Establishment of Inbred Strains of Chicken and Japanese Quail and their Potential as Animal Models

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Abstract: We started establishing inbred strains of chicken and Japanese quail in 1970. In class Aves, full sib mating is highly difficult due to inbreeding depression. In the chicken, we attempted to establish some inbred strains in three breeds, Black Minorca, White Leghorn and Fayoumi by fixing all the characters that differentiate individuals homozygously. In this paper, we describe some marker genes and characters fixed in the inbred strains of chicken and Japanese quail as well as a calculation of a putative coefficient of inbreeding in 8 chicken inbred strains using band sharing values detected by AFLP analysis. We established generalized glycogenosis type II quail, myotonic dystrophy quail, neurofilament deficient quail, visually impaired chicken, double oviduct chicken with partial kidney deficiency, chicken showing spontaneous lymphocytic thyroiditis with feathered amelanosis, and chicken with a hereditary nervous disorder. The processes of establishment and characteristics of these animal models are described with some interesting information obtained from these animal models. In generalized glycogenosis type II quail, the results of enzyme replacement therapy and gene therapy are described.

Key words: inbred strains, animal models, chicken, Japanese quail

Mammals, especially mice and rats have served as the most important laboratory animals. However, animals of other species are also expected to provide valuable information that cannot be obtained in mice or rats. In this aspect, we started establishing inbred strains of chicken and Japanese quail in 1970. The present paper describes the characteristics of the inbred strains thus established and their potential use as animal models of human diseases.

I. Definition of inbred strain in chicken and Japanese quail

In class Aves, full sib mating is highly difficult due to inbreeding depression. Therefore, closed colonies of chickens and Japanese quails that have coefficients of inbreeding higher than 50% or coefficients of relationship higher than 80% are regarded as inbred strains.

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Table 1-1. Red blood cell types detected by various plant lectins

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lectins</th>
<th>green pea</th>
<th>pea nut</th>
<th>parch sesame</th>
<th>tulip</th>
<th>potato</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-C</td>
<td>HiHi (PhPh)*</td>
<td>PnPn</td>
<td>sisi</td>
<td>tgl1g1</td>
<td>SiS1st1</td>
<td>st2s2</td>
</tr>
<tr>
<td>WL-F</td>
<td>hihi (phph)</td>
<td>pnpn</td>
<td>–</td>
<td>tgl1g1</td>
<td>st1st1</td>
<td>S2S2st2</td>
</tr>
<tr>
<td>WL-GM</td>
<td>hihi (phph)</td>
<td>PnPn</td>
<td>SiSi</td>
<td>tgl1g1</td>
<td>tgl2g2</td>
<td>st1s1</td>
</tr>
<tr>
<td>WL-N</td>
<td>hihi (phph)</td>
<td>PnPn</td>
<td>–</td>
<td>Tg1Tg1</td>
<td>Tg2Tg2</td>
<td>st1st1</td>
</tr>
<tr>
<td>PNP</td>
<td>hihi (phph)</td>
<td>Pn Pn</td>
<td>–</td>
<td>tgl1g1</td>
<td>–</td>
<td>st1s1</td>
</tr>
<tr>
<td>GSN/1</td>
<td>–</td>
<td>Pn Pn</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>S1S1st1</td>
</tr>
<tr>
<td>GSN/2</td>
<td>–</td>
<td>Pn Pn</td>
<td>–</td>
<td>–</td>
<td>Tg1Tg1</td>
<td>S1S1st1</td>
</tr>
<tr>
<td>GSP</td>
<td>hihi (phph)</td>
<td>pnpn</td>
<td>–</td>
<td>tgl1g1</td>
<td>–</td>
<td>S1S1st1</td>
</tr>
</tbody>
</table>

*genotype (a capital letter: dominant gene, a small letter: recessive gene.)

Table 1-2. Red blood cell types detected by various plant lectins

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lectins</th>
<th>PWA (poke weed)</th>
<th>MPA</th>
<th>leek</th>
<th>VAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-C</td>
<td>pw1pw1</td>
<td>pw2pw2</td>
<td>mp1mp1</td>
<td>atat</td>
<td>yava</td>
</tr>
<tr>
<td>WL-F</td>
<td>pw1pw1</td>
<td>pw2pw2</td>
<td>mp1mp1</td>
<td>atat</td>
<td>VaVa</td>
</tr>
<tr>
<td>WL-GM</td>
<td>pw1pw1</td>
<td>pw2pw2</td>
<td>mp1mp1</td>
<td>atat</td>
<td>VaVa</td>
</tr>
<tr>
<td>WL-N</td>
<td>pw1pw1</td>
<td>pw2pw2</td>
<td>mp1mp1</td>
<td>atat</td>
<td>–</td>
</tr>
<tr>
<td>PNP</td>
<td>pw1pw1</td>
<td>mp1mp1</td>
<td>Mpm2mp2</td>
<td>atat</td>
<td>VaVa</td>
</tr>
<tr>
<td>GSN/1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>atat</td>
<td>VaVa</td>
</tr>
<tr>
<td>GSN/2</td>
<td>pw1pw1</td>
<td>mp1mp1</td>
<td>–</td>
<td>atat</td>
<td>VaVa</td>
</tr>
<tr>
<td>GSP</td>
<td>Pw1Pw1</td>
<td>mp1mp1</td>
<td>–</td>
<td>atat</td>
<td>VaVa</td>
</tr>
</tbody>
</table>

II. Establishment of inbred strains in chicken

Except a few inbred strains established by full sib mating, most inbred chicken strains have been established by half sib mating or pedigree mating of White Leghorn, mainly to avoid inbreeding depression. As marker genes of inbreeding, the red blood cell types detected by isoimmune sera and some characters related to avian leukosis viruses, i.e. group specific antigen and chick helper factor of avian leukosis viruses and susceptibility to avian leukosis virus subgroup A-E, were examined.

