# Mapping QTLs for Muscle and White Adipose Tissue Mass in a Chicken Crossbred Population

M.K. Nassar\*, Z.S. Goraga\*, C. Neuschl\* and G.A. Brockmann\*

### Introduction

The yield of muscle mass and the amount of white adipose tissue deposition in the body are important traits that influence the nutritional and economic value of a chicken. The identification of genes contributing to differences in body composition enhances the subsequent search for genetic variation in chicken populations that are used for meat production. Understanding the genetic architecture of economically important traits could be useful for improving the efficiency of the production of meat-chicken and for improved product quality through the breeding process. As a first step in gene discovery, usually a linkage or association study is performed to map genomic loci contributing to the trait of interest. In chicken, several crosses between diverse breeds or lines were generated in which QTLs were mapped for body weight, carcass weight, muscle weight, and fat deposition. As different chicken strains can harbour different variants of genes that control body composition, additional crosses between chickens that differ extremely in the phenotype could add new information about genetic determinants of the traits.

The partially inbred line New Hampshire (NHI) and the inbred line White Leghorn (WL77) are such extremely different genetic resources. NHI and WL77 had been selected for high meat yield and low egg weight, respectively before they were inbred. NHI had about twice as heavy breast muscle and nine times more white adipose tissue mass. We generated a F<sub>2</sub> cross between NHI and WL77 and mapped QTLs responsible for the differences in body composition and to estimate the QTL effects. The identified QTLs will contribute to discover additional genetic factors determining muscle weight and fat deposition.

#### Material and methods

**Experimental population.** The inbreeding coefficients of the parental lines New Hampshire (NHI) and White Leghorn (WL77) were about 86% and close to 100%, respectively. One NHI male and two WL77 females were mated to produce  $F_1$ . Two  $F_1$  males and 22  $F_1$  females were randomly chosen to generate  $F_2$  progeny comprising of 127  $F_2$  males. These animals were used for the QTL analysis.

**Phenotyping.** Five to six males of the parental lines,  $14 ext{ } F_1$  and  $127 ext{ } F_2$  males were phenotyped. Body weights (BW) were recorded upon hatch and every 5 weeks. All animals were slaughtered and dissected after 10 hours fasting at the age of 20 weeks. Feathers, heads, skin, feet and shanks were removed. The weights of inner organs, carcass, and breast muscle

<sup>\*</sup> Humboldt-Universität zu Berlin, Breeding Biology and Molecular Genetics, Invalidenstr. 42 D-10115 Berlin, Germany.

were measured. All white adipose tissues were dissected and weighed, including: subcutaneous adipose tissue around the neck, around the heart, retroperitoneal below the kidneys, peritoneal sticking to the kidneys, mesenteric sticking to the gizzard, and abdominal around the gonads. The sum of all masses of white adipose tissues (WAT), the mass of the breast muscle (MW), carcass weight (CW), and body weight at 20 weeks were used in the linkage analysis.

**Genotyping.** 116 microsatellite markers spanning 23 autosomal chromosomes and the Z chromosome were used to genotype parental,  $24 F_1$  and  $127 F_2$  animals.

**Statistical analyses.** Hatch and family were included as fixed effects in the model for QTL mapping because they significantly affected the phenotype. Pedigree-specific linkage maps were constructed using CRIMAP (Green, Falls, and Crooks (1990)). QTLs were mapped with GridQTL (Seaton, Hernández-Sánchez, Grunchec et al. (2006)), in which the multiple regression last squares approach is implemented. 1000 permutations of the data were performed to derive experiment-specific significance thresholds for the one-QTL *vs.* no-QTL test statistics. The 95%-confidence interval of a single QTL was estimated using a parametric bootstrap analysis with 1000 iteration. For the detection of QTL, genome-wide scans were performed using the forward selection interval mapping approach (Carlborg, Brockmann, and Haley (2005)).

#### **Results and discussion**

**Phenotypes of parental lines and their F\_1 and F\_2 progeny.** Due to the origin of the breeds and the selection, NHI chickens were about twice as heavy as WL77 at the age of 20 weeks  $(2786 \pm 102 \text{ g } vs.\ 1442 \pm 111 \text{ g})$ . The  $F_1$  and  $F_2$  means shifted towards the mean value found in NHI animals (Table 1). The  $F_2$  population did not exceed the parental limits on average. Similar patterns of performance were found for other traits. A high phenotypic correlation was observed between body and carcass weights (r = 0.98, P < 0.0001) in the  $F_2$  progeny. Also, breast muscle weight highly correlated with carcass (r = 0.90, P < 0.0001) and body weight (r = 0.89, P < 0.0001). In addition, white adipose tissue mass was associated with body weight (r = 0.23, P = 0.0105) and correlated with carcass (r = 0.28, P = 0.0016) and breast muscle weight (r = 0.31, P = 0.0006).

