Identification and Minisatellite Linkage Analysis of SMXA Recombinant Inbred Strains of Mice by DNA Fingerprinting

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Abstract: SMXA recombinant inbred (RI) strains of mice were produced by systematic inbreeding from the F_2 generation of a cross between two progenitor inbred strains, A/J and SM/J, which differ considerably with respect to many characteristics, and consists of 28 inbred strains. In this study, we investigated the applicability of DNA fingerprinting with M13 phage DNA to the identification of these closely related strains. DNA fingerprints of the SMXA RI strains and their progenitors, SM/J and A/J, showed strain-specific patterns, with the same banding patterns within each strain. Linkage analysis by using strain distribution patterns of minisatellite loci with 108 genetic markers containing microsatellites, biochemical and immunological marker genes allowed 23 minisatellite loci to be assigned to 11 chromosomes. The results suggested that DNA fingerprinting with M13 phage DNA is applicable not only for strain identification but also for genetic monitoring of RI strains on almost all chromosomes.

Key words: DNA fingerprinting, genetic monitoring, minisatellite, SMXA RI mice

Introduction

DNA fingerprinting with a minisatellite probe detects on Southern blotting many DNA fragments (bands) that are inherited in Mendelian fashion. These highly polymorphic DNA fragments are derived from minisatellite loci which are widely dispersed on most chromosomes. Therefore DNA fingerprinting has been used widely for identifying individuals and for parentage testing in humans and wild and domestic animals

[2, 6–8, 13, 21]. Furthermore, since the banding pattern between individuals becomes more similar in proportion to their genetic relationship, the method recently has been applied to estimate genetic structure including coefficients of inbreeding and relationships in animal populations, and genetic distances between populations [4, 5, 11, 12, 14].

Thus, it is likely that DNA fingerprinting can be used for genetic monitoring to check the genetic homogeneity of laboratory animals, especially that of inbred

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strains [16], and for studying relationships between strains. We have applied DNA fingerprinting to several inbred strains and sublines of mice, and showed that the method is suitable for their genetic identification and for monitoring relationships [14, 15].

Nishimura, one of the authors, developed SMXA recombinant inbred (RI) strains [unpublished data] derived from a cross between the SM/J strain of mice characterized by small body size and low tumor incidence and the A/J mice which is characterized by high tumor incidence.

RI strains are derived by inbreeding the F₂ generation of a cross of two progenitor inbred strains [1]. Although such strains possess genes from only the two progenitor strains, they are distinguished from each other by numerous genetic differences. Each locus has a particular pattern of inheritance called the "strain distribution pattern" (SDP). If RI strains are taken as a set, we can analyze the segregation and gene mapping of a given trait based on the linkage of known marker genes [19, 20].

In this study, we applied DNA fingerprinting, with the M13 bacteriophage as a DNA probe, to SMXA RI strains of mice, and investigated the applicability of the method to genetic monitoring of these closely related strains. To map on the chromosomes minisatellite loci detected by DNA fingerprinting, linkage analysis was carried out using the known SDP of SMXA RI strains.

Materials and Methods

Animals: Mice examined were of two progenitor inbred strains, A/J and SM/J, and 28 RI strains SMXA (SMXA-1–SMXA-30) which have been maintained at the Institute for Experimental Animals, Hamamatsu University School of Medicine. Many characteristics, including body size and the number of lung tumors, differ between the A/J and SM/J progenitor strains. Three RI strains, SMXA-2, 13, and 28, were not used because they became extinct in the course of inbreeding. A mutant mouse with a *dilute*-like coat color appeared at the 10th generation in SMXA-21, but was separated from the strain, and inbred. We designated these progeny as SMXA-21d. A total of 180 mice (F₂₂–F₂₈) more than 90 days old, 3 males and 3 females of each strain, were used to generate DNA fingerprints. Mice were raised in a room conditioned to a tempera-

ture of 23 ± 2 °C and 50–60% humidity. They were fed a commercial diet for mice (F-1, Funabashi Farm Co. Ltd.) and tap water *ad libitum*.

