Genetic Analyses of Fancy Rat-Derived Mutations

Takashi KURAMOTO, Mayuko YOKOE, Kayoko YAGASAKI, Tatsuya KAWAGUCHI, Kenta KUMAFUJI, and Tadao SERIKAWA

Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

Abstract: To collect rat mutations and increase the value of the rat model system, we introduced fancy-derived mutations to the laboratory and carried out genetic analyses. Six fancy rats were shipped from a fancy rat colony in the USA and used as founders. After initial crosses with a laboratory strain, TM/Kyo or PVG/Seac, inbreeding started and 6 partially inbred lines, including 2 sublines, were produced as Kyoto Fancy Rat Stock (KFRS) strains. During inbreeding, we isolated 9 mutations: 5 coat colors, American mink (am), Black eye (Be), grey (g), Pearl (Pel), siamese (sia); 1 coat pattern, head spot (hs); 2 coat textures, Rex (Re), satin (sat); and an ear pinnae malformation, dumbo (dmbo). Genetic analyses mapped 7 mutations to particular regions of the rat chromosomes (Chr): am to Chr 1, sia to Chr 1, sat to Chr 3, Re to Chr 7, g to Chr 8, dmbo to Chr 14, and hs to Chr 15. Candidate gene analysis revealed that a missense mutation in the tyrosinase gene, Ser79Pro, was responsible for sia. From mutant phenotypes and mapping positions, it is likely that all mutations isolated in this study were unique to the fancy rat. These findings suggest that fancy rat colonies are a good source for collecting rat mutations. The fancy-derived mutations, made available to biomedical research in the current study, will increase the scientific value of laboratory rats.

Key words: bioresource, coat color, genetic mapping, inbreeding, mutation

Introduction

Genetic analyses of common diseases in humans have revealed that gene mutations are involved in diseases. Genome sequencing projects of various mammalian species followed by comparative genome analyses have revealed that a large number of genes are shared among species. Thus, it is thought that mutations found in model animals and animals carrying such mutations can contribute to the better understanding of human diseases.

The laboratory rat (Rattus norvegicus) has been widely used as an animal model of human diseases, because its size is suitable for manipulation [1, 27]. Sequencing of the rat genome has shown that the rat has about 20,000 predicted genes and shares as many as 90% with humans [9]. So far, at least 70 mutations have been identified as causative genes of specific diseases and rat strains carrying such mutations can be used as good animal models for these diseases; however, considering the high number of rat genes predicted [9], more mutations will be required to investigate the full range of diseases. Thus,
collecting rat mutations and making rats carrying these mutations available as bioresources would enhance the scientific value of rats as an animal model for human diseases.

There are several approaches to collecting rat mutations. They include discovering naturally occurring mutations and inducing mutations by random mutagenesis [21]. In addition, attempts have been made to collect mutations outside of the laboratory, from the field or fancy rat colonies; indeed, some inbred strains have been established from wild captured rats [11]. However, fancy rats have not been surveyed as a source of mutants, with a few exceptions [26].

Fancy rat colonies have potential as a source for collecting novel rat mutations, because various mutations are considered to persist only in fancy rats, largely coat and eye color mutations, and coat pattern mutations. Thus, when they are available in laboratory rats, most will provide opportunities to study the function of melanocytes, which are not only responsible for pigment synthesis in the skin and hair, but are also involved in inner ear and eye functions [30]. In addition, in human, dysfunctions of melanocytes result in skin disorders such as oculocutaneous albinism, piebaldism and skin cancers [12, 29], prompting us to introduce mutations found in the current fancy rat colonies to the laboratory and establish them as novel bioresources available for biomedical research.

In this study, we imported 6 fancy rats from a fancy rat colony in the USA to our laboratory. We tried to isolate fancy mutations and establish inbred strains carrying them. During inbreeding, we isolated 9 mutations, of which 7 were mapped to particular genomic regions of rat chromosomes. A coat color mutation, siamese, was identified as a missense mutation in the rat tyrosinase gene.

