Japanese Wild Mice: A Rich Resource for New Disease Models

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Abstract: Breeding of fancy mice has been a tradition in Japan. Recent progress in animal science has shed a new light on Japanese wild-derived mice as tools for discovery of new disease models because these mice, *Mus musculus molossinus*, are genetically far remote from the majority of available laboratory mice. After decades of effort, five inbred strains of mice have been established from pairs of wild mice trapped in Tohoku, northeastern Japan, namely KOR1/Stm, KOR5/Stm, KOR7/Stm, AIZ/Stm, and MAE/Stm. They carried numerous mutations, leading to a variety of diseases. During the inbreeding of KOR1, the first spontaneous mutation was found in the *ApoE* (apolipoprotein E) gene, and the mutant was later designated as spontaneous hyperlipidemic (SHL). Thereafter, a number of other mutations were discovered among wild-derived inbred strains, including atopic dermatitis, microphthalmia, dominant white spots, sebaceous gland abnormalities, and audible song-like vocalization. Furthermore, to examine the possible effects of the genetic background for these mutant genes, sets of congenic strains were generated, in which the mutant gene was introduced into at least 3 different strains of laboratory mice, including BALB/c and C57BL/6. These congenic strains have now been established as novel disease models. These wild-derived inbred strains serve as a treasure trove for novel disease models. Most of them have been deposited in the Riken BioResource Center (BRC), and some are also available from commercial breeders.

Key words: hyperlipidemia, KOR1, *M. m. molossinus*, mutant strains, wild mice

Introduction

Recent progress in genome sciences has enabled us to efficiently identify disease-related genes in both human and animal diseases [7, 21, 28]. To find these disease-related genes, excellent animal disease models are indispensable, since they provide important information regarding the genetic composition underlying many diseases, molecular insight into disease processes, and hints for therapeutic targets, irrespective of obvious species barriers. The majority of disease models have been reported in laboratory strains of mice and rats. Since laboratory strains are derived from a relatively narrow ancestry [29, 30], exploration of much broader resources should be a promising source of novel disease models. Japanese rodents are advantageous for study because of...
their geographic isolation from the ancestors of laboratory mice. This article is a summary of the use of fancy mice in Japan and a review of attempts to establish inbred strains from Japanese wild mice with a spectrum of mutations.

**Breeding of Fancy Mice, a Tradition in Japan**

Rats and mice are generally considered to be destructive vermin that threaten food supplies, dwellings, and furnishings. They have historically spread infectious diseases, such as bubonic plague and smallpox [22]. Despite their reputation, mice have commonly been kept as pets in Japan. Mice are featured in Japanese folk stories in positive roles, such as messengers from deities and harbingers of fortune, and they were bred as fancy mice in the Edo Period (1603–1867). In 1787, the Japanese booklet *Chingan-sodategusa*, “A guidebook for fancy mice breeding,” was published in Kyoto. The book describes various kinds of fancy mice with visible mutations, such as coat colors, body size, and behavior, most of which are evident in today’s mutant stocks. It also provides information on the maintenance of mutant mice, including methods for feeding and watering according to the season. The genetic knowledge contained in the booklet is generally accurate in the light of Mendelian genetics. An English translation of this book was published in 1935 as “An 18th Century Japanese Guidebook on Mouse Breeding” [26].

Fancy mice are featured in Japanese fine art. The followings are examples of mice featured in art from the author’s private collection. Figure 1 is a Kutani porcelain bowl with a painting of three mice with different coat colors playing on a koto, a Japanese harp. Several famous painters of *ukiyo* (Japanese Edo woodblock prints) were also fascinated with fancy mice. In an *ukiyo* print (Fig. 2), two mice are depicted in the hands of a geisha holding a basket for carrying mice in her left hand. The mice have mottled necks, suggesting that their coat color was favored. In another *ukiyo* print (Fig. 3), a woman plays with a mouse crawling on her arm, and a finely manufactured wood and wire mesh breeding cage is shown. These *ukiyo* prints suggest that mouse and rat breeding were favored during the Edo Period. Unfortunately, it is not possible to determine whether the animals are rats or mice, since the rodents were collectively referred to as *nezumi* in Japanese, and were not realistically depicted by the *ukiyo* painters.

