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Associations Between Microsatellite Markers and Traits Related to Performance, Carcass and Organs in Chickens

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Abstract: Associations between four microsatellite markers on chromosome 11 and five on chromosome 13 with performance, carcass and organs traits were investigated in chickens using a least-squares approach applied to single-marker analysis. Three hundred and twenty seven F₂ chickens from the EMBRAPA broiler x layer experimental population were evaluated for 16 traits: five related to performance, five to carcass and five to organs, plus the hematocrit. Two significance thresholds were considered: $p < 0.05$ and $p < 0.0056$; the last value resulted from the application of a multiple tests analyses correction. On chromosome 11, six associations ($p < 0.05$) between the genotypes of two markers with four growth related and one carcass trait were found. On chromosome 13, six associations ($p < 0.05$) between marker genotypes and three performance traits, eight associations ($p < 0.05$) between marker genotypes and two carcass traits and eight associations ($p < 0.05$) between marker genotypes and four organs traits were detected. These associations were indications of the presence of quantitative trait loci on these chromosomes, especially on chromosome 13. In this chromosome, the strongest evidence was for body weight at 41 days of age and percentage of carcass because the p -values exceeded the multiple test threshold ($p < 0.0056$), but also for breast percentage and heart weight due to the large number of markers (four) on chromosome 13 associated with each one of these traits. These associations should be further investigated by interval mapping analyses to find QTL positions and to allow the estimation of their effects.

Key words: Body weight, broiler, heart weight, poultry, single marker analysis

INTRODUCTION

Quantitative Trait Loci (QTL) are chromosome segments that affect a trait, but not necessarily a single locus (Falconer and Mackay, 1996). Several methods are available for QTL analysis and consist of scanning molecular markers across the genome with the objective of determining which markers are linked to QTL. The earliest method for QTL analysis included single-marker analysis (Gupta, 2002).

Single-marker analysis can be based on regression, t -test or analysis of variance. These approaches are easily implemented in statistical packages and softwares. Also, this method is appropriate to investigate associations between microsatellite marker genotypes and quantitative traits, does not require the previous construction of linkage maps and may be used to find and eliminate non-informative markers (Liu, 1998).

Several studies have been carried out with single-marker analysis in cattle (Milanesi *et al.*, 2008; Allan *et al.*, 2009) and plants, such as soybeans (Zhang *et al.*, 2009) and sorghum (Fernandez *et al.*, 2008). In chickens, Rosário *et al.* (2006a, 2006b) identified markers associated with performance and carcass traits on chromosomes 1 (GGA1), 3 (GGA3) and 4 (GGA4) in

a Brazilian F₂ reference population (males from a layer line and females from a broiler line). Atzmon *et al.* (2006) used single-marker analyses to investigate associations with traits related to growth and fatness in a commercial chicken line and found 44 significant associations out of the 456 marker-trait combinations. Applying a single marker approach to a multi generational chicken population, Atzmon *et al.* (2008) identified 729 associations with body weight, carcass traits and egg production, 150 of which were significant and of these, 54 were supported by the literature.

This study is part of a whole genome scan project that is being conducted by our group and was carried out to identify microsatellite markers GGA11 and 13 associated with growth, carcass and organs traits in the EMBRAPA F₂ experimental population.

MATERIALS AND METHODS

An experimental F₂ population developed by EMBRAPA Swine and Poultry Research Center through the crossbreeding of males from a broiler line (TT) and females from a layer line (CC) was used. These two lines had diverse breed origins and were selected according to the criteria described by Figueiredo *et al.*

(2003a, 2003b) and were described genotypically by Rosario *et al.* (2009) based on microsatellite markers from GGA1, GGA3 and GGA4. A total of 327 F₂ (TCTC) chickens from four full-sib families that showed the highest number of informative markers in the selective genotyping of GGA1 (Nones *et al.*, 2006), 3 and 5 (Ruy *et al.*, 2005) were chosen for this study.

F₂ chickens were raised as broilers, had free access to phase specific diets and water. They were kept in collective pens up to 35 days of age, weighed and individually caged up to 41 days to allow recording of feed intake. Body weights at hatching, at 35 (BW35), 41 (BW41) and 42 days were recorded, along with feed intake between 35 and 41 days (FI35-41), allowing the computation of weight gain (WG35-41) and feed conversion (FC35-41) between 35 and 41 days.