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**Materials and Methods**

**Animals**

In July, 2005, 6 fancy rats were imported from a fancy rat colony named Spoiled Ratten Rattery (SRR) kept by Ms. E. Brooks in Kansas City, Missouri, USA (http://www.spoiledratten.com/index.html). These rats (SRR01-06) were used as founders to establish fancy-derived strains. SRR01 was female and the others were males. It was known that these founders carried the following mutations (Table 1): SRR01 carried dumbo (dmbo), Rex (Re), and satin (sat); SRR02 carried sat and siamese (sia); SRR03 carried American mink (am), grey (g), and Pearl (Pel); SRR04 carried dumbo (dmbo); SRR05 carried Re; and SRR06 carried Black eye (Be). TM/Kyo and PVG/Seac rats were selected as mating partners to obtain progeny from the founder fancy rats, because they are homozygous for nonagouti (a/a) and hooded (h/h) recessive mutations. SRR01, SRR05, and SRR06 were crossed with the TM/Kyo strain and SRR02, SRR03, and SRR04 were crossed with the PVG/Seac strain. Following caesarean operations, F1 hybrids were introduced to specific pathogen-free (SPF) facilities in our institute. Brother-sister mating was carried out to establish fancy rat-derived strains for each founder. At each generation during inbreeding, rats showing the mutant phenotypes were selected. When different mutant phenotypes were found in an inbreeding line, sublines were separated.

To map the mutations isolated from fancy rats, a male rat representing each strain was used to make F1 hybrids with BN/SsNSlc (BN) or WTC/Kyo (WTC) female rats. Animal care and experimental procedures were approved by the Animal Research Committee, Kyoto University and were conducted according to the Regulation on Animal Experimentation at Kyoto University.

**Genetic mapping**

To map sat and sia mutations, SRR02 (F5) was mated with BN rats and 82 backcross progeny (BCP) were produced (cross 1). To map am, SRR03-am (F6) was mated with BN rats and 98 BCP were produced (cross 2). To map g and Pel, SRR03-g, Pel (F6) was mated with BN rats and 87 BCP were produced (cross 3). To map dmbo and hs, SRR04 (F5) was mated with BN rats and 99 BCP were produced (cross 4). To map Re, SRR05 (F3) was mated with BN rats and 50 BCP were produced (cross 5). To map Be, SRR06 (F6) was mated with BN and WTC rats, and 48 and 67 BCP were produced (crosses 6 and 7).

Genotyping was performed as described previously [16] with a set of highly informative simple sequence length polymorphism (SSLP) markers [20].
Table 1. Mutations isolated from fancy rats

