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Quantitative trait loci with sex-specific effects for internal organs weights and hematocrit value in a broiler-layer cross

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Abstract Rapid growth in broilers is associated with susceptibility to metabolic disorders such as pulmonary hypertension syndrome (ascites) and sudden death. This study describes a genome search for QTL associated with relative weight of cardio respiratory and metabolically important organs (heart, lungs, liver and gizzard), and hematocrit value in a Brazilian broiler-layer cross. QTL with similar or different effects across sexes were investigated. At 42 days of age after fasted for 6 h, the F_2 chickens were weighed and slaughtered. Weights and percentages of the weight relative to BW42 of gizzard, heart, lungs, liver and hematocrit were used in the QTL search. Parental, F_1 and F_2 individuals were genotyped with 128 genetic markers (127 microsatellites and 1 SNP) covering 22 linkage groups. QTL mapping analyses were carried out using mixed models. A total of 11 genome-wide significant QTL and five suggestive linkages were mapped. Thus, genome-wide significant QTL with similar effects across sexes were mapped to GGA2, 4 and 14 for heart weight, and to GGA2, 8 and 12 for gizzard %. Additionally, five genome-wide significant QTL with different effects across sexes were mapped to GGA 8, 19 and 26 for heart weight; GGA26 for heart % and GGA3 for hematocrit value. Five QTL were detected in chromosomal regions where QTL for similar traits were previously mapped in other F_2 chicken populations. Seven novel genome-wide significant QTL are reported here, and 21 positional candidate genes in QTL regions were identified.

Keywords Candidate gene · Gizzard · Heart · Liver · Microsatellite marker

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Introduction

Rapid growth in broilers is associated with susceptibility to metabolic disorders such as pulmonary hypertension syndrome (ascites) and sudden death. In broilers, muscle development is proportionally greater than that of important internal organs, in particular the heart and lungs. Rapid growth requires high metabolic rate, which generates high oxygen demand. Insufficient pulmonary vascular capacity and inability to deliver enough oxygen to meet the metabolic demand triggers a cascade of events including high cardiac output and increasing blood flow to the lungs causing pulmonary hypertension and accumulation of fluid in the celomic cavity (ascites) followed by death (Jaenisch et al. 2001; Druyan 2012).

Hypoxemia in ascites susceptible broilers lead to a series of symptoms that include increased hematocrit, heart weight and right ventricle to total ventricle ratio (Luger et al. 2001; Druyan 2012). There is an indication that increased hematocrit in ascitic chickens results from enhanced, but defective

erythropoiesis, leading to significant increase in immature erythrocytes (Luger et al. 2003).

Environmental factors that induce high oxygen consumption, such as low temperatures, and low oxygen supply at high altitudes, were reported to be involved in ascites incidence, but there is an experimental evidence for an important additive genetic component in susceptibility to ascites (Moghadam et al. 2001). Heritability estimates ranging from 0.1 to 0.7 were reported, in addition to differences among chicken breeds and genders. Fast growing males were found to be much more susceptible to ascites than females (Moghadam et al. 2001). The rapid success of selection experiments for ascites resistance (Wideman and French 2000) as well as the development of susceptible and resistant chicken lines by applying divergent selection for ascites mortality (Druyan et al. 2007; Pavlidis et al. 2007) indicated that a few major genes were involved in the genetic control of susceptibility to ascites.

Sudden death is an acute heart process associated with defibrillation of the right ventricle (Jaenisch et al. 2001). Incidence of ascites and sudden death represents important economic losses in the poultry industry, including mortality near to the end of the growing period and transportation, or leading to condemnation of carcasses in the slaughter house. Management practices adopted to reduce growth rate, also reduced oxygen demand and mortality rate in commercial flocks. However, this approach may compromise performance and the efficiency of broiler production (Druyan 2012). Finding new chromosomal regions associated with weight variation of cardio respiratory organs and the hematocrit value may provide breeders with tools to select against metabolic disorders in fast growing chickens.