Slaughtering was performed on day 42, after 6 h fasting. The following carcass traits were recorded after 6 h at -4°C: weights of eviscerated carcass (no organs, head, neck and feet), abdominal fat, breast (with skin and bones), drums and thighs and wings. Percentages, relative to body weight at 42 days, were computed for these last five traits, generating other five traits: weights of carcass as percentage of body weight (C%), abdominal fat as percentage of body weight (AF%), breast as percentage of body weight (B%), drums and thighs as percentage of body weight (D%) and wings as percentage of body weight (W%). Length of intestine (small plus large) (cm) and the weights of lungs (g), heart (g), liver (g) and gizzard (g) were also recorded. Blood samples were collected at slaughter for genomic DNA extraction using the DNAzo[®] reagent (Invitrogen™). DNA samples were diluted to a 20 ng DNA/μL concentration. Blood samples were also analyzed to obtain hematocrit values (microhematocrit method), in percentage (%).

Four microsatellite markers on GGA11 (*LEI0143*, *ADL0123*, *ADL0210* and *MCW0230*) and five on GGA13 (*ADL0147*, *LEI0251*, *MCW0213*, *MCW0110* and *MCW0104*) were amplified by PCR (Table 1). Reactions contained 100 ng DNA, buffer solution 50 mmol/L KCl and 10 mmol/L Tris-HCl (with pH = 8.5), 4.0 mmol/L magnesium chloride, 400 μM from each dNTP, 5 pmol/μL from each primer: direct (marked with HEX or FAM fluorescences) and reverse and 1.5 U of *Taq* DNA polymerase. The thermocycler program was: two minutes at 94°C, followed by 30 cycles of a minute at 94°C, a minute at annealing temperature (varying from 55-68°C, depending on the marker being amplified) and one minute at 72°C, followed by a final extension step of 10 min at 72°C.

PCR products were submitted to electrophoresis in agarose gels (2.0%), along with the molecular weight marker φX174 RF DNA/HaeIII (Invitrogen™). Fragment analyses and allele identification were performed in a MegaBACE 1000 (GE Healthcare) DNA sequencer.

Although PCR amplifications were made separated for each marker, genotypings were carried out with a mixture of amplified products from three to four markers, according to the expected length of amplified fragments and primer fluorescence, along with the molecular weight marker ET-ROX 400 (GE Healthcare). The Genetic Profiler (GE Healthcare) software was used in the graphical representation of fluorescence peaks and in the determination of alleles.

Marker genotypes and phenotypes (Table 2) from the F₂ chickens were used in the association analyses. The least-squares approach was applied with the GLM procedure of SAS (1999). The statistical model included the fixed effects of hatch, dam or family of full-sibs, marker genotype, sex and the genotype × sex interaction. The data were unbalanced in respect to hatch, due to the large number of classes. Therefore, the interactions involving hatch and family effects were not included in the model of analysis. Body weights at hatching and at day 42 (g) were used as covariates in the analyses. The first was used for BW35 and BW41 and the second for organ-related traits analyses. Additionally, the covariate BW35 was used in the analyses of WG35-41, FI35-41 and FC35-41.

Two significance thresholds were considered: $p < 0.05$ and $p < 0.0056$ in the F tests of genotype and genotype × sex effects. The $p < 0.0056$ value resulted from the application of a correction (α/n , where: $\alpha = 0.05$ and n = number of independent tests) for multiple tests analyses (Falconer and Mackay, 1996). The last value, however, was assumed to be too stringent, because some of the markers are linked, making the tests partially redundant.

RESULTS AND DISCUSSION

On GGA11, marker *LEI0143* was associated with BW35, BW41 (Table 3) and W% (Table 4) and marker *ADL0123* was associated with BW41, WG35-41 and FC35-41 (Table 3), totaling five different performance and carcass traits associated ($p < 0.05$) to marker genotypes from this chromosome. In addition, associations between AF% and gizzard weight and the genotype of *ADL0210* × sex interaction were also detected (Tables 4 and 5, respectively). Considering the correction for multiple tests analyses, none of these associations exceeded the significance threshold ($p < 0.0056$).

On GGA13, six associations ($p < 0.05$) between marker genotypes and performance traits were found (Table 3). Three markers were associated with BW41 (*MCW0213*, *LEI0251* and *MCW0104*), one marker was associated with both WG35-41 and FC35-41 (*MCW0110*) and one marker was associated with BW35 (*LEI0251*). Additionally, one association between WG35-41 and the genotype of *MCW0213* × sex interaction was also detected. The association between *MCW0213* and BW41 exceeded the multiple test significance threshold.