**New Light on Wild Mice as a Genetic Bio-Resource**

In 1983, a book entitled *Genetic Control of Laboratory Animals* (in Japanese) was published in honor of Professor Kondo’s retirement from Nagoya University. He emphasized that genetic issues will be increasingly important in the future [6], since it will be necessary to consider the genetic differences among humans when discussing medical issues.

Even in the 1960s, there were more than 200 major inbred strains of laboratory mice. The majority of them had been bred in the US since the early 20th century. Kondo claimed that the genetic diversity of these classical laboratory mice was limited because the majority of the strains originated from *M. m. domesticus* or *M. m. musculus*, the subspecies distributed in Western Europe, and their genetic diversity decreased significantly during long periods of selection and inbreeding. Therefore, he developed inbred strains from native Japanese fancy mice. In 1944, he visited a country house in Kasukabe, Saitama Prefecture, where he learned that farmers had been breeding fancy mice to sell at roadside stands since the Edo Period. The mice he obtained there were the
origin of the KK (Kasukabe-K line) mice [18], which later became a world-famous model for type 2 diabetes. Furthermore, he developed the inbred strain NC, which was derived from a pair of pet mice called ‘Nishiki nezumi’ purchased in a bird shop at about the same time. This line was called the Nishiki-Cinnamon mouse because of the distinct color of its coat, and, later, much attention was given to this line as a model for atopic dermatitis [4, 24, 27]. In the 1960s, Kondo started to capture wild house mice (M. m. molossinus) in the suburbs of Anjo, Aichi Prefecture, where the Faculty of Agriculture of Nagoya University was located. From these wild mice, the inbred strains Molossinus Anjo (MOA) and Molossinus Mizuho (MOM) were established.

**Wild Mice in the Tohoku Area**

Our motivation to study mouse genetics was initially triggered by the identification of polymorphism in mouse salivary and tear proteins among strains and sexes 30 years ago at Ohu University in Koriyama, Fukushima Prefecture. Some polymorphic salivary proteins are Spe1-r, Spe1-s, and Spe2, and some tear proteins are Tpe1 and Tpe2 [3, 9, 11–13]. To examine this issue in more detail, a variety of mice were collected from many institutions in Japan as well as from the Jackson Laboratory (Bar Harbor, ME, USA). Simultaneously, from the aforementioned book, *Genetic Control of Laboratory Animals*, it was clear that Kondo had collected Japanese wild mice in the Nagoya area and had established several wild mouse-derived inbred strains.

Inspired by Kondo’s pioneering works, we examined the saliva and tear protein types in wild mice of Fukushima Prefecture. The geographical distance, about 400 km, between Fukushima and Aichi seemed to assure sufficient genetic distance between the wild mice in both areas (Fig. 4). We first interviewed local residents to determine if *nezumi* (rats or mice) had been seen in the neighborhood. We asked the locals to capture the animals for us. To catch them alive, Sherman live traps were used. Starting from Fukushima, the collection fields were soon expanded to the entire Tohoku area (northeastern Japan). When male and female mice were caught in the same spot, breeding pairs were created to establish inbred strains. After 15 generations or more,
their pups, obtained either by Caesarean section or transplantation of fertilized eggs, were transferred to a specific pathogen-free (SPF) facility.

Breeding wild-derived mice requires more proficient skills than breeding laboratory mice. Some breeding guidelines are presented below. Wild mice are much more agile than laboratory mice and are likely to jump out when their cages are opened inadvertently. Therefore, it is recommended that they should be handled in large plastic containers (Fig. 5a). Even after repeated inbreeding, they retain the highly active physical capabilities that laboratory mice have lost. Wild-derived mice are generally smaller than laboratory mice; thus, their pups can readily escape through the standard mesh of a cage lid at about 3 weeks of age. To prevent escape, a cage cover with a fine mesh should be used. For effective breeding of wild-captured mice, paper cups and tissue paper are effective for hiding and nesting places (Fig. 5b). In general, a wild-derived strain with long domestication periods will no longer require these additional items.

