Journal of Animal and Veterinary Advances 10 (15): 1991-1993, 2011
ISSN: 1680-5593

A Missense Mutation in Mitochondrially Encoded NADH Dehydrogenase Subunit-5 Gene Associates with Chicken Fetal Growth under Hypoxia Condition

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Abstract: Hypoxia can restrict fetal growth and increase the mortality of chicken embryos. Under hypoxia conditions, the fetal growth of the high-altitude chicken was restricted but to a lesser extent compared to the fetal growth of the lowland chicken. Mutations in NADH dehydrogenase subunits may affect mitochondrial functions including the production of Adenosine Triphosphate (ATP) which is required in fetal growth. A missense substitution at nucleotide position 1627 of mitochondrially encoded NADH dehydrogenase subunit-5 gene (*MT-ND5*) has been found in a high-altitude chicken breed of the Tibet chicken which originates from the Qinghai-Tibet Plateau and has adapted itself well to hypoxia. The objective of the present study was to investigate association of the *MT-ND5* mutation with the fetal growth of the Tibet chicken. The genotypes of hens were identified using amplified created restriction sites and embryonic genotypes were determined by the genotypes of their mothers. Breeding eggs before incubation and fetuses at day 9 and 16 of incubation were weighed. The results showed that the *MT-ND5* gene variation was significantly associated with the growth of the Tibet chicken fetus under hypoxia condition but not under normoxia condition which implied that the *MT-ND5* gene could be one of putative candidate genes or linked to important genes that affect chicken fetal growth at high altitude.

Key words: Tibet chicken, fetal growth, *MT-ND5* gene, hypoxia, atitude, China

INTRODUCTION

Hypoxia is a deleterious physiological stress which can promote the production of Reactive Oxygen Species (ROS) (Duranteau *et al*., 1998) and lead oxidative stress (Askew, 2002). Excessive ROS may damage membrane lipids, protein and nucleotide in cells (Guerin *et al*., 2001) and affect mitochondrial enzyme activities and impair mitochondrial functions, consequently reduce the product of adenosine triphosphate (Paradies *et al*., 2002; Lei *et al*., 2006) which is required in fetal growth. Several investigators reported that hypoxia restricted the growth of chicken fetuses (Giussani *et al*., 2007; Mehta and Mehta, 2008; Zhang *et al*., 2008; Wei *et al*., 2007) and increased the mortality of chicken embryos (Zhang *et al*., 2006, 2008). Under hypoxia conditions, the fetal growth of the high-altitude chicken was also restricted but to a lesser extent compared to the fetal growth of the lowland chicken (Giussani *et al*., 2007; Wei *et al*., 2007).

NADH dehydrogenase (Complex I), consisting of 45 different subunits in mammalian (Carroll *et al*., 2006) is the largest among the four enzyme complexes in electronic transfer chain in mitochondria. It catalyzes the reduction of ubiquinone with NADH as reducing substrate which couples with the transfer of protons from mitochondrial matrix to the intermembranous space. The function of pumping protons across the inner mitochondrial membrane at Complex I, Cytochrome bc_1 (Complex III) and Cytochrome oxidase (Complex IV) establishes a proton gradient which can be used for ATP synthesis (Saraste, 1999).

Mutations in subunits of Complex I may affect functions of the enzyme and mitochondria usually result in some illness (Sharma *et al*., 2009; McKenzie *et al*., 2007). The Tibet chicken originates from the Qinghai-Tibet plateau and has adapted itself well to hypoxia. A missense substitution at nucleotide position 1627 (1627C>A) of mitochondrially encoded NADH dehydrogenase subunit-5 gene (*MT-ND5*; EF493865) resulting in a His-to-Asn change at amino acid position 543 has been found in the high-altitude chicken breed (Bao *et al*., 2007). The objective of the present study was to investigate association of the MT-ND5 mutation with fetal growth in the Tibet chicken.

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MATERIALS AND METHODS

Tibet chickens of the same week age were raised in the Experimental Chicken Farm of China Agricultural University. Breeding eggs from Tibet chickens, collected within 3 days post lay were separated into two groups based on the genotypes of the hens and each group was randomly divided into four subgroups. Two of the four were incubated under normoxia condition (21% of oxygen concentration, 21% O_2) for either 9 or 16 days and the rest two subgroups were incubated in 13% O_2 for 9 and 16 days, respectively. Eggs of any one subgroup were weighed prior to incubation. The incubations were carried out under the controlled condition of 37.8°C of temperature, 60% of humidity, 12 per day of egg rotation and 13 or 21% of oxygen concentration. At the end of the 9th or 16th day of incubation, egg shells were opened at the air-cell and the living fetuses were pulled out and killed. Following being apart from any other component of the eggs, the wet fetuses were weighed with a digital scale accurate to 0.001 g. After weighed, the fetuses were put into an air oven at 120°C for 2 h and then dried at 80°C until invariable. The dry fetuses were weighed after drying.