Table 1: Microsatellite markers, chromosome assignments and respective positions

Microsatellite marker	<i>Gallus gallus</i> autosome (GGA)	Position (cM) in the Consensus map ¹	Position (cM) in the EMBRAPA map ²
LEI0143	11	0	0
ADL0123	11	22	23
ADL0210	11	54	63
MCW0230	11	88	106
MCW0213	13	22	11
ADL0147	13	32	0
LEI0251	13	49	17
MCW0110	13	59	34
MCW0104	13	74	57

¹According to Schmid *et al.* (2005). ²According to Ambo *et al.* (2008)

Table 2: Descriptive statistics for the traits analysed in the F₂ population

Traits	Number of records	Mean	Standard deviation
Body weight at 35 days (g)	364	788.7	140.1
Body weight at 41 days (g)	364	1005.0	189.9
Feed intake between 35 and 41 days (g)	364	604.6	145.7
Weight gain between 35 and 41 days (g)	360	219.6	68.0
Feed conversion between 35 and 41 days (g)	360	2.95	0.88
Weight of carcass as percentage of body weight	364	64.81	2.04
Weight of abdominal fat as percentage of body weight	364	1.42	0.58
Weight of breast as percentage of body weight	364	16.33	1.04
Weight of drums and thighs as percentage of body weight	364	21.38	1.22
Weight of wings as percentage of body weight	364	8.23	0.48
Hematocrit value (%)	360	28.41	3.26
Length of intestine (cm)	364	150.6	14.8
Lungs weight (g)	363	8.09	2.21
Heart weight (g)	364	6.56	1.72
Liver weight (g)	364	26.31	5.36
Gizzard weight (g)	364	22.93	4.38

Yet on GGA13, eight associations ($p < 0.05$) between marker genotypes and two carcass traits were found (Table 4). The genotypes of four (MCW0213, ADL0147, LEI0251 and MCW0110) out of five markers were associated with C% and B%. Three associations between marker genotype $\times$ sex interaction with carcass traits were also detected: MCW0110 with B%, MCW0213 with W% and LEI0251 with AF%. The association between marker LEI0251 and C% exceeded the multiple test significance threshold.

And finally, eight associations ($p < 0.05$) between GGA13 marker genotypes and organ traits were detected, half of which with heart weight (Table 5). The genotypes of markers MCW0213, LEI0251, MCW0110 and MCW0104 were associated with heart weight, whereas that of MCW0110 was also associated with lungs, liver and gizzard weights and of LEI0251 also with gizzard weight. Six genotype markers $\times$ sex interactions for organ related traits were found, involving MCW0213 with hematocrit value and lungs weight; MCW0110 with lungs weight and length of intestine, ADL0147 with heart weight and LEI0251 with gizzard weight. Although some of these associations between markers and organ traits approached the multiple test significance threshold, none of them exceeded it.

The marker associated to the highest number of traits was MCW0110 on GGA13. This marker was positioned

at 34 cM in the EMBRAPA map (Table 1) and was associated to seven traits; it was followed by LEI0251 (17 cM, EMBRAPA map) associated to six traits and MCW0213 (11 cM, EMBRAPA map) with four traits, all from GGA13.

Several associations were identified between microsatellite markers on GGA11 and 13 and traits related to performance, carcass and organs, suggesting the presence of QTL for these traits. The strongest evidence was found for BW41 and C% on GGA13 because the p -values exceeded the multiple test threshold. Also for B% and heart weight due to the large number of markers on GGA13 (all but one) associated to these traits.

In the present study, one association between genotype of ADL0210 (positioned at 54 cM in the Consensus Map) $\times$ sex interaction and gizzard weight with was detected in GGA11. It could be inferred that the genotype of marker ADL0210 was associated to distinct effects on males and females for gizzard weight due to the genotype $\times$ sex interaction. Gizzard is responsible for grinding the feed, facilitating digestion and consequently the absorption of nutrients.

Results that were similar to those of the present study, where markers LEI0143 (GGA11) was associated with BW35 and BW42 and MCW0213 (GGA13) was associated with BW41 were reported by Atzmon *et al.*

Table 3: Probability of type I error in the association tests between microsatellite markers on GGA 11 and 13 and performance traits