We attempted to establish some inbred strains in three breeds, Black Minorca (BM-C), White Leghorn (WL-F, WL-GM, WL-N), and Fayoumi (PNP, GSN/1, GSN/2, GSP) by fixing all the characters that differentiate individuals homozygously. For example, we fixed red blood cell types detected by isoimmune sera, as well as by various plant lectins and a few virus agglutinins.

(1) Characters fixed in the inbred strains

Characters controlling the appearance

The type of comb, plumage color of chicks and adult chickens, rate of feathering, and shank color were fixed. Red blood cell types detected by various plant lectins

Marker genes of inbred strains on chicken red blood cell types detected by plant lectins produced from green pea [3, 19], peanut [20], parch sesame [23], bulb of tulip [27], tuber of potato [25], leek, PWA (pokeweed) [28], MPA (Macularia pomifera), and VAA (Viscum albumin) [29] are shown in Table 1-1 and 1-2. The appearance of the genotypes shown in Table 1-1, e.g. Tgl and St1, was not influenced by laying or non-laying conditions in females. The appearance of the genotypes shown in Table 1-2, e.g. tg2 and St2, was influenced by laying or non-laying condition in females. Red blood cell types detected by isoimmune sera

The comparison test of marker genes of inbred strains on red blood cell types detected by isoimmunized antisera was initially carried out at the laboratory of Dr. W.E. Briles in Northern Illinois University in 1987.
Table 2. Red blood cell types detected by isoimmune sera

<table>
<thead>
<tr>
<th>Strain</th>
<th>A</th>
<th>E</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>H</th>
<th>I</th>
<th>K</th>
<th>L</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-C</td>
<td>2</td>
<td>2</td>
<td>15</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>k</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>WL-F</td>
<td>1/2*</td>
<td>1/2</td>
<td>15</td>
<td>2</td>
<td>1/3</td>
<td>2</td>
<td>34/35</td>
<td>1</td>
<td>2</td>
<td>2/3</td>
</tr>
<tr>
<td>WL-GM</td>
<td>4</td>
<td>5/7</td>
<td>15</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>34</td>
<td>1</td>
<td>1</td>
<td>34</td>
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<tr>
<td>WL-N</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>k</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>PNP</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>GSN/1</td>
<td>4</td>
<td>1</td>
<td>21</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>GSN/2</td>
<td>4</td>
<td>1</td>
<td>21</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>35</td>
<td>3</td>
<td>1</td>
<td>2/4</td>
</tr>
<tr>
<td>GSP</td>
<td>4</td>
<td>1</td>
<td>21</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>k</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

RBC type 1 and 2 is segregating. Arabic numeral and Roman letter represent the name of allele.

Table 3. Red blood cell types detected by virus agglutinins

<table>
<thead>
<tr>
<th>Strain</th>
<th>Agglutinability</th>
<th>Porcine Parvo Virus</th>
<th>Vaccinia Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-C</td>
<td>±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>WL-F</td>
<td>++</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>WL-GM</td>
<td>–</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>WL-N</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PNP</td>
<td>±</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>GSN/1</td>
<td>–</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>GSN/2</td>
<td>–</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>GSP</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 4. Marker genes associated with avian leukosis viruses subgroups A–E

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gs-antigen</th>
<th>chf activity</th>
<th>susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-C</td>
<td>88*</td>
<td>HH</td>
<td>C/AB</td>
</tr>
<tr>
<td>WL-F</td>
<td>88</td>
<td>hh</td>
<td>C/O</td>
</tr>
<tr>
<td>WL-GM</td>
<td>88</td>
<td>hh</td>
<td>C/O</td>
</tr>
<tr>
<td>WL-N</td>
<td>88</td>
<td>hh</td>
<td>C/O</td>
</tr>
<tr>
<td>GSN/1</td>
<td>88</td>
<td>HH</td>
<td>C/ABE</td>
</tr>
<tr>
<td>GSN/2</td>
<td>88</td>
<td>HH</td>
<td>C/ABE</td>
</tr>
<tr>
<td>GSP</td>
<td>OG</td>
<td>HH</td>
<td>C/ABE</td>
</tr>
</tbody>
</table>

C/AB: resistant to A and B viruses, C/O: all susceptible to A–E viruses. * genotype.