We started to inbreed 12 strains of wild-derived mice from 7 collection areas within a 10-km area in Koriyama. After more than 10 years, only five strains have been successfully established as inbred strains, KoR1/Stm (F50), KoR5/Stm (F61), KoR7/Stm (F54), AIZ/Stm (F50), and MAE/StmRbrc (F49) (Fig. 6). Other strains could not be established as inbred strains because of their poor fertility. Among wild mice, a broad range of variation was found even in the same collection area, and such variation was often retained after extensive inbreeding. By comparing the behavioral activities of C57BL/6, a representative laboratory strain, and several wild-derived strains, it became evident that wild-derived mice are

![Fig. 4. Interrelationship among wild mice-derived inbred strains established in Japan. (a) Simplified phylogenetic relationship among Japanese wild-derived mice based on the analysis of 60 wild-derived strains of mice with 1,446 SNPs by the neighbor-joining method (Mekada, Yoshi, personal communication with permission). The bootstrap values represent the likeliness of genetic bifurcation. The bar shows the genetic distance. *JF1 is an inbred strain of mouse originally purchased in Denmark as “Japanese mice,” which was later shown to be closely related to M. m. molossinus [5], indicating that they are really of Japanese origin. ** HMI is a strain of M. m. castaneus trapped in Taiwan. (b) A map of northeastern and central Japan, showing the original capture sites of wild mice. The bar represents 100 km.]
more active than C57BL/6. The activities are not uniform even among wild-derived mice. In a comparison of KOR1/Stm, KOR5/Stm, and KOR7/Stm from Koriyama with MSM/Ms from Mishima, Shizuoka Prefecture, we found that the activity of MSM, in particular, the vertical jumping ability, was greater than that of other strains [17]. These behavioral activities are generally polygenic traits; therefore, mice with varied origin may provide tools for the study of the genetic control of behavior.

According to the Riken BRC database, the body weight and size of the KOR5/Stm mouse were the smallest among >123 strains of mice maintained at the BRC (http://www.brc.riken.jp/rmpd/mouse phenome_top.html). The body weight of the young adult of KOR5/Stm was about 1/3 that of AKR/J, although the body length of the former was slightly shorter than that of the latter (Fig. 7). Therefore, this strain is a promising tool to study genes responsible for body size.

Figure 4b shows the phylogenetic relationship of major inbred strains from Mus m. molossinus established in Japan as analyzed by the neighbor-joining method (Mekada, K. and Yoshiki, A., personal communication). Phylogenetic distance between these strains is roughly correlated with the geographic distance of the sites where the original pairs were captured (Fig. 4a). It is evident that the genetic distance is significant between the Japa-
nese wild mice, *M. m. molossinus*, and the HMI mice, a strain of *M. m. castaneus*, which was originally trapped in Taiwan. The laboratory strain mice, *M. m. domesticus* or *M. m. musculus*, are far more remote (not shown).

**Spontaneously Hyperlipidemic Mice**

In 1996, one female mouse of the KOR1/Stm strain showed abnormal coat texture at the abdomen and hair loss around the mammary papilla. Later, this mutant was found to spontaneously develop hyperlipidemia because of ApoE deficiency, and the mutant was designated as a spontaneously hypertlipidemic (SHL) mouse (KOR-Apoεhl) [10]. The mutant Apoε gene has a large deletion, and its mRNA and protein have not been detected. Since the KOR1-derived SHL mice were extremely active and difficult to handle, the original strain was considered unsuitable for experimental animals. Therefore, congenic strains were developed by introgression of the mutant locus into 3 common laboratory strains. The strains were selected by either their susceptibility (C57BL/6) or their resistance (BALB/c and C3H/He) to arteriosclerosis [20]. After 8 years of selective backcrossing, the congenic strains B6.KOR-Apoεhl, C.KOR-Apoεhl, and C3.KOR-Apoεhl were established [15]. The original SHL mice and 3 congenic strains developed hypercholesterolemia, hypertriglyceremia, skin xanthomatosis, and arteriosclerosis; however, the degree of each lesion varied according to their genetic background (Fig. 8). At 6 weeks of age, the total cholesterol (TC) values in KOR-Apoεhl, C3.KOR-Apoεhl, C.KOR-Apoεhl, and B6.KOR-Apoεhl were 1,300, 1,100, 900, and 700 mg/dl, respectively. A study of atherosclerotic lesions at the origin of the aortic valve at 9 months of age surprisingly revealed that the lowest degree lesions were found in the KOR-Apoεhl mice, which had the highest TC value. In contrast, the highest-degree lesions were observed in the B6.KOR-Apoεhl mice which had the lowest TC value (Fig. 8c and 8d). These observations suggest that KOR-Apoεhl mice with hyperlipidemia may possess modifier genes that can suppress the progression of arteriosclerosis. Both hyperlipidemia and arteriosclerosis are controlled by highly complicated quantitative trait loci, and thus any study on these lesions should be conducted with consideration of the host genetic background. The set of original SHL mice and their con-

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**Fig. 8.** Subcutaneous xanthoma developing in KOR-Apoεhl (a) and B6.KOR-Apoεhl (b). Histology of the aortic sinus of both strains revealed remarkable atherosclerosis at 39 weeks of age: KOR-Apoεhl (c) and B6.KOR-Apoεhl (d). See text for details. The bars indicate 500 µm.

