Morphometric Comparison of the Rat Mandible in F344 Substrains, F344/Du and F344/N

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Abstract: The shape of the mandible was compared by morphometric methods to ascertain the genetic differences between two substrains of F344 rats, F344/DuCrI/Crlj and F344/NSIc. Since these two substrains are clearly different in survival and the incidence of age associated disorders; thus, some genetic differences are suggested to be present between them. Although previous microsatellite analysis did not detect any differences between the two F344 substrains, the present study clearly detected interesting differences in the mandible morphology. At 2 months of age, the F344/Du mandible was characterized by a larger size, especially in length, than the F344/N mandible. The shape of the mandible seemed to be more variable in F344/N. This clear substrain difference suggests the importance of the substrain recognition in F344 rats, especially in experimental usage.

Key words: F344/Du, F344/N, mandible, morphometry, substrain difference

Introduction

Longevity sciences need practical and contributive animal models. The most convenient animal models, laboratory rodents, however, sometimes bring unexpected confusion due to their species, strain and sex specific biological characteristics, especially in their aging properties. Survival, a reliable and standard parameter for the establishment of aged individuals and

monitoring their aging process, was found to be different among F344 substrains, and between the USA and Japan [8]. Namely, the survival of F344/N is different from that of F344/Du in Japan, and the survival of F344/Du is different between Japan and the USA [9]. Microsatellite analysis, however, demonstrated that these two substrains in the two countries were the same [9, unpublished observation]. The morphometric analysis of the mandible shape, especially of laboratory

(Received 16 December 2005 / Accepted 7 July 2006)

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rodents, is known to be effective for the detection of strain and genetic differences, and their relationship [1, 2, 4–6]. Thus, the current research was conducted to compare the mandible shape between two F344 substrains available in Japan. Interestingly, the two substrains, F344/N (F344/NSlc and F344/NHsd) and F344/DuCrIcrIj, are identifiable in the living state from the look of their face [Tanaka and Miyaishi, unpublished observation].

All animal experiments were performed with the permission of the Committee for Animal Ethics NILS according to the Guideline of NILS Animal Experimentation with consideration of animal rights and welfare.

Materials and Methods

Animals

All animals were male and used at 2 months of age. Two substrains of F344, F344/Du and F344/N, were purchased from Charles River Laboratories Japan, Yokohama, Japan and SLC Japan, Hamamatsu, Japan, respectively. Thus, the substrains of F344 used were F344/DuCrIcrIj and F344/NSlc [9]. They are subsequently described only as F344/Du and F344/N, respectively. The animals had been housed in groups after weaning.

Preparation of the mandible

Animals were sacrificed by an overdose of anesthesia at either 58 or 59 days of age for F344/N, and 61 or 62 days of age for F344/Du on the delivery day, and the whole tissue around the oral cavity was dissected out. The dissected tissue was autoclaved at 121 degrees for 5 min. The soft tissue around the mandible was removed carefully. The mandible was incubated overnight in 0.5% papain (Merck, Germany) solution at 37 degrees and the attaching soft tissue was digested. After washing, a dried bone specimen of the mandible was prepared.

Morphometric analysis of the mandible

Eleven males from each of the two substrains were prepared for dry bone specimens. Three mandible specimens from F344/N were destroyed partially, mainly in the coronoid process (*Proc. coronoideus*), during the preparation and judged to be incomplete. Thus, man-

dible specimens of eleven F344/Du and eight F344/N were measured as follows. The tongue-side surface of the right mandible was measured at a 10 times magnification by a universal projector V-20B (Nihon Kogaku, Tokyo). The measurement was performed on thirteen sites as illustrated in Fig. 1. Seven sites, X_1 to X_7 , were the distances from the X axis, namely the heights of the sites. The remaining six sites, X_8 to X_{13} were the distances from the Y axis, namely the length of the sites.

Statistical procedures

The measurements obtained were first checked for their distribution by the F test, then compared using the t test and subjected to principal component (PC) analysis by SPSS (based on *Statistic Program for Social Science*; SPSS Co. Ltd.) for Windows, version 13.0.

Results

Morphometric measurement

The average and standard deviation (SD) of the 13 points (Fig. 1) of the F344/Du and F344/N mandibles are summarized in Table 1 with the animals' body weights. Among the 13 measurements points, heights X_2 , X_4 , X_7 ($P < 0.05$), X_1 , X_3 ($P < 0.01$), lengths X_{11} ($P < 0.01$) and X_{13} ($P < 0.001$), and body weights ($P < 0.001$) were significantly different between the two substrains.

Of the significantly different measurements, all except for X_1 were larger in F344/Du than in F344/N (Table 1).