Traits ¹	Effects	Microsatellite markers on GGA11				Microsatellite markers on GGA13				
		LEI0143	ADL0123	ADL0210	MCW0230	MCW0213	ADL0147	LEI0251	MCW0110	MCW0104
BW35 (g)	G ²	0.0113	0.1899	0.4972	0.4198	0.0614	0.3911	0.0353	0.3015	0.1886
	G×S ³	0.3718	0.4090	0.7934	0.3430	0.3314	0.9213	0.8266	0.2684	0.7361
BW41 (g)	G	0.0101	0.0341	0.5289	0.3212	0.0050	0.8063	0.0140	0.0578	0.0448
	G×S	0.6204	0.5295	0.4316	0.2582	0.6152	0.7942	0.6525	0.1630	0.7594
WG35-41 (g)	G	0.4962	0.0336	0.7661	0.2909	0.3921	0.2477	0.0522	0.0330	0.1981
	G×S	0.6925	0.8050	0.4137	0.1574	0.0333	0.1759	0.7017	0.7440	0.5356
FC35-41 (g/g)	G	0.8110	0.0315	0.5044	0.6261	0.6956	0.3268	0.3074	0.0452	0.1118
	G×S	0.7560	0.2089	0.1781	0.1997	0.2509	0.5036	0.1084	0.5956	0.4725
FI35-41 (g)	G	0.9902	0.5739	0.5012	0.0675	0.1319	0.2498	0.6107	0.3647	0.8066
	G×S	0.2106	0.5014	0.0719	0.9965	0.8055	0.7438	0.5743	0.4096	0.7758

¹Body weights at 35 (BW35) and 41 days (BW41), weight gain between 35 and 41 days (WG35-41), feed conversion (FC35-41) and feed intake (FI35-41) between 35 and 41 days. ²G = genotype. ³G×S = genotype × sex interaction

Table 4: Probability of type I error in the association tests between microsatellite markers on GGA 11 and 13 and carcass traits

Traits ¹	Effects	Microsatellite markers on GGA11				Microsatellite markers on GGA13				
		LEI0143	ADL0123	ADL0210	MCW0230	MCW0213	ADL0147	LEI0251	MCW0110	MCW0104
C%	G ²	0.0604	0.1628	0.9542	0.7597	0.0237	0.0264	0.0023	0.0362	0.3784
	G×S ³	0.9312	0.3161	0.3817	0.8210	0.3226	0.4297	0.4659	0.2122	0.1357
B%	G	0.7297	0.6242	0.6393	0.1737	0.0422	0.0147	0.0089	0.0250	0.1665
	G×S	0.3490	0.8459	0.2900	0.2700	0.3145	0.4789	0.3379	0.0241	0.7271
D%	G	0.0564	0.3801	0.4429	0.5886	0.8728	0.0927	0.2769	0.9440	0.0762
	G×S	0.3396	0.3755	0.0743	0.7727	0.6264	0.6900	0.9719	0.3596	0.0902
W%	G	0.0128	0.1429	0.7508	0.9509	0.2933	0.9742	0.8817	0.9930	0.6815
	G×S	0.7927	0.4342	0.5616	0.2437	0.0354	0.1986	0.8073	0.3860	0.6525
AF%	G	0.2517	0.4581	0.5014	0.8980	0.1098	0.5784	0.3374	0.3095	0.4085
	G×S	0.6549	0.7647	0.0129	0.0927	0.0538	0.5634	0.0380	0.0768	0.4572

¹Weights of carcass as percentage of body weight at 42 days (C%), breast as percentage of body weight at 42 days (B%), drums and thighs as percentage of body weight at 42 days (D%), wings as percentage of body weight at 42 days (W%) and abdominal fat as percentage of body weight at 42 days (AF%). ²G = genotype. ³G×S = genotype × sex interaction

(2008), who analyzed a multi generational resource chicken population using a single marker approach and found associations of both markers with body weight at 21 and 42 days.

In a comprehensive review of studies conducted to identify QTLs for traits related to growth, carcass and organs (among other traits) using interval mapping in chickens (Abasht *et al.*, 2006), no QTL for growth or carcass traits was mapped between *LEI0143* and *ROS0308* (0-69 cM according to the Chicken Consensus Map, Schmid *et al.*, 2005) on GGA11, but a suggestive linkage was found in this region for length of intestine at 9 weeks of age between *ROS0111* and *ADL0308* (37-69 cM, Consensus Map) by Navarro *et al.* (2005). Jennen *et al.* (2004) reported a suggestive linkage for abdominal fat weight in the interval between *ADL0287* (22 cM, Consensus Map) and *ADL0210*.

QTL for growth related traits such as: body weight at 2, 4, 6 and 8 weeks (Zhou *et al.*, 2006) and 7, 9 and 10 weeks of age (Jennen *et al.*, 2004), breast muscle and drums weights (Ikeobi *et al.*, 2004), skin fat weight (Ikeobi *et al.*, 2002) and heart weight (Navarro *et al.*, 2005), were mapped to GGA13 using interval mapping analyses. Associations between B%, AF% and heart weight and the genotype × sex interaction were also detected in the present study. One QTL for heart weight at 9 weeks of age was mapped between markers *ADL0147* (32 cM Consensus Map) and *ADL0225* (approximately 70 cM) by Navarro *et al.* (2005) and when

a model including the QTL × sex interaction was fitted, an overdominance effect was detected for males. Taken together, these results are a strong indication of the presence of one QTL for heart weight on GGA13 that shows distinctive effects on males and females.

