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Slc:Wistar outbred rats show close genetic similarity with F344 inbred rats

Satoshi NAKANISHI[1)], Tadao SERIKAWA[1, 2)], and Takashi KURAMOTO[1)]

[1)]*Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto 606-8501, Japan*
[2)]*Laboratory of Pharmacology, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan*

Abstract: Although Slc:Wistar rats are used widely in biomedical research as outbred rats, close similarities in growth curves, survival rates, and immunological and biochemical phenotypes have been reported between Slc:Wistar and F344 inbred rats. We reported previously that nine genetic variations that were fixed in Slc:Wistar rats had identical genotypes in F344 rats. Here, we examined the genetic characteristics of Slc:Wistar rats using 27 simple-sequence length polymorphism (SSLP) markers and compared them with other Wistar stocks available in Japan and with some F344 strains. Among 27 SSLP loci, 23 (85%) were fixed in the Slc:Wistar rats, which was the highest among the other Wistar stocks. The 23 fixed loci shared identical genotypes with corresponding loci in F344 rats. Further, the predominant allele types in the unfixed loci had allele frequencies as high as 80%, and these alleles were identical in the F344 rats. When the nine genetic variations reported previously are added, a total of 32 (89%) out of the 36 loci examined were fixed and identical in the Slc:Wistar and F344 rat genomes. These findings indicate the low genetic variation in Slc:Wistar rats and the high genetic similarity between the Slc:Wistar and F344 inbred rats. This study demonstrates the importance of characterizing outbred rats and the need to pay ample attention to the genetic characteristics the Slc:Wistar rats for their proper use.
Key words: F344 inbred rats, genetic characteristics, SSLP markers, Slc:Wistar, Wistar outbred rats

Introduction

Wistar rats are albino and outbred and originate from a stock established in the Wistar Institute (Philadelphia, PA, USA) in the early 1900's. Rats from the Wistar Institute were distributed globally and now there are many stocks available from different breeders in different countries. It is well known that Wistar rats from different breeders have different characteristics, although they are still called by the common stock name, "Wistar" [5, 11].

Slc:Wistar rats are used widely in Japan and are available from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). This stock is derived from a seed stock that was provided by the Institute of Medical Science (The University of Tokyo, Japan) in 1968. Slc:Wistar rats show rapid growth, good temperament, relatively small body size, and high survival rates in long-term experiments. They are used widely in toxicological, carcinogenic, pharmacological, and general biomedical studies [3, 9].

The similarity in phenotypes between Slc:Wistar and F344 inbred strains has been described in several previous studies. Organ distribution and histological types of spontaneous tumors observed in Slc:Wistar rats were reported to be very similar to these phenotypes in F344/

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Address corresponding: T. Kuramoto, Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto 606-8501, Japan
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DuCrj inbred rats [3]. Growth curves and survival rates in male and female Slc:Wistar rats were similar to those found in male and female F344/DuCrj inbred rats [8]. The similarity in immunological phenotypes was also observed. For example, spontaneous leukemia developed in Slc:Wistar rats could be transplanted into F344/NSlc rats [10]; spontaneous bladder carcinoma developed in F344/DuCrj rats could be transplanted into Slc:Wistar rats; and skin grafts from F344/NSlc to Slc:Wistar were also accepted [8]. Furthermore, the liver cytosolic fractions from Slc:Wistar and F344/DuCrj rats showed similar enzymatic activity against a selective monoamine oxidase A inhibitor [5]. Thus, several researchers have reported that Slc:Wistar outbred and F344 inbred rats show similar phenotypes in some developmental, immunological, and biochemical characteristics, implying that they may share similar genetic backgrounds.

