doi:10.1111/j.1365-2052.2005.01346.x

Genetic mapping of quantitative trait loci affecting susceptibility in chicken to develop pulmonary hypertension syndrome

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

*Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, Wageningen, The Netherlands. [†]Nutreco Breeding Research Center, Boxmeer, the Netherlands

Summary

Pulmonary hypertension syndrome (PHS), also referred to as ascites syndrome, is a growth-related disorder of chickens frequently observed in fast-growing broilers with insufficient pulmonary vascular capacity at low temperature and/or at high altitude. A cross between two genetically different broiler dam lines that originated from the White Plymouth Rock breed was used to produce a three-generation population. This population was used for the detection and localization of quantitative trait loci (QTL) affecting PHS-related traits. Ten full-sib families consisting of 456 G_2 birds 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 QTL. There was statistical evidence for QTL on chicken chromosome 2 (GGA2), GGA4 and GGA6. Suggestive QTL were found on chromosomes 5, 8, 10, 27 and 28. The most significant QTL were located on GGA2 for right and total ventricular weight as percentage of body weight (%RV and %TV respectively). A related trait, the ratio of right ventricular weight as percentage to total ventricular weight (RATIO), reached the suggestive threshold on this chromosome. All three QTL effects identified on GGA2 had their maximum test statistic in the region flanked by markers *MCW0185* and *MCW0245* (335–421 cM).

Keywords ascites, chicken, genome scan, metabolic disorder, pulmonary hypertension syndrome, 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, growth rate in broilers has increased at a rate of 4–5% per year over the past 30 years (Havenstein *et al.* 1994; Julian 2000). Fast growing broilers, however, are unable to deliver sufficient oxygen to satisfy the metabolic demands of their rapid growth rates. 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 a gradual increase under less extreme conditions has also been found (Albers & 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 avian respiratory and circulation systems are important in the susceptibility of broilers to PHS. Unlike mammals, avian lungs are relatively rigid and have limited movement during breathing because air goes through them into the air sacs and back through the lungs on expiration. In addition, the lungs of birds have more capillaries per area than mammals. Moreover, the avian heart is different from

Address for correspondence

M. A. M. Groenen, Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, PO Box 338, 6700 AH Wageningen, The Netherlands.
E-mail: martien.groenen@wur.nl

Accepted for publication 10 June 2005

[1]Present address: Biotechnology Research Institute, Suez Canal University, Ismailia 41522, Egypt. E-mail: tarik@egypt.net

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 fibres 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 PH, 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 PHS (Dewil *et al.* 1996). In susceptible lines, hypoxic conditions during incubation of the chicken egg can cause an increase in the incidence of disease in adult birds (Buys *et al.* 1997). Insufficient development of the lungs or of the pulmonary blood vessels in particular chicken broiler lines may form the basis of these observed differences. As thyroid function is an important regulatory mechanism of metabolic rate, thyroid functioning may be yet another system responsible for the observed differences in susceptibility to PHS. In addition, genetic predisposition plays a considerable role in the incidence of 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 makes PHS a trait that is particularly amenable for a whole-genome scan aimed at the mapping of quantitative trait loci (QTL). The objective of this study was a whole-genome scan aimed to detect and localize QTLs controlling ascites-related traits.

Material and methods

Experimental population and phenotyping

The experimental population used to detect PHS QTLs was based on three generations (G_1, G_2 and G_3) according to recommendations of Van der Beek (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 relatively high reproductive performance and is fast-feathering. The paternal line has relatively high growth performance and is slow-feathering. The population structure and number of birds are given in Table 1. Each G_2 male produced on average 23 G_3 offspring and each G2 female produced on average 15 G_3 offspring. To produce the G_3 birds, each G_2 male bird was on average mated to three females and each G_2 female to two males.

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

Generation[1]	Sires	Dams	Total	Observations
0	14	14	28	
1	10	10	20	Genotypes
2	183	281	464	Genotypes
3	2466	1736	4202	Phenotypes

[1]Male and female generation 0 birds were from different lines.

