Tarek El-Said Kamel Mohamed Rabie

Pulmonary Hypertension Syndrome in Chicken:

Peeking Under QTL Peaks

Proefschrift

Ter verkrijging van de graad van doctor
Op gezag van de rector magnificus
Van Wageningen Universiteit
Prof. Dr. Ir. L. Speelman
In het openbaar te verdedigen
Op maandag 13 december 2004
Des namiddags te half twee uur in de Aula

Rabie, Tarik, 2004

Pulmonary Hypertension Syndrome in Chicken:
Peeking Under QTL Peaks

Doctoral thesis, Wageningen University, The Netherlands, 2004
-With summary in Dutch and Arabic- 168 p.

ISBN 90-8504-132-5

PROPOSITIONS

1. The combined use of linkage disequilibrium and linkage analysis provides a powerful method to narrow down the confidence interval of the QTL.
(This thesis)

2. Genetic analysis of discrete traits can be easily accomplished if the phenotype is a clear reflection of the genotype. *(This thesis)*

3. Breeders should set aside lines in which growth rate is stabilized but selection for other broiler traits continues.

4. With controlled feeding of broilers becoming a growing trend it is important to sell it as welfare friendly management style to the general public. This means getting the right message across which is "controlling growth" not "restricted feed'....

5. Tell me and I'll forget; show me and I may remember; involve me and I'll understand. *Chinese proverb*

6. Agriculture policy in the EU today, ". . . is increasingly focusing on quality rather than quantity".

7. Pulmonary Hypertension Syndrome -a common condition in factory-raised broilers- causes severe distress.

Proposition for the PhD thesis
"Pulmonary Hypertension Syndrome In chicken: Peeking under QTL Peaks"

Tarik Rabie, Wageningen University, 13 December, 2004.

TABLE OF CONTENTS

CHAPTER 1

General Introduction

Pulmonary hypertension syndrome (PHS) is a leading cause of mortality and monetary losses to the poultry industry. PHS is the result of chronic pulmonary hypertension in the broiler chicken. Research within mammalian models has indicated that human isn't the only species that is susceptible to congestive heart failure—the heart's inability to maintain adequate blood flow to tissues. Chickens, particularly broilers, have a similar condition called ascites. Ascites is not a disease; it is a condition in which excess amount of ascitic fluids (a combination of lymph and blood plasma which has leaked from the liver) accumulate in the abdominal cavity which has prompted the common name "water belly". Ascites is more scientifically known as Pulmonary Hypertension Syndrome, PHS. Luger et al. (2003) found that in ascitic broilers, the composition of the abdominal cavity fluid was fairly similar to that of the plasma (in osmolarity, total protein, and albumin concentrations), suggesting a deficiency in the selective permeability of the blood vessels. These findings resemble those in cirrhotic human patients with ascites (Parving et al. 1977a,b; Henriksen et al. 2001). The leak of plasma fluid out of the blood vessels due to increased pulmonary hypertension and central venous congestion are symptoms found both in humans (Henriksen et al. 2001) and in broilers (Wideman 2000).

The problem has been around for about 20 years in birds grown at high altitudes. But in the last decade, it has become a problem everywhere. The incidence of ascites has increased worldwide over the past several years. This increase coincides with ongoing genetic and nutritional improvements in the areas of growth rate and feed efficiency. Meat-type chickens are selected for growth rate and muscle mass. Due to very successful genetic selection techniques, growth rate has increased at a rate of 4 to 5% per year over the past 30 years (Havenstein et al. 1994; Julian 2000). Along with this selection for rapid growth there has been an increased selection for muscle mass (particularly breast muscle mass) without a comparable increase in organ size (Julian 2000). Illustrative for this strong selection is the fact that a 2kg bird took 56 days to produce in 1976 while it can now be produced in less then 42 days.

The Cause of Ascites

Pulmonary hypertension occurs when the heart is unable to push sufficient blood through the lungs (*pulmonary*), thereby significantly increasing the blood pressure (*hypertension*). In broiler chickens, the condition often leads to death, because of their tendency to consume large quantities of feed and grow very rapidly, which in turn results in an extremely high demand for oxygen. Generally, the bird's cardiovascular system can accommodate this demand, with the heart efficiently pushing blood

through the lungs, where oxygen exchange occurs. When there is an increased demand for oxygen, the heart essentially pushes the blood through the lungs harder to increase the amount of oxygen available to the bird's metabolism. Because the lung volume and cardiovascular volume within the lung tissue is fixed, there comes a point at which the lung can no longer accommodate any more blood being supplied by the heart. The heart will enlarge due to both pressure and, with time, hypertrophy of the muscles due to the hard pumping activity. Further upstream, the blood vessels will also enlarge, causing the excessive buildup of fluid in the liver (passive liver congestion) and blood vessels on the intestines to become prominent. Because blood vessels are also quite leaky, fluid will leak out into the body cavity.

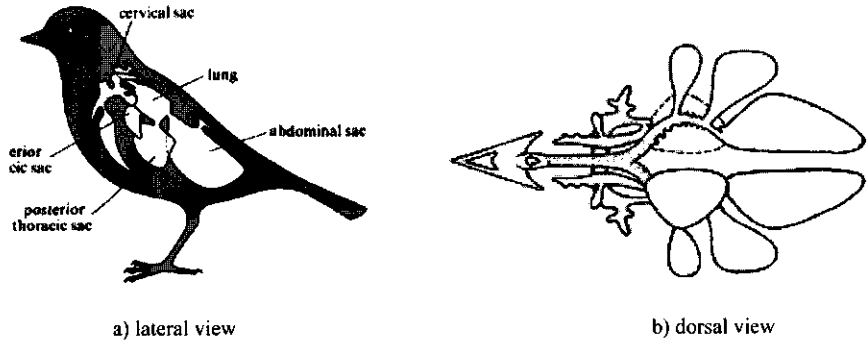
Cardiovascular System

Birds have a very efficient cardiovascular system that permits them to meet the metabolic demands of flight. The cardiovascular system not only delivers oxygen to body cells (and removes metabolic wastes) but also plays an important role in maintaining a bird's body temperature. The avian cardiovascular system consists of a respiratory system, and a circulation system (heart and blood vessels). The heart pumps blood and the blood vessels channel and deliver it throughout the body.

The Respiratory System

The anatomy and physiology of the avian respiratory system are important in the susceptibility of broilers to ascites. The anatomy of the avian respiratory system is quite complex compared to that of mammals. The avian and mammalian respiratory systems consist of the lungs which expand, gas exchange occurs and the air is exhaled. In birds the respiratory system also serves for the exchange of gases but is also important in eliminating heat from the body and has several non-respiratory functions such as the detoxification of metabolic products and vocalization. As in mammals, the upper respiratory system serves to filter air to trap dust. Unlike mammalian lungs, avian lungs are relatively rigid and do not move much during breathing. Emanating from the lungs are thin-walled air sacs that fill most of the body cavity not occupied by other viscera; most birds have nine. Some air sacs penetrate the interior of the bones and even under the skin (Figure 1). In mammals, inspired air goes into the lung as the lung expands and is expired when the lung contracts. Expanding of the lung in birds is very little because the air goes through them into the air sacs and back through the lungs on expiration. Thus not only can a greater volume of air pass through the lungs, but since it passes through twice, gas exchange is more

efficient. In addition, birds' lungs have more capillaries/area than mammals. Air sacs serve to cool the body during vigorous exercise through the internal evaporation of water. Birds have a resting respiratory rate of 50/min which increases to over 200 after flying around a room.

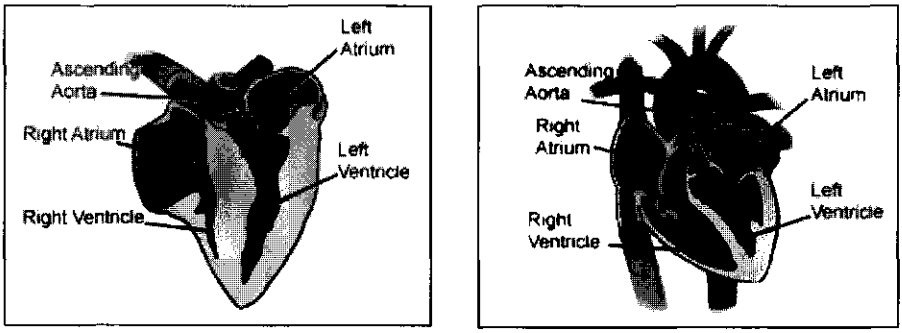


a) lateral view b) dorsal view

Figure 1. Air sacs and lungs in a bird

The Circulation System

The avian circulation system is very similar to that of mammals. One major difference is the formation of the aortic arch, Both mammalian and avian aortas develop embryologically from the bilaterally symmetrical fourth gill arch. In mammals the left arch persists: in birds the right. Thus in some ways the avian system is a mirror image of the mammal. Birds, like mammals, have a 4-chambered heart (2 atria & 2 ventricles), with complete separation of oxygenated and de-oxygenated blood. The right ventricle pumps blood to the lungs, while the left ventricle pumps blood to the rest of the body. The avian heart is different from mammals in that the left ventricle is thick-walled and right ventricle is thin-walled (Figure 2). The right atrioventricular valve is also different and composed of a muscle flap made up mainly of muscle fibers from the right ventricle wall. The anatomy of this valve makes birds very susceptible to valvular insufficiency (Julian et al. 1987; Julian 1990, 1993). The structure of the bird's circulatory system is basically like that of other vertebrates although there are some modifications. For example, the two jugular veins of the head are cross-connected so that a severe twist of the head doesn't cut off blood drainage.



a) A chicken's heart

b) A human's heart

Figure 2. Diagram of the heart showing the direction of blood flow through the heart (chicken *vs.* human)[1]

Typically, bird's veins and arteries are set up so that the veins and arteries lie next to each other. The warm blood leaving the heart and going to the extremities warms the cooler blood going to the heart from the extremities; thus some heat is returned to the body core and conserved. This is countercurrent heat exchange and is typical of cold environment birds. In warm environments, the venous return from the appendages is superficial so that heat is lost.

Contributing Factors

Pulmonary hypertension syndrome may result from physiological and/or environmental factors that either increase production or decrease removal of peritoneal lymph (Julian 1993; Currie 1999). The initial event can come in many forms, but the ultimate factors can be divided to external environmental factors such as altitude, cold temperature and incubation environment, and internal factors such as physiological changes which can be divided into many general categories as described by Balog (2003). Figure 3 shows the progression of events and factors contributing to ascites syndrome in broilers.

Management also plays a significant role in ascites development. Feed restriction, its form and contents, lighting, air quality and ventilation have all been implicated in ascites development. In addition, genetic predisposition plays a considerable role in the incidence of the PHS (Decuypere et al. 2000).

What Increases Oxygen Demand in Broiler Chickens?

The primary internal factors that result in an inadequate oxygen supply are rapid growth and efficient feed conversion. This has resulted in a broiler with such a high rate of metabolism that its heart and lungs are barely capable of providing enough oxygen to sustain the body. The increased body demand for oxygen prompts an increase in cardiac output. In a further attempt to increase the oxygen carrying capacity, the body also starts producing more red blood cells. This increase in the number of erythrocytes results in a concomitant increase in hematocrit (Maxwell et al. 1990; Julian and Mirsalimi 1992; Wideman al. 1998) and blood viscosity making it more difficult to be pumped through the lungs. Luger et al. (2001) found that hematocrit in ascitic broilers increased after exposure to cold and that the increase became significant approximately 2 wk before death. External cold temperatures, coupled with high energy diets, can result in ascites-related deaths as a consequence of insufficient pulmonary vascular capillary capacity for the blood flow necessary to meet a high metabolic oxygen requirement.

The birds that develop ascites as the result of exposure to low temperatures exhibit the same pathological signs as birds that develop ascites as the result of low oxygen levels, such as, increased hematocrit, hemoglobin, heart weight, and the ratio between right ventricle to total ventricle (Vogelaere et al. 1992; Kranen et al. 1998). The right ventricle of birds has developed as a volume pump, not as a pressure pump, since the heart rarely had to respond to changes in pressure (Akester 1984). Hypertrophy of the right ventricle therefore, will eventually result in decreased efficiency of the right atrio-ventricular valve. Valvular failure will cause the back flow of blood from the right ventricle to the atrium, gross dilation of the right ventricle and right ventricle failure and systematic circulatory congestion followed by liver congestion. This has been well documented in humans with liver cirrhosis or renal failure (Henriksen et al. 1981, 2001; Hedenborg et al. 1988). Therefore, high cardiac output associated with a high oxygen requirement increase pressure and blood flow through the lung (PHS) (Julian 2000). Thus, PHS in the young broiler is thought to result from unbalance between the metabolic demands of fast growth and cardiopulmonary performance (Odom 1993).

Environment and management

The most obvious environmental factor to play a role in ascites development in broilers is high altitude. The first studies of the effects of high altitude or hypoxia on ascites and heart disorders were being reported as early as 1950s and 1960s (Smith et al. 1954; Hall and Machicao 1968). The effect of high altitude (either natural or simulated) is a decrease in partial pressure of oxygen. The equivalent percentage of oxygen drops approximately 1.0% for every 500m rise in elevation (Julian 2000). Hall and Machiacao (1968) reported significant microscopic damage to the heart, lungs and kidneys of the birds reared at high altitude. These microscopic changes also were seen in one-week old broilers raised at high altitude (Maxwell 1990) and in birds exposed to simulated high altitude (Ploog 1973). When the PHS was first observed, it was seen mainly in areas of higher altitude and in fast growing broilers although it now occurs at all altitudes.

Factors acting at an early stage of the developing chicken embryo have been implicated to a further predisposition to ascites (Dewil et al. 1996). In susceptible lines, hypoxic conditions during incubation of the chicken egg have been shown to cause an increase in the incidence of the PHS in the adult bird (Buys et al. 1997). Management of broiler flocks has a tremendous impact on the ascites incidence within flocks. Unfortunately, good management and environmental practices that encourage high feed intake and rapid growth tend to exacerbate ascites.

Although the PHS is not fully understood, this heart failure is thought to be a consequence of a lowered oxygen tension in poultry houses and birds suffering from too little oxygen. Poor ventilation is a major contributory factor.

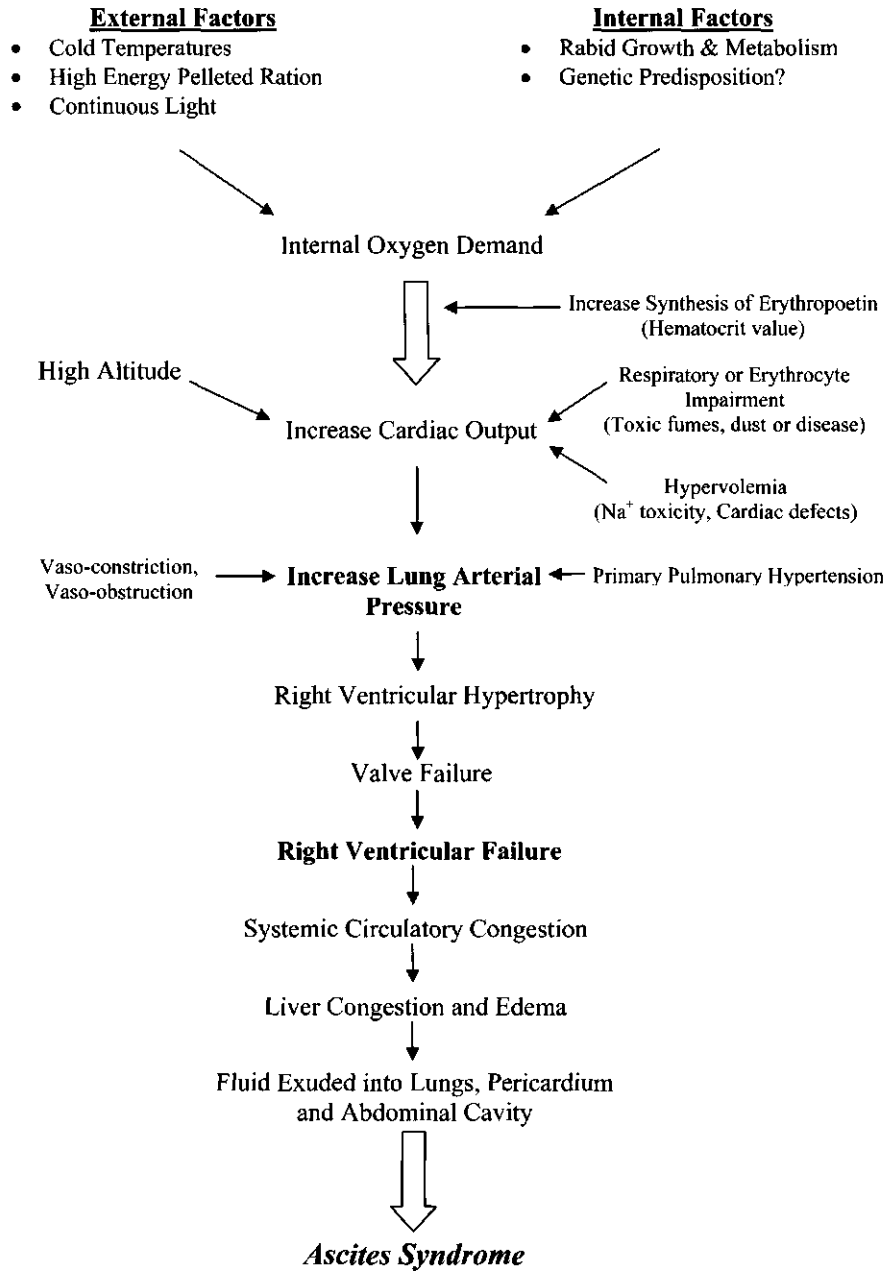


Figure 3. Progression of events and factors contributing to ascites syndrome in broilers (Balog 2003).

Pulmonary Hypertension in other species.

Pulmonary hypertension (PH) is a phenomenon that has also been described and studied in detail in humans (Newman et al. 2004) and experimental animals in particular the rat (e.g. see McMurtry et al. 2003). Although the causes for PH in humans and these experimental animals differ from that in chicken, the information available in these species nevertheless may point towards potential candidate genes involved in PHS in chicken. In human, Newman et al. (2004) demonstrated multiple independent pathways for pulmonary arterial hypertension. Figure 1 illustrates the complex interplay of the large number of genes within regulatory networks that potentially may play a role in the incidence of PH. Furthermore, additional genes that are active during the development of organs like the heart, lungs and blood vessels are other potential candidates in the susceptibility/resistance of animals to develop PH. This large number of potential candidate genes again emphasises the need for a further reduction of the size of the confidence intervals of the PHS QTL region identified in the experimental QTL mapping crosses as described in this thesis (see below).

Chicken as a model for QTL analysis.

The chicken (Gallus gallus) is an important species, and is the premier non-mammalian vertebrate model organism. The chicken has been an important experimental organism for developmental biology, and for several applications such as gene structure (Breathnach et al. 1977) and immunology (Glick 1994). Chick embryos are easily accessible from pre-gastrulation stages (the egg is laid with embryo already a two-layered structure of ~3000 cells), and throughout organogenesis (Brown et al. 2003). Moreover, it is possible to manipulate living embryos *in ovo* and follow subsequent development for up to several days. Retroviral gene delivery has been used widely in chick embryos, and the most common system is based on the Rous sarcoma virus (RSV) and uses replication-competent vectors (Hughes et al. 1987). In human interest in genetic diseases is rapidly shifting towards so-called "complex" or multi-gene traits. The term quantitative trait loci, QTL is describing such inherited characters. Although QTL analysis is also done in model species such as mouse and *Drosophila,* agricultural animals continue to be important experimental systems for quantitative genetics. In mice, the design of the QTL experiment mainly based on inbred lines but in poultry inbred lines are seldom used. In chicken crosses between divergent breeds (Tatsuda and Fujinaka 2001; Kerje et al. 2003) as well as crosses between commercial breeds (Van kaam et al. 1998; and this thesis) are used.

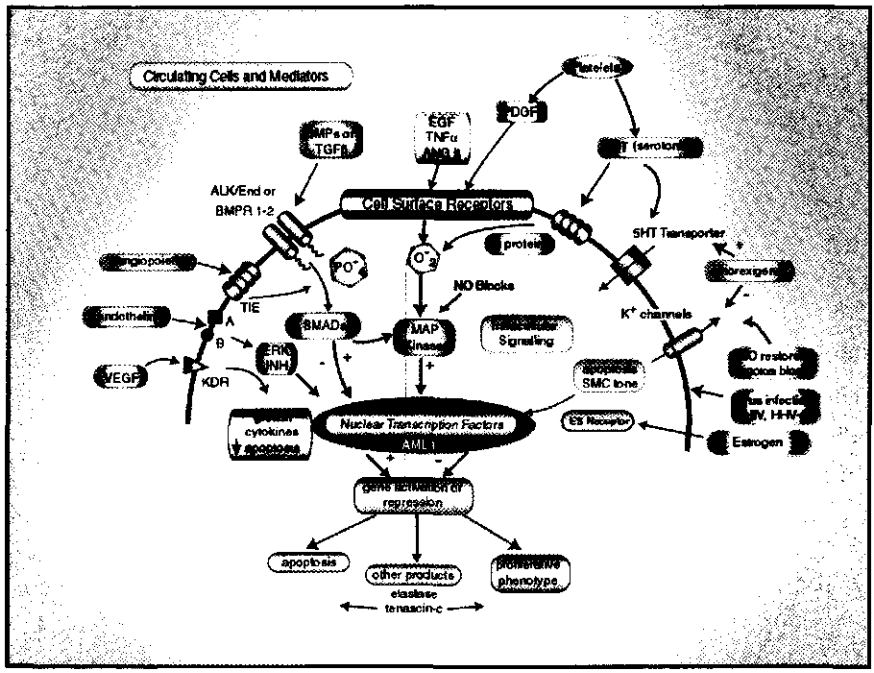


Figure 4. Some cellular processes implicated in the pathogenesis of PH. Extracellular mediators and cells (platelets) are highlighted in yellow, cell surface receptors and ion channels in purple, intracellular signaling in blue, and nuclear responses in green. Endothelin is vasoactive and a mitogen, acting through Ca2+ channels and ERK/Jun kinases. TIE is the angiopoi-etin receptor, a system found to be upregulated in pulmonary vascular disease. *Alk1* and *BMPR1-2* are receptors of the TGF-β super-family, and BMP indicates bone morphogenetic protein. Alk1 mutations cause hereditary hemorrhagic telangiectasia and some cases of PPH. Epidermal growth factor (EGF), tumor necrosis factor (TNF)-α, angiotensin II (ANGII), and platelet-derived growth factor (PDGF) are all proliferative stimuli that act through tyrosine kinase receptors and are partially transduced by intracellular oxidant species. In the intracellular domain, SMADs are regulatory proteins that activate nuclear transcription factors and interact with MAP kinases. AML1 is a nuclear transcription factor of potential importance. Elastase, downstream of AML1, has been implicated in vascular disease in experimental animals Newman et al. (2004).

The use of divergent lines increases the probability that F1 parents are heterozygous for the QTL and thereby increases the power of an experiment. He use of commercial lines on the other hand will shift the emphasis towards finding genes that explain the differences within a population and offers the opportunity of marker-assisted selection within the breed (Van Arendonk and Bovenhuis 2003). Among domestic animals, the chicken is ideal for genetic mapping and QTL analysis because of their high reproductive capacity, enabling several generations of large families to be generated in a reasonable timeframe. In addition, chicken is unique among agriculture species in that a number of selection lines are available.

The diploid karyotype of chicken is comprised of eight pairs of macro-chromosomes, thirty pairs of micro-chromosomes, and two sex chromosomes. The females are heterogametic (ZW) and males are homogametic (ZZ). Micro-chromosomes are estimated to comprise approximately 30% of the genome, but they include 40% of the genes (Brown et al. 2003). The relatively small size of the haploid chicken genome (1.2 x 10^9 bp) as compared to that of mammals (3 x 10^9 bp) is a big advantage for subsequent research aimed at the identification and characterisation of the genes underlying the QTL effects.

The development of genetic markers such as microsatellites, and single nucleotide polymorphism (SNP) have facilitated genome mapping. These genetic markers have been used to build genetic linkage maps. In chicken there are three different populations used as mapping population for genetic markers. The first one is the Compton (C) reference population which is a backcross of two partially inbred White Leghorn lines (Bumstead and Palyga 1992). The second population is the East Lansing (EL) reference population (Crittenden et al. 1993), and the third population is the Wageningen reference population (Groenen et al. 1998). The mapping results of all reference populations have been combined into a single consensus linkage map consisting of approximately 2100 markers and having a length of around 3800 cM (Groenen et al. 2000; Groenen and Crooijmans 2003). In the last few years, a large number of genetic markers enabling QTL analysis has been generated and mapped in experimental populations.

The development of high resolution genetic maps and the necessary powerful statistical methods have initiated QTL mapping experiments for a variety of traits, including those which affect growth (Groenen et al. 1997; Van Kaam et al. 1998, 1999a; Tatsuda and Fujinaka 2001; Kerje at al. 2003; Zhu et al. 2003), feed efficiency (Van Kaam et al. 1999a), carcass traits (Van Kaam et al. 1999b), resistance to salmonellosis (Hu et al. 1997), Marek's disease (Vallejo et al. 1998; Xu et al. 1998; Yonash et al. 2001), resistance to coccidiosis (Zhu et al. 2003), fatness (Jennen et al. 2004a), and pulmonary hypertension syndrome (PHS, this thesis). In chicken, crosses that have been used to detect QTL range from broiler X layer crosses (Sewalem et al. 2002) to crosses between two extreme broiler lines (Van Kaam et al. 1998). These approaches have been very successful in identifying QTL that explain differences between lines that have been selected for at least 50 generations. Preferably an identified QTL, is validated in the next generations or in additional crosses. E.g. De Koning et al. (2003) validated and confirmed QTLs for Body weight and residual feed intake on GGA4 within a commercial broiler line. QTL for fat deposition on GGA1 was validated and confirmed by Jennen et al. (2004b). The validation and confirmation of PHS-related traits are described in chapter 3 of this thesis. Depending on the statistical power of

the experiment the QTL regions can range from 20 to 100 cM in size, a region generally still containing hundreds of genes. Therefore, the second essential step towards the identification of the underlying causative gene(s) is the further fine mapping of the identified QTL regions which for the PHS related traits is described in chapter 5 of this thesis.

Chicken genomic resources

Chicken genomics is beginning to catch up with the genomics of other well-established eukaryotic organisms in term of resources. Large insert libraries such as a yeast artificial chromosome library (Toye et al. 1997) and bacterial artificial chromosome libraries (Crooijmans et al. 2000; Ren et al. 2003) were developed to make a physical map of the chicken genome. Recently these BAC libraries have been used to construct a physical BAC contig map of the chicken genome consisting of 180,000 BAC clones assembled into only 260 contigs (Wallis et al. 2004). The BAC clones also played an essential role in the integration of all mapping data (Aerts et al. 2003; Ren et al. 2003) and the assignment of these maps to specific chicken chromosomes (Masabanda et al. 2004). A radiation hybrid (RH) panel has also been constructed for chicken (ChickRH6; Morisson et al. 2002), which has been used to further improve the chicken gene maps (Chapter 5, Rabie et al. 2004; Jennen et al. 2004c; Shimogiri et al. 2004). During the last two years, there has also been dramatic progress in the development of chicken EST resources. In total there are now over 500,000 chicken ESTs available representing about 32,000 different clusters (Boardman et al. 2002). The recent completion of a draft sequence of the complete chicken genome (International Chicken Genome Sequencing Consortium 2004) is another milestone in the detailed characterization of the chicken genome. This information will dramatically increase our knowledge about the understanding of the structure and function of the chicken genes and will proof to be an invaluable tool towards the identification of the genes underlying the identified QTL.

Aim and outline of this dissertation.

The ultimate aim of the work described in this thesis is to identify genes controlling Pulmonary Hypertension Syndrome (PHS) in chicken. Because of its complex nature and being difficult (and thus expensive) to phenotype, it has been difficult to select for PHS in regular commercial breeding schemes. This makes it a trait that is particularly amenable for a whole genome scan aimed at the mapping of

quantitative trait loci (QTL). In chapter 2, the results are described of an experimental cross aimed at the characterization of the genes underlying resistance and susceptibility to PHS. Statistically significant and suggestive QTL for a large number of PHS related traits were identified located on a number of different chromosomes. Subsequently, a number of these QTL could be confirmed in further generations whereas several of the suggestive QTL could also be rejected (chapters 3 and 4). To further narrow down the region containing the QTL, with the ultimate aim of identifying the underlying causative gene(s), an advanced intercross was established. A combined linkage disequilibrium/linkage analyses analysis (LDLA) in generation 6-8 of this population made it possible to decrease the size of the confidence intervals of the two QTL on GGA2 and GGA4 (chapter 4). Comparative mapping offers the possibility to transfer information from one species to the other. In chapter 5, a medium resolution comparative map of chicken chromosome 4 and human chromosomes 4 and X is presented based on the chicken radiation hybrid panel (ChickRH6) in combination with BACs identified from the Wageningen chicken BAC library (Crooijmans et al. 2000). This comparative map was initially constructed to aid in the development of additional markers in the QTL region as well as to for the identification of potential candidate genes in that region. Although the availability of a draft sequence of the chicken genome has superseded this aim, the data described in chapter 5 was used by the international chicken genome sequencing consortium for the assembly of the sequence map for this chromosome. Finally, in Chapter 6 the results of this thesis are discussed within a broader context.

REFERENCES

Aerts J, Crooijmans R, Cornelissen S, Hemmatian K, Veenendaal T, Jaadar A, van der Poel J, Fillon V, Vignal A, Groenen M (2003) Integration of chicken genomic resources to enable whole-genome sequencing. *Cytogenet. Genome Res.* **102**, 297–303

Akester AR (1984) The cardiovascular system. In Physiology and Biochemistry of the Domestic Fowl. Vol. 5, ed, Freeman, B.M., Academic Press, London, P. 215.

Balog JM (2003) Ascites Syndrome (Pulmonary Hypertension Syndrome) in Broiler Chickens: Are We Seeing the Light at the End of the Tunnel? *Avian Poult. Biol. Rev.* **14**, 99-126

Boardman PE, Sanz-Ezquerro J, Overton IM, Burt DW, Bosch E, Fong WT, Tickle C, Brown WR, Wilson SA, Hubbard SJ (2002) A comprehensive collection of chicken cDNAs. *Curr. Biol.* **12**, 1965-1969

Bumstead, N, Palyga J (1992) A preliminary linkage map of chicken genome. *Genomics* **13,** 690-697.

Buys N, Dewil E, Decuypere E (1997) Embryonic characteristics and incubation conditions influence susceptibility to the ascites syndrome. *Xith International Congress of the World Veterinary Poultry Association, Budapest, Hungary,* p.139

Breathnach R, Mandel JL, Chambon P (1977) Ovalbumin gene is split in chicken DNA. *Nature* **270**, 314-319

Brown WR, Hubbard SJ, Tickle C, Wilson SA (2003) The chicken as a model for large scale analysis of vertebrate gene function. *Nat. Rev. Genet.* **4**, 87-98.

Crittenden LB, Provencher L, Santangelo L, Levin I, Abplanalp H, Briles RW, Briles WE, Dodgson JB (1993) Characterisations of Red Jungle Fowl by White Leghorn backcross reference population for molecular mapping in the chicken genome. *Poultry Sci.* **72**, 334-348.

Crooijmans RP, Verbalov J, Dijkhof RJ, Van der Poel JJ, Groenen MA (2000) Two dimensional screening of the Wageningen chicken BAC library. *Mamm. Genome* **11**, 360-363.

Currie RJW (1999) Ascites in poultry: Recent investigations. *Avian Pathol.* **28,** 313-326.

De Koning DJ, Windsor D, Hocking PM, Burt DW, Law A, Haley CS, Morris A, Vincent J, Griffin H (2003) Quantitative trait locus detection in commercial broiler lines using candidate regions. *J. Anim. Sci.* **81,** 1158-1165.