Quantitative trait loci (QTL) mapping is the first step for finding genomic regions associated with quantitative traits. However, only a few studies have mapped QTL for heart (Rabie et al. 2005; Zhou et al. 2006) and lungs related traits (Nones et al. 2006; Park et al. 2006), and for the hematocrit value (Navarro et al. 2005; Pinard-van der Laan et al. 2009), in independent populations, according to the Chicken QTL Database (www.animalgenome.org/cgi-bin/QTLdb/GG/index). Two candidate genes in GGA9 (*AGTR1* and *UTS2D*) were recently associated with susceptibility to ascites and ventricular hypertrophy in an F_2 cross between ascites—susceptible and resistant lines (Krishnamoorthy et al. 2014).

This study describes a whole genome search for QTL associated with relative weight of cardiorespiratory and metabolically important organs (heart, lungs, liver and gizzard) and hematocrit value in a Brazilian broiler-layer cross. Due to differences in metabolism between males and females, QTL with similar or different effects across sexes were also considered. Positional candidate genes were identified in QTL regions.

Material and methods

Experimental population and trait recording

The F_2 population used in this study originated from a wide cross between a broiler (TT) and a layer line (CC). These lines were selected at Embrapa Swine and Poultry, Concórdia, Brazil. When reared as broilers, they showed a fivefold difference in body weight at 41 days of age (Ledur et al. 2000). In the parental generation, seven TT males were mated to seven CC females to produce the F_1 generation. Twenty one F_1 females were then artificially inseminated with semen from seven F_1 males in a 3:1 ratio to produce the F_2 generation. The latter comprised seven paternal half-sib families composed of three full-sib families of approximately 100 individuals each, produced over 17 hatches, summing up to 2063 F_2 individuals.

The F_2 chickens were reared as broilers in floor pens up to 35 days of age when they were individually caged up to 41 days. At 42 days of age, after fasted for 6 h, they were transported to the slaughter house, weighed (BW42) and slaughtered. Weights of gizzard, heart, lungs and liver were recorded (in grams) immediately after slaughter. The covariate BW42 was used in the QTL mapping analyses of organs weights. Percentages of the weight of these organs relative to BW42 were calculated and also used in the QTL search. Body weight (+21.4 %) and hematocrit value were higher in males than in females, but gizzard % was higher in females (Table 1).

Blood samples were collected at slaughter for DNA analysis and hematocrit value determination by the microhematocrit method (Cardoso and Tessari 2003).

QTL mapping analyses

Parental (n=12), F_1 (n=10) and F_2 (up to 649) individuals from five to six full-sib families were genotyped with 127 microsatellite markers and one SNP covering 22 linkage groups (GGA1 to 15, 18, 19, 23, 24, 26 to 28). Total map length was 2630 cM, corresponding to approximately 63 % of genome coverage. For more details on genotyping and linkage map construction, refer to Ambo et al. (2009) and Campos et al. (2009).

Quantitative trait loci mapping analyses were carried out using mixed models implemented in the *Qxpak* software (Pérez-Enciso and Misztal 2004) according to the following model:

$$y_{ijk} = u + S_i + H_j + \mathrm{b_{ijk}} BW_{42} + c_{ijk} I_k + a_{ijk} A + \mathrm{d_{ijk}} D_k + e_{ijk},$$

where y is the phenotype, u is the general mean, S_i is the fixed effect of sex, H_j is the fixed effect of hatch, b is the coefficient corresponding to the covariate BW42, $\mathrm{c_{ijk}}$ is the coefficient associated with the infinitesimal random animal genetic effect, a_{ijk} is the coefficient associated with the

