CHICKEN FATNESS:
FROM QTL TO CANDIDATE GENE

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CHAPTER 1

GENERAL INTRODUCTION

Excessive body fatness has long been of interest to those concerned both with research on human obesity as well as on production in farm animals. It has been and still is a complicated trait and measures have been taken to try to minimize it. In the pig, for example, applied quantitative genetics has led, in Europe, to the decrease of backfat thickness from 45 to 9 mm (Merks 2000; Mourot and Hermier 2001). In broilers, on the other hand, selection for an increased growth rate (more exactly, reduction of the age at which the commercial slaughter weight is reached) resulted in a higher body fat content. It is well known that excessive fat in poultry depresses feed efficiency, has no commercial value, and is less appreciated by consumers. Therefore, considerable research effort has been applied around the world to study factors associated with fat deposition and methods to reduce it (Leclercq and Whitehead 1988). Although several candidate genes for this trait have been identified further research is needed to find the actual gene(s) causing chicken fatness.

Fat deposition in broilers

Current broiler strains contain about 15-20% of their body weight as fat (Griffin 1996). The main site of fat deposition (over 85%) in the animal body is adipose tissue (e.g. subcutaneous, intermuscular, abdominal fat). This tissue serves three functions: heat isolation, mechanical cushion, and most importantly, a source of energy in times when foodsupplies are limited (Nir *et al*. 1988). The remainder of the fat (about 2-2.5% of the total body weight) is present in the blood and other tissues as physiologically necessary fat (Leenstra 1986). The size of the different fat depots in the body are highly correlated. Changes in one depot will be accompanied by changes in other depots. However, the danger that broiler meat will become too lean is small. Abdominal fat is more variable than total fat and the fat content of meat. Therefore, considerable changes in abdominal fat are possible without large changes in inter- and intramuscular fat content (Cahaner *et al*. 1986).

Fat is stored as triglycerides in fat avian cells. The triglycerides are transported by the blood and are derived directly from the diet or synthesized in the liver (Griffin 1996). Both the number and size of the fat cells are variable and related to the amount of fat deposition. The amount of fat that can be

stored depends on energy intake, the amount of energy needed for maintenance (including activity) and for growth in general. If the energy intake exceeds these requirements fat will be deposited.

Influences on fat deposition

Both sex and age of the animal have a distinct effect on fat deposition. Females tend to be fatter than males and older birds have a higher fat content than younger birds. Fat depots grow by an increase in the number of fat cells (hyperplasia) and/or by an increase of the size of the fat cells (hypertrophy). Like in pig, three successive phases are observed in chicken: dominant hyperplasia until 4 or 5 weeks of age, hyperplasia and hypertrophy until 6 or 7 weeks of age, and predominant hypertrophy beyond 7 weeks of age (Leenstra 1986; Mourot and Hermier 2001).

Diet composition may directly or indirectly affect adipose tissue growth and fat deposition. An important factor is the effect of diet composition and texture on food intake during *ad libitum* feeding. Dietary manipulations favoring energy intake such as pelleting or changes in energy concentration are accompanied by an increase in fatness (Nir *et al*. 1988, 1994). Broilers fed diets containing polyunsaturated fatty acids, rather than saturated or monounsaturated fatty acids, show lower fat deposition (Sanz *et al*. 1999, 2000; Crespo *et al*. 2001).

Environmental factors, that influence maintenance requirements or activity, can also influence the fat content of broilers. Such factors are ambient temperature, housing systems and lighting regimes. Although the effect of rearing temperature on fat deposition is most prominent among environmental factors, its effect is small compared with the influence that nutritional or genetic factors have on fat deposition. The influence of environmental factors on fat deposition is therefore too small to be of interest in preventing excessive fat deposition (Leenstra 1986).

Differences in fat deposition between breeds and strains within breeds indicate the importance of genetic factors in fat deposition. Fatness is quite highly heritable in birds; h^2 ranges between 0.5 and 0.8 (Chambers 1990; Griffin 1996; Le Bihan-Duval *et al*. 1998, 1999, 2001). Experimental strains of leaner broilers have been produced by selection for low abdominal fat

(Leclercq *et al*. 1980; Cahaner *et al*. 1986) or indirectly for feed efficiency (Bordas and Mérat 1984) or low plasma very low density lipoprotein (Whitehead and Griffin 1984). In each case, selection has resulted in birds containing about 10% of their carcass weight as fat and if birds leaner than this are to be produced, a better understanding of the mechanisms underlying genetic variation in fatness in broilers is needed (Griffin 1996).

Lipoprotein metabolism

The liver is the major site of fatty acid synthesis (lipogenesis) in birds and much of the triglycerides that accumulates in avian adipose tissue is synthesized in the liver from carbohydrate or derived from the diet. Lipids are water insoluble and they are transported between tissues in the plasma in the form of lipoproteins. The metabolism of lipoproteins in the plasma directs lipids to specific tissues in response to nutritional or physiological need. Mechanisms involved in lipoprotein transport in birds and their contribution to regulation of adipose tissue growth in poultry have been extensively reviewed (Leclercq and Whitehead 1988).

Lipoprotein metabolism in chickens is similar to that in mammals and much of present understanding of lipoprotein metabolism in birds represents an extrapolation from very detailed knowledge of mammalian lipoprotein metabolism. Still, there are some differences between mammals and birds. There are major differences in the way in which dietary fat is transported from the intestine of birds and mammals (Figure 1.1) (Griffin and Hermier 1988).

In mammals, the intestinal mucosal cells incorporate almost all dietary fat into large lipoproteins 150-300 nm in diameter (chylomicrons). The intestinal lymphatic system is poorly developed in birds and dietary fat is secreted directly into the portal system in the form of triglyceride-rich lipoproteins with a mean diameter of about 150 nm (portomicrons). The lipid composition of these portomicrons is similar to mammalian chylomicrons. Most portomicrons secreted in the portal system pass straight through the liver to the extrahepatic tissues.

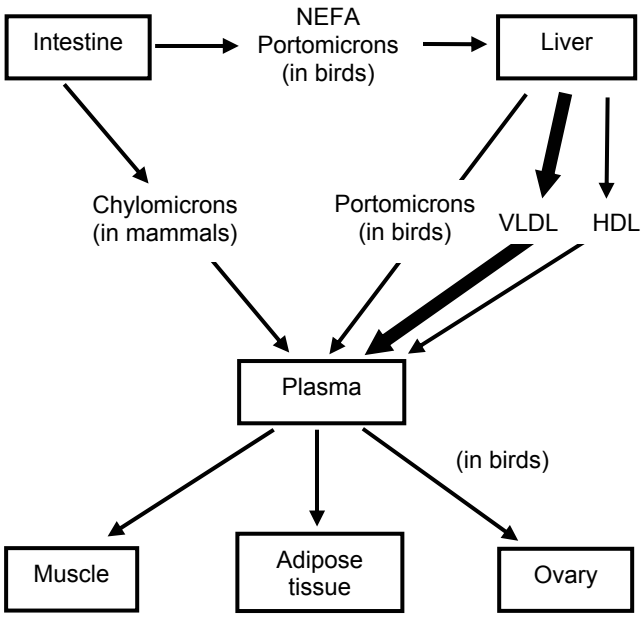

Figure 1.1 *Plasma lipid transport in birds and mammals (adapted from Griffin and Hermier 1988).*
NEFA = non-esterified fatty acids
VLDL = very low density lipoproteins
HDL = high density lipoproteins

The concentration of portomicrons in the plasma of chickens is low and most of the triglycerides in the plasma is present in very low density lipoproteins (VLDL). The triglyceride-rich lipoproteins secreted in the bloodstream from the intestine or liver are substrates for at least three enzymes: lipoprotein lipase (LPL), lecithin-cholesterol acetyltransferase (LCAT) and hepatic lipase. LPL, the most important one, is synthesized in a wide range of tissues, including adipose tissue, muscle, heart and ovarian follicles. In the plasma it catalyses the hydrolysis of triglycerides to fatty acids and glycerol (lipolysis). The fatty acids then enter the surrounding tissues and, in the case of adipose tissue, they are re-esterified and stored as triglycerides. Very high concentrations of insulin stimulate LPL activity, whereas dibutyryl cAMP can decrease both the synthesis and the activity of the enzyme in chicken adipocytes (Schauf *et al*. 1990; Hermier 1997).

Another factor that is important for lipid uptake is the high density lipoproteins (HDL), which are synthesized mainly by the liver. HDL particles circulating in the plasma facilitate uptake of lipids by transferring to VLDL and portomicrons (chylomicrons in human) a class of molecules called apolipoproteins, which are important for the attachment of HDL particles to membranes and for the activation of LPL. The HDL particles speed up the uptake of triglycerides and assist in collecting the cholesterol that is liberated from cell membranes into the plasma (Schauf *et al*. 1990). The major apolipoprotein of avian HDL is apolipoprotein A1 (APOA1) (Figure 1.2). In

mammals APOA1 is an activator of LCAT. This enzyme is responsible for esterification of cholesterol in the plasma: in mammals its main substrate is HDL. The presence of APOA1 in avian VLDL, intermediate density lipoproteins (IDL), and low density lipoproteins (LDL) suggests that these lipoproteins are substrates for cholesterol esterification (Griffin and Hermier 1988). It is reported that most of the cholesterol esters in chicken LDL are the result of LCAT activity.

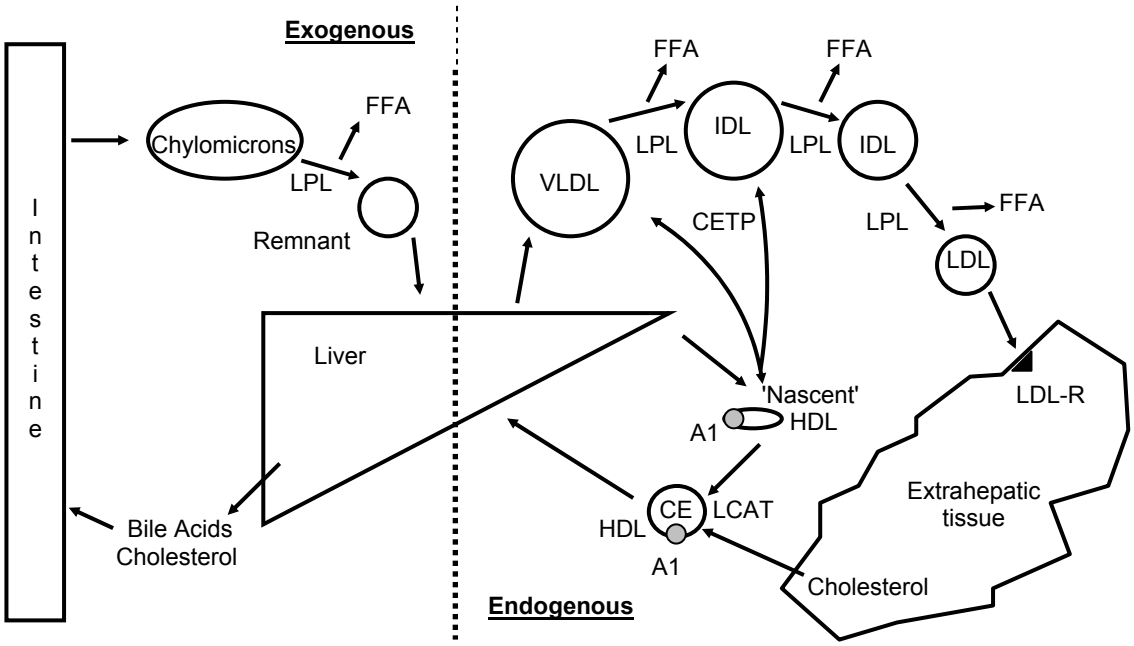

Figure 1.2 *Schematic illustration of human lipoprotein metabolism: endogenous and exogenous pathways.*
A1 = apolipoprotein A1; CE = cholesteryl esters; CETP = cholesteryl ester transfer protein; FFA = free fatty acids; HDL = high density lipoproteins; IDL = intermediate density lipoproteins; LCAT = lecithin-cholesterol acyltransferase; LDL = low density lipoproteins; LDL-R = LDL receptor; LPL = lipoprotein lipase; VLDL = very low density lipoproteins.

Control of fattening via lipoprotein metabolism

Intestinal portomicrons are usually present in very low amounts and HDL contain less than 5% triglycerides. Thus, adipose tissue growth in birds depends mainly on the availability of triglycerides transported by VLDL. Theoretically, it should be possible to alter VLDL metabolism in three compartments: liver, plasma, and adipose tissue (Hermier 1997).

From what is known on the role of the avian liver in lipogenesis, it may be inferred that any increase in hepatic fatty acid synthesis should lead to a higher lipid secretion. Data from several studies suggest that fattening results, at least partly, from an increased hepatic lipogenesis. However, all previous

studies failed to find any enzymatic criterion that could be used in a selection program against fatness in broilers. This is probably due to the fact that enzymatic activities are not a limiting factor of lipogenesis and VLDL synthesis.

When determined in lean and fat chickens, the plasma concentration of VLDL was twofold higher in the fat line, which indicated that VLDL concentrations reflect the availability of plasma triglycerides and therefore the susceptibility to fattening. As a consequence, divergent selection for plasma VLDL level resulted in two lines with a sixfold difference in plasma VLDL and a threefold difference in the relative abdominal fat pad weight. In the comparison of lean and fat chickens differences found in hepatic lipogenesis or activity of lipogenic enzymes are much smaller than the accompanying differences in plasma VLDL concentrations and are often not significant. Serum turbidity, a reflection of VLDL concentration, can be determined rather easily from blood samples, which allows direct selection. Therefore, this variable has been included in selection programs against excessive fattening of broilers in Great Britain (Hermier 1997).

Lipoprotein lipase is the rate-limiting enzyme in the hydrolysis of plasma triglyceride-rich lipoproteins. However, there is no experimental evidence that LPL activity is a determining factor in the regulation of fattening in birds. In fact positive correlations were found between adipose tissue, LPL activity, and growth of fat depots in broilers, but this does not prove that fatness results from higher LPL activity. Selection for low LPL activity should result in a reduction in the number of adipocytes, as well as a decreased propensity for fat storage. However, for practical purposes, LPL is not a good criterion, because the determination and expression of LPL activity cannot be routinely performed, and a biopsy sample may not be representative of whole-body activity.

If lipogenesis exceeds the capacity of VLDL secretion, triglycerides accumulate in the liver. In growing birds, the limitation of fattening relies on the control of VLDL production. Nutritional attempts, such as a partial replacement of dietary energy by protein, are very effective in reducing lipogenesis and subsequent fattening, but the cost is prohibitive. Hormonal treatments are not allowed. At the moment, one realistic procedure aiming to

reduce extrahepatic fattening in commercial broilers may consist in taking into account in the selection programs the factors that regulate the availability of triglycerides and thus the VLDL plasma concentration (Hermier 1997). Further research is needed to study these factors and to find the gene(s) involved in fat deposition in chicken.

Identification of genes controlling fat deposition

Genes causing fat deposition can be identified from physiological or biochemical understanding of its function. Based on the increased knowledge of the fat metabolism and its regulation in chicken (Hillgartner *et al*. 1995; Richards 2003), dozens of candidate genes controlling fatness in chicken can be identified. These candidates include genes involved in the synthesis, transport, and storage of fat, as well as other metabolic mechanisms. Examples of genes that have been linked with fatness in chicken and turkey include fatty acid synthetase (*FAS*) (Sourdioux *et al*. 1996, 1999), malic enzyme (*ME*) (Sourdioux *et al*. 1996, 1999; Daval *et al*. 2000), stearoyl-CoA desaturase 1 (*SCD1*) (Lagarrigue *et al*. 2000), and ATP citrate lyase (*ACLY*) (Daval *et al*. 2000). Figure 1.3 shows the pathway in which these genes are involved. Furthermore, studies on *APOA1* (Douaire *et al*. 1992; Lagarrigue *et al*. 2000; Daval *et al*. 2000) and the transcription factor steroid regulatory element binding protein 1 (*SREBP-1*) (Assaf *et al*. 2003), have suggested an important role of these genes in the regulation of fat deposition in chicken.

Although several candidate genes have been identified, many more are present in the chicken genome, but have not been investigated because complete knowledge of the genetic basis of fat deposition in chicken is not yet available. Therefore, a different approach is needed to elucidate the genetics that underlies this complex trait. Quantitative trait locus (QTL) mapping can be conducted without any previous knowledge of the underlying genes and can identify chromosomal regions controlling a complex trait. QTL mapping requires two key resources: genetically divergent strains and a linkage map covering all of the genome (Lander and Botstein 1989). Studies on obesity and other fat related traits in human and animal models have resulted in many QTL (Chagnon *et al*. 2003). Once a QTL has been mapped to a certain chromosomal region, the next step will be to identify the underlying gene itself.

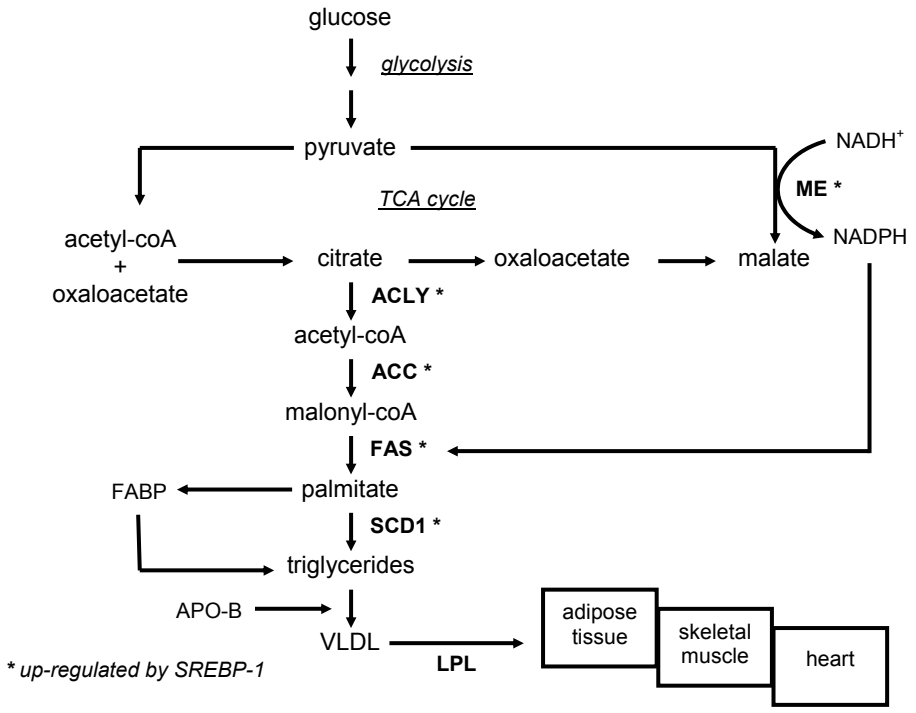

Figure 1.3 *The lipogenic metabolic pathway responsible for the production of triglycerides from glucose in the liver (adapted from Richards 2003). Specific enzymes (bold) are shown next to the steps in the reaction that they catalyze. Lipogenic enzymes that are up-regulated by transcription factor steroid regulatory element binding protein 1 (SREBP-1) are marked with an asterisk.*
ACC = acetyl-CoA carboxcylase; ACLY = ATP citrate lyase; APO-B = apolipoprotein B; FABP = fatty acid binding protein; FAS = fatty acid synthetase; LPL = lipoprotein lipase; ME = malic enzyme; SCD1 = stearoyl-CoA desaturase 1; VLDL = very low density lipoproteins.

Aim and outline of the thesis

This thesis aims at the identification of genes controlling fat deposition in chicken. The mapping of QTL for growth and fatness traits in chicken is described, as is the application of new approaches to identify the genes underlying these QTL. In Chapter 2 a total genome scan in chicken is performed, resulting in the localization of QTL for fat deposition. Several of these QTL are confirmed as well as new QTL are identified in an advanced intercross line (Chapter 3). The construction of high resolution comparative maps of GGA24 (Chapter 4) and GGA15 (Chapter 5 and 6) will help to identify potential candidate genes for fat deposition (Chapter 7). Finally, in Chapter 8 the results of this thesis are discussed.

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CHAPTER 2

DETECTION AND LOCALIZATION OF QUANTITATIVE TRAIT LOCI AFFECTING FATNESS IN BROILERS

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Abstract

A cross between two genetically different outcross broiler dam lines originating from the White Plymouth Rock breed, was used to produce a large three-generation broiler population. This population was used to detect and localize QTL affecting fatness in chicken. Twenty full sib animals in generation 1 and 456 full sib animals in generation 2 were typed for microsatellite markers and phenotypic observations were collected on three groups of generation 3 animals (~1,800 animals per group). Body weight, abdominal fat weight, and percentage abdominal fat was recorded at the age of 7, 9 and 10 wk. To study the presence of QTL, an across family weighted regression interval mapping approach was used in a full sib QTL analysis. Genotypes from 410 markers mapped on 25 chromosomes were available. For the three traits 26 QTL were found for 18 regions on 12 chromosomes. Two genomewise significant QTL (P<0.05) were detected, one for percentage abdominal fat at the age of 10 wk on chicken chromosome 1 at 241 cM (MCW0058 to MCW0101) with a test statistic of 2.75 and the other for BW at the age of 10 wk on chicken chromosome 13 at 9 cM (MCW0322 to MCW0110) with a test statistic of 2.77. Significance levels were obtained using the permutation test. Multiple suggestive QTL were found on chromosomes 1, 2, 4, 13, 15, and 18, whereas chromosomes 3, 7, 10, 11, 14, and 27 had a single suggestive QTL.

(*Key words*: quantitative trait loci, broiler, abdominal fat, dam lines, body weight)

Introduction

In recent decades, selection of meat-type broiler chickens for reduced slaughter age has greatly increased feed efficiency. However, these modern strains selected for more rapid growth exhibit excessive body fat deposition (Mallard and Douaire 1988; Griffin 1996). Fat is considered to be a by-product of very low commercial value. It is a costly body component from an energy point of view, and its deposition in large amounts can depress feed efficiency. Although several strategies of selection for leanness in meat production have been described, it is still not possible to measure fat easily (Mallard and Douaire 1988). The measurements of fatness are often laborious and expensive. Therefore, genetic information leading to the detection of QTL and preferably to the underlying genes for these traits will benefit poultry breeding programs.

Most QTL studies in chickens are based on F_2 populations obtained by crossing extreme lines. For example, in the experiments of Yonash *et al*. (1999), a cross between two White Leghorn lines, one susceptible and the other resistant to Marek's Disease, was used for QTL analysis. Recently, QTL for growth and fatness traits were mapped in F_2 populations based on crosses between fast and slow growing lines (Tatsuda and Fujinaka 2001a,b), between broilers and layers (Ikeobi *et al*. 2002; Sewalem *et al*. 2002), between red junglefowl and layers (Schütz *et al*. 2002; Carlborg *et al*. 2003), and between chicken lines selected for high and low fat content (Pitel *et al*. 2002). Nevertheless, crosses between less extreme lines (layer-layer and broiler-broiler crosses) also resulted in QTL for growth and fatness traits (Van Kaam *et al*. 1998, 1999a,b; McElroy *et al*. 2002; Tuiskula-Haavisto *et al*. 2002; Zhu *et al*. 2003).

In the current experiment an extended mapping population was used based on a cross between two genetically different outcross broiler dam lines originating from the White Plymouth Rock breed. Many microsatellite markers have been mapped in this large population resulting in a comprehensive microsatellite linkage map (Groenen *et al*. 1998) that is used as a reference map in the present study. Van Kaam *et al*. (1998, 1999a,b) was the first to analyze this large three-generation broiler population by means of a whole genome scan, and QTL were found for BW, carcass percentage and growth,

on chromosome 1; for feed intake traits on chromosome 2, 4, and 23; and for meat color on chromosome 2. The aim of the present study was to detect and localize QTL affecting fatness in the same three-generation design as described by Van Kaam *et al*. (1998, 1999a,b).

Material and methods

Experimental population and phenotyping

A three-generation population was created for the purpose of QTL detection, as previously described by Van Kaam *et al*. (1998, 1999a,b). The population structure and number of animals is given in Table 2.1. The design was based on a three-generation full-sib-half-sib design consisting of parents [generation (G) 1], full-sib offspring (G_2) and half-sib grand-offspring (G_3). The G_0 generation consisted of two broiler dam lines originating from the White Plymouth Rock breed. Unrelated G_1 animals were mated to produce 10 full-sib families with on average 46 G_2 offspring per family. The G_1 and G_2 animals were typed for microsatellite markers and phenotypic observations were collected for three groups of G_3 animals. Each group was raised in six hatches and housed in floor pens with approximately 20 animals/m^2. The animals were in the same pen starting from day 0, where they could access feed and water *ad libitum*; illumination was 23 h/d. A commercial broiler feed was used; it consisted of crumbled concentrates containing 12,970 kJ/kg and 21 % protein.

The three groups of G_3 birds were weighed at slaughter when they were 7 wk of age (group 1), 9 wk of age (group 2), and 10 wk of age (group 3). After slaughter the weight of abdominal fat pad (AFW) was measured and adjusted for BW [percentage abdominal fat (AF%)].

Table 2.1 Population structure with observations and numbers used in the analysis[1]

Generation	Males	Females	Total	Observations
0[2]	14	14	28	
1	10	10	20	Genotypes
2	177	279	456	Genotypes
3	963	968	1,931	Phenotypes at 7 wk of age
3	785	977	1,762	Phenotypes at 9 wk of age
3	870	900	1,770	Phenotypes at 10 wk of age

[1]Numbers exclude outliers and missing values.

[2]Male and female generation 0 animals were from different lines.

Genotyping

Microsatellite markers were genotyped as described previously (Crooijmans *et al*. 1997). The PCR amplifications were carried out in 12-µL reactions containing 10 to 60 ng genomic DNA, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* Tris-HCl (pH 8.3), 1 m*M* tetra-methylammoniumchloride, 0.1 % Triton X-100, 0.01 % gelatin, 0.2 m*M* each deoxyribonucleotide, 0.25 U Silverstar polymerase (Eurogentec, Liege, Belgium), and 2.3 pmol of each primer, one of which was labeled with a fluorescent dye (6-FAM, TET, or HEX) at the 5' end. The amplification reactions were as follows: 5 min 95°C followed by 35 cycles of 30 s at 94°C, 45 s at 55°C, and 90 s at 72°C, followed by a final elongation step of 10 min, at 72°C. Depending on the marker, annealing temperatures of 45, 50, or 60°C were used. The PCR amplification products for 14 to 21 markers from an individual DNA sample were pooled and analyzed on a 6 % denaturing polyacrylamide gel, Sequagel-6 (National Diagnostics, Atlanta, Georgia 30336), using an automatic sequencer (Applied Biosystems, Perkin Elmer, Foster City, CA 94404). Electrophoresis was performed for 3 h on 12 cm gels, and the results were analyzed using the Genescan and Genotyper software (Applied Biosystems, Perkin Elmer, Foster City, CA 94404).

In total 410 markers were tested, 256 were determined on all 10 families and 154 were only typed on 4 families. These markers were mapped on 25 autosomal chromosomes with an average marker interval of 7.9 cM. The linkage map used in the current study was calculated with CRIMAP (Green *et al*. 1990) using the marker genotypes for all these markers and all these families. The total linkage map covered 3,230.2 cM. Map distances given are sex-averaged distances in centimorgans on the Haldane scale (Haldane 1919). More detailed information on the marker data is given in Table 2.2.

Table 2.2 Information about the linkage groups

Chromosome[1]	Number of markers used	Map length (cM)	First marker	Last marker
1	82	625.0	MCW0168	MCW0108
2	70	465.0	ADL0228	ADL0146
3	42	378.4	MCW0037	MCW0261
4	34	281.9	LEI0073	ADL0143
5	24	199.2	MCW0263	ADL0298
6	17	126.4	ADL0323	LEI0192
7	15	182.0	LEI0064	ADL0169
8	19	106.3	MCW0275	LEI0044
9	13	88.7	ADL0191	MCW0134
10	11	88.7	ADL0112	MCW0149
11	8	99.7	LEI0143	MCW0230
12	2	1.0	LEI0099	MCW0198
13	9	54.8	MCW0104	MCW0213
14	6	87.1	MCW0296	MCW0225
15	8	48.4	MCW0031	MCW0211
17	7	90.5	ROS0020	ADL0202
18	6	53.7	MCW0045	MCW0219
19	5	26.0	MCW0266	MCW0278
23	5	34.0	LEI0090	MCW0165
24	2	11.2	LEI0069	LEI0155
26	7	59.2	ADL0330	LEI0074
27	3	16.6	MCW0076	MCW0328
28	8	72.7	LEI0135	ADL0299
E46C08W18	3	24.2	MCW0157	MCW0073
E47W24	4	20.7	MCW0119	ADL0324
Total	410	3230.2		

[1]Chromosome numbers are according to Schmid *et al*. (2000).