Decuypere E, Buyse J, and Buys N (2000) Ascites in broiler chickens: exogenous and endogenous structural and functional causal factors. *World Poltry Sci. J.* **56,** 367-377.

Dewil E, Buys N, Albers GAA, Decuypere E (1996) Different characteristics in chick embryos of two broiler lines differeng in susceptibility to ascites. *Br. Poultry Sci.* **37,** 1003-1013.

Glick B (1994) The bursa of fabricius: the evolution of a discovery. *Poultry Sci.* **73,** 979-983.

Groenen MAM, Crooijmans RPMA (2003) Structural genomics: integrating linkage, physical and sequence maps. *In Poultry genetics, breeding and technology, 3 Muir WM, Koehn RK, eds. (Cambridge, MA: CAB International),* pp 497–536

Groenen MAM, Cheng HH, Bumstead N, Benkel B, Briles E, Burt DW, Burke T, Crittenden LB, Dodgson J, Hillel J, Lamont SJ, Ponce de leon A, Soller M, Takahashi H, Vignal A (2000). A consensus linkage map of the chicken genome. *Genome Res.* **10**, 137-147.

Groenen MAM, Crooijmans RPMA, Veenendaal A, Cheng HH, Siwek M, van der Poel JJ (1998) A comprehensive microsatellite linkage map of the chicken genome. *Genomics* **49**, 265-274.

Groenen MAM, Crooijmans RPMA, Veenendaal A, van Kaam JBCHM, Vereijken ALJ, van Arendonk JAM & van der Poel JJ (1997) QTL mapping in chicken using a three generation full sib family structure of an extreme broiler x broiler cross. *Anim. Biotechnol.* **8,** 41-46.

Hall SA, Machicao N (1968) Mycocardities in broiler chickens reared at high altitude. *Avian Dis.* **12**, 75-84.

Havenstein GB, Ferket PR, Scheideler SE, Larson BT (1994) Growth livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. *Poultry Sci.* **73**, 1785-794.

Hedenborg G, Jonsson J, Norman A, Wisen O (1988) Bile constituents in ascitic fluid. *Scand. J. Clin. Lab. Invest.* **48**, 543–552.

Henriksen JH, Parving HH, Christiansen LA, Lassen NA, Ring-Larsen H, Winkler K (1981) The effect of ascitic fluid hydrostatic pressure on albumin extravasation rate in patients with cirrhosis of the liver. *Scand. J. Clin. Lab. Invest.* **41**, 601–609.

Henriksen JH, Siemssen O, Krintel JJ, Malchow-Moller A, Bendsten F, Ring-Larsen H (2001) Dynamics of albumin in plasma and ascitic fluid in patients with cirrhosis. *J. Hepatol.* **34**, 53–60.

Hu J, Bumstead N, Barrow P, Sebastiani G, Olien L, Morgan K, Malo D (1997) Resistance to salmonellosis in the chicken is linked to NRAMP1 and TNC. *Genome Res.* **7**, 693-704.

Hughes SH, Greenhous JJ, Petropoulos CJ, Sutrave P (1987) Adapter plasmids simplify the insertion of foreign DNA into helper-independent retroviral vectors. *J. Virol.* **61**, 3004-3012.

International Chicken Genome Sequencing Consortium (2004). Sequencing and comparative analysis of the chicken genome, *Gallus gallus*. *Nature*, in press

Jennen DGJ, Vereijken ALJ, Bovenhuis H, Crooijmans RPMA, Veenendaal A, van der Poel JJ, Groenen MAM (2004a) Detection and localization of quantitative trait loci affecting fatness in broilers. *Poultry Sci.* **83**, 295-301.

Jennen DGJ, Vereijken ALJ, Bovenhuis H, Crooijmans RPMA, van der Poel JJ, Groenen MAM (2004b) Confirmation of quantitative trait loci affecting fatness in chicken. *Gent. Sel. Evol.* In press

Jennen DGJ, Crooijmans RPMA, Morisson M, Grootemaat AE, van der Poel JJ, Vignal A, Groenen MAM (2004c) A radiation hybrid map of chicken chromosome 15. *Anim. Genet.* **35**, 63-65.

Julian RJ (1990) Cardiovascular disease, *In Poultry Diseases, 3rd edn, ed Jordan, F.T.W., Bailliére Tindall, London, England,* PP. 330-353.

Julian R J (1993) Ascites in poultry. *Avian Pathol.* **22**, 419–454.

Julian RJ (2000) Physiological, management nad environmental tiggers of the ascites syndrome: a review. *Avian Pathol.* **29**, 519-527

Julian RJ, Friars GW, French H, Quinton M (1987) The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broiler and roaster chickens. *Avian Dis.* **31**, 130-135.

Julian RJ, Mirsalimi SM (1992) Blood oxygen concentration of fast-growing and slow-growing broiler chickens, and chickens with ascites from right ventricular failure. *Avian Dis.* **36**, 730-732.

Kerje S, Carlborg Ö, Jacobsson L, Schütz K, Hartmann C, Jensen P, Andersson L (2003) The twofold difference in adult size between the red jungle fowl and white leghorn chickens is largely explained by a limited number of QTLs. *Anim. Genet.* **34**, 264-274.

Kranen RW, Veerkamp CH, Lambooy E, Van Kuppevelt TH, Veerkamp JH (1998) The effect of thermal preslaughter stress on the susceptibility of broiler chickens differing with respect to growth rate, age at slaughter, blood parameters, and ascites mortality, to hemorrhages in muscles. *Poultry Sci.* **77**, 737-744

Luger D, Shinder D, Rzepakovsky V, Rusal M, Yahav S (2001) Association between weight gain, blood parameters, and thyroid hormones and the development of ascites syndrome in broiler chickens. *Poultry Sci.* **80**, 965-971.

Luger D, Shinder D, Wolfenson D, Yahav S (2003) Erythropioesis regulation during the development of ascites syndrome in broiler chickens: A possible role of corticosterone. *J. Anim. Sci.* **81**, 784-790.

Masabanda J, Burt DW, O'Brien PCM, Vignal A, Fillon V, Walsh PS, Cox H, Tempest HG, Smith J, Habermann F, Schmid M, Matsuda Y, Ferguson-Smith MA, Crooijmans RPMA, Groenen MAM, Griffin DK (2004) Molecular cytogenetic definition of the chicken genome: The first complete avian karyotype. *Genetics* **166**, 1367-1373.

Maxwell MH (1990) Ultrastructural abnormalities in seven-day-old broilers reared at high altitude. *Res. Vet. Sci.* **49**, 182-189.

Maxwell MH, Spence S, Robertson GW, Mitchell MA (1990) Haematological and morphological response of broiler chickens to hypoxia. *Avian Pathol.* **19**, 23-40

McMurtry IF, Bauer NR, Fagan KA, Nagaoka T, Gebb SA, Oka M (2003) Hypoxia and Rho/Rho-kinase signaling. Lung development versus hypoxic pulmonary hypertension. *Adv. Exp. Med. Biol.* **543**, 127-137

Morisson M, Lemière A, Bosc S, Galan M, Plisson-Petit F, Pinton P, Delcros C, Fève K, Pitel F, Fillon V, Yerle M, Vignal A (2002) ChickRH6: a chicken whole-genome radiation hybrid panel. *Genet. Sel. Evol.* **34**, 521-533.

Newman JH, Fanburg BL, Archer SL, Badesch DB, Barst RJ, Garcia JGN, Kao PN, Knowles JA, Loyd JE, McGoon MD, Morse JH, Nichols WC, Rabinovitch M, Rodman DM, Stevens T, Tuder RM, Voelkel NF, Gail DB (2004) Pulmonary arterial hypertension future directions. *Circulation.* **109**, 2947-2952.

Odom TW (1993) Ascites syndrome: overview and update. *Poult. Dig.* **52**, 14-22.

Parving HH, Jensen HA, Westrup M (1977a) Increased transcapillary escape rate of albumin and IgG in essential hypertension. *Scand. J. Clin. Lab. Invest.* **37**, 223–227.

Parving HH, Ranek L, Lassen NA (1977b) Increased transcapillary escape rate of albumin in patients with cirrhosis of the liver. *Scand. J. Clin. Lab. Invest.* **37**, 643–648.

Ploog HP (1973) Physiologic changes in broiler chickens (*Gallus domesticus*) exposed to a stimulated altitude of 4267m (14000 ft). *M.Sc. thesis, The Pennsylvania State University, University Park, PA.*

Rabie TSKM, Crooijmans RPMA, Morisson M, Andryszkiewicz J, van der Poel JJ,Vignal A, Groenen MAM. (2004). A radiation hybrid map of chicken chromosome 4. *Mamm. Genome* **15**, 560-569

Ren C, Lee M-K, Yan B, Ding K, Cox B, Romanov MN, Price JA, Dodgson JB, Zhang H-B (2003) A BAC-based physical map of the chicken genome. *Genome Res.* **13**, 2754-2758.

Sewalem A, Morrice DM, Law A, Windsor D, Haley CS, Ikeobi CON, Burt DW, Hocking PM (2002) Mapping of quantitative trait loci for body weight at three, six, and nine weeks of age in a broiler layer cross. *Poultry Sci.* **81**, 1775-1781.

Shimogiri T, Bosak N, Morisson M, Okamoto S, Kawabe K, Maeda Y, Vignal A, Yasue H (2004) Assignment of CPS1, OTC, CRYD2, ARG2 and ASS genes to the chicken RH map. *Genet. Sel. Evol.* **36**, 593-599

Smith AH, Wilson WO, Pace N (1954) The effect of high altitude on growth of turkeys. *Growth* **18**, 27-35.

Tatsuda K, Fujinaka K (2001) Genetic mapping of the qtl affecting body weight in chickens using a f-2 family. *Brit. Poultry Sci.* **42**, 333-337.

Toye AA, Schalkwyk L, Lehrach H, Bumstead N (1997) A yeast artificial chromosome (YAC) library containing 10 haploid chicken genome equivalents. *Mamm. Genome* **8**, 274-276.

Vallejo RL, Bacon LD, Liu H-C, Witter RL, Groenen MAM., Hillel J, and Cheng HH (1998) Genetic mapping of quantitative trait loci affecting susceptibility to marek's disease virus induced tumors in F_2 intercross chickens. *Genetics* **148**, 349-360.

Van Arendonk JAM, Bovenhuis H (2003) Designs and methods to detect QTL for production traits based on mapped genetic markers. *In Poultry genetics, breeding and technology, 3 Muir WM, Koehn RK, eds. (Cambridge, MA: CAB International)*, pp 439–464.

Van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken LAJ, and van Arendonk JAM (1999a) Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency. *Poultry Sci.* **78**, 15-23

Van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken ALJ, van Arendonk JAM (1999b) Whole genome scan in chickens for quantitative trait loci affecting carcass traits. *Poultry Sci.* **78**, 1091-1099

Van Kaam JBCHM, van Arendonk JAM, Groenen MAM, Bovenhuis H, Vereijken ALJ, Crooijmans RPMA, van der Poel JJ, Veenendaal A (1998) Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. *Lives. Prod. Sci.* **54**, 133-150

Vogelaere P, Savourey G, Lecroat J, Braseur M, Bekaert S, Bittel J (1992) Reversal of cold induced haemoconcentration. *Eur. J. Appl. Physiol.* **64**, 244-249

Wallis JW, Aerts J, Groenen MAM, Crooijmans RPMA, Layman D, Graves T, Scheer D, Kremitzki C, Higgenbotham J, Gaige T, Mead K, Walker J, Albracht D, Davito J, Yang S-P, Leong S, Chinwalla A, Hillier L , Sekhon M, Wylie K, Dodgson J, Romanov MN, Cheng H, de Jong PJ, Zhang H, McPherson JD, Krzywinski M, Schein J, Mardis E, Wilson R, Warren WC (2004) A physical map of the chicken genome. *Nature*, in press

Wideman RF, Tackett CD (2000) Cardio-pulmonary function in broilers reared at warm or cool temperatures: effect of acute inhalation of 100% oxygen. *Poultry Sci.* **79**, 257–264.

Wideman RFJr, Wing T, Kirby YK, Forman MF, Marson N, Tackett CD, Ruiz-Feria CA (1998) Evaluation of minimally invasive indices for predicting ascites susceptibility in three successive hatches of broilers exposed to cool temperatures. *Poultry Sci.* **77**, 1565-1573.

Xu S, Yonash N, Vallejo RL, Cheng HH (1998) Mapping quantitative trait loci for binary traits using a hetrogenous residual variance model: an application to Marek's disease susceptibility in chickens. *Genetica* **104**, 171-178.

Yonash N, Cheng HH, Hillel J, Heller DE, Cahaner A (2001) DNA microsatellites linkage to quantitative trait loci affecting antibody response and survival rate in meat-type chickens. *Poultry Sci.* **80**, 22-28.

Zhu JJ, Lillehoj HS, Allen PC, Van Tassel CP, Sonstegard TS, Cheng HH, Pollock D, Sadjadi M, Min W, Emara MG (2003) Mapping quantitative trait loci associated with resistance to coccidiosis and growth. *Poultry Sci.* **82**, 9-16.

CHAPTER 2

Genetic mapping of quantitative trait loci affecting susceptibility in chicken to develop the Pulmonary Hypertension Syndrome (PHS)

T.S.K.M. Rabie[1], R.P.M.A. Crooijmans[1], H. Bovenhuis[1], A.L.J. Vereijken[2], A. Veenendaal[1], J.J. van der Poel[1], J.A.M. Van Arendonk[1], A. Pakdel[1], and M.A.M. Groenen[1]

1Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, PO Box 338, 6700 AH Wageningen, The Netherlands,

2 Nutreco Breeding Research Center, P.O. Box 220, 5830 AE, Boxmeer, The Netherlands

Submitted for publication

Abstract The pulmonary hypertension syndrome (PHS) also referred to as ascites syndrome is a growth-related disorder of broilers frequently observed in fast growing birds with insufficient pulmonary vascular capacity at low temperature and/or high altitude. A cross between two genetically different broiler dam lines originated from White Plymouth Rock breed was used to produce a three-generation broiler population. This population was used for the detection and localization of QTL affecting PHS related traits. Ten full sib families consisted of 20 G1 and 456 G2 birds were typed with 420 microsatellite markers covering 24 autosomal chromosomes. Phenotypic observations were collected on 4202 G3 birds and a full sib across family regression interval mapping approach was used to identify QTL.

There was statistical evidence for QTL on chicken chromosome 2 (GGA2), GGA4 and GGA6. Suggestive linkage was found on chromosomes 5, 8, 10, 27, and 28. The most significant QTL was located on GGA2 for the traits, right and total ventricular weight as percentage of body weight (%RV and %TV). A related trait, the ratio of right ventricular weight as percentage to total ventricular weight (RATIO), reached the suggestive threshold on this chromosome. All of three QTL effects identified on GGA2 had their maximum test statistic in the region in between 300-360 cM.

Keywords: *Chicken, pulmonary hypertension syndrome, metabolic disorder, genome scan quantitative trait loci.*

INTRODUCTION

Pulmonary hypertension syndrome (PHS, ascites) in poultry is a metabolic disorder related to rapid growth of the animal with insufficient pulmonary vascular capacity (Peacock et al. 1990; Julian 1998; Balog 2003). The incidence of ascites is influenced by several genetic and environmental factors (Julian 1993; Currie 1999). Due to very successful genetic selection techniques, growth rate has increased at a rate of 4 to 5% per year over the past 30 years (Havenstein et al. 1994; Julian 2000). Fast growing broilers are unable to deliver sufficient oxygen to satisfy the metabolic demands of their rapid growth rates. The PHS becomes particularly apparent under conditions of low oxygen supply e.g. at high altitudes (Smith et al. 1954, 1955, 1959; Olander et al. 1967; Cueva et al. 1974) or high oxygen consumption (high metabolism at low temperatures; Scheele et al. 1992), but has also seen a gradual increase under less extreme conditions (Albers and Frankenhuis 1990). High cardiac output associated with a high oxygen requirement increase pressure and blood flow through the lung (pulmonary hypertension, PH) (Julian 2000).

Both of avian respiratory and circulation systems are important in the susceptibility of broilers to PHS. Unlike mammalian, avian lungs are relatively rigid and do not move much during breathing. That is because the air goes through them into the air sacs and back through the lungs on expiration. In addition, the lungs of birds have more capillaries/area than mammals. Moreover, the avian heart is different from mammals in that the left ventricle is thick-walled and right ventricle is thin-walled. The right atrioventricular valve is also different and composed of a muscle flap made up mainly of muscle fibers from the right ventricle wall. The anatomy of this valve makes birds very susceptible to valvular insufficiency (Julian et al. 1987; Julian 1990, 1993). There are several clinical signs associated with ascites in broiler chicken such as pulmonary hypertension, right ventricular hypertrophy, central and portal venous congestion, hepatic damage, and transduction of fluid into the abdominal cavity (Riddell 1991; Yersin et al. 1992; Julian 1993; Wideman et al. 1995). In susceptible birds, this increase in workload for the right side of the heart can result in right ventricular failure and ascites. Factors acting at an early stage of the developing chicken embryo have also been implicated in an increased predisposition to the PHS (Dewil et al. 1996). In susceptible lines, hypoxic conditions during incubation of the chicken egg have been shown to cause an increase in the incidence of the disease in the adult bird (Buys et al. 1997). Insufficient development of the lungs or the pulmonary blood vessels in particular chicken broiler lines may form the basis of these observed differences. Because thyroid function is an important regulatory mechanism of metabolic rate, factors involved in thyroid functioning

present yet another system that might be responsible for the observed differences in susceptibility to PHS. In addition, the genetic predisposition plays a considerable role in the incidence of the PHS (Decuypere et al. 2000). The complexity of PHS, the large number of organs and thus the potentially large number of candidate genes involved in the disorder make PHS a trait that is particularly amenable for a whole genome scan aimed at the mapping of quantitative trait loci (QTL). The ability to dissect the genetics of QTL in chicken has been improved considerably in the past decade, through the development of detailed linkage maps based on microsatellite markers (Groenen et al. 2000; Groenen and Crooijmans 2003). Several studies have initiated QTL mapping experiments for a variety of traits which affect growth (Groenen et al. 1997; Van Kaam et al. 1998, 1999a; Tatsuda and Fujnaka 2001), feed efficiency (Van Kaam et al. 1999a), carcass traits (Van Kaam et al. 1999b), resistance to salmonellosis (Hu et al. 1997), Marek's disease (Vallejo et al. 1998; Xu et al. 1998; Yonash et al. 2001), resistance to coccidiosis and growth (Zhu et al. 2003), fatness (Jennen et al. 2004), and pulmonary hypertension syndrome (this study). The objective of this study was a whole genome scan aimed to detect and localize of QTLs in ascites related-traits.

MATERIALS AND METHODS

Expermintal Population and phenotyping

The experimental population used to detect the PHS QTLs were based on three generations (G1, G2 and G3), according to recommendations of Van der Beek et al. (1995), and has been described in detail previously (Van Kaam et al. 1998, 1999a,b). Briefly, two genetically different outcross dam lines (Hybro) originating from the White Plymouth Rock breed were chosen as the foundation of this experimental population. The maternal line has a relatively high reproductive performance and is fast-feathering. The paternal line has a relatively high growth performance and is slow-feathering. The population structure and number of birds are given in Table 1.

The experimental birds were hatched at 6 different weeks and kept in four different pens, where most of the birds were kept in one pen. Nine batches were allotted by hatching day and pen number. In order to identify individuals that were susceptible to ascites, a cold stress temperature schedule was applied. At the hatching time, the temperature was 30°C and then was gradually decreased to 10°C by 22 d of age. The temperature remained at 10°C until the end of the experiment. This was in contrast with a normal temperature schedule that starts at 33 to 34°C and then gradually decreases to 17-18°C by 35 day of age. Except for the adjusted temperature schedule, birds were kept under circumstances that closely resemble commercial

practice, i.e., they could access feed and water ad libitum; illumination was 23 h/d. A commercial broiler feed was used consisted of crumbled concentrates containing 12,970 KJ/Kg and 21% protein. The groups of birds were housed in floor pens with approximately 20 birds /m^2.

Table 1. Population structure with observations and numbers used in the analysis.

Generation	Sires	Dams	Total	Observations
0*	14	14	28	
1	10	10	20	Genotypes
2	177	279	456	Genotypes
3	2466	1736	4202	Phenotypes

*Male and Female generation 0 birds were from different lines.

In the 4202 G_3 chickens (1736 females and 2466 males), ascites-related traits were recorded just before or after slaughtering. Body weight (BW_{AS}), and hematocrit value (HCT) were measured 1 day before slaughtering at 5 wk of age. Liver abnormalities (LIVER) were scored as follows: 0 represented no abnormalities observed, 1 represented an abnormal liver, and 2 represented serious liver abnormalities. Liver abnormalities consisted of a lighter color, an irregular liver surface, or both. Accumulation of fluid in the heart sac (HEART) was scored with 0 if no fluid had accumulated, 1 if fluid accumulation was observed, and 2 if there was serious accumulation of fluid in the heart sac. Further, the weights of right ventricular (RV) and total ventricular (TV) were measured. From these measurements, the RV: TV (RATIO), RV as a percentage of total BW_{AS} (%RV), and TV as percentage of total BW_{AS} (%TV) were derived. Fluid accumulation in the chicken abdominal cavity (ABDOMEN) was scored as 0, 1, or 2. A score of 0 indicated no fluid, 1 indicated the presence of fluid in the abdomen, and 2 indicated a serious accumulation of fluid in this section. The breast color (BREAST) was scored as 0 representing normal color, 1 representing a color deviation, and 2 representing serious color deviation. In general, a deviation of breast color meant that the color was deep red. Finally, the total mortality (MORT-TOT) of the birds was recorded as 0 or 1. A score of 0 represented a bird that was alive at the end of the experiment, and a score of 1 indicated a bird that died before the end of the experiment. For the analysis of the phenotypic data a two step procedure was applied: first average adjusted progeny trait values were calculated by adjusting phenotypic observations for systematic environmental effects, and secondly a QTL analysis was undertaken using average adjusted progeny trait values as

dependent variables (Van Kaam et al. 1998). Data were adjusted for fixed effects of sex (males and Females), feathering, groups (Phenotypic observations were collected for three different groups of G_3 birds), and hatching day. Adjusted traits values were calculated using MTDFREML software (Boldman et al. 1995).

Marker Data and genotyping

Genotypes for microsatellite markers were determined using DNA derived from blood samples of 10 full sib families with approximately 45 offspring per family (20 G_1 and 456 G_2 animals). This population was also used as a linkage mapping population, which resulted in a highly informative microsatellite linkage map of the chicken genome (Groenen et al. 1998). As a result, 420 microsatellite markers were used where 266 microsatellites were typed across all 10 families, and an additional 154 microsatellites were typed on 4 of the families. The linkage map was calculated with CRIMAP (Green et al. 1990) and covered 3363.8 cM representing 24 of the 38 autosomal chromosomes. Map distances given in this study are sex-average distances in centimorgans on the haldane scale (Haldane 1919). The size of the chromosomes varied between 16 and 625 cM and the number of markers per chromosome varied between 3 and 82 markers. More information about the length of chromosomes, the number of markers on each chromosome and the average information content is given in Table 2.

Genotyping of the microsatellite markers was done as described by Crooijmans et al. (1997). The PCR amplifications were carried out in 12μL reactions as described previously by Jennen et al. (2004). The PCR amplification product from the same animal were diluted and pooled within sets of microdsatellite markers in such a way that each marker signal on ABI automated sequencer (Applied Biosystems, Perkin Elmer, Foster City, CA 94404) had a peak height of approximately 1000. Electrophoresis was performed on a 6% denaturing polyacrylamide gel, Sequagel-6 (National Diagnostics, Atlanta, Georgia 30336) for 3 h on 12 cm gels, using an automatic sequencer.

Full sib QTL Analysis

Full sib QTL analysis was undertaken using the regression interval mapping methodology as described by Van Kaam et al. (1998) in which a single QTL was fitted within each family. This method is an extension of the multi-marker regression method of (Knott et al. 1996) for outbred populations with a half sib family structure. Average adjusted progeny trait values of G2 were regressed on the probabilities of

inheriting the first allele of each parent. The across family full-sib model to fit a QTL at position k was:

$$Y_{ij} = family_i + b_{s,i}X_{s,ij} + b_{d,i}X_{d,ij} + e_{ij}$$

where:

Y_{ij} = Average adjusted trait value for the j^{th} offspring of the i^{th} family

$family_i$ = Family mean

$b_{s,i}$ = Regression coefficient for the sire (s) in family i

$X_{s,ij}$ = Probability that the j^{th} offspring from sire s in family i received allele 1

$b_{d,i}$ = Regression coefficient for dam (d) in family i

$X_{d,ij}$ = Probability that the j^{th} offspring from dam d in family i received allele 1.

e_{ij} = Random residual.

The family mean is used to account for polygenic differences between families. In order to test for the alternative hypothesis of the presence of QTL effects, versus the null hypothesis of the absence of QTL effect a test statistics was calculated at each centimorgan. The test statistic is the ratio of the explained mean square of the QTL effects under study in the numerator and the residual mean square of the full model in the denominator. The test statistic at position k was calculated as:

$$Test\ statistic_k\ (H_1{:}H_0) = \frac{\left(\frac{RSS\ (H_0) - RSS_k(H_1)}{df_{QTL}}\right)}{\left(\frac{RSS_k(H_1)}{df_{total} - df_{family} - df_{QTL}}\right)}$$

RSS (H_0) is the residual sums of squares of the reduced model, i.e. without fitting a QTL:

$$RSS\ (H_0) = \sum_{i=1}^{nf} \sum_{j=1}^{n} W_{ij}(Y_{ijk} - family_i)^2$$

and RSS_k (H_1) is the residual sums of squares after fitting a QTL at position k:

$$RSS_k(H_1) = \sum_{j-1}^{n} W_{ij}(Y_{ijk} - family_i - b_{s,ik}X_{s,ijk} - b_{d,ik}X_{d,ijk})^2$$

and *df* are the number of F_2 genotyped animals (df_{total}), number of family means (df_{family}) fitted in each family and number of QTL effects (df_{QTL}) fitted. Where *nf* is the number of families, and *n* is the number of offspring within the family. Weighting factor W_{ij} was calculated as described by van Kaam et al. (1998). The analyses were also performed for each family individually.

Significance thresholds

Significance thresholds were calculated by permutation testing (Churchill and Doerge 1994). This is an empirical method, which accounts for the location of the marker and phynotypic data. The genomewise significance threshold was calculated through permutation over all 24 chromosomes simultaneously in one analysis. For each trait, 1000 permutations at 5 cM intervals across the genome were performed. 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 as a 10% chromosomewise significance threshold. The suggestive linkage threshold is equivalent to one expected false positive result per trait in a whole genome scan.

In order to determine which parents were segregating for a QTL, permutation was also applied to single families 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 exceeding 10% chromosomewise threshold were assumed to be segregating for the QTL. The 10% chromosomewise thresholds were calculated per parent by performing 1000 permutations at 5 cM intervals.

Table 2. Information about the chromosomes length in centimorgans, number of markers, and the average information content for both sexes is given for all chromosomes.

Chromosome	Length in cM	Number of markers	First marker	Last Marker	Average Information Content	
					Sire	*Dame*
1	625.0	82	MCW0168	MCW0108	0.76	0.74
2	489.2	74	ADL0228	MCW0157	0.79	0.76
3	378.4	42	MCW0261	MCW0037	0.69	0.70
4	281.9	34	ADL0143	LEI0073	0.73	0.72
5	199.2	24	MCW0263	ADL0298	0.71	0.73
6	126.4	18	LEI0192	ABR0323	0.69	0.68
7	182.0	15	LEI0064	ADL0169	0.69	0.69
8	106.3	19	MCW0275	LEI0044	0.74	0.69
9	88.7	13	ADL0191	MCW0134	0.81	0.77
10	88.7	11	MCW0194	ADL0112	0.69	0.81
11	99.7	8	LEI0143	MCW0230	0.69	0.67
12	35.7	3	MCW0198	MCW0332	0.62	0.56
13	72.6	11	MCW0244	MCW0104	0.82	0.81
14	87.1	6	MCW0296	MCW0225	0.62	0.62
15	48.4	8	MCW0031	MCW0211	0.83	0.83
17	90.5	7	ROS0020	ADL0202	0.65	0.64
18	53.7	6	HUJ0010	MCW0219	0.67	0.76
19	54.5	6	MCW0266	MCW0349	0.62	0.67
20	20.7	4	MCW0119	ABR0324	0.83	0.80
23	34.0	5	MCW0165	MCW0249	0.54	0.67
24	16.3	3	LEI0155	LEI0069	0.61	0.53
26	59.2	7	ABR0330	LEI0074	0.64	0.71
27	52.9	6	MCW0350	MCW0328	0.70	0.71
28	72.7	8	LEI0135	ADL0299	0.69	0.73
Total	3363.8	420			0.72[1]	0.72[1]

[1]Overall average of information content.

RESULTS

Phenotypic data

Means and standard deviations for the traits measured under cold conditions are presented in Table 3. The average weight of broilers at 5 wk was 1604g (the average for males and females were 1676g and 1503g, respectively), and total mortality in the current experiment was 16%. Under cold conditions the mean and SD HCT of the birds was 35.4 ± 4.2% and 28.3 ± 2.3% under normal conditions. Genetic parameters for ascites related traits have been previously described by Pakdel et al. (2002).

Genotypic data

Table 2 shows the average information content per chromosome for males and females of all 24 chromosomes covered in the current study. The information content on single positions varied between 0.24 and 0.99 for sires and between 0.34 and 0.98 for dams. The average information content over all positions per chromosome was between 0.54 and 0.83 for sires and between 0.53 and 0.83 for dams. Average information content over all positions on all analyzed groups was 0.72 in both sexes. The average percentage of marker heterozygosity for G1 chickens varied from 42.3% to 83.3% per chromosome.

QTL Analysis

The QTL with suggestive and significant linkages for each trait are summarized in Table 4. Significant and suggestive QTLs were located on 5 macrochromosomes (2, 4, 5, 6, and 8) and 3 microchromosomes (10, 27, and 28). There was significant statistical evidence for 3 ascites-related traits on chromosome 2 and for one QTL on both chromosomes 4 and 6. Suggestive linkage was seen for a number of different traits on chromosomes 5, 8, 10, 27 and 28. In figure 1 the significant QTL on chromosome 2 for %RV and %TV and the suggestive QTL for RATIO are shown which are all three located between 300 and 360 cM. On Chromosome 4, a significant QTL was detected for BW_{AS} at position 129 cM, and the MORT-TOT was reached the suggestive threshold at position 126 cM. Furthermore, there were suggestive QTLs for BREAST, BW_{AS}, and TV on GGA8 located at positions 80, 91, 94 cM respectively. On GGA10, Both MORT-TOT and BW_{AS} showed suggestive linkage at the 3% chromosomewise level, and %RV showed suggestive linkage at 6% at chromosomewise level. The peak of the test statistic was located at 64 cM for MORT-TOT, at 88 cM for BW_{AS}, and at 79 cM for %RV. Details of the markers flanking each QTL, and their position on the chromosome, are presented in Table 4.