The results of the comparison test are shown in Table 2. Subsequently, we produced many types of antisera using these chickens, for which blood types had been identified, and fixed red blood cell types in each inbred strain using these antisera. Currently, the number of marker genes of red blood cell types that are homozygous is greater than those shown in Table 2.

RFLP patterns of B-G and B-F regions in B red blood cell system

B-G and B-F regions correspond to major histocompatibility systems in mammals. A strain difference of RFLP patterns was not found within each B-G region among all inbred strains [14]. On the other hand, an individual difference of the RFLP pattern of the B-F region was found in the GSP strain [13]. Thus, individuals in the GSP strain with the smallest numbers of band patterns were used as parents for the next generation.

Red blood cell types detected by virus agglutinins

The agglutinability of 8 inbred strains with porcine parvovirus and vaccinia virus is shown in Table 3.

Marker genes related to avian leukosis virus

In 7 inbred strains, the presence or absence of group specific antigen and chick helper factor of avian leukosis virus, and susceptibility to avian leukosis viruses subgroups A–E were fixed for each inbred strain as shown in Table 4.

Immunoreactivity

Immunoreactivity of 8 inbred strains against red blood cell antigens of chicken [24] and Japanese quail was examined. We selected high or low responders against red blood cell antigens of chicken and Japanese quail in each inbred strains. Examination of the immune responses of GSP, PNP, and WL-GM strains against BSA and Mycoplasma gallisepticum bacterin [49] indicated that GSP is a high responder and PNP and WL-GM are low responders. The death rate by anaphylactic shock after two challenge immunizations with BSA was investigated in BM-C, WL-F, WL-GM, PNP, GSN/1, GSN/2, and GSP strains. The death rate was
the highest in the GSP strain, and the lowest in the WL-GM strain. The death rate of GSP and WL-GM strains was fixed at 100% and 0%, respectively.

**Band patterns detected by DNA fingerprint or AP-PCR-SSCP methods**

We selected some individuals that showed the same band patterns detected by DNA fingerprint or AP-PCR-SSCP methods in each inbred strain.

**Marker genes that were fixed without selection**

- Ornithine transcarbamylase (OTC) gene: In chicken, OTC activity is highly variable within and between chicken breeds. The chicken OTC gene with a size of 26 kbp was found to consist of 10 exons and 9 introns. In intron 3, two polymorphic sites were found: one comprises a deletion of 401 nucleotides, and the other is a polymorphic region located 8 bases upstream from the deletion. BM-C, WL-F, WL-GM, and WL-N strains have 401 bp deletion but PNP, GSN/1, GSN/2, and GSP strains do not [41].

- 2',5'-oligoadenylate synthetase (OAS) gene: Chicken OAS gene has two alleles, OASA and OASB. The difference between these two alleles is in the 6th exon of the gene; a 96-nucleotide sequence in the ubiquitin-like 1 portion of OASA is deleted from OASB. No OASB gene was detected in 8 inbred strains.

**Visual impairment in GSN/1 strain**: When we noticed visual impairment in GSN/1 chickens, visual impairment had already been fixed in the strain. A pathological study revealed that the number of inherited ganglion cells were significantly smaller, and the sub-layers of the *stratum griseum et fibrosum superficiale*, in which axons of the optic nerve are distributed, were disorganized and irregular in all GSN/1 chicks. The spectral sensitivity of the electroretinogram (ERG) and visual evoked potential (VEP) were different from those of normal strain GSP.

**Agglutinability of RBC detected by plant lectin AIA produced from jackfruit**: This character was fixed in every inbred strain.

(2) **Putative coefficient of inbreeding**

We attempted to calculate a putative coefficient of inbreeding in 8 inbred strains using band sharing (BS) values detected by DNA fingerprint or AFLP analysis [15]. BS is the ratio of the frequency of the same polymorphic bands between two individuals. The average of BS is the sum of all polymorphic bands and is expressed by the following equation: \( BS = 2 \frac{Nab}{Na + Nb} \). \( Nab \) is the number of commonly shared polymorphic bands between individuals a and b. \( Na \) and \( Nb \) are the total numbers of polymorphic bands in individuals a and b, respectively. We examined average BS values in 8 inbred strains from AFLP polymorphic bands. The results shown in Table 5 indicate that the inbred strains with the exception of the WL-N strain are highly inbred.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Coefficient of inbreeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-C</td>
<td>92</td>
</tr>
<tr>
<td>WL-F</td>
<td>92</td>
</tr>
<tr>
<td>WL-GM</td>
<td>91</td>
</tr>
<tr>
<td>WL-N</td>
<td>61</td>
</tr>
<tr>
<td>PNP</td>
<td>95</td>
</tr>
<tr>
<td>GSN/1</td>
<td>97</td>
</tr>
<tr>
<td>GSN/2</td>
<td>95</td>
</tr>
<tr>
<td>GSP</td>
<td>96</td>
</tr>
</tbody>
</table>

**Table 5. Coefficient of inbreeding calculated by the band sharing value derived from AFLP**

(3) **Findings obtained from 8 inbred strains**

**Linkage between B red blood cell type and immunoreactivity against isoantigens**

When WL-F and PNP strains were immunized with 10% RBCs of BM-C strain, the primary immune response in the WL-F strain developed to a high level, but to a low level in the PNP strain. The relationship between the level of immune response against 10% RBCs of BM-C strain and B haplotype were examined in back-cross progenies obtained from mating F1(WL-F × PNP) × PNP.

