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Gene Assisted Selection For Meat Color In The Chicken

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Introduction

As poultry meat is now mostly consumed as parts or further-processed products, the importance of the sensorial and technological properties of the meat has been reinforced. The studies conducted in chicken within the last decade have showed an important effect of genetics in the control of several meat quality traits, such as the color, the water-holding capacity or the texture (Le Bihan-Duval et al., 1999, 2001, 2008). Even if the genetic parameter estimates suggest that "conventional" selection could be applied for improving meat quality, at the moment only a sib selection can be practiced, which implies lower efficiency and higher cost of the selection program. The identification of genetic markers constitutes another promising way. The research of genetic markers for chicken meat quality has been initiated a few years ago, by combining QTL detection and gene expression profiling (Le Bihan-Duval et al., 2007). This paper reports a first application, dealing with the identification of a gene controlling the variation of meat color in the chicken.

Material and methods

QTL detection. This research was conducted on a unique resource population obtained by a F2 inter-cross between two experimental lines of chickens divergently selected for body weight (Ricard, 1975). As detailed by Nadaf *et al.* (2007) and in table 1, at 9 weeks of age body weight and abdominal fatness were much higher in the High Growth (HG) line by comparison to the Low Growth (LG) line (3 and 12 fold differences, respectively). Interestingly, significant differences in breast meat quality were also reported, including for meat color which was significantly paler (higher L*) and less intense (lower redness a* and yellowness b*) in the HG chickens. A large variability of meat quality traits was also observed in the F2 inter-cross (table 1). For example, the difference between the F2 extreme birds was nearly 1 unit for meat ultimate pH and 8 units for meat yellowness.

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Several QTL for meat quality traits including breast meat pH, drip loss and color have been identified in this F2 inter-cross (Nadaf *et al*., 2007). However, the confidence intervals of the QTL remained large, which prevented any possibility of marker-assisted-selection. Several regions of interest have been refined, including a strong color QTL located on chromosome 11 (GGA11). As described below, the gene underlying this QTL region has been identified by combining positional and expressional data.

Results and discussion

In the preliminary study by Nadaf *et al.* (2007), genome-wide significant QTL for breast meat yellowness (BCo-Y) and redness (BCo-R) had been detected on GGA11, with respective positions of 62 cM and 68 cM. Respective confidence intervals were 56-69 cM and 58-69 cM. Thanks to the development of three additional markers in the distal part of this chromosome, a huge level of significance for BCo-Y QTL was confirmed and its position refined. All the F1 sires were found to be heterozygous for BCo-Y QTL, the allele transmitted by the LG line having a positive effect, consistent with the more intense color of the meat observed in this line. Similar refinement was obtained for BCo-R QTL, although the level of significance of this QTL remained lower. The BCo-Y QTL and BCo-R QTL overlapped which suggested a strong impact of this chromosomal region in the variability of the breast meat color in the F2 population.

Table 1: Body weight, body composition and meat quality traits in HG and LG lines and their F2 inter-cross at 9 weeks of age (mean ± standard deviation)

	HG	LG	p-value	F2
Chickens (n)	53	56		698
Growth and body composition				
Body Weight (g)	1922 ± 157	683 ± 67	<.0001	1127 ± 185
Abdominal Fat	2.5 ± 0.7	0.2 ± 0.2	<.0001	1.6 ± 0.9
Breast Yield	11. 4 ± 0.8	10.4 ± 0.8	<.0001	11.4 ± 0.5
Breast meat quality traits				
Drip loss (%)	2.3 ± 1.2	2.1 ± 1.5	Ns	1.2 ± 0.7
Lightness(L*)	48.3 ± 3.2	45.6 ± 1.8	<.0001	47.3 ± 2.4
Redness (a*)	-0.2 ± 0.8	1.6 ± 0.7	<.0001	1.0 ± 0.9
Yellowness (b*)	9.4 ± 1.2	13.3 ± 1.4	<.0001	11.7 ± 1.5
pH 15 min (pH15)	6.20 ± 0.22	6.33 ± 0.16	0.0004	6.33 ± 0.18
Ultimate pH (pHu)	5.74 ± 0.09	6.14 ± 0.14	<.0001	6.01 ± 0.15

After this step of refinement, a bioinformatics analysis of BCo-Y QTL region was conducted and *BCMO1* was identified as a good positional and functional candidate gene. This gene is encoding for the β-carotene 15, 15'-monooxygenase, a key enzyme in the conversion of the β-carotene (a yellow colour pigment) into vitamin A. The next step of the research was aimed at looking at the relationship between *BCMO1* gene expression and the variation of yellow color explained by the QTL. *BCMO1* mRNA level in breast muscle was found to be

higher in HG chickens compared to LG chickens across several ages, which suggested a higher level of conversion of the β-carotene in the former line. Then *BCMO1* mRNA levels were quantified within a full F2 family (i.e. on more than 130 birds) and these molecular data used for an expression QTL (eQTL) analysis. A strong cis e-QTL was detected and, by considering the mRNA level as a covariate, we confirmed that the variation of *BCMO1* gene expression was responsible for the variation of meat color due to BCo-Y QTL. To our knowledge, this is the first case (at least for the livestock species) in which a causative gene underlying a QTL has been identified thanks to an eQTL approach. The latter results also provided strong indications about the pathways of the causative mutations, which affected the level of transcription of the gene. Several putative mutations were identified within the gene by DNA sequencing. The functional effect of two SNPs in total linkage disequilibrium on the level of transcription of *BCMO1* gene was further confirmed by *in vitro* expression analyses. From all these results we conclude that this "double" mutation, localized in the promoter region of *BMCO1* gene, can be defined as a causative mutation responsible for variation of meat color in the chicken.

Conclusion

While several hundreds QTL have been reported in major livestock species including chicken (http://www.animalgenome.org/QTLdb), the identification of the genes and polymorphisms responsible for the variation of the phenotypes is still rare (Ron and Weller, 2007). The identification of causative mutations may allow a gene-assisted selection, all the more interesting in poultry since the number of lines is large, the family size relatively small and the generation interval short. The economic value of this first genetic marker for colouring meat in the chicken has now to be investigated, by estimating the frequency of the alternative alleles in commercial populations and their effect on the traits included in the selection index. A fast genotyping of the double mutation through HRM analysis (High-Resolution Melting) has been developed and will allow the screening of extensive populations.

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