The experimental birds were hatched at six different weeks and kept in four different pens. However, the majority of the birds were kept together in a single 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 hatching time, the temperature was 30 °C and then was gradually decreased to 10 °C by 22 days 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 33–34 °C and then gradually decreases to 17–18 °C by 35 days of age. Except for the adjusted temperature schedule, birds were kept under circumstances that closely resemble commercial practice, i.e. they could have *ad libitum* access to feed and water and illumination was 23 h/day. A commercial broiler feed was used; it 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.

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 haematocrit value (HCT) were measured 1 day before slaughtering at 5 weeks 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 colour, an irregular liver surface, or both. Accumulation of fluid in the heart sac (HEART) was scored as 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 ventricle (RV) and total ventricle (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 colour (BREAST) was scored as 0 representing normal colour, 1 representing a colour deviation and 2 representing serious colour deviation. In general, a deviation of breast colour meant that the colour 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). A total of 420 microsatellite markers were used including 266 microsatellites that were typed across all 10 families and an additional 154 microsatellites that were typed on four 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 are sex-averaged 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 varied between 3 and 82 markers per chromosome. 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 performed as described by Crooijmans *et al.* (1997). The PCR amplifications were carried out in 12-μl reactions as described previously by Jennen *et al.* (2004). The PCR amplification products from the same animal were diluted and pooled in such a way that each marker signal on ABI automated sequencer (Applied Biosystems, Perkin Elmer, Foster City, CA, USA) had a peak height of approximately 1000. Electrophoresis was performed on a 6% denaturing

Table 2 Chromosomes, length in centimorgans, number of markers, and the average information content for both sexes across all chromosomes.

Chromosome	Length in cM	Number of markers	First marker	Last marker	Average information content	
					Sire	Dam
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.

polyacrylamide gel, Sequagel-6 (National Diagnostics, Atlanta, GA, USA) 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:

$$Y_{ij} = \text{family}_i + b_{s,i}X_{s,ij} + b_{d,i}X_{d,ij} + e_{ij}$$

where Y_{ij} is the average adjusted trait value for the jth offspring of the ith family; family_i, family mean; $b_{s,i}$, regression coefficient for the sire (s) in family i; $X_{s,ij}$, probability that the jth 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 jth offspring from dam d in family i received allele 1; and 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 vs. the null hypothesis of the absence of QTL effects, 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:

$$\text{Test statistic}_k(H_1 : H_0) = \frac{\left(\frac{\text{RSS}_{k(H_0)} - \text{RSS}_{k(H_1)}}{\text{df}_{\text{QTL}}}\right)}{\left(\frac{\text{RSS}_{k(H_1)}}{\text{df}_{\text{total}} - \text{df}_{\text{family}} - \text{df}_{\text{QTL}}}\right)}$$

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

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

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

$$\text{RSS}_k(H_1) = \sum_{j-1}^{n} W_{ij}(Y_{ijk} - \text{family}_i - b_{s,ik}X_{s,ijk} - b_{d,ik}X_{d,ijk})^2$$

The df are the number of F_2 genotyped animals (df_{total}), number of family means ($\text{df}_{\text{family}}$) fitted and number of QTL effects (df_{QTL}) fitted, i.e. one in each family, 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.* (1999a). The analyses were also performed for each family individually.

Significance thresholds

Significance thresholds were calculated by permutation testing (Churchill & Doerge 1994). This is an empirical method, which accounts for the location of the marker and phenotypic 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 & 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. For each parent, a test comparing a model with a QTL vs. 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 the 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.

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 weeks was 1.604 g (the average for males and females were 1676 and 1503 g, 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%, which is in contrast to 28.3 ± 2.3% under normal conditions. Genetic parameters for ascites-related traits have been previously described by Pakdel *et al.* (2002).

Genotypic data

The average information content per chromosome for males and females of all 24 chromosomes covered in the current study are shown in Table 2. 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 analysed groups was

Table 3 Statistical description of the traits in cold conditions.