Full-sib QTL analysis

For the QTL analysis the regression interval mapping methodology described by Van Kaam *et al*. (1998, 1999a,b) was used. The analysis is an across-family weighted full-sib regression analysis. Because marker-QTL linkage phase can differ between families, QTL analysis was nested within families. Average breeding values of G_2 animals were regressed on the probabilities of inheriting the first allele of each G_1 parent. Average breeding values of G_2 animals were estimated based on the measurements of the G_3 animals. In the model, fixed effects for sex and week of hatching were included, as were family mean in order to account for polygenic differences between families. Differences in the number of G_3 animals contributing to G_2 average breeding values were taken into account by applying a weighing factor, based on the variance of the average breeding values. Test statistics were calculated at each centimorgan in order to test for the presence of QTL effects versus the absence of QTL effects. The test statistic was the ratio of the explained mean square of the QTL effects in the numerator and the residual mean square of the full model in the denominator.

Significance thresholds

Significance thresholds were calculated using the method of permutation testing (Churchill and Doerge 1994). This method is empirical and accounts for the distribution of the marker and phenotypic data. By using the genomewise significance thresholds, two types of significance thresholds were derived: significant and suggestive linkage (Lander and Kruglyak 1995). Significant linkage is defined as a 5% genomewise significance threshold, and suggestive linkage is equivalent to one expected false positive result per trait in a whole genome scan. All linkage groups were permutated together and common thresholds were applied. For each trait, 1,000 permutations at 50-cM intervals across the genome were performed.

Permutation was also applied to determine which parents were segregating for a QTL on those locations where a QTL was detected in the across-families analysis. Per parent, a test comparing a model with a QTL versus a model without a QTL was applied, accounting for the presence or absence of QTL effects in the mate. Parents with a test statistic above the 10% chromosomewise threshold were considered to be segregating for the QTL. The 10% chromosomewise thresholds were calculated per parent by performing 1,000 permutations at 1-cM intervals.

Results

Phenotypic data

The overall means and standard deviations of BW, AFW, and AF% are shown in Table 2.3 for G_3 individuals at (slaughter) age of 7, 9, and 10 wk. Although the means and variance increased with age, the coefficient of variation stayed the same for each trait. At every age males were heavier than females and had less abdominal fat; therefore the abdominal fat percentage was lower in the males compared to the females (data not shown).

Table 2.3 Means and standard deviations (SD) of phenotypic observations of generation 3 animals at the age of 7, 9, and 10 wk

Age (wk)	Live BW (g)	Abdominal fat weight (g)	Abdominal fat weight (%)
7	2,216 (335)	65 (21)	3.0 (0.9)
9	2,890 (414)	94 (32)	3.3 (1.1)
10	3,461 (547)	141 (43)	4.1 (1.3)

Full-sib QTL analysis

The QTL with suggestive and significant linkage for each trait are summarized in Table 2.4. Twenty-six QTL were detected; these were divided over 18 regions on 12 chromosomes. On chromosome 1, one significant and one suggestive QTL were found for AF% at 10 wk of age. The same regions harbored also suggestive QTL for AFW at 10 wk of age. A third region on chromosome 1 was represented by a suggestive QTL for AF% at 9 wk of age.

Table 2.4 Statistical tests (*F*-ratio), chromosomal position, and marker bracket for body weight (BW), abdominal fat weight (AFW), and percentage abdominal fat (AF%) at 7, 9 and 10 wk of age in a three-generation broiler population

Chromosome	*F*-ratio	Position[1] (cM)	Marker bracket
		BW at 7 wk of age	
2	2.04†	327	LEI0147 to MCW0096
13	2.03†	9	MCW0322 to MCW0110
14	2.14†	36	ADL0200 to LEI0098
		BW at 10 wk of age	
10	2.22†	88	ADL0038 to MCW0194
13	2.77*	9	MCW0322 to MCW0110
		AFW at 7 wk of age	
4	2.04†	22	LEI0063 to MCW0098
	2.26†	126	LEI0094 to LEI0122
11	2.15†	27	ADL0287 to ADL0210
13	2.10†	0	MCW0104 to MCW0322
		AFW at 9 wk of age	
4	2.04†	71	LEI0076 to MCW0276
		AFW at 10 wk of age	
1	1.98†	25	ADL0160 to HUJ0001
	2.33†	214	LEI0174 to ADL0361
7	2.08†	149	MCW0092 to MCW0316
15	2.21†	21	LEI0120 to MCW0231
18	2.22†	23	ADL0304 to MCW0217
		AF% at 7 wk of age	
2	1.96†	356	MCW0264 to ADL0164
3	2.14†	0	MCW0037 to MCW0148
15	2.07†	0	MCW0031 to MCW0226
		AF% at 9 wk of age	
1	2.03†	573	ADL0350 to MCW0107
4	2.37†	75	LEI0076 to MCW0276
15	2.22†	24	LEI0120 to MCW0231
27	2.04†	0	MCW0076 to MCW0146
		AF% at 10 wk of age	
1	1.98†	18	ADL0160 to HUJ0001
	2.75*	241	MCW0058 to MCW0101
15	2.14†	22	LEI0120 to MCW0231
18	2.49†	21	ADL0304 to MCW0217

[1]Position of QTL relative to the first marker in the set for this chromosome (Table 2.2).

*significant linkage at $P<0.05$; †suggestive linkage.

Also for BW at the age of 10 wk, one significant QTL was found on chromosome 13. This region also showed suggestive QTL for BW and AFW at the age of 7 wk. Multiple suggestive QTL were found on chromosomes 1, 2, 4, 13, 15, and 18, whereas chromosomes 3, 7, 10, 11, 14, and 27 had a single suggestive QTL (Table 2.4.).

Figure 2.1 shows two QTL for AF% at 10 wk of age on chromosome 1. The first QTL at 18 cM showing suggestive linkage, and the second QTL is at 241 cM, which is significant at the 5% level. To study the number of families contributing to these two QTL, allelic effects, their standard errors, and *t*-values were calculated for all families. Results suggest the segregation of the first QTL in 1 sire, of family 6, with an allelic effect of -0.33 % (SE 0.09). The second QTL segregated in 1 sire of family 9 and in 2 dams of families 5 and 9 with allelic effects of -0.71 % (SE 0.28), -0.83 % (SE 0.21), and -0.48 % (SE 0.14), respectively. The average allele substitution effect (α) of the second QTL in the 3 sires/dams was equal to 0.84 additive genetic SD.

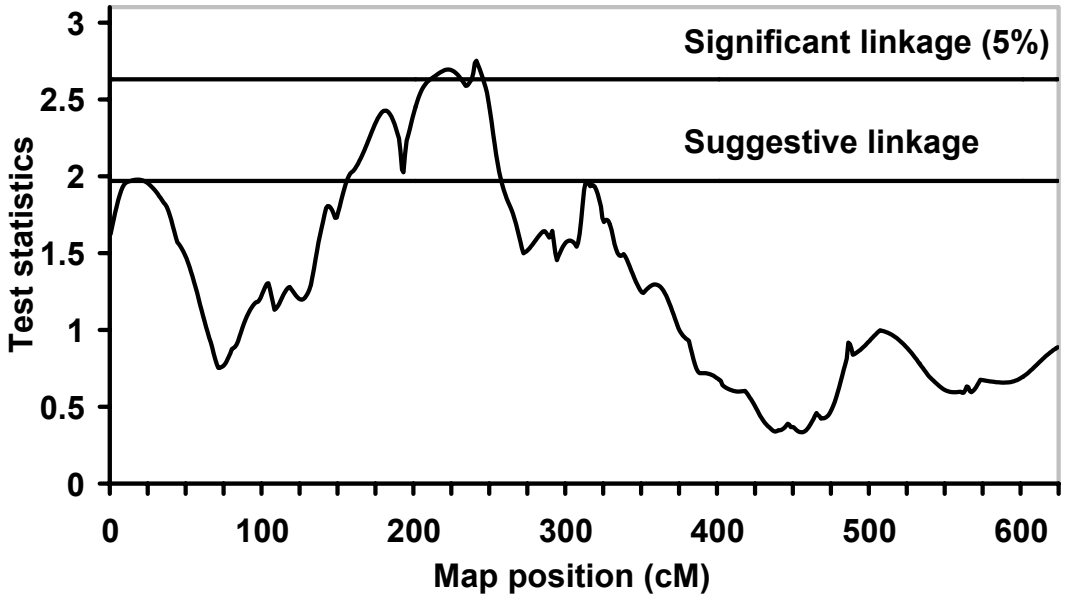

Figure 2.1 *Test statistic values from the full-sib QTL analysis for percentage abdominal fat at the age of 10 wk on chicken chromosome 1. Thresholds for significance linkage at the 5% level and for suggestive linkage are indicated.*

Also for the QTL for BW at the age of 10 wk on chromosome 13 (Figure 2.2), the number of families contributing to the QTL was studied. The QTL on chromosome 13 segregated in 2 sires of families 4 and 5 and in 2 dams of families 2 and 5 with allelic effects of -53 g (SE 14), -70 g (SE 22), 46 g (SE 18), and 49 g (SE 22), respectively. The average allele substitution effect (α) of the QTL in the 4 sires/dams was equal to 0.29 additive genetic SD.

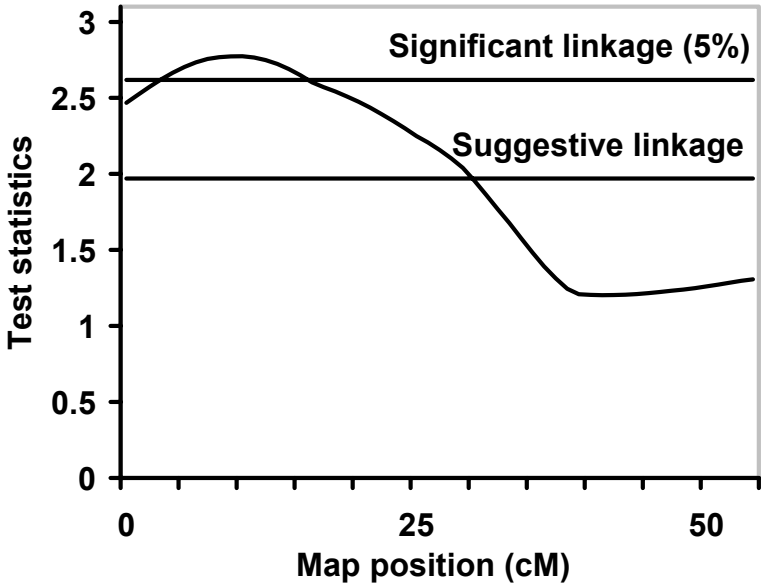

Figure 2.2 *Test statistic values from the full-sib QTL analysis for BW at the age of 10 wk on chicken chromosome 13. Thresholds for significance linkage at the 5% level and for suggestive linkage are indicated.*

Discussion

QTL for fatness traits

The most significant results in the current QTL study were found on chromosome 1 for AF% at the age of 10 wk and on chromosome 13 for BW at the age of 10 wk. These QTL explain 18.1 % and 26.6 % of the total genetic variance, respectively.

Van Kaam *et al*. (1998, 1999a) found QTL for BW at the age of 48 d on chromosome 1 with confidence intervals that overlap with the QTL for AFW and AF% at the age of 10 wk. Although a low genetic correlation was found between BW and AF% (Le Bihan-Duval *et al*. 1999, 2001; S. Zerehdaran

2003, Animal Breeding and Genetics Group, Wageningen University, Wageningen, The Netherlands, personal communication) suggesting that BW and AF% are affected by different genes, it can not be excluded that the region on chromosome 1 represents a pleiotropic QTL. In the present study however, no QTL for BW was found on chromosome 1. This is most likely caused by the differences in performance of the chickens in the current study compared with those used in the studies of Van Kaam *et al*. (1998, 1999a), which were kept under different housing conditions (i.e., free housing versus individual housing).

The QTL on chromosome 1 for AFW and AF% at the age of 10 wk confirm the QTL found by Ikeobi *et al*. (2002), who found a QTL for abdominal fatness in the same region. Three other regions detected in the present study on chromosomes 4, 13, and 15 are also reported by Ikeobi *et al*. (2002). Furthermore, four more QTL regions detected in the present study confirmed those found by others. The QTL on chromosome 7 for AFW at the age of 10 wk was also found by Tatsuda and Fujinaka (2001a), whereas the QTL for AF% at the age of 9 wk confirmed the one found by McElroy *et al*. (2002). The QTL for BW at the age of 7 and 10 wk on chromosome 13 observed in the present study is in the same region as the QTL for BW identified by Carlborg *et al*. (2003), McElroy *et al*. (2002), and Sewalem *et al*. (2002). The QTL for BW at the age of 7 wk on chromosome 2 was also found by Sewalem *et al*. (2002).

Although, several studies resulted in QTL at the same chromosomal regions, one should keep in mind that in these studies different breeds and measurements were used (Tatsuda and Fujinaka 2001a,b; Ikeobi *et al*. 2002; McElroy *et al*. 2002; Sewalem *et al*. 2002; Carlborg *et al*. 2003). For example, in the study of Ikeobi *et al*. (2002) a broiler-layer cross was used, and fatness traits were measured at 2 kg live weight when they were 9 wk of age. In the present broiler-broiler cross the chickens were already heavier at the age of 7 wk. Also at this age the birds contained more abdominal fat, but AFW was lower compared to the birds in the study of Ikeobi *et al*. (2002).

Potential Candidate Genes

The QTL regions found in the present study ranged from 50 to 100 cM, each containing up to 1,000 genes. Therefore, the chance of finding the gene(s) underlying the QTL was very low (<0.1%). However, studies on obesity and other fat related traits in human, mouse, and agricultural species provide useful information that can be used to identify potential candidate genes in the chicken. Based on the comparative maps among human, mouse, and chicken, a selection of potential candidate genes can be made, specific for the regions of interest.

In the current study the most interesting QTL for the fatness traits was the significant QTL for AF% at the age of 10 wk located on chicken chromosome 1. This QTL region on chromosome 1 shows conservation of synteny with parts of human chromosomes 12 and 22 (Schmid *et al*. 2000). Potential candidate genes mapped in the region on chicken chromosome 1 are peroxisome proliferative activated receptor-α (*PPARA*), insulin like growth factor-I (*IGF-I*), high mobility group I-C (*HMGIC*), lactate dehydrogenase B (*LDHB*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPD*). The *PPARA* gene is located on human chromosome 22, whereas the other four genes are located on human chromosome 12. The PPARA protein is a nuclear transcription factor and belongs to the steroid hormone receptor superfamily called PPAR. Studies in human and mouse indicated the important role of the PPARA protein in lipid homeostasis and the protection against obesity (Costet *et al*. 1998; Tai *et al*. 2002; Yamakawa-Kobayashi *et al*. 2002). Also in the chicken an association has been described between a polymorphism in the *PPARA* gene and fatness traits (Meng *et al*. 2002). The IGF-I and HMGIC protein, directly involved in the regulation of growth, were found to play a role in obesity and fat deposition in human and mouse (Sun *et al*. 1999; Anand and Chada 2000; Pérusse *et al*. 2001). The LDHB and GAPD protein catalyze reactions in the glycolysis and gluconeogenesis. These metabolic pathways yield intermediates that are important for the fat metabolism. Therefore, LDHB and GAPD could influence fat storage. However, no association has yet been found with obesity or other fat traits.

Another chicken chromosome showing conservation of synteny to parts of human chromosomes 12 and 22 is chicken chromosome 15 (Jennen *et al*. 2003). Potential candidate genes mapped on chromosome 15 are X-box binding protein 1 (*XBP1*), phosphotidylinositol transfer protein-β (*PITPNB*) and T-box 3 (*TBX3*). The *XBP1* and *PITPNB* genes are located on human chromosome 22 and the *TBX3* gene is located on human chromosome 12. The XBP1 and TBX3 protein are transcription factors involved in cell differentiation, whereas the PITPNB protein is able to transfer phospholipids between membranes. Whether these proteins are actually involved in the fat regulation is unknown. So far no association has been found with obesity or any other fat trait.

In conclusion, although it is tempting to look for potential candidate genes, one should be aware of the fact that the QTL have not yet been localized precisely. At present the number of identified genes (Schmid *et al*. 2000) is too limited to be able to align the chicken and human genetic maps accurately. Nevertheless, for several chicken chromosomes containing a QTL, detailed comparative maps between human and chicken have been published recently (Crooijmans *et al*. 2001; Buitenhuis *et al*. 2002; Jennen *et al*. 2003). Furthermore, the draft sequence of the chicken genome is expected to be completed by the end of the year 2003. This will increase the ability to align the chicken and human maps and consequently increase the chance to identify potential candidate genes.

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CHAPTER 3

CONFIRMATION OF QUANTITATIVE TRAIT LOCI AFFECTING FATNESS IN CHICKEN USING AN ADVANCED INTERCROSS LINE

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Abstract

In this report we describe the analysis of an advanced intercross line (AIL) to confirm the quantitative trait locus (QTL) regions found for fatness traits in a previous study. QTL analysis was performed on chromosomes 1, 3, 4, 15, 18, 24 and 27. The AIL was created by random intercrossing in each generation from generation 2 (G_2) onwards until generation 9 (G_9) was reached. QTL for abdominal fat weight (AFW) and/or percentage abdominal fat (AF%) on chromosomes 1, 3 and 27 were confirmed in the G_9 population. In addition, evidence for QTL effects for body weight at the age of 5 (BW5) and 7 (BW7) weeks and for percentage intramuscular fat (IF%) were found on chromosomes 1, 3, 15, 24, and 27. Significant evidence for QTL effects was detected on chromosome 1 for AFW, BW5 and BW7, and on chromosome 15 for BW5. Suggestive evidence was found on chromosome 1 for AFW, AF% and IF%, on chromosome 3 for AFW, AF% and BW7, on chromosome 15 for BW7, on chromosome 24 for IF%, and on chromosome 27 for BW5, AF% and IF%. For chromosomes 4 and 18 test statistics did not exceed the significance threshold.

(*Key words*: quantitative trait loci, advanced intercross line, chicken, fatness traits)

Introduction

Fat deposition is an important trait in chicken, which has been examined in several studies for the identification and localization of quantitative trait loci (QTL) (e.g. Tatsuda and Fujinaka 2001a; Ikeobi *et al*. 2002; McElroy *et al*. 2002; Pitel *et al*. 2002). We have previously identified QTL affecting fatness in a cross between two genetically different outcross broiler dam lines originating from the White Plymouth Rock breed (Jennen *et al*. 2004). This resulted in a genomewise significant QTL for percentage abdominal fat at the age of 10 weeks on chicken chromosome 1. This QTL explained about 18 % of the total genetic variance. Furthermore, suggestive QTL for fatness traits were found on chromosomes 1, 2, 3, 4, 7, 11, 13, 15, 18 and 27. Confirmation of these QTL is an essential step before attempts are made towards the fine mapping of the QTL and the identification of genes underlying the traits of interest.

Confirmation of the presence and location of the QTL of interest can be achieved by comparing the results from different QTL studies. In the comparison of two granddaughter designs Bennewitz *et al*. (2003) confirmed QTL affecting milk yield in cattle. In the study of two distinct layer x layer crosses Siwek *et al*. (2003) validated the presence of a QTL for the primary antibody response to keyhole lympet hemocyanin on chromosome 14 in both populations. Confirmation of QTL within a commercial broiler line was achieved by De Koning *et al*. (2003). In this study QTL for body weight and residual feed intake on chicken chromosome 4 were confirmed from results from other QTL studies reported in literature. Some, but not all of the fatness QTL found in our previous study were in the same chromosomal region as found by others (Tatsuda and Fujinaka 2001a; Ikeobi *et al*. 2002; McElroy *et al*. 2002). The QTL regions are quite large (50-100 cM) and only partially overlapping. Furthermore, different phenotypic measurements were used in the other studies (Tatsuda and Fujinaka 2001a; Ikeobi *et al*. 2002; McElroy *et al*. 2002). Therefore, confirmation of the presence and location of the QTL is still needed, by performing a confirmation experiment.

Confirmation experiments have been described in several studies. In a backcrossing experiment in pig the presence of one or more QTL for fatness and growth were confirmed on pig chromosome 4 (Marklund *et al*. 1999). Furthermore, a grand-granddaughter design in dairy cattle has been used successfully to confirm QTL affecting milk yield (Arranz *et al*. 1998; Coppieters *et al*. 1998). Another example to confirm QTL is the use of an advanced intercross line (AIL). In mice, for example, this resulted in the confirmation of QTL found in an earlier study (Iraqi *et al*. 2000; Wang M *et al*. 2003; Wang X *et al*. 2003). Basically, an AIL is used for the fine-mapping of a QTL region (Darvasi and Soller 1995). Such a population is created by repeated intercrossing for a number of generations. Because it is most suited for animals having a short generation interval, the AIL approach can be used in chickens.

The current report describes the use of an AIL to confirm the results of an earlier QTL mapping study. Therefore, from the previously used three-generation population (Jennen *et al*. 2004) a generation 9 (G_9) population was produced by random intercrossing in each generation from generation 2 (G_2) onwards. The results of the analysis of the G_9 population are presented in the present study.

Material and methods

Experimental population and observations

The three-generation full sib half sib design described by Van Kaam *et al*. (1998) was used in a previous study to detect QTL affecting fatness (Jennen *et al*. 2004). G_2 animals of this design were used to produce a G_9 population by random intercrossing in each generation. The population structure and number of animals is given in Table 3.1. In total, 12 full sib G_8/G_9 families were produced with on average 84 offspring. The G_9 population consists of 546 male and 460 female animals.

Table 3.1 Population structure and number of animals used for breeding, genotyping, and phenotyping

	Number of animals:		
Generation[1]	**Breeding**	**QTL analysis**	
G_0	28		
G_1	20	20	genotyped
G_2	71	456	genotyped
G_3	105	5363	phenotyped
G_4	122		
G_5	93		
G_6	108		
G_7	127		
G_8	100	24	genotyped
G_9	-	1006	genotyped & phenotyped

[1] G_0, etc. = Generation 0, etc.

The G_9 animals were raised in 15 hatches and housed in a litter system for broilers. Animal density was around 20 animals/m^2. The animals were in the same pen starting from day 0, where they received feed and water for *ad libitum* consumption and illumination was 23 hours a day. A commercial broiler feed containing 12,970 kJ/kg was used.

The birds were weighed at 5 weeks of age (BW5) and again at slaughter when they where 7 weeks of age (BW7). Around this age commercial broilers reach the slaughter weight of 2 kg. After slaughter the weight of the abdominal fat pad (AFW) was measured and percentage abdominal fat (AF%) was calculated. In addition, intramuscular fat content of part of the breast muscle, *pectoralis minor* was determined by means of extraction (Soxhlet method; NEN-ISO 1444; Dutch Center for Standardization NEN http://www.nen.nl. [Consulted: March 2003]) and percentage intramuscular fat (IF%) was calculated.

QTL regions

In the previous QTL analysis QTL for AFW and AF% were found on chromosomes 1, 2, 3, 4, 7, 11, 13, 15, 18 and 27 (Jennen *et al*. 2004). From the results of this analysis six chromosomes were chosen for further analysis in the G_9 population. Chromosomes 1, 4, 15, and 18 were selected because they showed significant evidence of QTL effects for both AFW and AF%. All other chromosomes had a suggestive QTL for either AFW or AF%, therefore, only the most promising chromosomes (chromosomes 3 and 27) were selected.

In addition, chromosome 24 was also analyzed in the G_9 population. On this chromosome an apolipoprotein gene cluster is located (Jennen *et al*. 2002), whose members are potential candidate genes for fatness traits.

Genotyping

Genotyping of the microsatellite markers was done as described previously (Crooijmans *et al*. 1997). PCR amplifications were carried out in 12 µl reactions containing 10-60 ng genomic DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH=8.3), 1 mM tetra-methylammoniumchloride (TMAC), 0.1 % Triton X-100, 0.01 % gelatin, 0.2 mM each dNTP, 0.25 U Silverstar polymerase (Eurogentec, Liège, Belgium) and 2.3 pmol of each primer, one of which was labeled with a fluorescent dye (FAM, TET and HEX) at the 5' end. The amplification reactions were as follows: 5 min 95°C followed by 35 cycles of 30 s 94°C, 45 s at 50 or 55°C and 90 s at 72°C, followed by a final elongation step of 10 minutes at 72°C. Per animal PCR amplification products for 7 to 10 markers were combined and analyzed simultaneously on a 6 % denaturing polyacrylamide gel, Sequagel-6 (National Diagnostics, Atlanta, Georgia 30336, USA) on an ABI377 automatic sequencer (Applied Biosystems, Perkin Elmer, Foster City, CA 94404, USA). Electrophoresis was performed for 2 hours on 12 cm gels, and the results were analyzed using the Genescan and Genotyper software (Applied Biosystems, Perkin Elmer, Foster City, CA 94404, USA).

A set of 8 microsatellite markers was used to check the inheritance from G_2 to G_9. For the QTL analysis, genotypes for 12 G_8/G_9 full sib families (1030 animals) were determined with 25 microsatellite markers. These 25 markers were located on chromosomes 1, 3, 4, 15, 18, 24, and 27. The linkage map used in the present study was calculated with CRIMAP (Green *et al*. 1990). Further analysis were performed, using the recombination fractions obtained from CRIMAP (Green *et al*. 1990) transformed to Haldane map distances (Haldane 1919). More information on the marker data is given in Table 3.2.

Table 3.2 Chromosomes and microsatellite markers that were used for the full sib QTL analysis in G_8/G_9. Map distances are given in cM on the Haldane scale

Chromosome	Microsatellite markers	Map distance (cM)
1	*MCW0044*	0
	MCW0289	8.7
	MCW0297	16.3
	ADL0364	23.8
	ADL0359	44.6
	MCW0018	73.6
	MCW0058	114.6
	MCW0101	122.2
3	*MCW0116*	0
	MCW0148	2
	MCW0037	3.1
4	*LEI0122*	0
	MCW0276	63.6
15	*MCW0031*	0
	LEI0120	6.4
	MCW0052	28.7
18	*MCW0045*	0
	MCW0217	25.5
	ADL0290	36.7
24	*ROS0123*	0
	MCW0301	19.3
	LEI0069	29.2
27	*MCW0076*	0
	MCW0328	11.2
	ADL0376	23.6

QTL analysis

Full sib QTL analysis was conducted using the regression interval mapping methodology as described by Van Kaam *et al*. (1998) in which a single QTL was fitted. The analysis is an across family weighted full sib regression analysis. Because, marker-QTL linkage phase can differ between families, QTL analysis was nested within families. Phenotypic values of G_9 animals were regressed on the probabilities of inheriting the first allele of each G_8 parent. Phenotypic values were adjusted for fixed effects sex (2 classes) and week of hatching (15 classes). In the model the family mean was included in order to account for polygenic differences between families. The model to fit a QTL at position k was:

$$y_{ij} = f_i + b_{s,ik}x_{s,ijk} + b_{d,ik}x_{d,ijk} + e_{ijk}$$

where:

y_{ij} = average adjusted progeny trait value for G_9 chicken j of family i;

f_i = polygenic effect of family i;

$b_{s,ik}$ = regression coefficient for the sire(s) of family i at position k;

$x_{s,ijk}$ = probability that G_9 chicken j in family i at position k received the chromosomal segment from haplotype 1 from the sire;

$b_{d,ik}$ = regression coefficient for the dam (d) of family i at position k;

$x_{d,ijk}$ = probability that G_9 chicken j in family i at position k received the chromosomal segment from haplotype 1 from the dam;

e_{ijk} = random residual.