The weights of heart and lungs are traits of extreme relevance because they are related to metabolism. The intense selection pressure for weight gain for many generations has lead to a proportional reduction of the cardio-respiratory system in broiler chickens, resulting in a higher incidence of ascites and sudden death, which causes great economic losses to the poultry industry (Rosário *et al.*, 2004). The difference in the estimates of genetic correlations between body weight at 38 and at 42 days and heart weight (0.60 and 0.28, respectively) obtained by Gaya *et al.* (2006) in a broiler line, gave support to this hypothesis. Furthermore, the estimate of heritability of heart weight obtained by the same authors was high (0.38). Based on these results they recommended that heart weight be used as a selection criterion in broiler chicken breeding programs. Ledur *et al.* (2006) estimated genetic and phenotypic parameters for the EMBRAPA experimental population. Heritability estimates were low for heart and lungs weights (0.11 and 0.10, respectively) and moderate for BW42 (0.24). Positive genetic correlations were obtained between heart weight and BW42 (0.55) and weights of liver (0.60) and lungs (0.67). Therefore, selecting for BW42 would increase heart and lungs weights but in a lower proportion.

Table 5: Probability of type I error in the association tests between microsatellite markers on GGA 11 and 13 and organs traits

		Microsatellite markers on GGA11				Microsatellite markers on GGA13				
Traits	Effects	LEI0143	ADL0123	ADL0210	MCW0230	MCW0213	ADL0147	LEI0251	MCW0110	MCW0104
Hematocrit value (%)	G ¹	0.2465	0.4358	0.9596	0.9239	0.5243	0.3597	0.7139	0.8313	0.3846
	G×S ²	0.6655	0.7305	0.7762	0.9566	0.0113	0.3487	0.3109	0.5806	0.7582
Length of intestine (cm)	G	0.4556	0.5492	0.4709	0.1174	0.4678	0.8071	0.0877	0.4379	0.2107
	G×S	0.3784	0.5992	0.7569	0.4241	0.1795	0.7109	0.2401	0.0383	0.4010
Lungs weight (g)	G	0.9331	0.6899	0.9955	0.2745	0.0532	0.1517	0.2639	0.0391	0.1426
	G×S	0.7352	0.3626	0.9727	0.3497	0.0075	0.2608	0.3439	0.0479	0.3117
Heart weight (g)	G	0.2211	0.2365	0.7637	0.8423	0.0210	0.7615	0.0228	0.0068	0.0338
	G×S	0.8518	0.8363	0.1372	0.9175	0.0634	0.0070	0.1893	0.2187	0.2809
Liver weight (g)	G	0.9645	0.9193	0.3004	0.4171	0.7560	0.1299	0.3970	0.0336	0.3440
	G×S	0.2689	0.7440	0.3839	0.3495	0.1486	0.8690	0.2251	0.1251	0.2468
Gizzard weight (g)	G	0.0669	0.2597	0.1159	0.2061	0.3840	0.9986	0.0065	0.0290	0.3977
	G×S	0.1906	0.2297	0.0315	0.9249	0.7782	0.9465	0.0057	0.5046	0.2279

¹G = genotype. ²G×S = genotype × sex interaction

The traits such as body weights, weight gain and feed conversion that were associated to markers genotypes from chicken chromosomes 11 and 13 in the present study are relevant to the poultry industry. Traits such as heart weight, feed conversion and carcass percentage and fat percentage are difficult and costly to measure in breeding programs. Therefore, the identification of genes involved in the control of these complex traits would be very useful in Marker Assisted Selection (MAS) to reduce such problems. The identification of a marker linked to heart weight, for instance, independently from body weight, could be used in MAS to help reduce metabolic problems (Ledur *et al.*, 2006).

Studies should proceed by searching the whole chicken genome for regions associated to performance, carcass and organs related traits in the EMBRAPA resource population. Moreover, a future direct search for genes responsible for these traits will be possible based on positional or functional candidate genes, allowing that molecular genetics may be used jointly with phenotypic selection in poultry breeding programs.

Conclusion: Associations between the genotypes of eight microsatellite markers and traits related to performance, carcass and organ traits were found, suggesting the presence of quantitative trait loci regions on chromosomes 11 and 13.

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