Table 1 Descriptive statistics of traits used in QTL mapping

Trait	Sex	N	Mean	SD [a]	Minimum	Maximum	CV [b]
BW42	M	539	1094.09	157.55	627.00	1688.00	14.40
	F	527	901.19	141.16	402.00	1370.00	15.66
Liver weight	M	539	29.17	4.58	20.00	49.00	15.71
	F	526	23.94	3.84	15.00	38.00	16.03
Heart weight	M	539	7.24	1.54	3.00	15.00	21.23
	F	525	5.84	1.38	2.00	10.00	23.61
Gizzard weight	M	539	26.51	4.48	16.00	43.00	16.89
	F	526	23.20	3.82	13.00	35.00	16.44
Lungs weight	M	538	9.20	2.27	3.00	16.00	24.64
	F	526	7.19	1.79	3.00	13.00	24.84
Hematocrit %	M	532	28.06	2.75	18.00	42.00	9.81
	F	516	27.92	2.93	16.00	44.00	10.48
Liver % [c]	M	539	2.68	0.34	1.84	4.68	12.71
	F	526	2.68	0.38	1.83	7.71	14.12
Heart % [c]	M	539	0.66	0.12	0.28	1.28	18.21
	F	525	0.65	0.13	0.32	1.20	20.60
Gizzard % [c]	M	539	2.44	0.39	1.50	3.71	15.93
	F	526	2.60	0.39	1.73	3.73	14.86
Lungs % [c]	M	538	0.84	0.17	0.28	1.36	19.88
	F	526	0.80	0.17	0.29	1.39	21.11

[a] SD=standard deviation

[b] CV=coefficient of variation

[c] Organs percentages relative to body weight at 42 days

additive effect (A) of the QTL, d_{ijk} is the coefficient associated with the dominance effect (D) of the QTL and e_{ijk} is the random residue. The model that considers QTL with sex-specific effects can be written in a similar way, but an additive and a dominance effect were estimated for each sex separately. The covariate BW42 was included in the models for organ weight traits, but not for the analyses of traits measured as percentages of body weight.

The analyses were initiated by fitting a model with an additive effect to all the data (all F_2 chickens included, irrespective of gender). If the statistical test for a QTL exceeded the suggestive threshold level, a second model with additive + dominance effect was fitted, otherwise the analyses were halted. This procedure was subsequently repeated for each sex separately. Additive and dominance effects were considered significant when they were at least twice the magnitude of their respective standard errors.

Significance thresholds were computed using 10,000 permutations (Churchill and Doerge 1994) for probability levels of 1 and 5 % genome-wide and for suggestive linkage (Lander and Kruglyak 1995), calculated following the Bonferroni correction and considering a genome length of 4200 cM (Schmid et al. 2005). Confidence intervals for QTL locations were obtained as described by Mangin et al. (1994). The method involves searching the first position, both to the right and to the left of the QTL location that has the likelihood ratio test, LRT $<(\mathrm{LRT}_{\mathrm{maximum}} - \chi^2)$. For instance, in the present study $\chi^2=3.84$ for LRT with one degree of freedom. Therefore, if the significant position corresponds to a $\mathrm{LRT}_{\mathrm{maximum}}=20$, then one would search for the first position, on both sides of the maximum point, that has LRT<16.16. The interval obtained in this way corresponds to the 95 % confidence interval for the QTL.

According to Sorensen et al. (2003), the total phenotypic variance between the parental lines explained by the QTL can be expressed as:

$$VP_{\exp} = 2p_Q\left(1-p_Q\right)a^2,$$

where p_Q is the frequency of the Q allele from the QTL, for which we assume the value 0.5 in the line-cross model, whereas α is the estimate for the additive effect of the QTL in the position in which it was mapped. From this information, the percent from total variance explained by the QTL may be estimated as follows:

$$\%VP_{\exp} = \frac{VP_{\exp}*100}{VP},$$

where $VP_{\exp}$ is the phenotypic variance explained by the QTL and VP is the total phenotypic variance in the model that includes the effects of the QTL.

Previous studies from our group reported QTL for performance, carcass and organs traits on GGA1 (Nones et al. 2006) and an association study of two SNPs (on *IGF1* and *KDM5A* genes) on GGA1 with organs traits and hematocrit value (Boschiero et al. 2013). For this reason, QTL for organs traits and hematocrit value on GGA1 are not presented in this study.

Analysis of positional candidate genes

In this study, we mapped QTL to marker intervals that ranged from 6.6 (GGA8) to 72.6 cM (GGA26), therefore it was not possible to associate gene polymorphisms with traits under study. However, we explored seven genomic regions within marker intervals where QTL were mapped, searching for potential positional candidate genes with known biological functions, based on OMIM® (http://www.omin.org) and NCBI (http://www.ncbi.nlm.nih.gov/) databases, that could be related to the respective traits under study. Microsatellite positions were obtained from the last version of chicken reference sequence (Gallus_gallus_4.0) based on the ArkDB database (http://www.thearkdb.org/arkdb/). Exact positions of markers *MCW0094* and *ROS0314* were not available in the databases, therefore we blasted primer sequences to obtain their positions. Subsequently, the BioMart tool (www.biomart.org) was employed to obtain a list of genes for each marker interval to which a QTL had been mapped.