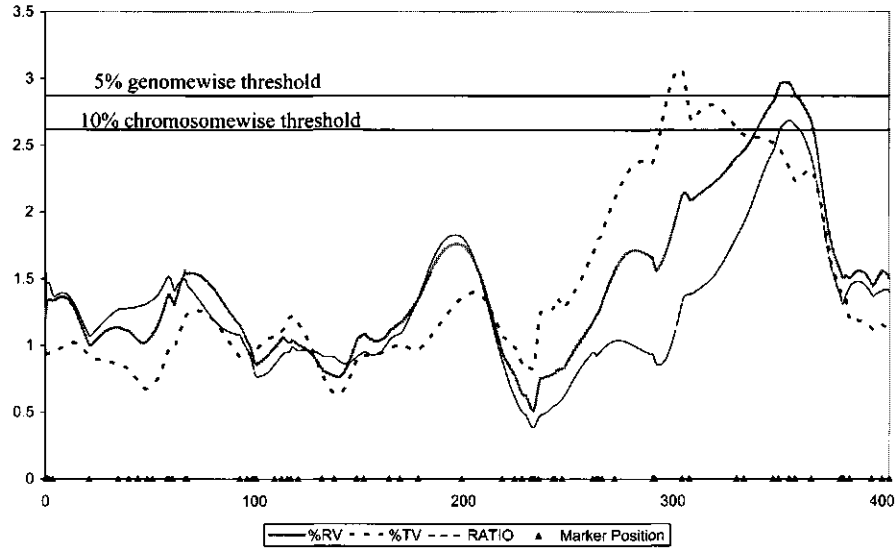


Figure 1. The test statistic values from the full sib QTL analysis on chromosome 2. 5% genomewise significance thresholds are included. Map positions are given using Haldane scale.

The number of families which are contributing to the identified QTL, and the allelic effects for each of the families were calculated. The results indicate the segregation of the QTL in a number of different families Five parents showed significant QTL effects for %RV, four parents for each of %TV, RV, TV, and BW_{AS}, three parents for each of ABDOMEN, BREAST, HCT, and RATIO, and two parents for each of HEART, LIVER and MORT-TOT. The allele substitution effect (α; Falconer 1989) average of these parents was 0.13 for ABDOMEN, 0.03 for BREAST, 1.12 for HCT, 0.07 for HEART, 0.04 for LIVER, 0.11 for MORT-TOT, 1.72 for %RV, 0.18 for %TV, 1.97 for RATIO, 0.18 for RV, 0.28 for TV, and 0.56 for BW_{AS}.

Table 4. Statistical tests (F-ratio), chromosomal positions, and markers bracket of QTL for ascites related traits.

Trait	Chromosome	Position[2] (cM)	Marker bracket[3]	F	Significance[1]
%RV					
	2	353	ADL0164-MCW0245	2.97	√
	10	79	ADL0231-ADL0102	2.09	**
	28	48	ADL0284-ADL0299	1.87	**
%TV					
	2	305	MCW0185-MCW0264	3.07	√
RATIO					
	2	356	ADL0164-MCW0245	2.69	***
	28	48	ADL0284-ADL0299	1.92	**
BW_{AS}					
	4	129	ADL0194-LEI0122	2.50	√
	8	91	ADL0301-LEI0044	2.21	***
	10	88	MCW0035-ADL0158	2.05	***
MORT-TOT					
	4	126	MCW0085-LEI0122	2.05	*
	10	64	ADL0231-MCW0035	2.35	***
LIVER					
	6	146	ABR0323	2.65	√
HEART					
	6	140	ADL0142-ABR0323	2.01	***
TV					
	8	94	MCW0271-LEI0044	1.99	**
	27	1	MCW0350-MCW0300	1.78	**
BREAST					
	5	68	MCW0090-MCW0038	2.05	**
	8	80	ADL0301	1.89	**

[1] Significance: √ = P≤0.05 genome-wise. *** = P≤0.05, ** = P≤0.10 and * = P≤0.17 chromosome-wise.

[2] Position of QTL (cM) relative to the first marker in the set for this chromosome (Table 2)

[3] The position of QTL is in between two flanking markers. One marker excesses in case the QTL position most likely identical as the marker position.

DISCUSSION

Design and Genome coverage

The identification of QTL affecting susceptibility to PHS was part of a much larger study aimed at the mapping of QTL for a variety of traits including growth and feed efficiency (Van Kaam et al. 1999a), carcass traits (Van Kaam et al. 1999b), Fatness (Jennen et al. 2004) and PHS (this study). The design of the study used is based on a so-called full sib half sib design with genotyping information on the G_2 and phenotypic information on different groups of G_3 animals (Van der Beek et al. 1995). This population was also used as a linkage mapping population, which resulted in a highly informative microsatellite linkage map of the chicken genome (Groenen et al. 1998). As a result, 266 microsatellites were typed across all 10 families, whereas an additional 154 microsatellites were typed on 4 of the families. Consequently the genome coverage and information content in this study is not uniform across all families and is somewhat higher on these 4 families. The markers used are located on 24 different chromosomes (Groenen et al. 2000; Schmid et al. 2000). The chicken genome consists of 39 pairs of chromosomes, which means that in the current study 15 chromosomes have not been covered. However, these 15 chromosomes constitute the smallest of the micro-chromosomes in chicken and probably account for less then 10 % of the chicken genome. Furthermore, even in the recently released sequence of the chicken genome, 10 of these chromosomes also are still not covered (International Chicken Genome Sequencing Consortium 2004). We estimate that in the current study approximately 90 % of the genome has been covered.

QTL affecting susceptibility to PHS

PHS in chicken is a complex disease in which hypoxemia eventually results in a number of measurable effects indicative of the disease, such as an enlargement of the right ventricle, the accumulation of ascitic fluid in the abdomen, general poor health of the internal organs, poor growth and eventually the death of the affected animal. In the current study, the measurements therefore concentrated on traits related to heart failure (RV, TV, RATIO, %RV, and %TV), general appearance of the internal organs and muscle (breast, liver, and heart), ascites (abdomen), poor growth and survival. Three significant QTLs affecting several of the traits outlined above were detected on chromosomes 2, 4 and 6. Suggestive QTLs were detected on chromosomes 8 and 10. In addition, several other regions indicated the presence of loci affecting PHS (Table

4), but these did not reach the genome-wise suggestive threshold (e.g. on chromosomes 5, 27 and 28).

As soon as the location of a QTL has been identified it is tempting to search for potential positional candidate genes in those regions. However, one has to realise that the confidence interval for the regions identified are still quite large. Furthermore, the number of organs and conditions involved in PHS and therefore the number of genes that potentially could play a role in the development of the disease is extremely large. Insufficient development of the lungs or the pulmonary blood vessels in particular chicken broiler lines e.g. may form the basis of these observed differences. Alterations in proportional growth as a result of selection for greater musculature may have had the effect of producing birds with relatively small respiratory and cardiovascular systems. Because thyroid function is an important regulatory mechanism of metabolic rate, factors involved thyroid functioning present yet another system that might be responsible for the observed differences in susceptibility to PHS. Although the genetic factors involved in susceptibility of chicken to develop PHS are poorly understood, the available data indicate that the genetic factors involved in the development and regulation of the chicken lung, thyroid and cardiovascular system are key factors that might play a role in this complex metabolic disorder. A large number of genes and gene families have already been identified that play a role in the development and functioning of these particular organs (*BMP*, *TGFB*, *SFTP*, *FGF*, *EGF*, *TRIP*, *TRAP* and *Nkx* gene families, *TITF1*, *SHH*).

In susceptible lines, hypoxic conditions during incubation of the chicken egg have been shown to cause an increase of the disease later on in the adult life. Further studies suggested that hypoxic conditions may be related to the porosity and structure of the egg shell or to the hypoxic condition during pepping and hatching. This clearly indicates that even genes acting at the level of the development of the structure of the egg need to be taken into account.

The most significant QTL for PHS identified in this study is located on the p arm of chromosome 2. Significant QTL effects were found on this chromosome for the traits %RV and %TV, whereas the RATIO reached the suggestive threshold (Figure 1). All of these QTL effects had their maximum test statistic in the region between *MCW0185* and *MCW0245* (302-364 cM). Although, %TV, %RV and RATIO reached the significance threshold in that region, the peak for these traits is very broad, reaching its maximum at position 305 cM, 353 and 356 cM respectively. A possible explanation for these results might be the presence of two separate QTLs on the region of 300 to 365 cM. However, the broad QTL peak for %TV, overlaps to a great

extend with those of %RV and RATIO. Given the high correlation between these traits a single QTL with a confidence interval from 300 cM to 365 cM is probably more likely. Although, two genes involved in early cardiogenesis are located in this region; *ZFPM2* (position 129.3 Mb on the sequence map) a member of the *FOG* family of transcription factors and the *GATA6* gene (101,8 Mb) no evidence is currently available that might suggest any involvement of theses genes in PHS. On chromosome 4, significant QTL for BW_{AS} and for MORT_TOT were identified at positions 129 cM and 126 cM respectively. The PHS QTL on GGA4 covers a region from 110-145 cM on the consensus linkage map of this chromosome which on the chicken sequence map of this chromosome is roughly the region between 32-50 Mb. There is a large number of genes in this region that are related to development of the heart and lung, including *EDNRA* (around position 32.4 Mb), *VEGFC* (45.1 Mb), *BMP2K* (45.5 Mb), *BMP3* (46.0 Mb), *NKX6-1* (47.4 Mb) and *IGFBP7* (49.7 Mb). The QTL effects observed on chromosome 6 were all related to the general condition of the internal organs (liver and heart) and the presence of ascites in the abdomen. No QTL effects were seen that were directly related to the heavy workload of the heart in PHS (RV, TV, RATIO, %RV and %TV). The significant QTL on this chromosome (LIVER) is located close to the telomere on the q arm. On chromosome 8 suggestive QTL for BREAST, BW_{AS}, and TV were detected at position 80, 91, and 94 cM respectively, indicative for the presence of a QTL for PHS at the distal part of the q-arm of this chromosome. Investigation of the genes located on QTL region at position 80-105 cM on this chromosome points to a possible gene has its effect on heart namely *TNNI3K* (position 29.7 Mb; *TNNI3* interacting kinase). Zhao et al. (2003) reported that *TNNI3K* is a cardiac-specific kinase and play important roles in the cardiovascular system. *TNNI3K* is highly expressed in the heart, but is undetectable in other tissues. Three suggestive QTL were detected in the middle of chromosome 10, for the traits MORT-TOT, BW_{AS}, and %RV. The peak of the test statistic was located at 64 cM for MORT-TOT, 88 cM for BW_{AS}, and 79 cM for %RV. Again, many potential candidate genes can be found on this chromosome including *IGF1R* (17.3 Mb), *NR2F2* (16.3 Mb) and *MEF2A* (17.9 Mb). Insulin-like growth factor 1 (*IGF1R*) was considered a potential candidate for the treatment of heart failure. McMullen et al. (2004) reported that Cardiac hypertrophy induced by over expression of *IGF1R* was completely blocked by a dominant negative *PI3K* (*p110alpha*) mutation, suggesting that *IGF1R* promotes compensated cardiac hypertrophy in a *PI3K* (*p110alpha*)-dependent manner.

PHS and its relevance to human disease

The study of the molecular mechanisms and genes underlying pulmonary hypertension syndrome (PHS) in chicken, might also have its implications for specific human diseases and more general will also improve our insight in the factors that play a role in cardiovascular functioning under high working load conditions. The research will be of particular interest in relation to high altitude pulmonary hypertension (OMIM 178400) in which acute pulmonary edema occurs in some persons at high altitude and which has been observed in particular human families, indicating genetic predisposition to the disease. PHS might also have implications for a better understanding of potential genetic factors that are involved in familial hypertrophic cardiomyopathy (OMIM 192600). Newman et al. (2004) emphasised that, the pulmonary artial hypertension (PAH) is a complex genetic disease, meaning that gene-gene and environmental-gene interactions may confer susceptibility to PAH. Finally, the detailed study of the influence of hypoxia in the developing chicken embryo will be of relevance to understand the effects of oxygenic stress in the developing human fetus and its possible role in the susceptibility to cardiovascular diseases at a later age in life.

ACKNOWLEDGEMENTS

The authors very grateful to the Egyptian Ministry of Higher Education and Scientific Research (Project No. 29110/416000) and Ministry of Science, Research, and Technology (MSRT) of Iran for financial support, and Nutreco Breeding Research Center for data collection.

REFERENCES

Albers G, Frankenhuis MT (1990) Ascites, a high altitude disease in the lowlands. *Poultry Misset.* **6**, 24-25

Boldman KG, Kriese LA, Van Vleck LD, Van Tassell CP, Kachman SD (1995) A manual for use of mtdfreml. A set of programs to obtain estimates of variances and covariances *[draft]. U.S. Department of agriculture, agricultural research service, USA*

Balog JM (2003) Ascites syndrome (Pulmonary Hypertension Syndrome) in broiler chickens: Are we seeing the light at the end of the tunnel? *Avian Poult. Biol. Rev.* **14**, 99-126

Buys N, Dewil E, Decuypere E (1997) Embryonic characteristics and incubation conditions influence susceptibility to the ascites syndrome. *Xith International Congress of the World Veterinary Poultry Association, Budapest, Hungary, p.139*

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138,** 963-971.

Cueva S, Sillau H, Valenzuela A, Ploog H (1974) High altitude induced pulmonary hypertension and righ heart failure in broiler chickens. *Res. Vet. Sci.* **16,** 370-374

Currie RJW (1999) Ascites in poultry: Recent investigations. *Avian Pathol.* **28,** 313-326.

Crooijmans RPMA, Dijkhof RJM, van der Poel JJ, Groenen MAM (1997) New microsatellite markers in chicken optimized for automated fluorescent genotyping. *Anim. Genet.* **28,** 427-437.

Decuypere E, Buyse J, Buys N (2000) Ascites in broiler chickens: exogenous and endogenous structural and functional causal factors. *World Poult. Sci. J.* **56,** 367-376

Dewil E, Buys N, Albers GAA, Decuypere E (1996) Different characteristics in chick embryos of two broiler lines differing in susceptibility to ascites. *Brit. Poultry Sci.* **37,** 1003-1013

Falconer DS (1989) Introduction to quantitative genetics. *Third edition. Longman Group UK, Harlow, Essex, United Kingdom.*

Green P, Falls K, Crooks S (1990) Documentation for CRI-MAP Version 2.4 (3/26/90).

Groenen MAM, Cheng HH, Bumstead N, Benkel B, Briles E, Burke T, Burt DW, Crittenden LB, Dodgson J, Hillel J, Lamont S, Ponce de Leon FA, Soller M, Takahashi H, Vignal A (2000) A consensus linkage map of the chicken genome. *Genome Res.* **10,** 137-147

Groenen MAM, Crooijmans RPMA (2003) Structural genomics: integrating linkage, physical and sequence maps. *In Poultry genetics, breeding and technology, 3 Muir WM, Koehn RK, eds. (Cambridge, MA: CAB International)*, pp 497–536

Groenen MAM, Crooijmans RPMA, Veenendaal A, Cheng HH, Siwek M, van der Poel JJ (1998) A comprehensive microsatellite linkage map of the chicken genome. *Genomics* **49,** 265-274.

Groenen MAM, Crooijmans RPMA, Veenendaal A, van Kaam JBCHM, Vereijken ALJ, van Arendonk JAM, van der Poel JJ (1997) QTL mapping in chicken using a three generation full sib family structure of an extreme broiler x broiler cross. *Anim. Biotechnol.* **8,** 41-46.

Haldane JBS (1919) The combination of linkage values and the calculation of distances between the loci of linked factors. *J. Genet.* **2,** 3-19

Havenstein GB, Ferket PR, Scheideler SE, Larson BT (1994) Growth livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. *Poultry Sci.* **73,** 1785-794.

Hu J, Bumstead N, Barrow P, Sebastiani G, Olien L, Morgan K, Malo D (1997) Resistance to salmonellosis in the chicken is linked to NRAMP1 and TNC. *Genome Res.* **7,** 693-704.

International Chicken Genome Sequencing Consortium (2004) Sequencing and comparative analysis of the chicken genome, *Gallus gallus, Nature*, in press

Jennen DGJ, Vereijken ALJ, Bovenhuis H, Crooijmans RPMA, Veenendaal A, van der Poel JJ, Groenen MAM (2004) Detection and localization of quantitative trait loci affecting fatness in broilers. *Poultry Sci.* **83,** 295-301.

Julian RJ (1990) Cardiovascular disease, *In Poultry Diseases, 3rd edn, ed Jordan, F.T.W., Bailliére Tindall, London, England,* pp. 330-353.

Julian RJ (1993) Ascites in poultry. *Avian Pathol.* **22,** 419-454.

Julian RJ (1998) Rapid growth problems: Ascites and skeletal deformities in broilers. *Poultry Sci.* **77,** 1773-1780

Julian RJ (2000) Physiological, management and environmental triggers of the ascites syndrome: a review. *Avian Pathol.* **29,** 519-527

Julian RJ, Frias GW, French H, Quinton M (1987) The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broiler and roster chickens. *Avian Dis.* **31,** 130-135.

Knott SA, Elsen JM, Haley CS (1996) Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theoret. Appl. Genet.* **93,** 71-80.

Lander ES, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* **11,** 241-247.

McMullen JR, Shioi T, Huang WY, Zhang L, Tarnavski O, Bisping E, Schinke M, Kong S, Sherwood MC, Brown J, Riggi L, Kang PM, Izumo S (2004) The insulin-like growth factor 1 receptor induces physiological heart growth via the phosphoinositide 3-kinase (p110alpha) pathway. *J. Biol. Chem.* **279,** 4782-93.

Newman JH, Fanburg BL, Archer SL, Badesch DB, Barst RJ, Garcia JGN, Kao PN, Knowles JA, Loyd JE, McGoon MD, Morse JH, Nichols WC, Rabinovitch M, Rodman DM, Stevens T, Tuder RM, Voelkel NF, Gail DB (2004) Pulmonary arterial hypertension future directions, Report of a National Heart, Lung and Blood Institute/ Office of Rare Diseases Workshop. *Circulation* **109,** 2947-2952.

Olander HJ, Burton RR, Adler HE (1967) The pathophysiology of chronic hypoxia in chickens. *Avian Dis.* **11,** 609-620.

Pakdel A, van Arendonk JAM, Vereijken ALJ, Bovenhuis H (2002) Direct and maternal genetic effects for ascites-related traits in broilers. *Poultry Sci.* **81,** 1273-1279.

Peacock AJ, Pickett C, Morris K, Reeves JT (1990) Spontaneous hypoxemia and right ventricular hypertrophy in fast growing broiler chickens reared at sea level. *Comp. Biochem. Physiol.* **97A,** 537-541

Riddell C (1991) Developmental, metabolic, and miscellaneous disorders, *In Diseases of poultry. 9th ed., eds Calnek, B.W., Barnes, H.J., Beard, C.W., Reid, W.M. and Yoder, H.W. Jr., Iowa State University Press, Ames, IA, pp. 839-841.*

Scheele CW, Decuypere E, Vereijken PFG, Schreurs FJG (1992) Ascites in broilers 2. Disturbances in the hormonal regulation of metabolic rate and fat metabolism. *Poultry Sci.* **71,** 1971-1984

Schmid M, Nada I, Guttenbach M, Steinlein C, Hoehn H, Schartl M, Haaf T, Weigend S, Fries R, Buerstedde JM, Wimmers K, Burt DW, Smith J, A'Hara S, Law A, Griffin DK, Bumstead N, Kaufman J, Thomson PA, Burke T, Groenen MA, Crooijmans RP, Vignal A, Fillon V, Morisson M, Pitel F, Tixier-Boichard M, Ladjali-Mohammedi K, Hillel J, Maki-Tanila A, Cheng HH, Delany ME, Burnside J, Mizuno S (2000) First report on chicken genes and chromosomes 2000. *Cytogenet. Cell Genet.* **90,** 169–218.

Smith AH, Abplanalp H, Harwood LM, Kelly CF (1959) Poultry at high altitudes. *California Agriculture, November, 8-9.*

Smith AH, Wilson WO, Pace N (1954) The effect of high altitude on growth of turkeys. *Growth* **18,** 27-35.

Smith AH, Wilson WO, Pace N (1955) Growth and reproduction in domestic birds at high altitudes. *Poultry Sci.* **35** (Suppl.1), 1175.

Tatsuda K, Fujinaka K (2001) Genetic mapping of the QTL affecting body weight in chickens using a F_2 family. *Brit. Poultry Sci.* **42,** 333-337.

Vallejo RL, Bacon LD, Liu H-C, Witter RL, Groenen MAM, Hillel J, Cheng HH (1998) Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced tumors in F_2 intercross chickens. *Genetics* **148,** 349-360

Van der Beek S, van Arendonk JAM, Groen AF (1995) Power of two- and three-generation QTL mapping experiments in an outbred population containing full-sib or half-sib families. *Theor. Appl. Genet.* **91**, 1115-1124

Van Kaam JBCHM, van Arendonk JAM, Groenen MAM, Bovenhuis H, Vereijken ALJ, Crooijmans RPMA, van der Poel JJ, Veenendaal A (1998) Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. *Livest. Prod. Sci.* **54,** 133-150

Van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken ALJ, van Arendonk JAM (1999a) Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency. *Poultry Sci.* 78, 15-23

Van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken ALJ, van Arendonk JAM (1999b) Whole genome scan in chickens for quantitative trait loci affecting carcass traits. *Poultry Sci.* 78, 1091-1099

Wideman RF Jr, Ismail M, Kirby YK, Bottje WG, Moore RW, Vardeman RC (1995) Furosemide reduces the incidence of pulmonary hypertension syndrome (ascites) in broiler exposed to cool environmental temperatures. *Poultry Sci.* **74,** 314-322.

Xu S, Yonash N, Vallejo RL, Cheng HH (1998) Mapping quantitative trait loci for binary traits using a hetrogenous residual variance model: an application to Marek's disease susceptibility in chickens. *Genetica* **104,** 171-178.

Yersin AG, Huff WE, Kubena LF, Elissalde MH, Harvey RB, Witzel DA, Giroir LE (1992) Changes in hematological, blood gas, and serum biochemical variables in broilers during exposure to simulated high altitude. *Avian Dis.* **36,** 189-196.

Yonash N, Cheng HH, Hillel J, Heller DE, Cahaner A (2001) DNA microsatellites linkage to quantitative trait loci affecting antibody response and survival rate in meat-type chickens. *Poultry Sci.* **80,** 22-28.

Zhao Y, Meng XM, Wei YJ, Zhao XW, Liu DQ, Cao HQ, Liew CC, Ding JF (2003) Cloning and characterization of a novel cardiac-specific kinase that interacts specifically with cardiac troponin I. *J. Mol. Med.* **81,** 297-304.

Zhu JJ, Lillehoj HS, Allen PC, Van Tassel CP, Sonstegard TS, Cheng HH Pollock D, Sadjadi M, Min W, Emara M G (2003) Mapping quantitative trait loci associated with resistance to coccidiosis and growth. *Poultry Sci.* **82,** 9-16.

CHAPTER 3

Confirmation of Quantitative Trait Loci Affecting Susceptibility of Broilers to Ascites

A. Pakdel,[1] T. Rabie,[1] T. Veenendaal,[1] R. P. M. A. Crooijmans,[1] M. A. M. Groenen,[1] A. L. J. Vereijken,[2] J. A. M. Van Arendonk,[1] and H. Bovenhuis,[1]

[1] *Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, PO Box 338, 6700 AH Wageningen, The Netherlands,*

[2] *Nutreco Breeding Research Center, P.O. Box 220, 5830 AE, Boxmeer, the Netherlands.*

Submitted for publication

ABSTRACT Pulmonary hypertension syndrome (PHS) followed by ascites is a major cause of economical losses to the broiler industry. The modern chicken small lung volume and body weight ratio is responsible for the inability of the respiratory system to respond to the broiler's elevated oxygen needs, which leads to ascites syndrome. Quantitative trait locus (QTL) mapping opens a way for breeders to manipulate quantitative trait genes. In a previous study, a three generation population was created for mapping of loci that are involved in the susceptibility of birds to ascites. The results revealed quantitative trait loci (QTL) with suggestive associations on chromosomes 8 and 10 for some of the ascites-related traits. The objective of this study is to confirm the QTL reported previously in subsequent generations. A two generation population including 19 full sib families has been created. In total 14 microsatellite markers were analyzed on all individuals. Twelve ascites-related traits were measured. Two QTLs exceeded the significance thresholds for the traits body weight at 2 and 5 weeks of age under ascites condition ($BW2_{AS}$, $BW5_{AS}$), right ventricular weight (RV), the ratio of right to total ventricular weight (RV:TV), and total mortality (MORT-TOT). The most significant QTL were located on chromosome 8 for traits $BW5_{AS}$ (*ADL0278-MCW0351*), RV (*ADL0278-MCW0351*) and RV:TV (*ROS0075-ADL0278*) and on chromosome 10 for traits $BW2_{AS}$ (*MCW0035-ADL0102*) and MORT-TOT (*ADL0158-LEI0112*).

***Key words**: Broilers, ascites, QTL mapping, cold stress.*

INTRODUCTION

Fast growth, high altitudes and cool temperatures are the primary triggers for ascites syndrome in commercial broilers (Julian 2000). This syndrome results in significant economic losses to the broiler industry due to high mortality around the world and has a negative impact on animal welfare. A number of studies suggested several traits that can be used as indicator traits for ascites. The large number of organs (e.g. heart, lung, liver, thyroid gland) which are involved in the disease makes ascites syndrome complicated. The physiology of this syndrome has been studied extensively (Julian 2000; Wideman and French 2000). In addition several studies showed that traits related to ascites syndrome have a relatively high heritability (Pakdel et al. 2002; De Greef et al. 2001; Maxwell and Robertson 1997; Lubritz et al. 1995). This indicates that genetic factors play a considerable role in susceptibility of birds to ascites and offers perspectives for selection against this syndrome. Pakdel et al. (2004a) estimated a weak but positive genetic correlation among ascites-related traits measured under cold conditions and BW measured under normal conditions and concluded that there is scope for simultaneous selection for birds with BW and susceptibility to ascites. In commercial broiler breeding, genetic improvements for traits like BW can easily be achieved through traditional selection that is based on phenotypic information. However, most of the ascites-related traits are difficult to measure in commercial breeding (e.g. the traits can not be measured on the live birds) and therefore the breeder must rely entirely on information from relatives. It is expected that the availability of molecular information is especially useful in selection for these type of traits (Van der Beek 1995). Using DNA marker techniques, QTL controlling ascites susceptibility can be identified and used in marker assisted selection. Moreover QTL knowledge for such traits may contribute to a better understanding of the physiological and biological background of ascites. In a previous study (Rabie et al. 2004), a three generation population was created for mapping of loci that are involved in the susceptibility of birds to ascites. The results revealed quantitative trait loci (QTLs) with suggestive association for some of the ascites-related traits on chicken chromosome 8 (GGA8) and GGA10. Confirmation of these QTLs is an essential step before an attempt is made towards the fine mapping of the QTL and identification of genes underlying the ascites-related traits. The objective of this study is to confirm the QTL reported previously for ascites-related traits in subsequent generations.

MATERIAL AND METHODS

Experimental Population

For this QTL detection study, a two generation full sib design with 19 families was created. The experimental population used in this study originates from the population that was used by Rabie et al. (2004). In the previous study (Rabie et al., 2004), a three-generation full sib half sib design was created for mapping of loci that are involved in the susceptibility of birds to ascites. G_2 animals of this design were used to produce subsequent generations by random intercrossing in each generation. In the present study, the parents are generation 6 (G_6) or generation 7 (G_7) birds and the full sib offspring are generation 7 or generation 8 (G_8) birds. In total 1,552 individuals were included in the study of which including 5 G_6/G_7 full sib families and 14 G_7/G_8 full sib families. The average number of offspring per family was 82 with a minimum of 61 and a maximum of 103 offspring per full-sib family. The birds in G_6, G_7 and G_8 were typed for genetic markers. Phenotypic observations were collected during in total 33 weeks. In order to identify individuals that were susceptible to ascites, a cold stress temperature schedule was applied. At the time of hatching, the temperature was 30°C and then was gradually decreased to 10°C by 22 d of age. The temperature remained at 10°C until the end of the experiment. Except for the adjusted temperature schedule, birds were kept under circumstances that closely resemble commercial practice, i.e. a standard commercial broiler feed containing 12,970 kJ/kg, artificially lighted housing for 23 h /d, and group housing with 20 birds/m^2.

Traits measured were body weight at 2 and 5 wk of age ($BW2_{AS}$ and $BW5_{AS}$), right ventricular weight (RV), total ventricular weight (TV) , the ratio of RV to TV (RV:TV), total mortality (MORT-TOT), blood pH (pH), partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), blood bicarbonate concentration (HCO_3), total carbon dioxide content (T_{CO_2}) and oxygen saturation in arterial blood (sO_2). For more information about the blood gas traits see Appendix.

QTL Regions

In the previous QTL analysis, QTL for ascites-related traits were found on different chromosomes of chickens (Rabie et al., 2004). From the results of that analysis chromosomes 8 and 10 were chosen for further analysis in the G_7 and G_8 populations. These two chromosomes were selected because they showed suggestive evidence of QTL effects for $BW5_{AS}$, TV, and MORT-TOT.

Genetic Markers

Genotypes for micro satellite markers were determined using DNA derived from blood samples from 10 G_6, 457 G_7 and 1085 G_8 animals. In total 14 informative markers were mapped to chromosomes 8 and 10. The Haldane mapping function (Haldane 1919) was used in the current study. Table 1 shows the name, the number of alleles and the relative location of 14 markers on the two linkage groups used in the present study.

Table 1. Linkage map of the chicken chromosomes 8 and 10 including marker names, marker positions in centimorgan and number of alleles.

Chromosome	Markers	Position in the current study	Number of alleles
GGA8	ADL0301	0	2
	MCW0271	13	6
	ROS0075	14	4
	ADL0278	14	5
	MCW0351	24	5
GGA10	MCW0194	0	4
	ADL0038	7	6
	MCW0067	11	3
	MCW0035	34	3
	ADL0102	40	4
	MCW0366	40	7
	ADL0158	53	4
	LEI0112	59	3
	LEI0103	61	4

Analysis of Phenotypic Data

For the analysis of the phenotypic data a two step procedure was applied; first average adjusted progeny trait values were calculated by adjusting phenotypic observations for systematic environmental effects, and secondly a QTL analysis was undertaken using adjusted trait values as dependent variables. Data were adjusted for the fixed effects sex and week of hatching. There was an additional fixed effect "date

of measurement" for blood gas traits. This fixed effect is related to the fact that measuring blood gas traits could not be done for all the birds on one day. Adjusted traits values were calculated using MTDFREML software (Boldman et al. 1995).

Full sib QTL Analysis

Full sib QTL analysis was undertaken using the regression interval mapping methodology as described by Van Kaam et al. (1998) in which a single QTL was fitted within each family. This method is an extension of the multi-marker regression method of (Knott et al. 1996) for outbred populations with a half sib family structure. Adjusted trait values of G_7 and G_8 were regressed on the probabilities of inheriting the first allele of each parent. The across family full-sib model to fit a QTL at position k was:

$$Y_{ij} = family_i + b_{s,i}X_{s,ij} + b_{d,i}X_{d,ij} + e_{ij}$$

where:

Y_{ij} = Adjusted trait value for the j^{th} offspring of the i^{th} family

$family_i$ = Family mean

$b_{s,i}$ = Regression coefficient for the sire (s) in family i

$X_{s,ij}$ = Probability that the j^{th} offspring from sire s in family i received allele 1

$b_{d,i}$ = Regression coefficient for the dam (d) in family i

$X_{d,ij}$ = Probability that the j^{th} offspring from dam d in family i received allele 1.

e_{ij} = Random residual.

The family mean is used to account for polygenic differences between families. In order to test for the alternative hypothesis of the presence of QTL effects, versus the null hypothesis of the absence of QTL effect a test statistics was calculated at each centimorgan. The test statistic is the ratio of the explained mean square of the QTL effect under study in the numerator and the residual mean square of the full model in the denominator. The test statistic at position k was calculated as:

$$Test\ statistic_k\ (H_1{:}H_0) = \frac{\left(\frac{RSS\ (H_0) - RSS_k(H_1)}{df_{QTL}}\right)}{\left(\frac{RSS_k(H_1)}{df_{total} - df_{family} - df_{QTL}}\right)}$$

RSS (H_0) is the residual sums of squares of the reduced model, i.e. without fitting a QTL:

$$RSS\ (H_0) = \sum_{j=1}^{n} (Y_{ijk} - family_i)^2$$

and RSS_k (H_1) is the residual sums of squares after fitting a QTL at position k:

$$RSS_k (H_1) = \sum_{j=1}^{n} (Y_{ijk} - family_i - b_{s,ik} X_{s,ijk} - b_{d,ik} X_{d,ijk})^2$$

and df are the number of F_7 and F_8 genotyped animals (df_{total}), number of family means (df_{family}) fitted and number of QTL effects (df_{QTL}) fitted in each family. In case significant evidence for the presence of a QTL was found, the analyses also were performed for each family individually with the purpose to test for the presence of QTL effects within each family.