The level of the immune response of the progenies with B haplotype of the WL-F strain was higher than those with B haplotype of the PNP strain. On the other hand, the level of the immune response of the progenies with A-E system of the WL-F strain was not different from that of those with A-E system of the PNP strain. These results indicate that the level of immunoreactivity against isoantigens is linked to the B haplotype.

**Susceptibility to Marek’s disease virus**

Genetic resistance to transplantable Marek’s disease virus-induced tumor cell line was examined in two inbred strains, PNP and GSP [50]. Mortality rates in
PNP and GSP strains at 7 weeks after challenge with the MSB1-41C cell line was 83.6% and 29.4%, respectively. The difference in mortality rate between PNP and GSP was significant.

Embryo death caused by blood group incompatibility

A few hens in one inbred strain were immunized every week with male RBCs obtained from another inbred strain, and every week the hens were artificially inseminated using semen obtained from the male which provided the RBCs used for immunization. Embryo death caused by blood group incompatibility was observed in specific combinations of the strains of donor and recipient. One example is shown in Fig. 1.

The results obtained from these experiments are as follows. Embryo death caused by blood group incompatibility appeared 2 or 3 months after the first immunization. Titers of antibody obtained from hens that showed embryo death and those obtained from hens that did not show embryo death were not different. Antibody against antigen of B haplotype seems not to be involved in embryo death, but we speculate that a specific antigen and a specific type of antibody are related to embryo death.

Mapping of chicken muscular dystrophic gene “am”

To construct a reference family, one male of the WL-F strain was mated with one female of the OPN strain (Fayoumi breed) with the muscular dystrophic gene ‘am’ introduced from the original muscular dystrophic strain 413 [11]. Approximately 50 progenies were obtained from backcross mating between the parental WL-F male and one female of the F1 hybrid. By linkage analysis of the ‘am’ gene using microsatellite marker genes and AFLP markers in the reference family, the ‘am’ gene was mapped to the second chromosome in the chicken.

Developmental biological findings using C/O (WL-M/O strain) and C/ABE strain (GSN/I or GSN/2 strain)

Epithelial-mesenchymal interactions are necessary for the normal development of various digestive organs. In chicken proventriculus, morphogenesis and differentiation of the epithelium depend upon inductive signals coming from the underlying mesenchyme. However, the nature of such signals is still unclear despite extensive analyses carried out using experimental tissue recombinations.

We have examined the possible involvement of bone
Table 6. Red blood cell types detected by isimmune sera and plant lectins on 5 strains of Japanese quail

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ly 6054</th>
<th>9035 soy</th>
<th>bean</th>
<th>peanut</th>
<th>Kuritake</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNN</td>
<td>2</td>
<td>-</td>
<td>SbSb*</td>
<td>pm pn</td>
<td>nns</td>
</tr>
<tr>
<td>AMRP</td>
<td>3</td>
<td>+</td>
<td>sbsb</td>
<td>PpPp</td>
<td>NpNs</td>
</tr>
<tr>
<td>TKP</td>
<td>3</td>
<td>+</td>
<td>SbSb</td>
<td>PpPp</td>
<td>NpNs</td>
</tr>
<tr>
<td>SBPN</td>
<td>3</td>
<td>-</td>
<td>sbsb</td>
<td>PpPp</td>
<td>nns</td>
</tr>
<tr>
<td>WEP</td>
<td>1</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ly, 6054, and 9035: Red blood cell types detected by immune sera. Soy bean, peanut, and kuritake: Red blood cell type detected by plant lectins. *genotype.

morphogenetic proteins (BMPs) in the formation of stomach glands in the chicken embryo. Analysis of the expression patterns of BMP-2, -4 and -7 showed that these BMPs are present in the proventricular mesenchyme prior to the initiation of the proventricular gland formation, and BMP-2 expression is restricted to the proventricular among anterior digestive organs. Virus-mediated BMP-2 overexpression resulted in an increase in the number of glands formed. Moreover, ectopic expression of Noggin, which antagonizes the effect of BMPs led to the complete inhibition of gland formation. In order to introduce BMP or Noggin genes using the avian subgroup A retrovirus into either epithelial cells or mesenchymal cells selectively, we used two kinds of chicken embryo, one originated from the C/O strain (WL-M/O), which is fully susceptible to any subgroup of avian retrovirus, and the other originated from the C/ABE (G/SN1 or G/SN2) strain, which is resistant to infection with subgroup A, B or E avian retrovirus [33].

III. Establishment of 5 candidate strains for inbred strains in Japanese quail

1. Characters fixed for establishment of inbred strains in Japanese quail

Red blood cell types

We established five partial inbred strains of Japanese quail by fixing both red blood cell types detected by isimmune sera and plant lectins and isozyme types of red blood cells and sera. Ly system [9] and unidentified system detected by isimmune sera were fixed, as well as red blood cell agglutinogen detected by plant lectins derived from peanut [22], soy bean [21], and a kind of mushroom, kuritake (Naematoloma sublatium) [26], which were fixed homozygously (Table 6).