An earlier study of the allele frequencies of nine functional polymorphisms (genetic variations associated with gene functions) in various outbred stocks revealed that all nine loci were fixed in the Slc:Wistar rats and their genotypes were identical to those in the F344 inbred strain [1]. Thus, although Slc:Wistar rats are treated as an outbred stock, they seem to have relatively small genetic variations and have a similar genetic background as the F344 inbred strain. To examine extensively the genetic characteristics of the Slc:Wistar rats, in this study, we determined the genotypes of 27 simple sequence length polymorphism (SSLP) marker loci in Slc:Wistar rats and calculated the allele frequencies of these loci. To compare allele frequencies, we used different Wistar outbred stocks available in Japan and F344 inbred substrains.

Materials and Methods

Genomic DNA samples

The genomic DNA samples used in this study were from those prepared in our previous study [1]. These samples were obtained from female Crj:WI (Glx/BRL/Han) IGS (n=31), Crlj:Wistar (n=31), Jcl:Wistar (n=32), BrlHan:WIST (n=32), and Slc:Wistar (n=31) outbred rats. Rats of each stock were thought to be derived from different litters. The genomic DNA samples from F344/NSlc, F344/DuCrlCrlj, F344/Stm, and F344/Jcl inbred strains were obtained from the National BioResource Project for the Rat (NBRP-Rat) in Japan [7].

Genotyping

Twenty-seven SSLP markers were used to genotype each rat (Fig. 1). Twenty of the SSLP makers are used in routine genetic monitoring in the NBRP-Rat [2], because they are highly polymorphic: average number of alleles per marker is 13.9 ± 3.5 (mean ± SD) [4]. Seven of the SSLP markers were selected especially for this study to allow us to survey genomic regions that the 20 SSLP markers could not cover. PCR products were analyzed with the MultiNA Microchip Electrophoresis System (Shimadzu, Kyoto, Japan). It can resolve more than about 5% difference in length of the PCR products. So, alleles of which size differences are less than the resolution are thought to be called as an identical allele type. To decease such false negative calling, some primer sets were redesigned and the size of PCR products was reduced. The allele types in each marker locus were determined based on the size of the PCR products and were called “a”, “b”, “c”, and so on, in ascending order of size. The allele frequency of each allele in the genomic DNA sample from each stock was calculated.

Results

Genetic homogeneity of Slc:Wistar outbred stock

All the SSLP markers generated specific and clear PCR products (Fig. 2). The alleles were called based on the sizes of the PCR products for each locus and the allele frequencies were calculated (Supplementary Table 1). When only one allele type was observed in a particular locus, the locus was regarded as a fixed locus. The numbers of fixed loci among the 27 examined were as follows: four (15%) in the BrlHan:WIST, five (19%) in the Crl:WI (Glx/BRL/Han) IGS, six (23%) in the Crlj:Wistar, 18 (67%) in the Jcl:Wistar, and 23 (85%) in the Slc:Wistar stocks. In the four unfixed loci in the Slc:Wistar stock, particular allele types were predominant (Fig. 3). These findings suggest that the Slc:Wistar stock has marked genetic homogeneity compared with the other Wistar outbred stocks tested.

Genetic characteristics of Slc:Wistar outbred stock was similar to those of the F344 strains

That the genetic backgrounds of Slc:Wistar rats and F344 inbred strains are very similar has been reported previously [1, 3, 8]. Therefore, we compared allele types that were fixed in the Slc:Wistar rats with the corresponding alleles in the F344 inbred strains. All 23 fixed loci