Significance Thresholds

The significance thresholds for the test statistics were calculated empirically using the permutation method outlined by Churchill and Doerge (1994). The genomewise threshold values were derived for each trait by permutating two linkage groups simultaneously. Significance tests for the presence of a QTL were performed at 5 cM interval. This was repeated 1000 times in order to construct the distribution of the test statistic under the null hypothesis.

Permutation was also applied to determine which parents were segregating for a QTL on those locations where a QTL was detected in the across family analysis. Per parent, a test comparing a model with a QTL versus a model without a QTL was applied. 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 1000 permutations at 5-cM intervals.

RESULTS

Twelve traits related to ascites have been analyzed in the current study. The statistical descriptions of the traits measured under cold stress conditions are presented in Table 2. The average weight of broilers at 5 wk of age was 1,119 g, the ratio of RV:TV which is an ascites heart index was 24 % and total mortality in the current experiment was 8%.

Information Content

The information content shows the ratio between the actual variance found in the data and the expected variance under full information (Spelman et al. 1996). The regions covered in the current study were 24.7 cM for GGA8 and 61.4 cM for GGA10. The average information content over 2 linkage groups was 0.77 for both males and females. There was a slight difference in the average information content between males and females for each linkage group. Figure 1 shows the sex averaged information content for the 2 linkage groups.

Table 2. Statistical description of the traits

Trait	Abbreviation	Number	Mean	Std Dev
BW at 2 wk of age (g)	$BW2_{AS}$	1507	370	111
BW at 5 wk of age (g)	$BW5_{AS}$	1382	1119	211
Right ventricular weight (g)	RV	1355	1.22	0.34
Total ventricular weight (g)	TV	1355	5.04	0.91
Ratio of right ventricular weight to total ventricular weight (%)	RV:TV	1355	24.05	4.83
Blood pH	pH	1260	7.38	0.05
Partial pressure of carbon dioxide	pCO_2	1261	45.48	6.41
Partial pressure of oxygen	pO_2	1261	52.42	10.44
Blood bicarbonate concentration	HCO_3	1261	27.10	3.11
Total carbon dioxide content	T_{CO_2}	1260	28.50	3.24
Oxygen saturation in arterial blood	sO_2	1259	84.18	6.47
Total mortality	MORT-TOT	1507	0.08	0.28

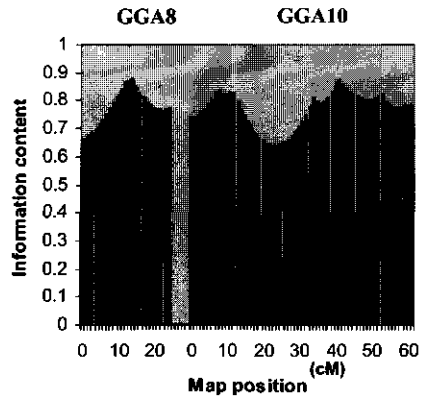


Figure 1. Information content for chicken chromosomes 8 (GGA8) and GGA10.

Full sib QTL analysis

Table 3 gives the test statistic for the presence of a QTL for each trait at the most likely location. A Quantitative trait locus for RV:TV was located at 15 cM on chromosome 8 as shown in Figure 2 and exceeded the threshold for significant linkage. Furthermore, in the same region QTL affecting the traits RV and $BW5_{AS}$ was found and exceeded the threshold for significance linkage (Figure 2). The test statistic for the trait RV peaked at 19 cM and for the trait $BW5_{AS}$ at 24 cM. To study the number of families contributing to these QTL, allelic effects were calculated for all families. Results suggest the segregation of the QTL in seven parents (of the 38) for trait RV:TV, in eight parents for trait RV and in eight parents for trait $BW5_{AS}$. The absolute average allele substitution effect (α) over these parents was 2.63% for RV:TV, 0.16 g for RV and 98.6 g for $BW5_{AS}$.

On chromosome 10, QTL for the traits $BW2_{AS}$ and MORT-TOT were found (Table 3). This QTL exceeded the threshold for significant linkage, reaching a 5% significance level for trait $BW2_{AS}$ and 7% for MORT-TOT. The peak of the test statistic was located at 34 cM for $BW2_{AS}$ and at 59 cM for MORT-TOT. Furthermore, another high test statistic value was found for MORT-TOT on GGA10 at 40 cM (Figure 3). Suggestive QTL were detected for the traits TV and pCO_2. The test statistic for TV peaked at 23 cM and for pCO_2 at 6 cM. Eleven parents showed significant QTL effects for $BW2_{AS}$ and eleven parents for MORT-TOT. The absolute average allele substitution effect (α) over these parents for $BW2_{AS}$ was 34.5 g and for MORT-TOT was 0.13%.

Table 3. Summary of interesting regions per trait. The linkage group, the most likely location in centimorgan, the test statistic and the genomewise significance level of the QTL at this position are indicated per trait.

Trait[1]	Chromosome	Location (cM)	Marker bracket	Test statistic	Significance[2]
$BW5_{AS}$	8	24	ADL0278 – MCW0351	1.66	**
RV	8	19	ADL0278 – MCW0351	1.63	**
RV:TV	8	15	ROS0075 – ADL0278	1.84	***
pO_2	8	12	ADL0301 – MCW0271	1.55	ns
sO_2	8	12	ADL0301 – MCW0271	1.29	ns
$BW2_{AS}$	10	34	MCW0035 – ADL0102	1.72	***
TV	10	23	MCW0067 – MCW0035	1.52	*
MORT-TOT	10	59	ADL0158 – LEI0112	1.69	**
pCO_2	10	6	MCW0194 – ADL0038	1.6	*
HCO_3	10	34	MCW0035 – ADL0102	1.28	ns
T_{CO_2}	10	35	MCW0035 – ADL0102	1.29	ns
PH	10	35	MCW0035 – ADL0102	1.49	ns

[1] $BW5_{AS}$ = BW at 5 wk of age under ascites condition; RV = right ventricular weight; RV:TV = ratio of right ventricular weight to total ventricular weight; pO_2 = partial pressure of oxygen; sO_2 = Oxygen saturation in arterial blood; $BW2_{AS}$ = BW at 2 wk of age under ascites condition; TV = total ventricular weight; MORT-TOT = Total mortality; pCO_2 = Partial pressure of carbon dioxide; HCO_3 = Blood bicarbonate concentration; T_{CO_2} = Total carbon dioxide content; PH = Blood pH

[2] Accounting for the multiple testing over the 3 chromosomes regions: *** = P≤0.05, ** = P≤0.10, * = P≤0.20 and ns = non significant.

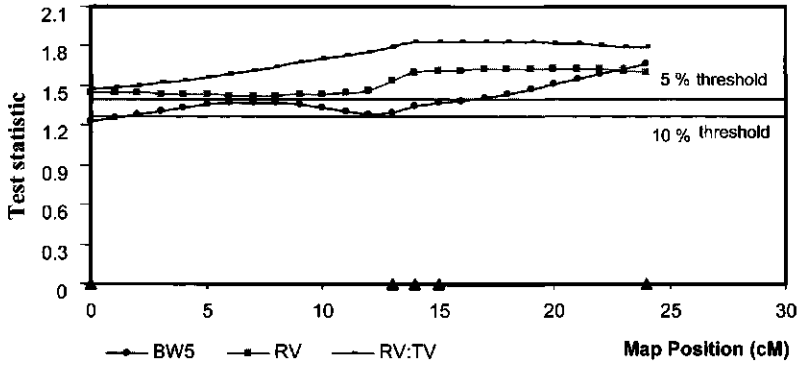


Figure 2. Test statistic values from the analysis of traits $BW5_{AS}$, RV and RV:TV for quantitative trait loci on chromosome 8. 5 and 10 % thresholds for trait RV:TV are included. Map positions are given using Haldane scale. The triangulars indicate the position of markers on chromosome.

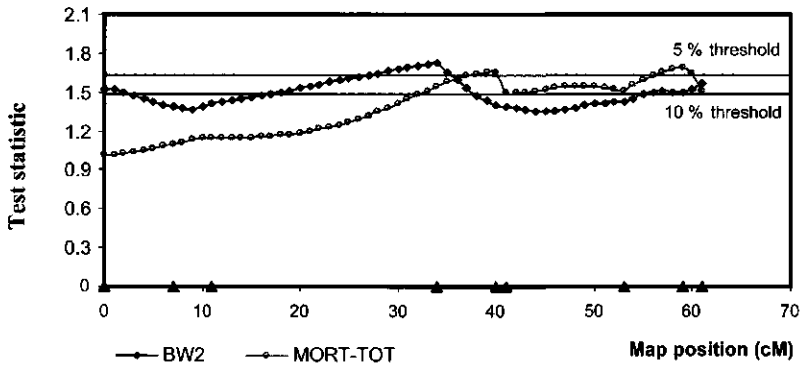


Figure 3. Test statistic values from the analysis of traits $BW2_{AS}$ and MORT-TOT for quantitative trait loci on chromosome 10. 5 and 10 % thresholds for trait $BW2_{AS}$ are included. Map positions are given using Haldane scale. The triangulars indicate the position of markers on chromosome.

DISCUSSION

This article aims at confirming QTLs controlling ascites resistance in broilers. A full sib regression interval mapping approach was applied, because it includes the segregation of alleles from both parents. This research has resulted in the identification and confirmation of several regions on two different chromosomes that may involved in susceptibility of chicken to develop ascites. Twelve ascites-related traits and more than 1500 individuals where analyzed in the current study.

Compared to the previous work (Rabie et al. 2004), both experiments were conducted under cold stress conditions which can stimulate metabolic rates and increase oxygen requirements, adversely affecting the incidence of ascites (Lubritz et al. 1995). Many of the ascites-related traits like BW_{AS}, RV, TV, RV:TV and MORT-TOT were common in both experiments. However, in the previous study some score traits related to ascites syndrome (e.g. fluid accumulation in the abdominal cavity, heart and liver abnormality and etc.) were measured, whereas these traits were not measured in the current study and blood gas traits were investigated in its place.

The most significant results in the present study were found on GGA8 for the traits BW_{AS} at five weeks of age and RV:TV and on GGA10 for the traits BW_{AS} at two weeks of age and total mortality. These QTLs explain 5.7% and 5% of the total phenotypic variance for the traits $BW5_{AS}$ and RV:TV respectively, and 6.8% and 5.7% of the total phenotypic variance for the traits $BW2_{AS}$ and MORT-TOT respectively.

Weight ratio of the right ventricle wall relative to both ventricles and septum (RV:TV) that quantifies the degree of hypertrophy of the heart, is a good indicator for ascites syndrome (Huchzermeyer and de Ruyck 1986; Pakdel et al. 2004b). It is generally agreed that birds susceptible to the ascites syndrome are expected to have higher RV:TV than resistant birds. The QTL results of current study show that there are QTLs on GGA8 associated with traits $BW5_{AS}$, RV and RV:TV. These results suggest that there are genes in this region which regulate growth rate at 5 wk of age, the weight of right ventricular of the heart and ratio of RV:TV and therefore susceptibility of birds to ascites syndrome.

Among the factors causing ascites syndrome, inadequate oxygen supply relative to metabolic demand of tissues is a main factor. Low oxygen accessibility (high altitude) or high oxygen consumption (high performance or low ambient temperature) can increase the incidence of ascites (Julian 1993; Odum 1993). The traits that indicate such oxygen deficiency in susceptible birds are partial pressure of oxygen (pO_2) and/or oxygen saturation in arterial blood (sO_2). The results of current study showed suggestive linkage on chromosome 8 for pO_2 and sO_2. Because these two traits are genetically highly correlated (0.78, data not shown) and the most likely location of the QTL were the same in the current study, it seems reasonable to assume that the same QTL affected these traits.

In the present study two significant loci were detected for the growth traits ($BW2_{AS}$ and $BW5_{AS}$). The two QTL affecting growth rate in chickens were identified on GGA8 and GGA10. The QTL detected for BW at 2 wk ($BW2_{AS}$) on GGA10 did not significantly affect weight at 5 wk of age. This result suggest that the gene or genes involved in early growth and are responsible for skeletal growth and digestive organs are different with that affect the later growth of muscle tissue.

The present research has resulted in the identification and confirmation of five regions on two chromosomes which contain genes that are involved in susceptibility of chicken to develop ascites. To identify the genes involved in the ascites syndrome, additional microsatellite markers need to be mapped in these regions. Once the susceptible alleles or closely linked markers are identified, marker-assisted selection can be applied to select birds against ascites syndrome.

ACKNOWLEDGEMENTS

The authors are very grateful to the Islamic Republic of Iran, Ministry of Science, Research, and Technology (MSRT) and Egyptian Ministry of Higher Education and Scientific Research for financial support and Nutreco Breeding Research Center for data collection.

REFERENCES

Boldman KG, Kriese LA, Van Vleck LD, Van Tassell CP, Kachman SD (1995) A manual for use of mtdfreml. *A set of programs to obtain estimates of variances and covariances [draft]. U.S. Department of agriculture, agricultural research service*, USA.

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963-971.

De Greef KH, Janss LLG, Vereijken ALJ, Pit R, Gerritsen CLM (2001) Disease-induced variability of genetic correlations: Ascites in broilers as a case study. J. *Anim. Sci.* **79**, 1723-1733.

Haldane JBS (1919) The combination of linkage values and the calculation of distances between the loci of linked factors. *J. Genet.* **2**, 3-19.

Huchzermeyer FW, de Ruyck AMC (1986) Pulmonary hypertension syndrome associated with ascites in broilers. *Vet. Rec.* **119**, 94

Julian RJ (1993) Ascites in Poultry. *Avian Pathol.* **22**,419-454.

Julian RJ (2000) Physiological, management and environmental triggers of the ascites syndrome: A review. *Avian Pathol.* **29**, 519-527.

Knott SA, Elsen JM, Haley CS (1996) Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theor. Appl. Genet.* **93**, 71-80.

Lubritz DL, Smith JL, McPherson BN (1995) Heritability of ascites and the ratio of right to total ventricle weight in broiler breeder male lines. *Poultry Sci.* **74**, 1237-1241.

Maxwell MH, Robertson GW (1997) World broiler ascites survey 1996. *Poultry Int.* **36**, 16-30.

Odum TW (1993) Ascites syndrome: Overview and update. *Poultry Dig.* **52**, 14-22.

Pakdel A, Van Arendonk JAM, Vereijken ALJ, Bovenhuis H (2002) Direct and maternal genetic effects for ascites-related traits in broilers. *Poultry Sci.* **81**, 1273-1279.

Pakdel A, Van Arendonk JAM, Vereijken ALJ, Bovenhuis H (2004a) Genetic correlations among ascites-related traits and performance traits in broilers. *Submitted for publication*

Pakdel A, Van Arendonk JAM, Vereijken ALJ, Bovenhuis H (2004b) Genetic and phenotypic correlations for ascites-related traits in broilers measured under cold and normal conditions. *Submitted for publication.*

Rabie TSKM, Crooijmans RPMA, Bovenhuis H, Vereijken ALJ, Veenendaal T, van der Poel JJ, Pakdel A, van Arendonk JAM, Groenen MAM (2004) Genetic mapping of quantitative trait loci affecting susceptibility in chicken to develop the Pulmonary Hypertension Syndrome (HPS). *Submitted for publication*

Spelman RJ, Coppieters W, Karim L, van Arendonk JAM, Bovenhuis H (1996) Quantitative trait loci analysis for five milk production traits on chromosome six in the dutch holstein-friesian population. *Genetics* **144**, 1799-1807.

Van der Beek S (1995) The use of genetic markers in poultry breeding. *Doctoral thesis, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.*

Van Kaam JBCHM, van Arendonk JAM, Groenen MAM, Bovenhuis H, Vereijken ALJ, Crooijmans RPMA, van der Poel JJ, Veenendaal A (1998) Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. *Livest. Prod. Sci.* **54,** 133-150

Wideman RF, French H (2000) Ascites resistance of progeny from broiler breeders selected for two generations using chronic unilateral pulmonary artery occlusion. *Poultry Sci.* **79**, 396-401.

APPENDIX

pO_2, pCO_2 and Calculated Values for HCO_3, T_{CO_2}, sO_2:

pCO_2 is measured by direct potentiometry. In the calculation of results for pCO_2, concentration is related to potential through the Nernst equation. When a cartridge includes sensors for both pH and pCO_2, bicarbonate (HCO_3), total carbon dioxide (T_{CO_2}) are calculated.

$$\text{Log } HCO_3 = pH + \log pCO_2 - 7.608$$
$$T_{CO_2} = HCO_3 + 0.03\ pCO_2$$

pO_2 is measured ampermetrically. sO_2 (oxygen saturation) is the amount of oxyhemoglobin expressed as a fraction of the total amount of hemoglobin able to bind oxygen (oxyhemoglobin plus deoxyhemoglobin).

$$sO_2 = 100\frac{X^3 + 150X}{X^3 + 150X + 23400}$$

where $X = pO_2 \times 10^{[0.48(pH-7.4)-0.0013(HCO3-25)]}$

CHAPTER 4

Validation and fine-scale mapping of quantitative trait loci affecting Pulmonary Hypertension Syndrome (PHS) in broilers using advanced intercross line.

T.S.K.M Rabie[1], R.P.M.A. Crooijmans[1], H. Bovenhuis[1], A.L.J. Vereijken[2], A. Veenendaal[1], J.J. van der Poel[1], J.A.M. Van Arendonk[1], and M.A.M. Groenen[1].

[1] *Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, PO Box 338, 6700 AH Wageningen, The Netherlands,*

[2]*Nutreco Breeding Research Center, P.O. Box 220, 5830 AE, Boxmeer, the Netherlands.*

Will be submitted for publication

ABSTRACT Pulmonary hypertension syndrome (PHS) is one of the major quantitative traits that affects both of production and welfare in chicken. In this study, we confirmed and fine-mapped QTL for PHS-related traits on chicken chromosomes 2 and 4 using an advanced intercross line (AIL) derived from a cross between two genetically different broiler dam lines originating from the White Plymouth Rock breed. Combined linkage disequilibrium and linkage analysis (LD/LA) was used to refine the position of these two PHS related QTL previously identified in a total genome scan of generation 2. In total 47 microsatellite markers were used at an average marker spacing of 2 cM. The use of a high marker density in combination with the combined LD/LA analysis on generation 7 and 8 of the AIL, resulted in the reduction of the size of the confidence interval on GGA4 from the original 50-60 cM to around 10 cM.

***Key words**: Broilers, pulmonary hypertension, QTL ,validation, fine mapping.*

INTRODUCTION

Pulmonary hypertension syndrome (PHS) is a cascade of events that result in cardiac anomalies including an enlarged heart, right ventricular hypertrophy, variable liver changes, and accumulation of fluid in the abdominal cavity (ascites) (Riddell 1991). In broiler chickens, growth rate has increased at a rate of 4 to 5% per year over the past 30 years as a result of very successful genetic selection techniques (Havenstein et al. 1994; Julian 2000). Along with this selection for rapid growth, a selection for muscle mass has been increased without a comparable increase in organ size (Julian 2000). Sometimes fast growing broilers are unable to deliver sufficient oxygen to satisfy the metabolic demands of their rapid growth rates. High cardiac output associated with a high oxygen requirement increase pressure and blood flow through the lung (pulmonary hypertension, PH) (Julian 2000). The etiology of the PHS appears to be multifactoral. Both genetic and environmental factors such as altitude, temperature, lighting, ventilation, and nutritional factors influence the development of ascites (Julian 1993; Currie 1999). PHS is a complex trait and a large number of organs (e.g. heart, liver, lung, thyroid gland) are affected. QTL information therefore, will enable the identification of some of the major genetic factors underlying this trait and thus contribute to a better understanding of the physiological and biological background of PHS. Recently, several QTL related to the development of PHS have been identified (Rabie et al 2004). However, the confidence intervals of these QTL are large (40-80 cM) necessitating the further fine mapping of these regions.

Several studies have proposed theories and methods to refine the mapping position of QTL (Darvasi 1998; Riquet et al. 1999; Meuwissen and Goddard 2000). Of these methods, the variance component method using combined linkage and linkage disequilibrium (LD) has been considered as a promising approach for fine mapping (Meuwissen and Goddard 2001; Lund et al. 2003; Abdallah et al. 2003; Lee et al. 2004). The main advantage of LD mapping over linkage analysis is that it makes use of all historical recombination in populations of unrelated individuals, giving more precise estimates of gene location. To efficiently utilize LD a much higher marker density is required then that is used for within family linkage analyses. Therefore, polymorphic markers (microsatellites and/or single nucleotide polymorphism markers, SNPs) need to be developed within the QTL regions to increase mapping resolution.

The recent availability of a draft genome sequence (International chicken genome sequencing consortium 2004), as well as a high density SNP map (International Chicken Polymorphism Map Consortium 2004) of chicken has generated a rich

resource of both SNP and microsatellite markers. The objective of the current study is to validate and fine-map the QTL regions for PHS-related traits on GGA2 and GGA4 (Rabie et al. 2004) using microsatellite markers in generation 7 and 8 of an advanced intercross population.

MATERIALS AND METHODS

Experimental Population and phenotyping

An advanced intercross line (AIL) was produced from the experimental cross original used to identify QTL for a number of PHS related traits (Rabie et al. 2004). From generation two onwards, between 100 to 120 animals per generation were randomly intercrossed to produce the next generation. Animals in generation 2 were predominantly chosen from the families that, based on the regression analysis in G2/G3, were segregating for PHS related QTL. Each generation, inheritance was checked by typing the animals with a set of 8 microsatellites. Phenotypes were measured on the offspring in generations 7 and 8 whereas genotyping was done on both parents and offspring (generations 6 to 8). In total 10 full sib families (910 birds) were used with an average number of offspring per family of 89 ranging from 78 to 106 offspring per family. In order to identify individuals that were susceptible to PHS, the same cold stress temperature schedule was applied as described by Rabie et al. (2004).

Traits measured were: body weight at 3 weeks of age under cold stress ($BW3_{AS}$), body weight at 5 wks of age under cold stress ($BW5_{AS}$), right ventricular weight (RV), total ventricular weight (TV), RATIO (RV: TV), RV as a percentage of total $BW5_{AS}$ (%RV), TV as percentage of total $BW5_{AS}$ (%TV), total mortality (MORT-TOT), blood pH (pH), partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), blood bicarbonate concentration (HCO_3), total carbon dioxide content (T_{CO_2}) and oxygen saturation in arterial blood (sO_2). pCO_2 was measured by direct potentiometry. When a cartridge includes sensors for both pH and pCO_2, bicarbonate (HCO_3), and total carbon dioxide (T_{CO_2}) were also calculated using the following equations:

$\text{Log } HCO_3 = pH + \log pCO_2 - 7.608$

$T_{CO_2} = HCO_3 + 0.03\ pCO_2$

pO_2 is measured amperimetrically. sO_2 (oxygen saturation) is the amount of oxyhemoglobin expressed as a fraction of the total amount of hemoglobin able to bind oxygen (oxyhemoglobin plus deoxyhemoglobin) using the equation

$$sO_2 = 100\frac{X^3 + 150X}{X^3 + 150X + 23400}$$

where $X = pO_2 \times 10^{[0.48\,(pH-7.4) - 0.0013(HCO_3 - 25)]}$

Microsatellite Markers

Details about the PCR conditions and map location of the 19 microsatellites that were used for the initial validation of the QTL on GGA2 and GGA4 (see Figure 1) have been described previously (Crooijmans 2000; Groenen et al. 2000). For the fine mapping 34 additional microsatellites were developed located within the regions containing the putative QTL. CA-type microsatellites consisting of at least 10 repeats were identified in the draft chicken genome sequence (release GalGal2; International chicken genome sequencing consortium 2004) between positions 116.58 Mb and 130.97 Mb on GGA2 and between 36.32 Mb and 56.05 Mb on GGA4 using a small perl script. Primers flanking microsatellites were designed using PRIMER3 program (http://frodo.wi.mit.edu/). Criteria for primer development are the size of amplification product and the fluorescent dye (FAM, HEX, and TET) in such a way that all selected markers can be run simultaneously on an ABI-automated sequencer. Potential markers were tested for polymorphism on parents of Wageningen resource population (20 G_2 birds; individually). Amplification conditions for each marker were optimized by varying the annealing temperature. Details of the 47 microsatellites used in this study for fine-mapping are shown in Table 1.

Genotyping

Genotyping of the 47 microsatellite markers (22 markers for GGA2 and 25 markers for GGA4) was performed as described by Crooijmans et al. (1997). The PCR amplifications were carried out in 12µl reactions as described previously by Jennen et al. (2004). The PCR amplification product of different markers within each marker set from the same bird were diluted and pooled in such a way that each marker signal on ABI-377 automated sequencer (Applied Biosystems, Perkin Elmer, Foster City, CA 94404) had a peak height of approximately 1000. Electrophoresis was performed on a 6% denaturing polyacrylamide gel, Sequagel-6 (National Diagnostics, Atlanta, Georgia 30336) for 2 h on 12 cm gels, using an automated sequencer. Genotypes for all microsatellite markers were checked for allele inheritance by Crimap (Green et al. 1990). Map distances given in this study are sex-average distances in centimorgans on the haldane scale (Haldane 1919).

Analysis of Phenotypic Data

For the analysis of the phenotypic data a two step procedure was applied: first average adjusted progeny trait values were calculated by adjusting phenotypic observations for systematic environmental effects, and secondly a QTL analysis was undertaken using adjusted trait values as dependent variables. Data were adjusted for the fixed effects such as sex, week of hatching and date of measurement for blood gas traits. The last effect is related to the fact that measuring blood gas traits could not be done for all the birds on one day. Adjusted traits values were calculated using MTDFREML software (Boldman et al., 1995).

QTL Analysis

Full sib QTL Analysis

Full sib QTL analysis was undertaken as described previously (Rabie et al. 2004) using the regression interval mapping methodology as described by Van Kaam et al. (1998) in which a single QTL was fitted within each family. This method is an extension of the multi-marker regression method of (Knott et al. 1996) for outbred populations with a half sib family structure. Significance thresholds were calculated by permutation testing (Churchill and Doerge 1994). The significance threshold was calculated through permutation over both chromosomes simultaneously in one analysis. For each trait, 1000 permutations at 5 cM intervals across the genome were

performed. Because lower significance thresholds can be used in a validation experiment, a significance threshold of 10% was used.

Combined Linkage Disequilibrium Linkage Analysis (LDLA)

The most likely haplotypes for each pedigree were determined by SimWalk2 program (Sobel and Lange 1996), under the assumption of no linkage disequilibrium. Recombination fractions between the markers were estimated from physical distances in cM using an average of 340 Kb per cM. Using the haplotype set identified by SimWalk2, IBD probabilities of pairs of haplotypes at putative QTL positions (midpoint of each marker bracket) were predicted using the method as described by Meuwissen and Goddard (2001). The final step consisted of the calculation of the likelihood at the putative QTL positions using a variance component method.

The significance was determined by calculating twice the difference between the log likelihood for the null hypothesis (no QTL) and the log likelihood for the alternative hypothesis (QTL present). This difference was tested against the standard contingency table X^2 with one degree of freedom.

RESULTS

Previously we reported the localization of significant QTL for a number of traits related to PHS on GGA2 and GGA4 within a cross between two commercial broiler lines (Rabie et al. 2004). On GGA4 this region was located between markers *MCW0085* and *MCW0284* which on the chicken consensus map (Groenen et al. 2000) is between positions 112-167 cM. On GGA2 the region containing these PHS related QTL was even larger from 280-379 cM between markers *MCW0185* and *MCW0282* on the consensus linkage map.

To confirm the previously identified QTL for the PHS related traits and to further narrow down the regions containing these QTL, an advanced intercross line (AIL) was produced.

Validation study

The AIL was produced by randomly intercrossing 100 to 120 animals per generation to produce the next generation from generation two (G_2) onwards. Animals in G_2 were predominantly chosen from the families that, based on the regression analysis in G_2/G_3, were segregating for PHS related QTL. To validate the previously identified QTL on GGA2 and GGA4, a within family regression analysis was performed on 10 full sib families (G_6/G_7 and G_7/G_8 respectively). The results of the regression analysis are shown in Figure 1 with the 10% significance threshold indicated by a vertical line.

On GGA2 only the pCO_2 reached the 10% significance level, whereas on GGA4 two traits ($BW5_{AS}$ and HCO_3) reached the 10% significance level. As expected for a within family regression analysis QTL peaks were broad and confidence intervals are in the order of 60 cM.

Fine mapping using LDLA

To further narrow down the QTL regions on GGA2 and GGA4 a combined linkage disequilibrium and linkage analysis was performed (Lund et al. 2003). In this approach a marker is estimated to be identical by descent (IBD) rather then identical by state (IBS) based on the known pedigree and that information is combined with the available linkage data. Depending on the informativety of the markers used, this analysis requires a marker density of one marker every 1-5 cM. Because the number of markers available in these regions was not sufficient additional markers were first developed from the recently available draft chicken genome sequence.

Because microsatellites on average have higher information content then SNPs, it was decided to develop additional microsatellite markers on GGA2 and GGA4. The number of microsatellite markers successfully developed on GGA2 and GGA4 were 14 and 20, respectively. Six markers out of these 34 markers appeared to be monomorphic leaving 28 markers to be used for the present study. Combined with the original 19 markers brings this to 47 markers in the LDLA analysis. The marker spacing for these 47 markers varies from 1-7 cM with an average spacing of 5 cM.

Surprisingly, none of the traits reached the 5% significance threshold on GGA2. Although, pCO_2 almost reached this threshold (X^2=3.82; p=0.051) for marker brackets *ADL0114-ADL0271* and *MCW0417-MCW0314* these two marker brackets are not adjacent to each other. On GGA4 for the traits $BW5_{AS}$ and TV several marker brackets within the region between markers *MCW0398* and *MCW0402* reached this threshold with the highest observed X^2 of 7.82 (p=0.0052) for $BW5_{AS}$ between markers *LEI0122* and *MCW0396* (Figure 2).

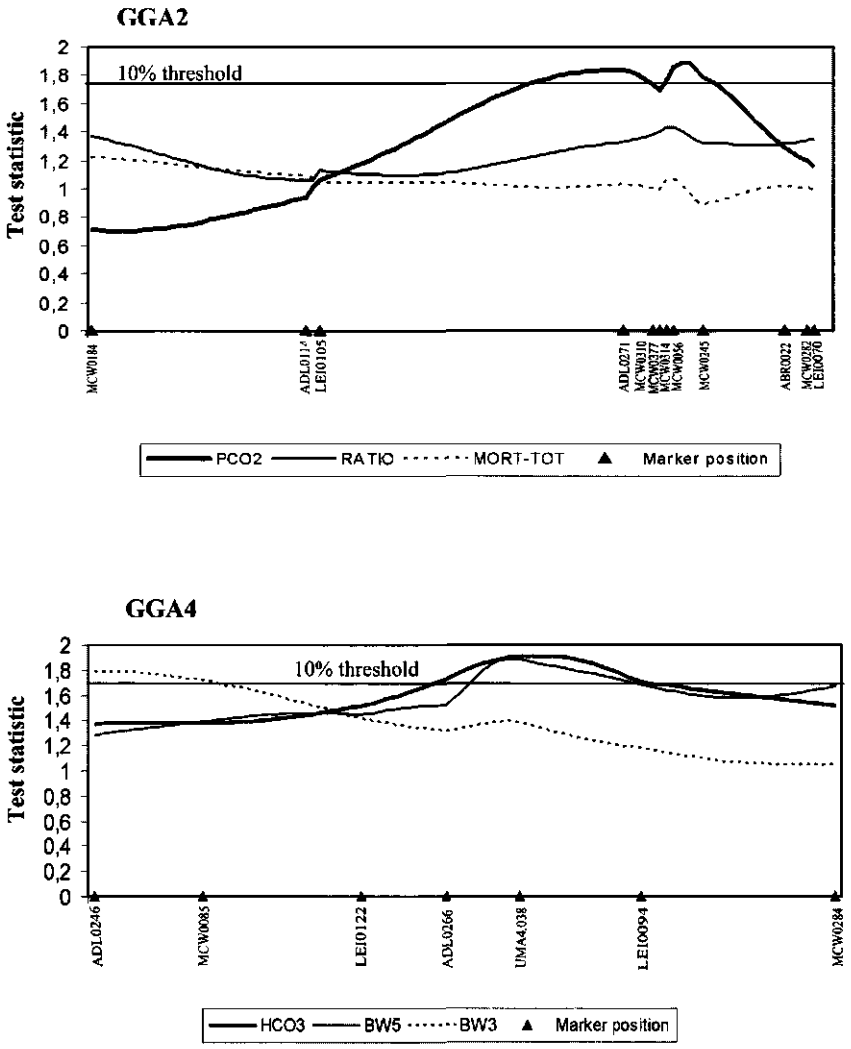


Figure 1. Validation of PHS-related traits on chicken chromosomes 2 and 4.