<table>
<thead>
<tr>
<th>Mutation (symbol)</th>
<th>MP term (MP id)</th>
<th>Characteristic</th>
<th>Origin(ab)</th>
<th>KFRS strain</th>
<th>Mode of inheritance</th>
<th>Mapped position in rats</th>
<th>Candidate gene name (Gene symbol)</th>
<th>Mutant phenotype of candidate gene in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>American mink (am)</td>
<td>diluted coat color (0000371)</td>
<td>Light brown body hair [26]</td>
<td>Unknown. Different from the original mink described by Robinson</td>
<td>KFRS3A/Kyo</td>
<td>recessive</td>
<td>1 MB</td>
<td>Herman- sky-Pulilak syndrom 5 (Hps5)</td>
<td>Mice Homozygous for Hps5 mutation (ruby-2) have hypopigmented eyes and hair [33]</td>
</tr>
<tr>
<td>Black eye (Be)</td>
<td>diluted coat color (0000371)</td>
<td>Cream coat with pigmented eyes</td>
<td>Laboratory colony at Edinburgh University in Scotland in 1998 → Breeder in England</td>
<td>KFRS6/Kyo</td>
<td>dominant</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>dumbo (dmbo)</td>
<td>abnormal outer ear morphology (0002177)</td>
<td>Ears are set lower on the head, and are larger and rounder.</td>
<td>Fancy rats somewhere in the northwest US</td>
<td>KFRS4/Kyo</td>
<td>recessive</td>
<td>14 MB</td>
<td>H6 homeobox 1 (Hmx1)</td>
<td>Mice carrying Hmx1 mutations exhibit enlarged ear pinnae with a distinctive ventrolateral shift [23]</td>
</tr>
<tr>
<td>grey (g)</td>
<td>diluted coat color (0000371)</td>
<td>Light grey body hair</td>
<td>Maybe Russian blue. From fancies of east coast US.</td>
<td>KFRS3B/Kyo</td>
<td>recessive</td>
<td>8 MB</td>
<td>RR27A, member RAS oncogene family (Rab27a), myosin VA (Myo5a)</td>
<td>Gene defects produce abnormal pigmenta- tion and a gray or diluted coat color in ashen or dilute mice [22, 32] and dop rats [8].</td>
</tr>
<tr>
<td>head spot (hs)</td>
<td>head head spot (0002939)</td>
<td>White spotting on the head</td>
<td>Unknown</td>
<td>KFRS4/Kyo</td>
<td>recessive</td>
<td>15 MB</td>
<td>endothelin receptor type B (Ederb)</td>
<td>Mice homozygous for the Ederb mutation show irregular white spotting, depending on the genetic back- ground [25]</td>
</tr>
<tr>
<td>Pearle (Pel)</td>
<td>diluted coat color (0000410), embryonic lethality (0008762)</td>
<td>Lighter coat color expressed on mink or grey. Homozygotes die in the embryonic period (E10-E12)</td>
<td>English fancy [26]</td>
<td>KFRS3A/Kyo, KFRS3B/Kyo</td>
<td>dominant</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Rex (Re)</td>
<td>wavy hair (0000410), nude (0003815), wavy vibrisses (0001279)</td>
<td>Heterozygotes show wavy body hair, while homozygotes lose body hair after the first molt. Both heterozygotes and homozygotes show wavy vibrisses.</td>
<td>England → Breeder in California</td>
<td>KFRS5/Kyo</td>
<td>dominant</td>
<td>7 MB</td>
<td>keratin 71 (Krt71)</td>
<td>Mouse mutations in the Krt71 gene, caracul (Ca), cause wavy coat hairs in Cu+/ heterozygous mice [14]</td>
</tr>
<tr>
<td>satin (sat)</td>
<td>abnormal coat appearance (0001510), curly vibrisses (0001274)</td>
<td>Longer hair and shiny-looking “greasy” hair. Vibrisses are bent downward.</td>
<td>Fancy rats kept by a breeder in California</td>
<td>KFRS2/Kyo</td>
<td>recessive</td>
<td>3 MB</td>
<td>fibroblast growth factor 7 (Fgf7)</td>
<td>Mice lacking the Fgf7 gene develop a matted coat [10]</td>
</tr>
<tr>
<td>siamese (sia)</td>
<td>diluted coat color (0000371)</td>
<td>Homozygotes show light body hair, but their ears, nose, tail, and scrotum are dark, as in Siamese cats. Eyes are slightly pigmented and appear red.</td>
<td>Laboratory in France in the 1980s → Breeder in UK → breeders in California</td>
<td>KFRS2/Kyo</td>
<td>recessive</td>
<td>1 MB</td>
<td>tyrosinase (Tyr)</td>
<td>Mice homozygous for Tyr+ show light coat color and darkened ears, nose, and scrotum. [18]</td>
</tr>
</tbody>
</table>

4b: Mutant phenotypes are classified by mammalian phenotype ontology. 5b: Provided by Ms. E. Brooks. 6a: RGSC v3.4. 6b: Expected theoretical maximum distance between am or Re and non-recombinant markers. Physical distance corresponding to 1 cM was expected to be 1 Mb.
Direct sequencing of the Tyr gene of Black-eyed and Siamese rats