Test statistics were calculated at each centimorgan, in order to test for the presence of QTL effects vs. the absence of QTL effects. The test statistic was the ratio of the explained mean square of the QTL effects in the numerator and the residual mean square of the full model in the denominator.

Significance thresholds

Significance thresholds were calculated using the method of permutation testing (Churchill and Doerge 1994). This is an empirical method, which accounts for the distribution of the marker and phenotypic data. Experiment wise significance thresholds were obtained by permutating all linkage groups together and applying common thresholds. For each trait, 1000 permutations at 50 cM intervals across the linkage groups were performed. In this study significant linkage is equivalent to 0.05 expected false positives per trait in a scan over all linkage groups that were analyzed in this study. Suggestive linkage is equivalent to one expected false positive result.

Results and discussion

QTL analysis G_8/G_9

From the results of the QTL analysis for AFW and AF% in the previous study (Jennen *et al*. 2004) chromosomes 1, 3, 4, 15, 18, and 27 were chosen for further analysis in the G_9 population. In addition, chromosome 24, containing potential candidate genes for fatness traits, was also analyzed in the G_9 population.

For the QTL analysis, three sets of in total 25 microsatellite marker were used on 12 G_8/G_9 full sib families (1030 animals) resulting in over 25.000 genotypes. Where possible, for each QTL region three microsatellite markers were chosen, i.e. two at the border of the 95% confidence interval (CI) of the QTL and one in the middle. However, on chromosome 3 the three microsatellite markers were situated much closer together than the 95% CI and on chromosome 4 only two microsatellite markers were used. The QTL region on chromosome 1 was approximately four times as big as the other QTL regions, therefore, more markers (eight) were chosen. On chromosome 24 three microsatellite markers were chosen closest to the apolipoprotein genes. For the selected chromosomal regions the marker order and map distance in cM on the Haldane scale is shown in Table 3.2. Marker order is the same as in the consensus linkage map reported by Groenen *et al*. (2000) and map distances recalculated for cM on the Kosambi scale (Kosambi 1944) are comparable to those in the consensus linkage map.

The results of the full sib QTL analysis are summarized in Table 3.3. Evidence was found for QTL effects for AFW, AF%, BW5, BW7, and/or IF% on chromosomes 1, 3, 15, 24, and 27. For chromosomes 4 and 18 test statistics did not exceed the significance threshold for any of the traits measured in this experiment. On chromosome 1 two distinct QTL regions were identified (Figure 3.1). In the region 0-30 cM suggestive evidence was found for AFW and AF% and in the region 40-120 cM significant evidence was found for AFW, BW5, and BW7 and suggestive evidence for AF% and IF%. On chromosome 15 significant and suggestive QTL effects were found for BW5 and BW7, respectively. Furthermore, suggestive evidence was found on chromosome 3 for AFW, AF% and BW7, on chromosome 24 for IF%, and on chromosome 27 for BW5, AF% and IF%.

Table 3.3 QTL for abdominal fat weight (AFW), percentage abdominal fat (AF%), body weight at 5 (BW5) and 7 (BW7) weeks of age, and percentage intramuscular fat (IF%) in the G_8/G_9 population of chickens derived from a broiler x broiler cross. Positions are given in cM on the Haldane scale

Trait	Chromosome	Position (cM)	Marker bracket	Significance
AFW	1	10	*MCW0289-MCW0297*	†
		82	*MCW0018-MCW0058*	*
	3	0	*MCW0116-MCW0148*	†
AF%	1	11	*MCW0289-MCW0297*	†
		84	*MCW0018-MCW0058*	†
	3	0	*MCW0116-MCW0148*	†
	27	11	*MCW0076-ADL0376*	†
BW5	1	68	*ADL0359-MCW0018*	*
	15	19	*LEI0120-MCW0052*	*
	27	9	*MCW0076-MCW0376*	†
BW7	1	83	*MCW0018-MCW0058*	*
	3	0	*MCW0116-MCW0148*	†
	15	2	*MCW0031-LEI0120*	†
IF%	1	114	*MCW0018-MCW0101*	†
	24	10	*ROS0123-MCW0301*	†
	27	23	*MCW0328-ADL0376*	†

* significant linkage; † suggestive linkage.

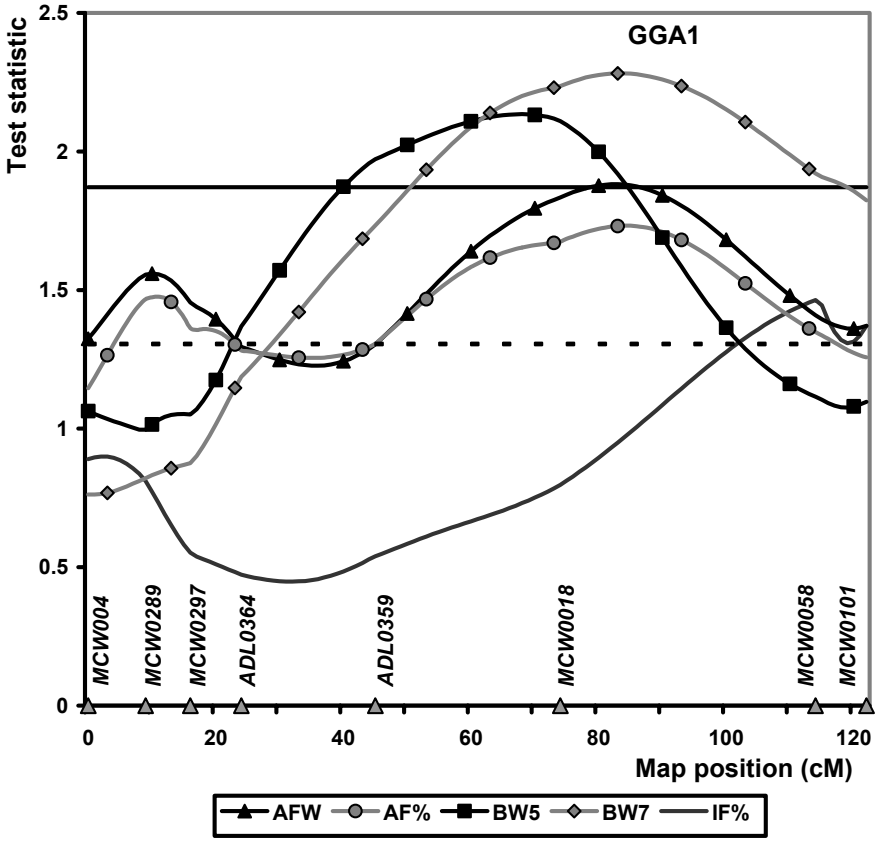

Figure 3.1 *Test statistic values from the full sib QTL analysis for abdominal fat weight (AFW), percentage abdominal fat (AF%), body weight at the age of 5 and 7 weeks (BW5 and BW7), and percentage intramuscular fat (IF%) on chicken chromosome 1. Average thresholds for significance linkage at the 5% level (——) and for suggestive linkage (- - - -) are included. Map positions are given in cM on the Haldane scale.*

Power of the QTL analysis

Power was calculated using methods described by Van der Beek *et al*. (1995), assuming a QTL heterozygosity of 0.5 and an average distance between informative markers of 20 cM. In the three-generation experiment, the power of the design to detect a QTL with an effect of $0.3\sigma_p$ is approximately 0.33 with α is 0.05. Using the same parameters, the power in the two generation (G_8/G_9) full sib design was 0.85, showing that the design of the present study is very powerful and that true and false QTL should be distinguishable. Nevertheless, one may fail to confirm the QTL in a

subsequent experiment for several reasons as indicated by Marklund *et al*. (1999). For example the original observation may be a type I error or a large QTL effect may be caused by several linked QTL each with a small effect, and the linkage may break up in subsequent generations.

In the present study we consider a suggestive QTL from the previous study to be confirmed when in the present study the test statistics exceed the significance threshold for suggestive linkage. A comparison between the results of both studies is shown in Table 3.4.

Table 3.4 Significance levels of QTL detected for regions included in G_8/G_9 population. Traits are abdominal fat weight (AFW) and percentage abdominal fat (AF%) at 7 weeks of age, body weight at 5 (BW5) and 7 (BW7) weeks of age, and percentage intramuscular fat (IF%) at 7 weeks of age

	AFW		AF%		BW5		BW7		IF%	
Chr	G_2/G_3	G_8/G_9	G_2/G_3	G_8/G_9	G_2/G_3	G_8/G_9	G_2/G_3	G_8/G_9	G_2/G_3	G_8/G_9
1	†[1]	*	*[1]	†	nd	*	-	*	nd	†
3	-	†	†[3]	†	nd	-	-	†	nd	-
4	†[2, 3]	-	†[2]	-	nd	-	-	-	nd	-
15	†[1]	-	†[1, 2, 3]	-	nd	*	-	†	nd	-
18	†[1]	-	†[1]	-	nd	-	-	-	nd	-
24	-	-	-	-	nd	-	-	-	nd	†
27	-	-	†[2]	†	nd	†	-	-	nd	†

* significant linkage; † suggestive linkage; nd not determined.

[1]effect detected at 10 weeks of age.

[2]effect detected at 9 weeks of age.

[3]effect detected at 7 weeks of age.

QTL for abdominal fatness

On chromosome 1 the QTL for AFW as well as AF% were confirmed (Table 3.4). Moreover, for both traits the analysis revealed two distinct peaks on this chromosome at a distance of around 75 cM (Figure 3.1). An additional two QTL regression analysis was undertaken by fitting two QTL for both AFW and AF%. The results of this analysis suggest that two distinct QTL for fat deposition are present on this chromosome. The first QTL (between *MCW0289-MCW0297)* is suggestive for both traits whereas, the second QTL

(between *MCW0018-MCW0058*) is significant for AFW and suggestive for AF%. Support for the QTL between *MCW0289-MCW0297* is also given by Ikeobi *et al*. (2002), who reported a QTL for abdominal fatness, which co-locates with ours. The 95% CI (determined by bootstrapping) of the first and second QTL are 25 and 60 cM respectively. This is considerately smaller than the CI of the G_2/G_3 QTL (~145 cM). The reduction of the CI is due to the increased number of informative meioses as a result of the larger number of animals used in the G_9.

The suggestive QTL for AF% on chromosomes 3 and 27 were also confirmed in this study and a suggestive evidence for AFW was detected on chromosome 3. The QTL on chromosome 27 is supported by McElroy *et al*. (2002) who found suggestive linkage for fat weight (p=0.06) with a single marker (*MCW0233*), close to our QTL. For the QTL region on chromosome 3 no other fatness QTL have been reported in the literature.

The suggestive QTL for AFW and AF% previously found on chromosomes 4, 15, and 18 could not be confirmed, suggesting that the previously found QTL were falsely identified. However, power was calculated using a marker distance of 20 cM, while on chromosome 4 marker distance is 63.6 cM (Table 3.2). Therefore, power to detect a QTL on this chromosome with an effect of $0.3\sigma_p$ is approximately 0.52 with α is 0.05. This is considerately lower than the previous calculated power of 0.85, as is the chance to detect a QTL. Therefore, we cannot completely exclude the possibility of the presence of a QTL for abdominal fatness on chromosome 4. Furthermore, Ikeobi *et al*. (2002) found significant QTL for fatness traits on chromosome 15 in the same region as the previous identified QTL in the G_2/G_3 population (Jennen *et al*. 2004). These results suggest that there might be genes located on this chromosome, which are involved in the regulation of fat deposition.

QTL for body weight

Evidence for QTL effects for BW5 and/or BW7 was found on chromosomes 1, 3, 15, and 27 (Table 3.3). In the three-generation design of our previous study (Jennen *et al*. 2004) we did not find any evidence for the presence of QTL with an effect on BW on these chromosomes. However, in our group several QTL studies were performed using the same three generation design, with the same genetic background, but different G_3 offspring (Van Kaam *et al*. 1998, 1999a,b). In two of these studies a suggestive QTL for BW7 was identified on chromosome 1 near microsatellite markers *MCW0058* and *LEI0071* (Van Kaam *et al*. 1998, 1999a), which is confirmed by the results of the present study.

The presence of QTL for BW on chromosomes 1, 15 and 27 is supported by the results of several other QTL studies. In the same region on chromosome 1 Tatsuda and Fujinaka (2001b) detected QTL for BW at 13 and 16 weeks of age. Furthermore, Carlborg *et al*. (2003) found QTL on chromosomes 15 and 27, whereas, Sewalem *et al*. (2002) did on chromosomes 1 and 27.

QTL for intramuscular fat

In addition to the traits AFW, AF% and BW, which have been analyzed in several QTL studies, IF% was also analyzed in this study. So far no QTL mapping studies have been conducted for this trait in poultry. We found suggestive evidence for IF% on chromosomes 1, 24, and 27. Considering the fact that on chromosomes 1 and 27 also evidence was found for BW, AFW and/or AF% (Table 3.3; Figure 3.1), it is likely that the underlying gene has pleiotropic effects. The estimation of genetic parameters on the present data (Zerehdaran *et al*. 2004) showed that IF% and BW were genetically highly correlated (0.87-0.91), whereas genetic correlation between AFW and IF% was almost zero (0.02). These correlations suggest (Falconer and Mackay 1996) that the metabolic pathways for growth and fat deposition in the muscles are influenced by the same genes in the same direction, whereas the metabolic pathway for fat deposition in abdomen might be influenced by other genes.

Candidate genes

The ultimate goal of QTL mapping is to identify the underlying genes responsible for the observed QTL effects. Studies on obesity and other fat related traits in human, mouse and other agricultural species provide useful information, which can be used to identify potential candidate genes in chicken. In previous studies (Van Kaam *et al*. 1998; Jennen *et al*. 2004) potential candidate genes for abdominal fatness and growth have been proposed. For intramuscular fatness so far no potential candidate genes have been reported in poultry.

On chromosomes 1 and 27 suggestive evidence was found for IF% as well as for all other traits (Table 3.3), suggesting the presence at these locations of genes having peiotropic effects on growth and fat deposition. Potential candidate genes located in the QTL regions on chromosomes 1 and 27 are respectively insulin-like growth factor 1 (*IGF1*) and growth hormone (*GH*). Both genes are involved in the regulation of growth and directly or indirectly influence several metabolic pathways, including fat metabolism. IGF1 was found to play a role in obesity and fat deposition in human (Sun *et al*. 1999; Pérusse *et al*. 2001). Furthermore, high and low growth rates of divergently selected chickens were associated with high and low levels of hepatic IGF1 mRNA respectively (Beccavin *et al*. 2001). GH plays an important role in growth control. Its major role in stimulating body growth is to stimulate the liver and other tissues to secrete IGF1. Further, it stimulates both the differentiation and proliferation of myoblasts and also the amino acid uptake and protein synthesis in muscle and other tissues (Rebhan *et al*. 1997).

Within the QTL region for IF% on chromosome 24 three members of the apolipoprotein gene family (*APOA1*, *APOA4*, and *APOA5*) are located (Jennen *et al*. 2002). These proteins are components of high-density lipoprotein, which are involved in the plasma lipoprotein metabolism and transport. Douaire *et al*. (1992) and Lagarrigue *et al*. (2000) showed significant differences for *ApoA1* mRNA levels between fat and lean birds. Therefore, the apolipoproteins are potential candidate genes for the IF% QTL detected on chromosome 24.

Conclusion

The results of this study show the use of an AIL for the confirmation of QTL found in an earlier generation. Moreover, on chromosome 1 we were able to identify two distinct regions for fat deposition. This is the first step towards the fine mapping of the QTL for fat deposition. The identification of conserved chromosomal segments (i.e. haplotype blocks), which are associated with the observed QTL effects will be needed to further reduce the size of the QTL regions. To identify these haplotype blocks more densely spaced markers are needed. Therefore, new markers (i.e. SNPs) need to be developed. This is an essential step before moving towards the next phase of identifying the underlying genes responsible for the observed QTL effects.

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CHAPTER 4

A COMPARATIVE MAP OF CHICKEN CHROMOSOME 24 AND HUMAN CHROMOSOME 11

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Summary

To improve the physical and comparative map of chicken chromosome 24 (GGA24; former linkage group E49C20W21) bacterial artificial chromosome (BAC) contigs were constructed around loci previously mapped on this chromosome by linkage analysis. The BAC clones were used for both sample sequencing and BAC end sequencing. Sequence tagged site (STS) markers derived from the BAC end sequences were used for chromosome walking. In total 191 BAC clones were isolated, covering almost 30% of GGA24, and 76 STS were developed (65 STS derived from BAC end sequences and 11 STS derived within genes). The partial sequences of the chicken BAC clones were compared with sequences present in the EMBL/GenBank databases, and revealed matches to 19 genes, ESTs and genomic clones located on human chromosome 11q22-q24 and mouse chromosome 9. Furthermore 11 chicken orthologues of human genes located on HSA11q22-q24 were directly mapped within BAC contigs of GGA24. These results provide a better alignment of GGA24 with the corresponding regions in human and mouse and identify several intrachromosomal rearrangements between chicken and mammals.

(*Key words*: *chicken, comparative map, genome mapping, human*)

Introduction

The human genome holds a wealth of information about human development, physiology, medicine and evolution. With the complete sequence at hand it is possible to locate and identify all of its genes. The human sequence is not only important for human genetics, but other organisms will also benefit from it. Using comparative mapping studies it is possible to identify homologous chromosome segments in distantly related vertebrates, allowing exchange of information between species.

In chicken, a model organism, many segments homologous with human and mouse are found (Burt *et al*. 1999; Groenen *et al*. 2000; Schmid *et al*. 2000). The first detailed comparative map between chicken chromosome 10 and human chromosome 15 described by Crooijmans *et al*. (2001) shows very high conservation of synteny, but also reveals a high level of intrachromosomal rearrangements.

Chicken chromosome 24 (GGA24; former linkage group E49C20W21), one of the microchromosomes, appears to share homology with one specific human and one specific mouse chromosome. The orthologues of the five genes (*APOA1*, *ETS1*, *OPCML*, *OCT11* and *RPS25*) mapped to GGA24 map to human chromosome 11q23 and mouse chromosome 9, indicating that this chicken chromosome shows conservation of synteny with these chromosomes. In chicken 18 orthologues of human genes from other HSA11 regions (i.e. 11p15, 11q13 and 11q21-q22) are found on GGA1, GGA5, GGA19 and GGA26 (Schmid *et al*. 2000).

This paper describes the first results of a detailed analysis of chicken chromosome 24, using a bi-directional approach, starting from loci known to be located on GGA24 in addition to genes known to be located in the identified orthologous regions in man.

Material and methods

Chicken chromosome 24 BAC clones

For building the BAC contigs, the Wageningen BAC library was screened for all microsatellite markers and genes located on chicken chromosome 24 (GGA24) using two rounds of polymerase chain reaction (PCR) (Crooijmans *et al*. 2000). A detailed description of all loci used is available at the web site of the Animal Breeding and Genetics Group in Wageningen (http://www.zod.wau.nl/vf/). New oligos were designed for *LEI0155* to optimise the amplification product of this marker.

Forward primer: 5'-AGGAGTGGTACGTGTAGCTC-3'

Reverse primer: 5'-GATCCGTAAAGCAAAGCTGC-3'

All identified BAC clones were tested for purity by PCR amplification of the marker directly on two single colonies (colony PCR).

BAC-end sequencing

BAC clones were cultured overnight (o/n) in 2 ml LB containing 12.5 μg/ml chloramphenicol (Cm) at 37 ºC. BAC DNA was isolated with REAL prep 96 kit (Qiagen GmbH, Hilden, Germany), and dissolved in 32 μl 5 mM Tris-HCl pH 8.0. Cycle sequencing was carried out in a 40 μl reaction volume containing 16 μl BAC DNA (~250 ng), 8 μl Half Big Dye terminator (Genpak Ltd, New Milton, UK), 8 μl Big Dye Terminator Ready Reaction mix (Applied Biosystems, Foster City, CA, USA), 1 μl M13 forward or M13 reverse sequence primer (10 pmol/μl) and 7 μl distilled water. Amplification reactions were as follows: 5 min 96 ºC followed by 45 cycles of 30 s 96 ºC, 20 s 50 ºC, 4 min 60 ºC. After precipitation with isopropanol, the amplification product was dissolved in 2.5 μl 83% de-ionized formamide and 17 % loading buffer (Applied Biosystems). PCR products were seperated using a denaturating 5% Long Ranger Gel (BioWhittaker Molecular Applications, Rockland, ME, USA) on an automated sequencer ABI377 (Applied Biosystems). Electrophoresis was performed for 7 hours on 36 cm gels. The results were analysed using ABI sequence software (Applied Biosystems).

Sample sequencing.

BAC DNA was isolated from a 4 ml o/n culture (4 ml LB + 12.5 μg/ml Cm) (Crooijmans *et al*. 2000). BAC DNA was digested with *Eco*RI and ligated into the *Eco*RI site of pTZ18R. Ligation products were transformed into DH5α. In total twelve subclones per BAC clone were randomly selected and plasmid DNA was isolated using Qiaprep 96 miniprep kit (Qiagen GmbH). Cycle sequencing was performed in 10 μl containing 200-500 ng plasmid DNA, 2 μl Half Big Dye terminator (Genpak Ltd), 2 μl Big Dye Terminator Ready Reaction mix (Applied Biosystems) and 1 μl M13 forward or M13 reverse sequence primer (0.8 pmol/μl). Amplification was carried out using the following PCR program: 5 min 96 ºC followed by 30 cycles of 30 s 96 ºC, 10 s 45 ºC, 4 min 60 ºC. The excess dye terminator was removed by isopropanol precipitation. Sequence reactions were analyzed on a 96 well 36 cm 5% denaturating Long Ranger Gel (BioWhittaker Molecular Applications) according to ABI (Applied Biosystems). All sequences obtained were first analyzed with PREGAP4 of the STADEN software package (Bonfield *et al*. 1995; http://www.mrc-lmb.cam.ac.uk/pubseq) to eliminate vector sequences, *E. coli* sequences and poor quality sequences. The network BLAST client software (blastcl3) of the National Center for Biotechnology Information (NCBI) was used to compare the final sequences with sequences deposited in public databases.

Fluorescent In Situ Hybridization (FISH).

Miniprep DNA of selected BAC clones, derived from chicken genes with a HSA11q22-24 orthologue, was used in FISH experiments to evaluate the map location of these clones. Two-colour FISH was performed using *Not*I digested BAC DNA, either labeled by random priming with biotin-16-dUTP or with digoxigenin-11-dUTP (Roche Diagnostics, Almere, Netherlands) (Trask *et al*. 1991). BAC clone bW020E08, identified with microsatellite marker *LEI0069* and known to be located on GGA24, was used in the two-colour FISH as positive control.

Results and discussion

Increasing the number of genes mapped to GGA24 would allow a more accurate alignment of the human, mouse and chicken maps. Therefore, the construction of a complete BAC contig of GGA24 was started.

For the initial screening of the Wageningen BAC library seven microsatellite markers and three genes (Figure 4.1) known to be located on GGA24 (Groenen *et al*. 2000) were used. For each marker one of the BAC clones was selected and used for BAC end sequencing. The resulting sequences were subsequently used for the development of STS markers for chromosome walking.

In order to further increase the number of starting-points for chromosome walking and the number of mapped genes, chicken orthologues of genes from HSA11q22-24 were used for developing additional STS markers to screen the chicken BAC library. Chicken orthologues from 10 human genes, were identified using a BLAST search with the mRNA sequences of human genes known to be located on HSA11q22-24. For these 10 genes (Table 4.1; Figure 4.1, underlined) at least one BAC clone was isolated which subsequently was used to map that particular gene in chicken. Eight genes, *APOA4*, *CD3G/D*, *CRYAB*, *DKFZP434F162*, *NCAM1*, *ORP150*, *PAFAH1B2* and *TAGLN* were directly mapped to BAC clones present in the BAC contigs located on GGA24. The other two genes, *APLP2* and *ATM* were mapped by FISH to GGA1q and not to GGA24 (data not shown). This is in good agreement with the known location of three other genes from HSA11q21-q22 (*PGR*, *TYR*, *FUT4*) and that are closely linked to the human *ATM* gene. All three genes previously have been mapped to GGA1 in chicken (Schmid *et al*. 2000). These results indicate that one of the breakpoints of the translocations that separate these chromosomal regions in man and chicken is located between *ATM* and *CRYAB*. The other breakpoint is more difficult to identify, because more complex rearrangements seem to have occurred within the region of the *OPCML*, *APLP2* and *ETS1* genes (Figure 4.1).

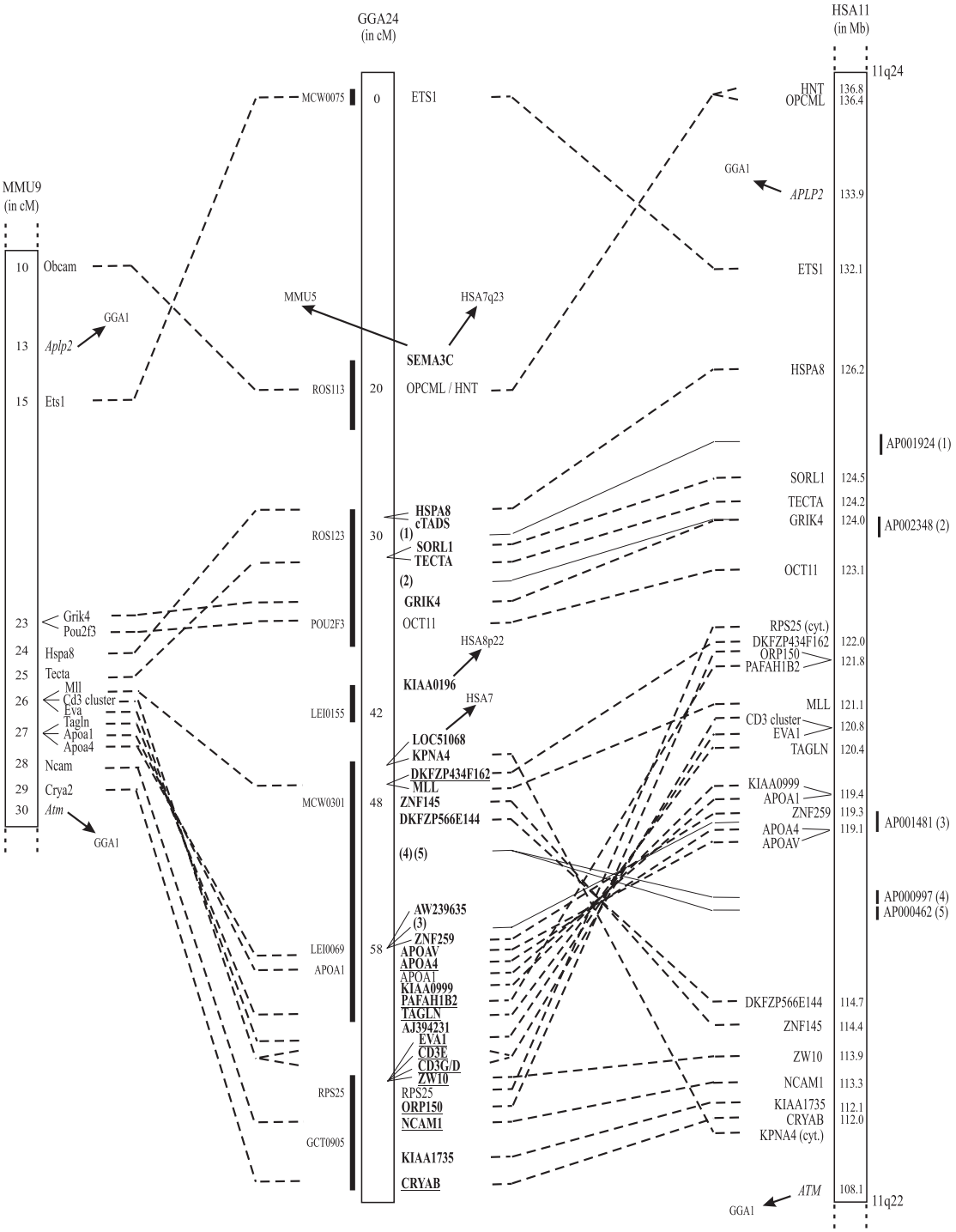
MMU9
(in cM)
10 Obcam
13 Aplp2
15 Ets1
23 Grik4
Pou2f3
24 Hspa8
25 Tecta
26 Mll
Cd3 cluster
Eva
27 Tagln
Apoa1
Apoa4
28 Ncam
29 Crya2
30 Atm
GGA1
GGA24
(in cM)
MCW0075
ROS113
ROS123
POU2F3
LEI0155
MCW0301
LEI0069
APOA1
RPS25
GCT0905
0 ETS1
20 OPCML / HNT
30 HSPA8
cTADS
(1)
SORL1
TECTA
(2)
GRIK4
OCT11
42
KIAA0196
LOC51068
KPNA4
DKFZP434F162
MLL
48 ZNF145
DKFZP566E144
(4) (5)
58 AW239635
(3)
ZNF259
APOAV
APOA4
APOA1
KIAA0999
PAFAH1B2
TAGLN
AJ394231
EVA1
CD3E
CD3G/D
ZW10
RPS25
ORP150
NCAM1
KIAA1735
CRYAB
MMU5
HSA7q23
SEMA3C
HSA8p22
HSA7
HSA11
(in Mb)
11q24
HNT 136.8
OPCML 136.4
GGA1
APLP2 133.9
ETS1 132.1
HSPA8 126.2
AP001924 (1)
SORL1 124.5
TECTA 124.2
GRIK4 124.0
AP002348 (2)
OCT11 123.1
RPS25 (cyt.)
DKFZP434F162 122.0
ORP150
PAFAH1B2 121.8
MLL 121.1
CD3 cluster
EVA1 120.8
TAGLN 120.4
KIAA0999
APOA1 119.4
ZNF259 119.3
APOA4 119.1
APOAV
AP001481 (3)
AP000997 (4)
AP000462 (5)
DKFZP566E144 114.7
ZNF145 114.4
ZW10 113.9
NCAM1 113.3
KIAA1735 112.1
CRYAB 112.0
KPNA4 (cyt.)
ATM 108.1
GGA1
11q22

Figure 4.1 *Comparative map of GGA24 (middle), part of MMU9 (left) and part of HSA11q22-q24 (right). Chromosomes are not to scale. Estimated positions for mouse and human are given, according to NCBI Map Viewer build24 (http://www.ncbi.nlm.nih.gov/), in cM and Mb, respectively. The loci located on the linkage map of the chicken genome (Groenen, et al. 2000) are shown on the left of the vertical bar. The numbers inside the vertical bar of GGA24 represent the relative positions in cM of the chicken loci. On the left of the vertical bar from GGA24 the six BAC contigs are shown in solid bars (not to scale). The genes (human name) and ESTs located on GGA24 are shown on the right site. Numbers between parentheses correspond to the human genomic clones on the right site of the HSA11 bar (connected via thin lines). Genes mapped in this study are in bold and genes mapped by chromosome walking are also underlined. Genes used in FISH studies are in italics.*

An additional three genes, *ZW10*, *CD3E* and *EVA1*, can also be placed on GGA24, because they are located on the same chicken cosmid clone (accession number AJ250458) as the *CD3G/D* gene. The cosmid clone was used to develop a STS marker for the *CD3G/D* gene (Table 4.1).

