Causal Mutation And Associated Traits For The "silver" Japanese Quail

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Introduction

Mutations in *MITF* (microphtalmia-associated transcription factor) have detrimental effects on melanocytes and lead to decreased pigmentation and other effects well described in mice but little investigated in other species (Steingrímsson et al. (1994)). In the mouse, the semi dominant mutation $Mitf^{Mi\text{-}wh}$ produces heterozygous mice with a diluted coat colour and homozygous mice which are all white (Steingrímsson et al. (2004)). Similarly in the Japanese quail (*Coturnix japonica*), the plumage colours of the "white" (*B/B*) and "silver" (*B/+*) quail (Figure 1) were found to be associated with a semi dominant mutation *B* (Homma et al. (1969)), which was located in *MITF* (Mochii et al. (1998)). The causal mutation, however, was not determined, and other phenotypic effects associated with the mutation were not studied, except for osteopetrosis (Kawaguchi et al. (2001)). The "silver" Japanese quail might be an interesting model for the comparative study of the effects of *MITF* in birds and mammals. The objectives of this study were to find the mutation in the coding sequence of *MITF* which is responsible for the "silver" plumage colour and to study the associated effects of the mutation on growth, feed consumption, body composition and other characteristics of the Japanese quail.

Figure 1: "Silver" quail (B/+) on the left and "white" quail (B/B) on the right

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Material and methods

Birds and husbandry. The "silver" mutation *B* was introduced from Gifu University (Japan), and a line segregating for *B*, but +/+ for all other known major plumage colour mutations, was set up in Nouzilly (France). The 90 quail used in the study were the single hatch progeny of 17 single pair matings between *B*/+ quail. All quail were raised together and given *ad libitum* commercial feed and drinking water.

Sequencing and genotyping. The structure of *MITF* in quail was determined by comparative alignment with chicken data for *MITF*, using the quail mRNA sequence (AB005229). The two genomic changes (Mochii et al. (1998)) associated previously with "silver" plumage were located in exons 8 (non-synonymous change) and 11 (2bp deletion). Amplifications of the two regions led to a product of 1055 bp for the first one and to a product of 278 or 280 bp according to the genotype for the second one. The 2bp deletion polymorphism was genotyped in all quail and PCR product sizes were 90 bp for +/+, 88 bp for B/B, and 88 and 90 bp for B/+ quail.

Traits and statistical analyses. All quail underwent a feed trial between the ages of 9 and 12 weeks, during which individual feed intake, body weight and body temperature, as well as egg size and egg number laid by females, were recorded. They were sacrificed and weighed at 24 weeks, and a dissection was performed to measure the weights of abdominal adipose tissue, liver, heart, right *Pectoralis* muscles, and of tibias from the three genotypes. Except for female traits, analyses of variance and covariance had family, sex and genotype (B/B, B/+ and +/+) as main effects. The covariable was body weight (for body temperature) and carcass weight (for dissection traits). All analyses were run using the SAS software.

Results and discussion

Localization of the silver mutation in *MITF*. The two putative regions for the mutation in *MITF* were sequenced in 3 +/+ and 3 B/B Japanese quail, and the sequences were deposited in the GenBank nucleotide data base under accession numbers GQ386796-GQ386799. The non-synonymous change was not associated to the plumage colour. On the other hand, a perfect association was obtained between the genotype for the 2 bp deletion and the plumage colour (white, silver or wild-type) of the F2 quail population which showed that the stop codon resulting from this deletion was the most probable cause of the B mutation.

Phenotypic effects associated with the silver mutation. The results of the feed trial are given in Table 1. The genotype at *MITF* was associated with variations in body weight ($P<0.01$ or $P<0.05$), feed intake ($P<0.05$), and body temperature after fasting ($P<0.001$), but it did not affect egg laying, at least during the 3-week feed test. Homozygous "white" quail were lighter than the other two quail classes, and they also had a decreased feed intake and a consistently lower body temperature. Decreased body weight was also reported in homozygous white mice (Grobman and Charles (1947)) and in Japanese quail with non wild-type plumage, like the "roux" quail (Minvielle et al. (1999)). Published results on body temperature or feed intake in *MITF*-mutated mice could not be found in the Literature. A significant association between body temperature and plumage colour variation was already reported in Japanese quail, for the "yellow" mutation in the *ASIP* gene (Minvielle et al. (2007)), but the decrease (-0.3 to -0.5 °C) in body temperature associated with "silver" was more severe.