Trait	Abbreviation	Number of birds	Mean	SD
Haematocrit value (%)	HCT	3547	35.40	4.21
BW at 5 weeks (g) under ascites conditions	BW_{as}	3693	1604	263
Right ventricular weight (g)	RV	3660	1.95	0.68
Total ventricular weight (g)	TV	3658	6.97	1.17
Ratio of right ventricular weight as percentage to total ventricular weight (%)	RATIO	3658	27.94	8.07
Right ventricular weight as percentage of BW_{as} (%)	%RV	3646	0.125	0.050
Total ventricular weight as percentage of BW_{as} (%)	%TV	3644	0.439	0.070
Total mortality	MORT-TOT	2494	0.16	0.37
Fluid in the abdomen	ABDOMEN	3697	0.08	0.38
Colour of the breast	BREAST	3697	0.03	0.18
Liver abnormalities	LIVER	3697	0.07	0.29
Fluid in the heart sac	HEART	3696	0.59	0.62

0.72 in both sexes. The average percentage of marker heterozygosity for G_1 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 five macrochromosomes (GGA2, 4, 5, 6 and 8) and three microchromosomes (GGA10, 27 and 28). There was significant statistical evidence for three 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 Fig. 1, the significant QTL on chromosome 2 for %RV and %TV and the suggestive QTL for RATIO are shown, and all three are located between markers *MCW0185* (335 cM) and *MCW0245* (421 cM). On chromosome 4, a significant QTL was detected for BW_{as} at position 129 cM between markers *ADL0194* and *LEI0122*. Furthermore, there were suggestive QTLs for BW_{as}, BREAST and TV on GGA8 located between markers *ADL0301* and *LEI0044*. On GGA10, both MORT-TOT and BW_{as} showed suggestive linkage at the 3% chromosomewise level, and %RV showed suggestive linkage at the 6% chromosomewise level. The peak of the test statistic was located between

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

Trait	Chromosome	Position[1](cM)	Marker bracket[2]	*F*-ratio	Significance[3]
%RV	2	408	*MCW0166-MCW0245*	2.97	***
	10	79	*ADL023-MCW0102*	2.09	*
	28	48	*ADL0284-ADL0299*	1.87	*
%TV	2	351	*MCW0185-MCW0264*	3.07	***
RATIO	2	411	*MCW0166-MCW0245*	2.69	**
	28	48	*ADL0284-ADL0299*	1.92	*
BW_{as}	4	129	*ADL0194-LEI0122*	2.50	***
	8	101	*MCW0271-LEI0044*	2.21	**
	10	88	*MCW0035-ADL0158*	2.05	**
MORT-TOT	10	64	*ADL0231-MCW0035*	2.35	**
LIVER	6	146	*ABR0323*	2.65	***
HEART	6	140	*ADL0142-ABR0323*	2.01	**
TV	8	105	*MCW0271-LEI0044*	1.99	*
	27	1	*MCW0350-MCW0300*	1.78	*
BREAST	5	68	*MCW0090-MCW0038*	2.05	*
	8	80	*ADL0301*	1.89	*

[1]Position of QTL (cM) relative to the first marker in the set for this chromosome (Table 2).
[2]Position of QTL is between two flanking markers. When only one marker is shown, the QTL position is most likely identical to the marker position.
[3]***$P \leq 0.05$ genomewise. **$P \leq 0.05$ and *$P \leq 0.10$ chromosomewise.