Altogether, 76 STS were developed (65 from BAC end sequences and 11 from genes) and 191 BACs identified, resulting in six BAC contigs with an estimated chromosome coverage of GGA24 of almost 30%. The number of BAC clones per marker varied from 1 to 12 with an average of 4.9 BACs per marker, which is in agreement with the estimated 5.5 times genome coverage of the library (Crooijmans *et al*. 2000).

To further increase the number of genes mapped to GGA24, 13 different BAC clones from the six GGA24 contigs were used for sample sequencing. The sequences obtained by the sample sequencing and the BAC end sequences were compared with sequences in the EMBL or GenBank databases using the BLAST algorithm. Sequence identity is on average 96.3% with chicken sequences and 89.2% with human sequences. Most BLAST hits showed homology to genes, ESTs and genomic clones from HSA11q22-q24 and MMU9 (Figure 4.1; Table 4.2), which resulted in several cases in similar gene orders in chickens and humans. For example, the gene order within the chicken contig of *ROS123* was *HSPA8* (126.2 Mb), *SORL1* (124.5 Mb), *TECTA* (124.2 Mb), *GRIK4* (124.0 Mb), and *OCT11* (123.1 Mb), with the human sequence notation in parentheses. The genes *TECTA* and *SORL1* were located in chicken on the same BAC clone (bW052D04). Multiple BLAST hits with HSA11 were also found with sample sequences of the chicken BAC clones bW003K05 and bW020E08, both within the contig of markers *MCW0301* and *LEI0069* (Table 4.2).

Table 4.1 Characteristics of STS markers developed in chicken genes

Accession number of chicken	Gene	Accession number of human[1]	Human cytogenetic map position	PCR size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
AF030341	*APLP2*	NM_001642	11q24	222	TTAGCTGCTTCTCTTTGCCG	CCAATGCCTAAAGTGTGCAC
M96012	*APOA1*	-	11q23-q24	269	ATCGCCCAGTTCGAGTCCTC	AGCGTGTCCAGGTTGTCAGC
Y16534	*APOA4*	NM_000482	11q23	179	AGATGAAGCAGAAGCTGGAG	CACCAGTTTATTCTGCCACG
AB026548	*ATM*	NM_000051	11q22-q23	132	AAGCCAGTGATGACCAGAGC	CCTGCTGTATGAGTAGGTTC
	CD3G/D	NM_000073	11			
		NM_000732	11			
AJ250458	*CD3E*	NM_000733	11	163	CAGTGATTCCTTCACACGAC	AGCAGAGTGCTACAAGCAGC
	ZW10	NM_004724	11q23			
	EVA1	NM_005797	11q24			
S53164	*CRYAB*	NM_001885	11q22.3-q23.1	119	CAGTTGCAGATGACAACTGC	GAAGGGCACAGACAACATAG
AJ392094	*DKFZP434F162*	NM_015517	11	109	AACATCTGGATACGCACAGC	TGGACTTTGCGGTAGTGCAG
M15923	*NCAM1*	NM_000615	11q23.1	223	TATTCTCTGCAGTGGCAGGG	TTCACATTGACGGTGGCTTC
AW773175	*ORP150*	NM_006389	11q23.1-q23.3	~500	TTTGACCGTACTCTTGGAGG	GTCAATATCATCCAGCAGCC
AJ394959	*PAFAH1B2*	NM_002572	11q23	238	CAATGTCCAGCTCCTGGATG	TGACAGAACTGATCTGATGG
U77715	*OCT11*	-	11q23.3	147	GAGGAGATCTCCTTGATAGC	TCACCAGTCTGGAATTGTAG
AF179465	*RPS25*	NM_001028	11q23.3	162	GAGAGACAAGTTGAACAACC	TTACCGAGCAGTTCCTGCAG
M83105	*TAGLN*	NM_003186	11q23.2	223	TCCAGCTCTCCTCTTCTCTC	CATTTGCAAGGATCTCTGCC

[1]Accession number of human genes used in BLAST search to identify chicken orthologous genes.

Table 4.2 BAC sample sequencing hits and accession numbers

Locus[1]	BAC clone	Accession number [2]	BLAST hit[3]	Accession number[4]	Human orthologue	bp homology	Identity (%)	HSA map position		MMU map position
								Cytogenetic map	Sequence map[5] (Mb)	
ST24BE043	bW007K13	BH024319	**COLL-3**	AF022946	SEMA3C	128	99	7q21-q31	78.4	5
ST24BE065	bW114D24	BH024320	**HSPA8**	AJ004940	HSPA8	353	96	11q23.3-q25	126.2	9 (24 cM)
	bW114D24	BH024321	**cTADS**	AF035677	unknown	89	84	unknown	-	
ROS123	bW008L04	BH024322	human BAC clone	AP001924		366	90	11q	125.1	
ST24BE068	bW052D04	BH024323	**LR11**	Y08109	SORL1	202	98	11q23.2-q24.2	124.5	
	bW052D04	BH024324	**TECTA**	AJ012287	TECTA	67	95	11q22-q24	124.2	9 (25 cM)
ST24BE049	bW003F15	BH024325	human BAC clone	AP002348		44	90	11q	123.9	
ST24BE034	bW031P07	BH024326	GRIK4	NM_014619		169	86	11q22.3	124.0	9 (23 cM)
ST24BE067	bW034P01	BH024327	KIAA0196	AI147681		83	85	8p22	122.2	
ST24BE052	bW107D17	BH024328	**chicken EST**	AI980520	LOC51068	217	99	7	61.2	
	bW058J09	BH024329	KPNA4	NM_002268		86	89	11q22	-	
ST24BE023	bW009G24	BH024330	**MLL**	AJ011003	MLL	471	98	11q23	121.1	9 (26 cM)
MCW0301	bW074B23	BH024331	ZNF145	AF060568		65	89	11q23.1	114.4	
ST24BE001	bW029M15	BH024332	DKFZP566E144	NM_015523		144	90	11q23.1-q23.2	114.7	
ST24BE071	bW003K05	BH024333	human BAC clone	AP000462		124	93	11q	117.7	
	bW003K05	BH024334	human clone	AP000997		256	91	11q23.2	118.0	
LEI0069	bW020E08	BH024335	**chicken EST**	AW239635	unknown	93	93	unknown	-	
	bW020E08	BH024336	human BAC clone	AP001481		197	94	11q	119.0	
	bW020E08	BH024337	**chicken EST**	BG710416	ZNF259	148	99	11q14.2-q14.3	119.3	
ST24BE002	bW061J01	BH406521	**chicken EST**	BG711386	APOAV	413	97			
ST24BE003	bW059F14	BH024338	KIAA0999	AB023216		83	87	11	119.4	
ST24BE028	bW000I04	BH024339	**chicken EST**	AJ394231	unknown	448	96	unknown	-	
CD3G/D	bW026F18	BH406568	**CD3E**	Y08917	CD3E	190	100			
ST24BE038	bW110M02	BH024340	KIAA1735	AB051522		77	85	11	112.1	

[1]Order of loci according to the chicken linkage map (Groenen *et al.* 2000).

[2]Accession number of BAC sample sequence.

[3]BLAST hits to chicken genes and ESTs are in bold, the other hits are directly to human.

[4]Accession number of BLAST hit.

[5]Sequence map position is according to NCBI Map Viewer build24 (http://www.ncbi.nlm.nih.gov/).

In total, sequence identity was found to 34 human, mouse and chicken genes, ESTs and genomic clones. Most of the genes and ESTs found on GGA24 are located on human chromosome 11. In three cases homology was found with genes that are located on other human chromosomes (Figure 4.1; *SEMA3C* on HSA7q21-q31, *LOC51068* on HSA7, *KIAA0196* on HSA8p22). It is possible that in this case a human paralogous gene was identified, rather than the orthologous gene. For example *SEMA3C*, located on MMU5 and HSA7q21-q31 is a member of a large gene family. Although no other member of this family is known to map to HSA11, the true orthologue could have been deleted during evolution in the lineage leading to human. Whether the other two genes are part of a gene family or just represent small regions of homology to other human chromosomes is not known yet. If these three genes do represent true orthologues, then other orthologues from the same human regions would be expected to be present on GGA24. Although *LOC51068* and *SEMA3C* are located on the same chromosome (HSA7), they are in fact from two different regions, 17.2 Mb apart.

For two chicken ESTs (accession number AJ394231, AW239635) and one chicken gene (*cTADS*) no human orthologue was identified. The gene *cTADS* (accession number AF035677) belongs to the immunoglobulin superfamily, which has several members located on HSA11q23, i.e. *NCAM*, *OPCML* and *THY1*. Identification of a mammalian orthologue to *cTADS* may have failed because of the low sequence conservation between avian and mammalian genes and the overall low sequence similarity that exists among the immunoglobulin superfamily members. Another possibility is the loss of the mammalian orthologue to *cTADS* during evolution (Ruble and Foster 2000).

For *OPCML* identification of more than one human homologue occurred. Initially Smith *et al*. (2000) found sequence identity in the microchromosomal cosmid clone AJ231833 to 5' part of the human *OPCML* gene, but the same sequence identity is also found with the human gene, *HNT*. Both human genes encode cell adhesion molecules belonging to the same gene family and are located close to each other on HSA11q24 (*OPCML* at 136.4 Mb and *HNT* at 136.8 Mb). The high sequence identity between both genes suggests that

the gene, originally identified as the chicken *OPCML* gene, might also be the *HNT* gene.

In addition to the ESTs and genes, sequence identities were found with human genomic clones on HSA11 (Figure 4.1, no. 1-5). One of these genomic clones contains a gene of which the chicken orthologue is located on GGA24: *ZNF259* on AP001481 (clone no. 3). The identified regions are likely to contain conserved (non-coding) regulatory elements.

Data presented in this paper allow a more accurate alignment of GGA24 with the corresponding regions in human and mouse. Furthermore this alignment clearly shows that intrachromosomal rearrangements have occured within the lineage leading to chicken and mammals. A good example of such rearrangements is the inversion of the genes in the region between *KPNA4* and *ORP150* (Figure 4.1). Moreover, within this region many rearrangements between genes are observed.

Acknowledgments

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GenBank accession numbers: The nucleotide sequence data has been submitted to GenBank under accession numbers: BAC end sequences (BH024322, BH024325-BH024328, BH024331, BH024332, BH024338, BH024339 and BH406513-BH406568) and BAC sample sequences (BH024319-BH024321, BH024323, BH024324, BH024329, BH024330, BH024333-BH024337 and BH024340).

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CHAPTER 5

COMPARATIVE MAP BETWEEN CHICKEN CHROMOSOME 15 AND HUMAN CHROMOSOMAL REGION 12q24 AND 22q11-q12

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Abstract

The physical and comparative map of GGA15 was improved by the construction of 9 BAC contigs around loci previously mapped on GGA15 by linkage analysis. In total 240 BAC clones were isolated, covering 30-35% of GGA15, and 120 STS were developed (104 STS derived from BAC end sequences and 18 STS derived within genes). Seventeen chicken orthologues of human genes located on human chromosome 22q11-q12 were directly mapped within BAC contigs of GGA15. Furthermore, the partial sequences of the chicken BAC clones were compared with sequences present in the EMBL/GenBank databases and revealed matches to 26 genes, ESTs, and genomic clones located on HSA22q11-q12 and HSA12q24. These results provide a better alignment of GGA15 with the corresponding regions in human and mouse, and improve our knowledge of the evolution and dynamics of the vertebrate genome.

Introduction

Although birds and mammals diverged over 300 million years ago, several chromosomal segments of similar gene content are conserved between human and chicken (Burt *et al.* 1999). Comparative mapping studies have been shown to be very useful to identify such homologous chromosome segments in human and chicken. Recently, detailed comparative maps between human and chicken chromosomes have been published (Nanda *et al*. 2000; Crooijmans *et al.* 2001; Buitenhuis *et al.* 2002; Jennen *et al.* 2002).

In our group, a bi-directional approach is used to improve the comparative map of chicken and human (Crooijmans *et al.* 2001; Buitenhuis *et al.* 2002; Jennen *et al.* 2002). First, a BAC contig is built starting from loci whose positions on the chicken genome are known. Second, genes known to be located in the identified syntenic regions in human and mouse are used to map additional genes in these regions.

The linkage map of chicken chromosome 15 (GGA15) is 71 cM in size and contains 19 markers (Groenen *et al*. 2000). Four chicken orthologues of human genes (*CRYBB1*, *CRYBA4*, *IGL@*, *MIF*), located in the human on chromosome 22q11 (HSA22q11), have been mapped to this chicken microchromosome (Schmid *et al*. 2000).

The aim of this study is to improve the comparative map between GGA15 and HSA22q11 on the chicken genome.

Material and methods

Chicken chromosome 15 BAC clones

The Wageningen chicken BAC library was screened by PCR (Crooijmans *et al*. 2000) for all microsatellite markers and STS markers within genes located on GGA15. A detailed description of all loci used can be found at the ARKdb farm animal database (http://www.thearkdb.org/). This includes microsatellite marker *MCW0052* located within the gene *IGVPS*. Primers corresponding to all other genes mapped to GGA15 (*CRYBB1*, *CRYBA4*, *MIF*) were designed based on database sequences (Table 5.1). All identified BAC clones were tested for purity by PCR amplification of the marker directly on two single colonies (colony PCR).

Table 5.1 Characteristics of markers developed in chicken genes

Gene	Accession number		Human cytogenetic map position	PCR size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
	Chicken	Human[1]				
ADRBK2	AJ397769	NM_005160	22q12.1	95	CTGACCATGAATGACTTCAG	CAGTGTCTGCTTTTCTGCAC
CDC45L	AJ393544	NM_003504	22q11.21	97	TATTGGAATTCTGCAGCGCC	TTCAAATGCTATTCGCATGC
CRYBA4	U18260	NM_001886	22q12.1	172	TGGTGTGTTTGCGCTTGGAG	TTGCAGCTCCTAATTCCTCC
CRYBB1	U09951	NM_001887	22q12.1	318	CCCTGTACGAGTCTGCTGAC	CGACGGATGGACTGGATCTG
CRYBB2	S52930	NM_000496	22q11.23	122	TCCAACTGAAGCCCTCGCAC	AGCTTTATTCGGTTGCGGTG
CRYBB3	U28146	NM_004076	22q11.23	198	GCCAGCACGTCTTTGAGAAG	AAGAACACCAAACCGATGGC
GSTT1	U13676	NM_000853	22q11.23	152	CTTTAACCACTGCTCTCCAC	GCAATTATGTGAAGGCTGTG
HIRA	X99375	NM_003325	22q11.21	138	AGGATTTACTGGGTCCAGTC	CTGTGAAAAGCCTCTGGAAG
MIF	M95776	NM_002415	22q11.23	97	AGTACATAGCCGTGCACATC	TAGAACGGTTACGACATCTC
PITPNB	AI979795	NM_012399	22q12.1	115	GGTCAACTTTATTCGGTGGC	TGAGTATATTGTCCCTTCTC
PNUTL1	AJ393439	NM_002688	22q11.21	124	GGAAGCCCATCACTGACTAC	AAGGGCGAGATGAAGTAGAG
PPIL2	AW198371	NM_014337	22q11.21	97	AATACAGAGACCCGAGAGAC	CTTTCTTCTTTTCAGGAGCC
RANBP1	AF179468	NM_002882	22q11.21	138	GCCCTTTTGAAGATGTCTTC	AATCCTTCGGAGGCTGTTTC
SMARCB1	AJ398441	NM_003073	22q11.23	134	AATCGCGATTCGCAACACGG	CAGTGTTGGCCAAGCGTCTC
TFIP11	AJ396682	NM_012143	22q12.1	155	TCCTGCTTGGAACTGTGAAG	TATCATTTCACCTCCTGCCG
UFD1L	AF228284	NM_005659	22q11.21	127	GTTCTCCTTCAACATGTTCG	GCCTTTCTCCACATCTGACC
XBP1	AJ394086	NM_005080	22q12.1	120	CGCAGCACTCAGACTACGTG	GAATCTGAAGAGTCACTGCC
TBX3	AF033669	NM_016569	12q24.1	159	CATGTACTGTGCTGTTTAGAG	CTTCCTACTGCAGGAGTAGTC

[1]Accession number of human genes used in BLAST search to identify chicken orthologous genes.

Sequencing

BAC-end sequencing and sample sequencing were performed as described by Jennen *et al.* (2002). Sequences obtained were first analysed with PREGAP4 of the STADEN software package (Bonfield *et al*. 1995; http://www.mrc-lmb.cam.ac.uk/pubseq). The network BLAST client software (blastcl3) of the NCBI was used to compare the sequences of good quality reads with sequences deposited in public databases.

The BAC-end sequences were also used to develop new STS markers for chromosome walking. Sample sequences and BAC-end sequences, including STS markers have been submitted to GenBank and have been assigned the accession numbers BZ592394-BZ592544.

Mapping of genes

Genes of interest were either mapped to BACs that were already present within known BAC contigs or mapped by SNP typing as described by Buitenhuis *et al.* (2002). The SNP was first detected in the parents of the Wageningen mapping population (Groenen *et* al. 1998). A specific restriction enzyme for the SNP was used to map the gene as a PCR-RFLP on one selected family from the Wageningen mapping population.

Analysis of chromosomal rearrangements

Chromosomal rearrangements were analyzed by using GRIMM (Tesler 2002; http://www-cse.ucsd.edu/groups/bioinformatics/GRIMM). GRIMM enables the analysis of rearrangements in multichromosomal genomes and provides a new algorithm for analyzing comparative maps for which gene directions are unknown.

Gene data sets based on the comparative map between human, mouse, and chicken were used for the calculation of the minimum possible number of rearrangements steps (the multichromosomal distance) between chicken and human, chicken and mouse, and human and mouse. The data sets were used with an unsigned gene order, because the gene orientation in chicken is unknown.

Results

Construction of GGA15 BAC contigs

BAC contigs of GGA15 were constructed around loci known to be located on this chromosome. The Wageningen chicken BAC library was initially screened with 17 markers. One BAC clone per marker was selected for end sequencing. The BAC-end sequences were used to design specific STS markers for chromosome walking. In total, 104 STS markers were designed and 240 BAC clones isolated, which resulted in the construction of nine BAC contigs.

Identification and mapping of genes

Since GGA15 showed conservation of synteny with HSA22q11 (Schmid *et al*. 2000), chicken orthologues of human genes from HSA22q11-q12 were identified to further increase the number of starting points for chromosome walking. Chicken orthologues from 14 human genes were identified by using a BLAST search with the mRNA sequences of human genes known to be located on HSA22q11-q12. For these 14 genes, STS markers were developed to screen the BAC library (Table 5.1). Nine genes, *ADRBK2*, *CRYBB2*, *CRYBB3*, *GSTT1*, *PITPNB*, *RANBP1*, *SMARCB1*, *TFIP11*, and *XBP1*, were mapped to BACs that were already present within the BAC contigs of GGA15.

The other five genes, *CDC45L*, *HIRA*, *PNUTL1*, *PPIL2*, and *UFD1L*, were mapped to BACs that formed a single contig, which had not yet been assigned to a chromosome. With PCR-RFLP, this BAC contig could also be mapped genetically to GGA15. Restriction enzyme *Hha*I was used to map the BAC clone bW041F24 positive for locus *HIRA* on the chicken linkage map. *HIRA* and, therefore, the complete BAC contig, were mapped close to microsatellite marker *MCW0031* (recombination fraction = 0; LOD score = 12.64).

The chicken orthologue of *TBX3*, which in human is located on HSA12q24, was initially used within another project and, by using PCR-RFLP was found to be located on GGA15. With restriction enzyme *Tsp* 509 I, BAC clone bW110C15 positive for locus *TBX3* was mapped on the chicken linkage map close to *ACW0169* (recombination fraction = 0.04; LOD score = 10.54).

To further increase the number of genes mapped to GGA15, 19 different BAC clones from GGA15 contigs were used for sample sequencing. The sequences obtained by sample sequencing and BAC-end sequencing were compared with sequences in Genbank and with the UMIST Chicken EST sequences (Boardman *et al*. 2002; http://www.chick.umist.ac.uk/) by using the BLAST algorithm. In total, sequence identity was found to 66 genes, ESTs, and genomic clones from chicken, human, and other vertebrates. The BLAST hits showed homology to 10 sequences from HSA22q11-q12 and to 16 sequences from HSA12q24. Homology to two genes and two anonymous genome segments from HSA3 was also found (Figure 5.1a; Table 5.2), clearly marking a conserved segment. Furthermore, a sample sequence of BAC clone bW086M10 showed homology to a genomic clone from HSA1 (Figure 5.1a; Table 5.2), but did not show homology to any annotated gene. On average, sequence homology with chicken sequences was 96.9%, and with human sequences, 81.5%.

Table 5.2 BAC sample sequencing hits and accession numbers

Locus[1]	BAC clone	Accession number [2]	BLAST hit[3]	Accession number[4]	Human orthologue[5]	bp homology	% identity	HSA map position		MMU map position
								Cytogenetic map	Sequence map[6] (Mb)	
ST15BE059	bW128N01	BZ592498	**chicken EST**	AL584815		590	96			
		BZ592499	**chicken EST**	*334464.5*	RANBP1	659	97	22q11.21	17.0	16 (10.9 cM)
		BZ592500	**chicken EST**	*334464.5*	RANBP1	702	95	22q11.21	17.0	16 (10.9 cM)
		BZ592501	**chicken EST**	AL584937		590	95			
		BZ592502	**chicken EST**	AF179468	RANBP1	119	100	22q11.21	17.0	16 (10.9 cM)
		BZ592503	**chicken EST**	AF179468	RANBP1	58	100	22q11.21	17.0	16 (10.9 cM)
		BZ592504	**chicken EST**	*043373.1*		392	98			
MCW0031	bW109B14	BZ592505	**chicken EST**	*043373.1*		461	99			
		BZ592506	**chicken EST**	AL584937		555	93			
		BZ592507	**chicken EST**	*336214.1*	HTF9C	183	100	22q11.21	17.0	16 (10.9 cM)
			chicken EST	AJ445064	HTF9C	175	100	22q11.21	17.0	16 (10.9 cM)
		BZ592405	**chicken EST**	AJ397158		248	98			
			chicken EST	*333056.2*		635	96			
ST15BE009	bW044P19	BZ592432	**chicken EST**	*022848.1*		149	98			
ST15BE074	bW050B14	BZ592508	KIAA0692	AK024061		152	86	12	cyt	
		BZ592509	**chicken EST**	*002176.1*		134	98			
		BZ592510	**chicken EST**	*016090.1*		423	96			
		BZ592511	**chicken EST**	*331940.5*	GOLGA3	330	96	12q24.33	103.9	5 (62.0 cM)
		BZ592512	**chicken EST**	*331940.5*	GOLGA3	470	96	12q24.33	103.9	5 (62.0 cM)
		BZ592452	**chicken EST**	*333056.2*		439	95			
			chicken EST	*050929.1*		302	96			
ST15BE131	bW058E06	BZ592482	**chicken EST**	BM440415		239	92			
			chicken EST	*355017.8*		588	96			
ADL0206	bW091F08	BZ592513	**chicken EST**	*344548.1*		275	96			
ST15BE055	bW037I08	BZ592514	rat BAC clone	AC115194		146	84			
		BZ592515	**chicken EST**	*038082.1*		124	96			
		BZ592516	**chicken EST**	*056038.1*	ULK1	149	99	12q24.3	cyt	5
		BZ592517	**chicken EST**	*050266.1*		579	95			
LEI0083	bW017J22	BZ592518	**chicken EST**	*319066.1*		328	98			
		BZ592519	SFRS8	AA374595		68	88	12q24.13	130.4	5
		BZ592520	**chicken EST**	AJ442844	SFRS8	178	94	12q24.13	130.4	5
		BZ592521	human BAC clone	AC020724		260	95	12	130.2	
			chicken EST	*355445.1*		594	99			
		BZ592404	**chicken EST**	*018980.1*		265	97			

Table 5.2 (Continued)

Locus[1]	BAC clone	Accession number [2]	BLAST hit[3]	Accession number[4]	Human orthologue[5]	bp homology	% identity	HSA map position		MMU map position
								Cytogenetic map	Sequence map[6] (Mb)	
ST15BE002	bW034D23	BZ592448	human BAC clone	AC020724		227	89	12	130.2	
			SFRS8	NM_004592		126	89	12q24.13	130.4	5
ST15BE118	bW054N17	BZ592481	**chicken EST**	*019152.1*		545	97			
ST15BE089	bW034E12	BZ592522	KIAA1944	AB075824		80	88	12q24.33	128.2	
LEI0120	bW049K11	BZ592523	**chicken EST**	*021433.1*		246	99			
ST15BE005	bW020E20	BZ592427	human BAC clone	AC018873		85	92	12	128.4	
ADL0039	bW113E05	BZ592524	**chicken EST**	AJ447520	BCL7A	104	94	12q24.13	120.9	5
		BZ592525	**chicken EST**	*044129.1*	BCL7A	256	99	12q24.13	120.9	5
		BZ592526	**chicken EST**	AJ453931	ARHF	229	96	12q24.31	121.1	
		BZ592527	**chicken EST**	BQ037409		271	97			
		BZ592408	**chicken EST**	*044925.2*		587	98			
ST15BE093	bW017B07	BZ592461	**chicken EST**	AJ444684		193	95			
			chicken EST	AJ396350		60	98			
			chicken EST	*334980.3*		504	98			
		BZ592528	**chicken EST**	BI394105		63	95			
		BZ592529	**chicken EST**	*340810.4*		199	95			
ST15BE017	bW014A05	BZ592425	**chicken genomic clone**	AF079888[7]	VPS29	860	95	12q24	110.0	5
			chicken EST	*051914.1*	VPS29	103	99	12q24	110.0	5
MCW0052	bW122C13	BZ592530	**chicken genomic clone**	AF079888[7]	VPS29	520	93	12q24	110.0	5
		BZ592531	**chicken genomic clone**	AF079888[7]	VPS29	596	97	12q24	110.0	5
		BZ592532	**chicken genomic clone**	AF079888[7]	VPS29	462	96	12q24	110.0	5
		BZ592533	**chicken EST**	*051914.1*	VPS29	406	97	12q24	110.0	5
		BZ592534	**IGL@**	M97945	IGL@	254	91	22q	20.0	16 (13.0cM)
		BZ592535	**IGL@**	M15141	IGL@	212	97	22q	20.0	16 (13.0cM)
		BZ592536	**IGL@**	M24403	IGL@	544	95	22q	20.0	16 (13.0cM)
		BZ592537	**Cidd**	X95885	DGCR2	42	97	22q11.21	16.1	16 (10.37cM)
		BZ592409	**chicken EST**	BI392070	DGCR2	118	98	22q11.21	16.1	16 (10.37cM)
			chicken EST	*356683.3*		117	98			
ST15BE091	bW033N17	BZ592538	human BAC clone	AC092037		97	87	3	50.5	
		BZ592539	human BAC clone	AC067763		65	87	3	50.3	
		BZ592540	**chicken EST**	*056914.3*	DOCK3	389	96	3p21.3	50.3	9
		BZ592541	**chicken EST**	AJ443413	KIAA0800	90	94	3p21.31	50.5	9
		BZ592542	**chicken EST**	T20324		172	93			
			chicken EST	*056914.3*	DOCK3	442	99	3p21.3	50.3	9
		BZ592543	**chicken EST**	*056914.3*	DOCK3	368	98	3p21.3	50.3	9

Table 5.2 (Continued)

Locus[1]	BAC clone	Accession number [2]	BLAST hit[3]	Accession number[4]	Human orthologue[5]	bp homology	% identity	HSA map position		MMU map position
								Cytogenetic map	Sequence map[6] (Mb)	
MCW0231	bW107C20	BZ592402	**chicken EST**	*053822.1*		263	100			
ST15BE125	bW086M10	BZ592483	human BAC clone	AC073981		152	84	1	161.4	
GCT0054	bW043G23	BZ592544	**chicken EST**	*356750.1*		674	97			
			chicken EST	AI982143		546	94			
		BZ592439	human BAC clone	Z99774		57	92	22q11.22-12.2	23.8	
		BZ592440	**chicken EST**	BQ038575		551	92			
ST15BE122	bW017H24	BZ592489	**p95PKL**	AF112366	GIT2	367	98	12q24.1	109.4	5
			chicken EST	*332621.1*	GIT2	367	97	12q24.1	109.4	5
GCT0014	bW047O08	BZ592477	**chicken EST**	BM491459		498	98			
			chicken EST	*043045.1*		413	98			

[1]Order of loci according to the chicken linkage map (Groenen *et al.* 2000) and Figure 5.1.

