An Integrated Rat Genome Map Based on Genetic and Cytogenetic Data

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Abstract: In this study we combined three major rat genome maps, by adding 66 markers to the Kyoto Laboratory Animal Science map (KLAS map), and constructed an integrated map. The resultant integrated map consists of 5,682 redundant markers, spanning a genetic length of 2,028 cM. Eighty genetic markers were anchored to the cytogenetic map, fixing all the genetic maps in the physically correct orientation. This map encapsulates the progress in rat mapping studies in past years and offers useful information for QTL analysis. The map figures are available at <http://www.anim.med.kyoto-u.ac.jp/>.

Key words: cytogenetic map, genetic map, genome map, integrated map, rat

Introduction

The laboratory rat, *Rattus norvegicus*, is a major animal model for physiological and pathophysiological studies. Significant progress in genetic fields has led to many important pathophysiological findings regarding numerous rat disease models. Many mapping results were published in past years, and partial genome maps were constructed by several groups [3, 5, 6, 9–11, 13]. Among these linkage maps, two should be emphasized: the Wellcome Trust Centre map (WTC) [2] and the Whitehead Institute/MIT map (WI/MIT) [14]. Each of these two maps densely covers the vast majority of the entire rat genome by using simple sequence length polymorphism (SSLP) markers. For both maps, detailed information is available via the Internet. All primers can be obtained commercially, and the WI/MIT map is

connected to a unique database. This database provides SSLP profiles of many rat inbred strains used worldwide for scientific research.

Advancements have also been achieved by anchoring genetic markers to the cytogenetic maps and by anchoring cytogenetic markers to the genetic maps. Early publications in this field reported data regarding a single chromosome [15], whereby specific genes were mapped genetically and cytogenetically. More extensive studies have been reported by two groups [1, 12]. These groups fixed the linkage maps in correct orientation for each chromosome, excluding chromosome Y, by determining the genetic and cytogenetic localization of anchor markers. Unfortunately, these maps lack dense coverage of informative markers, which is necessary for any studies concerning genetic dissection of complex diseases and analysis of congenic lines.

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In response, we combined three different genetic maps in this study. Two maps (WTC and WI/MIT) were marker-rich, whereas the Kyoto Laboratory Animal Sciences Map (KLAS map) is a linkage map illustrated with the cytogenetic map. The KLAS map was revised with markers that had been localized in at least one of the two other maps. Cytogenetic data obtained from Szpirer *et al.* [12] were also included in this study.

Materials and Methods

Retrieved Maps

Data from the WI/MIT and WTC maps were obtained from corresponding Internet web sites in May, 1999: WTC: <http://www.well.ox.ac.uk/~bihoreau/key.html> (767 markers) and WI/MIT: <http://waldo.wi.mit.edu/rat/public/> (4,616 markers; only the data from F₂ SHRSP × BN were utilized). Marker information in these maps was taken to construct a linking map that combines the linkage information in these two dense genetic maps with cytogenetic data [1, 12].

KLAS map (Kyoto Laboratory Animal Science map)

Five backcrosses, (ACI × F344) × F344, (ACI × WTC) × WTC, (BN × WTC) × WTC, (SHR × BN) × BN and (ZI × TM) × ZI were raised at the Institute of Laboratory Animals, Kyoto University. The sixth backcross (TM × KDP) × KDP [16] was supplied by Dr. K. Komeda of the Animal Research Center, Tokyo Medical College. Each cross contained at least 48 animals. To develop the linkage map, the most informative backcross panels showing the largest number of polymorphic markers were selected on each chromosome. In several chromosomes, for which more crosses than one were used to construct linkage maps, two or more common markers on different crosses were genotyped to combine independent linkage groups into one linkage map. Detailed information on the crosses used is shown in Table 1. To revise the KLAS map, 64 markers, shown in Fig. 1, were newly genotyped as described previously [11]. Furthermore, two SSLP markers with the following primer sequences were developed in this study: *Calm2p3* 5'-AGCTCGTTGAGTGATTGATC-3' and 5'-GAAGAGTTTtagCTCACCATCCCCAT-3'; *Hprt* 5'-AAAGTAGAGAATGACAGAGC-3' and 5'-AAAAAGAAAAAGAAAAGATG-3'. The segregation

Table 1. Backcrosses used in linkage analysis for each chromosome in the KLAS map

Chromosome	Backcrosses
1	(SHR × BN) × BN (ACI × WTC) × WTC
2	(SHR × BN) × BN
3	(SHR × BN) × BN (BN × WTC) × WTC
4	(BN × WTC) × WTC
5	(SHR × BN) × BN (ACI × WTC) × WTC
6	(SHR × BN) × BN (ACI × WTC) × WTC
7	(BN × WTC) × WTC (ZI × TM) × ZI
8	(SHR × BN) × BN (TM × KDP) × KDP
9	(SHR × BN) × BN
10	(BN × WTC) × WTC (ACI × F344) × F344
11	(SHR × BN) × BN
12	(SHR × BN) × BN
13	(SHR × BN) × BN (ACI × WTC) × WTC
14	(BN × WTC) × WTC
15	(SHR × BN) × BN
16	(SHR × BN) × BN
17	(SHR × BN) × BN (BN × WTC) × WTC
18	(SHR × BN) × BN (TM × KDP) × KDP
19	(ACI × WTC) × WTC
20	(SHR × BN) × BN
X	(ACI × WTC) × WTC

