline mutation in the rat homologous to the human tuberous sclerosis gene (*TSC2*) as the predisposing *Eker* gene.[2–5)] Recently, we found a novel hereditary RC model in the Sprague-Dawley (SD) rat in Japan. We have named this novel RC model the Nihon rat.[6)] We found that the homozygous mutant condition is lethal at around the 10th day of fetal life. In heterozygotes, RCs develop from early preneoplastic lesions, which begin to appear as early as 3 weeks of age, to adenomas (at 8 weeks of age). By the age 6 months; penetrance for this *RC* gene is virtually complete.[7)] The Nihon rat is also an example of a Mendelian dominantly inherited predisposition for development of RCs, but of predominantly clear cell type, and bears a single gene mutation like the Eker rat.[6, 7)] In the present study, we performed a genetic linkage analysis to the *Nihon* mutation, as a first step toward its identification.

The *RC* gene of Nihon rat (the *Nihon* gene) is being carried in the SD strain of rats. A genealogically unrelated strain, inbred Brown Norway (BN) (Charles River Breeding Laboratory), which does not carry the mutations, was used in mating with the Nihon male (Nihon/+, SD strain) to produce F1 hybrids. Nihon rats are diagnosed by detecting microscopic and/or macroscopic kidney tumors following a unilateral nephrectomy. Then, one heterozygote (Nihon/+) F1 male (designated BX0011-203 in this study) was in turn mated to BN(+/+) females to produce backcross progenies. Among 113 backcross [F1{(SD (Nihon/+)×BN(+/+)}×BN(+/+)] animals, 63 (♂: 30, ♀: 33, 56%) were histologically found to have multiple renal tumors (adenomas) at 13 weeks of age and 50 (♂: 24, ♀: 26, 44%) were negative, which is not significantly different from the expected 50%. Control animals never developed adenomas/RCs at this age.

Tail DNAs from 113 backcross animals at 13 weeks of age were extracted by digestion with 2% sodium dodecyl sulfate (SDS)/proteinase K followed by extraction with phenol as reported.[8)] DNAs were tested for linkage, noting whether uniquely SD bands of DNA were preferentially found in *Nihon* gene-bearing rats, with marker DNAs showing polymorphisms between SD and BN strains by polymerase chain reaction (PCR).

Rat DNA markers ("MAP PAIRS"), covering whole rat chromosomes, were purchased from Research Genetics, Inc. (Carlsbad, California). PCR was performed in 10 μl of a reaction mixture containing 50 ng of the genomic DNA, 1 pmol/μl of the primers, 0.5–1.25 m*M* $MgCl_2$

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Table I. Linkage Analysis with *Tsc2*, *IL-3*, *LLGL1* and *MYHSE* Loci

Locus	No. of animals with tumor		No. of animals without tumor		% Recombinant	Lod score	χ^2	*P* value
	SD/BN	BN/BN	SD/BN	BN/BN				
Tsc2	51	12	17	33	25.7	6.10	25.6	$P<0.0001$
IL3	61	2	3	47	4.4	25.16	93.6	$P<0.0001$
LLGL1	62	1	0	50	0.9	31.56	109.0	$P<0.0001$
MYHSE	58	5	1	49	5.3	23.87	90.6	$P<0.0001$

Lod scores; $Z_{max}=n\log_{10}(r)+(113-n)\log_{10}(1-r)-113\log_{10}(0.5)$, n=number of recombinants, r=recombination fraction.
χ^2 tests: StatView J-4.5 (Abacus Concepts, Inc., CA).

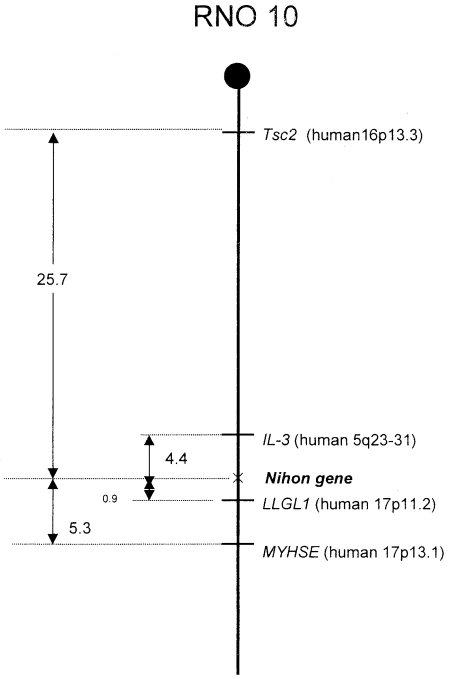

Fig. 1. Linkage between the *Nihon* gene and *Tsc2*, *IL-3*, *LLGL1* and *MYHSE* loci on rat chromosome 10. On the left are distances derived from 113 backcross animals. Numbers in parentheses are the human chromosome in which each gene is located.