Results and discussion

This study describes a whole genome search for QTL associated with relative weight of cardiorespiratory and metabolically important organs (heart, lungs, liver and gizzard) and hematocrit value in a Brazilian broiler-layer cross. A total of 11 genome-wide significant QTL and five suggestive linkages were mapped. From these, six genome-wide significant QTL and five suggestive linkages showed similar effects across sexes and five genome-wide significant QTL had different effects across sexes.

Positional candidate genes were identified in marker intervals where QTL were mapped, except for gizzard weight and percentage. A total of 654 genes (BioMart Portal 2014) were mapped to seven intervals associated to QTLs. The number of genes in each interval is reported in Table 2. Positional candidates (n=21) were selected based on current known biological functions.

QTL with similar effects across sexes

A total of six genome-wide significant QTL and five suggestive linkages with similar effects across sexes were mapped to GGA2, 3, 4, 8, 12 and 14 for organs weights and % (Table 3). For relative heart weight, QTL exceeding 1 % genome-wide threshold were mapped to GGA2, 4 and 14. Negative additive effects indicated that the QTL allele increasing the trait value came from the layer line in all three cases. This is not surprising considering that the broiler line was selected for fast growth and to increase muscle development, which may have reduced the relative proportion of internal organs, including the cardiorespiratory tract (Deeb and Lamont 2002).

The QTL on GGA2 was mapped to the *MCW0185-MCW0264* interval. Interestingly, the two most significant QTL mapped by Rabie et al. (2005) for right and total ventricular weight as percentage of body weight were located in this same marker interval. Those two traits were related to the susceptibility to develop pulmonary hypertension syndrome and the authors indicated two genes involved in early cardiogenesis as possible positional candidates in that marker interval: *ZFPM2* and *GATA6*. Our search within this interval retrieved three other potential candidate genes, involved in cardiac development and function: *DTNA, SNAI2* and *CHD7* (Table 4). One non-synonymous SNP in the *DTNA* gene was identified in a human family with left ventricular noncompaction and congenital heart disease (Ichida et al. 2001). SNAI2 participates in Wnt signaling that was shown to restrict cardiomyocyte proliferation and control heart size in the mouse (Heallen et al. 2011). Vissers et al. (2004) found *CHD7* mutations in individuals with CHARGE syndrome (congenital anomalies in humans including malformations of the heart). This gene is expressed in branchial arches of chicken embryos (Aramaki et al. 2007), which is the primordial tissue that give rise to the heart.

The QTL mapped for heart weight to GGA4 (*LEI0085-MCW0174* interval) was in a similar region previously associated to heart weight in another broiler-layer cross (Zhou et al. 2006), close to *ADL0260*, which is in the same chromosomal region as *LEI0085*. These two markers map between 82 and 83 Mb. A candidate gene (*WHSC1*), which encodes a histone methyltransferase that regulates the expression of transcription factors in mammalian embryonic heart (Vallaster et al. 2012), was located at 82.8 Mb (Table 4).

A novel QTL for heart weight is reported here on GGA14 (Table 4). A possible candidate gene in this region was *DNAJA3, which* encodes a mitochondrial chaperone. Mice *DNAJA3* deficient developed dilated cardiomyopathy, died prematurely due to progressive respiratory chain deficiency and decreased copy number of mitochondrial DNA in cardiomyocytes (Hayashi et al. 2006).