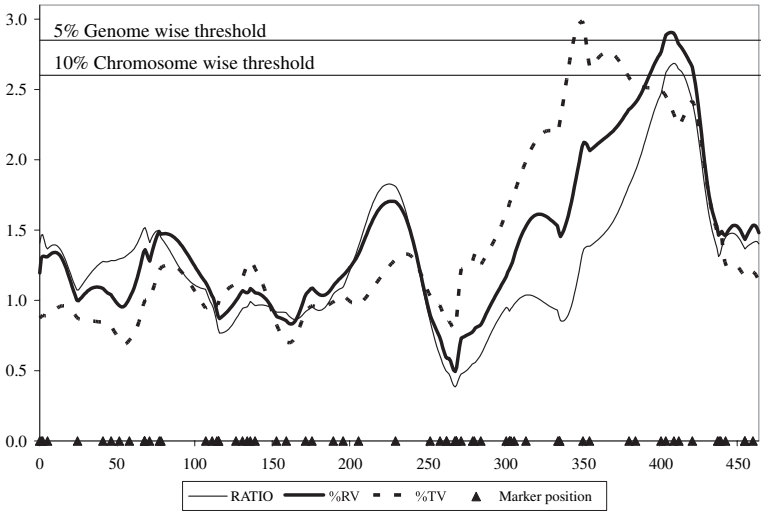

Figure 1 The test statistic values from the full-sib QTL analysis on chicken chromosome 2 for % RV (perrv) and % TV (pertv). Genomewise significance thresholds at 5% are included. Map positions are given using the Haldane scale.

markers *ADL0231* and *MCW0035* for MORT-TOT, between *MCW0035* and *ADL0158* for BW_{as} and between markers *ADL0231* and *ADL0102* for %RV. Details of the markers flanking each QTL, and their positions on the chromosomes are presented in Table 4. The number of parents contributing to the genomewise significant QTL and their allele substitution effects were determined based on a single family analysis. Five parents contributed to the QTL affecting %RV on GGA2, three to the QTL affecting %TV on GGA2, four to the QTL affecting BW_{as} on GGA4 and six to the QTL affecting liver on GGA6.

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). Besides mapping of QTLs, the 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 markers were typed in four of the 10 families whereas the remaining six families were typed only for 266 markers. Consequently the genome coverage and information content in this study is not uniform across all families and is somewhat higher on these four 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 <10% of the chicken genome. Furthermore, even in the recently released sequence of the chicken genome, 10 of these chromosomes are still not described (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

The PHS in chicken is a complex disease in which hypoxaemia eventually results in several 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 genomewise 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, the confidence intervals 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 of the pulmonary blood vessels in particular chicken broiler lines may form the basis of these observed differences. Alterations in proportional growth as a result of selection for greater musculature may have the effect of producing birds with relatively small respiratory and cardiovascular systems. As

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. 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 disease. 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*, *TITF1*, *SSH* and members of the *NKx* gene family).

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 might be related to the porosity and structure of the eggshell or to the hypoxic condition during pepping and hatching. This clearly indicates that even genes acting at the level of the development of egg structure 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 (Fig. 1). All of these QTL effects had their maximum test statistic in the region between *MCW0185* and *MCW0245* (335–421 cM). Although %TV, %RV and RATIO reached the significance threshold in that region, the peak for these traits was very broad, reaching its maximum at position 351, 408 and 411 cM respectively. A possible explanation for these results might be the presence of two separate QTLs in the region between *MCW0185* and *MCW0245*. However, the broad QTL peak for %TV overlaps to a great extent with those of %RV and RATIO. Given the high correlation between these traits, a single QTL with a confidence interval between *MCW0185* and *MCW0245* is more likely. Although, two genes involved in early cardiogenesis are located in this region, *ZFPM2* (position 129.3 Mb on the sequence map), which is 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, a significant QTL for BW_{as} was identified at position 129 cM. The PHS QTL on GGA4 covers the region between markers *MCW0085* and *LEI0122* on the consensus linkage map of this chromosome, which on the chicken sequence map of this chromosome is roughly the region between 32 and 50 Mb. There are 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 to 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 BW_{as}, BREAST and TV were detected between markers *ADL0301* and *LEI01044*, 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 within the QTL region at position 80–105 cM on this chromosome points to a possible gene that 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 playing 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 between markers *ADL0231* and *ADL0158*. The peak of the test statistic was located at 64 cM for MORT-TOT, 88 cM for BW_{as} and 49 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 receptor* (*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 PHS in chickens might also have implications for specific human diseases and more generally will also improve our insight into 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 PH (OMIM 178400) in which acute pulmonary oedema occurs in some people 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) emphasized that pulmonary atrial hypertension (PAH) is a complex genetic disease, meaning that gene–gene and environmental–gene interactions may confer susceptibility to PAH. Finally, a detailed study of the influence of hypoxia in the developing chicken embryo will be of relevance to understanding the effects of oxygenic stress in the developing human foetus and its possible role in the susceptibility to cardiovascular diseases at a later age in life.