Direct sequencing was performed as described previously [17]. Rat Tyr exons were amplified with the following 6 sets of primers: rTyr-1&2 (exon 1,463 bp), 5'-TGTTTGAGCAGATCTTGTACG-3' and 5'-TGTTTTGCCAAAGTGAGGTAA-3'; rTyr-3&11 (exon 1,633 bp) 5'-GCGGAACGTGAAAGTTTGGA-3' and 5'-AAGGTTCCTTTTCTGTGCTGA-3'; rTyr-12&13 (exon 2,398 bp), 5'-TTTCATTCATATGTAAGTCCCTTG-3' and 5'-GCTTAGCATTGCAAAACTACA-3'; rTyr-14&15 (exon 3,384 bp), 5'-TTGTTTATTAAAATTAGGCTTACCTC-3' and 5'-TCTCAAAATAGAGAACCACCAAA-3'; rTyr-16&17 (exon 4,488 bp), 5'-AAGGTTCCTTTTCTGTGCTGA-3' and 5'-AAGGTTCCTTTTCTGTGCTGA-3'; rTyr-18&10 (exon 5,489 bp), 5'-GCACTCAAACCCAAGCATCT-3' and 5'-TTCCCTAGAAGACTGGGACGTG-3'.

Examination of fetuses at cesarean section

Six wild-type SRR03 females (+/+) and six Pel-heterozygous female SRR03 (Pel/+ ) rats were mated with the Pel-heterozygous SRR03 males (Pel/+ ). At P20, fetuses were removed by cesarean section. The numbers of corpora lutea, live fetuses, and embryo-fetal deaths were counted. Embryo-fetal deaths were categorized into early death (implantation sites, resorbed embryos, and placental remnants) and late death (early macerated fetuses, late macerated fetuses, and dead fetuses). The number of implantations was calculated from the sum of the number of live fetuses and the number of embryo-fetal deaths.

Statistical analysis

To determine the mode of inheritance and linkage relationship, chi-square tests were performed. When the P value of chi-square for 1:1 was more than 0.05, the mutation was thought to be an autosomal single gene. When the P value of chi-square for linkage was less than 0.05, the linkage relationship between loci was thought to be significant. For statistical analysis of embryo-fetal deaths found in the Pearl mutant, Student’s t-test was performed using Microsoft Excel.

Results

Fancy rat-derived strains

We isolated 9 mutations during inbreeding and assigned “Mammalian Phenotype terms (MP)” to their mutant phenotypes to make it easy to understand them [31] (Table 1). They involved 5 coat color mutations (am, Be, g, Pel, and sia), 1 coat pattern mutation (hs), 2 coat texture mutations (Re and sat), and an ear pinnae malformation mutation (dmbo). The Pearl phenotype manifested in conjunction with homozygous status for am or g.

During inbreeding, the line originating from SRR01 became extinct. Although inbreeding was not fully completed, we tentatively named the derived lines Kyoto Fancy Rat Stock (KFRS). Each strain was defined with a number representing the names of the founder rats, and sublines were defined by the addition of a letter after the number. Six lines, including sublines, were produced and their strain names, mutations they carried, and generations at the end of February, 2010 were as follows: KFRS2/Kyo carrying sat and sia (F18), KFRS3A/Kyo carrying am and Pel (F19), KFRS3B/Kyo carrying g and Pel (F20), KFRS4/Kyo carrying dmbo and hs (F18), KFRS5A/Kyo carrying Re (F19), and KFRS6/Kyo carrying Be (F17) (Fig. 1 and Table 1).

Mode of inheritance and genetically mapped region of fancy mutations

In cross 1, 40 had satin-type body hair and 42 had normal body hair. Thirty-six had a siamese coat color, while forty-six had normal coat color. These findings indicated that both the sat and sia mutations were autosomal recessive. The linkage map including sat was D3Got76 – 1.2 cM – D3Got69, sat – 1.2 cM – D3Mco2. The sat locus spanned the 9.1-Mb region defined by D3Got76 and D3Mco2. The linkage map including sia was D1Rat273 – 2.4 cM – sia – 2.4 cM – D1Rat138. The sia locus spanned the 4.9-Mb region defined by D1Rat273 and D1Rat138.

In cross 2, 47 had American mink-type body hair and 51 had normal body hair, indicating the am mutation was autosomal recessive. The am showed no recombination with D1Rat214 and D1Mgh35 in 98 meioses, which indicated that am was located <3.0 cM away from these
markers with 95% probability [7].