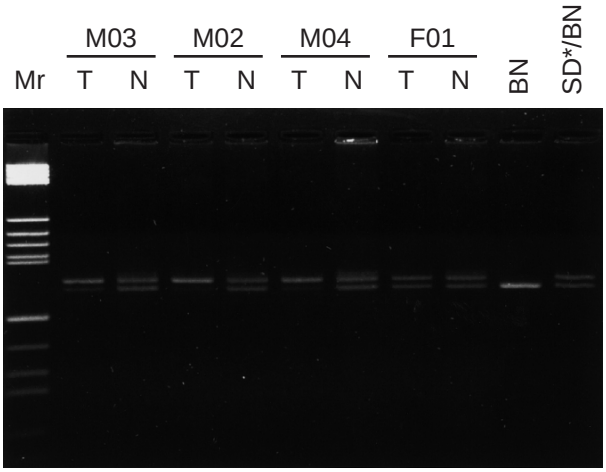

Fig. 2. Electrophoresis of PCR products of *MYHSE* gene. Four RCs were obtained from 4 different Nihon rats at 26 weeks of age. Three (M02, M03 and M04) out of 4 RCs clearly showed LOH. SD strain of rats (genetic background of Nihon rat) are outbred and fortunately, the *MYHSE* locus shows polymorphism. M, male; F, female; T, tumor; N, normal; BN, BN strain; SD*/BN, Fi Nihon rat (SD/BN); Mr, DNA size markers. Primer: MYHSE.

(ToYoBo, Osaka), 100–200 μm dNTPmix (ToYoBo) and 0.5 units of *Taq* polymerase (ToYoBo). Amplification for 25–35 cycles, each consisting of denaturation for 60 s at 92°C, annealing for 60 s at 55°C, and extension for 90 s at 72°C was performed with a thermal programmer (Quick Thermo Personal; Nippon Genetics Co., Ltd., Tokyo). The products of the PCR were electrophoresed on 3–3.5% low melting point (LMP) agarose gels (Gibco BRL) and visualized with ethidium bromide. First, we checked 121 DNA markers covering rat chromosomes, then we chose 63 DNA markers clearly showing polymorphism between Nihon (SD) and BN strains and started to screen. At the 1st screening using 67 backcross animals, *D10Rat27* showed the smallest recombination fraction (7/67=10%) among DNA markers. As reported previously, the Nihon rat bears a single autosomal gene mutation, like the Eker rat.[7] Therefore, we focused on rat chromosome 10. The *Tsc2* (5′-CCTGGGGATCTATATCACCT-3′ and 5′-GTCTAAAGCCTCCTCCTGAC-3′; unpublished data), *IL-3* (5′-CTGCTTAGAGCCTTCACACA-3′ and 5′-AGGAATTCGTCCAGGTTTACT-3′), *LLGL1* locus (5′-TGTGTGTATGCGCATGTGAC-3′ and 5′-GAAGGTGGCATCTACAGGGA-3′) and *MYHSE* (5′-TCATCTGGTGGGGACATA-

AC-3′ and 5′-GATGAACCAGCACATGGAAG-3′), which are located on rat chromosome 10, clearly showed polymorphism between Nihon (SD) and BN strains and the recombination fractions were 0.257 (29/113; χ^2=25.6, P<0.0001, Lod score=6.10), 0.044 (5/113; χ^2=93.6, P<0.0001, Lod score=25.16), 0.009 (1/113; χ^2=109.0, P<0.0001, Lod score=31.56) and 0.053 (6/113; χ^2=90.6, P<0.0001, Lod score=23.87), respectively. The linkage association between the 4 loci on rat chromosome 10 (*Tsc2*, *IL3*, *LLGL1* and *MYHSE*) and the *Nihon* mutation is summarized in Table I and Fig. 1.

As mentioned above, the Nihon rat is a genetically outbred SD strain and fortunately, the *MYHSE* locus showed polymorphism even among Nihon rats (Fig. 2). Therefore, we detected loss of heterozygosity (LOH) in RCs of the Nihon rats (3 out of 4 RCs) at the *MYHSE* locus and importantly all three were loss of the wild-type allele, as judged from the DNA patterns of BN and F1 (Nihon/BN) rats (Fig. 2), fitting Knudson's "two hit" model. The Nihon rat might have a mutation of a novel tumor suppressor gene related to renal carcinogenesis. Interestingly, the *Eker* (*Tsc2*) gene is located on the proximal part of the same chromosome 10.[3, 4] Thus, RC-related genes are located on rat chromosome 3 (*Tsc1* & *WT1*), rat chromosome 4 (*VHL* & *c-Met*) and rat chromosome 10 (*Tsc2* & *Nihon*).

In conclusion, the predisposing inherited gene in the Nihon rat was mapped to rat chromosome 10 between *IL3* (human 5q23-31) and *LLGL1* (human 17p11.2)/*MYHSE* (human 17p13.1) loci, away from 4.4 cM and 0.9 cM/5.3 cM, respectively (Fig. 1). At the present time, we do not know the human chromosome to which this corresponds (e.g., human chromosome 5 or 17). However, very recently, the predisposing gene of Birt-Hogg-Dube syndrome associated with RCs was mapped to human chromosome 17p11.2 or 17p12-q11.2.[9, 10]

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