Table1: Phenotypic characterization of parental lines,  $F_1$  and  $F_2$  animals of the cross NHIxWL77

Population	n	$LSM \pm SE$					
		BW, g	CW, g	MW, g	WAT, g		
New Hampshire (NHI)	6	$2786 \pm 102^{a}$	$1915 \pm 78^{a}$	$342 \pm 14^{a}$	$28 \pm 7^{a}$		
White Leghorn (WL77)	5	$1442 \pm 111^{c}$	$909 \pm 86^{c}$	$178 \pm 16^{b}$	$3 \pm 2^{c}$		
F <sub>1</sub> males	14	$2551 \pm 66^{ab}$	$1679 \pm 51^{ab}$	$312 \pm 9^{ab}$	$23 \pm 4^{ac}$		
F <sub>2</sub> males	127	$2420 \pm 22^{b}$	$1666 \pm 17^{b}$	$309 \pm 3^{a}$	$25 \pm 2^{ab}$		

a,b,c Significant differences between populations for the same trait ( $P \le 0.05$ ) (one way ANOVA followed by Tukey-Kramer test).

**QTL mapping.** The total genetic length of the marker linkage map used for QTL analysis was 2676 cM, and average marker spacing was 32.9 cM. A total of 18 QTLs on eight chromosomes surpassed the genome-wide suggestive threshold (Table 2). As expected,

QTLs for carcass weight, which was highly correlated with body weight, were detected roughly at the same genomic regions as the detected body weight QTLs on GGA1, 4, 11, 13, 17 and 20. Confidence intervals coincided for both traits. For the QTL on GGA1 Ikeobi, Woolliams, and Morrice (2002); Kerje, Carlborg, Jacobsson et al. (2003); Abasht, Dekkers, and Lamont (2006) and Rowe, Pong-Wong, Haley et al. (2009) also reported effects for body weight, fat weight and carcass traits.

Interestingly, due to the high correlation between body weight and weights of carcass, breast muscle and white adipose tissues, a genomic region on GGA4 was detected where the most likely QTLs positions (at 164 to 169 cM) and confidence intervals coincided for all four traits (Table 2). Hence, these QTLs might have a pleiotropic effect on muscle, white adipose tissue and bone development or is a growth factor supporting linear growth. The QTL explained 29.7, 26.3 and 20.3% of the phenotypic F<sub>2</sub> variance of body weight, carcass weight and breast muscle weight at 20 weeks, respectively. The closest marker to the peak F-value is *UMA4.034* at 165 cM (75 Mb). The allele derived from the NHI line increased body weight, carcass weight, and muscle weight at the QTLs on GGA4, whereas it decreased white adipose tissues mass. QTLs for body weight and composition were mapped repeatedly to the same chromosome region in different crosses (<a href="http://www.animalgenome.org/cgi-bin/QTLdb/GG/index">http://www.animalgenome.org/cgi-bin/QTLdb/GG/index</a>).

Table 2: OTLs and their effects

Trait	GGA	cM <sup>1</sup> (95% confidence	F-value <sup>2</sup>	Additive	Dominance	
		interval)		effect (SE) <sup>3</sup>	effect (SE) <sup>3</sup>	$\% P^4$
BW	1	147 (127.5 - 541.0)	10.05*	72.1 (30.3)	153.7 (45.9)	15.6
	4	166 (158.0 - 173.0)	23.02**	184.8 (27.3)	22.6 (36.7)	29.7
	11	56 (0.0 - 57.0)	$6.53^{\dagger}$	58.0 (24.9)	95.8 (38.1)	10.7
	13	88 (72.0 - 93.5)	9.37*	-61.9 (31.9)	189.9 (46.3)	14.7
	17	26 (14.0 - 39.0)	$8.02^{\dagger}$	-23.7 (27.4)	175.8 (45.9)	12.8
	20	0 (0.0 - 15.0)	$5.58^{\dagger}$	8.3 (30.9)	-196.2 (58.8)	9.3
CW	1	144 (127.5 - 486.0)	13.31**	59.6 (24.0)	151.2 (37.1)	20.7
	4	164 (156.0 - 173.0)	18.22**	135.4 (22.4)	5.3 (28.9)	26.3
	11	57 (0.0 - 57.0)	$6.61^{\dagger}$	39.5 (19.1)	81.6 (28.8)	11.5
	13	84 (50.0 - 92.0)	$7.55^{\dagger}$	-54.9 (24.7)	120.5 (34.8)	12.9
	17	25 (17.0 - 39.0)	12.41**	-25.9 (21.7)	178.8 (37.7)	19.6
	20	3 (0.0 - 15.0)	$5.63^{\dagger}$	-9.6 (23.9)	-140.5 (42.8)	9.9
MW	1	138 (15.5 - 398.5)	9.99*	12.6 (4.5)	21.2 (6.9)	16.0
	4	165 (0.0 - 176.0)	13.39**	22.3 (4.3)	2.2 (5.5)	20.3
	27	57 (0.0 - 58.0)	10.94**	19.4 (4.3)	4.6 (5.9)	17.2
WAT	1	17 (5.0 - 384.0)	$8.64^{\dagger}$	9.2 (2.3)	2.2 (4.0)	13.8
	3	67 (47.0 - 354.0)	$6.41^{\dagger}$	-4.6 (3.1)	-14.8 (5.6)	10.6
	4	169 (31.5 -184.0)	$5.77^{\dagger}$	-6.4 (2.3)	-6.7 (3.3)	9.7