**Fig. 9.** Candidates of new disease models: spontaneous atopic dermatitis (a), spontaneous microphthalmia (b), spontaneous dominant white spots, which exhibit embryonic lethality (c), spontaneous sebaceous gland abnormalities (d), and spontaneous audible song-like vocalizations (e). The vocalizations are shown in musical notation (f).
genic mouse strains are highly promising resources for
developing a strategy for tailor-made medicine for these
diseases. Recently, this set has been used to evaluate the
effects of functional food on the lipid metabolism [1, 2,
8, 16, 19].

**Other Disease Models in Wild-Derived Strains**

SHL mice were found at the F16 generation of suc-
cessive breeding of KOR1. After the 20th generation,
several other disease phenotypes were discovered among
descendants of KOR1, namely, spontaneous atopic der-
matitis (Fig. 9a), microphthalmia (Fig. 9b), dominant
white spots (homozygotes, embryonic lethal) (Fig. 9c),
sebaceous gland abnormalities (Fig. 9d), and audible
song-like vocalizations (Fig. 9e and 9f). Currently, we
are attempting to clone the causative genes of these dis-
eases and to generate congenic strains by introducing
causative loci into several laboratory strains of mice. As
a typical example, a causative gene for spontaneous
atopic dermatitis in mice has been identified. Briefly,
mutant mice showing high levels of serum IgE and an
atopic dermatitis (AD)-like skin disease were found in
a colony of the KOR inbred strain. A study of (BALB/c ×
KOR mutant) N2 intercross mice suggested that the
AD-like lesion is inherited by a single recessive locus
and that hyper-IgE-emia and dermatitis were tightly as-
sociated. The mutant locus was named as atopic derma-
titis from Japanese mice (adjm). The gene responsible
for the AD-like phenotypes was later identified by posi-
tional cloning and found to encode the mouse homologue
of the human TNFR-associated factor 3-interacting pro-
tein 2 (TRAF3IP2). The mutant gene carried a single
point mutation leading to the substitution of a stop codon
for glutamine at amino acid position 214. This protein
has been shown to function as an adaptor protein in sig-
naling pathways mediated by the TNFR superfamily
members CD40 and B cell-activating factor in epithe-
lial cells and B cells as well as in the IL-17-mediated
signaling pathway. Our results suggest that malfunction
of the Traf3ip2 protein also causes hyper-IgE-emia
through the CD40- and B cell-activating factor-mediated
pathway in B cells as well as skin inflammation
through the IL-17-mediated pathway. This study dem-
strates that the Traf3ip2 protein plays an important
role in AD and suggests the protein as a therapeutic tar-
get to treat AD [14].

Other mutations found in wild-derived inbred strains
include C8b deficiency found in MAE/Stm [25] and CD8
deficiency in KORS/Stm (Kitaura, unpublished data).
Keratoconus, conical shaping of the cornea probably due
to keratitis, was found in MSM/Ms, a wild-derived in-
bred strain that originated in Mishima, and the respon-
sible locus was mapped on chromosome 13 [23].

**Conclusion**

Guided by pioneering works by Kondo, we have es-
stablished several inbred strains from Japanese wild mice
and shown that they are a rich resource for disease mod-
eels irrespective of disadvantages, such as difficulty in
handling and relatively poor fertility. They have been
deposited in the Riken BRC (http://www.brc.riken.jp),
analyzed for phenotype and made available for inter-
ested researchers. Furthermore, 3 congenic SHL strains
are commercially available from Japan SLC, Inc. (http://
www.jslc.co.jp), and about 2,000 mice are used annu-
ally. In addition, as the SHL mice are derived from a
spontaneous mutant animal, they have the advantage that
they are not subject to the regulations of the Cartagena
Protocol on Biosafety, which applies to transgenic ani-
imals. We hope that substantial original research out-
comes are generated using wild-derived mice.

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