Table 1. Characteristics of exist chicken microsatellite markers and newly developed markers from draft chicken sequence.

Marker[1]	*Dye*[2]	*Length bp*[3]	*Forward primerf*	*Reverse primer*	*PCR*[4]	*Allele*[5]	*cM*	*Mb*[6]	*diff_Mb*[7]	*Contig*
GGA2										
MCW0185	TET	200-226	GATCTACTGTCATTTTAGTTT	TGAATAGATTTCAGTGAGTGC	55	3	302	105,35		Contig89.63
ADL0114	FAM	165-185	GGCTCATAACTACCTTTTTT	GCTCTACATTCCTTCAGTCA	50	5	324	111,34	5,99	Contig304.19
ADL0271	FAM	137-144	ATGAATGAAACCCATCTAAC	TCATCAGAAGCCCAAGCACA	55	2	354	118,05	6,70	Contig21.101
MCW0420	HEX	175-179	GTTTTTAGCCCTCCTCCTAC	CCATGCGTATCCATTACTGC	55	3		119,65	1,60	contig21.172
MCW0310	FAM	318-320	CCAACTAGGTGGAGGAACTTC	GATCCCAGGTAGGGCTGGAAG	55	2	359	120,55	0,90	Contig21.214
MCW0387	TET	356-364	GCCAGTTTTTACCCTCCAAG	CAGAGCTCAATCATGACATC	50	4		121,04	0,49	Contig21.223
MCW0422	HEX	284-292	AACCCTTAGAGAAGACAAAC	ATACTGCTCTGAACATTGTC	50	3		121,10	0,06	Contig21.225
MCW0414	TET	220-222	TCTCCTAACAAGGTGGAGAC	TGAGAAAAGGATTTCTCACC	50	2		121,54	0,44	Contig21.244
MCW0413	FAM	300-304	TGTCCTCAGACACTCATAAC	CTTTCTAAGGAGGTGATGTC	50	3		121,91	0,38	Contig21.260
MCW0412	TET	393-403	CTGACTCTCAAGCTTACTGG	ATACATAGAAGTCCCGTCTG	60	4		122,03	0,12	Contig21.268
MCW0056	TET	175-207	TGGTAACCTCTAACCTTGACG	AGTGAAGGAGACTCCACAGCCTCT	50	4	360	122,89	0,85	Contig21.301
MCW0166	HEX	194-210	GATCAGAAAGAACTGGAACTG	AGGAGTTAGTTGAACCAGAAC	55	7	358	123,02	0,13	Contig21.305
MCW0416	FAM	279-281	GGAATGTATCTCCCTCTCTC	GCTCTATTTGCTGCACAAGC	60	2		123,47	0,10	Contig21.311
MCW0417	FAM	190-210	GCGTGGAGAAGAATAAGCTG	CGTCCTCTACTTTGGCTTTC	60	5		123,63	0,17	Contig21.315
MCW0314	TET	278-284	GCCAGGCTACACCTCTTCTAG	GTTGGTATGATGGTATGATGC	55	2	360	123,65	0,02	Contig21.316
MCW0388	HEX	121-123	ATCCCCAAACCGTAATATTC	ATCTAGCTGGCATTTGCTTC	50	2		124,51	0,47	Contig21.356
MCW0245	FAM	284-290	ATCTATGGCCACCTCAAACTG	GATCTGTGCTGAACACAGCAG	55	2	364	125,21	0,71	Contig21.403
MCW0415	TET	293-303	CACTGCCCAAACTAGGCTTC	CAGCACTGTCAGTCTTATGG	50	4		127,07	1,85	Contig205.13
MCW0418	FAM	130-136	CTGGAAGGTCATCCTCAAAC	CAAGGTTTCCAGATCCACAC	55	4		127,49	0,43	Contig205.53
MCW0419	TET	129-133	AATGGTGGCAAGGTTATTAG	ATTTATATGATGGGTTGGTC	55	2		129,27	1,78	Contig72.298
MCW0421	TET	161-165	CTTTACCTGCCACCATTAGC	CTGTGATGCACAGTGAGTCC	50	3		129,88	0,61	Contig72.240
ABR0022	FAM	202-216	CACACCAGTGAAGTAGCCCTC	TAACACAGTGCTGAGGACAGC	55	3	374	130,33	0,46	Contig72.196
MCW0282	HEX	287-308	GATCCTAAGGTTCTACTACAG	AGTATTTCACTAGTGAACTACC	45	3		131,57	1,24	Contig72.107
LEI0070	HEX	185-223	TGCGGAGAGCAATTAGTCTGC	GGAAAACAATCACTGCCTCG	55	6	379	132,66	1,09	Contig72.7

(Continued)

Table 1 Continued

Marker[1]	*Dye*[2]	*Length bp*[3]	*Forward primerf*	*Reverse primer*	*PCR*[4]	*Allele*[5]	*cM*	*Mb*[6]	*diff_Mb*[7]	*Contig*
GGA4										
ADL0246	TET	149-171	GCAGGCTGATAGAAAAATGC	CTGCAAGCTGCTCTGGTATT	55	5	112	37,66		Contig3.127
MCW0408	HEX	228-245	GGTGCTACACGAAGGTACTG	TTTCTGAGCTGCTTGTCCTC	55	2		38,27	0,61	Contig3.371
MCW0085	FAM	272-282	GTGCAGTTATATGAAGTCTCTC	GGTATCAGGGCTTCTGAAACA	50	5	120	38,79	0,52	Contig3.388
MCW0410	TET	144-162	CACGAAGAAGAGAACCTTCC	CACCTCCTGTGTTGGTCCAG	55	2		39,84	1,05	Contig3.424
MCW0398	TET	203-207	GTTCTTCCATCAGAGCACAG	GGAGCGTAGACTGTATCAGG	55	2		40,56	0,73	Contig3.448
LEI0122	HEX	289-300	AATCCCTATAGAACTTTGTGC	GATCTTACTGGATTACCATTC	55	6	132	40,91	0,34	Contig3.453
MCW0396	FAM	245-253	CTCACTTTCTGCAGTTACCC	CTGGTGACACCTTCAAACTG	50	3		42,14	0,61	Contig3.492
MCW0400	FAM	142-144	GGATTTATCCCATGCCTCAG	GGGACAGAGAGAAGCAGTGG	55	2		42,50	0,36	Contig3.513
MCW0397	TET	258-272	TGAGTCAGGCTTGATTCTGC	ACCACCCCTCACATGGATTC	50	3		43,07	0,57	Contig3.533
MCW0401	HEX	257-267	GAGTGGAATTACCGGAGAAC	CTAGCTACTGTTAGGTGGAG	50	3		44,31	1,24	Contig3.579
MCW0390	FAM	275-279	TACTACACAACCCCCTCTAC	GACTAATTCAGGGTGCTCTC	50	3		44,52	0,21	Contig3.591
MCW0402	HEX	205-230	ACTGTGCCTAGGACTAGCTG	CCTAAGTCTGGGCTCTTCTG	55	4		44,72	0,19	Contig3.595
ADL0266	HEX	109-136	GTGGCATTCAGGCAGAGCAG	AATGCATTGCAGGATGTATG	55	5	138	45,52	0,81	Contig3.619
MCW0391	TET	352-354	AGGATTACCAGCTCCCAGAC	CTTTTCACTGCTCCGTAGAC	50	2		46,20	0,67	Contig3.646
MCW0405	TET	296-321	GGAGCTGAGATTTGTTGAGC	GCTGCAAGGTGAAGGAAAAC	55	3		47,01	0,81	Contig3.670
UMA4.038	FAM	179-215	CATTTGCAAGTGCCATACAG	GCCCTGGTAAACTGGTGTCC	45	4	144	47,35	0,34	Contig3.688
MCW0404	FAM	344-354	GCACAGACTAAACCTTGCTC	GTTAGTAAGCAGGGGGTCTG	55	2		47,98	0,64	Contig3.707
MCW0403	FAM	198-202	GGTACGGAAGAACTGATAGG	GACATGGTAGAACTGCAAGG	55	2		48,13	0,14	Contig3.710
MCW0393	HEX	125-135	GGGAGAGGTGAGACAGATAG	TCTAGAGGAGGCTTTGCTAC	50	3		48,24	0,03	Contig3.724
MCW0394	HEX	350-360	ATCAAGTCCTCCGATACTGC	GAACAACTGGCTAGGCTAAG	50	2		50,15	1,90	Contig3.800
LEI0094	TET	253-285	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC	55	5	153	50,33	0,18	Contig3.808
MCW0395	FAM	326-332	TGCTTGTGCAGAGATGAAGC	AGTAAGTACAGAGCCACTGC	50	3		50,50	0,17	Contig3.815
MCW0411	FAM	382-384	GAAGGTCTCCCAGCTATAAG	TTTGGTGTGGGTAGAAGGTG	50	2		50,89	0,38	Contig3.824
MCW0409	TET	379-397	GCACACTGAGCTACCTTTAG	GTTCTGGAGAAGACTGCTTG	50	3		51,35	0,46	Contig3.831
MCW0284	TET	238-246	CAGAGCTGGATTGGTGTCAAG	GCCTTAGGAAAAACTCCTAAGG	50	2	167	53,90	2,55	Contig3.901

[1] Markers in Italic were new developed using the draft chicken genome sequence. Others were drived from consensus linkage map (Groenen et al. 2000).

[2] Fluroscent amidite dye attached to the 5' end of the forward primer, which allows for detection on the ABI-373/377 DNA sequencer. [3] Fragment lengths of the alleles found after PCR according to the ABI genescan-500 standard. [4] The optimal annealing temperature in the PCR reaction. [5] The number of alleles found during genotyping. [6] The position of marker in Mb according to the draft chicken genome sequence data. [7] The distance between the two following marker in Mb.

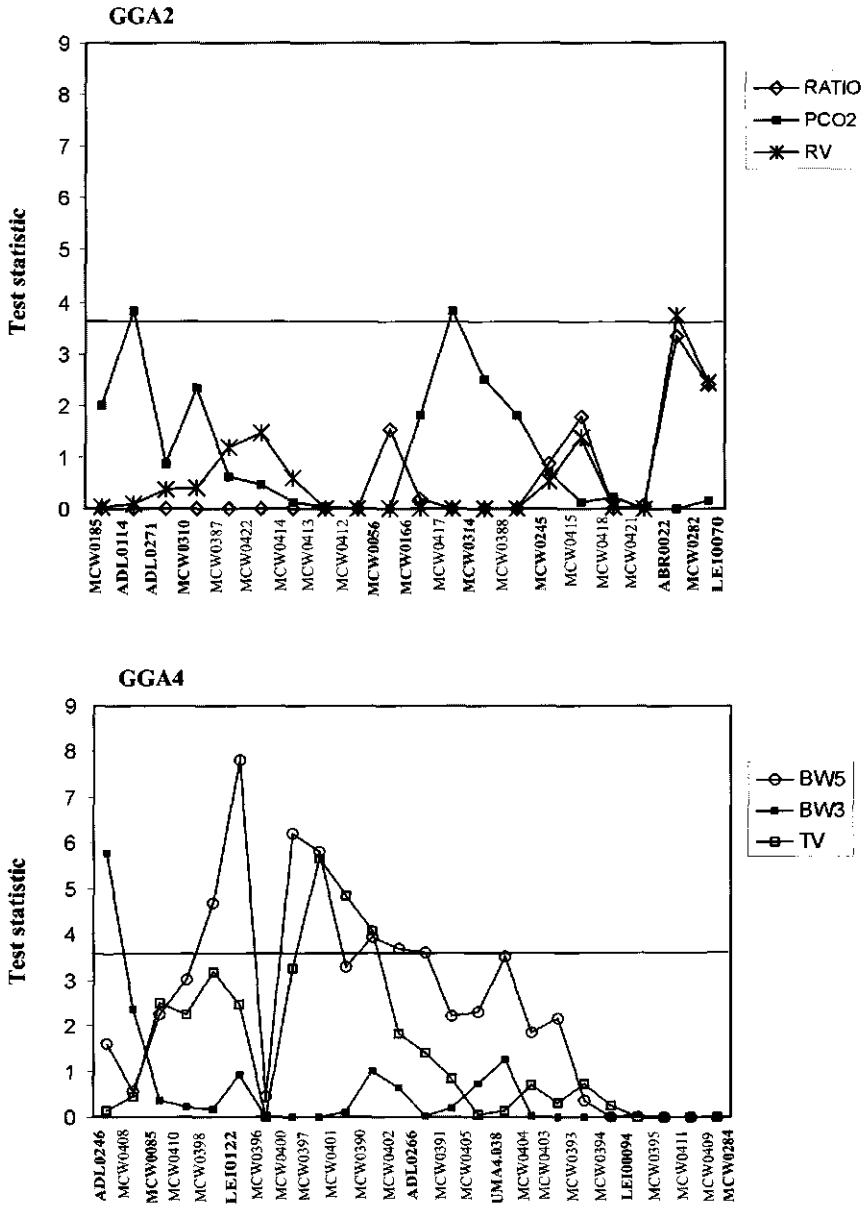


Figure 2. Fine mapping of PHS-related traits on chicken chromosomes 2 and 4. (5% threshold, X^2=3.82; p=0.051)

DISCUSSION

QTL mapping in chicken and other farm animals has seen a dramatic increase due to the similar dramatic increase in molecular resources and techniques needed to perform total genome scans. Consequently, hundreds of QTL have been described in chicken for a variety of traits (for a recent overview see the ChickAce database at https://acedb.asg.wur.nl/) although the majority of these traits a related to growth and body composition and traits related to disease are scarce. Recently, we have described the results of a total genome scan aimed at the identification of QTL for PHS (Rabie et al. 2004). Due to the complexity of this trait and to the choice of a commercial broiler x broiler cross the statistical power to detect QTL which influencing this trait is limited. Nevertheless, several regions with evidence for significant or suggestive QTL could be identified in that study. Here we confirm the segregation of two of these QTL on GGA2 and GGA4, although for GGA2 this confirmation is based on another trait related to PHS then in the original G_2/G_3 cross. PHS is a complex disorder characterised by a cascade of events affecting several organs, most notably the heart. All these effects are related to an inadequate oxygen supply relative to metabolic demand of tissues is the main factor. The traits that indicate such oxygen deficiency in susceptible birds are pO_2, HCO_3 sO_2 and pCO_2. In the original genome scan in the G_2/G_3, PHS related measurements were restricted to the condition of the heart and the liver, the appearance of ascites in the abdomen and reduced growth. To further improve the detection of birds affected by PHS, it was decided in the current study to also measure the traits related to oxygen saturation in the blood.

One has to realize that none of these traits on its own completely describes PHS. Often different combinations of significant or suggestive QTL for these traits are found within a particular region (Rabie et al. 2004) providing further evidence of the presence of a QTL at that particular chromosomal location. The PHS QTL previously identified on GGA2 was based on a significant F statistic for TV, %RV and RATIO. These three QTLs were located in a large region on GGA2 between positions 300 and 380 cM of the consensus linkage map (Groenen et al. 2000). Furthermore the maximum of these three QTL was at different locations within this large region. Due to the large confidence intervals, it could not be concluded whether this region consisted of a single QTL or multiple closely linked QTL. In the current study, the trait pCO_2 showed significance at the 10% threshold between location 340-380 cM on GGA2, the region where %RV and RATIO had there maximum in the G_2/G_3. No effect was seen for any of the traits measured in the current study between 300 and 340 cM. The most likely explanation for these results are the lower statistical power

of the current experiment and the fact that pCO_2 probably is a better indicator for PHS then the heart related measurements TV, %RV and RATIO. However, it can not be excluded that this region on GGA2 consists of two linked QTL regions (see LDLA below).

On GGA4 the QTL for PHS could also be confirmed as the traits $BW5_{AS}$ and HCO_3 both reached the 10% significance level with their maximum between 130 and 155 cM. However, the curves of the F statistic are flat and the QTL can be located anywhere in the region on GGA4 that was analysed in the current study (112-167 cM, between markers *ADL0246* and *MCW0284*). In the analysis of the G_2/G_3 the trait $BW5_{AS}$ also reached the significance threshold (5% in that study) at this location whereas MORT-TOT reached and TV approached the suggestive threshold at the same location as well.

The further reduction of a previously identified QTL and the identification of the underlying genetic cause are the real challenges in QTL mapping, in particular for complex traits like PHS. In the current study, we applied combined linkage disequilibrium and linkage analysis to further reduce the size of the confidence interval of the confirmed QTL on GGA2 and GGA4 (Figure2). On GGA2 only pCO_2 approached a p-value of 0.05 between marker brackets *ADL0114-ADL0271* and *MCW0381* and *MCW0314* around positions 324-354 and 358-362 of the consensus map respectively. Together with the previous results from the G_2/G_3 (Rabie et al. 2004) this might indicate that there are indeed two different linked QTL within this location.

The LDLA analysis on GGA4 gave significant results for $BW5_{AS}$ and TV. The results from the analysis for $BW5_{AS}$ indicates that the most likely location of this QTL is between markers *MCW0398* and *MCW0402* which translated to the chicken consensus linkage map is between positions 130 and 140 cM on GGA4. This region overlaps with the location of the QTL for TV between markers *MCW0397* and *MCW0402* whose location (calculated relative to markers on the consensus linkage map) on GGA4 is between positions 134 and 140 cM. The location between positions 130 and 140 cM corresponds to the region between 40.56 Mb and 44.72 Mb on the sequencer map of this chromosome (chicken sequence build GalGal2). Whereas the region on GGA4 corresponding to positions 112 to 167 cM on the consensus map 187 genes contains 187 ensembl gene predictions (see http://www.ensembl.org/Gallus_gallus/) the region that has been identified only contains 18 such gene predictions.

Although a reduction in the size of the QTL region could only be clearly obtained for the QTL on GGA4, the results show that the combined use of linkage disequilibrium and linkage analysis provides a powerful method the further narrow down the confidence interval of the QTL. As discussed above, the nature of the QTL and the possibility of multiple closely linked QTL complicate the analysis. Furthermore, when the marker density approaches densities close to 1 marker per cM as is the case for some of the areas within the regions studied here, even modest error rates have a dramatic effect on the reconstruction of the haplotypes used in LDLA analysis (data not shown). Further analysis therefore is required. The inclusion of SNP markers within these regions will further assist in the identification of shared haplotype fragments between the animals in the different families thereby further utilizing the full power of the advanced intercross for the fine mapping of these regions.

ACKNOWLEDGEMENTS

The authors very grateful to the Egyptian Ministry of Higher Education and Scientific Research (Project No. 29110/416000) for financial support, Jolanda de Gooyert and Anita Grootemaat for technician support, and Nutreco Breeding Research Center for data collection.

REFERENCES

Abdallah JM, Goffinet B, Cierco-Ayrolles C, Perez-Enciso M. (2003) Linkage disequilibrium fine mapping of quantitative trait loci: a simulation study. *Genet. Sel. Evol.* **35**, 513-32.

Boldman KG, Kriese LA, Van Vleck LD, Van Tassell CP, Kachman SD (1995) *A manual for use of mtdfreml. A set of programs to obtain estimates of variances and covariances* [draft]. U.S. Department of agriculture, agricultural research service, USA

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138,** 963-971.

Crooijmans RPMA (2000) Gene hunting: molecular analysis of the chicken genome. *Doctoral thesis, Animal breeding and genetics group, Wageningen University, The Netherlands.*

Crooijmans RPMA, Dijkhof RJM, van der Poel JJ, Groenen MAM (1997) New microsatellite markers in chicken optimized for automated fluorescent genotyping. *Anim. Genet.* **28,** 427-437.

Currie RJW (1999) Ascites in poultry: Recent investigations. *Avian Pathol.* **28,** 313-326.

Darvasi A (1998) Experimental strategies for the genetic dissection of complex traits in animal models. *Nat. Genet.* **18**, 19-24.

Green P, Falls K, Crooks S (1990) Documentation for CRI-MAP Version 2.4 (3/26/90).

Groenen MAM, Cheng HH, Bumstead N, Benkel B, Briles E, Burke T, Burt DW, Crittenden LB, Dodgson J, Hillel J, Lamont S, Ponce de Leon FA, Soller M, Takahashi H, Vignal A (2000) A consensus linkage map of the chicken genome. *Genome Res.* **10,** 137-147

Haldane JBS (1919) The combination of linkage values and the calculation of distances between the loci of linked factors. *J. Genet.* **2,** 3-19

Havenstein GB, Ferket PR, Scheideler SE, Larson BT (1994) Growth livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. *Poultry Sci.* **73**, 1785-794.

International Chicken Genome Sequencing Consortium (2004) Sequencing and comparative analysis of the chicken genome, Gallus gallus. *Nature*, in press

International Chicken Polymorphism Map Consortium (2004) A polymorphism map for chicken with 2.8 million SNPs. *Nature*, In press

Jennen DGJ, Vereijken ALJ, Bovenhuis H, Crooijmans RPMA, van der Poel JJ, Groenen MAM (2004) Confirmation of quantitative trait loci affecting fatness in chicken. *Gent. Sel. Evol.* In press

Julian RJ (2000) Physiological, management nad environmental tiggers of the ascites syndrome: A review. *Avian Pathol.* **29**, 519-527

Julian RJ (1993) Ascites in poultry. *Avian Pathol.* **22,** 419-454.

Knott SA, Elsen JM, Haley CS (1996) Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theoret. Appl. Genet.* **93,** 71-80.

Lee SH, Julius HJ, van derWerf (2004) The eciency of designs for fine-mapping of quantitative trait loci using combined linkage disequilibrium and linkage. *Genet. Sel. Evol.* **36**, 145–161

Lund MS, Sørensen P, Guldbrandtsen B, Sorensen DA (2003) Multitrait fine mapping of quantitative trait loci using combined linkage disequilibria and linkage analysis. *Genetics* **163**, 405–410

Meuwissen THE, Goddard ME (2000) Fine mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. *Genetics* **155**, 421-430.

Meuwissen THE, Goddard ME (2001) Prediction of identity by descent probabilities from marker haplotypes. *Genet. Sel. Evol.* **33**, 605–634.

Rabie TSKM, Crooijmans RPMA, Bovenhuis H, Vereijken ALJ, Veenendaal A, van der Poel JJ, Van Arendonk JAM, Pakdel A, Groenen MAM (2004) Genetic mapping of quantitative trait loci affecting susceptibility in chicken to develop the Pulmonary Hypertension Syndrome (PHS). *Submitted For publication*

Riddell C (1991) Developmental, metabolic, and miscellaneous disorders, In Diseases of poultry. *9th ed., eds Calnek, B.W., Barnes, H.J., Beard, C.W., Reid, W.M. and Yoder, H.W. Jr., Iowa State University Press, Ames, IA, pp. 839-841.*

Riquet J, Coppieters W, Cambisano N, Arranz J-J, Berzi P, Davis SK, Grisart B, Fanir F, Karim L, Mni M, Simon P, Taylor JF, Vanmanshoven P, Wagenaar D, Womack JE, Georges M (1999) Fine mapping of quantitative trait loci by identity by descent in outbred populations: application to milk production in dairy cattle. *Proc. Natl. Acad. Sci.* **96**, 9252–9257.

Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN (1997) Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. *Nat. Genet.* **16**, 154-160

Sobel E, Lange K (1996). Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *Am.J.Hum.Genet.* **58**, 1323-1337.

Van Kaam JBCHM, van Arendonk JAM, Groenen MAM, Bovenhuis H, Vereijken ALJ, Crooijmans RPMA, van der Poel JJ, Veenendaal A (1998) Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. *Livest. Prod. Sci.* **54,** 133-150

CHAPTER 5

A Radiation Hybrid Map of Chicken Chromosome 4

Tarik S.K.M. Rabie[1], Richard P.M.A. Crooijmans[1], Mireille Morisson[2], Joanna Andryszkiewicz[1], Jan J. van der Poel[1], Alain Vignal[2], Martien A.M. Groenen[1]

[1] *Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, PO Box 338, 6700 AH Wageningen, The Netherlands,*

[2]Laboratoire de génétique cellulaire, Institut national de la recherche agronomique, 31326 Castanet-Tolosan, France.

Mammalian Genome: 15, 650-569 (2004)

Abstract The mapping resolution of the physical map for chicken Chromosome 4 (GGA4) was improved by a combination of radiation hybrid (RH) mapping and bacterial artificial chromosome (BAC) mapping. The ChickRH6 hybrid panel was used to construct an RH map of GGA4. Eleven microsatellites known to be located on GGA4 were included as anchors to the genetic linkage map for this chromosome. Based on the known conserved synteny between GGA4 and human Chromosomes 4 and X, sequences were identified for the orthologous chicken genes from these human chromosomes by BLAST analysis. These sequences were subsequently used for the development of STS markers to be typed on the RH panel. Using a logarithm of the odds (LOD) threshold of 5.0, nine linkage groups could be constructed which were aligned with the genetic linkage map of this chromosome. The resulting RH map consisted of the 11 microsatellite markers and 50 genes. To further increase the number of genes on the map and to provide additional anchor points for the physical BAC map of this chromosome, BAC clones were identified for 22 microsatellites and 99 genes. The combined RH and BAC mapping approach resulted in the mapping of 61 genes on GGA4 increasing the resolution of the chicken–human comparative map for this chromosome. This enhanced comparative mapping resolution enabled the identification of multiple rearrangements between GGA4 and human Chromosomes 4q and Xp.

Key words: *Chicken, radiation hybrid, comparative map, chromosome 4*

INTRODUCTION

Comparative genomics plays an important role in the understanding of genome dynamics during evolution and as a tool for the transfer of mapping information from species with gene-dense maps to species whose maps are less well developed (O'Brien et al. 1993, 1999). For farm animals, therefore, the human and mouse have been the logical choice as the model species used for this comparison. Medium-resolution comparative maps have been published for many of the livestock species, including pig, cattle, sheep, and horse, identifying large regions of conserved synteny between these species and man and mouse. More detailed analyses subsequently showed the presence of many internal rearrangements resulting in altered gene orders within these syntenic blocks (Sun et al. 1997, 1999; Rink et al. 2002; Larkin et al. 2003). In chicken, the first comparative maps indicated an extraordinary conservation of synteny between this species and mammals, even though these species diverged around 300–350 Myr ago (Smith et al. 1997; Groenen et al. 1998; Nanda et al. 1999; Burt et al. 1999). However, subsequent detailed mapping studies on a number of chicken chromosomes indicated that the number of intrachromosomal rearrangements was considerably higher then thus far anticipated (Suchyta et al. 2001; Crooijmans et al. 2001; Buitenhuis et al. 2002; Jennen et al. 2002, 2003), clearly showing the need for an increased gene density on the chicken maps. Although a considerable number of genes have been mapped on the chicken linkage map, achieving the required high gene density necessary to identify the different conserved blocks within the regions of conserved synteny is not very practical because of the required polymorphism in the markers used. An alternative mapping approach that circumvents this problem is by using the radiation hybrid mapping technique (Walter et al. 1994). Originally, Goss and Harris (1975) first developed a technology for physical map generation using irradiation and fusion gene transfer (IFGT). This technique, however, was rarely used until advances in molecular genetics allowed efficient polymerase chain reaction (PCR) screening of the RH panels. Therefore, it was recently rediscovered (Cox et al. 1990; Walter et al. 1994) as an effective approach to building ordered maps of sequence-tagged sites. Since then radiation hybrid (RH) cell lines have proven to be a powerful resource for gene mapping, particularly in mammals, and they have been used to develop detailed physical gene-dense maps in human (Gyapay et al. 1996), zebrafish (Geisler et al. 1999), mouse (McCarthy et al. 1997), pig (Yerle et al. 1998), and horse (Kiguwa et al. 2000). Recently, a RH panel has also been constructed for chicken (Morisson et al. 2002), which has been used in the present study to improve the gene density on chicken Chromosome 4 (GGA4).

Genes mapped on the chicken linkage map for GGA4 (Groenen et al. 2000; Schmid et al. 2000) indicated that most of this chromosome showed synteny with

human Chromosome 4 and the q arm of the human X chromosome. In addition, genes located on a number of different human chromosomes (HSA2, 3, and 5) mapped to the end of the linkage group of GGA4, most likely representing the tip of the q arm of this chromosome. These results were further confirmed by zoo-FISH experiments between HSA4 and GGA4 (Chowdhary and Raudsepp 2000). These results indicated that the region from GGA4q1.1 to GGA4q2.6 is syntenic with HSA4.

Recently, large collections of chicken gene sequences have become available in the form of expressed sequenced tags (EST) (Tirunaguru et al. 2000; Abdrakhmanov et al. 2000; Boardman et al. 2002). Clustering of these ESTs followed by sequence comparisons to human genes indicates that the chance of finding a chicken ortholog for a particular human gene is around 2 out of 3. This resource of chicken EST sequences was used in the current study to improve the gene density on GGA4 both by using the ChickRH6 panel and the chicken BAC library constructed in Wageningen (Crooijmans et al. 2000).

MATERIALS AND METHODS

Selection of markers and genes.

For type II markers, 23 chicken microsatellite markers covering the p and q arms of GGA4 were selected from the published chicken genetic map (Groenen et al. 2000). Primer information for microsatellite markers located on GGA4, such as a primer sequence and PCR conditions, can be found at ARKdb farm animal database (http://www.thearkdb.org/) and ChickAce (https://acedb.asg.wur.nl/).

For type I markers, 127 primer pairs derived from EST sequences representing chicken orthologs to genes located on HSA4 (102 genes) and HSAX (25 genes) were selected. Potential chicken orthologous sequences were first identified by a BLAST database search (BLAST v2.0 software; http://www.ncbi.nlm.nih.gov/blast) with the human mRNA sequences representing all the genes known to be located on HSA4 and HSAX. The BLAST analysis was performed against a local chicken EST database containing all publicly available chicken EST sequences. Homologous chicken ESTs were subsequently used in a BLAST search against all human mRNAs (E-values at least e -50) to distinguish between orthologous and paralogous sequences. Only those chicken EST sequences that most likely represented the chicken ortholog of a gene located in human on HSA4 and HSAX were used for further analysis. Primer pairs were designed preferably within a single exon. For those cases where the resulting PCR product would be too small (<100 bp), primers were designed in adjacent exons

spanning the intervening intron. In these cases, preferably the smaller introns were chosen. Primers were designed with the PRIMER3 program (http://frodo.wi.mit.edu/) (Table 1). Amplification conditions for each marker were optimized by varying the annealing temperature to produce a single amplicon of the predicted length with chicken genomic DNA and no amplification with genomic hamster DNA. Only primer pairs that gave a clear amplification product with the chicken and not with the hamster DNA were used for RH typing.

RH panel screening.