Acknowledgements

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

References

Albers G. & Frankenhuis M.T. (1990) Ascites, a high altitude disease in the lowlands. *Poultry Misset* **6,** 24–5.

Balog J.M. (2003) Ascites syndrome (pulmonary hypertension syndrome) in broiler chickens: are we seeing the light at the end of the tunnel? *Avian and Poultry Biology Reviews* **14,** 99–126.

Boldman K.G., Kriese L.A., Van Vleck L.D., Van Tassell C.P. & Kachman S.D. (1995) *A Manual for Use of Mtdfreml. A Set of Programs to Obtain Estimates of Variances and Covariances [draft].* US Department of Agriculture, Agricultural Research Service, Washington DC.

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, 139 pp.

Churchill G.A. & Doerge R.W. (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138,** 963–71.

Crooijmans R.P.M.A., Dijkhof R.J.M., Van der Poel J.J. & Groenen M.A.M. (1997) New microsatellite markers in chicken optimized for automated fluorescent genotyping. *Animal Genetics* **28,** 427–37.

Cueva S., Sillau H., Valenzuela A. & Ploog H. (1974) High altitude induced pulmonary hypertension and right heart failure in broiler chickens. *Research in Veterinary Science* **16,** 370–4.

Currie R.J.W. (1999) Ascites in poultry: recent investigations. *Avian Pathology* **28,** 313–26.

Decuypere E., Buyse J. & Buys N. (2000) Ascites in broiler chickens: exogenous and endogenous structural and functional causal factors. *World's Poultry Science Journal* **56,** 367–76.

Dewil E., Buys N., Albers G.A.A. & Decuypere E. (1996) Different characteristics in chick embryos of two broiler lines differing in susceptibility to ascites. *British Poultry Science* **37,** 1003–13.

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

Groenen M.A.M., Crooijmans R.P.M.A., Veenendaal T., Cheng H.H., Siwek M. & van der Poel J.J. (1998) A comprehensive microsatellite linkage map of the chicken genome. *Genomics* **49,** 265–74.

Groenen M.A.M., Cheng H.H., Bumstead N. *et al.* (2000) A consensus linkage map of the chicken genome. *Genome Research* **10,** 137–47.

Haldane J.B.S. (1919) The combination of linkage values and the calculation of distances between the loci of linked factors. *Journal of Genetics* **2,** 3–19.

Havenstein G.B., Ferket P.R., Scheideler S.E. & Larson B.T. (1994) Growth livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. *Poultry Science* **73,** 1785–94.

International Chicken Genome Sequencing Consortium (2004) Sequencing and comparative analysis of the chicken genome, *Gallus gallus*. *Nature* **432,** 695–716.

Jennen D.G.J., Vereijken A.L.J., Bovenhuis H., Crooijmans R.P.M.A., Veenendaal A., van der Poel J.J. & Groenen M.A.M. (2004) Detection and localization of quantitative trait loci affecting fatness in broilers. *Poultry Science* **83,** 295–301.

Julian R.J. (1990) In: *Cardiovascular disease, poultry Diseases,* 3rd edn (Ed. by F.T.W. Jordan), pp. 330–53. Bailliére Tindall, London, UK.

Julian R.J. (1993) Ascites in poultry. *Avian Pathology* **22,** 419–54.

Julian R.J. (1998) Rapid growth problems: ascites and skeletal deformities in broilers. *Poultry Science* **77,** 1773–80.

Julian R.J. (2000) Physiological, management and environmental triggers of the ascites syndrome: a review. *Avian Pathology* **29,** 519–27.

Julian R.J., Frias G.W., 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 Diseases* **31,** 130–5.

Knott S.A., Elsen J.M. & Haley C.S. (1996) Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theoretical and Applied Genetics* **93,** 71–80.

Lander E.S. & Kruglyak L. (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genetics* **11,** 241–7.

McMullen J.R., Shioi T., Huang W.Y. *et al.* (2004) The insulin-like growth factor 1 receptor induces physiological heart growth via the phosphoinositide 3-kinase(p110alpha) pathway. *Journal of Biological Chemistry* **279,** 4782–93.