[2]Genbank accession number of BAC sample sequence.

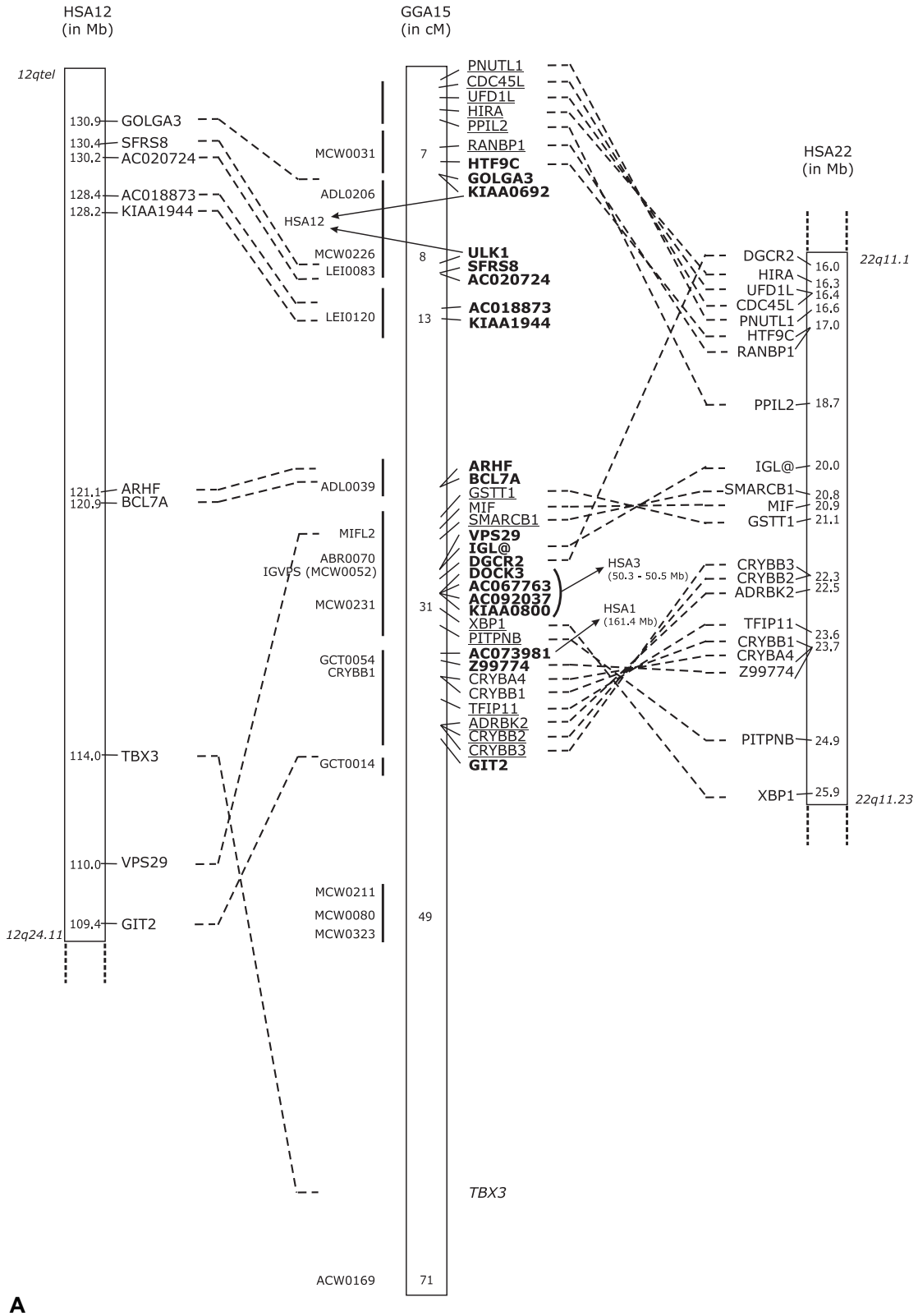
[3]BLAST hits to chicken genes and ESTs are in bold; the other hits are directly to human and other vertebrates.

[4]Genbank accession number or UMIST cluster number (in italics) of BLAST hit.

[5]Human orthologues of chicken sequences; anonymous ESTs and genome segments are not shown.

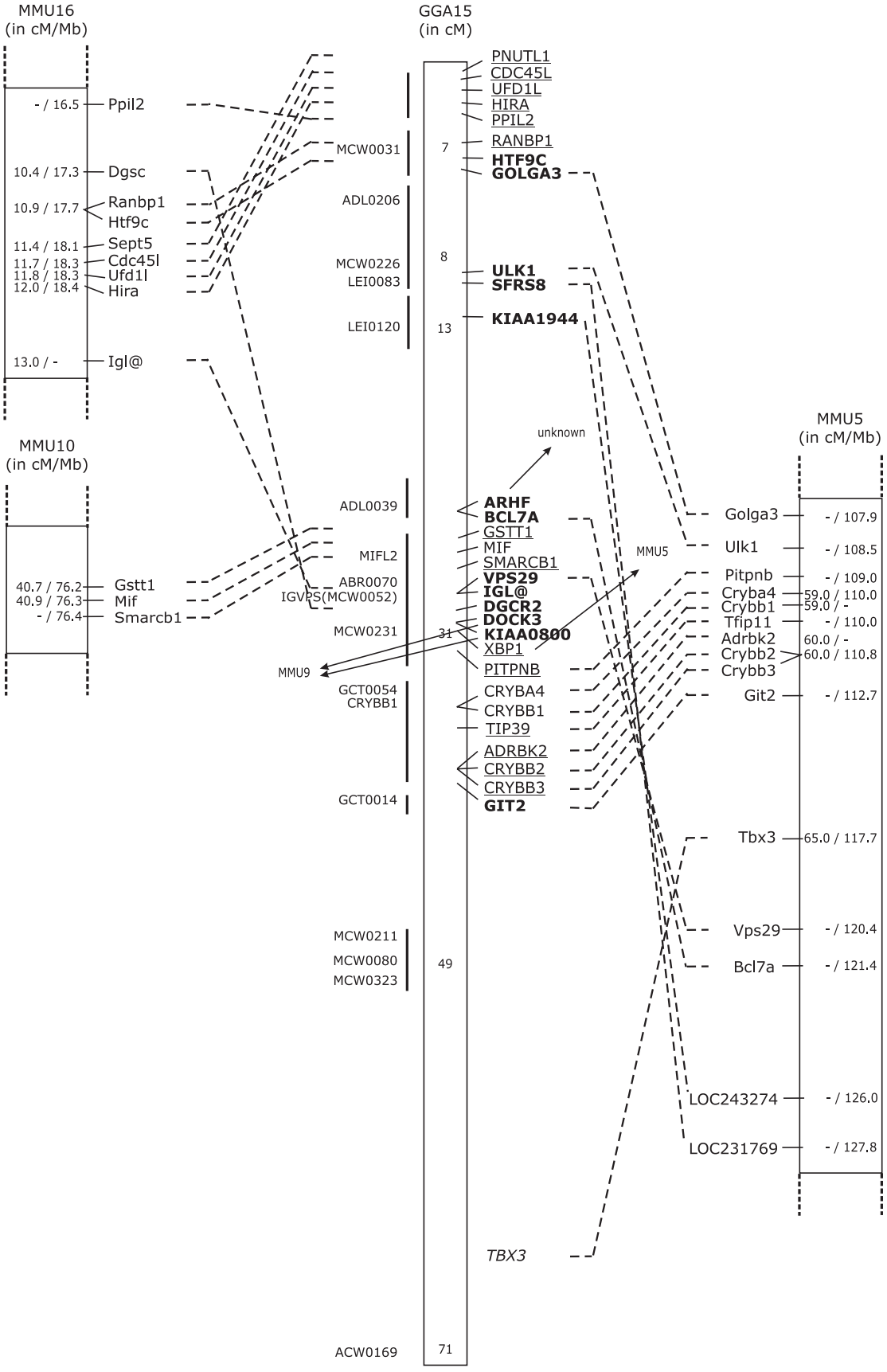
[6]Sequence map position is according to NCBI Map Viewer build30 (http://www.ncbi.nlm.nih.gov/).

[7]AF079888 is known as Gallus gallus immunoglobulin light chain gene, 5' DNaseI hypersensitive site sequence.



A

Figure 5.1 *Comparative map of (**a**) GGA15 and part of HSA12 and 22, and (**b**) GGA15 and part of MMU5, 10, and 16. Estimated positions for mouse and human are given in cM and/or Mb, according to the Map Viewer from Entrez Genomes (http://www.ncbi.nlm.nih.gov/). The loci located on the linkage map of the chicken genome (Groenen et al. 2000) are shown on the left of the vertical bar. The numbers inside the vertical bar of GGA15 represent the relative positions in cM of the chicken loci. On the left of the vertical bar from GGA15 the 9 BAC contigs are shown in solid bars (not to scale). The genes (human name) and genomic clones (human accession number) located on GGA15 are shown on the right site. Genes mapped in this study by sequence comparison using the BLAST algorithem are in bold; genes mapped by chromosome walking are underlined; and genes used in PCR-RFLP studies are in italics.*



B

Figure 5.1 *(Continued)*

Analysis of chromosomal rearrangements

GRIMM was used for the calculation of the multichromosomal distance between chicken and human, chicken and mouse, and human and mouse. Based on the comparative map between human, mouse, and chicken as shown in Figure 5.2, the following data sets were generated (format as needed for GRIMM):

>chicken
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 $
>human
8 9 10 11 12 30 16 29 $
18 4 3 2 1 7 6 5 17 15 14 13 28 27 26 25 24 23 21 $
19 20 $
22 $
>mouse
5 18 6 7 1 2 3 4 17 $
13 14 15 $
19 20 $
8 9 21 23 24 25 26 27 28 29 30 16 12 11 10 $
22 $

These data sets were used with an unsigned gene order, because the gene orientation in chicken is unknown. The gene order for human and mouse is given per chromosome. Calculations resulted in a multichromosomal distance between chicken and human of 11, between chicken and mouse of 12, and between human and mouse of 6.

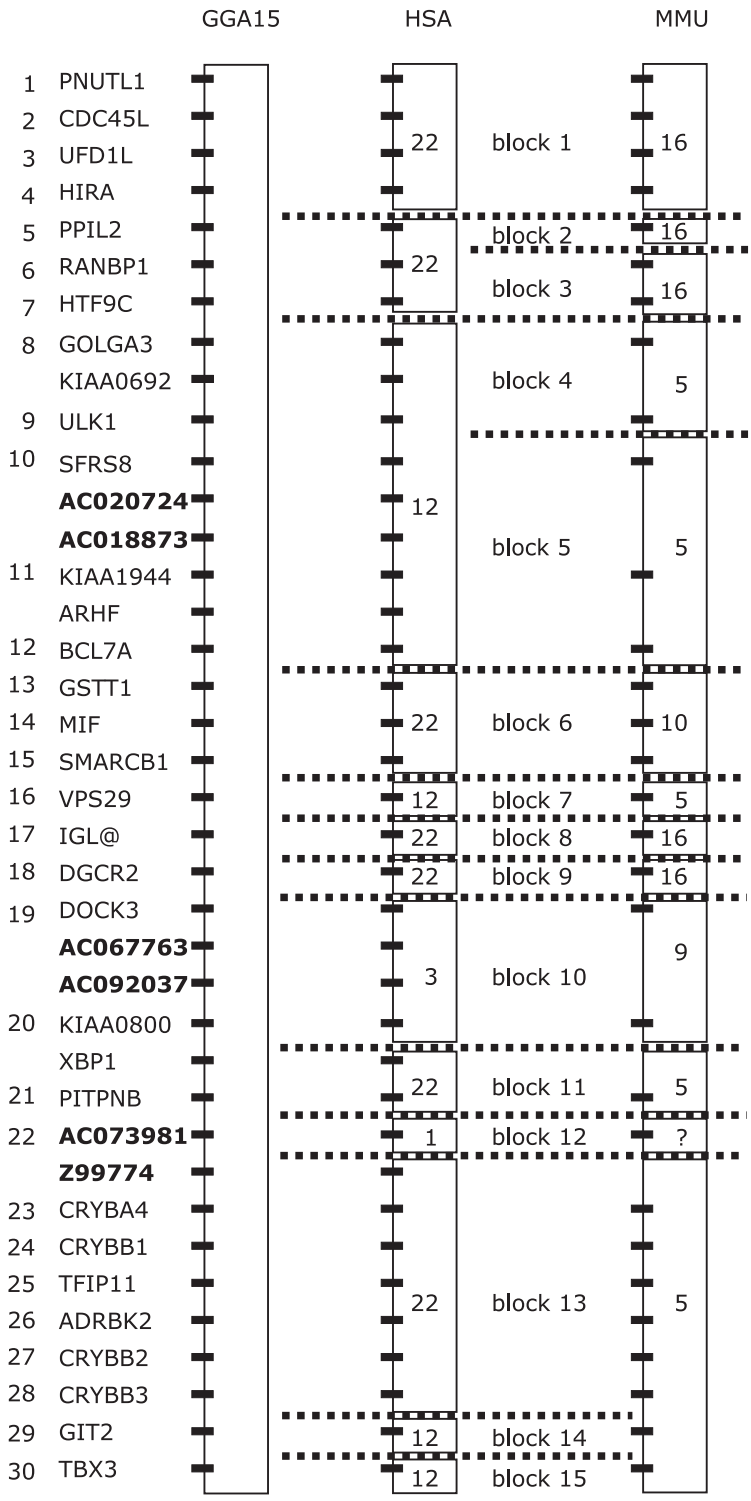


Figure 5.2 *Comparative map of chicken chromosome 15 (GGA15) to human (HSA) and mouse (MMU). A number is assigned to each gene and genomic clone (1-30), which were mapped in all three species. Chromosome segments in which the gene order in all three species is the same, are indicated by block 1-15. Positions of chromosomal rearrangements are indicated by dotted lines, with the chicken gene order as a start. The numbers of the human and mouse chromosomes are shown inside the vertical bars of HSA and MMU respectively.*

Discussion

GGA15 BAC contigs

The linkage map of GGA15 is 71 cM, which is about 1.8% of the total chicken genome (~4000 cM) (Groenen *et al*. 2000; Groenen and Crooijmans 2003). On the basis of the physical size of the chicken genome of $1.2x10^9$ bp, 1 cM on average corresponds to 300 kb. Although there are some indications that this ratio is somewhat different for macrochromosomes versus microchromosomes, the estimated physical size for GGA15 would be around 21 Mb. From the average insert size of the BACs of 134 kb (Crooijmans *et al*. 2000) and correcting for the overlap between the different BACs, we calculated that the BACs would cover around 30-35% of GGA15.

However, because no markers were identified between microsatellite marker *MCW0323* and AFLP marker *ACW0169* (distance 22 cM) to screen the Wageningen BAC library, no BAC clones could be found in this region, except for the BAC clone positive for *TBX3*. Therefore, only the first 49 cM of GGA15 (between *ADL0206* and *MCW0323*) are covered, with BAC clones in nine contigs. For this region the coverage is estimated to be almost 50%. On average, 4.8 BAC clones were obtained per marker, which is comparable to the previously reported number (Crooijmans *et al*. 2001; Buitenhuis *et al*. 2002; Jennen *et al*. 2002).

The beta crystallin gene cluster

In human, *CRYBB1* and *CRYBA4* form a gene cluster with *CRYBB2*, *CRYBB2P1*, and *CRYBB3*, the beta crystallin gene cluster. Orthologues of all human members of this gene cluster, except for the pseudogene *CRYBB2P1*, are present in mouse as a gene cluster on chromosome 5 (MMU5) (Hulsebos *et al*. 1995a, 1995b). From the comparative map, we expected the chicken orthologues of human *CRYBB2* and *CRYBB3* to form a gene cluster with chicken *CRYBB1* and *CRYBA4*. The results above confirm our expectation of the four chicken beta crystallin genes to form a gene cluster. Figure 5.3 shows that all four beta crystallin genes are located on overlapping BAC clones in the same BAC contig; *CRYBB1* and *CRYBA4* are located on BAC clones bW043G23 and bW093I01, whereas *CRYBB2* and *CRYBB3* are located on bW031O19, bW058F23 and bW070F04. In chicken, *CRYBB1* and *CRYBA4*

are linked head to head, with 2147 nt of intervening sequence (Duncan *et al*. 1995). Also in human both genes are tightly linked head to head, with 3890 bp in between (NCBI Map Viewer build30; http://www.ncbi.nlm.nih.gov/). It can be expected that chicken *CRYBB2* and *CRYBB3* are also tightly linked, because the distance between the human genes is 2235 bp.

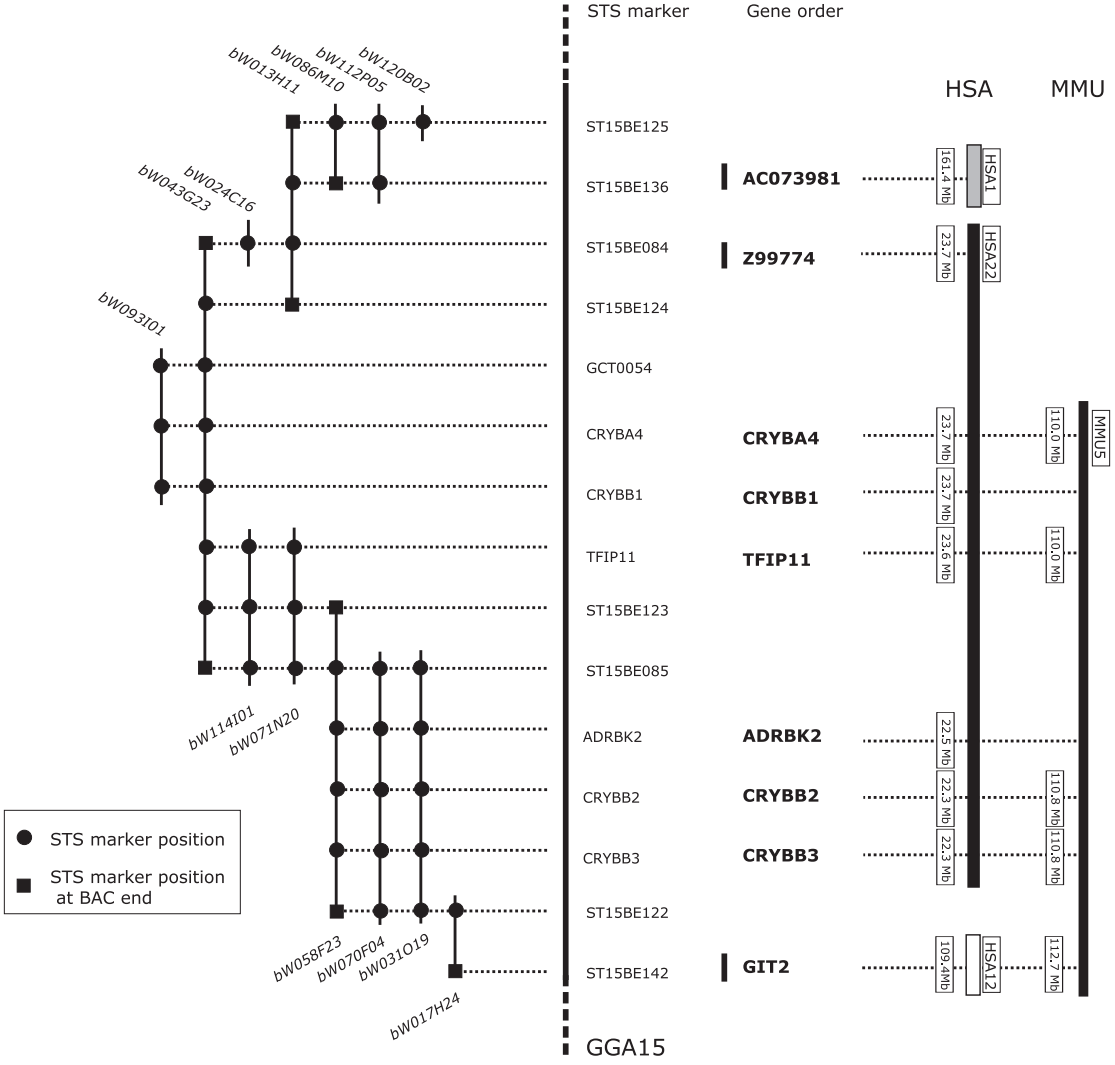


Figure 5.3 *BAC contig of GGA15 constructed around the beta crystallin gene cluster. The BAC contig with its BAC clones and STS markers is shown on the left and the comparative map with human and mouse on the right. Coloured bars give the positions of the genes and genomic clones in human and mouse. Human and mouse map positions are given in Mb.*

Furthermore, in our study the distance between *CRYBB1* and *CRYBB2* is equal to one BAC clone (~134 kb), which is about 10 times smaller than in human. This in not according to the whole chicken genome size, which is on average three times smaller than the human. Nevertheless, it could be that genome sizes differ more on specific spots.

Chicken versus human and mouse

Although, GGA15 initially showed only conservation of synteny with HSA22, our results also showed conservation of synteny with HSA12. However, from an ancestral point of view, our findings are not at all surprising. Several reports on the evolution of the mammalian genome (Murphy *et al*. 2001; Haig 1999; O'Brien and Stanyon 1999; Chowdhary *et al*. 1998) describe two ancestral chromosomes, which are both a combination of human chromosomes 12 and 22. These ancestral chromosomes were reconstructed by using chromosome paints and comparative maps of several primates, rodents, and other mammalian species. The first ancestral chromosome is a combination of HSA12p-q and HSA22qtel (12pq-22qtel) and shows conservation of synteny with segments of GGA1 (Murphy *et al*. 2001; Schmid *et al*. 2000). The second ancestral chromosome comprises HSA12qtel and HSA22q (22-12qtel) and is syntenic to GGA15 (this paper).

In order to reconstruct the common ancestor of mouse, human, and chicken, we compared the gene order in chicken with the human and mouse maps. This comparison clearly shows a large number of intra- and interchromosomal rearrangements. For example, the BAC clone bW122C13 within the contig of markers *ABR0070*, *MCW0052*, and *MCW0231* contains the gene *DGCR2* and gene cluster *IGL@* (including *IGVPS*), both located in human on HSA22q, and the gene *VPS29* which is located in human on HSA12q24 (Figure 5.1a). The presence of *DGCR2* and *IGL@* from HSA22 as well as *VPS29* from HSA12 on the same BAC clone suggests that a chromosomal breakpoint is located between *IGL@* and *VPS29*. This chromosomal breakpoint can also be found in mouse, where *IGL@* is located on MMU16 and *VPS29* is located on MMU5 (Figure 5.1b).

Another example of a chromosomal breakpoint can be seen in the BAC contig containing the beta crystallin gene cluster (Figure 5.3). Conserved blocks of genes are shown in all three species within this region. In human, the chromosomal breakpoint can be found between the genes *CRYBB3* (HSA22q11) and *GIT2* (HSA12q24). This breakpoint is absent in mouse, which is in good agreement with the mammalian ancestor 22-12qtel.

Both examples indicate that during evolution the breakpoints occurred after the separation of the mammalian and bird lineages. Moreover, the second example clearly shows that the breakpoint occurred in the human lineage after the human and mouse lineages separated, suggesting that the origin of the chromosome breaks was in human.

A more detailed comparison of the conserved chromosome segments between GGA15 and the human and mouse chromosomes is shown in Figure 5.2. The order of the conserved segments is based on a combination of genetic mapping, chromosome walking results, and sequencing. We can identify at least 15 blocks, which is the minimum number of conserved segments between GGA15 and the human and mouse chromosomes. The number of genes/genomic clones per block varies from 1 (blocks 2, 7, 8, 9, 12, 14, and 15) to 7 (block 13). The dotted lines indicate the points where chromosomal rearrangements took place. For analyzing these rearrangements, we used GRIMM. Between human and mouse the lowest multichromosomal distance (6) is calculated. The distances between chicken and human (11) and between chicken and mouse (12) are comparable. These results are in agreement with the fact that human and mouse evolved from a common ancestor and, therefore, are more closely related than either one is to chicken. This is in contrast to the findings of Burt *et al.* (1999), who looked at the whole chicken genome and concluded that the genomes of chicken and human are more alike than those of mouse and human. However, the conclusion of Burt *et al.* (1999) was based on a limited number of mapped genes and conserved segments.

Using the same approach as Burt *et al*. (1999) and assuming that the GGA15 data are representative for the whole chicken genome, we predict the total number of conserved segments in the chicken-human-mouse comparison to be at least 800 and the total number of chromosomal rearrangements to be in the same size order. Crooijmans *et al*. (2001) estimated, in the comparative mapping study between GGA10 and HSA15, the total number of conserved segments to be at least 600.

From these numbers, we estimate the rate of chromosomal change in the chicken lineage to range from 1.6 to 2 rearrangements per Myr since the divergence 300 Myr ago, which is slightly higher than the estimate of 1.5 in the human and mouse lineages (Pevzner and Tesler 2003).