DNA fingerprinting: DNA was extracted from liver. The liver was homogenized in a solution of TNE (10 mM Tris-HCl pH 7.5, 0.1M NaCl and 1 mM EDTA) and centrifuged at 3,000 g for 5 minutes after filtration. The pellet was suspended in TNE and dissolved in a solution of 0.5% N-lauroylsarcosine sodium salt, 10 mM Tris-HCl pH 8.0 and 10 mM EDTA. After phenol extraction, DNA was precipitated by the addition of 2 volumes of ethanol and dissolved in a solution of 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA. The DNA was digested overnight with restriction enzymes (*Hinf* I, *Pst* I), electrophoresed in a 1.2%, 20- or 40-cm long agarose gel at 2V/cm for 80 h, and transferred to nitrocellulose membranes (S & S). Single-strand M13 phage DNA was labeled by primer extension for use as a probe [21]. Hybridization was carried out in a solution of 6 × SSC, 40% formamide, 15 mM EDTA, and 0.25% skim milk at 37 °C. Washing stringency was 55 °C in 2 × SSC and 0.1% SDS. The membranes were autoradiographed at –80 °C for 2–4 days with an intensifying screen.

Linkage analysis: Linkage analysis was carried out by comparing the SDPs for minisatellite loci with known SDPs for biochemical, immunological and microsatellite loci which had been mapped previously on chromosomes in SMXA RI strains (Nishimura, M., Hirayama, N., Serikawa, T., Katoh, H., and Kojima, A.) with Map Manager (Manly, K.F.).

Results

DNA fingerprinting in SMXA RI strains

DNA fingerprinting was used to study the genetic homogeneity of SMXA RI strains of mice, novel inbred strains which have been established recently. DNA fingerprints for SMXA-8 and SMXA-9 are shown in Fig. 1. All mice within the same strain showed a similar banding pattern regardless of sex. Thus, these mice are genetically homogeneous within the same strain. In the same way, we examined the other SMXA RI strains. All mice within the same strain showed a similar banding pattern. Thus, all of the SMXA RI strains could be regarded as inbred strains. Since band-

ing patterns did not differ between sexes, we could not detect the DNA fragment originating in the sex chromosome.

As polymorphic DNA fragments in SMXA RI strains were detected by DNA fingerprinting, the usefulness of DNA fingerprinting for identification of SMXA RI strains was explored. Figs. 2 and 3 show DNA fingerprints, obtained with *Hinf I* and *Pst I* restriction enzymes, for SMXA-1–SMXA-16 and their progenitors A/J and SM/J. A/J and SM/J showed strain-specific banding patterns but shared some common DNA fragments. Most of the DNA fragments scored in all SMXA RI strains could be traced back to those scored in either the A/J or SM/J progenitor strain. These fragments of DNA fingerprints are therefore inherited over 20 generations as stable genetic traits. In DNA fingerprints obtained by using *Hinf I* as a restriction enzyme (Fig. 2), SMXA RI strains showed several fragments

which both progenitor strains, A/J and SM/J, lacked. In contrast, a few fragments present in both progenitor strains were lacking in all SMXA RI strains. These fragments (Fig. 2) might be regarded as mutants generated during selective brother-sister mating to establish each SMXA RI strain. There was no difference in DNA fingerprints between SMXA-21 and SMXA-21d except for a fragment thought to be a mutation (not the *dilute*-like mutation). Except for these mutants, the number of fragments showing clear polymorphism totaled 40:23 in DNA fingerprints obtained with *Hinf I* as the restriction enzyme (Fig. 2) and 17 in DNA fingerprints obtained with *Pst I* (Fig. 3). Of these 40 fragments, either of the two bracketed fragments was always found in each of the RI strains, indicating that

Fig. 1. Examples of genetic monitoring for SMXA RI strains of mice by DNA fingerprinting. Fragment sizes are indicated to the right.

Fig. 2. DNA fingerprints of SMXA RI strains of mice (1–16) and their progenitor strains generated by M13 probe with *Hinf I* digest. Short solid lines (—) on the left indicate sites showing polymorphic bands through RI sites. Allelic bands are shown in brackets. The 23 scored bands define 22 distinct loci termed *Ms13-Hf1* to *Ms13-Hf22*. Arrowheads indicate mutant fragments that occurred spontaneously during successive inbreeding.