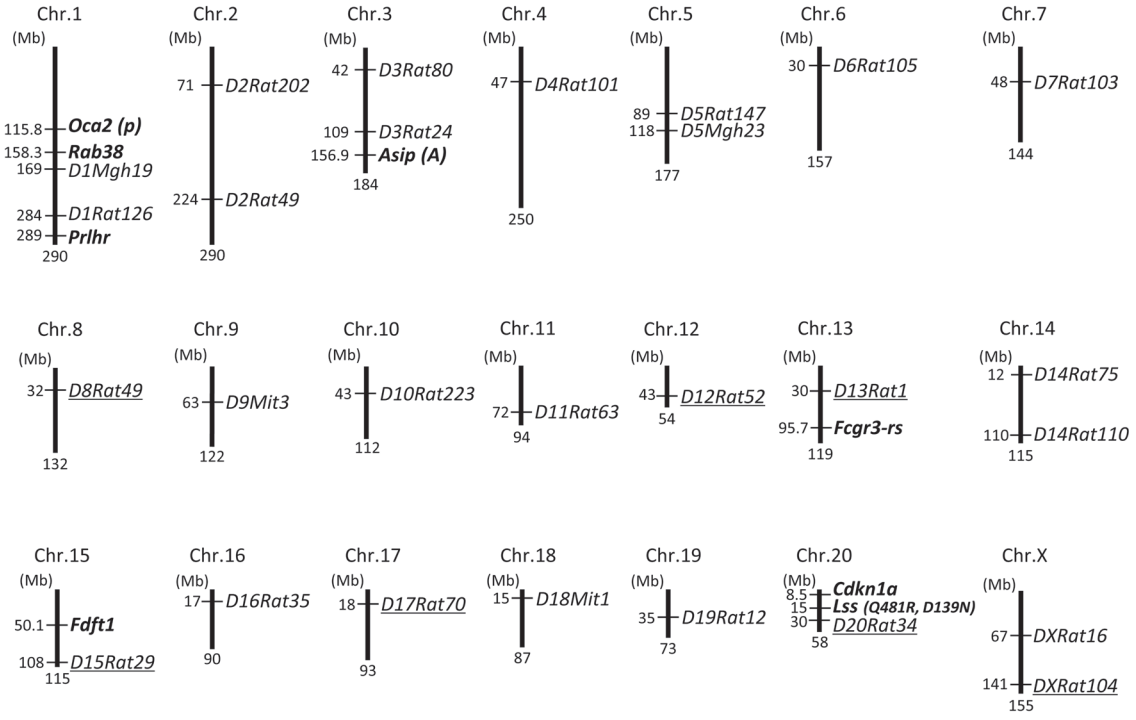

Fig. 1. Chromosomal locations of the 27 simple sequence length polymorphism (SSLP) markers and nine functional polymorphic loci in the rat genome.
Twenty of the SSLP makers are used in the routine genetic monitoring in NBRP-Rat. Seven SSLP markers that were selected for this study are underlined. The nine functional polymorphic loci from a previous study [1] are in bold. Physical positions of markers and physical length of chromosomes are referred to the rat genome assembly: Rnor_5.0.

in the Slc:Wistar rats had the same genotypes as the corresponding loci in the F344/NSlc, F344/Stm, and F344/Jcl strains, and only one of the loci (*DXRat16*) was different in F344/DuCrlCrlj (Fig. 3). In addition, for the four unfixed loci in the Slc:Wistar rats, the predominant allele types (allele frequencies reached about 80%) were identical in the F344 inbred strains. With the addition of the nine functional polymorphic loci we reported previously [1], a total of 32 (89%) out of the 36 loci examined so far were found to be fixed in the Slc:Wistar rats and identical between the Slc:Wistar and F344 rats. These findings strongly suggest that the genetic backgrounds of the Slc:Wistar rats and F344 inbred strains are very similar.

Discussion

It is important to know the genetic characteristics of the rats to be used in experimental studies so that the most suitable rats for a particular experimental purpose can be selected. In this study, we surveyed 27 SSLP loci and found that 23 (85%) of them were fixed in the Slc:Wistar rats, and this was much higher than the percentage found among the four other Wistar outbred stocks. The genotypes of the 23 fixed loci in the Slc:Wistar rats were all the same as of the genotypes of the corresponding loci in the F344/Jcl, F344/NSlc, and F344/Stm inbred strains. These findings confirmed the markedly small genetic diversity in Slc:Wistar rats and the close similarity in genetic characteristics among the Slc:Wistar rats and the F344 inbred rat strains.