Fig. 1. The revised KLAS map, accompanied by the new version of the cytogenetic map (A), was combined with two other genetic maps, the WTC (B) and WI/MIT (C) maps. Both the cytogenetic and the genetic map in the KLAS map (Andoh *et al.* 1998) are drawn according to the recommendations of the Subcommittee on Rat Gene and Chromosome Nomenclature (Levan *et al.* 1995). Newly mapped markers on the KLAS map are labeled with an asterisk. Bold characters indicate relations to the WI/MIT or WTC map; underlined markers are anchored in the cytogenetic map. Distances between loci are indicated in cM. The genetic length of each chromosome is drawn below the KLAS map. U-d connotes upside down, indicating that these chromosomes had to be inverted to physically correct orientation.

Chromosome 5

Chromosome 6

Chromosome 7

Chromosome 8

Chromosome 9

Chromosome 10

patterns of these markers in the backcross panels were analyzed with the computer program, MAPMAKER/EXP (ver. 3.0 b) [4]. The recombination frequencies were converted to centiMorgan (cM) by means of the Kosambi mapping function. Altogether 322 markers

were used to construct the KLAS map.

Combining the maps

Three maps, WTC, WI/MIT and KLAS, were combined manually with markers that were common in at

least two maps. Only the KLAS map contains anchored markers accessible to both genetic and cytogenetic maps, so that via this KLAS map, the WTC

and WI/MIT maps were connected to the cytogenetic map.

Results

The new version of the KLAS map contained 322 SSLP markers and spanned 2,028 cM altogether. The average distance between neighboring markers was approximately 6.8 cM. The largest gap was found between *Pbpc2* and *D1Mgh12*, comprising 36.1 cM, on Chromosome 1. The chromosome length varied from 206.5 cM (Chr. 1) to 42.4 cM (Chr 20). The lod scores for linkage between two contiguous loci were 2 or more. The revised KLAS map integrated genetic markers derived from the WTC and WI/MIT maps, as well as several cytogenetic markers [12]. One-hundred-twenty-eight genetic markers were shared between the KLAS and WTC maps, and 103 between the KLAS and WI/MIT maps. For this reason, it was possible to determine the correct orientation of all three genetic maps. Chromosomes 3, 6, 7, 8, 9, 10, 11, 16, 18 and 19 of the WI/MIT map were inverted to the correct orientation as mentioned previously [1, 12]. Chromosomes 1, 2, 3, 6, 9, 10, 11, 12, 14, 17, 18, 19, 20 and X of the WTC map were also inverted to their cytogenetically correct orientation. Maps for all chromosomes are shown in Fig. 1 and are available at the following Web site: <http://www.anim.med.kyoto-u.ac.jp/>. The length of each chromosome was compared among the three genetic maps (Fig. 2). In total, the KLAS map spans a genetic length of 2,028 cM, the WTC map, 1,998 cM, and the last

version of the WI/MIT map, 1,586 cM (calculated from the map of F₂ (SHRSP × BN)).

Discussion

Since rat genetic maps were first published in the early 1990s, the number of mapped genes and markers has greatly increased. Recently more than 5,000 markers have been accurately localized in the rat linkage maps. Accompanying the development of SSLP markers, the genetic length of the rat genome map has been extended. The revised KLAS map in this study spans 2,028 cM. This length is comparable to the WTC map (1.5% larger), but it is 22% longer than the WI/MIT map (F₂ SHRSP × BN). The apparent length differences between the KLAS and WI/MIT maps have been found for chromosomes 1, 2, 4, 6, 10 and X (Fig. 2). Interestingly, our map, which utilizes the smallest number of markers, is the longest. The genetic length of linkage maps is influenced by many factors. Possible reasons for this genetic length difference among the three maps are: First, the KLAS map was constructed with backcross panels, in which only female meioses are included in calculation, whereas the WTC and WI/MIT maps were constructed with intercross panels, in which both male and female meioses are included in calculation. Since the recombination rate in female gametes is greater than that in male gametes on aver-

Fig. 2. Comparison of the length of all chromosomes in the three genetic maps.

age [8], the KLAS map would be longer than the other two maps. Second, to obtain maximum recombination data, we selected the backcross(es) on each chromosome showing the greatest number of polymorphic SSLP markers between the parental strains. For example, (SHR $\times$ BN) $\times$ BN and (ACI $\times$ WTC) $\times$ WTC were chosen to construct a linkage map of Chromosome 1, whereas (BN $\times$ WTC) $\times$ WTC were chosen for Chromosome 4. By means of this procedure, recombination events may be detected more efficiently in the KLAS map than in the WTC and WI/MIT maps.

In this study, the revised KLAS map was combined with two other genetic maps, the WTC and WI/MIT maps, and accompanied by the new version of the cytogenetic map. Comprehensive genetic studies in rats, such as genetic dissection of multifactorial traits, would be more easily performed with the benefit of this integrated map.

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