The ChickRH6 panel (Morisson et al. 2002) consists of a total of 90 hybrids. Chicken and hamster genomic DNA and TE buffer were used as positive and negative controls, respectively. Ten to 25 ng of each panel DNA was amplified in a 384-well plate in a 6-ll mixture containing 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1 mM tetramethylammoniumchloride (TMAC), 0.1% Triton X-100, 0.01% gelatin, 0.2 mM of each dNTP, 0.125 U Silverstar polymerase (Eurogentec, Liege, Belgium) and 1.2 pmol of each primer. Amplification products were separated by electrophoresis on 1.5% ethidium bromide-stained agarose gels in 0.5 TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA, pH 8.3), and reactions were scored for the presence or absence of the specific amplification product. Each marker was typed independently in duplicate.

BAC library screening.

The BAC library was screened for type I and type II markers by two-dimensional PCR (Crooijmans et al. 2000). In the first round, positive plates were identified followed by the PCR analysis of the row and column pools of the positive plates. At least one BAC clone from each of the markers was identified.

PCR conditions.

For both RH panel and BAC screening, the PCR reactions were started with 5 min at 95°C followed by 35 cycles for 30 sec at 95°C, 45 sec at 45°C, 50°C or 55°C annealing temperature, and 60 sec at 72°C, followed by a final elongation step at 72°C for 10 min, and finally stop step at 4°C. PCR reactions were performed in PCR system Biometra_ using 384-well plates.

Table 1. Primer information for genes used in this study.

Gene symbol[1]	*Human cytogenetic map position*	*Accession Number*		*PCR Product size (bp)*	*Temp*[3].	*Rf*[4]	*BAC Clone*
		Human[2]	*Chicken*				
ABCE1	4q31	002940	JMB20j18r1	118	50	0.27	bW028B23
ADH5	4q21-q25	000671	TC6849	168	50	-	bW029H01
AFAP	4p16	021638	NP344463	120	50	0.13	bW011H18
AGXT2L1	4q25	031279	46351.2	148	50	0.17	bW028C02
ALP	4q35	014476	345746.3	178	50	0.14	bW037A03
ANK2	4q25-q27	001148	47969.1	267	50	0.13	bW036M09
AP1S2	Xp22.31	003916	337157.1	140	50	-	bW003B19
APG-1	4q28	014278	JMB35g8r1	146	50	0.13	bW015H15
ARGBP2	4q35.1	003603	54771.8	104	50	0.13	bW020H21
ARHGEF9	Xq11.1	015185	334327.1	162	50	0.41	bW008I04
ATP7A	Xq13.2-q13.3	000052	34574.1	360	50	-	-
BCMP1	Xp11.4	031442	500086.1	115	50	-	bW039C07
BMPR1B	4q22-q24	001203	349923.1	157	50	0.26	bW015M22
BTK	Xq21.33-q22	000061	345575.2	154	50	-	-
CAMK2D	4q25	001221	346674	192	50	-	bW007B01
CCNA2	4q25-q31	001237	56394	104	50	-	-
CCNG2	4q13.3	004354	44206	145	50	-	bW013P18
CCNI	4q13.3	006835	TC6787	129	50	0.11	bW080M14
CDKL2	4q21.1	003948	56394	139	50	0.12	bW034M24
CENTG1	4q21.21	017593	47690.1	118	50	0.40	bW010H02
CLCN3	4q33	001829	JMB7c4r1	147	50	0.57	bW064B10
CLCN5	Xp11.23-p11.22	000084	346697.1	324	50	-	bW046O02
CLOCK	4q12	004898	TC7301	144	50	-	-
CMG2	4q21.22	058172	345485.2	328	45	-	-
CNGA1	4p12-cen	000087	NP346095	242	50	0.07	bW121F14
COPS4	4q21.3	016129	TC4353	121	50	0.13	bW104P05
COVA1	Xq25-q26.2	006375	334353.3	131	50	0.51	bW023C04
CPE	4q32.3	001873	TC4299	137	50	0.36	bW023N17
CRMP1	4p16.1-p15	001313	TC4156	147	50	-	-
CUL4B	Xq23	003588	38850.1	148	50	-	bW039K18
D4S234E	4p16.3	014392	JMB7123745	110	45	-	bW094K13
DC2	4q24	021227	TC7399	130	55	-	bW006A08
DCK	4q13.3-q21.1	000788	337391.2	131	50	0.10	bW095E06
DCX	Xq22.3-q23	000555	57758.2	196	50	-	bW012F01
DDX3X	Xp11.3-p11.23	001356	56851.1	126	50	-	-
DIAPH2	Xq22	006729	347324.1	184	50	0.73	bW012P24
DJ473B4	Xq26.3	019556	341971.1	121	45	-	-
DKC1	Xq28	001363	337406.3	150	50	0.58	-
DKK2	4q25	014421	342800.1	125	50	0.18	bW026B08
DLG3	Xq13.1	021120	58956.1	129	50	-	bW017B24
ED1	Xq12-q13.1	001399	337200.2	166	50	-	bW010A11
EIF2S3	Xp22.2-p22.1	001415	335006.2	157	55	-	bW037A22
EIF4E	4q21-q25	001968	TC6391	202	50	0.13	-
ELF2	4q28	006874	BI394288	183	50	-	bW031P04

(Continued)

Table 1. Continued

Gene symbol[1]	*Human cytogenetic map position*	*Accession Number*		*PCR Product size (bp)*	*Temp*[3].	*Rf*[4]	*BAC Clone*
		Human[2]	*Chicken*				
ELOVL6	4q25	024090	AI981662	203	50	0.14	bW011K04
EPHA5	4q13.1	004439	334182.2	326	50	-	bW016J03
FACL2	4q34-q35	021122	339343.2	130	50	-	-
FAT	4q34-q35	005245	331914.2	422	50	0.31	bW061E08
FBXO8	4q34.1	012180	JMB30p13r1	184	50	-	bW048H08
FGB	4q28	005141	TC6789	204	50	-	bW013I12
FGF2	4q26-q27	002006	TC7431	280	50	0.16	bW014I21
FLNA	Xq28	001456	53860.1	107	50	0.18	bW041F20
FMR1	Xq27.3	002024	34198.1	138	45	0.60	bW010I17
GAB1	4q28.3	002039	1489.1	210	50	0.09	-
GABRA4	4p12	000809	17087.1	122	50	0.20	bW040D21
GALNT7	4q32.1	017423	5917	127	55	0.12	bW060F11
GDI1	Xq28	001493	TC6728	153	50	-	bW030F13
GLRB	4q31.3	000824	25861	171	50	0.50	-
GPM6A	4q34	005277	TC7231	109	50	0.18	-
GRIA2	4q32-q33	000826	43814.1	140	50	-	bW036I02
GRID2	4q22	001510	38183	249	50	0.19	bW056K10
GRPEL1	4p16	025196	JMB32n8r1	192	55	0.18	bW012N03
GUCY1A3	4q31.1-q31.2	000856	34342	269	50	0.52	bW010I19
HAND2	4q33	021973	TC7847	216	45	-	bW053D06
HD	4p16.3	002111	31503.1	195	50	0.17	bW017P21
HDAC8	Xq13	018486	335214.3	113	50	0.48	bW028E14
HERC3	4q21	014606	JMB7132077	110	55	0.10	bW035K18
HNRPD	4q21.1-q21.2	031370	AI981884	151	50	0.13	bW055D20
HNRPDL	4q13-q21	005463	TC7164	127	45	-	-
HNRPH2	Xq22	019597	TC4142	697	50	-	-
HSA6591	4	014487	345459.4	113	45	-	-
ING1L	4q35.1	001564	342599.1	260	50	-	bW012O02
KAL1	Xp22.32	000216	NP346520	139	50	0.07	-
KLHL2	4q21.2	007246	45313.2	125	50	-	bW023N17
LDB2	4p16	001290	NP345067	150	50	0.16	bW032K09
LPHN3	4q12	015236	26237	305	50	0.14	-
LGR3	4q31.3	021634	35188	127	55	0.67	bW024G14
LGR7	4q32.1	021634	35188.1	127	50	0.48	bW028J09
MAB21L2	4q31	006439	AL586521	441	50	-	bW056M04
MADH1	4q28	005900	TC5227	159	50	0.13	bW063C11
MGC10646	4q21.1	032693	TC6571	153	50	-	-
MGC11324	4q21.3	032717	336345.1	165	50	0.16	bW001O18
MID1	Xp22	000381	341078.2	137	50	-	bW129O12
MORF4	4q33-q34.1	006792	BI391102	222	50	0.12	bW045D14
NDST3	4q28.1	004784	19176	178	50	0.20	bW040N15
NDST4	4q25-q26	022569	58090.1	161	50	0.12	bW015N23
NFKB1	4q24	003998	TC4333	168	50	0.13	bW021B20
NR3C2	4q31.1	000901	349338	124	50	0.21	bW023A13
NUP54	4q21.1	017426	AI982010	135	50	0.11	bW096F06
PAPSS1	4q24	005443	34885	148	50	0.18	bW020L08
PCDH10	4q28.3	020815	508333	218	50	0.33	bW026F07

(Continued)

Table 1. Continued

Gene symbol[1]	*Human cytogenetic map position*	*Accession Number*		*PCR Product size (bp)*	*Temp*[3].	*Rf*[4]	*BAC Clone*
		Human[2]	*Chicken*				
PCDH7	4p15	002589	4735.1	184	50	-	-
PDGFC	4q32	016205	NP344712	125	55	0.52	bW014N12
PDGFRA	4q12	006206	TC8274	223	50	0.20	bW029L03
PDHA2	4q22-q23	005390	332935.5	156	50	-	bW001E08
PDZGEF1	4q32.1	014247	5249	212	50	0.11	bW040J23
PGRMC2	4q26	006320	BI391557	152	55	-	bW015H15
PITX2	4q25-q27	000325	TC5315	106	50	-	-
PKD2	4q13.2	016457	AJ395970	250	50	0.14	bW016H13
PMSCL1	4q28.1	005033	JMB7120551	109	50	-	bW023K21
POLR2B	4q12	000938	TC7941	159	50	0.18	bW017P12
PPAT	4q12	002703	TC8440	187	50	0.13	bW018F15
PRSS12	4q28.1	003619	46899.1	166	50	0.13	bW014K04
RAB33B	4q28	031296	37969.1	282	50	0.29	bW039B02
RRAGB	Xp11.21	006064	335137.4	119	45	0.41	bW074F04
REST	4q12	005612	44465	292	50	-	bW017P12
RPS3A	4q31.2-q31.3	001006	TC4166	248	50	-	-
SEC3	4q11	018261	43272	127	50	-	bW016D22
SGCB	4q12	000232	TC6012	219	50	0.13	bW084D22
ShrmL	4q13.3	020859	331830.9	164	50	0.13	-
SLC25A4	4q35	001151	TC6688	183	50	-	-
SLC4A4	4q21	003759	346067	220	50	0.12	bW039D14
SLIT2	4p15.2	004787	2748.1	143	50	-	-
SMARCA5	4q31.1-q31.2	003601	TC6224	159	50	-	bW122E10
SMCIL1	Xp11.22-p11.21	006306	349580.1	150	50	-	-
SNX25	4q35.1	031953	33244	101	50	0.19	bW075K07
STIM2	4p15.2	020860	AI981296	148	50	0.18	bW056N05
TEC	4p12	003215	JMB38k23r1	135	50	0.17	bW082I06
TLL1	4q32-q33	012464	20997	176	50	0.37	bW034I19
TPARL	4q12	018475	340757.2	129	50	0.19	bW022H06
TRIM2	4q31.23	015271	21084	215	50	-	-
TUBB4Q	4q35	020040	TC4138	212	50	0.11	bW088E02
UBE2D3	4q22.2	003340	TC6035	159	45	0.20	bW012N06
UGDH	4p15.1	003359	TC5502	147	50	0.13	bW001M13
UGT8	4q26	003360	33803.1	276	50	0.16	bW015N23
USP38	4q31.1	032557	JMB21p18r1	207	50	0.29	bW068F11
VEGFC	4q34.1-q34.3	005429	44227.1	188	50	0.19	bW027L11

[1]Primer information such as a primer sequence can be found at (https://acedb.asg.wur.nl/).

[2]Accession number (NM_) of human genes used in BLAST, searching for identifies chicken orthologous genes.

[3]Annealing Temperature in PCR.

[4]Retention frequency (*Rf*).

Statistical analysis and map construction.

The CarthaGene program (Schiex and Gaspin 1997) was used to analyze and construct the RH map for GGA4. CarthaGene is a maximum-likelihood multipoint RH and genetic data-mapping tool (available at http://www.inra.fr/bia/T/CarthaGene/). Markers disrupting good map ordering were identified in the best sets of map orders produced and by examining the consistency of patterns for two-point LOD scores (higher than 5) of markers in their assigned order against each other across the chromosome to get the final frame work groups (Figure 1). Therefore, final map distances were calculated by using CarthaGene order as input for the RH map for maximum likelihood.

RESULTS

RH mapping.

We initially started with the 127 STS markers representing likely orthologs of genes from human Chromosomes 4 and X and 23 chicken microsatellite markers that had previously been mapped to GGA4. These markers were tested for successful amplification on chicken genomic DNA and on the DNA from the RH panel. Successful amplification was defined as a single amplification product as visualized on agarose gel electrophoresis and the absence of that fragment using the hamster genomic DNA as a control. This was eventually achieved for 77 genes (60.63%) and 15 of the microsatellite markers (65.22%). These markers were typed on the RH panel and used for further linkage analysis.

The first step in building the chicken Chromosome 4 RH map was to group markers within separate linkage groups using a LOD threshold of 5.0. This allowed us to create an initial set of 9 RH linkage groups, containing 61 of the genes and 11 of the microsatellite markers. For 22 markers (randomly distributed along HSA4 and HSAX), no linkage was observed with any of the other markers, even after lowering the LOD threshold to 2.0. For the linkage groups 1–9 (Figure 1), the locus order was investigated by using the build option of the CarthaGene program. The retention frequency (*Rf*) was calculated for all markers used and was found to vary enormously. Although the average *Rf* for the markers was 24%, the lowest *Rf* observed was 7% for the *KAL1* gene whereas the highest *Rf* (82%) was observed for marker *ADL0317* (Table 1).

BAC library.

To further increase the number of genes on GGA4 and to increase the number of anchor points between the RH maps of Chromosome 4 and the BAC contig map currently under construction, the Wageningen BAC library (Table 1) was screened with 127 of the STS markers described above. For 99.99 markers at least one BAC clone was identified (77.95%). Because several of the STSs appeared to be positive for the same BAC(s), an additional 5 genes could be assigned to the RH linkage maps (linkage groups 3, 4, 5, 7, and 8).

Furthermore, an additional RH linkage group (linkage group 6) could be anchored to the genetic linkage map of GGA4 (Figure 1).

The BAC clone bW040D21 identified for microsatellite marker *LEI0388* was also positive for the marker developed within the gene *GABRA4*, allowing the positioning of RH linkage group 6 containing the genes *STIM2*, *GABRA4*, *TEC*, *SGCB*, *PDGFRA*, *TPARL*, and *PPAT* to the region around position 180 cM on the genetic linkage map for GGA4.

The five genes (*KLHL2*, *PGRMC2*, *REST*, *UGT8*, and *D4S234E*) that were added to RH linkage groups 3, 4, 5, 7 and 8, respectively, based on the identified BACs are labeled in Figure 1 by an asterisk. In order to avoid inflation of the map size of these linkage groups, we chose to project these additional markers at their most likely location without altering the multipoint distance between framework markers (Figure 1).

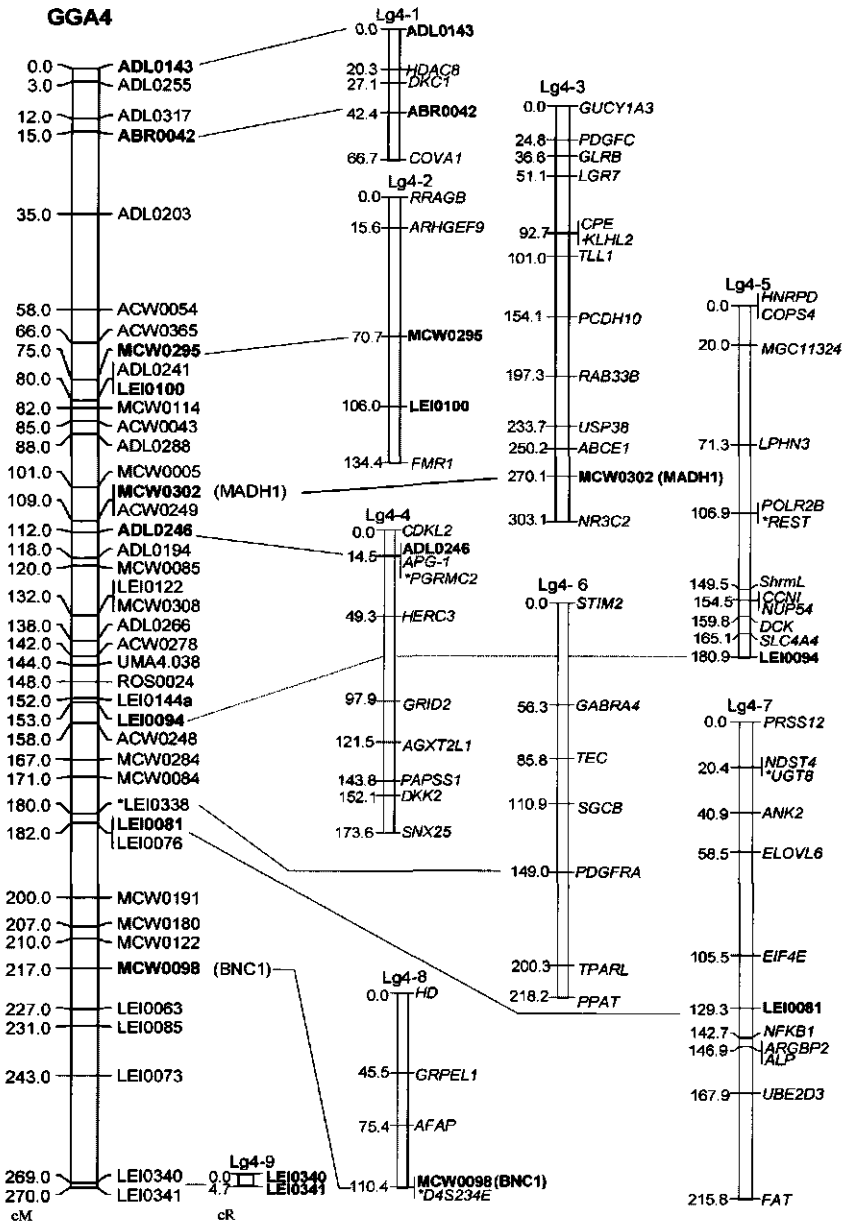


Figure 1. Alignment of RH maps of chicken Chromosome 4 with the framework genetic linkage map of GGA4 map. Microsatellites that are also located on the genetic linkage map are indicated in bold. Genes indicated by an asterisk have been mapped by using BAC information. RH linkage groups shown are supported by a two-point LOD score >5. The map within each group was identified as the best order using the "Nice-map" analysis within CarthaGene.

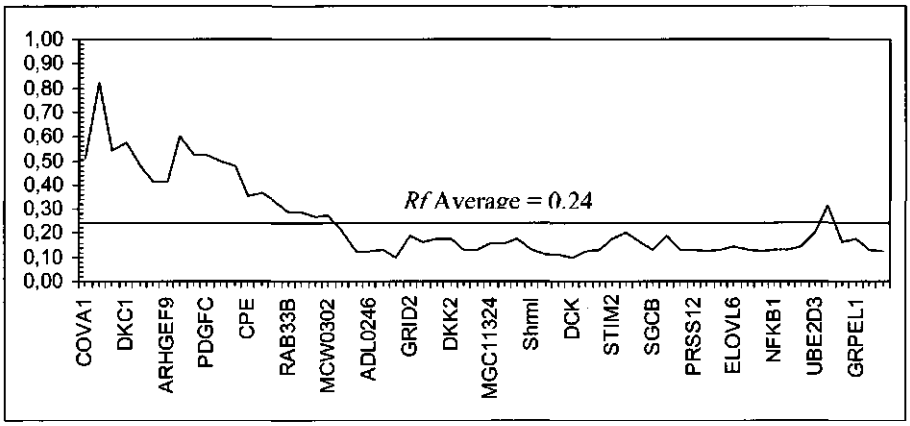


Figure 2. Distribution of retention frequency along chicken chromosome 4 for the markers used. The average retention frequency was 24%, the highest and the lowest values (82% and 7%) were observed for the marker *ADL0317* and for *KAL1* gene, respectively.

DISCUSSION

RH mapping.

Because GGA4 showed conservation of synteny mainly to HSAX and HSA4 (Schmid et al. 2000), chicken ESTs orthologous to genes located in man on HSAX and HSA4 were identified. The combined RH mapping and BAC identification approach eventually resulted in the successful mapping of 61 genes to GGA4, 54 of which were homologous to genes located on HSA4 and 7 of which were homologous to genes located on HSAX. Although the resulting RH map consists of 9 independent linkage groups, all are linked by a marker to the genetic linkage map of GGA4 and therefore could be ordered with respect to each other. However, since most of these linkage maps are connected to the genetic linkage map by only a single marker, the relative orientation of them is not known. There were some initial difficulties at arriving at the gene order for the 9 radiation hybrid maps for GGA4. For example, although *PDGFRA* has a low two-point LOD score with *SGCB*, *GABRA4*, and *PPAT* (only 2.0, 0.2, and 3.3, respectively), it has a high two-point LOD score with *TEC* and *TPARL* (7.0 and 8.0, respectively), and clearly indicating that it is located on the same linkage group. The average observed *Rf* for the markers mapped to GGA4 is 24%,

which is similar to the results described by Morisson et al. (2002) who used two microsatellite markers from GGA4 (*MCW0085* and *MCW0099*). The *Rf*s for these two markers were 20% and 7%, respectively. In another study using this RH panel (Jennen et al. 2004), which focused on GGA15, the observed average *Rf* was also found to be in the same range (18%). However, the observed *Rf* of the markers used in this study varied considerably along the chromosome (Figure 2). The *Rf* was particularly high in the region around position 15 and 80 cM of the consensus linkage map (around map positions 27 and 134 cR of linkage groups 1 and 2, respectively). The differences in *Rf* are observed for markers that are not just randomly distributed along GGA4 but that are located in the same region on the chromosome. This indicates that the observed retention frequencies reflect the actual retention of these chromosomal fragments in the cell lines from the radiation hybrid cell panel and are not caused by nonspecific amplification of the marker.

BAC library.

The BAC clones that were identified by screening the Wageningen BAC library resulted in the mapping of several additional genes on GGA4, i.e., *KLHL2*, *PGRMC2*, *REST*, *UGT8*, and *D4S234E*. Furthermore, the identification of BAC bW040D21 for markers *LEI0338* and *GABRA4* resulted in the positioning of RH linkage group 6 around position 180 on the genetic linkage map of GGA4, thereby placing another 7 genes on this chromosome (*STIM2*, *GABRA4*, *TEC*, *SGCB*, *PDGFRA*, *TPARL*, and *PPAT*) (marker details can be found at https://acedb.asg.wur.nl/). BACs that have been identified with markers mapped on GGA4 are also key elements that help to integrate the cytogenetic and linkage maps with the physical BAC contig map. BAC end sequencing of the Wageningen BAC clones is currently in progress and this will aid in the further integration of the RH, linkage, and BAC mapping data with chicken genome sequence contigs.

Comparative map.

A comparison of the genes mapped on GGA4 to the chromosomal location of the orthologous genes in human is shown in Figure 3. The available comparative mapping data clearly show that the major part of GGA4 shows conserved synteny to the q arm of HSAX and the q arm of HSA4. In addition, regions syntenic to the p arm of HSA4 and to small regions on HSA21q, HSA3p, HSA2p, and HSA5q have also been described (Groenen et al. 2000; Schmid et al. 2000; Matsishima et al. 2000). However, in those cases where such conserved synteny is based on only a single gene, one has to pay particular caution as many of these links may be the result of incorrect

previous identifications of orthologs versus paralogs. The conserved synteny between GGA4 and HSA5, for example, is based on the FISH mapping of the *CTNN1* gene to the distal tip of the q arm of GGA4 (Suzuki et al. 1999). Interestingly, the related *CTNN2* gene has also been mapped to HSA2p12–11.1, already indicating that the gene mapped in chicken probably is the ortholog of CTNN2 (Groenen and Crooijmans 2003). This led us to reexamine the FISH mapping results by Suzuki et al. (1999) and a BLASTN search was done with the sequence of the cDNA clone used in the FISH experiment (accession number D11090). The results clearly showed that this cDNA was the orthologous gene of the human *CTNNA2* gene that is located on GGA4 (with a sequence identity of 82%). The other example on GGA4 is the *TGFBR2* gene, which is located on HSA3 and on MMU9. No other genes currently mapped on GGA4 are located on these chromosomes in man and mouse. A close reexamination of the data for the consensus linkage map showed that the localization of this gene on GGA4 on the consensus map (Groenen et al. 2000) has been erroneous.

Finally, the link between the distal part of GGA4p to HSA21q needs to be regarded as doubtful. This presumed syntenic link was established by mapping the microsatellite *MCW0047* to position 23 cM on the linkage map of GGA4 (Crooijmans et al. 1995; Groenen et al. 2000). This microsatellite was derived from a genomic clone (accession number M20817) containing part of the *HMGN1* gene. However, a close examination of a BLASTN database search using this sequence showed that this sequence also has sequence identity of 94–97% to several other human clones derived from a number of different human chromosomes (11, 12, 13, 14, 15, 17, 21, and 22) including HSAX. Although the exact map location of the genes that were mapped in this study to GGA4 is not known yet, the approximate positions of these genes already clearly indicate that multiple rearrangements have occurred within the syntenic regions on chicken Chromosome 4 and human Chromosomes 4, X, and 2 (Figure 3).

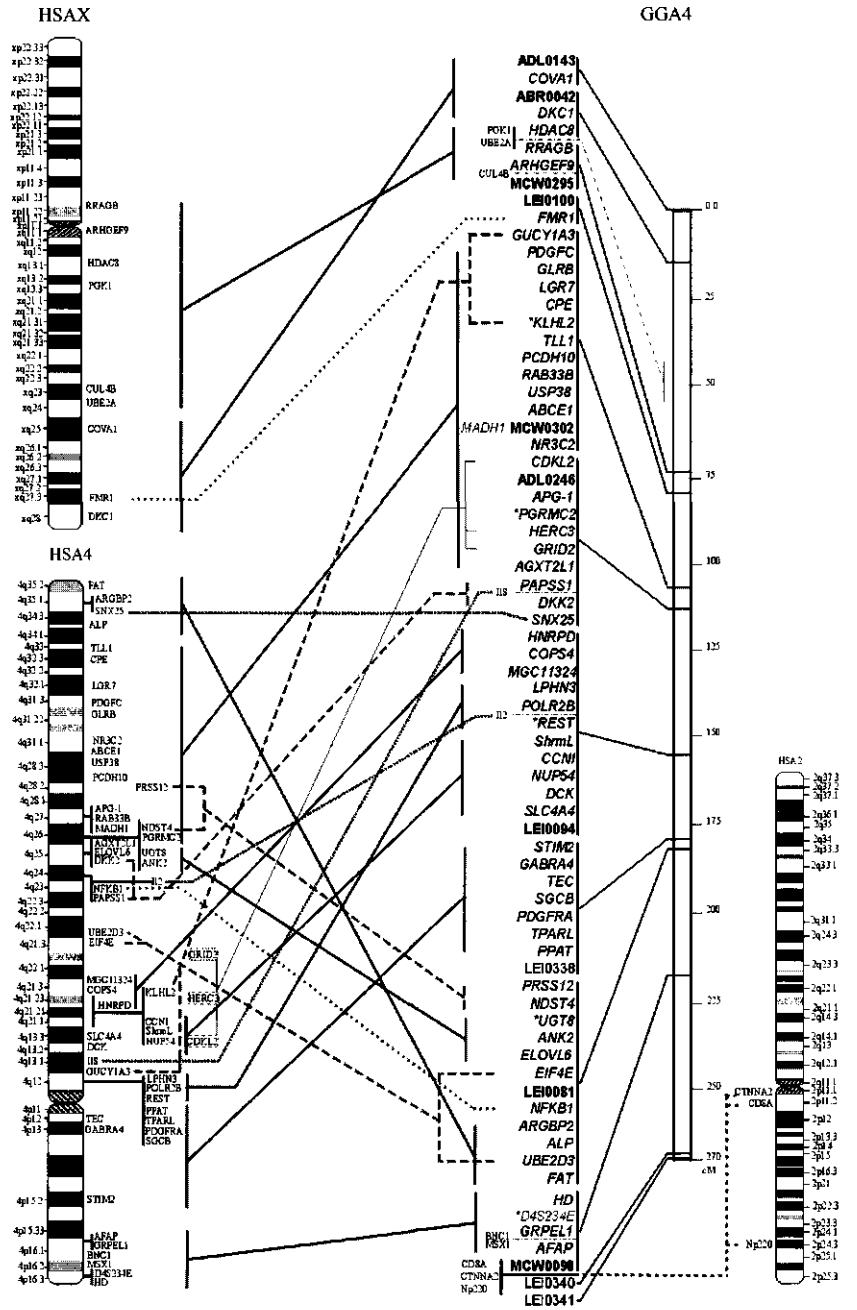


Figure 3. Comparative map between chicken Chromosome 4 and human Chromosomes 2, 4, and X. Estimated positions for human genes are given in cM, according to the Map Viewer from Entrez Genomes (http://www.ncbi.nlm.nih.gov/). The numbers located on the right of the vertical bar of GGA4 represent the relative positions in cM of the chicken loci. Microsatellite markers are in bold; genes labeled with an asterisk are mapped based on the BAC screening. Genes mapped by RH are in italics.

The comparative data that are currently available for GGA4 indicate that this chromosome contains at least 20 CSOs (Conserved Segments Ordered) when compared to human. These findings are in agreement with previous studies on chicken Chromosomes 10 and 15 where a large number of rearrangements were observed within regions of conserved synteny between man and chicken (Crooijmans et al. 2001; Jennen et al. 2003). In these studies the average size of these CSOs was between 4 and 6 cM, which is even smaller than that observed for GGA4. This, however, is due to the density of the genes currently mapped on these chromosomes, and it is expected that the number of conserved segments will increase as the number of genes mapped in chicken increases.

Based on the currently available data, we expect that the number of conserved regions between chicken and human might be as high as 300 (20 CSOs with a size of GGA4 approximately 7% of the chicken genome) to 1000 (19 CSOs with a size of GGA15 approximately 1.8% of the chicken genome; Jennen et al. 2004) with an average size of just a few cM. A more accurate estimation of this number has to await the completion of the physical and sequence maps of the chicken genome expected to become available in 2004.

ACKNOWLEDGMENTS

The authors would like to thank the Egyptian Ministry of Higher Education and Scientific Research for financial support (Project No. 29110/416000).

REFERENCES

Abdrakhomanov I, Lodygin D, Geroth P, Arakawa H, Law A, Plachy J, Korn B, Buerstedde J-M (2000) A large database of chicken bursal ESTs as a resource for the analysis of vertebrate gene function. *Genome Res.* **10**, 2062-2069

Boardman PE, Sanz-Ezquerro J, Overton IM, Burt DW, Bosch E, Fong WT, Tickle C, Brown WR, Wilson SA, Hubbard SJ (2002) A comprehensive collection of chicken cDNAs. *Curr. Biol.***12**, 1965-1969

Buitenhuis AJ, Crooijmans RPM., Bruijnesteijn van Coppenraet, Veenendaal A, Groenen MAM, van der Poel JJ (2002). Improvement of the comparative map of chicken chromosome 13. *Anim. Genet.* **33**, 249-254.

Burt DW, Bruley C, Dunn IC, Jones CT, Ramage A, Law AS, Morrice DR, Paton IR, Smith J, Windsor D, Sazanov A, Fries R, Waddington D (1999). The dynamics of chromosome evolution in birds and mammals. *Nature* **402**, 411-413.