Newman J.H., Fanburg B.L., Archer S. L. *et al.* (2004) Pulmonary arterial hypertension, future directions: report of a National Heart, Lung and Blood Institute/Office of Rare Diseases Workshop. *Circulation* **109,** 2947–52.

Olander , Burton R.R. & Adler H.E. (1967) The pathophysiology of chronic hypoxia in chickens. *Avian Diseases* **11,** 609–20.

Pakdel A., van Arendonk J.A.M., Vereijken A.L.J. & Bovenhuis H. (2002) Direct and maternal genetic effects for ascites-related traits in broilers. *Poultry Science* **81,** 1273–9.

Peacock A.J., Pickett C., Morris K. & Reeves J.T. (1990) Spontaneous hypoxemia and right ventricular hypertrophy in fast growing broiler chickens reared at sea level. *Comparative Biochemistry and Physiology* **97A,** 537–41.

Riddell C. (1991) Developmental, metabolic, and miscellaneous disorders. In: *Diseases of Poultry,* 9th edn (Ed. by B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reid & H.W. Jr Yoder), pp. 839–41. Iowa State University Press, Ames, IA.

Scheele C.W., Decuypere E., Vereijken P.F.G. & Schreurs F.J.G. (1992) Ascites in broilers. 2. Disturbances in the hormonal regulation of metabolic rate and fat metabolism. *Poultry Science* **71,** 1971–84.

Schmid M., Nanda I., Guttenbach M. *et al.* (2000) First report on chicken genes and chromosomes 2000. *Cytogenetics and Cell Genetics* **90,** 169–218.

Smith A.H., Wilson W.O & Pace N. (1954) The effect of high altitude on growth of turkeys. *Growth* **18,** 27–35.

Smith A.H., Wilson W.O & Pace N. (1955) Growth and reproduction in domestic birds at high altitudes. *Poultry Science* **35** (Suppl.1), 1175.

Smith A.H., Abplanalp H., Harwood L.M. & Kelly C.F. (1959) Poultry at high altitudes. *California Agriculture,* **13,** 8–9.

Van der Beek S. (1995) *The use of genetic markers in poultry breeding.* Doctoral thesis, Wageningen University, Wageningen, The Netherlands.

Van Kaam J.B.C.H.M., van Arendonk J.A.M., Groenen M.A.M., Bovenhuis H., Vereijken A.L.J., Crooijmans R.P.M.A., van der Poel J.J. & Veenendaal A. (1998) Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. *Livestock Production Science* **54,** 133–50.

Van Kaam J.B.C.H.M., Groenen M.A.M., Bovenhuis H., Veenendaal A., Vereijken A.L.J. & van Arendonk J.A.M. (1999a) Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency. *Poultry Science* **78,** 15–23.

Van Kaam J.B.C.H.M., Groenen M.A.M., Bovenhuis H., Veenendaal A., Vereijken A.L.J. & van Arendonk J.A.M. (1999b) Whole genome scan in chickens for quantitative trait loci affecting carcass traits. *Poultry Science* **78,** 1091–9.

Wideman R.F. Jr, Ismail M., Kirby Y.K., Bottje W.G., Moore R.W. & Vardeman R.C. (1995) Furosemide reduces the incidence of pulmonary hypertension syndrome (ascites) in broiler exposed to cool environmental temperatures. *Poultry Science* **74,** 314–22.

Yersin A.G., Huff W.E., Kubena L.F., Elissalde M.H., Harvey R.B., Witzel D.A. & Giroir L.E. (1992) Changes in hematological, blood gas, and serum biochemical variables in broilers during exposure to simulated high altitude. *Avian Diseases* **36,** 189–96.

Zhao Y., Meng X.M., Wei Y.J., Zhao X.W., Liu D.Q., Cao H.Q., Liew C.C. & Ding J.F. (2003) Cloning and characterization of a novel cardiac-specific kinase that interacts specifically with cardiac troponin I. *Journal of Molecular Medicine* **81,** 297–304.