In cross 3, 46 had grey-type body hair and 41 had normal body hair, indicating the g mutation was autosomal recessive. The linkage map including g was $D8Rat36 - 6.9\, cM - D8Rat182, g - 14.9\, cM - D8Rat131$. The g locus spanned the 37.9-Mb region defined by
D8Rat36 and D8Rat131.

In cross 4, 55 had dumbo-type ears and 44 had normal ears. Forty-five had white spots on their head and forty-four had no head spots. These findings indicated that both dmbo and hs mutations were autosomal recessive. The linkage map including dmbo was D14Arb10 – 1.0 cM – D14Rat37, dmbo – 6.1 cM – D14Rat57. The dmbo locus spanned the 5.7-Mb region defined by D14Rat10 and D14Rat57. The linkage map including hs was D15Got78 – 5 cM – hs – 12 cM – D15Rat26. The hs locus spanned the 6.6-Mb region defined by D15Got78 and D15Rat26.

In cross 5, 24 had Rex-type body hair and 26 had normal body hair, indicating that the Re mutation was autosomal dominant. Re showed no recombination with D7Mtl1 and D7Rat80 in 50 meioses, indicating that Re was located <5.8 cM from these markers with 95% probability [7].

Using crosses 6 and 7, we carried out genetic analysis of the Be mutation. In rat fanciers, it is known that the Re mutation masks the coat color only in combination with the albino mutation. This combination produces rats with a pale creamy white coat color and black eyes. To elucidate the inheritance pattern of the black eye, we first crossed a SRR06 male with BN/SsNSlc (a/a, b/b, C/C) rats. Since all (BN/SsNSlc × SRR06)F1 rats had a black coat and pigmented eyes, we backcrossed F1 females to SRR06 males. In cross 6, 27 had a white coat with black eyes, and 21 had a colored coat with black eyes. The phenotype of the white coat with black eyes was completely cosegregated with a missense mutation (data not shown).

Identification of siamese as a missense mutation in the Tyrosinase gene

Tyrosinase (Tyr) was thought to be a good candidate for sia, because mouse himalayan mutation (h) at the Tyr locus showed an extremely similar coat color phenotype to the siamese rat. Direct sequencing of the Tyr gene of the SRR06 genome demonstrated that SRR06 also harbored the Arg299His missense mutation (data not shown).

To elucidate the inheritance pattern of the black eye on the albino background, we crossed a SRR06 male with albino WTC/Kyo (a/a, B/B, c/c) rats. All (WTC/Kyo × SRR06)F1 rats had a white coat and black eyes. We then backcrossed the F1 females to WTC/Kyo males. In cross 7, 29 had a white coat with black eyes, and 38 had a colored coat with black eyes. These findings indicated that the Be mutation was a single autosomal mutation and manifested dominantly only in the presence of the albino mutation in the homozygous state.

Table 2. Number of embryo-fetal deaths found in Pearl mutants

<table>
<thead>
<tr>
<th>Stage of embryo-fetal death</th>
<th>Cross to produce embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+ × Pel/+</td>
</tr>
<tr>
<td>Implantation site</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Resorbed embryo</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>Placental remnant</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Early macerated fetus</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Late macerated fetus</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Dead fetus</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Total</td>
<td>0.2 ± 0.4</td>
</tr>
</tbody>
</table>

*: P<0.05, **: P<0.01.

Embryonic lethality of the Pearl (Pel) mutation

There were no significant differences in the numbers of corpora lutea [12.5 ± 1.6 vs. 12.7 ± 0.8 (mean ± SD), P=0.42] and implantations (12.2 ± 1.6 vs. 11.7 ± 0.5, P=0.25) between wild-type (+/+ ) and Pearl (Pel/+ ) females both crossed with Pearl (Pel/+ ) males. Meanwhile, embryo-fetus deaths were significantly higher in (Pel/+ × Pel/+ )F1 embryos than in (+/+ × Pel/+ )F1 embryos: 3.5 ± 0.8 vs. 0.2 ± 0.4, P<0.01 (Table 2). Embryo-fetus deaths found in (Pel/+ × Pel/+ )F1 embryos included resorbed embryos (3.0 ± 0.6) and placental remnant (0.5 ± 0.5). The proportion of embryo-fetus deaths with regard to the number of corpora lutea in (Pel/+ × Pel/+ )F1 was 27.5%, which agreed with 25% embryo-fetus death when homozygous lethality occurred in Pel/Pel embryos.
153