For gizzard weight, three suggestive linkages were detected on GGA2, 4 and 8, whereas for gizzard % three QTL were found on GGA2, 8 and 12 and a suggestive linkage on GGA3 (Table 3). QTLs for gizzard weight and percentage were mapped to the same interval on GGA8, had positive additive effects and are likely to be the same QTL. The QTL for gizzard % on GGA12 exceeded the 1 % genome wide threshold and showed positive dominance effects, suggesting the

Table 2 Genomic positions of markers from intervals where QTL were mapped

Marker interval	GGA	Position gga4 (ArkDB) [a]	Number of genes (Biomart) [b]	First marker Blast position [c]	Second marker Blast position [c]
MCW0185-MCW0264	2	105,848,755-112,648,761	66	105,848,938	112,648,761
MCW0040-LEI0166	3	103,716,571-Unknown	71	103,716,571	105,017,790
LEI0085-MCW0174	4	82,437,148-84,297.286	19	82,437,147	84,297,286
MCW0351-LEPR (LEPR-MCW0351)	8	27,238,562 (*LEPR*)-27,623,626	14	27,238,562	27,623,626
MCW0123-ADL0263	14	11,341,581-13,932,426	110	11,341,581	13,932,425
MCW0094-MCW0287	19	Unknown-7,262,555	202	3,373,697	7,262,555
ROS0314-LEI0074	26	Unknown-4.777.041	172	1,367,176	4,776,827

[a] Position according to Ensembl (ENSEMBL71 Galgal4 Chick Assembly: 4)

[b] Ensembl version 71 database

[c] Position according to NCBI database

superiority of the heterozygote over the midparent. The other QTL for gizzard % on GGA2 and 8 exceeded the 5 % genome-wide threshold and showed positive additive effects, indicating that the QTL allele that increased gizzard % came from the broiler line. To our knowledge, no previous studies have mapped QTL for relative gizzard weight to any of these chromosomal regions; therefore, they are novel positions for this trait. No potential positional candidate genes with known biological function for gizzard weight or % were identified. A suggestive linkage was detected for liver % in the *LEI0098-MCW0123* interval on GGA14, also a novel position for this trait.

The percentage of the phenotypic variance explained by the QTL with similar effects across sexes varied from 0.88 to 2.56 % (Table 3). Six out of 11 QTL and suggestive linkages described in Table 3 were mapped to similar intervals that we previously associated with QTL for body weight (Ambo et al. 2009) and carcass traits (Baron et al. 2010). Examples of potential pleiotropic QTL is a QTL for breast % mapped to GGA2 and a QTL for shank % mapped to GGA4 in the same intervals to which QTL for heart weight were mapped in the present study. They are likely to be pleiotropic QTL with positive additive effects on percentage of breast and shank, but negative additive effects on heart weight.

QTL with different effects across sexes

A total of five genome-wide significant QTL with different effects across sexes were mapped to GGA3, 8, 19 and 26 (Table 5) for three traits. One QTL for relative heart weight

Table 3 Quantitative trait loci with sex-similar effects

Trait	GGA	Position (cM)	Confidence interval	LRT [a]	Flanking markers	Model	Additive effect (SE)	Dominance effect (SE)	PV [b] (%)
Heart weight [c]	2	239	232 - 251	17.75**	*MCW0185-MCW0264*	A	−0.13 (0.07)		1.68
	4	168	158 - 177	28.68**	*LEI0085-MCW0174*	AD	−0.33 (0.14)	−0.20 (0.35)	1.69
	14	26	11 - 41	20.83**	*MCW0123-ADL0263*	A	−0.31 (0.10)		2.05
Gizzard weight [c]	2	3	1 - 27	14.92†	*LMU0013-MCW0247*	AD	0.54 (0.20)	−0.72 (0.29)	1.33
	4	140	121 - 178	13.89†	*MCW0240-LEI0063*	A	−1.55 (0.39)		1.43
	8	68	50 - 83	13.24†	*ABR0345-ADL0172*	A	0.96 (0.27)		1.04
Gizzard (%)	2	20	1 - 34	18.42*	*MCW0247-LEI0086*	AD	0.11 (0.03)	−0.07 (0.05)	1.84
	3	46	1 - 76	14.29†	*MCW0169-MCW0222*	A	0.12 (0.04)		0.88
	8	60	41 - 77	20.29*	*ABR0345-ADL0172*	A	0.13 (0.03)		1.44
	12	86.2	75 - 86.2	18.65**	*ADL0044-GCT0055*	AD	−0.06 (0.03)	0.21 (0.05)	2.56
Liver (%)	14	13	1 - 39	10.12†	*LEI0098-MCW0123*	A	−1.33 (0.37)		1.08

