Mapping Of A QTL Region On GGA1

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Introduction

The strategies available to increase genetic information in a genomic region of interest include increasing the number of individuals, genotyping additional markers and using advanced generations. In a Brazilian chicken F_2 population (broiler x layer), Nones *et al.* (2006) mapped QTLs for body weight, heart and lungs weights, abdominal fat weight adjusted for body weight and intestine length in a specific region of GGA1 (between markers *ADL0234* and *LEI0071*). Studies in other populations also indicated the presence of QTLs for these traits in this region (Sewalen *et al.* (2002); Jennen *et al.* (2005) and Liu *et al.* (2007)). Based on these results, our objective was to fine map QTLs previously identified by Nones *et al.* (2006) between markers *ADL0234* and *LEI0071*, by selecting and genotyping additional markers in this region.

Material and methods

Experimental population and data recording. The F_2 population was originated from the crossbreeding of males from a broiler line and females from a layer line. A total of 642 F_2 chickens were used in this study. Details are in Nones *et al.* (2006). Phenotypes were: body weight at 35, 41 and 42 d (BW35, 41 and 42), weights of heart (HW), lungs (LW) and abdominal fat (AFW), and the length of small plus large intestine in cm (IL). In the analyses of adjusted fat weight (AFWadj), BW42 was used as a covariate.

Genotyping and linkage map. Genotypes of six microsatellite markers and two SNPs were added to the ten microsatellites previously genotyped by Nones *et al.* (2006) in the target region (*ADL0234-LEI0071*). Microsatellite markers were selected in this region from the chicken Consensus map (Schmid *et al.* (2005)). The size of alleles was determined in a MegaBACE sequencer (GE Healthcare). The two SNPs were determined in our population based on sequences of two genes found in this interval (http://www.ncbi.nlm.nih.gov): g.47920G>A (SNP1) which was identified in the *IGF1 gene* (Boschiero *et al.* (2009)) and SNP2 (unpublished results). SNP genotyping was carried out with TaqMan® probes (Applied Biosystems), in a real-time PCR LightCycler 480 System II® (Roche). The partial linkage map was constructed using multipoint linkage analyses with the CRI-MAP software (Green *et al.* (1990)).

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QTL mapping analysis. QTL interval mapping using regression methods (Haley *et al.* (1994)) and the line-cross genetic model of the QTL Express software (Seaton (2002)) was applied. The fixed effects of sex, hatch, and family were included in the model. A total of 10,000 permutations were computed to determine suggestive linkage and 5% and 1% genome-wide significance levels (Lander and Kruglyak (1995)). Confidence intervals for QTL positions were estimated with bootstrapping according to Visscher *et al.* (1996).

Results and discussion

Descriptive statistics of traits measured are in Table 1. Although the extent of the linkage map of this region increased from 82.3 cM (Nones *et al.* (2005)) to 112.2 cM (Figure 1), the average marker spacing decreased from 9.1 cM to 7.0 cM when eight additional markers were added. The marker *ADL0192* was positioned farther than expected in our population. A total of six significant QTLs were mapped to the target region of GGA1 in the present study (Table 2), five of which in a single interval (*LEI0146*-SNP1), comprising 8.5 cM.

Table 1: Descriptive statistics for the traits evaluated in the F_2 chickens (n= 642)

Traits	Mean	SD	Minimum	Maximum
BW35 (g)	806	136	458	1688
BW41 (g)	1027	182	550	1698
BW42 (g)	991	180	526	1688
HW (g)	6.4	1.6	2.0	12.0
LW (g)	8.1	2.2	3.0	16.0
AFW (g)	16.3	7.7	1.0	55.0
IL (cm)	153.0	14.8	111.0	197.0

[1]BW35, BW41= body weight at 35 and 41 days of age respectively, HW= heart weight, LW= lungs weight, AFW= abdominal fat weight and IL= intestine length.