Total DNA was isolated from hen blood samples referring to the protocol described by Huang *et al*. (2004). The genotypes of hens were identified according to the method described by Bao *et al*. (2007) and the fetal genotypes were determined by the hen genotypes because mitochondrial DNA is maternal inherited (Berlin and Ellegren, 2001).

Values were expressed as the mean±standard error. Data were analyzed using the t-test of Excel. XP (Microsoft Corp., Redmond, WA) or Duncan's test of SPSS 13.0 (SPSS Inc., Chicago, IL). Statistical significances were set at $p<0.05$ or $p<0.01$.

RESULTS AND DISCUSSION

The weight of breeding egg affects the fetal growth (Mortola and Awam, 2010). In the present study, all breeding eggs were weighed before incubation and the values were shown in Table 1. No differences were found in egg weight among the eight groups and between the breeding eggs of the A genotype and the C genotype implying that the egg weight was not an important factor to be considered necessarily in the present study.

Data on the fetal weights with different genotypes at day 9 and 16 of incubation were shown in Table 2 and 3, respectively. Whether at day 9 or day 16 of incubation, the weights of chicken fetuses (including the wet and dry fetuses) under the hypoxia condition decreased by 22.7-44.3% compared to the corresponding controls under the normoxia condition which tallies with the general view that hypoxia retards the growth of the chicken embryo. There were no differences in the fetal weights (including the wet and dry fetal weights) between the A genotype and the C genotype embryos incubated in the same oxygen concentrations at day 9 of incubation. At day 16 of incubation, no difference was found in the fetal weights (including the wet and dry fetal weights) between the A genotype and the C genotype embryos under the normaxia condition (21% O_2) whereas under the hypoxia condition of 13% O_2, the fetal weights (including the wet and dry fetal weights) of the C genotype were higher than those of the A genotype (Wet fetus: $p = 0.016$; Dry fetus: $p = 0.024$).

Table 1: Measurements of the weights of breeding eggs before incubation[1,2]

Group name	Phase of incubation[3]	Oxygen concentration	Genotype[4]	Weights of breeding eggs before incubation (g)
NA-9	9	21% O_2	A	46.2±1.1 (n = 14)
NC-9	9	21% O_2	C	46.4±1.0 (n = 14)
HA-9	9	13% O_2	A	45.1±0.9 (n = 14)
HC-9	9	13% O_2	C	46.0±0.7 (n = 14)
NA-16	16	21% O_2	A	45.5±1.3 (n = 12)
NC-16	16	21% O_2	C	46.2±0.7 (n = 12)
HA-16	16	13% O_2	A	47.0±1.4 (n = 12)
HC-16	16	13% O_2	C	45.8±1.0 (n = 12)

[1]Values represent the mean±SE; [2]The egg weight of the A genotype is 46.0±0.6 and the egg weight of the C genotype is 46.1±0.4; [3]At the end of the 9th or 16th day of incubation, egg shells were opened at the air-cell and the fetuses apart from any other components of breeding eggs were weighed; [4]NADH dehydrogenase subunit-5 gene is coded by mitochondrial DNA

Table 2: The weights of wet and dry fetuses with different genotypes at day 9 of incubation[1] (n = 14)

Group name	Oxygen concentration	Genotype[2]	Wet fetus (g)	Dry fetus (g)
NA-9	21% O_2	A	1.40±0.03[a]	0.094±0.002[a]
NC-9	21% O_2	C	1.41±0.03[a]	0.092±0.002[a]
HA-9	13% O_2	A	1.07±0.03[b]	0.068±0.002[b]
HC-9	13% O_2	C	1.09±0.02[b]	0.067±0.002[b]

[1]Values represent the mean±SE; [2]NADH dehydrogenase subunit-5 gene is coded by mitochondrial DNA; [a, b]values within a column with different letters are different ($p<0.05$)

Table 3: The weights of wet and dry fetuses with different genotypes at day 16 of incubation[1] (n = 12)

Group name	Oxygen concentration	Genotype[2]	Wet fetus (g)	Dry fetus (g)
NA-16	21% O_2	A	12.23±0.19[a]	1.974±0.046[a]
NC-16	21% O_2	C	11.84±0.32[a]	1.924±0.060[a]
HA-16	13% O_2	A	8.36±0.27[c]	1.099±0.048[c]
HC-16	13% O_2	C	9.15±0.16[b]	1.246±0.042[b]

[1]Values represent the mean±SE; [2]NADH dehydrogenase subunit-5 gene is coded by mitochondrial DNA.[a-c]values within a column with different letters are different

CONCLUSION

The results suggested that the genotypes were significantly associated with the fetal growth in the period from day 9-16 of incubation under the hypoxia condition (13% O_2) but not under the normoxia condition which

implied that the *MT-ND5* gene could be one of putative candidate genes or linked to important genes that affect chicken fetal growth at high altitude.

ACKNOWLEDGEMENT

The research was supported by the 973 Projects of China (grant No. 2006CB102101).

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