The remaining values, X_5 , X_6 , X_8 , X_9 , X_{10} and X_{12} , were not significantly different (Table 1).

Fig. 1. Thirteen points for the measurements. Tongue-side view.

Table 1. Measurements of thirteen points on the mandible and body weights of F344/N and F344/Du

Substrain	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	Body weight
	#													##
F344/N n=8														
Average	1.294	3.787	5.321	7.009	8.724	9.603	11.114	7.021	17.386	18.865	20.403	22.959	22.800	175.5
SD	0.072	0.040	0.084	0.090	0.174	0.194	0.150	0.189	0.126	0.474	0.113	0.203	0.121	5.0
F344/Du n=11														
Average	1.217	3.840	5.440	7.122	8.782	9.578	11.246	7.137	17.515	18.902	20.640	23.123	23.203	190.6
SD	0.039	0.052	0.069	0.122	0.185	0.071	0.063	0.111	0.224	0.337	0.157	0.154	0.210	4.3
#mm, ##g														
	**	*	**	*	NS	NS	*	NS	NS	NS	**	NS	***	***
	N	Du	Du	Du			Du				Du		Du	Du

*, ** and ***: Significant difference between two substrains at $P < 0.05$, 0.01 and 0.001. NS: Not significant.

Principal component analysis

The thirteen measurements presented in Table 1 correlate to each other, and among seventy-eight combinations (${}_{13}C_2$), thirty-one were significant (data not shown). So, it is impossible to analyze the substrain differences of the mandible morphology merely based on the data represented in Table 1. To clarify synthetically the morphometrical differences of mandibles between the two substrains, F344/Du and F344/N, a principal component analysis of multivariate analyses was judged to be the most suitable method [1, 2, 4–6]. Principal component analysis was applied to the mandible measurements for the comparison of its size and shape.

As summarized in Table 2, the eigenvalues and cumulative contribution ratios (%) of three principal components (PC) were larger than 1.0 and 75.1%, respectively. The cumulative contribution ratios from the 1st (PC1) to 3rd (PC3), 75.1%, means these first three PCs extracted account for more than 75% of morphometrical variation information.

Table 3 summarizes the contributing vectors of each variable to PC1, PC2 and PC3, respectively.

As is shown in Table 3, for PC1, among the six coefficients representing the whole mandibular length, the largest five were: X₈, X₉, and X₁₁ to X₁₃.

For PC2, four length coefficients X₈ to X₁₁, and X₁₂ and X₁₃ were negative or small, while the coefficients representing the mandibular height were positive and larger.

For PC3, X₂, X₃, X₇–X₉, X₁₀ and X₁₁ had negative coefficients, regardless of height or length. The positive and large coefficients were X₉ and X₁₀. They are concerned with the lengths to the convex at the base and peak/apex of the coronoid process.

Principal component scores

The average and SD of the scores of PC1, PC2 and PC3 are summarized in Table 4. The averages with SD bars of PC1 and PC2 are shown in Fig. 2, in which PC1 is the X axis and PC2 is the Y axis. For PC1, the average and SD scores of F344/Du (0.601 $\pm$ 0.731) were significantly ($P < 0.001$) larger than those of F344/N (–0.827 $\pm$ 0.678) (Table 4). However, the averages of PC2 and PC3 were not significantly different between the two substrains (Table 4).

Discussion

The three principal components (PC1, PC2 and PC3) extracted through principal component analysis accounted for more than 75% of the total variation of mandible morphology (Table 2). Above all, PC1 had the highest calculated contribution, up to 38.6%, to the mandible morphology (Table 2). Among the contributing vectors of PC1, coefficients concerned with the mandible length (X₈ to X₁₃) were all positive and mostly larger, while those concerned with the height (X₁ to X₇) consisted of negative or mostly smaller values (Table 3). Since the larger (or smaller) almost all calculated eigenvectors resulted in the larger (or smaller)

Table 2. Eigenvalue and its contribution

Principal component	Eigenvalue	Cumulative contribution (%)
1	5.027	38.667
2	3.424	65.009
3	1.322	75.181

Table 3. Coefficients for eigenvectors for 3 principal component

Variable	1	2	3
PC			
X1	-0.607	0.406	0.207
X2	0.553	0.607	-0.161
X3	0.770	0.109	-0.231
X4	0.506	0.663	0.311
X5	0.011	0.812	0.373
X6	-0.370	0.798	-0.150
X7	0.199	0.826	-0.154
X8	0.703	-0.531	-0.099
X9	0.771	-0.005	0.488
X10	0.248	-0.378	0.768
X11	0.831	-0.112	-0.212
X12	0.798	0.065	-0.185
X13	0.923	0.136	0.037

PC1, PC1 was judged to extract the size factor. Thus, the substrain plotted on the right side in Fig. 2 is implied to have a larger size as a whole than that plotted on the left side. This means that PC1 is acceptable as a size factor, especially in length.