Table 1: Effects of genotype on body weights, feed intake, egg production and body temperature (mean ± SD) of 9-week old Japanese quail, and comparisons between genotypes

Trait	Genotype				Contrast	
	White (*B*/*B*)	Silver (*B*/+)	Wild-type (+/+)	Signif.	(*B*/*B*) - (+/+)	(*B*/*B*) - (*B*/+)
64-d body weight (g)	148.5 ± 20.2	154.5 ± 15.6	158.2 ± 15.1	**	-14.9***	-11.9**
85-d body weight (g)	159.8 ± 23.5	161.4 ± 16.6	164.5 ± 16.2	*	-11.3**	-8.4*
Feed intake (g/d)	20.5 ± 5.2	21.1 ± 4.0	22.4 ± 5.1	**	-3.43**	-2.45*
Egg number on 3-wk test	17.0 ± 6.1	19.1 ± 2.9	18.9 ± 4.3	NS	NS	NS
Egg weight (g)	9.87 ± 0.87	9.61 ± 0.71	9.73 ± 0.52	NS	NS	NS
64-d body temperature (°C)	40.82 ± 0.40	41.16 ± 0.32	41.32 ± 0.29	***	-0.53***	-0.33**
85-d body temperature (°C)	40.67 ± 0.25	41.23 ± 0.35	41.34 ± 0.33	***	-0.57***	-0.44***

*:P<0.05; **:P<0.01; ***:P<0.001; NS: not significant.

The results of the gross dissection of quail carcasses are listed in Table 2. The genotype at *MITF* had a significant effect (P<0.01 or P<0.05) on the weights of all measured carcass parts but the liver. On an equal body weight basis, the contents in abdominal adipose tissue were about twice as large in homozygous "white" quail than in the other two quail classes. On the contrary, the contents in skeletal *Pectoralis* muscles were larger in "silver" and wild-type" quail, and the heart was smaller in "white" quail. Body composition has not been extensively studied in *MITF* mutant mice, but our observations on heart size of "white" quail are consistent with the previous report of a lower heart weight in homozygous *MITF* mutant mice (Tshori et al. (2006)). They indicate that *MITF* is likely to be also associated to cardiac growth in birds. Depressed growth of *Pectoralis* muscles in "white" quail might be an indication that *MITF* is also involved in skeletal muscle development, but there are no published data available on skeletal muscle growth and *MITF* variation in mice for comparison purposes. Finally, the 30% increase in tibia weights observed in "white" quail confirmed the previous reports that B/B quail (Kawaguchi et al. (2001)) and mice homozygous for the strong semi dominant *MITF* mutations (Steingrímsson et al. (2002)) showed "osteopetrosis", i.e. a thickening of the bones which become abnormally dense due to an inherited defect in bone resorption.

Table 2: Effects of genotype on body composition (mean ± SD) of 24-week old Japanese quail, and comparisons between genotypes

Trait	Genotype				Contrast	
	White (*B*/*B*)	Silver (*B*/+)	Wild-type (+/+)	Signif.	(*B*/*B*) - (+/+)	(*B*/*B*) - (*B*/+)
Carcass weight (g)	159.8 ± 27.9	163.2 ± 19.4	168.3 ± 18.7	**	-16.6**	NS
Abdominal adipose tissue[1] (g)	2.3 ± 3.2	1.4 ± 0.9	1.5 ± 1.3	*	1.5**	1.2**
Pectoralis muscle weight[1,2] (g)	11.7 ± 2.2	13.0 ± 1.5	13.4 ± 1.6	**	-1.9***	-1.6***
Liver weight[1] (g)	3.4 ± 1.1	3.6 ± 1.4	3.5 ± 1.0	NS	NS	NS
Heart weight[1] (g)	1.2 ± 0.2	1.4 ± 0.2	1.5 ± 0.2	*	-0.2**	-0.2**
Tibia weight (g)	0.91 ± 0.19	0.72 ± 0.10	0.70 ± 0.08	***	0.17***	0.15***

[1]:Carcass weight was used as a covariable for the analyses of abdominal adipose tissue, *Pectoralis* muscle weight, liver weight, and heart weight.
[2]:Total weight of the right *Pectoralis major* and *Pectoralis minor* muscles.
*:P<0.05; **:P<0.01; ***:P<0.001; NS: not significant.

Conclusion

The similarities of phenotypes (white or silver plumage and coat colours) and of associated traits (body and heart growth, osteopetrosis) in quail and mice with mutations at the *MITF* gene are such that the "silver" Japanese quail should be useful for comparative studies of the effects of this gene in birds and mammals.

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