Isozyme patterns

Isozyme patterns for transferrin, prealbumin, hemoglobin 1, esterase D, and glucosephosphatase isomerase were fixed in a certain type in each strain.

Restriction fragment length polymorphism (RFLP) patterns of mitochondrial DNA

RFLP patterns of mitochondrial DNA were analyzed using 15 restriction endonucleases that recognize six base pairs. Only Bam HI showed RFLPs [40]. The results are shown in Table 7.

Immunoreactivity

We found strain differences in immunoreactivity against several bovine antigens among four quail strains tested, as shown in Table 8 [38]. The inbreeding coefficient of these strains is still uncertain, and remains to be calculated by BS value using the AFLP method.

Susceptibility to avian erythroblastosis virus (AEV)

The susceptibility of 12 strains of Japanese quail to the R strain of AEV was examined. PNN strain showed high susceptibility and developed various types of tumors including erythroblastosis, hemangioma and myeloblastic leukemia [31].

IV. Establishment of animal models in chicken and Japanese quail

We established generalized glycogenosis type II quail, myotonic dystrophy quail, neurofilament deficient quail, visually impaired chicken, double oviduct chicken with partial kidney deficiency, chicken showing spontaneous lymphocytic thyroiditis with feather amelanosis, and chicken with a hereditary nervous disorder.

The process of establishment and characteristics of
Table 8. Antibody titers of 5 lines of Japanese quails immunized with 5 kinds of Bovine proteins

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG (ELISA value, mean ± S.D) against</td>
</tr>
<tr>
<td>PNN</td>
<td>1.79 ± 0.17</td>
</tr>
<tr>
<td>AMRP</td>
<td>0.61 ± 0.48</td>
</tr>
<tr>
<td>TKP</td>
<td>1.41 ± 0.54</td>
</tr>
<tr>
<td>SBPN</td>
<td>0.56 ± 0.43</td>
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</tbody>
</table>

BSA: Serum albumin, β-Lg: β-Lactoglobulin.

these animal models is described below with some interesting information obtained from these animal models.

(1) Generalized glycogenosis type II (Acid maltase deficiency) quail

Generalized glycogenosis type II is an inherited glycogen storage disease. It has been reported in various species of animals including humans [4]. Glycogen storage disease is caused by inborn errors of glycogen metabolism. In humans, eight types of glycogen storage disease have been identified, each being associated with a particular enzyme deficiency. Generalized glycogenosis type II typically involves lysosomal accumulation of glycogen, as the activity of the lysosomal acid maltase is severely depressed.

Affected quails show the first symptom, wing dysfunction, at about 6 weeks of age. The diagnosis of the condition is made on the basis of low acid maltase activity and excessive accumulation of glycogen in tissues [16, 32, 35]. The gene controlling glycogenosis was fixed by selecting quail with the wing abnormality and a low level of acid maltase.

1) Breeding history

In 1974, one 6-month-old male in a flock of PNN strain was found to have difficulty in raising its wings. To examine the hereditary character of the condition, the quail was mated with a female derived from a healthy flock of WE strain. No quails showing abnormality of wing movement were detected in 8 F1 quails, 14 quails in backcross mating or 21 F2 quails. During the course of establishing a closed colony from these offspring, the appearance of the abnormality was observed. The wing abnormality was first found in one female quail (3 months old) in 1977 and then in 2 male quails (7 and 8 months old) in 1978, suggesting the hereditary nature of the disease.

Thereafter, we started to select quails with the wing abnormality and a low level of acid maltase. At the beginning of selection, glycogenosis appeared mostly in aged males but it tended to develop in younger individuals and in both sexes around the third generation. Egg production, fertility and hatchability in affected quails were normal, and this made it possible to undertake further selection, enabling us finally to establish the RW strain [35]. Currently, there are two types concerning the appearance of the wing abnormality, the RWNE strain that develops the wing abnormality before 6 weeks of age and the RWNL strain which develops the abnormality after 4 months of age.

2) Histopathology

Histopathological changes were observed in quails of the RW strain [5, 16, 35]. The cells in the liver, heart, and skeletal muscles showed cytoplasm with decreased staining by hematoxylin and eosin. In older quails, cytoplasmic vacuoles or translucent spaces were often seen in liver cells and cardiac muscle fibers. Some fibers of skeletal muscles showed hypertrophy or granular degeneration. Deposition of PAS-positive material was seen in the liver, heart, skeletal muscles and to a lesser extent in the brain, intestine and gizzard. The material was digested by diastase in all affected tissues, and is considered to be glycogen. The glycogen begin to appear in the cytoplasm as small granules at 2 weeks of age and with increasing age the granules appear to coalesce and become larger droplets, forming the cytoplasmic vacuoles seen in sections stained with PAS after diastase digestion. The superficial pectoralis muscle is most severely affected in the skeletal muscle. On electron microscopy, glycogen-filled vacuoles with ly-
sosomal membranes were seen in cardiac muscle, liver, wing muscle and pectoral muscle of embryos with glycogenosis type II on incubation days 3, 5, and 10 [18]. In all examined tissues and organs of affected quails, glycogen content was markedly increased. In superficial pectoral muscles, which were severely atrophic and replaced with adipose tissue, glycogen content increased to three and a half times that of the controls. In femoral and heart muscles, it was twenty times that of the controls and in the liver, two hundred times that of the controls.