Chowdhary BP and Raudsepp T (2000) HSA4 and GGA4: Remarkable conservation despite 300-Myr divergence. *Genomics* **64**, 102-105

Cox DR, Burmeister M, Price ER, Kim S, Myers RM (1990) Radiation hybrid mapping: A somatic cell genetic method for constructing high-resolution maps of mammalian chromosomes. *Science* **250**, 245–250.

Crooijmans RPMA, van der Poel JJ, Groenen MAM (1995) Functional genes mapped on the chicken genome. *Anim. Genet.* **26**, 73-78

Crooijmans RPMA, Verbalove J, Dijkhof RJM, Van Der Poel JJ, Groenen MAM (2000) Two-dimensional screening of the wageningen chicken BAC library. *Mamm. Genome* **11**, 360-363.

Crooijmans RPMA, Dijkhof RJM, Veenendaal T, van der Poel JJ, Nicholls RD, Bovenhuis H, Groenen MAM (2001). The gene orders on human chromosomes 15 and chicken chromosome 10 reveal multiple inter- and intrachromosomal rearrangements. *Mol. Biol. Evol.* **18**, 2102-2109

Geisler R, Rauch G-J, Baier H, Van Bebber F, Bro L, Dekens MPS, Finger K, Fricke C, Gates MA, Geiger H, Geiger-Rudolph S, Gilmour D, Glaser S, Gnügge L, Habeck H, Hingst K, Holley S, Keenan J, Kirn A, Knaut H, Lashkari D, Maderspacher F, Martyn U, Neuhauss S, Neumann C, Nicolson T, Pelegri F, Ray R, Rick JM, Roehl H, Roeser T, Schauerte HE, Schier AF, Schönberger U, Schönthaler H-B, Schulte-Merker S, Seydler C, Talbot WS, Weiler C, Nüsslein-Volhard C, Haffter P (1999) A radiation hybrid map of the zebrafish genome. *Nat. Genet.* **23**, 86-89.

Goss S and H Harris (1975) New method for mapping genes in human chromosomes. *Nature* **255**, 680–684.

Groenen MAM and Crooijmans RPMA (2003) Chapter 26. Structural genomics: integrating linkage, physical and sequence maps. *In Poultry genetics, breeding and technology, Muir WM, Koehn RK, eds. (Cambridge, MA: CAB International),* pp 497-536.

Groenen MAM, Cheng HH, Bumstead N, Benkel B, Briles E, Burt DW, Burke T, Crittenden LB, Dodgson J, Hillel J, Lamont SJ, Ponce de leon A, Soller M, Takahashi H, Vignal A (2000). A consensus linkage map of the chicken genome. *Genome Res.* **10**, 137-147.

Groenen MAM, Crooijmans RPMA, Veenendaal A, Cheng HH, Siwek M, van der Poel JJ (1998) A comprehensive microsatellite linkage map of the chicken genome. *Genomics* **49**, 265-274.

Gyapay G, Schmitt K, Fizames C, Jones H, Vega-Czarny N, Spillett D, Muselet D, Prud'Homme J-F, Dib C, Auffray C, Morissette J, Weissenbach J, Goodfellow N (1996) A radiation hybrid map of the human genome. *Hum. Mol. Genet.* **5**, 339–346.

Jennen DGJ,Crooijmans RPMA, Kamps B, Acar A, Veenendaal A,van der Poel JJ, Groenen MAM (2002) A comparative map of chicken chromosome 24 and human chromosome 11. *Anim. Genet.* **33**, 205-210

Jennen DGJ, Crooijmans RPMA, Kamps B, Açar R, van der Poel JJ, Groenen MAM (2003) Comparative Map between chicken chromosome 15 and human chromosomal region 12q24 and 22q11-q12. *Mamm. Genome* **14**, 629-639

Jennen DGJ, Crooijmans RPMA, Morisson M, Grootemaat AE, van der Poel JJ, Vignal A, Groenen MAM (2004) A radiation hybrid map of chicken chromosome 15. *Anim. Genet.* **35**, 63-65.

Kiguwa SL, Hextall P, Smith AL, Critcher R, Swinburne J, Millon L, Binns MM, Goodfellow PN, McCarthy LC, Farr CJ, Oakenfull EA (2000) Characterization of whol genome radiation hybrid panel: Chromosome 1 and 10 preliminary maps. *Mamm. Genome* **11**, 803-805.

Larkin DM, van der AE, Rebeiz WM , Schweitzer PA, Bachman S, Green C, Wright CL, Campos EJ, Benson LD, Edwards J, Liu L, Osoegawa K, Womack JE, de Jong PJ, Lewin HA (2003) A cattle-human comparative map built with cattle BAC-ends and human genome sequence. *Genome Res.* **13**, 1966-1972.

Matsishima Y, Matsumura K, Ishii S, Suzuki T, Matsuda Y, Kitagawa Y (2000) Chromosomal assignment of the chicken NP220 gene encoding a large DNA-binding nuclear protein and its targeted disruption in chicken DT40 cells. *Cytotechnology* **33**, 109-115

McCarthy LC, Terrett J, Davis ME, Smith AL, Critcher R, Schmitt K, Hudson J, Spurr NK, Goodfellow PN (1997) A first-generation whole genome–radiation Hybrid map spanning the mouse genome. *Genome Res.* **7**, 1153–1161.

Morisson M, Lemière A, Bosc S, Galan M, Plisson-Petit F, Pinton P, Delcros C, Fève K, Pitel F, Fillon V, Yerle M, Vignal A (2002) ChickRH6: a chicken whole-genome radiation hybrid panel. *Genet. Sel. Evol.* **34**, 521-533.

Nanda I, Koehler M, Schartl M, Burt DW, Koehler M, Nothwang H, Grutzner F, Paton IR, Windsor D, Dunn I, Engel W, Staeheli P, Mizuno S, Haaf T, Schmid M (1999) 300 million years of conserved synteny between chicken Z and human chromosome 9. *Nat. Genet.* **21**, 258-259

O'Brien SJ, Womack JE, Lyons LA, Moore KJ, Jenkins NA, Copeland NG (1993) Anchored reference loci for comparative genome mapping in mammals. *Nat. Genet.* **3**, 103–112

O'Brien SJ, Raymond MM, Murphy WJ, Nash WG, Wienberg J, Stanyon R, Copeland NG, Jenkins NA, Womack JE, Graves JAM (1999) The promise of comparative genomics in mammals. *Science* **286**, 458-481.

Rink A, Santschi EM, Eyer KM, Roelofs B, Hess M, Godfrey M, Karajusuf EK, Yerle M, Milan D, Beattie CW (2002) A first generation EST RH comparative map of porcine and human genome. *Mamm. Genome* **13**, 578-587

Schiex T, Gaspin C (1997) CARTHAGENE: constructing and joining maximum likelihood genetic maps. *Proceedings of International Conference on Intelligent Systems for Molecular Biology* **5**, 258-67.

Schmid M, Nada I, Guttenbach M, Steinlein C, Hoehn H, Schartl M, Haaf T, Weigend S, Fries R, Buerstedde JM, Wimmers K, Burt DW, Smith J, A'Hara S, Law A, Griffin DK, Bumstead N, Kaufman J, Thomson PA, Burke T, Groenen MA, Crooijmans RP, Vignal A, Fillon V, Morisson M, Pitel F, Tixier-Boichard M, Ladjali-Mohammedi K, Hillel J, Maki-Tanila A, Cheng HH, Delany ME, Burnside J, Mizuno S (2000) First report on chicken genes and chromosomes 2000. *Cytogenet. Cell Genet.* **90,** 169–218.

Smith EJ, Lyons LA, Cheng HH, Suchyta SP (1997). Comparative mapping of the chicken genome using the East Lansing reference population. *Poult. Sci.* **76**, 743–747.

Suchyta SP, Cheng HH, Burnside J, Dodgson JB (2001). Comparative mapping of chicken anchor loci orthologous to genes on human chromosomes 1, 4 and 9. *Anim. Genet.* **32**, 12-18.

Sun HS, Cai L, Davis SK, Taylor JF, Doud LK, Bishop MD, Hayes H, Barendse W, Vaiman D, McGraw RA, Hirano T, Sugimoto Y, Kirkpatrick BW (1997) Comparative linkage mapping of human chromosome 13 and bovine chromosome 12. *Genomics* **39**, 47–54

Sun HS, Yerle M, Pinton P, Chardon P, Rogel-Gaillard C, Lyons LA, Laughlin TF, Tuggle CK (1999) Physical assignments of human chromosome 13 genes on pig chromosome 11 demonstrate extensive synteny and gene order conservation between pig and human. *Anim. Genet.* **30**, 304-308.

Suzuki T, Kurosaki T, Shimada K, Kansaku N, Kuhnlein U, Zadworny D, Agata K, Hashimoto A, Koide M, Koike M, Takata M, Kuroiwa A, Minai S, Namikawa T, Matsuda Y (1999) Cytogenetic mapping of 31 functional genes on chicken chromosomes by direct R-banding FISH. *Cytogenet. Cell Genet.* **87**, 32-40

Tirunaguru VG, Sofer L, Cui J, Burnside J (2000) An expressed sequence tag database of T-cell-enriched activated chicken splenocytes: sequence analysis of 5251 clones. *Genomics* **66**, 144-151

Walter MA, Spillett DJ, Tomas P, Weissenbach J, Goodfellow PN (1994) A method for constructing radiation hybrid maps of whole genomes. *Nat. Genet.* **7**, 22–28.

Yerle M, Pinton P, Robic A, Alfonso A, Palvadeau Y, Delcros C, Hawken R, Alexander L, Beattie C, Schook L, Milan D, Gellin J (1998) Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. *Cytogenet. Cell Genet.* **82**, 182-188.

CHAPTER 6

GENERAL DISCUSSION

GENERAL DISCUSSION

The objective of the study described in this thesis was to identify genes controlling Pulmonary Hypertension Syndrome (PHS) in chicken, a metabolic disease increasingly being observed in fast growing birds. Depending on the geographical location and housing conditions, reducing the incidence of this disease would have a major impact on animal welfare and also reduce economic losses to the poultry industry. The breeding industry has for a long time recognized that susceptibility to the disease not only is affected by the environment but that genetic factors play a role as well. However, because PHS is difficult and expensive to measure in practical breeding circumstances, selection against the incidence of the disease has not been implemented in commercial breeding. The availability of a genetic test for (some of) the major genetic components that confer resistance to the disease would therefore be highly desirable. Based on these considerations it was already decided in the mid nineties to develop a large experimental population and analyze it in relation to PHS. In this thesis the first results of this ongoing work have been described. In this final chapter we look back at this work which was performed during a time where the available information of the chicken genome increased at a rate that was unexpected at the start of the project. The implications of the work described in this thesis as well as the implications of the recent availability of the enormous genome resources in chicken on future research will be discussed. This chapter is divided into three major sections: The first section discusses the initial detection and confirmation of QTL for PHS as was described in Chapters 2 and 3 of this thesis as well as the subsequent fine-mapping of the confirmed QTL on GGA2 and GGA4. The second section focuses on the role of comparative mapping in this approach followed by the last section about the selection of potential candidate genes underlying a QTL and the use of haplotype analysis and linkage disequilibrium mapping to accomplish that goal.

Quantitative trait loci and fine scale mapping study.

The QTL mapping population used in this study was established in the mid nineties and is based on a cross between two broiler dam lines originating from the White Plymouth Rock breed. This directly highlights an important difference between this cross and the majority of other experimental crosses in chicken used for QTL mapping (Tatsuda and fujinaka 2001) where generally two extreme breeds are crossed. Because

our aim was to identify genes affecting PHS that are segregating in commercial broiler lines, it was decided to use a cross between two such lines. It is expected that this choice results in a lower power of the experiment something that is clearly apparent from the lower test statistics observed in the current study (chapter 2, 3 and 4) and other studies (Van Kaam et al. 1998, 1999a,b; Jennen et al. 2004a,b) based on the same population. Whether the original assumption that many relevant genes will be fixed between more extreme breeds still has to await the molecular cloning of the genes underlying the identified QTL in the different types of QTL crosses.

An additional drawback of the mapping of QTL for PHS is the complexity of the trait. PHS evolves as a complex cascade of events in which many different organs are involved (see chapter 1, figure 3). The phenotype therefore is measured through a number of different characteristics most of them related to the condition of the heart, mortality and impaired growth (chapter 2). To further improve the description of the disease at a later stage (during the validation and fine mapping experiments) additional measurements were added directly related to the oxygen saturation (chapters 3 and 4). In retrospect, there is not one single measurement that best describes PHS and one suggestion might be to try to combine the different measurements in a single derived trait. However, how to best weigh the different measurements is far from trivial and will require further investigation.

The initial QTL mapping experiment resulted in the identification of several significant QTLs on chromosomes 2, 4 and 6 and suggestive linkage was found on chromosomes 5, 8, 10, 27, and 28 (Chapter 2). However, the estimated map position of the QTL lack precision, and the QTL often spread across more than 20-60 cM which effectively for the smaller chromosomes like GGA27 and GGA28 means almost the complete chromosome.

Before putting a lot of effort in trying to narrow down these regions, it is essential to validate the QTLs in subsequent generations. Eventually this was done on generations 6-8 of this cross. Of the 8 QTL regions initially identified, 4 showed evidence of a QTL related to PHS in generation 6-8 (chapter 3). The 4 regions that could not be confirmed most likely represent false positives in the initial genome scan, although it can not be ruled out that some of these regions consisted of multiple linked QTL each with a small effect that were separated in the subsequent generations. After validation of the QTLs, the chromosomal region associated with the trait should be narrowed down by using a fine mapping approach. Although it is tempting to look directly for potential candidate genes in the confirmed regions, the size and therefore the large number of genes makes this approach not very successful.

In chapter 2 we nevertheless made an attempt to identify such potential candidates, and although several candidates were identified the number was still to large to be meaningful.

To narrow down the region containing a QTL additional animals with a recombinant haplotype in that particular region are needed. This can be accomplished by either generating new recombination events *de novo* by producing more offspring or, alternatively, by taking advantage of historical recombinants by analyzing another (distantly related) population. For the fine mapping of the PHS QTL it was chosen to use an advanced intercross line (AIL; Darvasi and Soller 1995). An AIL is produced by random intercrossing (avoiding sibling pairing) in each generation from G_2 onwards, until the desired advanced intercross G_n is obtained. For QTL fine-mapping purpose, individuals in the latest generation are phenotyped and genotyped. In this way, many recombination events applicable for high-resolution mapping of QTL accumulate in a relatively small population over multiple generations. In their original publication, Darvasi and Soller (1995) proposed this method for crosses between two inbred lines. Using an AIL based on a cross between two outbred populations constitutes a challenge and requires a substantially larger number of markers. Although, initially much effort was put into the targeted development of markers, the availability of a draft sequence of the chicken genome and a high density SNP map greatly has circumvented this necessity. An import choice remains in which type of marker to use (microsatellites versus SNPs). SNP markers are abundant with on average in chicken 1 SNP per 200 bp (International chicken polymorphism map consortium 2004). SNPs have the advantage that they are more suitable for association studies because of their relatively low mutation rate they are almost exclusively IBD. A disadvantage is that they are not as informative as microsatellites. Because of the higher information content it was decided to use additional microsatellites for the fine mapping of the QTL (chapter 4). However, in future experiments this choice will also be dependent on the typing method used. Because the number and throughput of SNP typing methods is still increasing while genotyping costs are going down, it is to be expected that the balance will shift to SNPs in future experiments.

Comparative mapping

The objectives for comparative mapping between chicken and other species, in particular man, have greatly changed because of the completion of the draft sequence of the chicken genome. Initially, and for the largest part of the duration of the work described in this thesis, comparative mapping between chicken and human was used as a way to predict the genes located in the identified QTL regions. In addition, this also provided the means to target marker development in those regions as well. This approach using both the RH panel and the Wageningen BAC library resulted for GGA4 in a gene-dense map and a high resolution comparative map between GGA4 and human chromosome 4 and X (chapter 5). This map was used to assign and align sequence contigs derived from the whole genome shotgun sequencing approach to chicken chromosome 4 (International chicken genome sequencing consortium 2004). This in turn made it possible to select and design a large number of additional microsatellite markers in the QTL region on GGA4 (chapter 4).

Although a draft sequence of the chicken genome is now available further improvement of the chicken RH and linkage maps is still of high importance. It is estimated that the current draft of the chicken genome sequence covers around 90-95 % of the genome (International chicken genome sequencing consortium 2004). Furthermore, 10 % of the sequence contigs have not yet been assigned to a chicken chromosome or chromosomal location. The same is true for the physical map of the chicken genome where 34 of the 260 contigs have not yet been assigned a chromosomal location (Wallis et al. 2004). Comparative mapping between chicken and mammalian genomes still is an important tool in chicken (and human) genome research. Although no longer needed to assist in the targeted development of new markers it is an important resource for the annotation of chicken genes and the identification of regulatory sequences. Recently, several studies (Van Laere et al. 2003; Freking et al. 2002) have shown that mutations in regulatory sequences can have a big impact on quantitative traits such as muscle growth and fat deposition further emphasizing the need to identify such elements. Having a detailed annotation of the chicken genome both with regard to genes as well as regulatory elements and the availability of a large collection of SNPs (International chicken genome sequencing consortium 2004) will greatly enhance our possibilities to identify the underlying mutations for the identified QTL.

From mapping to candidate genes

The ultimate goal of QTL mapping is to identify the genes underlying these polygenic traits and to gain a better understanding of physiology and biochemistry of the trait. Although it is already possible to use a marker bracket of tightly linked markers around the QTL in breeding schemes (Marker assisted selection, MAS) the efficient use of the identified QTLs in breeding programes requires the ultimate positional cloning of the corresponding genes (Schwerin et al. 2004). For the successful implementation of marker-assisted selection in other populations than those originally used to identify the QTL, segregation of QTL needs to be verified within these lines. Furthermore, depending on the size of the marker bracket the linkage phase of the marker and the positive QTL need to be re-examined at regular time intervals as well. This inevitable results not only in an increase of the genotyping costs but also in the increase of the phenotyping costs which in the case of PHS in chicken will not be economically feasible. Therefore, it will be essential to eventually identify the gene and the underlying causative mutation and to develop a specific genotyping assay for that mutation before it can be efficiently implemented in commercial breeding (Gene assisted selection, GAS). The large number of potential determinants of PHS, and the large number of potential candidate genes for PHS makes this an especially challenging goal for this trait.

In chapter 2, eleven potential candidate genes have been identified based on the location of the detected QTL on chicken chromosomes 4, 8 and 10 and based on the known function of that gene in mammals. However, it is apparent that additional candidates are located within these regions and that it is essential to further reduce the size of the QTL interval (chapter4). A further reduction in the size of these QTL intervals combined with a detailed analysis of the haplotypes associated with the positive and negative QTL effect will be required for the identification of the gene involved. Although an understanding of linkage disequilibrium is a fundamental component of the candidate-gene approach, it is important to note that there is very limited information, at present, with regard to LD patterns across the chicken genome (Chapter 4, this thesis). Therefore, assumptions about the ability to detect associations on the basis of LD and about the predictive power of haplotypes need to be carefully valuated (Goddard et al. 2000; Ardlie et al. 2002). The identification and selection of the polymorphisms (SNPs) for genotyping can be a formidable task in the design of a candidate-gene study. With a complete genome sequence and a polymorphism map with over 2.8 million SNPs this approach has now become feasible in chicken. However, the ultimate proof that a particular mutation among a group of associated polymorphisms (SNPs) is the causative mutation for the QTL observed still remains a daunting task.

REFERENCES

Ardlie KG, Kruglyak L, Seielstad M (2002) Patterns of linkage disequilibrium in the human genome. *Nat. Rev. Genet.* **3**, 299–309

Darvasi A, Soller M (1995) Advanced intercross lines, an experimental population for fine genetic mapping. *Genetics* **141**, 1199-1207.

Freking BA, Murphy SK, Wylie AA, Rhodes SJ, Keele JW, Leymaster KA, Jirtle RL, Smith TP (2002) Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar over dominance in mammals. *Genome Res.* **12**, 1496-1506

Goddard KAB, Hopkins PJ, Hall JM, Witte JS (2000). Linkage disequilibrium and allele-frequency distributions for 114 single-nucleotide polymorphisms in five populations. *Am. J. Hum. Genet.* **66**, 216–234.

International Chicken Genome Sequencing Consortium (2004). Sequencing and comparative analysis of the chicken genome, *Gallus gallus*. *Nature*, in press.

International Chicken Polymorphism Map Consortium (2004). A polymorphism map for chicken with 2.8 million SNPs. *Nature,* in press.

Jennen DGJ, Vereijken ALJ, Bovenhuis H, Crooijmans RPMA, Veenendaal A, van der Poel JJ, Groenen MAM (2004a) Detection and localization of quantitative trait loci affecting fatness in broilers. *Poultry Sci.* **83**, 295-301.

Jennen DGJ, Vereijken ALJ, Bovenhuis H, Crooijmans RPMA, van der Poel JJ, Groenen MAM (2004b) Confirmation of quantitative trait loci affecting fatness in chicken. *Gent. Sel. Evol.* In press

Schwerin M, Kühn Ch, Brunner R, Goldammer T, Bennewitz J, Reinsch N, Xu N, Thomsen H, Looft C, Weimann C, Hiendleder S, Erhardt G, Medjugorac I, Förster M, Brenig B, Reinhardt F, Reents R, Russ I, Averdunk G, Blümel J, Kalm E (2004) QTL mapping and mining functional candidate genes affecting health. The German ADR QTL Dairy Cattle Project. *Anim. Sci. Pap. Rep.* **22**, 95-100

Tatsuda K, Fujinaka K (2001) Genetic mapping of the QTL affecting body weight in chickens using a F_2 family. *Brit. Poultry Sci.* **42,** 333-337.

Van Kaam JBCHM, van Arendonk JAM, Groenen MAM, Bovenhuis H, Vereijken ALJ, Crooijmans RPMA, van der Poel JJ, Veenendaal A (1998) Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. *Livest. Prod. Sci.* **54,** 133-150

Van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken ALJ, van Arendonk JAM (1999a) Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency. *Poultry Sci.* **78**, 15-23

Van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken ALJ, van Arendonk JAM (1999b) Whole genome scan in chickens for quantitative trait loci affecting carcass traits. *Poultry Sci.* **78**, 1091-1099

Van Laere AS, Nguyen M, Braunschweig M, Nezer C, Collette C, Moreau L, Archibald AL, Haley CS, Buys N, Tally M, Andersson G, Georges M, Andersson L (2003) A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. *Nature* **425**, 832-6

Wallis JW, Aerts J, Groenen MAM, Crooijmans RPMA, Layman D, Graves T, Scheer D, Kremitzki C, Higgenbotham J, Gaige T, Mead K, Walker J, Albracht D, Davito J, Yang S-P, Leong S, Chinwalla A, Hillier L , Sekhon M, Wylie K, Dodgson J, Romanov MN, Cheng H, de Jong PJ, Zhang H, McPherson JD, Krzywinski M, Schein J, Mardis E, Wilson R, Warren WC (2004) A physical map of the chicken genome. *Nature*, in press

SUMMARY

SUMMARY

Pulmonary hypertension syndrome (PHS) is characterized a cascade of events resulting in cardiac anomalies including an enlarged heart, right ventricular hypertrophy, variable liver changes, and accumulation of fluid in the abdominal cavity (ascites). PHS is not a disease; it is a condition in which excess amount of ascitic fluids (a combination of lymph and blood plasma which has leaked from the liver) accumulate in the abdominal cavity which has prompted the common name "water belly". Several genetic and environmental factors such as altitude, temperature, lighting, ventilation, and nutritional factors seem to influence the development of the PHS. The work described in this dissertation is a first step towards the identification of genetic factors controlling PHS.

An experimental population was specifically generated with the aim to map QTL for a number of different economically important traits including PHS. The population is based on a so-called full-sib/half-sib design based on three generations (G_1, G_2 and G_3) derived from a cross between two genetically different outcross broiler dam lines originated from the White Plymouth Rock breed. ***Chapter 2*** describes the initial mapping of a several QTL for traits related to PHS using a total genome scan. The total genome scan was performed on ten full sib families consisting of 20 G_1 and 456 G_2 birds which were typed with 420 microsatellite markers covering 24 autosomal chromosomes. Phenotypic observations were collected on 4202 G_3 birds and a full sib across family regression interval mapping approach was used to identify the QTL. Statistical evidence for QTL was found on chicken chromosomes (GGA) 2, 4 and 6. Suggestive linkage was found on chromosomes 5, 8, 10, 27, and 28. Phenotypic measurements mostly were related to the condition of the heart (%RV, %TV, RATIO), appearance of the internal organs, ascites and survival. The most significant QTL that were identified are located on GGA2 for the traits, right and total ventricular weight as percentage of body weight (%RV and %TV). A related trait, the ratio of right ventricular weight as percentage to total ventricular weight (RATIO), reached the suggestive threshold on this chromosome.

The observed test statistics were not very high, which was to be expected in a G_2 cross between two related breeds. The subsequent confirmation of the observed QTL in the next generations therefore, is an essential step before attempting to further narrow down the identified QTL intervals. The validation of the QTL on chromosomes 8, 10 and 28 and the confirmation of those on GGA8 and GGA10 is described in ***Chapter 3***.

Validation was performed in generations G6 to G8 of the experimental cross. The population consisted of 19 full sib families (a combination of G_6/G_7 and G_7/G_8). In total 14 microsatellite markers were analyzed and twelve PHS-related traits measured on more than 1500 individuals. Significant results were found for the traits body weight at 2 and 5 weeks of age, right ventricular weight, RATIO (RV:TV) and total mortality. The most significant QTL were located on chromosome 8 for traits body weight at 5 wk of age (*ADL0278-MCW0351*), right ventricular weight (*ADL0278-MCW0351*) and RATIO (RV:TV) (*ROS0075-ADL0278*) and on chromosome 10 for traits body weight at 2 wk of age (*MCW0035-ADL0102*) and total mortality (*ADL0158-LEI0112*). The QTL on GGA28 could not be confirmed.

The validation and confirmation of the significant QTL on GGA2 and GGA4 are described in ***Chapter 4***. This chapter also describes the further fine mapping of these two QTL using a combined linkage disequilibrium/linkage analysis approach (LDLA). Validation and fine mapping was performed on an advanced intercross line (AIL) created by random intercrossing in each generation from G_3 onwards until G_8. In total 47 microsatellite markers were used, located within approximately 25 Mb and 16 Mb on GGA2 and GGA4, respectively. QTL originally detected in G_2/G_3 generations were confirmed on the two chromosomes, for the traits RATIO on GGA2 and for $BW5_{AS}$ on GGA4. Additional QTLs were detected for the trait pCO_2 on GGA2, and for $BW3_{AS}$ on GGA4. These two traits had not previously been measured in generation 3 of the original study. The combined LDLA approach enabled a 3-4 fold reduction of the size of the QTL interval.

The improvement of the physical map for GGA4 and the generation of a detailed comparative map between this chicken chromosome and human chromosomes 4 and X are described in ***Chapter 5***. The mapping resolution of the physical map for GGA4 was improved by a combination of radiation hybrid (RH) mapping and BAC mapping. The ChickRH6 hybrid panel was used to construct an RH map of GGA4. Eleven microsatellites known to be located on GGA4 were included as anchors to the genetic linkage map for this chromosome. Based on the known conserved synteny between GGA4 and human chromosomes 4 and X, sequences were identified for the orthologous

chicken genes from these human chromosomes by BLAST analysis. These sequences were subsequently used for the development of STS markers to be typed on the RH panel. Using a logarithm of the odds (LOD) threshold of 5.0, nine-linkage groups could be constructed which were aligned with the genetic linkage map of this chromosome. The resulting RH map consisted of the 11 microsatellite markers and 50 genes. To further increase the number of genes on the map and to provide additional anchor points for the physical BAC map of this chromosome BAC clones were identified for 22 microsatellites and 99 genes. The combined RH and BAC mapping approach resulted in the mapping of 61 genes on chicken GGA4 considerably increasing the resolution of the chicken-human comparative map for this chromosome. This enhanced comparative mapping resolution enabled the identification of multiple rearrangements between chicken chromosome 4 and human chromosomes 4q and Xp.

Finally, the results of this thesis are discussed within a broader context in ***Chapter 6*** and in particular in relation to the recently published draft sequence of the chicken genome and the polymorphism map consisting of 2.8 million single nucleotide polymorphisms.

SAMENVATTING

SAMENVATTING

Het pulmonale hypertensie syndroom (PHS) wordt gekarakteriseerd door een cascade van gebeurtenissen resulterend in hart afwijkingen (vergroot hart, rechter ventriculaire hypertrofie), variabele leverafwijkingen en ophoping van vocht in de abdominale holten (ascites). Door een overmaat van ascitesvocht (lymfevocht en bloedplasma uit de lever) in de buikholten is PHS ook wel bekend onder de naam waterbuik. Verschillende genetische als ook omgevingsfactoren zoals hoogte, temperatuur, licht, ventilatie en voedingsfactoren lijken van invloed te zijn op de ontwikkeling van PHS. Het onderzoek beschreven in dit proefschrift is een eerste stap om de genetische factoren te identificeren die aanleiding geven tot PHS.

Dit proefschrift richt zich op de identificatie van genen betrokken bij PHS bij vleeskippen. Om deze genen te identificeren wordt gebuik gemaakt van een "quantitative trait loci" (QTL) analyse. Om QTL voor verschillende economisch belangrijke kenmerken waaronder PHS op te sporen is een experimentele populatie opgezet. Deze populatie is opgezet volgens een zogenaamd "full sib/half sib" ontwerp en is een kruising tussen twee genetisch verschillende vleeskuiken moederlijnen, afkomstig van het White Plymounth Rock ras. In ***hoofdstuk 2*** wordt het detecteren en localiseren van QTL voor de kenmerken gerelateerd aan PHS beschreven door gebruik te maken van een zogenaamde complete genoom analyse. De totale genoom analyse werd uitgevoerd door 10 "full sib" families, bestaande uit 20 generatie 1 (G1) en 456 G2 kippen te typeren voor 420 microsatelliet merkers afkomstig van 24 autosomale chromosomen. Verder werden van 4202 G3 kippen fenotypische waarnemingen verzameld. QTL analyse werd uitgevoerd door gebruik te maken van een "full sib" over familie regressie interval methode. Significante QTL werden gedetecteerd op de chromosomen (GGA) 2, 4 en 6. Suggestieve koppeling werd gevonden voor de chromosomen 5, 8, 10, 27 en 28. De fenotypische waarnemingen waren meestal gerelateerd aan hart condities (%RV, %TV en RATIO), condities van de inwendige organen, ascites en overleving. Het QTL gevonden op GGA2 voor de kenmerken rechter en totale hartkamer gewicht als percentage van het lichaamsgewicht (%RV en %TV) had de hoogste statistische significantie. Een gerelateerd kenmerk, de verhouding van het rechter hartkamer gewicht als percentage van het totale hartkamer gewicht (RATIO), bereikte een suggestieve drempel op ditzelfde chromosoom. Doordat de populatie bestond uit een kruising van twee

gerelateerde rassen kon de verwachte lagere test statistieken worden verklaard. Het is daarom essentieel om de gevonden QTL te valideren in verdere generaties voordat begonnen kan worden aan het verkleinen van de gevonden QTL gebieden. De validatie van de QTL op chromosoom 8, 10 en 28 en de bevestiging daarvan op GGA8 en GGA10 wordt beschreven in ***hoofdstuk 3***.

De validate voor deze chromosomen is uitgevoerd in generatie G6 tot G8 van de experimentele populatie. Deze populatie bestaat uit 19 families met volle broers/zussen (een combinatie van G6/G7 en G7/G8). In totaal zijn alle individuen getypeerd voor 14 microsatelliet merkers. Verder zijn aan meer dan 1500 vleeskuikens 12 ascites gerelateerde kenmerken gemeten. Significante resultaten zijn gevonden voor de kenmerken lichaamsgewicht op 2 en 5 weken leeftijd, gewicht rechter hartkamer, RATIO (RV:TV) en uitval. Het meest significante QTL ligt op chromosoom 8 voor de kenmerken lichaamsgewicht op 5 weken leeftijd ($BW5_{AS}$) (*ADL0278-MCW0351*) van de genetische kaart, gewicht rechter hartkamer (*ADL0278-MCW0351*) en RATIO (*ROS0075-ADL0278*). Voor GGA10 voor de kenmerken lichaamsgewicht op twee weken leeftijd (*MCW0035-ADL0102*) en uitval (*ADL0158-LEI0112*). Het QTL op GGA28 kon niet worden gevalideerd.