Fig. 3. DNA fingerprints of SMXA RI strains of mice (1–16) and their progenitor strains generated by M13 probe with *Pst I* digest. The 16 scored bands define 15 distinct loci termed *Ms13-Ps1* to *Ms13-Ps15*. *Ms13-Ps16* is omitted from this figure owing to the small fragment size (2.3 Kb).

these fragments have a relation to an allele. We could detect two pairs of fragments having the allelic relation mentioned. These two pairs of fragments were also found in DNA fingerprints of other inbred mouse strains (C3H/HeJah, C57BL/6Jah, DDD/Jah, NC/Jah, RR/Jah) (data not shown). Thus, we detected 38 minisatellite loci in total. In the present study, we have designated them as *Ms13-Hf1*, *Ms13-Hf2*, ... (minisatellite locus, M13 phage probe and restriction enzyme *Hinf I*) according to fragment size, and *Ms13-Ps1*, *Ms13-Ps2*, ... (restriction enzyme *Pst I*). Strain distribution patterns (SDPs) of the 38 minisatellite loci are given in Table 1. None of the SMXA RI strains had the same distribution pattern as any other, except for SMXA-21 and SMXA-21d (which can be differentiated by using the mutant fragment). Thus, DNA fingerprinting is a suitable method for identifying SMXA RI strains.

Linkage analysis of minisatellite locus

RI strains are very useful for gene mapping, especially for establishing a preliminary map position for a novel gene which is responsible for a trait differing in progenitor strains. In order to localize the above 38 minisatellite loci on the mouse linkage map, linkage analysis was carried out by comparing the SDPs of minisatellite loci with known SDPs for other loci which had been mapped previously on chromosomes in SMXA RI strains. Significant linkages with known loci were found for 23 of the 38 minisatellite loci detected (Table 2). These 23 loci were dispersed over 11 autosomes.

Discussion

Minisatellites detected by DNA fingerprinting have not been influenced by selective pressure, because they do not express as any traits. Their loci therefore have extremely high polymorphism, and they are widely dispersed on chromosomes. For these reasons, DNA fingerprinting is likely to be useful for strain identification and genetic monitoring, not only of standard inbred strains but also of sublines of mice. As RI strains of mice consist of a set of inbred strains derived from two inbred strains, the genetic diversity between RI strains should be less than that between less closely related inbred strains of standard laboratory mice. In the present investigation, nevertheless, DNA fingerprinting with the M13 phage probe was applicable to genetic monitoring and identification of SMXA RI strains. In such species as the laboratory rabbit, golden hamster and mastomys, which have not been studied in detail for genetic variation, it has been difficult to confirm the establishment or genetic homogeneity of an inbred strain, because few genetic markers can be applied for genetic monitoring. DNA fingerprinting can be applied widely even to such species [3, 17, 18], and should be suitable for genetic monitoring of their inbred strains.

Minisatellite loci are widely dispersed on most chromosomes, but their mapping is not easy. Jeffreys *et al.* [9] and Julier *et al.* [10] assigned minisatellite loci to some specific chromosomes by linkage analysis with BXD RI strains of mice. In this study, 23 of the 38 minisatellite loci detected in SMXA RI strains could be assigned to chromosomes by linkage analysis. As shown in Table 2, *Car-1*, *D3Mit11* and *Cdm*, marker

Table 1. Strain distribution patterns of minisatellite loci in SMXA recombinant inbred strains