The comparison between the results of Burt *et al.* (1999) and our results indicates that a higher gene density as well as the exact gene order and orientation are needed to better understand the evolutionary events that took place in the lineage leading to human, mouse, and chicken.

Acknowledgments

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CHAPTER 6

A RADIATION HYBRID MAP OF CHICKEN CHROMOSOME 15

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Summary

We have constructed a radiation hybrid (RH) map of chicken chromosome (GGA) 15. This map can be used as a resource to efficiently map genes to this chromosome. The map has been developed using a 6000 rad chicken-hamster whole-genome radiation hybrid panel (ChickRH6). In total, six microsatellite loci, 18 sequence tagged sites (STSs) from BAC end sequences, and 11 genes were typed on the panel. The initial framework map comprised eight markers and an additional 23 markers were then added to generate the final map. The total map length was 334 centiRay$_{6000}$ (cR$_{6000}$). The estimated retention frequency for the data set was 18%. Using an estimated physical length of 21 Mb, the ratio between cR$_{6000}$ and physical distance over GGA15 was estimated to be 0.063 Mb/cR$_{6000}$. The present map increases the marker density and the marker resolution on GGA15 and enables fast mapping of new chicken genes homologous to genes from human chromosomes 12 and 22.

(*Key words*: *chicken, chicken chromosome 15, framework map, radiation hybrid panel*)

Radiation hybrid (RH) mapping has proven to be an efficient way for the construction of physical maps with a resolution intermediate between genetic maps and bacterial artificial chromosome (BAC) contigs. The mapping is performed by simple polymerase chain reaction (PCR), therefore, RH markers need not to be polymorphic. Whole-genome RH panels are available for several domestic animals, e.g. cow (Womack *et al*. 1997), pig (Yerle *et al*. 1998), and horse (Kiguwa *et al*. 2000). A chicken whole-genome RH panel was created by Morisson *et al*. (2002), by using 6000 rad of gamma rays. This panel, called ChickRH6 consists of 90 hybrid clones. We report here the first application of this panel for the construction of a RH map of chicken chromosome (GGA) 15. This chromosome, containing QTL for fatness traits (Ikeobi *et al*. 2002; Jennen *et al*. 2004) has been used previously in a comparative mapping study (Jennen *et al*. 2003).

A detailed description of the microsatellite markers used for the construction of the RH map of GGA15 have been published by Groenen *et al*. (2000). Genes previously mapped on GGA15 (*CRYBB1*, *HIRA*, *PITPNB*, and *TBX3*) and STSs derived from BAC end sequences of GGA15 BAC contigs were described by Jennen *et al*. (2003). From the comparative mapping information described by Jennen *et al*. (2003) seven chicken orthologues of human genes located in human on chromosomes 12 and 22 were used in this study. Primers were designed from sequences of these chicken orthologues, available in public databases (Table 6.1). Primer pairs that gave a clear amplification product in chicken and not in the hamster DNA control were used for RH typing. Ten to 25 ng of each panel DNA in a 384-well plate was amplified in a 6 µl reaction mixture containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH=8.3), 1 mM tetra-methylammoniumchloride (TMAC), 0.1 % Triton X-100, 0.01 % gelatine, 0.2 mM of each dNTP, 0.125 U Silverstar polymerase (Eurogentec, Liège, Belgium) and 1.2 pmol of each primer. Amplification was carried out under the conditions as follows: denaturation at 95 °C for 2 min, then 35 cycles at 95 °C for 30 s, optimal annealing temperature (45 to 60 °C) for 45 s and 72 °C for 60 s. Scoring of the panel was performed as a plus-minus screening on an ethidiumbromide stained 1.5% agarose gel in 0.5X TBE. Each marker was typed in duplicate independently.

Table 6.1 Characteristics of sequence tagged site markers developed in chicken genes

Gene	Accession number		Human cytogenetic map position	PCR size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
	Chicken	Human[1]				
ACADS	BU305889	NM_000017	12q22-qter	>800[2]	CATTAGTGCATTCCTTGTTC	ATGAGCGATGTTTGTTGCAG
TBX5	AF069396	NM_000192	12q24.1	343	AAACTTCACCAGCGGAAGAG	TGGAACATGCTATGGGTGTC
TCF1	X67690	NM_000545	12q24.2	422	TGCTGCCATCCACTCATAAC	TGTCTTGGCATTTTCTGCTG
LIMK2	D26310	NM_005569	22q12.2	112	AAACTGGGTCCAGTGGATTC	CCACACATTACCTAGGACTC
NF2	AJ393948	NM_000268	22q12.2	167	GATGAGGTCTGAAGAGACAG	CCTTCTGTTCCATCAGTCGC
SERPIND1	AF061728	NM_000185	22q11.21	176	TAAAGCAGAGAACACCACCG	TGCTATTGAGTCCATTCACG
SNRPD3	AJ397202	NM_004175	22q11.23	135	TTTGGCCTTGACCAGTATGC	TGTGTAAGAGGAGTTCTGTC

[1]Accession number of human genes used in BLAST search to identify chicken orthologous genes.

[2]Fragment contains introns of unknown size.

A framework map was constructed, using the Carthagene program (Schiex and Gaspin 1997). From the previously constructed BAC contigs (Jennen *et al*. 2003) eight markers (one per BAC contig) were used to compute a 1000:1 framework map (LOD score greater than 3.0). The framework map consisted of six microsatellite loci (*MCW0031*, *MCW0226*, *LEI0120*, *ADL0039*, *MCW0231*, and *MCW0080*), one STS from BAC end sequence (*ST15BE142*), and one gene (*TBX3*) (Figure 6.1). Additional markers, that have a LOD score greater than 5.0 with at least one framework marker, were placed on the map. Four previously mapped markers, including the gene *CRYBB1* did not meet this criterion and were excluded from the map. In order to avoid inflation of the map size, we chose to project additional markers at their most likely location without altering the multipoint distance between framework markers (Figure 6.1). Twenty-three markers were consistently integrated into the framework map. Finally, the radiation hybrid map of GGA15 contained 31 markers with an average retention frequency of 18%. This is in good agreement with the retention frequencies found for microchromosomes by Morisson *et al*. (2002). The total map length was 334 centiRay$_{6000}$ (cR$_{6000}$). With an estimated physical length of GGA15 of 21 Mb (Jennen *et al*. 2003), the ratio between cR$_{6000}$ and physical distance over GGA15 was estimated to be 0.063 Mb/cR$_{6000}$.

Our data clearly show that the previous assignment of *LIMK2* to GGA2 (Groenen and Crooijmans 2003) was not correct and that the gene is located on GGA15. As a result of sequencing errors, *LIMK2* was incorrectly linked to microsatellite marker *MCW0189*, which is located on GGA2. Furthermore, six previously unmapped genes, i.e. *ACADS*, *NF2*, *SERPIND1*, *SNRPD3*, *TBX5*, and *TCF1* were also mapped to GGA15. By mapping seven new genes, we also improved the comparative map of GGA15 with human and mouse. The number of conserved segments increased to at least 19 segments in the chicken-human-mouse comparison (Figure 6.2). Using the same approach previously described (Jennen *et al*. 2003), we estimated, for the whole chicken genome, the total number of conserved segments to be at least 1000 and the rate of chromosomal change in the chicken lineage to range from 2-3 rearrangements per million years since the divergence 300 Ma. This is slightly higher than the previous estimates (Jennen *et al*. 2003).

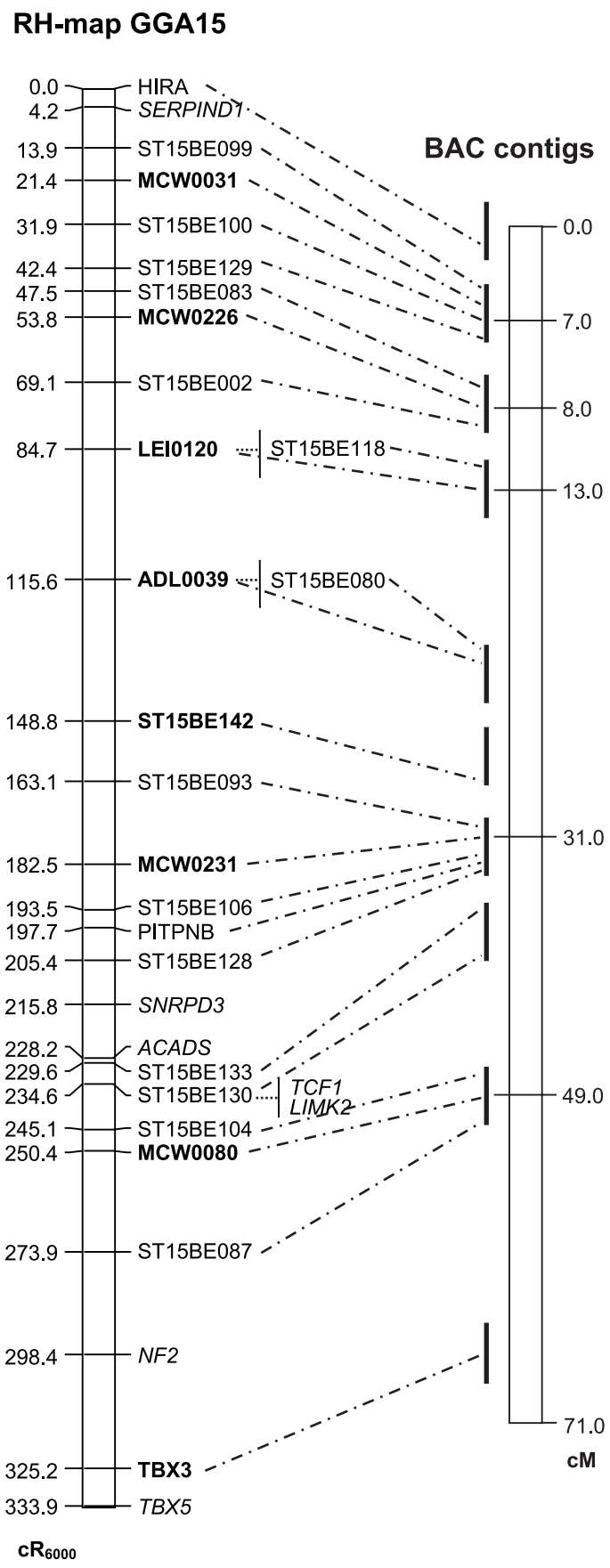

Figure 6.1 *Radiation hybrid (RH) map of chicken chromosome 15 (GGA15) comprising 31 loci. The markers of the 1000:1 framework map (LOD score greater than 3.0) are highlighted in bold. Previously unmapped genes are shown in italics.*
The RH map is aligned with the GGA15 BAC contigs (not to scale). The relative positions of the anchor loci on the GGA15 linkage map is given in cM according to Groenen and Crooijmans (2003).

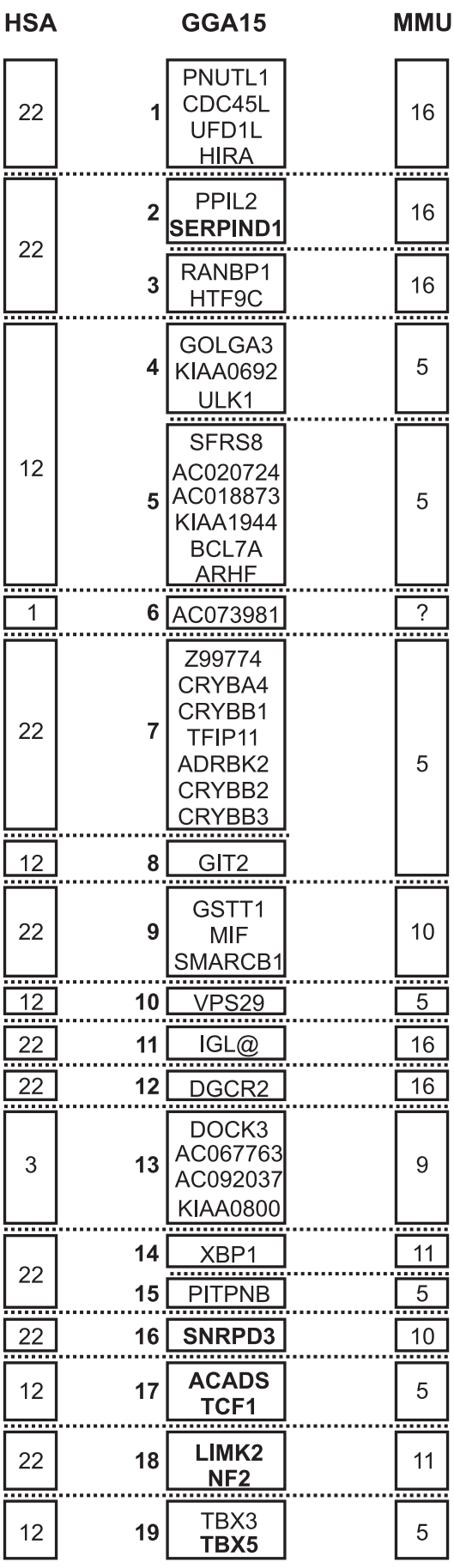

Figure 6.2 *Comparative map of chicken chromosome 15 (GGA15) to human (HSA) and mouse (MMU). Previously unmapped genes are shown in bold. Chromosome segments in which the gene order in all three species is the same, are indicated by block 1-19. Positions of chromosomal rearrangements are indicated by dotted lines, with the chicken gene order as a start. The numbers of the human and mouse chromosomes are shown inside the vertical bars of HSA and MMU respectively.*

Acknowledgments

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CHAPTER 7

IDENTIFICATION AND SNP ANALYSIS OF CANDIDATE GENES FOR FATNESS TRAITS IN CHICKEN

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Abstract

In chicken, fat deposition is an economically important trait and a high number of quantitative trait loci (QTL) have already been identified for this trait. In this study candidate genes were identified within three QTL regions on GGA1, GGA15, and GGA27, and single nucleotide polymorphisms (SNPs) were identified within these genes. In total, 29 chicken genes were analysed. Sequence tagged site (STS) markers were developed for these genes, followed by the identification of SNPs by a direct sequencing approach of the polymerase chain reaction (PCR) amplification products from a panel of eight individuals. The panel consisted of four parental pairs of the families that have been shown to be segregating for the fatness QTL. Subsequently, the SNPs were typed in eight offspring of these families. Amplification products from 20 candidate genes were sequenced and 94 SNPs were identified in 13,379 bp of consensus sequence, an average of one SNP in every 142 bp. Twelve SNPs were found within coding sequences of six candidate genes (*TEF*, *PPARA*, *TCF1*, *GNB3*, *LRP1*, and *BRD1*). The two SNPs within the exon of *BRD1* were non-synonymous and resulted in a *Leu*-to-*Arg* (L-to-R) and *Gln*-to-*Glu* (Q-to-E) substitution. By using the program SIFT (sorts intolerant from tolerant) the amino acid substitution of L-to-R was predicted to be deleterious, thus, affecting the protein function.

Introduction

Genetic selection in meat-type chickens has provided the industry with flocks which reach the target slaughter weight in an ever shorter period of time. However, this fast growth has been accompanied by an increased fat deposition. In chicken, this excessive fat depresses feed efficiency, has no commercial value, and is less appreciated by consumers. Therefore, considerable research effort has been applied to study factors associated with fat deposition and methods to reduce it.

Based on the increased knowledge of the fat metabolism and its regulation in chicken (Hillgartner *et al*. 1995; Richards 2003), candidate genes controlling fatness in chicken can be identified. Examples of genes that have been examined in poultry include apolipoprotein A1, fatty acid synthetase, malic enzyme, stearoyl-CoA desaturase 1, ATP citrate lyase, and transcription factor steroid regulatory element binding protein 1 (Douaire *et al*. 1992; Sourdioux *et al*. 1996; Sourdioux *et al*. 1999; Daval *et al*. 2000a; Lagarrigue *et al*. 2000; Assaf *et al*. 2003).

Although, a large number of genes involved in fat metabolism in chicken has already been identified and studied, many remain that have not yet been investigated in relation to this trait or that have not yet been identified in the chicken genome. To further identify genes involved in fat deposition in chicken several studies have been performed aimed at the localization of quantitative trait loci (QTL) for this trait (Tatsuda and Fujinaka 2001; Ikeobi *et al*. 2002; McElroy *et al*. 2002; Pitel *et al*. 2002). In a previous study we have identified and confirmed several regions containing QTL for fat deposition (on chromosomes 1, 3, 4, 15, 18, and 27) in a cross between two genetically divergent outcross broiler dam lines originating from the White Plymouth Rock breed (Jennen *et al*. 2004b,c). Currently the number of genes mapped on the chicken genome is very limited, approximately 400 (Schmid *et al*. 2000), which makes the direct identification of potential candidate genes mapped in these regions not very succesful. Comparative gene mapping, however, provides a solution by identifying homologous chromosomal regions in other information-rich species. Because significantly more is known about gene location, structure and function in human and mouse, potential candidate genes are to be found within the regions of interest.

The objectives of this study were to identify candidate genes in the QTL regions on GGA1, GGA15, and GGA27, and to detect single nucleotide polymorphisms (SNPs) within these genes.

Material and methods

Candidate gene selection

Candidate genes were selected based on their map position and their known function in fat metabolism or adipose tissue development. Cloned genes identified as having a major effect on fatness in other species are particularly strong candidates. Candidate genes were derived from literature, e.g. "The Human Obesity Gene Map: The 2002 Update" (Chagnon *et al*. 2003) and by using the web-based data mining tool GeneSeeker at http://www.cmbi.kun.nl/GeneSeeker/ (accessed June 2002) (Van Driel *et al*. 2003) with the keywords "fat" and "adipose". Chicken orthologues of 29 human genes were identified using a BLAST search with the mRNA sequences of the human genes (Table 7.1).

STS markers

Primers corresponding to the candidate genes were designed based on publically available chicken sequences (mRNA, EST, or genomic DNA) (Table 7.2) using Primer3 (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi) (Rozen and Skaletsky 2000). Primers were designed in such a way that the PCR amplification products were at least 200 bp in size. Preferably primers were selected within one exon. However, if the exons were to small (<200 bp) also introns were included. The exon-intron boundries were identified by aligning the chicken sequences to the human genomic sequences.

Table 7.1 Candidate genes for fatness in chicken

Official gene symbol and name[1]		Accession number		Map position			Mapping references
		Chicken	Human	Chicken	Human	Mouse	
HSD3B1	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	D43762	NM_000862	1	1p13.1	3 (49.1 cM)	Smith *et al*. 1997
PPARG	peroxisome proliferative activated receptor gamma	AF163811	NM_005037	1	3p25	6 (52.7 cM)	this study
GAPD	glyceraldehyde-3-phosphate dehydrogenase	M11213	NM_002046	1	12p13	6 (56 cM)	Burt 1994
LDHB	lactate dehydrogenase B	AF069771	NM_002300	1	12p12.2-p12.1	6 (62 cM)	Schmid *et al*. 2000
HOXC6	homeo box C6	X80114	NM_004503	1	12q12-q13	15 (57.4 cM)	Ladjali-Mohammedi *et al*. 2001
HMGIC	high mobility group I-C	AF058287	NM_003483	1	12q15	10 (67.5 cM)	Ruyter-Spira *et al*. 1998
IGF1	insulin like growth factor 1	M32791 & M74176	NM_000618	1	12q22-q23	10 (48 cM)	Klein *et al*. 1996
TEF	thyrotrophic embryonic factor	U09221	NM_003216	1	22q13.2	15 (46.7 cM)	this study
PDGFB	platelet-derived growth factor beta polypeptide	AB031025	NM_002608	1	22q13.1	15 (56.8 cM)	this study
PPARA	peroxisome proliferative activated receptor alpha	AF163809	NM_005036	1	22q13.31	15 (48.8 cM)	Schmid *et al*. 2000
VDR	vitamin D receptor	AF011356	NM_000376	4	12q12-q14	5 (56 cM)	this study
PITPNB	phosphotidylinositol transfer protein beta	AI979795	NM_012399	15	22q12.1	5	Jennen *et al*. 2004a
XBP1	X-box binding protein 1	AJ394086	NM_005080	15	22q12.1	11	Jennen *et al*. 2003
IAPP	islet amyloid polypeptide	L16955	NM_000415	15	12p12.3-p12.1	6 (62 cM)	this study
ACADS	acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain	BU305889	NM_000017	15	12q22-qter	5 (65 cM)	Jennen *et al*. 2004a
PLA2G1B	phospholipase A2, group IB, pancreas	BU488507	NM_000928	15	12q23-q24.1	5	this study
TBX3	T-box 3	AF033669	NM_016569	15	12q24.1	5 (65 cM)	Jennen *et al*. 2003
TBX5	T-box 5	AF069396	NM_000192	15	12q24.1	5 (65 cM)	Jennen *et al*. 2004a
TCF1	transcription factor 1, hepatic	X67690	NM_000545	15	12q24.2	5 (65 cM)	Jennen *et al*. 2004a
SOX9	SRY (sex determining region Y)-box 9	AB012236	NM_000346	18	17q24.3-q25.1	11 (69.5 cM)	this study
ACLY	ATP citrate lyase	AJ245664	NM_001096	27	17q21-q22	11	Daval *et al*. 2000b
GH	Growth hormone	D10484	NM_000515	27	17q24.2	11 (65 cM)	Liu *et al*. 2001
GNB3	guanine nucleotide binding protein, beta polypeptide 3	BU332711	NM_002075	unknown	12p13	6 (60.2 cM)	
LRP1	low density lipoprotein-related protein 1	X74904	NM_002332	unknown	12q13-q14	15	
SCARB1	scavenger receptor class B, member 1	BU363175	NM_005505	unknown	12q24.32	5 (68 cM)	
HSD17B1	hydroxysteroid (17-beta) dehydrogenase 1	AB002410	NM_000413	unknown	17q11-q21	11 (60.25 cM)	
ACE	angiotensin 1 converting enzyme 1	L40175	NM_000789	unknown	17q23	11 (65 cM)	
BRD1	bromodomain containing 1	BU268302	NM_014577	unknown	22q13.33	15	
SMTN	smoothelin	AF272975	NM_006932	unknown	22q12.2	11	

[1]Official gene symbol and name according to the HUGO Gene Nomenclature Committee (HGNC) (Wain *et al*. 2002)

(http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl).

Table 7.2 SNPs identified in the Wageningen broiler/broiler cross by direct sequencing of PCR products

Gene	Accession number	Bases screened	No. of SNPs	SNP position[1] and substitution[2]	PCR primers Forward primer (5'-3')	Reverse primer (5'-3')
HSD3B1	D43762	286	1	183 C/T	ATATTGCAGGACAGCCTTGG	GCCTCATGAAGCAAGGTTTG
PPARG	AF163811	237	1	214 A/C	TGCTTGCTTCACTGAGGTTG	GTATGCAAAGTCCTCGTTAC
GAPD	M11213	662	8	112 A/G; 165 C/T; 199 G/T; 203 A/G; 314 A/G; 335 A/G; 390 C/T; 391 A/G	TGTGACTTCAATGGTGACAG	CAGATCAGTTTCTATCAGCC
HOXC6	X80114	245	4	41 A/C; 55 A/G; 65 C/G; 146 C/G	ATGTTGGGTGCTGTGTGAAG	GGTAGCATTCTCCTCAATGG
IGF1	M74176	832	0	nil	CAAGCAGTGCTCAACAACTC	CAAGAAATCACAAAAGCAGCAC
TEF	U09221	931	15	**126 C/T**; 355 C/T; 361 G/T; 369 A/C; 370 A/G; 466 A/G; 467 G/T; 477 G/T; 493 C/T; 581 C/T; 624 A/C/G; 705 A/G; 711 A/G; 712 A/G; 716 C/T	CACCGCAAAACGAGAGAAAT	CCGTATTCTCTTTCTCAAGG
PDGFB	AB031025	1153	4	717 A/G; 740 C/T; 949 A/G; 980 G/T	GCCAGCACAGAAGAGAAAAC	TTCGGTAGGAGAAGGTCCAC
PPARA	AF163809	227	3	**82 A/T**; **84 A/G**; **106 C/T**	TTGTGCATGGCAGAGAAGAC	TCCATAGGCTACCAGCATCC
PITPNB	AI979795	351	4	116 A/G; 125 A/G; 230 A/G; 235 C/T	GAAGCTAGCAAAAATGAAAC	GCAATCATTCTTACAAAAGG
XBP1	AJ394086	1251	8	192 C/T; 195 G/T; 341 A/G; 351 A/G; **433 C/T**; 672 C/T; 956 C/G; 959 C/G	CTCCTTTTTCCGAGTGATGC	GAATCTGAAGAGTCACTGCC
IAPP	L16955	346	0	nil	TCACCAACTGGAGAAACGGA	GACAAGCAGACTTCTGGAGTTC
PLA2G1B	BU488507	973	9	414 C/T; 418 C/T; 575 A/G; 606 A/G; 607 C/T; 715 C/T; 720 A/C; 759 G/T; 763 A/G	GCTGCCATCGCTGCTATCTC	TGCTACACGTGATCTCCTCA
TBX3	AF033669	507	0	nil	CATGTACTGTGCTGTTTAGAG	TCTCCGTGCCTCTTTTATGG
TCF1sts1	X67690	534	2	**18 C/T**; **78 A/G**	TGACTTCAACCCAGCCCCAG	TGGTGGAGGCTGTTACCTGT
TCF1sts2		381	0	nil	TGCTGCCATCCACTCATAAC	TGTCTTGGCATTTTCTGGCTG
ACLY	AJ245664	647	7	386 A/T; 391 C/T; 407 C/T; 411 C/G; 416 A/G; 509 C/T; 527 C/T	GTGACCACAGGCAGAAGTTC	GTTGCAGGCCCAATGATAGT
GH	D10484	1122	17	20 A/G; 31 A/G; 44 A/C; 52 C/G; 331 C/T; 634 A/G; 684 G/T; 739 C/T; 750 C/T; 778 A/C; 817 G/T; 866 A/G; 887 A/G; 931 A/T; 947 G/T; 955 A/C; 1007 C/T	CTAAAGGACCTGGAAGAAGGG	AACTTGTCGTAGGTGGGTCTG
GNB3	BU332711	1138	3	819 A/T; 903 A/G; **998 C/T**	CTGCACTGGCTCAGATGATG	CGTTCTGCTTTCAGGGAGTC
LRP1	X74904	110	1	**39 C/T**	ATATTGCGGACGACAACAAG	TGTGCCAGTTGGTCCAGTAG
BRD1	BU268302	339	2	**17 G/T**; **316 C/G**	GCATGGATGGTGAATGTCAG	CAGTTTGCTTTGTGGCACTG
SMTN	AF272975	1107	5	282 A/G; 626 A/T; 808 A/G; **894 A/G**; 945 A/G	CAGCGGGAGAAGGAGCGGGA	ATGCTGCCTGTCTTCTTGGA
	Total=	13379	94			

[1]Position of SNPs relative to the first nucleotide after the forward PCR primer.

[2]All SNPs are bi-allelic, with the exception of the *TEF* SNP which is underlined. SNPs within exons are highlighted in bold

Screening for SNPs

The gene specific STS markers were used for the identification and subsequently typing of SNPs. The SNPs were first identified in a panel of eight individuals. The panel consisted of four parental pairs of the families that have been shown to be segregating for the fatness QTL (Jennen *et al*. 2004b). The SNPs were typed in eight G_2 offspring per selected family using a direct sequencing approach. PCR amplification was performed as previously described (Crooijmans *et al*. 2000). Before sequencing, the PCR amplification products were purified on a P-100 Biogel column (Bio-Rad Laboratories, Hercules, CA, USA). The quantities of the purified amplification products were measured on a 1.5% EtBr stained agarose gel with a SmartLadder (Eurogentec, Seraing, Belgium). Sequence reactions of the amplification products (100 ng) were performed with the forward or the reverse PCR primer (0.8 pmol/µl). Before analysis of the sequences, sequence reactions were purified on Sephadex G-50 Superfine columns (Amersham Pharmacia Biotech, Dübendorf, Switzerland) in MAHVN 4510 plates (Millipore, Molsheim, France) according to the Millipore protocol. Sequencing conditions and sequence analysis were performed on an automated sequencer ABI377 (Applied Biosystems Inc. Foster City, CA, USA) as described (Crooijmans *et al*. 2001). All sequences obtained were analysed with the PREGAP4 program of the STADEN software package before analysis with the GAP4 program to identify SNPs (http://www.mrc-lmb.cam.ac.uk/pubseq) (Bonfield *et al*. 1995).