De validatie en bevestiging van de significante QTL op GGA2 en GGA4 zijn beschreven in ***hoofdstuk 4***. In dit hoofdstuk is tevens de verdere verfijning van deze twee QTL gebieden beschreven, gebruik makend van de gecombineerde "linkage disequilibrium/ linkage analysis" methode (LDLA). Validatie en verfijning van de QTL gebieden is uitgevoerd in onderling gekruiste lijnen (de "advanced intercross line"; AIL) welke gemaakt zijn door willekeurig ondelinge kruisingen toe te passen vanaf de derde generatie (G3) totdat de achtste (G8) werd bereikt. In totaal zijn 47 microsatelliet merkers gebruikt voor het QTL gebied op GGA2 (ongeveer 15 Mb groot) en GGA4 (ongeveer 16 Mb groot). Het QTL origineel gedetecteerd in de G2/G3 generatie werd bevestigd op de twee chromosomen voor de kenmerken RATIO op GGA2 en $BW5_{AS}$ op GGA4. Bovendien zijn additionele QTL gedetecteerd op GGA2 voor het kenmerk pCO_2 en $BW3_{AS}$ voor GGA4. Deze laatste twee kenmerken zijn niet gemeten in generatie 3 van de oorspronkelijke studie. De gecombineerde LD/LA methode resulteerde in een 3 tot 4 voudige reductie van de grootte van het QTL interval.

De verbetering van de fysische kaart van GGA4 en het genereren van een gedetaileerde vergelijkende kaart tussen dit kippen chromosoom en de humane chromosomen 4 en X zijn beschreven in ***hoofstuk 5***. De resolutie van de fysische kaart voor GGA4 is verbeterd door een combinatie van "radiation hybrid" (RH)

kartering en "bacterial artificial library" (BAC) kartering. Het ChickRH6 hybrid panel is gebruikt voor het maken van een RH kaart van GGA4. Elf microsatelliet merkers, genetisch gelokaliseerd op GGA4, zijn gebruikt als ankers tussen de genetische en de RH kaarten. Met behulp van het vergelijken van sequencies in de EMBL/Genbank databanken ("BLAST") zijn, gebaseerd op de bekende geconserveerde synteny tussen GGA4 en de humane chromosomen 4 en X, sequenties geïdentificeerd voor orthologe kippen genen van deze humane chromosomen. Deze sequencies zijn gebruikt voor de ontwikkeling van STS merkers die vervolgens getypeerd zijn op het RH panel. Door gebruik te maken van de LOD drempels van 5,0 werden 9 koppelingsgroepen geconstrueerd welke gekoppeld werden met de genetische koppelingskaart van dit chromosoom. De hieruit resulteerende RH kaart bestond uit 11 microsatelliet merkers en 50 genen. Om het aantal genen op de kaart uit te breiden en om additionele ankerpunten naar de fysische BAC kaart van dit chromosoom te krijgen zijn BAC klonen geïdentificeerd voor 22 microsatelliet merkers en 99 genen. De gecombineerde RH en BAC kartering heeft geresulteerd in plaatsing van 61 genen op GGA4 en hiermee is de resolutie van de kippen/humane vergelijkende kaart voor dit chromosoom aanzienlijk verbeterd. Door deze verbeterde resolutie van de vergelijkende kaart zijn verschillende herrangschikkingen geïdentificeerd tussen kip GGA4 en de humane chromosomen 4q en Xp.

Tenslotte worden in ***hoofdstuk 6*** de resultaten van dit proefschrift bediscussieerd en in een bredere context geplaatst waarin de recent gepubliceerde voorlopige sequentie van het kippen genoom en de polymorfisme kaart welke bestaat uit 2,8 miljoen "single nucleotide polymorphisms" (SNPs) zijn meegenomen.

ABBREVIATIONS KEY

ABBREVIATIONS KEY

ABDOMEN	Fluid accumulation in the abdomen
BREAST	Color of the chicken breast
$BW2_{AS}$	Body weight at 2 weeks of age under ascites condition
$BW3_{AS}$	Body weight at 3 weeks of age under ascites condition
$BW5_{AS}$	Body weight at 5 weeks of age under ascites condition
HCO_3	Blood bicarbonate concentration
HCT	Hematocrit value
HEART	Fluid in the heart sac
LIVER	Liver abnormalities
MORT-TOT	Total mortality
pCO_2	Partial pressure of carbon dioxide
pH	Blood pH
pO_2	Partial pressure of oxygen
QTL	Quantitative trait locus
RV	Right ventricular weight
RATIO (RV:TV)	Ratio of right ventricular weight to total ventricular weight
sO_2	Oxygen saturation in arterial blood
T_{CO_2}	Total carbon dioxide content
TV	Total ventricular weight
%RV	Right ventricular weight as percentage of BW
%TV	Total ventricular weight as percentage of BW

LIST OF PUBLICATIONS

LIST OF PUBLICATIONS

Tarik S.K.M. Rabie, Richard P.M.A. Crooijmans, Mireille Morisson, Joanna Andryszkiewicz, Jan J. van der Poel, Alain Vignal, Martien A.M. Groenen (2004). A Radiation Hybrid Map of Chicken Chromosome 4. *Mammalian Genome* **15**, 650-569

T.S.K.M. Rabie, R.P.M.A. Crooijmans, H. Bovenhuis, A.L.J. Vereijken, A. Veenendaal, J.J. van der Poel, J.A.M. Van Arendonk, A. Pakdel, M.A.M. Groenen (xxxx). Genetic mapping of quantitative trait loci affecting susceptibility in chicken to develop the Pulmonary Hypertension Syndrome (PHS). *Submitted*

T.S.K.M Rabie, R.P.M.A. Crooijmans, H. Bovenhuis, A.L.J. Vereijken, A. Veenendaal, J.J. van der Poel, J.A.M. Van Arendonk, M.A.M. Groenen (xxxx). Validation and fine-scale mapping of quantitative trait loci affecting Pulmonary Hypertension Syndrome (PHS) in broilers using advanced intercross line. *Will be Submitted*

A. Pakdel, **T. Rabie**, T. Veenendaal, R. P. M. A. Crooijmans, M. A. M. Groenen, A. L. J. Vereijken, J. A. M. Van Arendonk, H. Bovenhuis (xxxx). Confirmation of Quantitative Trait Loci Affecting Susceptibility of Broilers to Ascites. *Submitted*

I. Hermes, M. Khatab, **T.S. Rabie** (1996). Effect of feeding layers different sources and levels of calcium at graded animal fat levels on some productive traits. *Eg. J. Anim. Prod.* 459-469.

J.J. van der Poel, R.P.M.A. Crooijmans, R.J.M. Dijkhof, A. Veenendaal, S.J.B. Cornelissen, K. Hemmatian, A.J. Buitenhuis, D.G.J. Jennen, M. Siwek, **T. Rabie,** A.J.A. van Kampen, J. Aerts, M.A.M. Groenen (2002). BAC contig mapping: towards a physical map of the chicken genome. *7th World Congress on Genetics Applied to Livestock Production, Montpellier, France.* **30**, 201-204.

Tarik Rabie, Richard Crooijmans,Addie Vereijken, Gerard Albers, Tineke Veenendaal, Rosilde Dijkhof, Jan van der Poel, Henk Bovenhuis, Martien Groenen (2002). Fine mapping of quantitative trait loci (QTL) affecting susceptibility to ascites in chicken. *28th Conference of the International Society of Animal Genetics (ISAG), Göttingen, Germany.* P.189

Rabie, T.S.K.M., R.P.M.A. Crooijmans, A.L.J. Vereijken, G. Albers, T. Veenendaal, R.J.M. Dijkhof, A.F.B. van der Poel, A. Pakdel, J.A.M. van Arendonk, H. Bovenhuis, M.A.M. Groenen (2002). Fine mapping of Quantitative trait loci (QTL) affecting susceptibility to Ascites in chicken. *53rd Annual meeting of European Association for Animal Production (EAAP), Cairo, Egypt.* P.12

Rabie, T.S.K.M., R.P.M.A. Crooijmans, A.L.J. Vereijken, G. Albers, T. Veenendaal, R.J.M. Dijkhof, A.F.B. van der Poel, A. Pakdel, J.A.M. van Arendonk, H. Bovenhuis, M.A.M. Groenen (2003). Validation of quantitative trait loci (QTL) affecting susceptibility to ascites in chicken. *3rd European Poultry Genetics Symposium, Wageningen, the Netherlands.* P.76

Tarik S.K.M. Rabie, Richard P.M.A. Crooijmans, Mireille Morisson, Joanna Andryszkiewicz, Jan van der Poel, Alain Vignal, Martien A.M. Groenen (2004). A radiation hybrid map of chicken chromosome 4. *Plant and Animal Genome XII, San Diego, CA, USA.* P.234

A. Pakdel, **T. Rabie**, T. Veenendaal, R. P. M. A. Crooijmans, M. A. M. Groenen, A. L. J. Vereijken, J. A. M. Van Arendonk, H. Bovenhuis (2004). Confirmation of QTL Affecting Ascites Susceptibility in Broilers. *XXII World's Poultry Congress, Istanbul, Turkey.* P.176

Richard Crooijmans, Laurie Gordon, Sandra Corenelissen, **Tarik Rabie**, Jan van der Poel, Mireille Morisson, Alain Vignal, Lisa Stubbs, Martien Groenen (2002). Comparative mapping and sequencing between chicken chromosome 28 and human chromosome 19p. *28th Conference of the International Society of Animal Genetics (ISAG), Göttingen, Germany.* P.37

Laurie Gordon, Richard Crooijmans, Joomyeong Kim, Hummy Badri, Mari Christensen, Sandra Cornelissen, Matthew Groza, **Tarik Rabie**, Mary Tran, Martien Groenen, Lisa Stubbs (2002). A Sequence-ready Comparative Map of Chicken Genomic Segments Syntenically Homologous to Human Chromosome 19. *DOE Genome Contractor & Grantee Workshop IX, Oakland, California, USA.* No.57

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

First of all, I would like to take this opportunity to express my heartily thanks to my parents for they emotional support and giving special great power that lead me to success and to complete this study. Without they invaluable support, nothing of this work would have been possible.

I would like to express my heartily gratitude and appreciations to my wife, for giving me a moral support; she managed to keep me in a high spirit all the time. Also for her endurance over the years of my PhD studies, she shared me the difficulties and the happiness during our staying in Wageningen. I devote this work also to my lovely children *Lo'ay, Amaar*, and *Reaam*, they initiated me in the father's world and they taught me how to draw "animals" and "maps". I would like to express my heartily thanks to my sister, her husband *Mohamed* and their children, *Mo'men, Amro*, and *Samaa'* for they emotional support.

My special thanks are given to my supervisor *Professor Dr. Martien Groenen*, for his excellent guidance from the first day onwards. As a knowledgeable supervisor; he was one of very few people who teach me how to be a good scientist and he put me in the first step of the field of molecular biology. I would like to express my gratitude for his valuable comments and criticism on manuscripts that lead to the completion of this work.

I owe a great deal to my co-supervisor *Dr. Ir. Richard Crooijmans*, for his help, supervision and guidance for this work and his advice throughout the practical work.

I would like to express my thanks to *Dr. Ir. Henk Bovenhuis* for valuable discussion and for helping in solving statistical problems.

I appreciated a very helpful man, *Professor Dr. Ir. Johan van Arendonk*, for his opening personality, cordiality and support. I appreciate him as a successful manager, in addition his professional capacity.

I am also grateful to the members and the colleagues in the Animal Breeding and Genetics Group, Wageningen University, The Netherlands, for providing a congenial atmosphere. Many thanks are due to *Dr. Ir. Jan van der Poel*, for his kindness and sociality, *Bart (J)*, my roommate for the good time that we spent together, and *Danyel Jennen*, for his kind friendship. Meanwhile, sincere thanks are due to all people who supported me in preparing this dissertation.

I would like to thank my friends and their families here in Wageningen specially *Budhi, M. Yakoub, Hamada, and Abdul Aziz.* Also *Nabeil, Ahmed, Khaled, Dr. M. El-Lithy, Dr. Hamed El-Mashad, Prof. Dr. Magdy El-Nagar*, and special thanks are due to *Dr. Tamer Gameil*, *Dr. Loay*, and *Dr. El-Sayed Ewis* and their families for the good time that we spent together either in Wageningen or in Egypt. Many thanks are due to *Dr. Zizy* for her kind friendship with my family at the last few months of our staying in Wageningen. I'm grateful to *Harm* and *Hanneke* as nice neighbors at "Haarweg 199", also for the good time that we spent together, and for playing "Guitar" with our children.

Special thanks are given to *Dr. Adel El-Sayed* and his lovely family, for everything; kindness, friendship, brotherhoods, and the excellent time that we spent together in Wageningen and Veenendaal.

Back home to my beloved country Egypt, Special gratitude goes to the Egyptian Government for sponsoring my study and dealing with the financial administration.

I am grateful to the academic stuff of the Department of Animal Production and Fisheries, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt for nominating me to get a scholarship from the Egyptian Ministry of higher Education and Scientific Research. I would like to express my deeply thanks to all of my Egyptian professors, colleagues and friends, specially *Professor Dr. Hani Sabri*, Animal Production Department, Faculty of Agriculture, Suez Canal University, for his personal insight and friendly communications during my studying in Wageningen.

I want to express my deeply thanks to *Professor Dr. Helmy Omran*, supervisor of Biotechnology Research Institute (BRI), Suez Canal University, for his personal insight, encouragement, valuable comments, advice, and his diplomatic support.

Special thanks are due to *Dr. Haiam Abdel Haleim*, *Dr. Mervat Ali*, *Dr. Ahmed Yehia.* I would like to express my heartily thanks to *Dr. Abdulla Ghazy* for his excellent friendship and brotherhoods.

Last but not least, I would like to express my special thanks to Eng. *Tarek Helmy*, Vienna, Austria, for his brotherhoods and friendly support. And my gratitude to *Dr. Magda Hozaien*, Miami, USA, and her family for they intense contact and for the good time that we spent together either in science or in social life.

CURRICULUM VITAE

CURRICULUM VITAE

The author of this dissertation, ***Tarik El-Said Kamel Mohamed Rabie***, was born on September 3rd, 1969 in Alexandria, Egypt. After finishing high school in 1986, he joined the Faculty of Agriculture, Suez Canal University, at Ismailia city, Egypt. He obtained his Bachelor of Science degree (B.Sc.) in 1990, on the field of animal and poultry production from Animal Production and Fisheries Department, Faculty of Agriculture, Suez Canal University where he became the first among all his colleagues and he graduated with the general grade Very Good with Honor degree. From 1990 he has been chosen to be a Researcher at the same department. The end of the year, he became a staff member of the same department in the branch of Animal and Poultry Production and he worked as demonstrator for 5 years. During this period, Tarik was engaged in teaching in the university (Faculty of Agriculture and Faculty of Veterinary Medicine). In addition, he awarded a fellowship to study computer sciences at CEMARP (Canada-Egypt McGill Agriculture Research Program). He got an "Excellent" grade for all courses which he attended.

In January 1996, he obtained his Master of Science degree (MSc.) in the field of poultry production, poultry nutrition, Faculty of Agriculture, Suez Canal University. From that date, he is working as assistant lecturer; also he was involved in teaching in the University. In 1997/1998, the author was awarded a grant of full fellowship by Egyptian Ministry of Higher Education and Scientific Research to study PhD at Animal Breeding and Genetics Group, Wageningen University, the Netherlands. In 2000, the author was awarded the *Professor Dr. Ahmed Zewail's Prize* for Scientific Creativity and Innovation. In September 2000, he joined the Animal Breeding and Genetics Group at Wageningen University. His research work was on "The Identification and Analysis of Ascites Resistance Genes in Chicken". He finished his PhD project in 4 years with financial support of Egyptian Government. Within this period, in 2002, Tarik was chosen to be a member of Biotechnology Research Institute (BRI), Suez Canal University, Ismailia, Egypt. After return back to Egypt, Tarik will start his work as Assistant Professor at both of Animal Production Department, Faculty of Agriculture, and Biotechnology Research Institute, Suez Canal University, Ismailia, Egypt.

Mailing Address
Suez Canal University
Faculty of Agriculture
Department of Animal Production.
Ismailia, 41522
Egypt.
e-mail: tarik@egypt.net

Prof. Dr. Ahmed Zewail: Egyptian Professor who awarded the Nobel Prize in Chemistry 1999.

PhD EDUCATION PROGRAMME
(WIAS)

Completed PhD Education Plan		**Graduate School *WIAS***	
Name	Tarik Rabie		
Group	Animal Breeding and Genetics Group		
Supervisor(s)	Prof. Dr. Martien Groenen		
Daily supervisor(s)	Dr. Richard Crooijmans		
Period	September 2000 until September 2004		
The Basic Package *(minimum 2 cp)*		**year**	**cp***
➢ WIAS Common Course *(mandatory)*		2001	2,0
➢ Course on philosophy of science and ethics *(mandatory)*		2002	1,0
SUBTOTAL			**3,0**
Scientific Exposure *(conferences, seminars and presentations, minimum 5 cp)*		**year**	**cp**
➢ ***International conferences*** *(minimum 2 cp)*			
ISAG Conference,Göttingen, Germany		2002	1,0
EAAP Conference, Cairo, Egypt		2002	0,8
3rd European Poultry Genetics Symposuim, The Netherlands		2003	0,6
Plant and Animal Genome Conference XII, San Diego, CA-USA		2004	1,0
➢ ***Seminars and workshops***			
WIAS Science Day (2001; 2002; 2003; and 2004)		2001-2004	0,8
Genetic Retrait Rolduc, NOW-MW, Kerkrade		2001/2003	0,8
2nd WIAS workshop on PhD Supervision		2001	0,1
Shaping the embryo; Dynamics of early vertebrate development		2003	0,2
Animal Reproduction		2003	0,1
➢ ***Presentations*** *(minimum 4 original presentations of which at least 1 oral)*			
Oral presentation in EAAP meeting in Cairo, Egypt		2002	0,5
Poster presentation in ISAG meeting in Göttingen, Germany		2002	0,5
Oral presentation in the Faculty of Agriculture in Ismailia, Egypt		2002	0,5
Oral presentation in the Biotechnology Research Institute (BRI) in Egypt		2002	0,5
Oral presentation in the Department of Animal Production in Ismailia, Egypt		2002	0,5
Poster presentation in 3rd European Poultry Genetics Symposuim in the NL		2003	0,5
Poster presentation in XII Plant and Animal Genome in San Diego,CA, USA		2004	0,5
Poster presentation in WIAS Science day in Wageningen, the Netherlands		2004	0,5
SUBTOTAL			**9,4**
In-Depth Studies *(minimum 4 cp)*		**year**	**cp**
➢ ***Disciplinary and interdisciplinary courses***			
Principles of QTL mapping, Salzburg, Austria		2003	2,0
➢ ***Undergraduate courses***			
Recombinant DNA and Genetics Manipulation		2000	2,0
Molecular Genome Analysis		2001	2,0
Elementary Molecular Biology Techniques		2001	1,0
Genome Analysis		2001	2,0
SUBTOTAL			**9,0**
Professional Skills Support Courses *(minimum 2 cp)*		**year**	**cp**
WIAS Course Techniques for Scientific Writing (advised)		2001	0,8
Dutch Language course 1st Level		2001	1,0
Time planning and Project management		2003	1,0
SUBTOTAL			**2,8**
Research Skills Training *(apart from carrying out the PhD project, optional)*		**year**	**cp**
Preparing own PhD research proposal (optional, maximum 4 cp)			4,0
SUBTOTAL			**4,0**
Didactic Skills Training *(optional)*		**year**	**cp**
➢ ***Supervising MSc theses*** *(maximum 1 cp per MSc student)*			
Physical mapping of chicken Chromosome 4 (GGA4)		2003	1,0
Fine Mapping of QTL on GGA2 & GGA4		2004	1,0
SUBTOTAL			**2,0**
Education and Training Total (minimum 21 cp)			**30,2**

* One credit point (cp) equals a study load of approximately 40 hours

The research described in this thesis was financially supported by the Egyptian Ministry of Higher Education and Scientific Research, Egyptian Government (Project No. 29110/416000).

Printed by: Ponsen & Looijen BV, Wageningen, the Netherlands

The cover was designed by *Rania Dewaidar*.

و من ثم وبواسطة استخدام هذه الخرائط المقارنة أمكن التعرف على العديد من ترتيب وإعادة ترتيب مواقع الجينات بين كل من كروموزوم 4 في الدواجن و كروموزومي 4 و X في الإنسان.
و عليه فالفصل الأخير من هذه الرسالة (***الفصل السادس***) يتناول مناقشة كل النتائج المتحصل عليها في هذه الرسالة مجتمعة باستفاضة آخذا في الاعتبار الاستفادة من توفر المعلومات الأولية الخاصة بكلاً من الـChicken genome sequence و كذلك الخريطة متعددة الأشكال و المكونة من 2.8 مليون نيوكلوتيدة متعددة الأشكال Single Nucleotide Polymorphism

RATIO على الكروموزوم الثاني و صفة وزن الجسم عند عمر خمسة أسابيع على الكروموزوم الرابع للدواجن. هذا بالإضافة إلى اكتشاف QTLs لصفات جديدة لم يتم قياسها من قبل من ثم لم تكتشف من قبل في الجيل الثالث مثل pCO_2 على الكروموزوم الثاني و كذلك وزن الجسم عند عمر ثلاثة أسابيع على الكروموزوم الرابع للدواجن.
إن استخدام طريقة التحليل الجديدة (LD/LA) قد قلل من مسافة الـQTL إلى 75% من المسافة الأصلية المكتشفة في الجيل الثالث.

الفصل الخامس من هذه الرسالة يناقش تحسين الخريطة الفيزيقية للكروموزوم الرابع للدواجن و كذلك تشكيل خريطة جينات مقارنة متقدمة بصورة مفصلة بين الكروموزوم الرابع للدواجن و الكروموزوم الرابع و الكروموزوم الجنسي(X) في الإنسان. و ذلك باستخدام كلا من هجن الـDNA المشعة (RH) مع مزرعة الكروموزومات البكتيرية الصناعية (BAC).

استخدمت مجموعة هجن الـDNA المشعة المنتقاه المعروفة باسم ChickRH6 لإنشاء خريطة الهجن المشعة (Radiation hybrid map) للكروموزوم الرابع للدواجن. و استخدم لهذا الغرض عدد 11 مميز من نوع ميكروستلايت المعروفة الموقع على هذا الكروموزوم و المنتقاه من الخريطة الجينية للدواجن الخاصة بهذا الكروموزوم. بناءا على المعلومات القليلة المتوفرة عن مدى توافق كلا من كروموزوم 4 في الدواجن و كروموزومي 4 و X في الإنسان، قد انتخبت مجموعة تتابع النيوكلوتيدات (Sequence) للجينات المعروفة في الإنسان بواسطة استخدام طريقة المضاهاة بواسطة برنامج التحليل الخاص BLAST. و قد استخدم الـSequence الخاص بالجينات المنتخبة في تصميم ما يسمى بالـSTS للدواجن بواسطة استخدام عند الحد الحرج للوغاريتم المسمى LOD. و من نتائج هذه الدراسة انه تم بنجاح إنشاء و تكوين عدد 9 مجموعات جينية مختلفة مرتبطة ارتباط وثيق بخريطة الجينات المرتبطة (Linkage map) بكروموزوم 4 في الدواجن. و بهذا فان استخدام الـRH قد نتج عنه تكوين خريطة الـRH مكونة من 11 مميز من نوع ميكروستلايت بالإضافة إلى 50 جين لم تكن معروفة في الدواجن من قبل.
و لكي نتمكن من زيادة عدد الجينات في هذه الخريطة التي تم الحصول عليها ، تم استخدام مكتبة الـBAC و بذلك تم التعرف على 22 مميز من نوع مايكروستلايت بالإضافة إلى 99 جين في الدواجن. تم إدماج الخرائط المتحصل عليها من كلا من الـRH و الـBAC لكي ينتج عنهما وضع 61 جين جديد في خريطة الجينات الخاصة بالكروموزوم الرابع للدواجن و الذي أدى إلى إنتاج خريطة فيزيقية عالية الوضوح و الجودة و كذلك خريطة الجينات المقارنة بين الدواجن و الإنسان(Comparative map).

RV% و TV%. و قد وجد أن الـQTL لصفة الـRATIO معنوية جدا و قد تجاوزت الحد الحرج للمعنوية لهذا الكروموزوم.

يجب التأكيد على وجود الـQTL في الأجيال المتعاقبة قبل الاستفاضة في الدراسات المتقدمة بناءا على النتائج المتحصل عليها في الفصل الثاني و لذلك يجب دراسة صلاحية الـQTL الواقعة على كلا من الكروموزومات 8، 10 و 28 . يتناول ***الفصل الثالث*** هذه الدراسات باستفاضة و التي تمت على الجيل السابع و الثامن على 19 عائلة ناتجة من تداخلات ما بين الجيل السادس والجيل السابع و بين الجيل السابع والجيل الثامن. تناول هذا الفصل دراسة اثنتا عشر صفة مرتبطة بـالـPHS هذا بواسطة استخدام 14 مميز من نوع ميكروستلايت. تمت الدراسة على 1500 طائر. و من النتائج المتحصل عليها في هذه الدراسة و جود نتائج معنوية لصفة وزن الجسم عند عمر اسبوعين و خمسة أسابيع، وزن الصمام الأيمن للقلب، صفة الـRATIO بالإضافة إلى صفة نسبة النفوق الكلية . معظم الـQTL المعنوية تقع على الكروموزوم الثامن للدواجن مثل الـQTL الخاصة بصفة وزن الجسم عند عمر خمسة أسابيع تقع عند 24سنتيمورجان ، وزن صمام القلب الأيمن يقع عند 19 سنتيمورجان و RATIO عند 15 سنتيمورجان. و قد و جدت أيضا العديد من الـQTLs على الكروموزوم العاشر لكلا من صفة وزن الجسم عند عمر اسبوعين عند موقع 34 سنتيمورجان و نسبة النفوق الكلية عند 59 سنتيمورجان. و لم نتمكن من إثبات صلاحية الـQTLs الواقعة على الكروموزوم 28 للدواجن. و بهذا قد تم إثبات و تأكيد صلاحية الـQTLs على كلا من الكروموزوم الثامن و العاشر للدواجن.

يتناول ***الفصل الرابع*** دراسة صلاحية و تأكيد معنوية الـQTLs الواقعة على كلا من الكروموزوم الثاني و الرابع للدواجن كخطوة تمهيدية يعقبه دراسة الخرائط الشديدة الوضوح
(Fine Mapping) وذلك باستخدام طريقة التحليل الحديثة و المسماه دمج مابين طريقتي تحليل عدم اتزان التكرار الجيني و التحليل باستخدام الارتباط
(Linkage disequilibrium/Linkage analysis "LDLA")

و قد صممت التجربة باستخدام خلط الخطوط الداخلية المتقدم Advanced Intercross Line (AIL) و ذلك بالخلط العشوائي لكل جيل ابتداءا من الجيل الثالث حتى الوصول الى الجيل الثامن. استخدم في هذه الدراسة 47 مميز من نوع الميكروستلايت موزعين على مسافة 25 سنتيمورجان على الكروموزوم الثاني وعلى مسافة 16 سنتيمورجان على الكروموزوم الرابع للدواجن. و من نتائج هذه الدراسة تأكيد وجود الـQTLs الرئيسية - و التي اكتشفت في الجيل الثاني و الثالث- على كلا من الكروموزومين الثاني و الرابع في الجيل الثامن لكلا من الصفات

هذه الرسالة تناقش عرض الزيادة المفرطة في الضغط الرئوي في دجاج اللحم
(Pulmonary Hypertension Syndrome "PHS")

يوصف الـPHS بأنه عرض و ليس مرض و هو المسبب الأساسي لانعدام كفاءة القلب و الذي يتمثل في تضخم القلب خاصة تضخم صمام القلب الأيمن و كذلك تغيرات فسيولوجية في الكبد مع تراكم كميات غير طبيعية من السوائل (خليط من بلازما الدم و السائل الليمفاوي) في التجويف البطني للطائر و الذي يعرف عند العامة بالاستسقاء (Ascites). العديد من العوامل الجينية و البيئية تتحكم في نشوء و تطور الـPHS و نأخذ على سبيل المثال و ليس الحصر الارتفاع عن مستوى سطح البحر, درجة الحرارة المحيطة، الإضاءة، التهوية بالإضافة إلى التغذية.

الغرض الأساسي في هذه الرسالة يتمثل في اكتشاف و التعرف على العوامل الجينية و كذلك تحديد عدد من الجينات المتحكمة في الـPHS. لهذا الغرض قد تم إنشاء العشيرة التجريبية لكي تكون النواة الرئيسية لدراسة مواقع الصفات الكمية (QTL) للعديد من الصفات التي لها أهمية اقتصادية كبيرة مثل الـPHS. أساس هذه العشيرة هو عائلات الأشقة الكاملة وأنصاف الأشقة المنبثقة من ثلاث أجيال متتابعة والتي تم إنشائها من الخلط بين خطين وراثيين أميين مختلفان وراثيا اصلهما من سلالة البلايموث روك الابيض (White Plymouth Rock).

و يعتبر ***الفصل الأول*** من هذه الرسالة بمثابة مقدمه عامة يتناول فيها ماهية هذا العرض (PHS) و العوامل المسببة له و العديد من النقاط التي توضح أهمية الدواجن كنموذج دراسي لمواقع الصفات الكمية كالـPHS للاستفادة منه في التعامل معه في الإنسان.

الفصل الثاني من هذه الرسالة يوضح الخريطة التمهيدية للعديد من الـQTL للصفات المرتبطة بالـPHS عن طريق المسح الجينومي (Genome Scan). قد استخدم عدد 420 مميز من نوع ميكروستلايت (Microsatellite) موزعة عشوائيا على 24 كروموزوم جسمي لإجراء Genome Scan على عدد 10 عائلات مكونة من 20 طائر من الجيل الأول و 456 طائر من الجيل الثاني. القياسات الظاهرية للطيور جمعت من 4202 طائر من الجيل الثالث و تم تحليل بيانات الثلاث أجيال بواسطة طريقة Regression Interval Mapping. و من النتائج المتحصل عليها وجود QTL على الكروموزومات 5، 8، 10، 27 و 28 و من المدهش إن أغلب هذه الصفات مرتبطة بالقلب مثل صفة النسبة المئوية لوزن الصمام الأيمن للقلب إلى وزن الجسم (%RV) ، النسبة المئوية لوزن صمامات القلب الإجمالي إلى وزن الجسم (%TV)، بالإضافة إلى صفة النسبة المئوية لوزن صمام القلب الأيمن إلى الوزن الإجمالي لصمامات القلب (RATIO). معظم الـQTL المعنوية إحصائيا تقع على الكروموزوم الثاني للدواجن للصفات

الملخص العربي

الزيادة المفرطة في الضغط الرئوي في دجاج اللحم: نظرة إلى ما تحت منحنيات مواقع الصفات الكمية

رسالة دكتوراه

مقدمة من

طارق السعيد كامل محمد ربيع

جامعة فاخننجن – قسم تربية ووراثة الحيوان

معمل التقنية الحيوية والبيولوجيا الجزيئية

فاخننجن – هولندا

إهداء

أهدي هذه الرسالة إلى .....

إلى نبع العطاء .....

إلى أبي و أمي

إلى شركائي في مشوار الحياة .....

إلى زوجتي و أولادي "لؤي .. عمار .. رئام"

إلى شقيقتي و أسرتها الكريمة