CONTRIBUTION OF FUNCTIONAL GENOMICS TO THE IDENTIFICATION OF GENES CONTROLLING GRPWTH, BODY COMPOSITION AND BREAST MEAT QUALITY IN CHICKENS

M.J. Duclos*[1], C. Jenkins[1], E. Lebihan-Duval[1], J. Simon[1], T.E. Porter[2], S.E. Aggrey[3], and L.A. Cogburn[4]

[1]INRA, Station de Recherches Avicoles, 37380 Nouzilly, France

[2]University of Maryland, Department of Animal and Avian Sciences, College Park, MD 20742-2311, USA

[3]University of Georgia, Department of Poultry Sciences, Athens,GA 30602-2772, USA

[4]University of Delaware, Department of Animal and Food Sciences, Newark, DE, 19717-1303, USA

Summary

We have chosen a functional genomic approach, based on the comparison of 4 experimental genotypes divergently selected either on growth rate or body composition, to dissect the mechanisms underlying growth, metabolism, muscle structure and body composition; all of which ultimately control meat quality. Microarrays are being used to compare global gene expression profiles within each of the two sets of divergent genotypes (fast growing compared to slow growing and Fat compared to Lean chickens with the same body weight). Simultaneously, a Quantitative Traits Loci (QTL) search is conducted on the two F2 populations resulting of the crosses of the genotypes. The combination of the results from both strategies is expected to identify double functional and positional candidates, as a step toward the identification of the genes explaining the differences between genotypes.

Key words: broiler chickens, muscle, meat quality, fatness, genetics

Introduction

Meat-type chickens show high growth rate and high food efficiency; they are derived from several breeds or strains. In practice, this variability is also used for the diversification of poultry productions, with intensive productions using the fastest growing genotypes and, in some countries, alternative productions using slower growing genotypes. In each type of production the growth rate has to be optimised to reach an acceptable level of productivity and the quality optimised to match the specific targets of each production.

The quality of poultry products can be decomposed into several attributes: mainly, the sensory (colour, tenderness, flavour, juiciness) and the physical (muscle yield, water holding capacity, cooking loss) attributes of chicken carcasses and meat, which vary with growth rate and body composition. The first concern is to produce a carcass of good quality, specifically with a limited percentage of fat, also depending on age at slaughtering. Selection for high growth rate leads to higher fattening but it is possible to select for growth and against fat deposition at the same time. Given the general trend for consuming more chicken cuts and further processed products rather that whole carcasses, an important aspect today is the technological quality of the meat (pH, colour and water retention capacity). We have observed that selection for higher growth rate and increased muscle yield and against abdominal fatness led to an improved technological ability (Le Bihan-Duval *et al.*, 2001).

We think that there is now a need for a better understanding of the mechanisms underlying growth, metabolism, muscle structure and body composition; all of which ultimately control meat quality. We have chosen a functional genomic approach, based on the comparison of experimental genotypes selected either on growth rate or body composition to dissect these mechanisms. This genomic approach is used to study several tissues implicated in those traits: the muscle as the main target tissue, the liver which is the site of fat synthesis, adipose tissue which is the site of fat storage and hypothalamus and pituitary, two major glands of the endocrine system which control tissue growth and metabolism. The present report will only focus on muscle tissue.

The chicken models

Divergent selection is a powerful tool to generate models for the study of a particular character and underlying mechanisms. Such models are available for the study of growth and body composition. On one hand, chickens have been divergently selected for high or low growth rates (HG and LG, respectively, Ricard, 1975). At hatch, the HG chicks are already heavier than LG chicks, but the difference is limited; however, within a few days post hatch, the HG chicks become markedly heavier than the LG chicks. For example, at 6 weeks of age, HG chickens from the 38th generation were about 3 fold heavier than LG chickens reared under the same conditions (Beccavin *et al.,* 2001). The two genotypes also differed on abdominal fat content, which was much higher in HG than in LG chickens (Ricard, 1975). To achieve their larger format and increased muscle mass, the HG chickens also showed more numerous muscle fibres and of larger size (this has been quantified in the anterior latissimus dorsi muscle, Rémignon *et al.,* 1995). On the other hand, Fat and Lean chickens have also been selected in the same way (Leclercq *et al.,* 1980). The two genotypes have been maintained at a similar body size, but show a large difference in abdominal fat contents; the Fat chickens have about 3 times more abdominal fat than the Lean chickens. The Fat chickens also show a significantly lower breast muscle yield than the Lean chickens, and a lower ultimate pH of their meat, likely as a result of increased muscle glycogen content (Berri *et al.,* 2005). Therefore, selection on body composition can affect muscle metabolism and thereby meat quality, through mechanisms which remain to be documented.

The genomic approach

The recent advances in genomics, the availability of micro-arrays to measure global gene expression profiles, and more recently the availability of the chicken genome sequence have opened new possibilities to dissect out complex traits. Under a consortium, that includes several laboratories, our current program is aimed at identifying genes involved in growth, body composition and meat quality in broiler chickens. Our approach is based on the comparison of global gene expression profiles within the two sets of divergent genotypes described above (HG vs LG and Fat vs Lean). In the mean time, a Quantitative Traits Loci (QTL) search is being conducted on the two F2 populations resulting from the crosses of the genotypes (HG x LG, Fat x Lean). The combination of the results from both strategies is expected to identify double functional and positional candidates, as a step toward the identification of the genes explaining the differences between genotypes.