For PC2, among the six coefficients concerned with the length, four (X_8 to X_{11}) were negative and the remaining two were small, and coefficients concerned with the height, X_1 to X_7 , were all positive and large, especially X_5 to X_7 . This indicates that PC2 is acceptable as a relation of the whole mandible height to the length, namely a shape factor. The substrain plotted on the upper side in Fig. 2 is implied to have a higher mandible than that plotted on the lower side.

Following the above interpretations, the mandible is larger in length in F344/Du than in F344/N while its shape is nearly the same in both substrains. The average scores of F344/N in PC2 had a larger SD (Table 4, Fig. 2), suggesting that F344/N is highly variable in shape. This might be due to the large negative score (-2.697) of the third F344/N individual (data not shown).

Table 4. Averages of three principal component scores in 2 substrains

Substrain		PC 1	PC 2	PC 3
F344/N				
n=8	Average	-0.827	-0.265	0.207
	SD	0.678	1.274	1.002
F344/Du				
n=11	Average	0.601	0.193	-0.150
	SD	0.731	0.753	1.019

* NS

* and NS: Significant difference at $P < 0.001$ and not significant between two substrains.

For PC3, negative coefficients appeared regardless of the height or the length, suggesting that this might be a shape factor, but not a size factor. The largest coefficient was X_{10} , the length to the peak of the coronoid process and the second largest coefficient was X_9 , the length to the convex at the base of the coronoid process. Thus, PC3 might represent the coronoid process spread. Namely the larger coefficient might represent a sharp and long coronoid process towards the posterior. Thus, both PC2 and PC3 do not seem to be related to the substrain differences between F344/Du and F344/N. As a conclusion, only PC1 correlated with the size factor, especially length, and was responsible for the substrain difference at the age studied.

The measurements of 13 points and the bodyweight showed some substrain differences. The significant differences shown in Table 1, except for X_1 , were consistent with the result of PC1. The interrelationships among the variables X_1 to X_{13} are so complicated that it seems too difficult to compare the mandible morphology between the substrains. In this case, the principal component analysis seems to have been most effective.

The larger bodyweight and PC1 may partly be related with the acute growth in F344/Du. However, they do not explain the most prominent substrain difference, the longer survival of F344/Du. This may be due to a lower incidence of leukemia (about 50%, personal communication) and its later onset in F344/Du. The leukemia incidence in male F344/N is 83.6% [7] and its onset is earlier than F344/Du (personal communication). In addition, in both substrain the incidence of leukemia is far higher than that of original F344, 24% [3]. It is easy to suppose a correlation between

Fig. 2. Scatter plot of the average and SD (bars) of PC1 and PC2 of the two substrains.

the survival and the leukemia incidence, but it is very difficult to imagine a correlation among the survival, mandible size and growth rate.

To ascertain the substrain differences in detail without the possible modification of growth, research on full-grown adults, aged between 12 and 24 months is needed. At that age, both the length and the height factors of the mandible might have stopped changing, and the mandible shape is expected to become stable and constant.

Some biological characteristics were noted to be different between the two F344 substrains, F344/Du and F344/N in Japan [7, 8], including survival, disorders with aging and some morphological differences; but the two substrains were not different in microsatellite analysis [9]. The morphometric analysis of laboratory animals using mandible shape and size as principal components has detected and demonstrated clear strain differences in inbred strains of mice [1, 2], a recombinant inbred strain group of mice [6], inbred strains of rats [4], and outbred strains of rabbits [5]. In the current study, this analysis detected clear differences between the substrains of F344, F344/Du and F344/N, even in their growing stage. This means that this analysis method is highly sensitive to the differences which although small in measurement are profoundly important. In addition to microsatellite analysis or biochemical marker genes, morphometric analysis, such as mandible analysis, seems to be necessary for the genetic monitoring of substrains. Thus, substrain differences should be noted carefully in experimental usage, since many phenotypes including the present and

previous results strongly suggest the presence of some genetic differences between the two F344 substrains in Japan.

Acknowledgments

This report was supported in part by a Research Grant for Longevity Sciences (14C-5) from the Ministry of Health, Labour and Welfare. The authors wish to express their sincere thanks to Dr. Hiroaki AOYAMA, Institute for Environmental Toxicology and Dr. Megumi SASAKI, Department of Gerontological Policy, NILS, for their advice on the statistics and to Dr. Takako YAMAMOTO-SAWAMURA, Ms. Haruko SUZUKI and Keiko SONE for their help in the preparation and measurement of specimens.

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