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Complete Regeneration of Muscular Dystrophy Chickens by Mating of Male and Female Offspring Derived from Germline Chimeras

Akira Fujiwara[1,2], Tamao Ono[2], Kohzy Hiramatsu[2] and Hiroshi Kagami[2]

[1] Nippon Institute for Biological Science, Laboratory Animal Research Station, Kobuchisawa, Yamanashi 408–0041, Japan
[2] Faculty of Agriculture, Shinshu University, 8304 Minamiminowa, Kamiina, Nagano 399–4598, Japan

In previous studies, two types of offspring were generated from germline chimera between NH-413 strain (donor) and White Leghorn L-M strain (recipient). Phenotype and symptom of type-I offspring were quite similar to that of the NH-413 strain. In the other offspring of type-II, feather color showed mixture of white and brown and the symptom was not dominantly indicated.

In the present studies, sexually matured males and females of the type-I were mated each other. Form these mating, chickens manifesting completely same phenotype to that of the donor NH-413 strain; brown feather color and symptoms of muscular dystrophy, were regenerated. Therefore, complete regeneration of the muscular dystrophy chickens could be achieved by mating males and female offspring derived from the germline chimeras. Fertility, hatchability and survival rate of these regenerated offspring were significantly improved as compared to that of the original NH-413 strain.

The established strategies should be one of the useful systems to regenerate chickens with muscular dystrophy.

Key words: chicken, complete regeneration, germline chimera, muscular dystrophy

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Introduction

The chicken is now one of the most versatile experimental systems available (Stern, 2005). Various technologies such as *in vivo* electroporation, the isolation of embryonic stem cells, and the sequencing of the chick genome (Wallis *et al.,* 2004) could be available. However, numbers of useful experimental animal model in chickens are very small as compared to that of mammalian species. A chicken strain of NH-413 has found to be possessing candidate genes relating to Fukuyama type muscular dystrophy (Saito *et al.,* 2005). Although the strain is a good chicken model for the muscular dystrophy, the breeding and reproduction is very difficult with the conventional system.

In recent years, avian embryo engineering technologies of transplanting donor avian pluripotent cells from the stage X blastoderms or PGCs into the recipient embryos was established to generate germline chimeras. These embryos manipulation system has been considered as one of the most powerful for genetic conservation of rare birds or production of transgenic birds (Tajima *et al.,* 1993; Pain *et al.,* 1996; Kagami *et al.,* 1997). In the previous studies, a novel strategy has been developed to generate muscular dystrophy chickens by germline chimeras (Fujiwara *et al.,* 2008). Donor blastoderms were obtained from the NH-413 chicken strain which have genes responsible for Fukuyama type muscular dystrophy (Saito *et al.,* 2005). Recipient embryos were obtained from Line-M chicken. The chimeras had the donor derived brown plumage in the down in some extent. The chimeric chickens were back-crossed to donor strain; the NH-413 strain. The phenotype of some of the offspring (type-I) was very similar to that of the donor strain as muscular dystrophy. In the other offspring of type-II, feather color showed mixture of white and brown and the symptom was not dominantly indicated.

In the present studies, sexually matured males and females of the type-I were mated each other to achieve complete regeneration of muscular dystrophy chickens.

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Correspondence: Prof. H. Kagami, Faculty of Agriculture, Shinshu University, 8304, Minamiminowa, Kamiina, Nagano 399–4598, Japan. (E-mail: kagami@shinshu-u.ac.jp)

Material and Methods

Classification of the offspring from germline chimera

In the previous studies, germline chimeras were generated. Donor blastoderms from the NH-413 chicken strain which had genes responsible for Fukuyama type muscular dystrophy (Saito *et al.,* 2005) was injected into recipient embryos from Line-M chicken. The chimeras showed the mixed feather color of donor derived brown

Table 1. **The fertility, hatchability, pigmentation and survival rate of NH-413 strain and Type-I***

	Number of fertilization eggs	Fertility	Hatchability	Percentage of brown pigmentation	Survival rate
NH-413 strain	50	41.2± 3.5[a]	36.2± 5.1[a]	100	24.4± 6.9[a]
Type-I	64	65.4±12.8[b]	58.6±12.0[b]	100	50.8±12.9[b]

* Value represent the mean±SE in chickens.
[a: b]: Value without common superscripts are significantly different (p-test: $p < 0.01$).

and recipient derived white (Fujiwara *et al.,* 2008). The chimeric chickens were back-crossed to donor strain; the NH-413 strain. The phenotype of some of the offspring was very similar to that of the donor strain as muscular dystrophy. These offspring was classified as type-I. In the other offspring, feather color showed mixture of white and brown and the symptom was not dominantly indicated. These offspring was classified as type-II. In the present studies only type-I offspring was used for further experiment.

Test mating of the males and females of type-I offspring

Fertility, hatchability and survival rate of the type-I offspring was recorded and these parameters were compared to that of the original NH-413 strain. Hatched males and female type-I offspring were raised until sexually matured. Phenotype and symptoms of these offspring was observed. Males and females of theses type-I offspring was mated each other. Phenotype and symptom of the hatched offspring was observed.