Transcriptome

Global gene expression profiles were measured using a chicken cDNA microarray (http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi?acc=GPL1731) developed at the University of Delaware The microarrays were printed on glass slides with non redundant clones representing about 14000 UniGenes, 97% of which are localised on the chicken genome assembly.

They can be used to work on RNA samples from all chicken tissues (Cogburn *et al.,* 2004). Their use on muscle samples has recently been validated (Jenkins *et al.,* 2005). Total RNAs were reverse transcribed and indirectly labelled with one of two fluorophores emitting at different wave lengths (Cy3/Cy5 or Alexa dyes). This allowed the simultaneous hybridization of two samples differently labelled on the same slide. To favour between lines comparisons, we compared animals from both genotypes at the same age, in a balanced block design; half of the samples from one genotype (3/6) were labelled with the first dye, and the other with the second dye. The comparisons were performed at 1, 3, 5, 7, 9 and 11 weeks of age. The signals were read using a dual wave length laser scanner; image analysis was performed using the GenePix Pro software and the data were normalised following the lowess procedure described by Le Meur *et al.* (2004). Further analysis of the data was performed using Significance Analysis of Microarrays (Tusher *et al.,* 2001), for between lines comparisons at the same age or a linear model with ANOVA analysis. A sample of differentially expressed genes is then chosen for confirmation by quantitative reverse transcription polymerase chain reaction (RTPCR).

QTL analysis

The F2 resource populations consisted of 5 sire families for each cross, each family containing an average of 140 offspring, for the HG X LG cross and from 94 to 147 for the Fat X lean cross. All the birds have been reared under the same conditions and with the same diet. A number of phenotypic parameters have been recorded during growth and following slaughter at the age of 9 weeks. Blood samples have been collected at the age of 7 weeks for preparing DNA and for the measure of some plasma parameters. Table 1 summarizes the characters which have been quantitatively measured, in relation with growth performance, body composition, metabolism and meat quality. Genotyping has been performed on 130 polymorphic markers distributed over the chicken genome. Genetic parameters of the different traits were estimated by REML using the VCE software (Groeneveld, 1997) while QTL positions were estimated by Maximum Likelihood using QTLMAP software (Le Roy *et al.,* 1998).

Table 1: Phenotypic characters measured in the F2 progeny resulting from the HG*LG or Fat * Lean cross.

Growth parameters
• Live Body Weight at 1, 3, 5, 7, 9 weeks of age • Growth curve parameters (Gompertz) • Breast muscle, abdominal fat and leg weights and yields • Tarsal length and width
Meat quality parameters (Breast meat)
• Acidification rate (pH at 15 min and 24 h post-mortem) • Colour (L*, a*, b*) • Water holding capacity.
Physiological parameters
• Circulating IGF-I levels • Glycaemia, plasma free fatty acids • Body temperature

Presentation and discussion of preliminary results

The comparison of global gene expression profiles has started with the comparison of the HG and LG genotypes. In the Pectoralis major muscle, on average several hundreds of genes are expressed at different levels at a given age. Some differentially expressed genes are common to different ages, and the total number of genes showing differential expression amounts to about

1900. Classification of those genes by GeneOntology annotations revealed that they belong to categories involved in cell growth, transcription, metabolism, apopotosis and muscle development. Focussing on those genes which are common between two stages or more, we have identified some which we chose for confirmation by quantitative RTPCR. The differential expression was confirmed for the majority of them.

A first identification of QTLs has been reported (Aggrey *et al.,* 2005). The traits analyzed were breast meat yield, fat yield, percent thigh, thigh weight, Pectoralis major and Pectoralis minor. Body weight at 9 wks was used as a covariance. QTLs for abdominal fat weight were found on chromosomes 1, 3, 4, 5, 6 and Z; Pectoralis major and Pectoralis minor on chromosomes 1, 2, 4, 5, 20 and Z; breast meat yield on chromosomes 1, 3, 4. 6, 10, 20 and Z; and thigh weight on chromosomes 1, 5, 13, 20, 26, and Z. There were significant QTL by sex interactions for both Pectoralis muscles on chromosomes 1, 2, 4, 20 and Z; and for thigh weight on chromosome 5, 13, and 20. Further analyses are underway for meat quality traits, such as pHu and colour, but preliminary data already indicate the presence of QTL controlling those traits. For each QTL region we looked for the presence of differentially expressed genes. As an example, within a QTL region for growth on chromosome 1, which contained about 120 gene loci in the Ensembl gene assembly (http://www.ensembl.org/Gallus_gallus/index.html), we identified about 60 EST from our chicken cDNA microarray, and three genes which were consistently expressed at different levels between the HG and LG genotype across two stages or more. These genes will deserve further attention.

Conclusion and perspectives

We have built a strategy to identify genes underlying growth, body composition and meat quality traits and developed the corresponding tools. We obtained preliminary results on fast and slow growing chickens showing its validity and power. This strategy is being applied simultaneously to the two genetic models, which should permit to identify the genes which are specific to one model or common to both for similar criteria; i.e., ideally, this should permit to identify and discriminate between general mechanisms and other mechanisms which may be influenced by the "genetic environment". In the future, a major task will be to identify the mutation(s) underlying a favourable QTL allele, so that it can be used by the breeders in marker assisted selection schemes.

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