<sup>&</sup>lt;sup>1</sup> Chromosomal location is given as pedigree specific cM-position; first marker on each chromosome was set at 0 cM.

<sup>&</sup>lt;sup>2</sup> \*\* highly significant at 1% genome-wide level (F ≥ 10.80), \* significant at 5% genome-wide level (F ≥ 8.81), † significant at 5% chromosome-wide level, which is assumed suggestive at the genome-wide level.

<sup>&</sup>lt;sup>3</sup> The direction of QTL effects is given as NHI allele effect compared to WL77.

<sup>&</sup>lt;sup>4</sup>Phenotypic F<sub>2</sub> variance (%) explained by the QTL.

Chicken breast meat is an important carcass part preferred by consumers. Significant and highly significant breast muscle weight QTLs were detected on GGA1 and 4, the above mentioned and on GGA27 that explained 16-20.3% of the phenotypic variation. On GGA1 and 4 QTLs for muscle weight were identified in others chicken cross too (e.g. Nadaf, Pitel, Gilbert et al. (2009)). No QTL influencing breast muscle have been discovered before on GGA27.

High fat levels are considered an indicator of low commercial value. Three suggestive QTLs were founded for white adipose tissue mass on GGA1, 3, and 4. The test statistic of the QTL for total adipose tissues on GGA1 at 17 cM (F=8.64) was very close to 5% genome-wide threshold (F=8.81). It had an additive effect of 9.2 g and explained 13.8% of the phenotypic variance of total adipose tissue mass in 20 weeks old chickens of the (NHIxWL77)  $F_2$  population (Table 2). These results may be of interest to chicken geneticists. Jennen, Vereijken, Bovenhuis et al. (2004) mapped also a suggestive fatness QTL on GGA1 at 25 cM in a broiler population. Lagarrigue, Pitel, Carre et al. (2006) reported suggestive fatness QTLs on GGA3 at 84 cM.

#### **Conclusions**

Using an intercross between the partially inbred New Hampshire and the inbred White Leghorn chicken lines, we have mapped unique QTLs for carcass weight on GGA11, 13, 17 and 20, and for breast muscle weight on GGA27, which have not been reported before. Several QTLs in the current study confirmed QTLs found in other cross-bred QTLs studies. The highest QTL effect was found in an 18 cM region on GGA4. This is a good basis for fine mapping and subsequent identification genes contributing to muscle development and white adipose tissues deposition.

## Acknowledgement

Mostafa K. Nassar acknowledges support of Yousef Jameel Foundation.

#### References

Abasht, B., Dekkers, J.C.M., and Lamont, S.J. (2006). Poult. Sci. 85:2079-2096.

Carlborg, O., Brockmann, G.A., and Haley, C.S. (2005). Mamm. Genome 16:481-494.

Green, P., Falls, K., and Crooks, S. (1990) CRI-MAP Program Version 2.4. Washington University School of Medicine, St Louis, MO.

Ikeobi, C.O.N., Woolliams, J.A., and Morrice, D.R. (2002). Anim. Genet. 33:428-435.

Jennen, D.G.J., Vereijken, A.L.J., Bovenhuis, H. et al. (2004). Poult. Sci. 83:295-301.

Kerje, S., Carlborg, O., Jacobsson, L. et al. (2003). Anim. Genet. 34:264-274.

Lagarrigue, S., Pitel, F., Carre, W. et al. (2006). Genet. Sel. Evol. 38:85-97.

Nadaf, J., Pitel, F., Gilbert, H. et al. (2009). Physiol. Genomics 38: 241–249.

Rowe, S.J., Pong-Wong, R., Haley, C.S. et al. (2009). Genet. Sel. Evol. 41:6.

Seaton, G., Hernández-Sánchez, J., Grunchec, J.A. et al. (2006). In Proc 8th WCGALP, Abstr. No 27\_633-916.