According to the method of Schmid and Mahler, anaerobic glycolysis in vitro was examined in terms of lactate production. Lactate production with the addition of glycogen was not different between the pectoral muscle of controls and affected quails. The activities of phosphorylase, PGlu-M, PGlu-I, PFK, PK and LDH of diseased muscle were within the same ranges as those of the controls. Judging from these results, the main pathway of glycolysis was in good order.

With maltose as the substrate, the activity of acid maltase in the affected muscle decreased to 0 and 12 μmol/g/min. With 4-MUG as the substrate, the activity of acid maltase was decreased in the femoral muscle, superficial pectoral muscle, heart muscle and liver of affected quail. However, there was no difference in the neutral maltase activity in those tissues [32]. The nature of acid maltase of glycogenosis type II quails was also examined [42].

3) Genetic analysis of glycogenosis type II (AMD) in Japanese quail

The mating of a normal female and an affected male produced normal 20 F1 males and 20 F1 females. Matings of F1 birds (20 pairs) produced 127 F2 progenies.

The histopathological test of superficial pectoral muscle obtained from 14-week-old progenies exhibited 96 were normal and 31 were affected. Mating of F1 cocks and AMD hens produced 63 progenies, and the histopathological test of superficial pectoral muscles obtained from 14-week-old progenies exhibited 33 were normal and 30 were affected. The segregation ratios are not significantly deviated from the 3:1 and 1:1 ratios expected on the basis of a single autosomal recessive mutation.

We attempted to establish an early-onset strain by mating quails that showed wing dysfunction at 3 weeks of age. However, we occasionally found some birds with normal behavior at this age in every generation. From original early-onset AMD quails (RWNE strain), we isolated late-onset AMD quails (RWNL strain) with a normal wing function in spite of the presence of pathological changes in the pectoral and wing muscle. So far, late-onset AMD quails have not exhibited the severe wing dysfunction found in early-onset AMD quails even after 35 weeks of age. The RWNE strain produced 55 quails of E-type AMD and 26 quails of L-type AMD (2:1). The L × L mating (RWNL strain) produced 43 quails of only L-type AMD. The reproduction data of RWNE and RWNL strains are shown in Table 9. The fertility and hatchability of the RWNE strain are 52.9% and 39.7%, respectively. Low hatchability in the RWNE strain was due to high mortality (42.3%) before hatching. Fertility and hatchability of the RWNL strain are 55.6% and 70.0%, respectively. The matings of E × L or L × E produced 22 quails of E-type AMD and 20 quails of L-type AMD (1:1). These results indicate that a dominant modifier gene influences the severity of AMD in Japanese quail. Namely, the quails that have a dominant modifier gene homozygously die before hatching, the heterozygous quails show E-type AMD, and the recessively homozygous quails show L-type AMD.

Histopathological analysis could not detect the cause of death in quail embryos that had a homozygous dominant modifier gene. These 3 types of AMD quail resemble the human infantile form, childhood form, and adult form, respectively. These results show that AMD quail could be a good model for all types of AMD in

<table>
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<tr>
<th>Strain</th>
<th>Fertility</th>
<th>Hatchability</th>
<th>Mortality in late embryo stages</th>
<th>Mortality in early embryo stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWNE</td>
<td>52.9</td>
<td>39.7</td>
<td>42.3</td>
<td>10.3</td>
</tr>
<tr>
<td>RWNL</td>
<td>55.6</td>
<td>70.0</td>
<td>20.0</td>
<td>10.0</td>
</tr>
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</table>
In 1998, the full length acid α-glucosidase (GAA I) cDNA of Japanese quail was isolated from a cDNA library derived from Japanese quail liver. The cDNA is 3,569 base pairs long and has an open reading frame capable of coding 932 amino acids. Compared to normal quail, the levels of GAAI mRNA were significantly reduced in the muscle, liver, heart muscle and brain of AMD quails. A second GAA II cDNA was identified after probing the cDNA library from the ovarian large follicles of quails with a PCR product derived from cultured quail skin fibroblasts. This clone with a 3.1 kbp insert, has GAA activity as well (3 to 10 fold increase in AMD quails). The cDNA of GAA II showed 63% homology to human GAA and 51% homology to the GAA I [12].

4) Gene therapy in AMD quail

Enzyme replacement therapy

Human recombinant GAA restored acid maltase in acid maltase-deficient human fibroblasts, quail fibroblasts, and quail myoblasts to its normal level within 24 hr [53]. These results demonstrate the potential of the AMD quail to test receptor-mediated enzyme replacement therapy for Pompe disease. Therefore, six 4-week-old AMD quails were intravenously injected with 14 or 4.2 mg/kg of precursor form of recombinant human GAA every 2–3 days for 18 days (seven injections). On day 18, two high dose-treated birds (14 mg/kg) scored positive flip tests and flapped their wings. GAA activity increased in most of the tissues examined. In the heart and liver, glycogen levels dropped to normal, and histopathology of the pectoralis muscle became normal, except for increased glycogen granules. These results are the first to show that an exogenous protein can target muscle and improve muscle function. These data also suggest that enzyme replacement with recombinant human GAA is a potential therapy for human Pompe disease [10].