BrlHan:WIST originates from Wistar Hannover that was kept in Central Institute for Laboratory Animal Breeding (Hannover, Germany). In 1989, it was moved to RCC Ltd., (Fullinsdorf, Switzerland) and then shipped to CLEA Japan Inc. in 1998. Crl:WI (Glx/BRL/Han) IGS also originates Wistar Hannover. It was rederived by Glaxo Wellcome from Wistar Hannover stock supplied by RCC Ltd.. It was transferred to Charles River UK in 1996, to Charles River USA in 1997, and to Charles River Japan in 2007. Crlj:Wistar was shipped to Charles River Laboratories UK in 1947 from Wistar Institute. It was transferred to Charles River USA in 1975 and to Charles River Japan in 1981. Jcl:Wistar was transferred

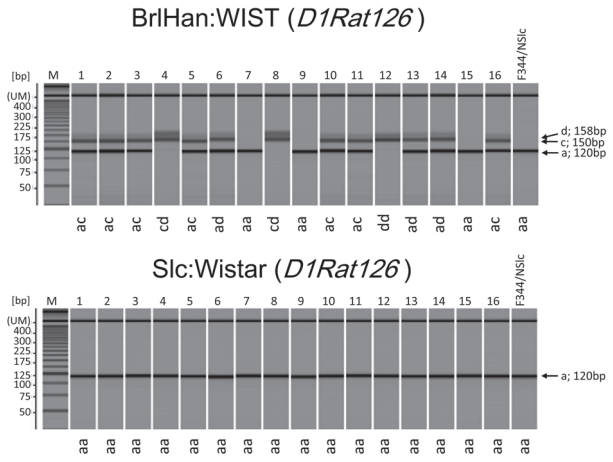

Fig. 2. Gel images of the PCR products and genotypes in a representative locus (*D1Rat126*) of BrlHan:WIST and Slc:Wistar outbred rats.
PCR products from 16 BrlHan:WIST outbred rats (upper) and 16 Slc:Wistar outbred rats (lower) are shown. The allele types were called based on the sizes of the PCR products. In the *D1Rat126* locus, three different PCR products were detected. The smallest (~120 bp), intermediate (~150 bp), and largest (~158 bp) products were called "a", "c", and "d", respectively. The allele "b" (~140 bp) was not observed in BrlHan:WIST rats. Note that all 16 Slc:Wistar rats were homozygous for the "a" allele. M is the 25-bp DNA ladder.

from Carworth (UK) to CLEA Japan in 1970. Although these stocks have been kept by random breeding or rotation system to ensure that genetic diversity is maintained, genetic drift is inevitable over time. Thus, percentages of the fixed loci in Wistar stocks examined here appeared to depend to time after they were transferred.

The Slc:Wistar stock has been bred by the rotation system among four groups. Well-grown and healthy rats from the third or fourth litters are selected from the stock for the production of the next generation (Breeding Control Standards, Japan SLC Inc., 2011). Until now, no other stocks or strains have been added to the Slc:Wistar stock. Thus, why the Slc:Wistar rats share extensive genetic similarity with the F344 inbred rat strains needs to be considered. Possible explanations may include unexpected genetic contamination, random genetic drift, and artificial selective breeding for good growth and health. However, in such cases, the resultant genetic background might be expected to be a mosaic of F344 and Wistar rats. Therefore, it is likely that the Wistar seed stock that was transferred from the Institute of Medical Science (The University of Tokyo, Japan) to Japan SLC, Inc. (Hamamatsu, Japan) already had ge-