FANCY RAT- DERIVED MUTATIONS

Discussion

From mapped positions and phenotype resemblances to existing mutations of rats or mice, we selected candidate genes for the fancy mutations (Table 1). For Myo5a and Ednrb, rat mutations have been identified: dilute-opisthotonus (dop) mutation in Myo5a [8] and spotting lethal (sl) mutation in Ednrb [15]. We confirmed the absence of these mutations in KFRS3B/Kyo and head spot-homozygous KFRS4/Kyo rats (data not shown). Therefore, all the fancy mutations isolated were likely to be unique and our study has made them available to the laboratory.

The Ser79Pro missense mutation was completely cosegregated with the siamese phenotype and was specific to KFRS2/Kyo. Missense mutations around the 79th amino acid of TYR provoke albinism in mice and humans, suggesting that this region plays an important role in hair and skin pigmentation [3, 24]. Therefore, we concluded that the S79P missense mutation is responsible for the siamese phenotype in rats. Tyrosinase is the key enzyme involved in the melanin biosynthetic pathway and is responsible for the rate limiting step [5]. Mutations in the TYR gene cause human oculocutaneous albinism 1 (OCA1) [24]. Although there are more than 100 mutations in the mouse Tyr locus, such as albino (Arg77Leu), himalayan (His420Arg), and chinchilla (Ala482Thr) [3], increasing the range of Tyr mutations will provide a wealth of information on the biology of tyrosinase and lead to better understanding of the pathogenesis of OCA1.

In addition to previous work on the Pearl phenotype [26], we revealed that approx. 25% embryos were largely resorbed, suggesting that Pel/Pel embryos die in the early stage of organogenesis (gestation days 10 to 12) [6]. There is a close relationship between Pel and agouti (A) [26]. In the current study, we carried out preliminary genetic analysis using 46 g-homozygous rats from cross 3. However, we failed to find a linkage relationship between Pel and D3Mit2, a SSLP marker located 2 cM apart from A, which suggests that multiple genetic determinants might be involved in the expression of Pel.

To our knowledge, this study is the first report on the systemic introduction of fancy-derived mutations to the laboratory. Fancy rats are considered to be a good source for developing a new bioresource of rats. They allow us to isolate rat mutations effectively. Usually, the rate at which new mutations arise spontaneously is exceedingly low: it is known that, on average, only one gamete in 100,000 is likely to carry a detectable mutation at any single locus naturally occurring mutation rate [28], which means that the discovery of mutations depends on chance. In this study, we could isolate 9 unique mutations from only 6 founder rats, and it took only a few generations to isolate them. Moreover, fancy rats are usually kept by outbreeding, so when they are subjected to inbreeding in the laboratory, hidden mutations sometimes manifest. Actually, we observed the cataract and sterile phenotypes, which were unknown in the SRR, at several generations after starting inbreeding (data not shown).

Fancy rat colonies are thought to be maintained relatively independently of laboratory rats and have unique breeding histories different from the laboratory rats [2]. Therefore, it is expected that the fancy-derived KFRS strains will retain their unique genetic background different from laboratory rats, although almost half of them are derived from laboratory rats. The IS/Kyo strain originates from a cross of a wild captured male rat with Wistar female rats [13] and shows a clearly different
genetic background from other strains [20]. Systematic phenotypic analysis of IS/Kyo rats uncovered their unique traits, such as hypotension and high body temperature, which implies that a wild-derived genome might confer these unique traits [19]. Following several generations, all KFRS strains will be established as full inbred strains. Thus, we consider that the systematic genotype and phenotype analyses of these KFRSs will reveal their genetic background and untapped unique traits, which make them potential disease models. Finally, phenotypically annotated KFRSs will contribute to increase the scientific value of rats.

Acknowledgments

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