Results

Total 64 fertilized eggs obtained by mating the type-I males and females offspring were collected and incubated. Total 50 fertilized eggs from the NH-413 strain were incubated and used as control (Table 1). Egg fertility of the type-I was 65.4% and it was significantly high ($p < 0.01$) as compared to that of 41.2% in control eggs. Hatchability of the chick offspring from type-I eggs was 58.6% and it was significantly high ($p < 0.01$) as compared to that of 36.2% in control eggs. Survival rate of the chicks from type-I eggs was 50.8% and it was significantly high ($p < 0.01$) as compared to that of 24.4% in control eggs. Therefore, all of these three parameters of fertility, hatchability and survival rate in offspring from type-t mating were significantly improved ($p < 0.01$) as compared to that of the original NH-413 strain (Fig. 1). All down (100%) of the hatched chicks showed brown color and was similar to that of original NH-413 strain. After sexual maturity, the feather color of these type-I male and female (Fig. 2) is brown and was similar to that of original NH-413 strain. Symptoms of the muscular dystrophy was observed in these chickens.

When these male and female of type-I was mated, chick offspring was obtained. Feather color of these hatched chicks was all brown and was just same as to that of original NH-413 strain. Symptoms of the muscular dystrophy was also just same as that of the original NH-413 strain. After sexual maturity these chicken hardly fell down very easily (Fig. 3). Therefore, complete regeneration of the muscular dystrophy chickens by mating of type-I male and female offspring derived from germline chimeras was achieved.

Discussion

Chicken muscular dystrophy with abnormal muscles was first reported by Asmundson and Julidan (1956). The disorder is transmitted co dominantly by a single gene, whose phenotype is modified by other "background" genes (Asmundson and Julian, 1956; Wagner and Peterson, 1970).

In previous study, when the blastodermal cells of muscular dystrophy NH-413 strain was incorporated into the recipient embryo of L-M strain, somatic chimerism was observed by existence of the brown feather pigmentations from NH-413 strain. In the generated chimera, muscular dystrophy symptom was not so sever as compared to that of original NH-413 strain. By mating between NH-413 strain and generated chimeras, 2 types offspring: type-I and type-II was previously obtained (Fujiwara *et al.,* 2008). Type-I had similar phenotype to NH-413 strain. But, Type-II were not similar to NH-413 strain at all. In the present studies, only type-I adult males and females was used as parents to obtain their offspring. As summarized in Fig. 4, complete regeneration of the muscular dystrophy chickens by the mating was achieved. Moreover, the fertility, hatchability , donor-derived pigmentation and survival rate of the offspring have significantly improved as compared to that of the original NH-413 strain. It was suggested that the offspring from the germline chimera possessed genes responsible for the dystrophy symptoms but it also accumulated genes responsible reproductive performance. Therefore, these parameters could be improved remarkably. The established strategy should be one of the most powerful tools for breeding and regeneration of the muscular dystrophy chickens. These chimeric technologies could also contribute for the generation of the transgenic chickens.

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Fig. 1. **NH-413 strain (since 1976 in NIBS).**
The chickens has symptom of Muscular dystrophy disease. Therefore, conventional breeding and maintenance are very difficult.

Fig. 2. **Type-I progeny: A: male, B: female.**
Phenotype of these type-I chicken was similar to NH-413 strain.

Fig. 3. **Offspring by mating type-I male and female.**
Sever symptoms of the muscular dystrophy in male (right) and female (left) offspring was also just same as that of the original NH-413 strain. After sexual maturity these chicken hardly fell down very easily. Complete regeneration of the muscular dystrophy chickens was achieved.

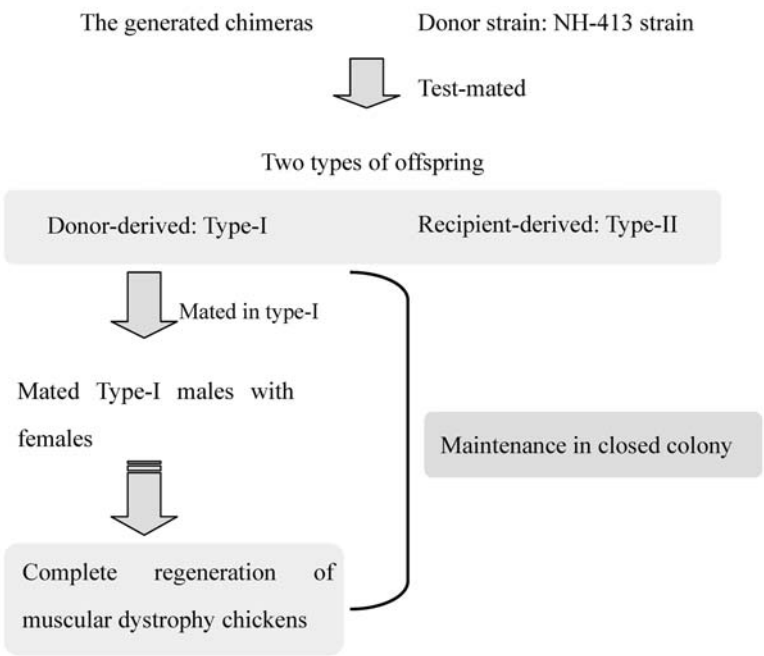

Fig. 4. **Outline of present studies.**
Sexually matured males and females of the type-I offspring from a germline chimera were mated each other. Form these mating, chickens manifesting completely same phenotype to that of the donor NH-413 strain; brown feather color and symptoms of muscular dystrophy, were regenerated. Complete regeneration of the muscular dystrophy chickens could be achieved.

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