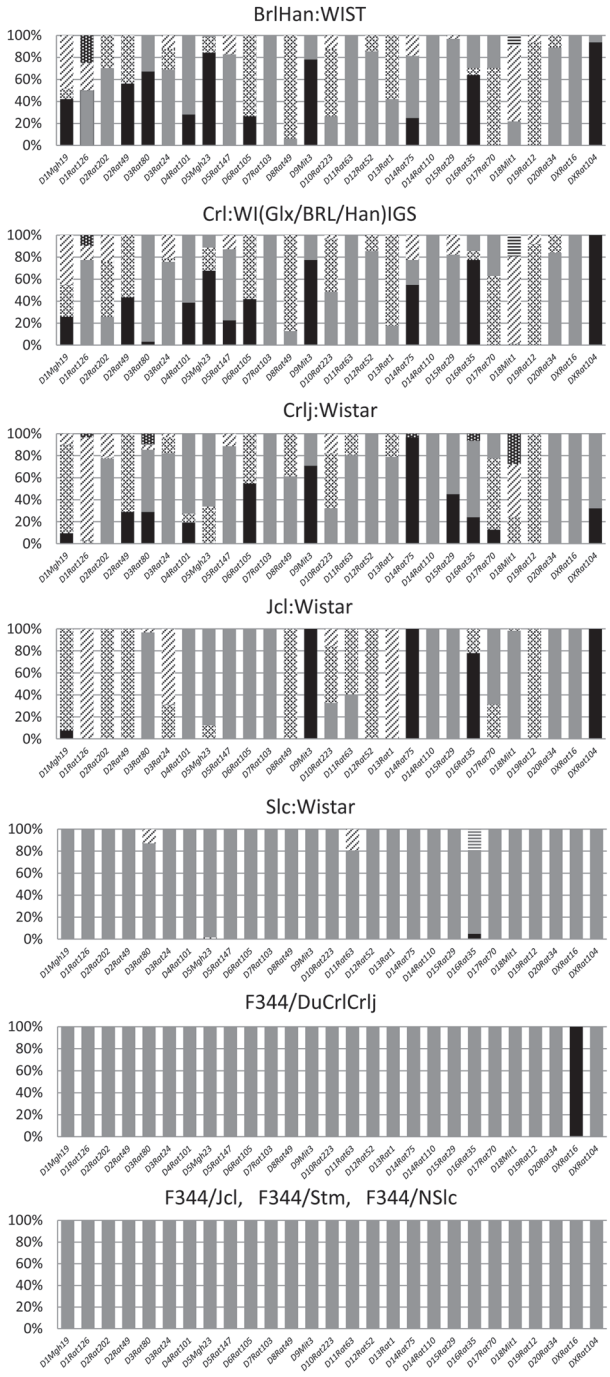

Fig. 3. Distribution of the 27 simple sequence length polymorphism marker alleles in Wistar outbred stocks and F344 strains.
The distribution of each allele is shown for each locus in each stock. Alleles that are identical to the corresponding alleles in F344/Jcl, F344/NSlc, and F344/Stm are shown as solid grey bars. Other allele types are shown as shaded, diagonal, tiled, or solid black bars. Almost all the loci in the Slc:Wistar rats were fixed and identical with of the corresponding loci in the F344 inbred strains. The dominant allele types in the unfixed loci were also identical to the corresponding loci in the F344 strains.

netic characteristics that were very similar to those of the F344 rat strain. Alternatively, an undetected mix-up occurred during the transportation of the seed stock or during the microbiological cleaning of the stock.

Only small numbers of genetic markers were available in rats before the systematic development of SSLP markers occurred in the 1990's [6]. Thus, the genetic characterization of the Slc:Wistar stock was difficult even after the close phenotypic similarity between the Slc:Wistar and F344 rats was pointed out [8]. Now, using 27 SSLP markers, we have demonstrated clearly that the Slc:Wistar rats and F344 inbred strain share markedly similar genetic characteristics but about 10% of loci is different between them. The Slc:Wistar rats are neither the so-called outbred nor an inbred strain. Therefore, we suggest that the supplier of the Slc:Wistar rats should consider closing of the colony to prevent confusions that arise in using of the stock as being outbred Wistar rats.

In summary, we examined the genetic characteristics of Slc:Wistar rats using 27 SSLP markers and found that they were very similar to the genetic characteristics of F344 inbred rats. This finding raises the need to be cautious about using Slc:Wistar rats as outbred animals.

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