[a] LRT=likelihood ratio test; †suggestive genome-wide; *5 % genome-wide; **1 % genome-wide

[b] PV (%)=percentage of the phenotypic variance explained by the QTL

[c] Body weight at 42 days was used as covariate in the analyses of these traits

Table 4 Positional candidate genes identified in the QTL regions

Trait [a]	GGA	Flanking markers [b]	Candidate gene[l]	Description	Gene function in humans or model organism [c]
Heart weight and %	2	*MCW0185-MCW0264* (105.8 – 112.6)	*DTNA* (107.1)	Dystrobrevin alfa	Congenital heart defects
			SNAI2 (108.1)	SNAIL, Drosophila homolog of	Cardiomyocyte proliferation and controls heart size
			CHD7 (112.6)	Chromodomain helicase DNA-binding protein 7	Cardiovascular malformations
	4	*LEI0085-MCW0174* (82.4 – 84.3)	*WHSC1* (82.8)	WHS candidate 1 gene	Histone methyltransferase, regulates the expression of transcription factors in embryonic heart
	8	*MCW0351–LEPR* (27.2 – 27.6)	*LEPR* (27.2)	Leptin receptor	Cardiac signal transduction pathway
	14	*MCW0123-ADL0263* (11.3 – 13.9)	*DNAJA3* (12.7)	DNAJ/HSP40 homolog, subfamily A, member 3	Mitochondrial chaperone involved in dilated cardiomiopathy
	19	*MCW0094-MCW0287* (3.4 – 7.3)	*MYL2* (3.8)	Myosin light chain 2, regulatory, cardiac, slow	Cardiac muscle contraction
			YWHAG (4.2)	tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein, gamma isoform	Hypertrophic cardiomyopathy
			RASL10B (4.4)	Ras-like family 10, member B;	Regulates atrial natriuretic peptide secretion from atrial cardiomyocytes
			UNC45B (4.5)	UNC45, *C. elegans* homolog of, B	Myoblast fusion and sarcomere formation in heart muscle
			NEK8 (5.8)	Never in mitosis gene A-related kinase 8	Renal and cardiovascular development
			SLC6A4 (6.2)	Solute carrier family 6 neurotransmitter transporter, serotonin), member 4	Negative regulation of organ growth
			PIGL (6.5)	Phosphatidylinositol glycan, class L	Congenital heart defects
			AKAP10 (6.8)	A-kinase anchor protein 10	Susceptibility to cardiac conduction defect
	26	*ROS0314-LEI0074* (1.4 – 4.8)	*DSTYK* (2.0)	Dual serine/threonine and tyrosine protein kinase	Development of major organs, including heart
			IL10 (2.5)	Interleukin 10	Heart disease
			PFKFB2 (2.6)	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 2	Regulates fructose-2,6-bisphosphate levels in the heart
			ADORA3 (3.2)	Adenosine A3 receptor	Protects the ventricular heart cell against injury during a subsequent exposure to ischemia
			NGF (4.0)	Nerve growth factor	Cardiac sympathetic innervation
			PPARD (4.0)	Peroxisome proliferator – activated	Myocardial fatty acid oxidation, cardiac function
Hematocrit	3	*MCW0040-LEI0166* (103.7 - 105.0)	*DNMT3A* (104.2)	DNA Methyltransferase 3A	Catalyzes the epigenetic modification of DNA methylation, upregulation of hematopoietic stem cell multipotency genes and downregulation of differentiation genes

[a] No candidate genes for gizzard weight and % were found in this study

[b] Positions in Mb according to NCBI, Gallus_gallus-4.0. Note that the positions of markers *LEPR-MCW0351* are inverted in our map compared to the genomic sequence. This may have resulted from the small distance between the two markers (0.4 Mb)