Gene therapy of AMD quail by adeno virus containing the human acid maltase cDNA

We constructed a recombinant adeno virus containing the human acid maltase cDNA downstream of the CAG promoter, composed of modified chicken β-actin promoter and CMV IE enhancer (AxCANAM). When cultured fibroblasts from AMD quail were infected with AxCANAM, acid maltase activity in the cells increased in proportion to the multiplicity of infection. When AxCANAM was injected unilaterally into the superficial pectral muscle of AMD quail, PAS staining showed that glycogenosomes disappeared and stainability of acid phosphatase was reduced in the injected area as compared with the contralateral muscle of the same bird. Biochemically, acid maltase activity increased and glycogen content decreased in the injected muscle [47].

(2) Myotonic dystrophy in Japanese quail

The disorder is clinically apparent as early as 28 days of age; it is characterized by generalized myotonia, muscle stiffness, and muscle weakness [1]. Affected birds are identified by their inability to lift their wings vertically upward and by their inability to right themselves when placed on their dorsum. This symptom is very similar to that of AMD quail. AMD in Japanese quail is controlled by an autosomal recessive gene. However, myotonic dystrophy is controlled by an autosomal dominant gene and is lethal in the homozygous type [2].

Electromyographic studies in mutant quail showed high-frequency repetitive discharges comparable to those of myotonic runs. These discharges persisted after nerve resection. The distinctive histopathologic changes in the various muscles examined were ring fibers, sarcoplasmic masses, and internal migration of sarcolemmal nuclei. A slight decrease in the size of type IIB muscle fibers and a slight increase in the size of type IIA fibers were observed in the M. pectoralis thoracicus of affected quails. The typical muscle lesions and multisystem involvement, which are manifested by testicular degeneration and atrophy in affected male quail specimens and bilateral lenticular cataracts in 6 of 13 affected quails, suggest the resemblance of this inherited muscular disorder to myotonic dystrophy in humans. The relationship between the development of muscle lesions and age, as well as immunohistochemical and ultrastructural features of this mutant quail have also been studied [45, 46].

(3) Japanese quail with neurofilament deficiency

We found a new behavioral mutant showing either head or body quivering, or both, in Japanese quail. This trait was characterized by neurofilament deficiency in the axons of the cervical spinal cord and the optic and sciatic nerves, and was named "hypotrophic
axonopathy”. This character was controlled by an autosomal recessive gene [30]. This is the first mutant suggesting compatibility to a vertebrate without neurofilaments (NFs). The existence of this mutant shows that NFs are not necessary for the maintenance of life.

A mutant strain (Quv) was successfully established. Electron microscopically and immunohistochemically, neurofilaments were not detected in the axons or neuronal cell bodies in Quv. Axons in Quv were composed mainly of microtubules, which were increased in number in relation to the axonal size. Gel electrophoresis and Western blot analysis indicated that low, middle and high molecular mass neurofilament subunits are markedly deficient in the brain, cervical spinal cord and sciatic nerve of Quv. Immunohistochemically, the spinal cord of Quv has no immunoreactive products corresponding to low molecular mass NF. However, middle and high molecular mass NF antisera stains axons.

We hypothesize that the deficiency of NFs in Quv results from an alteration of filament assembly caused by defective expression of low molecular mass NF [51, 52]. NF-L genes of the normal and mutant quails were cloned and sequenced. The NF-L gene in the mutant was found to have a nonsense mutation at the deduced amino acid residue 114, indicating that the mutant is incapable of producing even a trace amount of NF-L protein under any condition [37]. The morphological features of the myelinated fibers in Quv have also been studied in detail [8, 39, 54–56].

The neurotoxic effects of acrylamide (AC), β, β'-iminodipropionitrile (IDPN), and 2,5-Hexaniedione (2,5-HD) were investigated in Quv. AC produced axonopathy with a distal-proximal progression in both normal and Quv quails. In AC-intoxicated normal quails, the nerve fiber pathology was characterized by typical Wallerian-like degeneration, consisting of axonal degeneration, myelin breakdown, macrophage migration, Schwann cell proliferation and regeneration of nerve fibers. Ultrastructurally, AC-induced NF accumulation was detected in the axon of myelinated nerve fibers. In AC-intoxicated Quv quails, axonal degeneration with accumulation of membraneous organelles occurred; however, sequential events of Wallerian-like degeneration were not as prominent as in AC-intoxicated normal quails. These results demonstrated that NF-deficient Quv quails are sensitive to neurotoxic effects of AC. On the other hand, the different pathology of AC-intoxicated normal and Quv quails indicates that the presence or absence of NFs influences the appearance and extent of AC axonopathy [43, 44].