		SMXA																														
<i>Locus</i>	scored ^a	1	3	4	5	6	7	8	9	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	3				
		0	1	2	4	5	6	7	8	9	0	1	2	4	5	6	7	8	9	0	1	1d	2	3	4	5	6	7	9	0		
<i>Ms13-Ps</i>	1	AS	A	A	S	A	S	A	A	A	A	S	S	A	A	A	A	S	S	A	A	S	S	S	A	A	S	S	A			
	2	S	A	A	A	S	A	A	S	A	S	A	S	S	A	A	A	A	S	A	S	S	S	A	S	S	S	A	A	S		
	3	S	S	A	A	S	A	S	S	S	A	S	S	S	S	A	A	S	A	S	S	S	S	S	A	A	S	S	A	S		
	4	A	S	A	A	A	S	S	S	S	A	A	S	S	S	A	A	A	A	A	A	A	S	A	S	S	S	A	A	S		
	5	A	A	A	A	S	A	A	S	A	S	S	A	S	A	A	A	A	S	A	S	S	A	A	S	S	S	S	A	S		
	6	A	S	A	A	A	S	S	S	A	A	S	A	S	S	A	A	S	S	S	S	S	S	A	A	S	A	S	S	S		
	7	S	A	S	A	A	A	S	A	S	A	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	A	A	S		
	8	S	S	S	S	S	S	A	S	S	A	S	A	A	S	A	A	A	S	A	A	S	S	S	A	S	A	A	A	S		
	9	S	S	A	A	S	S	S	S	A	A	S	S	A	S	S	A	A	S	S	S	S	S	A	A	A	S	A	S	S		
	10	S	S	S	A	S	S	A	A	S	A	A	S	A	S	A	S	S	A	S	S	S	A	A	S	A	A	S	A	A		
	11	A	S	A	S	A	S	S	A	S	A	S	S	S	A	S	A	S	A	S	A	S	A	A	S	A	A	A	A	S		
	12	S	A	A	A	S	A	A	A	A	S	A	S	S	S	A	A	A	S	S	S	S	S	S	S	A	S	S	S	S		
	13	A	S	S	S	S	A	S	A	S	A	A	S	A	A	S	S	S	A	S	A	A	S	S	S	A	S	A	A	S		
	14	A	S	A	A	S	S	S	S	A	A	A	S	A	S	S	A	A	S	S	S	S	S	A	A	A	S	S	S	S		
	15	S	S	S	S	A	A	S	A	A	S	S	S	S	S	S	S	S	S	A	S	S	S	S	A	A	A	A	A	A		
	16	A	S	S	A	S	S	S	S	A	S	S	S	A	S	A	S	S	A	S	S	S	S	S	S	S	S	A	S	S		
<i>Ms13-Hf</i>	1	S	S	S	A	S	A	S	A	A	A	A	S	S	S	S	S	S	S	S	S	A	A	S	S	S	A	A	S	A	A	
	2	A	S	S	A	S	A	S	A	A	A	A	S	S	S	S	A	S	A	S	A	S	A	A	A	S	S	A	S	A	A	
	3	S	S	A	S	A	S	A	A	A	S	S	S	S	A	S	S	A	A	A	S	S	A	A	A	S	S	A	A	S		
	4	S	A	A	A	S	A	A	S	S	A	A	S	A	A	A	A	A	A	S	A	A	A	A	A	A	A	A	A	S	A	
	5	A	A	A	S	A	S	A	A	A	A	A	A	A	A	A	A	A	S	S	S	A	A	S	S	S	S	A	S	S	S	
	6	S	A	A	A	A	A	S	A	A	A	A	A	A	A	A	S	A	A	A	A	S	S	A	A	A	A	S	S	S	A	
	7	A	S	S	S	S	S	S	A	A	A	S	S	S	S	A	S	S	S	A	S	S	S	S	A	A	S	S	S	S	A	
	8	S	S	A	A	S	S	S	A	S	A	S	A	S	S	A	A	A	S	S	S	S	S	S	S	A	S	S	S	A	A	
	9	A	A	A	S	S	A	S	S	S	S	A	S	A	A	A	S	S	S	S	A	A	S	S	A	A	S	A	A	A	A	
	10	A	A	A	S	S	S	S	A	A	S	S	S	A	A	A	S	A	S	S	A	A	S	A	S	A	S	A	A	A	A	
	11	S	A	A	A	A	S	A	S	S	A	S	A	S	S	S	S	S	S	S	A	A	S	S	S	S	A	S	S	A	A	
	12	A	S	A	A	S	S	S	S	A	A	S	S	A	S	S	A	A	S	S	S	S	S	S	A	A	A	S	S	S	S	
	13	S	S	S	A	S	S	S	A	S	S	S	A	A	S	S	S	S	S	S	S	A	A	S	S	S	S	S	S	S	S	
	14	A	S	A	S	S	A	S	A	A	S	A	A	S	A	A	S	S	S	S	S	A	A	S	S	S	A	S	A	A	A	
	15	S	A	S	S	S	A	A	A	A	S	A	S	A	A	A	A	S	A	A	S	S	S	A	A	S	S	S	S	S	S	
	16	AS	A	S	A	S	A	A	A	A	S	A	S	A	A	A	A	S	A	A	S	S	S	A	A	S	A	S	A	S	A	
	17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	A	A	A	S	S	S	S	S	S	
	18	S	A	A	S	S	S	A	A	A	S	A	S	A	S	A	A	A	S	A	A	A	S	A	A	A	A	A	A	S	A	A
	19	A	A	A	S	A	A	S	S	S	A	A	A	A	A	A	S	A	A	A	A	A	A	S	A	A	A	A	S	S	S	
	20	S	S	A	S	A	S	S	A	A	A	S	A	S	S	A	A	S	A	S	A	S	S	S	S	S	S	A	S	S	A	
	21	A	S	A	S	A	S	S	A	A	A	A	A	A	A	A	A	A	S	S	S	S	A	S	S	S	A	S	S	A	A	
	22	A	A	A	S	A	S	A	A	A	A	A	S	S	A	A	A	A	S	S	A	A	S	S	S	A	A	S	A	S	A	