[c] OMIM® (http://www.omin.org) and NCBI (http://www.ncbi.nlm.nih.gov/) databases

with different modes of action on males and females was mapped to GGA8, in the *MCW0351-LEPR* interval. In males, it acted in a dominant fashion with positive effects, indicating that heterozygote males had higher trait value than the midparent. In females, the same QTL presented positive additive effects, suggesting that the allele that increased trait value came from the broiler line (Table 5). The *LEPR* gene was selected as a candidate gene in this interval (Table 4). Disruption of the leptin signaling pathway within the heart was shown to cause left ventricular hypertrophy in mice (Raju et al. 2006). We mapped a QTL for heart weight on GGA8 in the *MCW0351-LEPR* interval. A QTL for total ventricular weight was previously reported in the same chromosomal region (*MCW0271-LEI0044* interval) (Rabie et al. 2005). These authors indicated *TNNI3K* as a positional candidate gene, a cardiac-specific kinase that mediates cell signaling to modulate cardiac response to stress (Tang et al. 2013).

Another QTL for relative heart weight mapped to GGA19 was female-specific with negative additive effects, suggesting that the allele conferring higher heart weight originated from the layer line. Eight potential candidate genes for relative heart weight were found on GGA19: *MYL2, YWHAG, RASL10B, UNC45B, NEK8, SLC6A4, PIGL* and *AKAP10* (Table 4). Four of them seem to be more directly related to heart weight and percentage: *MYL2, YWHAG, UNC45B* and *PIGL*. In humans,

Table 5 Quantitative trait loci with sex-specific effects

Trait	GGA	Position (cM)	Confidence interval	LRT [a]	Flanking markers	Model	Sex	Additive effect (SE)	Dominance effect (SE)	PV (%) [b]
Heart weight [c]	8	89.6	84 – 89.6	42.41**	*MCW0351 - LEPR*	AD	M	0.00 (0.11)	0.73 (0.17)	2.92
							F	0.28 (0.11)	0.03 (0.16)	
	19	12	1 - 28	25.86**	*MCW0094-MCW0287*	A	M	0.09 (0.12)		2.39
							F	−0.42 (0.13)		
	26	5	1 - 33	28.96**	*ROS0314-LEI0074*	AD	M	−0.10 (0.14)	0.18 (0.24)	2.76
							F	0.37 (0.13)	−0.59 (0.22)	
Heart (%)	26	13	1 - 42	22.57*	*ROS0314-LEI0074*	AD	M	−0.02 (0.02)	0.02 (0.04)	1.96
							F	0.06 (0.02)	−0.10 (0.03)	
Hematocrit	3	273	263 - 273	24.68*	*MCW0040-LEI0166*	AD	M	−0.76 (0.28)	−1.07 (0.41)	2.08
							F	−0.47 (0.27)	1.08 (0.38)	

[a] LRT=likelihood ratio test; †suggestive genome-wide; *5 % genome-wide; **1 % genome-wide

[b] PV (%)=percentage of the phenotypic variance explained by the QTL

[c] Body weight at 42 days was used as covariate in the analyses of these traits

mutations in the *MYL2* gene were associated to dilated cardiomyopathy (Poetter et al. 1996). In zebrafish, knocking down *YWHAG* resulted in increased diameter of the heart tube (Komoike et al. 2010). *UNC45B* encodes a chaperone that aids in myoblast fusion and sarcomere formation of myosin isoforms required for cardiac muscle morphogenesis and contraction in zebrafish (Wohlgemuth et al. 2007). *PIGL* was pointed out as a strong candidate for the CHIME syndrome, which is characterized by colobomas, heart defects and other signs in humans (Ng et al. 2012).

QTLs for heart weight and percentage, also female-specific, with positive additive and negative dominance effects, were mapped in GGA26. Females that were heterozygous for these two QTLs had lighter hearts than the homozygous females. No QTL for heart weight or percentage in males was found in this study. Six positional candidate genes for heart weight and percentage were identified on GGA26: *DSTYK, IL10, PFKFB2, ADORA3, NGF and PPARD* (Table 4). *DSTYK* was suggested to have a role in the development of major organs in zebrafish, including the heart (Sanna-Cherchi et al. 2013). Knocking down this gene caused developmental defects compatible with the global loss of FGF signaling in that species. *IL10* gene polymorphism was associated with lower production of this anti-inflammatory cytokine in human patients with acute myocardial infarction than in controls and older participants. Therefore, Lio et al. (2004) indicated that high production of interleukin 10 was protective for acute myocardial infarction and a determinative parameter for longevity in humans. A cultured chicken ventricular myocyte model was used to investigate the cardioprotective role of *ADORA3 gene*. The activation of this gene protects the ventricular heart cell against injury during a subsequent exposure to ischemia (Liang and Jacobson 1998). *NGF* contributes to development and maintenance of heart sympathetic innervation (Ieda et al. 2004). Deletion of *PPARD* in mice decreased the expression of heart fatty acid oxidation genes, leading to cardiac hypertrophy and heart failure (Cheng et al. 2004).