When IDPN was injected into normal quails, axonal swelling was observed histologically in the ventral motor neurons, ventral root, commissura grisea and spinal ganglion in the cervical and sacral spinal cord. Electron microscopically, the changes consisted of increased NFs, with scattered mitochondria, smooth endoplasmic reticulum and microtubules. The myelin sheaths of the involved nerves were thinner than the normal axons. These lesions were similar to those induced by IDPN intoxication in mammalian experimental animals. In NF-deficient quails injected with IDPN, no axonal changes were detected. These findings suggested that IDPN selectively attacks the NFs [17].

The 2,5-HD-exposed normal quails showed leg paralysis about 4 weeks after initiation of dosing. Some treated normal quails fell into dysstasia and died of nutritional disturbances. Histologically, 2,5-HD-treated normal quails had NF-rich axonal swellings and degenerations in the distal parts of the peripheral nerves, spinal cord, and cerebellar peduncles. In contrast, 2,5-HD-injected Quv quails showed tonic convolution, ataxic gait, severe quivering, and excitation about 2–3 days after administration. Some treated Quv birds died immediately after systemic tonic convolution, probably because of asphyxia. Although all treated Quv quails showed neurologic signs, there were no recognizable 2,5-HD induced lesions in the nervous system. After about 4–6 weeks of dosing, 2,5-HD induced distal axonopathy in normal quails and acute neurotoxicity in Quv quails [6, 7].

(4) Inherited visual impairment in GSN/1 chickens

GSN/1 chickens were developed by us in a Fayoumi breed as a strain with visual impairment. Pathological study revealed that the number of the retinal ganglion cells was significantly smaller in GSN/1 chicks than in GSP control chicks. The amplitude of b waves of electroretinograms (ERGs) for green stimuli was similar to that for red stimuli in GSP normal controls for each stimulus intensity. In GSN/1 chicks, the amplitude of b waves of ERGs for green stimuli was smaller than that for red stimuli.
In conclusion, impaired bipolar cell function specific to color was suggested in GSN/1 chicks. Clear visual evoked potentials (VEPs) responses to red and green, dim stimuli were observed in GSP chicks. However, VEPs responses to green dim stimuli were not clear in GSN/1 chicks. The mode of inheritance of the character is under investigation.

(5) PNP/DO chicken line showing right oviduct and metanephric hypoplasia

Females in the PNP/DO line have varying length of an elongated right oviduct besides a normal left oviduct and ovary. PNP/DO line produces 80 to 100% of female with right oviduct in every generation. This character was suggested to be controlled by a few pairs of autosomal recessive genes [48].

Regarding the metanephric hypoplasia, affected embryos exhibited defects of all three lobes of metanephros uni- or bi-laterally. In affected embryos, hypertrophy of mesonephros that accompanied the metanephros defect was also observed. The character of metanephric hypoplasia seems to be inherited, and the mode of inheritance is under investigation.

(6) Fayoumi chicken showing spontaneous lymphocytic thyroiditis with feather amelanosis

Several chickens showing amelanotic changes of feathers after molting were discovered in a closed colony, YL line of Fayoumi chicken. Histologically, lymphocytic thyroiditis with a possible autoimmune basis was present in the affected chickens. The thyroid glands were mildly or severely infiltrated by lymphoid cells and plasma cells. Most of the lesions developed diffusely around capillaries, and in a severe case more than half of the gland was replaced by infiltrating cells. The most characteristic findings were the presence of plasma cells as well as germinal centers containing many activated macrophages and mitotic cells, suggesting acute lymphocytopenia and antibody production due to prolonged antigenic stimuli.

The chickens with lymphocytic thyroiditis lacked pigment completely or partially in barb ridges and barbule cells of many regenerating contour feathers collected from the wings and tail. At the tip of the barb ridges, melanocyte abnormalities were occasionally observed, such as irregularities in the cell shape and bulbous swelling with thick and short dendrites suggestive of dendritic retraction and random distribution of these cells. The mean concentrations of plasma levels of T4 and T3 in affected chickens were lower than those of control chickens.

The sera from normal chickens were negative for the autoantibody to thyroglobulin, while those from chickens with thyroiditis specifically reacted with the thyroglobulin antigen. The feather amelanosis seen in chickens closely resembled that in chickens of the delayed amelanotic line (DAM or Smith chicken) [36].

(7) Fayoumi chicken with a hereditary nervous disorder

A hereditary nervous disorder in Fayoumi chicken was found in 1976. Affected birds suddenly bent their necks downwords and placed their heads beneath the bodies. Some birds simultaneously took a backward step. The duration of the convulsive seizure varied, but in most cases it lasted for 15–30 s. Once the seizure occurred it was not interrupted and ran its course. Following the seizures, the birds opened their eyes, slowly regained their footing and recovered completely. The seizures were instigated by unexpected loud noises, sudden movement, bright light and other audiovisual stimuli.

The main histopathological lesions consisted of degeneration of small arteries with cellular infiltration in the cerebrum of all chickens examined and demyelination and/or loss of nerve fibers with edema and Schwann cell proliferation in the cervical spinal nerves of most birds. Genetic analysis revealed inheritance controlled by an autosomal recessive gene [34].

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