^a: Progenitor strain having DNA fragment in each locus, A; A/J, S; SM/J.

genes in chromosome 3, are located 4, 69 and 82cM from the centromere, respectively. This suggested that *Ms13-Hf10*, *Ms13-Hf13* and *Ms13-Hf16* linked these marker genes are also dispersed in this chromosome. This is equally true of chromosomes 9, 11 and 12. Thus minisatellite loci detected with the M13 phage probe are widely dispersed not only on most chromosomes

but also in each chromosome. The other 15 loci which were not assigned in this study would be assigned to each chromosome if the SDP of SMXA RI strains increased. The results suggested that genetic monitoring by DNA fingerprinting with M13 phage DNA can be used in monitoring almost all chromosomes.

Table 2. Linkage between minisatellite loci and other genetic markers typed in SMXA RI strains

<i>Ms locus</i> ^a	Chromosome	Marker gene (cM) ^b	Recombinants/ tested RI strains	Estimated map distance, cM (99% confidence limits)
<i>Ms13-Ps8</i>	1	<i>D1Mit8</i> (26)	5/27	6.4 (1.1–32.6)
<i>Ms13-Ps13</i>	1	<i>D1Mit8</i> (26)	1/27	1.0 (0.0– 9.7)
<i>Ms13-Hf14</i>	1	<i>D1Mit8</i> (26)	2/27	2.1 (0.1–13.7)
<i>Ms13-Hf10</i>	3	<i>Car-1</i> (4)	1/27	1.0 (0.0– 9.7)
<i>Ms13-Hf13</i>	3	<i>D3Mit11</i> (69)	5/27	6.4 (1.1–32.6)
<i>Ms13-Hf16</i>	3	<i>Cdm</i> (82)	5/26	6.8 (1.2–35.8)
<i>Ms13-Ps10</i>	5	<i>D5Mit6</i> (40)	0/27	0.0 (0.0– 6.1)
<i>Ms13-Hf3</i>	7	<i>D7Mit17</i> (45)	6/27	8.3 (1.7–43.8)
<i>Ms13-Hf18</i>	9	<i>D9Mit10</i> (43)	5/27	6.4 (1.1–32.6)
<i>Ms13-Ps5</i>	9	<i>D9Mit14</i> (54)	6/27	8.3 (1.7–43.8)
<i>Ms13-Ps12</i>	9	<i>D9Mit8</i> (66)	5/27	6.4 (1.1–32.6)
<i>Ms13-Hf8</i>	9	<i>M10</i>	0/27	0.0 (0.0– 6.1)
<i>Ms13-Hf4</i>	10	<i>D10Mit14</i> (74)	6/27	8.3 (1.7–43.8)
<i>Ms13-Ps1</i>	11	<i>D11Mit16</i> (4)	5/27	6.4 (1.1–32.6)
<i>Ms13-Hf5</i>	11	<i>D11Mit16</i> (4)	4/27	4.8 (0.7–24.6)
<i>Ms13-Hf22</i>	11	<i>D11Mit16</i> (4)	5/27	6.4 (1.1–32.6)
<i>Ms13-Hf21</i>	11	<i>D11Mit14</i> (56)	6/27	8.3 (1.7–43.8)
<i>Ms13-Ps7</i>	12	<i>Ly-18</i> (13)	4/26	5.0 (0.7–26.6)
<i>Ms13-Hf9</i>	12	<i>D12Mit5</i> (30)	3/27	3.3 (0.3–18.5)
<i>Ms13-Hf1</i>	14	<i>Es-10</i> (18)	6/27	8.3 (1.7–43.8)
<i>Ms13-Hf2</i>	14	<i>Es-10</i> (18)	4/27	4.8 (0.7–24.6)
<i>Ms13-Ps4</i>	16	<i>GT-53</i> (35)	5/27	6.4 (1.1–32.6)
<i>Ms13-Ps15</i>	18	<i>D18Mit17</i> (21)	2/27	2.1 (0.1–13.7)

^a: minisatellite locus. ^b: map distance from the centromere.

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