Mapping of genes

Candidate genes were mapped on one to three families of the Wageningen mapping population (Groenen *et al*. 1998) by SNP typing. Per family the two parents and eight offspring were used. Recombination fractions and LOD scores were calculated for each pair of loci using Cri-MAP v2.4 (Green *et al*. 1990).

In addition, candidate genes were mapped to BACs that had already been positioned within known BAC contigs. Towards this end, the Wageningen chicken BAC library was screened by PCR (Crooijmans *et al*. 2000) using the STS markers.

Results and discussion

Screening for SNPs

Candidate genes were identified within three QTL regions on GGA1 (143-248 cM), GGA15 (7-49 cM), and GGA27 (32-47 cM). Map positions of the QTL regions are based on the consensus linkage map (Groenen *et al*. 2000). The regions on GGA1 and GGA15 show conservation of synteny with segments of HSA12 and HSA22, whereas GGA27 shows conservation of synteny with HSA14q11-12 and HSA17q21-24 (Schmid *et al*. 2000; Jennen *et al*. 2003). Chicken orthologues of 29 human genes were identified using a BLAST search (sequence identity >80%; E values $<1e^{-5}$). The majority of the human genes are located on HSA12 (15 genes), HSA17 (5 genes), or HSA22 (7 genes) (Table 7.1). STS markers were developed for these chicken genes. For 20 chicken genes (Table 7.2) the PCR amplification was successful and the amplification products were used to screen for SNPs and to identify BAC clones containing these genes. Twenty-one PCR fragments were produced of which three are located within the 3' UTR region of the candidate gene (*HSD3B1*, *HOXC6*, and *PPARG*) and eight are located within a single exon (*IGF1*, *PPARA*, *IAPP*, *TBX3*, *TCF1sts1*, *TCF1sts2*, *LRP1*, and *BRD1*). The remaining PCR fragments contain introns as well.

The number of SNPs identified within one fragment ranges from 0 to 17. In total 94 SNPs were identified in the 13,379 bases analysed, equating a frequency of approximately one SNP per 142 bp. This frequency is lower as found in the work of Smith *et al*. (2002), who recorded in commercial broiler and layer populations 47 SNPs in a survey of some 6,000 bp of chicken sequence (i.e. one SNP per 128 bp). However, a direct comparison between the results of these studies is only valid, when both studies would use a panel of diploid individuals of equal size. In the study of Smith *et al*. (2002) 10 animals were used for SNP discovery, whereas in this study only eight animals were used. As the number of SNPs to be observed is strongly dependent on the number of individuals sampled, these numbers should be normalized for the assayed sample size (Cargill *et al*. 1999; Jungerius *et al*. 2003). Normalization resulted in one SNP per 473 bp for this study and one SNP per 453 bp for the study of Smith *et al*. (2002).

All the SNPs detected in the present study were substitutions. Of the 94 SNPs detected, one SNP was tri-allelic A/C/G, whereas the rest was bi-allelic (Table 7.2). The most frequent polymorphisms were A/G or C/T transitions (~ 66%).

Two chicken genes (*GH* and *IGF1*) used in the present study were also typed for SNPs in other studies (Schmid *et al*. 2000; Amills *et al*. 2003). A direct sequencing approach of PCR amplified DNA was applied to the Roslin broiler/layer cross enabling the identification of nine SNPs in the chicken growth hormone (GH) gene (Schmid *et al*. 2000). In the present study eight of these SNPs were also detected, as well as nine additional ones. The SNPs were detected using the same STS marker and approach. In the study of Amills *et al*. (2003) one SNP (A/C substitution) was detected in the promotor region of the IGF1 gene (position 570 of the sequence with accession number M74176). This SNP was not segregating in our cross.

Mapping of genes

Candidate genes were mapped on the chicken linkage map using CriMAP v2.4 (Green *et al*. 1990). Because of the limited number of animals used (eight per family) for SNP typing, LOD scores are low. Therefore, only the chicken orthologues of *PLA2G1B*, *TEF*, and *PDGFB* were mapped on the chicken linkage map. *PLA2G1B* was mapped close to microsatellite marker *MCW0052* (recombination fraction = 0; LOD score = 3.31) located on GGA15 and both *TEF* and *PDGFB* were mapped close to microsatellite marker *ADL0234* (recombination fraction = 0; LOD score = 2.11) located on GGA1. The map location of *TEF* and *PDGFB* on GGA1 is confirmed, because both genes were mapped to BAC clones that were already known to be located within BAC contigs on GGA1. Furthermore, the chicken orthologues of *PPARG*, *VDR*, *IAPP*, and *SOX9* were also mapped to BAC clones known to be located within BAC contigs on GGA1, GGA4, GGA15, and GGA18, respectively. *VDR* and *SOX9* are mapped within the fatness QTL regions located on GGA4 and GGA18 (Jennen *et al*. 2004b), respectively. Therefore, they are interesting candidate genes for the QTL identified in these particular regions.

Effect of SNPs

Most of the identified SNPs (~87%) are located within non-coding regions of the candidate genes. However, 12 SNPs were found within coding sequences of six candidate genes (*TEF*, *PPARA*, *TCF1*, *GNB3*, *LRP1*, and *BRD1*). Ten of these SNPs are synonymous, i.e. do not result in an amino acid change. On the other hand, the two SNPs within the exon of *BRD1* are non-synonymous and resulted in a *Leu*-to-*Arg* (L-to-R) and *Gln*-to-*Glu* (Q-to-E) substitution. Non-synonymous SNPs are of interest due to their potential effect on protein function and, ultimately the phenotype. The effect on protein function of the substitution of the two amino acids in chicken *BRD1* was predicted using SIFT (http://blocks.fhcrc.org/sift/SIFT.html) (Ng and Henikoff 2001, 2002, 2003). SIFT is a sequence homology-based tool that sorts intolerant from tolerant substitutions and classifies substitutions as tolerated or deleterious (probability <0.05) (Ng and Henikoff 2001, 2002, 2003). Given the amino acid sequence of the translated PCR fragment of *BRD1*, SIFT searched for homologous protein sequences and calculated probabilities for each possible amino acid change (data not shown). This resulted in the prediction of the substitution of L-to-R to be deleterious, thus, affecting the protein function. The Q-to-E substitution was tolerated. At present, it is unclear where *BRD1* mappes on the chicken genome and how it is related to fat deposition in chicken.

Application of SNPs

Although not all SNPs have a direct effect on protein function, the identified SNPs can be used as genetic markers in mapping studies. In contrast to the highly polymorphic microsatellite markers, SNPs are less informative due to their bi-allelic nature. However, a cluster of SNPs within a gene can be evaluated to determine molecular haplotypes that can be used for genotyping (Emara and Kim 2003). Johnson *et al*. (2001) showed that 2-5 SNPs, refered to as haplotype tag SNPs or htSNPs, can be used to define the common haplotypes observed at each locus. In this study, several good examples of htSNPs within a gene can be found, for example, the genes *PLA2G1B* and *TEF*. For both genes haplotypes were estimated using Merlin (Abecasis *et al*. 2002). Within *PLA2G1B* nine SNPs were identified, which

account for three haplotypes (Figure 7.1a). These haplotypes can be identified by typing only two of the nine SNPs. For *TEF* five haplotypes were estimated, which can be identified by typing three of the 15 SNPs (Figure 7.1b). Once identified, the next step will be to test the polymorphisms in the candidate genes for an effect on the fatness traits.

(A) *PLA2G1B*

haplotype	SNP 1	SNP 2	SNP 3	SNP 4	SNP 5	SNP 6	SNP 7	SNP 8	SNP 9
1	C	T	G	G	C	C	C	T	A
2	T	C	A	A	T	T	A	G	G
3	T	C	G	A	C	C	A	G	G

(B) *TEF*

haplotype	SNP 1	SNP 2	SNP 3	SNP 4	SNP 5	SNP 6	SNP 7	SNP 8	SNP 9	SNP 10	SNP 11	SNP 12	SNP 13	SNP 14	SNP 15
1	G	C	G	T	T	G	G	A	A	C	T	G	A	G	G
2	G	C	G	C	T	A	G	A	C	C	C	T	C	G	G
3	A	C	A	C	T	G	A	C	C	T	C	T	C	A	G
4	G	T	A	C	C	G	G	A	C	C	C	T	C	A	A
5	G	T	A	C	G	G	G	A	C	C	C	T	C	G	G

Figure 7.1 *Polymorphisms detected and haplotypes within the genes (A) PLA2G1B and (B) TEF for eight animals. Per gene the haplotype tag SNPs (htSNPs; boxed) describe all of the common haplotypes observed.*

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CHAPTER 8

GENERAL DISCUSSION

Genetic improvement of domesticated animal species, by selection on economically important quantitative traits, such as yield and product quality, has been carried out very successfully for several decades. In chicken several strategies of selection for leanness in meat production have been described (Leclercq and Whitehead 1988) and selection has reached a limit of about 10% fat (Griffin 1996). Until recently, genetic improvement has been accomplished by selection on phenotype, with little knowledge of the genetic nature of the selected traits. Such selection is, therefore, limited by the accuracy and availability of phenotypic information. In chicken it is still not possible to measure fat easily. The birds have to be killed for the measurements of fatness, which is laborious and expensive. Most recently, however, tools have become available to elucidate the genetics that underlies complex quantitative traits through the identification of chromosomal regions that harbor quantitative trait loci (QTL) or preferably the causal genes. This has opened the door for the use of molecular genetics to enhance breeding programs (Andersson 2001).

This thesis concerns the identification of genes controlling fat deposition in broilers. The strategy used to identify these genes, the so-called positional candidate gene approach (Collins 1995), consists of four steps: (1) identification and localization of QTL; (2) construction of high resolution and comparative maps; (3) prediction of candidate genes from these maps; (4) establishing a causal link between the genetic trait and the candidate genes from functional evidence. In this chapter these four steps will be discussed. Furthermore, the main conclusions of this thesis and future directions are presented.

QTL studies

Over the last decade, enormous effort has been applied to identify and localize QTL involved in fatness and growth traits in chicken. The results of six studies for fatness traits have been summarized in Table 8.1. In total 17 chromosomes were identified, 14 of which also contained QTL for body weight (BW) (Table 8.2). Significant QTL for multiple fat traits (abdominal and skin fat) were found on GGA1, GGA3, GGA5, GGA7, GGA13, GGA15, and GGA28. On these chromosomes, except on GGA28, significant evidence was also found for BW.

Table 8.1 Genomic regions with QTL for fatness traits in chicken

Chr	Pos (cM)[1]	Sig[2]	Phenotype[3]	Age	Crosses[4]		References
1	33-54	†	AFW	10 wk	WPR x WPR	F3	Chapter 2
		†	AF%	10 wk			
	133-151	*	AF	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
	151-162	†	AFW	7 wk	WPR x WPR	F9	Chapter 3
		†	AF%	7 wk			
	205-225	†	AFW	10 wk	WPR x WPR	F3	Chapter 2
	205-241	*	AFW	7 wk	WPR x WPR	F9	Chapter 3
		†	AF%	7 wk			
	205-248	†	IF%	7 wk	WPR x WPR	F9	Chapter 3
	241-248	*	AF%	10 wk	WPR x WPR	F3	Chapter 2
	443-527	†	SF	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
	521-565	†	AF%	9 wk	WPR x WPR	F3	Chapter 2
2	69	*	FW		CB x CB	F2	McElroy *et al*. 2002
	226-270	†	SFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
		†	SF	9 wk			
	320-350	†	AF%	7 wk	WPR x WPR	F3	Chapter 2
	360-474	†	FD	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
3	32-51	*	AFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
		†	AF	9 wk			
	153-225	*	SFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
		*	SF	9 wk			
	314-316	†	AFW	7 wk	WPR x WPR	F9	Chapter 3
		†	AF%	7 wk			
	316-317	†	AF%	7 wk	WPR x WPR	F3	Chapter 2
4	132-153	†	AFW	7 wk	WPR x WPR	F3	Chapter 2
	138-243	†	SFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
	182	†	AFW	9 wk	WPR x WPR	F3	Chapter 2
		†	AF%	9 wk			
	217-227	†	AFW	7 wk	WPR x WPR	F3	Chapter 2
5	50	†	FW		CB x CB	F2	McElroy *et al*. 2002
	25-79	†	SF	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
	73	*	AFW		h-AFC x l-AFC	F2	Pitel *et al*. 2002
	79-83	**	AFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
		**	AF	9 wk			
		†	SFW	9 wk			
		*	FD	9 wk			
6	91-113	†	SFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002

Table 8.1 (continued)

Chr	Pos (cM)[1]	Sig[2]	Phenotype[3]	Age	Crosses[4]		References
7	0-101	**	AFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
		**	AF	9 wk			
		*	SFW	9 wk			
		**	FD	9 wk			
	127-129	†	AFW	10 wk	WPR x WPR	F3	Chapter 2
		*	AF%	16 wk	S x WPR	F2	Tatsuda & Fujinaka 2001a
9	61-128	†	AF	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
11	22-54	†	AFW	7 wk	WPR x WPR	F3	Chapter 2
13	32-70	†	AFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
		*	SFW	9 wk			
		†	SF	9 wk			
	67-74	†	AFW	7 wk	WPR x WPR	F3	Chapter 2
15	0-49	*	AFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
		†	AF	9 wk			
		*	FD	9 wk			
	7-8	†	AF%	7 wk	WPR x WPR	F3	Chapter 2
	13-31	†	AFW	10 wk	WPR x WPR	F3	Chapter 2
		†	AF%	10 wk			
		†	AF%	9 wk			
18	7-24	†	AFW	10 wk	WPR x WPR	F3	Chapter 2
		†	AF%	10 wk			
23	1	†	FW		CB x CB	F2	McElroy *et al*. 2002
24	30-48	†	IF%	7 wk	WPR x WPR	F9	Chapter 3
27	19	†	FW		CB x CB	F2	McElroy *et al*. 2002
	32-36	†	AF%	9 wk	WPR x WPR	F3	Chapter 2
	36-59	†	AF%	7 wk	WPR x WPR	F2	Chapter 3
	47-59	†	IF%	7 wk	WPR x WPR	F9	Chapter 3
28	21-60	*	AFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
		*	AF	9 wk			
		†	SFW	9 wk			
		*	SF	9 wk			
Z	118-165	†	AFW	9 wk	WL x CB	F2	Ikeobi *et al*. 2002
		†	AF	9 wk			

[1]Estimated chromosomal position of QTL flanking markers based on concensus linkage map (Groenen *et al*. 2000).

[2]Significance level: **significant linkage at $p < 0.01$; *significant linkage at $p < 0.05$; [†]suggestive linkage.

[3]AF=abdominal fatness; AFW=abdominal fat weight; AF%=percentage abdominal fat; IF%=percentage intramuscular fat; FD=fat distribution; FW=fat weight; SF=skin fatness; SFW=skin fat weight.

[4]CB=commercial broiler; h-/l-AFC=high/low abdominal fat content; S=Satsumadori; WL=White Leghorn layer; WPR=White Plymouth Rock broiler.

Table 8.2 Genomic regions with QTL for body weight in chicken[1]

Chr	Pos (cM)	Sig	Phenotype[2]	Age	Crosses		References[3]
1	72-122	*	BW	8 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
		*	BW	46 d			
		*	BW	112 d			
		*	BW	200 d			
	72-133	†	BW	9 wk	WL x CB	F2	Sewalem *et al*. 2002
	151-169	*	BW	3 wk	WL x CB	F2	Sewalem *et al*. 2002
	169-205	*	BW	6 wk	WL x CB	F2	Sewalem *et al*. 2002
	179-205	*	BW	5 wk	WPR x WPR	F9	Chapter 3
	205-241	†	BW	7 wk	WPR x WPR	F9	Chapter 3
	205-242	†	BW	13 wk	S x WPR	F2	Tatsuda & Fujinaka 2001b
		*	BW	16 wk			
	240	†	BW	48 d	WPR x WPR	F3	Van Kaam *et al*. 1998
	241-242	†	BW	48 d	WPR x WPR	F3	Van Kaam *et al*. 1999
	386	*	BW	1 d	RJ x WL	F2	Carlborg *et al*. 2003
	424-527	†	BW	8 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
		*	BW	46 d			
		*	BW	112 d			
		*	BW	200 d			
	426-443	*	BW	9 wk	WL x CB	F2	Sewalem *et al*. 2002
	443-527	*	BW	3 wk	WL x CB	F2	Sewalem *et al*. 2002
		*	BW	6 wk			
	527	†	BW	8 d	RJ x WL	F2	Carlborg *et al*. 2003
2	0-77	†	BW	112 d	RJ x WL	F2	Carlborg *et al*. 2003
	2-60	*	BW	16 wk	S x WPR	F2	Tatsuda & Fujinaka 2001b
	60-119cM	*	BW	13 wk	S x WPR	F2	Tatsuda & Fujinaka 2001b
	172	*	BW	8 d	RJ x WL	F2	Carlborg *et al*. 2003
	235-252	†	BW	46 d	RJ x WL	F2	Carlborg *et al*. 2003
		†	BW	112 d			
	247	*	prBW		CB x CB	F2	McElroy *et al*. 2002
	282-302	†	BW	7 wk	WPR x WPR	F3	Chapter 2
	292-302	**	BW	6 wk	WL x CB	F2	Sewalem *et al*. 2002
		**	BW	9 wk			
	384-452	†	BW	46 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
		*	BW	200 d			
3	31	*	poBW		CB x CB	F2	McElroy *et al*. 2002
	154	*	poBW		CB x CB	F2	McElroy *et al*. 2002
	314-316	†	BW	7 wk	WPR x WPR	F9	Chapter 3
4	37	**	BW		CB x CB	F3	De Koning *et al*. 2003
	74	†	BW	48 d	WPR x WPR	F3	Van Kaam *et al*. 1998
	138-153	*	BW	112 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
	138-243	†	BW	3 wk	WL x CB	F2	Sewalem *et al*. 2002
		**	BW	6 wk			
		**	BW	9 wk			
	200-207	**	BW	40 wk	RIR x RIR	F2	Tuiskula-Haavisto *et al*. 2002
5	32-71	†	BW	112 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
		*	BW	200 d			
	50	†	prBW		CB x CB	F2	McElroy *et al*. 2002
		†	poBW				
	25-79	†	BW	3 wk	WL x CB	F2	Sewalem *et al*. 2002
6	58-91	†	BW	6 wk	WL x CB	F2	Sewalem *et al*. 2002
		†	BW	9 wk			
	59	†	BW	46 d	RJ x WL	F2	Carlborg *et al*. 2003
		†	BW	112 d			
7	0-77	*	BW	112 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
		†	BW	200 d			
	0-101	**	BW	3 wk	WL x CB	F2	Sewalem *et al*. 2002
		*	BW	6 wk			
		†	BW	9 wk			
	109-165	†	BW	8 d	RJ x WL	F2	Carlborg *et al*. 2003

Table 8.2 (continued)

Chr	Pos (cM)	Sig	Phenotype[2]	Age	Crosses		References[3]
8	25-94	†	BW	3 wk	WL x CB	F2	Sewalem *et al*. 2002
		*	BW	6 wk			
		*	BW	9 wk			
	46	*	BW	8 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
		†	BW	46 d			
		†	BW	112 d			
9	0-61	†	BW	6 wk	WL x CB	F2	Sewalem *et al*. 2002
		†	BW	9 wk			
	61-107	†	BW	200 d	RJ x WL	F2	Carlborg *et al*. 2003
10	48-55	†	BW	10 wk	WPR x WPR	F3	Chapter 2
11	22-69	*	BW	46 d	RJ x WL	F2	Carlborg *et al*. 2003
		*	BW	112 d			
	93	†	BW	8 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
		*	BW	46 d			
12	48-55	†	BW	8 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
		†	BW	46 d			
		*	BW	112 d			
		†	BW	200 d			
13	15	*	prBW		CB x CB	F2	McElroy *et al*. 2002
		*	poBW				
	22-67	*	BW	46 d	RJ x WL	F2	Carlborg *et al*. 2003
	32-70	**	BW	3 wk	WL x CB	F2	Sewalem *et al*. 2002
		**	BW	6 wk			
		*	BW	9 wk			
	59-67	†	BW	7 wk	WPR x WPR	F3	Chapter 2
		*	BW	10 wk			
14	16-37	†	BW	7 wk	WPR x WPR	F3	Chapter 2
	20-37	*	BW	1 d	RJ x WL	F2	Carlborg *et al*. 2003
15	7-13	†	BW	7 wk	WPR x WPR	F9	Chapter 3
	13-35	*	BW	5 wk	WPR x WPR	F9	Chapter 3
	13-49	†	BW	46 d	RJ x WL	F2	Carlborg *et al*. 2003
18	7-35	†	BW	46 d	RJ x WL	F2	Carlborg *et al*. 2003
20	6	*	BW	46 d	RJ x WL	F2	Carlborg *et al*. 2003
		*	BW	112 d			
26	26	†	prBW		CB x CB	F2	McElroy *et al*. 2002
		†	poBW				
27	11-47	*	BW	112 d	RJ x WL	F2	Carlborg *et al*. 2003; Kerje *et al*. 2003
		*	BW	200 d			
	36-59	†	BW	5 wk	WPR x WPR	F2	Chapter 3
	47	†	BW	6 wk	WL x CB	F2	Sewalem *et al*. 2002
		*	BW	9 wk			
Z	118-165	**	BW	3 wk	WL x CB	F2	Sewalem *et al*. 2002
		†	BW	6 wk			

[1]Abbreviations see Table 8.1.

[2]BW=body weight; poBW=body weight post transport; prBW=body weight prior transport.

[3]RIR=Rhode Island Red layer; RJ=Red Junglefowl.

Intramuscular fat

In addition to the abdominal fatness traits and BW, an analysis of the percentage intramuscular fat (IF%) was described for the first time in this thesis (Chapter 3). Intramuscular fat has been described to have a positive influence on meat quality, with respect to tenderness and flavor of the meat (Wood *et al*. 1999; Mourot and Hermier 2001). Traditional selection is difficult for this trait, because heritability is low (h^2 = 0.08) (Zerehdaran *et al*. 2004) and IF% is not easily recorded. In the QTL analysis described in Chapter 3, suggestive evidence for a QTL for IF% was found on GGA1, GGA24, and GGA27. In the same chromosomal regions on GGA1 and GGA27 evidence was also found for QTL for BW, abdominal fat weight (AFW), and/or percentage abdominal fat (AF%) (Table 8.1 and 8.2), suggesting that the underlying gene might have pleiotropic effects. However, Cahaner *et al*. (1986) found that considerable changes in the size of adipose tissue are not accompanied by substantial changes in inter- or intramuscular fat in the chicken. Furthermore, the estimation of genetic parameters in the study of Zerehdaran *et al*. (2004) showed that IF% and BW were genetically highly correlated (0.87-0.91), whereas genetic correlation between AFW and IF% was almost zero (0.02). These correlations suggest (Falconer and Mackay 1996) that the metabolic pathways for growth and fat deposition in the muscles are influenced by the same genes in the same direction, whereas the metabolic pathway for fat deposition in abdomen might be influenced by other genes. Increasing our knowledge regarding the metabolic pathways for growth and fat deposition and their regulation in chicken will make it possible to identify the genes responsible for the observed QTL effects.

High resolution maps and comparative mapping

High resolution mapping of QTL regions

Once a QTL has been localized, the next step is to predict candidate-genes for the genetic traits. However, the mapping resolution is low (generally 20 cM or more), making it difficult to move from mapping a QTL to identifying the actual gene. The use of fine-mapping methods generates a route towards eventually cloning the genes underlying the QTL in question. For fine-mapping it is essential to increase the number of recombinants. This can be done by

producing more offspring in a F_2 or backcross (BC) population or by using advanced intercross lines (AIL) to increase the cross-over density in the analyzed generation (Darvasi 1998). In Chapter 3 the development of such an AIL has been described. Alternatively, instead of producing recombinants *de novo*, one can attempt to exploit "historical" recombinants, i.e., exploit linkage disequilibrium (LD) that might exist around the QTL of interest (Coppieters *et al*. 1999). To be efficient, however, a dense marker map is required. Therefore, additional polymorphic markers, e.g. single nucleotide polymorphisms (SNPs), need to be developed. Tools such as expressed sequence tag (EST) collections, bacterial artificial chromosome (BAC) libraries, radiation hybrid (RH) panels, and comparative maps provide the necessary information to find markers, thereby, increasing the marker density (Andersson 2001).

Comparative mapping

At the end of the 20th century, a preliminary comparative map between chicken and human has been made based on linkage data of mapped genes (Groenen *et al*. 2000). There were less than 400 genes mapped in chicken for which a human orthologue had been identified (Schmid *et al*. 2000). This number of mapped genes with a human orthologue is low; therefore, several studies directed their effort to further increase this number. Nanda *et al*. (2000) showed homology between chicken chromosome Z (GGAZ) and human chromosome 9 (HSA9). Chromosome painting has revealed homology between HSA4 and GGA4 (Chowdhary and Raudsepp 2000). Further, Crooijmans *et al*. (2001) and Buitenhuis *et al*. (2002) improved the comparative map of chicken and human using the Wageningen chicken bacterial artificial chromosome (BAC) library (Crooijmans *et al*. 2000). In this approach a BAC contig was build by chromosome walking starting from loci whose position on the chicken genome is known. In addition, genes known to be located in the identified syntenic regions in man and mouse were used to map additional chicken genes in these regions.

Radiation hybrid cell lines have proven to be a powerful resource for gene mapping and they have been used to develop detailed physical gene dense maps in mammals. Morisson *et al*. (2002) created a chicken whole-

genome RH panel, called ChickRH6, consisting of 90 hybrid clones. The mapping of microsatellite markers on this panel, whose location on the linkage map is known and for which also BAC clones are available will guarantee the integration of all available maps in chicken. Preliminary mapping experiments have resulted in an RH map for GGA10 consisting of 45 loci, which is in good agreement with the linkage and BAC contig map for this chromosome (Groenen and Crooijmans 2003).

Mapping results of GGA15 and GGA24

In this thesis the same approach described by Crooijmans *et al*. (2001) and Buitenhuis *et al*. (2002) was used to improve the physical and comparative maps of GGA15 and GGA24. Initially, GGA15 showed only conservation of synteny with HSA22q11-q12, but conservation of synteny was also found with HSA12q24 and to some lesser extend with HSA3 (Chapter 5). Using RH mapping, the comparative map between GGA15 and its human and mouse counterparts was studied at a much higher resolution (Chapter 6). The order of the BAC contigs and mapped genes was further improved and additional genes were mapped on GGA15. In total, 43 genes were mapped on this chicken chromosome. In Chapter 4 the construction of the comparative map between GGA24, HSA11q22-q24, and part of mouse chromosome 9 (MMU9) is described. The number of genes mapped on GGA24 increased from five to 34.

Increasing the number of genes mapped to GGA15 and GGA24, has resulted in the identification of at least 19 conserved segments between these chicken microchromosomes and their human and mouse counterparts. At this moment, however, only 5-10% of the actual genes located on GGA15 and GGA24 are known. It is to be expected that further research of the large conserved segments of both chromosomes will reveal a substantial number of intra- or interchromosomal rearrangements not described in this thesis. The structure of the rearrangements between these species in the QTL regions is of importance for the identification of candidate genes.

Candidate genes and single nucleotide polymorphisms

Selecting a candidate gene

In the positional candidate gene approach, described in this thesis, candidate genes are selected based on the location of the detected QTL. Generally, comparative maps are used to identify homologous chromosomal regions in other information-rich species. Because significantly more is known about gene location, structure and function in human and mouse, potential candidate genes can be found in the region of interest. Using this approach, however, a well-determined definition of the trait of interest is needed. The fatness traits in mammals and chicken are physiologically different and measured in another way. In human, excess body fat (obesity) is measured externally. These measurements are indicators for multiple fat depots in a person (Bray *et al*. 1998). Measurements in chicken can be performed internally and represent specific fat depots, e.g. abdominal fat pad or intramuscular fat (Chapter 2 and 3). Nevertheless, the processes involved in fat deposition in mammals and chicken are similar (see Chapter 1) and therefore, most likely the underlying genes as well.