Finally, a QTL for hematocrit value with different effects across sexes was detected in GGA3. This QTL showed different modes of action in males and females: positive dominance effects were detected on females, suggesting that the heterozygote female had higher hematocrit value than the midparent. Negative additive effects were predominant in males, in addition to negative dominance effects, indicating that the allele that increased trait value was coming from the layer line and also that the heterozygote male had lower hematocrit than the midparent. Since higher hematocrit value was found in broilers with ascites (Luger et al. 2001), the QTL allele that increased hematocrit value could be related to ascites incidence. The fact that fast growing males are more prone to ascites than females (Moghadam et al. 2001) is in accordance with our findings. A strong potential candidate gene for hematocrit value is *DNMT3A* (Table 4). It was demonstrated that this DNA methyltransferase is involved in the upregulation of hematopoietic stem cell multipotency genes and downregulation of differentiation genes in mice (Celik et al. 2015). There is indication that increased hematocrit in ascitic chickens results from enhanced, but defective erythropoiesis, leading to significant increase in immature erythrocytes (Luger et al. 2003).

The percentage of the phenotypic variance explained by the sex-different QTL varied from 1.96 to 2.92 % (Table 5). These QTL with sex specific effects could help explain phenotypic differences in metabolic disorders between males and females.

In the present study, QTLs for weight of organs from the digestive tract, such as gizzard and liver, showed no specific gender effects, whereas QTL for heart weight and hematocrit

value had sex specific effects. Different effects across sexes for relative heart weight and hematocrit may help explain higher incidence of ascites in males than in females. For gizzard %, QTL with similar effects across sexes with various modes of action were mapped to three chromosomes, indicating that there is additive genetic variation within and non-additive genetic variation between the lines involved in this cross for this trait. Accordingly, estimates that were obtained from 3823 F_2 chickens from the reciprocal crosses between TT and CC lines (Ledur et al. 2006), revealed that heritability estimates were high for gizzard weight (0.56), intermediate for liver weight (0.27) and low for heart weight (0.11) and hematocrit value (0.11).

Our results indicate the layer line as a possible source of alleles to increase relative heart weight, irrespective of gender. In contrast, if we consider males and females separately, QTL with additive effects on heart weight or percentage were detected on GGA8, 19 and 26 only in females. These three QTL could contribute to increase the cardiovascular capacity and should be further investigated.

A QTL allele coming from the layer line increased hematocrit value. This finding together with the fact that no QTL for lungs weight or percentage was detected, are not very promising to help mitigate the physiological problems associated with ascites in broilers. The estimate of genetic correlation of lungs weight with hematocrit value was high and negative (−0.58) (Ledur et al. 2006). Therefore, increased lungs weight would be associated with lower hematocrit value and perhaps lower susceptibility to ascites. The genetic correlation of heart weight with hematocrit value in that same study was also negative, but low (−0.12), indicating a weaker indirect relationship between heart weight and hematocrit value.

Overall, four novel genome-wide significant QTL with similar effects across sexes are reported here for heart weight on GGA14, for gizzard weight on GGA8, and for gizzard % on 8 and 12. Four novel genome-wide significant QTL with different effects across sexes are also reported here for heart weight on GGA19 and 26, for heart % on GGA26 and for hematocrit value on GGA3. The 21 candidate genes retrieved from marker intervals in which QTL were mapped should be further explored in association studies with traits of interest. Likewise, mutations in these genes that were previously described in other organisms should be further investigated in chickens.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and institutional guidelines for the care and use of animals were followed.

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