Studies on obesity and other fat related traits in human, mouse and agricultural species provided useful information to identify candidate genes for fatness traits in chicken. In literature, e.g. "The Human Obesity Gene Map: The 2002 Update" (Chagnon *et al*. 2003) and by using the web-based data mining tool GeneSeeker (Van Driel *et al*. 2003) with the keywords "fat" and "adipose", a large number of potential candidate genes were identified (Chapter 7). These candidates include genes involved in the synthesis, transport, and storage of fat, as well as hormones and transcription factors influencing these processes. Table 8.3 gives an overview of potential candidate genes presently mapped in the fatness QTL regions found in this thesis (Chapter 2 and 3). With several candidate genes identified, the next step will be to test for association between polymorphisms (e.g. SNPs) in these candidate genes and the fatness traits.

Table 8.3 Potential candidate genes mapped in the chicken fatness QTL regions

Chr	Phenotype[1]	Candidate genes
1	AFW, AF%, BW, IF%	*GAPD*, *HMGIC*, *HOXC@*, *HSD3B1*, *IGF1*, *LDHB*, *PDGFB*, *PPARA*, *PPARG*, *SREBP-2*,*TEF*, *UCP2*
2	AF%, BW	*LYN*, *PRKDC*, *RYR2*, *SDC2*
3	AFW, AF%, BW	*POMC*, *TNFRSF1A*
4	AFW	*ANXA5*, *CD8A*, *FGF2*, *IL2*, *KDR*, *VDR*
7	AFW	*HOXD@*
11	AFW	-
13	AFW, BW	*CDX1*
15	AFW, AF%, BW	*ACADS*, *DGCR2*, *IAPP*, *PITPNB*, *PLA2G1B*, *SERPIND1*,*TBX3*, *TBX5*, *TCF1*, *XBP1*
18	AFW, AF%	*FAS*, *SOX9*
24	IF%	*APOA1*, *APOA4*, *APOA5*, *PAFAH1B2*
27	AF%, BW, IF%	*ACE*, *ACLY*, *GH*, *HOXB@*

[1]AFW=abdominal fat weight; AF%=percentage abdominal fat; BW=body weight; IF%=percentage intramuscular fat.

Single nucleotide polymorphisms

As their name implies, SNP are single base changes or nucleotide variations that can occur in genes (promoter, exons, or introns) or between genes (intergenic regions). The SNP within the coding sequences are categorized as either synonymous (does not result in an amino acid change) or non-synonymous (results in an amino acid change). Non-synonymous SNPs (nsSNPs) are of particular interest because they can potentially impact protein function and phenotype of an individual. In contrast, synonymous SNPs do not result in a different protein and they probably have minimal effects on gene expression. Nevertheless, they could affect gene function by altering the stability, splicing, or localization of the mRNA. Both synonymous and non-synonymous SNPs are excellent genetic markers for studying the genetics of complex phenotypic traits (Tabor *et al*. 2002; Emara and Kim 2003).

In Chapter 7 direct sequencing of polymerase chain reaction (PCR) amplified DNA was used for the identification as well as typing of SNPs. The SNPs were first identified in the parents of the families segregating for the fatness QTL on GGA1, GGA15, and GGA27. A total of 94 SNPs were identified, of which twelve were located within the coding regions of six candidate genes (*TEF*, *PPARA*, *TCF1*, *GNB3*, *LRP1*, and *BRD1*). The two SNPs found in the PCR fragment of *BRD1* (bromodomain containing 1) were non-synonymous and one of them was predicted to be deleterious. This genetic mutation is an excellent candidate for showing association of *BRD1* with fat deposition in chicken.

Conclusions and future directions

This thesis concentrated on the analysis of QTL controlling fatness in chicken. Because of the large confidence intervals, the identification of the underlying genes was challenging. By using molecular genetics tools (i.e. BAC libraries, RH panels, and comparative mapping) ~25 potential candidate genes were mapped within the fatness QTL regions. These genes and the polymorphisms within these genes will be of interest for future research.

At present no causal link could be established, but nevertheless, the identified SNPs can be used to refine the map position of the QTL. A promising fine-mapping approach is the combination of both linkage and LD (Farnir *et al*. 2002; Meuwissen *et al*. 2002; Pérez-Enciso 2003; Lee and Van der Werf, 2004). In this approach LD mapping can take into account the larger number of recombinations that occurred within the AIL as has been outlined in Chapter 3 compared to the more limited number of recombinants in a three generation design (Chapter 2). Combining linkage and LD has proven to result in a mapping resolution accurate enough to narrow down the QTL confidence interval to a few cM of the genomic region (Meuwissen *et al.* 2002).

In addition, evidence for strong candidate genes can be found by extending the study presented in this thesis through the incorporation of functional data such as gene expression obtained from microarrays (Burt and Hocking 2002). This has already resulted in the identification of several interesting differentially expressed genes, including those that control lipid metabolism, in commercial broilers and divergently selected broiler lines (Cogburn *et al*. 2003).

Eventually, increasing our knowledge will lead to the identification of those genes and genetic markers associated with the fatness traits that subsequently can be incorporated in selection programs to enhance the genetic improvement of breeding stocks through marker-assisted selection.

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SUMMARY

Excessive body fatness has long been of interest to those concerned both with research on human obesity as well as on production in farm animals. It has been and still is a problem in some species of farm animals and measures have been taken to try to minimize it. It is well known that excessive fat in poultry depresses feed efficiency, has no commercial value, and is less appreciated by consumers. Therefore, considerable research effort has been applied around the world to study factors associated with fat deposition and methods of decreasing it. So far, no clear candidate genes for this trait have been identified and further research is needed to find the actual gene(s) causing chicken fatness.

In the past decades advances in molecular genetics and the genomics of model organisms and man have contributed to considerable progress in the area of livestock gene mapping. A number of resources and approaches are now well established in the chicken, including genetic markers and maps, quantitative trait loci (QTL) mapping, comparative mapping, expressed sequence tag and bacterial artificial chromosome resources, and physical maps. Furthermore, the complete chicken genome sequence has recently become available as well.

This thesis concerns the identification of genes controlling fat deposition in broilers. The strategy used to identify these genes is the so-called positional candidate gene approach. This approach moves from the identification and localization of QTL (**Chapter 2 and 3**), through the construction of high resolution and comparative maps (**Chapter 4, 5, and 6**), towards the prediction of potential candidate genes mapped in the QTL regions (**Chapter 7**). Finally, a causal link between the genetic trait and the candidate genes can be established from functional evidence.

A cross between two genetically different outcross broiler dam lines originating from the White Plymouth Rock breed, was used to produce a large three generation broiler population. In **Chapter 2**, it is described how this population was used to detect and localize QTL affecting fatness in chicken. Twenty full sib animals in generation 1 and 456 full sib animals in generation 2 were typed for microsatellite markers and phenotypic observations were collected on three groups of generation 3 animals (~1800 animals/group).

Body weight, abdominal fat weight and percentage abdominal fat was recorded at the age of 7, 9 and 10 weeks. To study the presence of QTL, an across family weighted regression interval mapping approach was used in a full sib QTL analysis. Genotypes from 410 markers mapped on 25 chromosomes were available. For the three traits 26 QTL were found for 18 regions on 12 chromosomes. Two genomewise significant QTL ($p<0.05$) were detected. One for percentage abdominal fat at the age of 10 weeks on chicken chromosome 1 at 241 cM (*MCW0058* - *MCW0101*) with a test statistic of 2.75 and the other for body weight at the age of 10 weeks on chicken chromosome 13 at 9 cM (*MCW0322* - *MCW0110*) with a test statistic of 2.77. Significance levels were obtained using the permutation test. Multiple suggestive QTL were found on chromosomes 1, 2, 4, 13, 15 and 18, whereas chromosomes 3, 7, 10, 11, 14 and 27 had a single suggestive QTL.

Chapter 3 describes the analysis of an advanced intercross line (AIL) to confirm the quantitative trait locus (QTL) regions found for fatness traits in a previous study. QTL analysis was performed on chromosomes 1, 3, 4, 15, 18, 24, and 27. The AIL was created by random intercrossing in each generation from generation 3 (G_3) onwards until generation 9 (G_9) was reached. QTL for abdominal fat weight (AFW) and/or percentage abdominal fat (AF%) on chromosomes 1, 3 and 27 were confirmed in the G_9 population. In addition, evidence for QTL effects for body weight at the age of 5 (BW5) and 7 (BW7) weeks and for percentage intramuscular fat (IF%) were found on chromosomes 1, 3, 15, 24, and 27. Significant evidence for QTL effects was detected on chromosome 1 for AFW, BW5 and BW7, and on chromosome 15 for BW5. Suggestive evidence was found on chromosome 1 for AFW, AF% and IF%, on chromosome 3 for AFW, AF% and BW7, on chromosome 15 for BW7, on chromosome 24 for IF%, and on chromosome 27 for BW5, AF% and IF%. For chromosomes 4 and 18 test statistics did not exceed the significance threshold.

To improve the physical and comparative map of chicken chromosome 24 (GGA24; former linkage group E49C20W21) bacterial artificial chromosome (BAC) contigs were constructed around loci previously mapped on this chromosome by linkage analysis. The results of this study are reported in **Chapter 4**. The BAC clones were used for both sample sequencing and BAC end sequencing. Sequence tagged site (STS) markers derived from the BAC end sequences were used for chromosome walking. In total 191 BAC clones were isolated, covering almost 30 % of GGA24, and 76 STS were developed (65 STS derived from BAC end sequences and 11 STS derived within genes). The partial sequences of the chicken BAC clones were compared with sequences present in the EMBL/GenBank databases, and revealed matches to 19 genes, ESTs and genomic clones located on human chromosome 11q22-q24 and mouse chromosome 9. Furthermore 11 chicken orthologues of human genes located on HSA11q22-q24 were directly mapped within BAC contigs of GGA24. This resulted in a better alignment of GGA24 with the corresponding regions in human and mouse and identify several intra-chromosomal rearrangements between chicken and mammals.

In **Chapter 5** the improvement of the physical and comparative map of GGA15 is described. The map was improved by the construction of 9 BAC contigs around loci previously mapped on GGA15 by linkage analysis. In total 240 BAC clones were isolated, covering 30-35% of GGA15, and 120 STS were developed (104 STS derived from BAC end sequences and 18 STS derived within genes). Seventeen chicken orthologues of human genes located on human chromosome 22q11-q12 were directly mapped within BAC contigs of GGA15. Furthermore, the partial sequences of the chicken BAC clones were compared to sequences present in the EMBL/GenBank databases, and revealed matches to 26 genes, ESTs and genomic clones located on HSA22q11-q12 and HSA12q24. These results provide a better alignment of GGA15 with the corresponding regions in human and mouse, and improve our knowledge of the evolution and dynamics of the vertebrate genome.

To further improve the existing comparative map of GGA15 a radiation hybrid (RH) map of this chromosome was constructed. **Chapter 6** shows how the RH map can be used as a resource to efficiently map genes to GGA15. The map has been developed using a 6,000 rad chicken-hamster whole-genome radiation hybrid panel (ChickRH6). In total, six microsatellite loci, 18 STSs from BAC end sequences, and 11 genes, including eight new ones, were typed on the panel. The initial framework map comprised of eight markers and an additional 23 markers were then added to generate the final map. The total map length was 334 centiRay$_{6,000}$ (cR$_{6,000}$). The estimated retention frequency for the data set was 18%. Using an estimated physical length of 21 Mb, the ratio between cR$_{6,000}$ and physical distance over GGA15 was estimated to be 0.063 Mb/cR$_{6,000}$. The present map increases the marker density and the marker resolution on GGA15 and enables fast mapping of new chicken genes homologous to genes from human chromosomes 12 and 22.

In **Chapter 7** candidate genes were identified within three QTL regions on GGA1, GGA15, and GGA27, and single nucleotide polymorphisms (SNPs) were identified within these genes. In total, 29 chicken genes were analysed. Sequence tagged site (STS) markers were developed for these genes, followed by the identification of SNPs by a direct sequencing approach of the polymerase chain reaction (PCR) amplification products from a panel of eight individuals. The panel consisted of four parental pairs of the families that have been shown to be segregating for the fatness QTL. Subsequently, the SNPs were typed in eight offspring of these families. Amplification products from 20 candidate genes were sequenced and 94 SNPs were identified in 13,379 bp of consensus sequence, an average of one SNP in every 142 bp. Twelve SNPs were found within coding sequences of six candidate genes (*TEF*, *PPARA*, *TCF1*, *GNB3*, *LRP1*, and *BRD1*). The two SNPs within the exon of *BRD1* were non-synonymous and resulted in a *Leu*-to-*Arg* (L-to-R) and *Gln*-to-*Glu* (Q-to-E) substitution. Whether these amino acid substitutions affect protein function, was predicted by using the program SIFT (sorts intolerant from tolerant). This resulted in the prediction of the substitution of L-to-R to be deleterious, thus, affecting the protein function.

Finally, the results of the positional candidate gene approach outlined in this thesis are discussed in **Chapter 8**. Within the QTL regions ~25 potential candidate genes are mapped. These genes and the polymorphisms within these genes will be of interest for future research. At present no causal link was established, but nevertheless, the identified SNPs can be used to refine the map position of the QTL by analysing linkage and linkage disequilibrium. In addition, extending the study presented in this thesis through the incorporation of functional data such as gene expression obtained from microarrays will increase our knowledge of the fat metabolism and the genes involved. Eventually, this will lead to the identification of those genes and genetic markers associated with the fatness traits that subsequently will be incorporated in selection programs to enhance the genetic improvement of breeding stocks through marker-assisted selection.

SAMENVATTING

Overtollig vet is zowel in mens als dier een probleem waar al veel onderzoek naar gepleegd is. In pluimvee is algemeen bekend dat overtollig vet een efficiënte voedselopname beperkt, dat het geen commerciële waarde heeft en minder gewaardeerd wordt door de consument. Daarom wordt er wereldwijd onderzoek gedaan om de factoren geassocieerd met vetophoping te bestuderen. Echter, tot nu toe zijn de genen, verantwoordelijk voor vetophoping bij de kip, nog niet gevonden en is verder onderzoek vereist.

In de laatste decennia heeft de vooruitgang in de moleculaire genetica en genomics van muis en mens bijgedragen tot een aanzienlijke vooruitgang op het gebied van “gene mapping” in landbouwhuisdieren. Ook voor de kip heeft dit geresulteerd in een groot aantal moleculaire toepassingen en is sinds kort de complete kippen-genoom-sequentie beschikbaar.

Dit proefschrift richt zich op de identificatie van genen betrokken bij de vetophoping in vleeskuikens. Om deze genen te identificeren wordt gebruikt gemaakt van de zogenaamde “positionele kandidaat-gen benadering”. In deze benadering worden allereerst de genomische gebieden, waar potentiële kandidaat-genen te vinden zijn (de “quantitative trait loci”; QTL), in kaart gebracht (**Hoofdstuk 2 en 3**). Vervolgens worden van deze QTL regio’s vergelijkende genetische kaarten geconstrueerd (**Hoofdstuk 4, 5 en 6**) om de feitelijke kandidaat-genen te vinden (**Hoofdstuk 7**). Uiteindelijk kan er een oorzakelijk verband gelegd worden tussen het genetische kenmerk en de kandidaat-genen.

Een kruising tussen twee genetisch verschillende vleeskuiken moederlijnen, afkomstig van het White Plymouth Rock ras, is gebruikt om een grote drie-generatie-experimentele-vleeskuiken populatie te produceren. In **Hoofdstuk 2** wordt beschreven hoe deze populatie is gebruikt om de QTL, die de vetophoping in kip veroorzaakt, te detecteren en te lokaliseren. Twintig dieren in de eerste generatie en 456 dieren in de tweede generatie werden getypeerd voor 410 microsateliet merkers gelegen op 25 chromosomen. Fenotypische waarnemingen werden verzameld van drie groepen van de derde generatie (~1800 dieren/groep). Het lichaamsgewicht, abdominaal vetgewicht (AFW) en percentage abdominaal vet (AF%) werd genoteerd op de leeftijd van 7, 9 en 10 weken. Voor de drie kenmerken werden 26 QTL

voor 18 regio's op 12 chromosomen gevonden. Twee significante ($p<0,05$) QTL werden gedetecteerd. Eén voor het percentage abdominaal vet in de leeftijd van 10 weken op kippen chromosoom 1 bij 241 cM (*MCW0058* – *MCW0101*) met een statistische testwaarde van 2,75 en een ander voor lichaamsgewicht in de leeftijd van 10 weken op kippen chromosoom 13 bij 9 cM (*MCW0322* – *MCW0110*) met een statistische testwaarde van 2,77. Significantie niveau's werden verkregen met behulp van de permutatie test. Meerder suggestieve QTL werden gevonden op chromosoom 1, 2, 4, 13, 15 en 18. Chromosoom 3, 7, 10, 11, 14 en 27 hadden een enkele suggestieve QTL.

Hoofdstuk 3 beschrijft de analyse van onderling gekruiste lijnen (de "advanced intercross lines"; AIL) om de QTL regio's, die in een vorige studie gevonden waren, te bevestigen. De QTL analyse werd uitgevoerd op chromosoom 1, 3, 4, 15, 18, 24 en 27. De AIL werd gecreëerd door willekeurige onderlinge kruisingen in iedere generatie vanaf de derde (G_3) totdat de negende (G_9) werd bereikt. QTL voor AFW en/of AF% op chromosoom 1, 3 en 27 werden bevestigd in de negende generatie. Verder werd bewijs geleverd voor de QTL effecten voor lichaamsgewicht op de leeftijd van 5 (BW5) en 7 (BW7) weken en voor het percentage intramusculair vet (IF%) gevonden op chromosoom 1, 3, 15, 24 en 27. Significant bewijs voor QTL effecten werden gedetecteerd op chromosoom 1 voor AFW, BW5 en BW7, en op chromosoom 15 voor BW5. Suggestief bewijs werd gevonden op chromosoom 1 voor AFW, AF%, en IF%, op chromosoom 3 voor AFW, AF% en BW7, op chromosoom 15 voor BW7, op chromosoom 24 voor IF% en op chromosoom 27 voor BW5, AF% en IF%. Voor chromosoom 4 en 18 kwamen de statistische testwaarden niet boven de significantie grenzen.

Om de fysisch en vergelijkende kaarten van kippen chromosoom 24 (GGA24) te verbeteren, werden "bacterial artificial chromosome (BAC) contigs" gebouwd rond de merkers, die voorheen op dit chromosoom geplaatst waren middels linkage analyse. De resultaten van deze studie worden in **hoofdstuk 4** beschreven. Van de BAC clones werd de DNA sequentie bepaald. "Sequence tagged sites" (STS) merkers afkomstig van BAC end sequenties,

werden gebruikt voor chromosoom wandelen. In totaal werden 191 BAC clones geïsoleerd, die bijna 30% van GGA24 bedekken, en werden 76 STS ontwikkeld (65 STS afkomstig van BAC end sequenties en 11 STS afkomstig van genen). De sequenties van de kippen BAC clones werden vergeleken met sequenties, aanwezig in de EMBL/GenBank databanken, en bevatten overeenkomsten met 19 genen, ESTs en genomisch clones, gelegen op humaan chromosoom 11q22-q24 en muis chromosoom 9. Verder werden 11 kippen orthologen van humane genen, gelegen op HSA11q22-q24 direct geplaatst binnen de BAC contigs van GGA24. Dit resulteerde in een betere vergelijkinging van GGA24 met de corresponderende regio's in mens en muis en identificatie van verschillende chromosomale veranderingen tussen kip en zoogdieren.

In **hoofdstuk 5** wordt de verbetering van de fysische en vergelijkende kaart van GGA15 beschreven. De kaart werd verbeterd door de constructie van 9 BAC contigs, rondom de merkers, die voorheen geplaatst waren op GGA15 door linkage analyse. In totaal werden 240 BAC clones geïsoleerd, 30-35% van GGA15 bedekkend en werden 120 STS ontwikkeld (104 STS afkomstig van BAC end sequenties en 18 STS afkomstig van genen). Zeventien kippen orthologen van humane genen, gelegen op chromosoom 22q11-q12 werden meteen geplaatst binnen de BAC contigs van GGA15. Verder werden de sequenties van de kippen BAC clones vergeleken met de sequenties, aanwezig in de EMBL/GenBank databanken, resulterend in overeenkomsten met 26 genen, ESTs en genomisch clones, gelegen op HSAq11-q12 en HSA12q24. Deze resultaten geven een betere vergelijking van GGA15 met de corresponderende regio's in mens en muis en verbeteren onze kennis van de evolutie van het genoom van vertebraten.

Om de bestaande vergelijkende kaart van GGA15 verder te verbeteren werd een "radiation hybrid" (RH) kaart gebouwd. **Hoofdstuk 6** toont hoe de RH kaart gebruikt kan worden voor het efficiënt plaatsen van genen op GGA15. De kaart is ontwikkeld door gebruik te maken van een 6000 rad kippen-hamster RH panel (ChickRH6). In totaal werden 6 microsatelliet merkers, 18 STS merkers van BAC end sequenties, en 11 genen, inclusief 8 nieuwe,

getypeerd op het panel. De initiële kaart bestaat uit 8 merkers waaraan nog eens 23 merkers werden toegevoegd om de uiteindelijke kaart te genereren. De totale map lengte was 334 centiRay$_{6000}$ (cR$_{6000}$). De geschatte retentie frequentie voor de data set was 18%. Met behulp van de geschatte fysieke lengte van 21 Mb, werd de verhouding tussen cR$_{6000}$ en de fysieke afstand over GGA15 geschat op 0,063 Mb/cR$_{6000}$. De huidig kaart verhoogt de merkerdichtheid en -resolutie van GGA15 en maakt een snelle plaatsing mogelijk van nieuwe kippen genen homoloog aan genen van de humane chromosomen 12 en 22.

In **hoofdstuk 7** werden kandidaat genen geïdentificeerd binnen 3 QTL regio's op GGA1, GGA15 en GGA27 en werden veel voorkomende merkers, de SNP merkers ("single nucleotide polymorphism") binnen deze genen geïdentificeerd. In totaal werden 29 kippen genen geanalyseerd. Voor deze genen werden STS merkers ontwikkeld, gevolgd door de identificatie van SNPs in de sequenties van de amplificatie-producten van een panel van 8 individuen. Het panel bestond uit 4 ouderparen van de families waarin de QTL voor vetophoping segregeert. Vervolgens werden de SNPs getypeerd in 8 nakomelingen van deze families. Amplificatie-producten van 20 kandidaat genen werden gesequenced en 94 SNPs werden geïdentificeerd in 13.379 bp van de consensus sequentie, een gemiddelde van één SNP per iedere 142 bp. Twaalf SNPs werden binnen coderende sequences van 6 kandidaat genen (*TEF*, *PPARA*, *TCF-1*, *GNB3*, *LRP1* en *BRD1*) gevonden. De twee SNPs binnen het exon van *BRD1* resulteerden in een *Leu*-tot-*Arg* (L-tot-R) en *Gln*-tot-*Glu* (Q-tot-E) substitutie. Of deze aminozuur substituties de eiwitfunctie beïnvloeden werd voorspeld met behulp van het programma SIFT ("sorts intolerant from intolerant"). Dit resulteerde in de voorspelling dat de substitutie van L-tot-R schadelijk is, en dus de eiwitfunctie beïnvloed.

In **hoofdstuk 8** worden de resultaten van de positionele kandidaat-gen benadering, zoals besproken in dit proefschrift, bediscussieerd. Binnen de QTL regio's zijn ongeveer 25 potentiële kandidaat genen geplaatst. Deze genen en de polymorfismes binnen deze genen zijn interessant voor toekomstig onderzoek. Tot nu toe is er nog geen oorzakelijk verband gelegd,

maar desondanks kunnen de geïdentificeerde SNPs gebruikt worden om de QTL regio's te verfijnen door de analyse van "linkage" en "linkage disequilibrium". Verder zal voortzetting van de studie, zoals beschreven in dit proefschrift, door incorporatie van functionele data, zoals genexpressie verkregen van microarrays, de kennis van het vet metabolisme en de betrokken genen vergroten. Dit zou uiteindelijk kunnen leiden tot de indentificatie van die genen en genetische merkers, die geassocieerd worden met de vet kenmerken. De incorporatie hiervan in selectieprogramma's zou vervolgens kunnen leiden tot een genetische verbetering van de vleeskuikenlijnen door merker geassisteerde selectie.

LIST OF PUBLICATIONS

Related to this thesis

Jennen, D.G.J., R.P.M.A. Crooijmans, B. Kamps, R. Açar, A. Veenendaal, J.J. van der Poel, and M.A.M. Groenen. 2002. A comparative map of chicken chromosome 24 and human chromosome 11. Anim. Genet. 33, 205-210

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Jennen, D.G.J., R.P.M.A. Crooijmans, A.E. Grootemaat, J.J. van der Poel, and M.A.M. Groenen. ####. Identification and SNP analysis of candidate genes for fatness traits in chicken. Submitted for publication

Not related to this thesis

Groenen, M.A.M., R.M.J. Dijkhof, A. Veenendaal, A. Visser, D.G.J. Jennen, R. Acar, S.J.B. Cornelissen, A.J. Buitenhuis, J.J. van der Poel, and R.P.M.A. Crooijmans. 2002. Physical mapping of the chicken genome. Pages 97-104 in Genomics and biotechnology in livestock breeding, K. Schellander, N. Li, A.M. Neeteson, and K. Wimmers., eds., Shaker-Verlag, Aachen, Germany

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CURRICULUM VITAE

Danyel Gerardus Jacobus Jennen werd op 6 januari 1973 geboren te Elsloo (Lb). In 1991 werd het VWO-diploma behaald aan S.G. Groenewald te Stein. In datzelfde jaar werd begonnen met de studie Bioprocestechnologie aan de toenmalige Landbouwuniversiteit te Wageningen. Afstudeervakken en stages werden achtereenvolgens uitgevoerd bij de sectie Moleculaire Genetica van Industriële Micro-organismen, het “Institut für Industrielle Genetik” aan de Universiteit te Stuttgart (Duitsland) en Stichting Proefstation voor de Champignoncultuur te Horst. Na zijn studie, die hij in september 1996 voltooide, werkte hij enige tijd als technisch medewerker bij de afdeling Anthropogenetica aan de Rijksuniversiteit Leiden en als wetenschappelijk onderzoeker bij het Instituut voor Agrotechnologisch Onderzoek (ATO-DLO) te Wageningen. In februari 1999 begon hij met zijn promotieonderzoek, zoals dat beschreven staat in dit proefschrift, bij de Leerstoelgroep Fokkerij en Genetica aan de Wageningen Universiteit. Sinds februari 2004 is hij werkzaam bij het “Institut für Tierzuchtwissenschaft (ITZ)” aan de Rheinische Friedrich-Wilhelms Universiteit te Bonn (Duitsland).

TRAINING AND SUPERVISION PLAN WIAS

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The Basic Package

WIAS Common Course

Course on philosophy of science and/or ethics

Scientific Exposure

International conferences

XXXVIII International conference on Animal Genetics Göttingen (ISAG)

3rd European Poultry Genetics Symposium Wageningen (EPGS)

Seminars and workshops

Workshop on Bioinformatics (Purmerend)

Advances in quantitative genetic analysis in farm animals (WIAS seminar)

The genetics of resistance to infectious diseases (WIAS seminar plus)

Quantitative Genomics (WIAS seminar)

WIAS Science Day 1999, 2000, 2001, 2002, 2003

NWO-MW Genetics 'Retraite Rolduc' 1999, 2000, 2001, 2003

Presentations

ISAG meeting Göttingen (2 poster presentations)

Seminar University Maastricht (oral)

Seminar University Liège, Faculty of Vetinary Medicine (oral)

WIAS Science Day 2003 (oral)

EPGS Wageningen (poster and oral)

In-Depth Studies

Inleiding Fokkerij E250-219

Winterschool Bioinformatics (EPS)

Object-georiënteerd Perl

Professional Skills Support Courses

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