This study confirmed the QTLs previously mapped by Nones *et al.* (2006), but some of them, in different positions. A possible explanation for this difference was that, although a larger set of markers was used, the present map spanned only part of the chromosome region studied by Nones *et al.* (2006). There was no reduction of intervals between markers flanking QTLs for BW35, BW41, LW and IL. QTLs for BW35, BW41 and IL were previously identified by Nones *et al.* (2006) in the interval between *LEI0068* and *MCW0297* (spanning 6.2 cM, Figure 1), whereas in the present study, the QTLs for BW were mapped between markers *LEI0146* and SNP1 (8.5 cM), and the QTL for IL was mapped between the two SNPs, in an interval spanning 14.3 cM. Between markers *LEI0068* and *MCW0297* two other markers were included (*MCW0289E-MCW0353*). The QTL for LW was mapped between *MCW0297* and *LEI0146* (4.5 cM) by Nones *et al.* (2006) and now between markers *LEI0146* and SNP1 (8.5 cM). The inclusion of marker *ADL0364* between *MCW0297* and *LEI0146* may have caused this change. The lengths of intervals in which QTLs were mapped for HW and AFWadj, on the other hand, decreased from 29.5 (*LEI0146-LEI0174*) (Nones *et al*. (2006)) to 8.5 cM (*LEI0146*-SNP1). In the present study, two SNPs were included in this interval. Therefore, the addition of eight markers in the target region was effective in reducing marker intervals for two of the QTLs previously mapped. The QTL for AFWadj explained 4.6% of the phenotypic variance (Table 2), indicating that it may be of interest to

the poultry industry. Additionally, the QTL for HW, explained 4.2% of the phenotypic variance. Searching the genome sequence for candidate genes in this interval could be recommended.

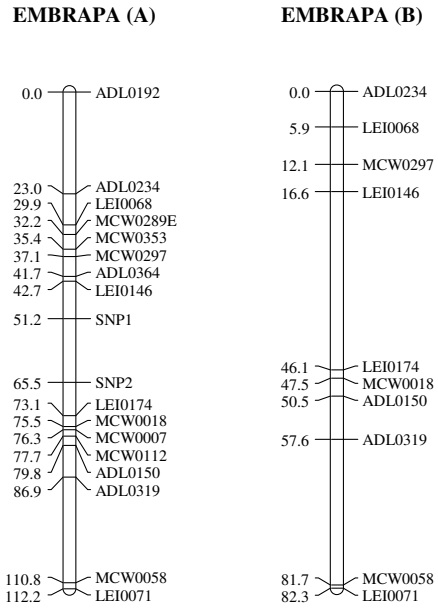

Figure 1: (A) Linkage map of EMBRAPA population of the target region of GGA1. (B) EMBRAPA's linkage map of target region (Nones *et al.* (2005)). Positions are in centiMorgans (cM). First marker of each map was considered at 0 cM to facilitate comparisons, but marker *ADL0234* was positioned at 145.7 cM (Nones *et al.* (2005)). MapChart was used for graphical representation (Voorrips (2002)).

Table 2: Quantitative trait loci fine mapping in the line-cross analysis on GGA1

Trait[1]	F-ratio	Position (cM)[3]	CI (cM)	Additive effect (SE)	Flanking markers	PV (%)[4]
BW35 (g)	22.3**	47	1-99	31.27 (6.62)	*LEI0146*-SNP1	3.1
BW41 (g)	20.0**	46	0-99	38.39 (8.58)	*LEI0146*-SNP1	3.0
HW (g)	28.2**	45	24-112	0.39 (0.07)	*LEI0146*-SNP1	4.2
LW (g)	22.7**	43	40-47	0.53 (0.11)	*LEI0146*-SNP1	3.4
AFWadj (g)[2]	30.6**	47	40-60	-1.51 (0.27)	*LEI0146*-SNP1	4.6
IL (cm)	17.7*	54	0-87	3.28 (0.78)	SNP1-SNP2	0.5

**genome-wide 1% significance; *genome-wide 5% significance. [1]BW35, BW41 = body weight (g) at 35 and 41 days of age, respectively, HW= heart weight, LW= lungs weight, IL= intestine length. [2]Analyses include BW42 as a covariate. [3]Position from the first marker in the set for the chromosome in our map. [4]PV= per cent of phenotypic variance explained by the QTL [(MSR- MSF)/MSR]* 100. Where %PV = percentage of phenotypic variation, MSR = residual mean square in the reduced model, MSF = residual mean square in the full model. CI= confidence interval.

Conclusion

All the QTLs previously mapped in the target region were confirmed. This study confirmed six QTLs for body weights, heart and lungs weight, adjusted abdominal fat weight and length of intestine on GGA1, five of those were mapped to one marker interval. The possibility of multiple linked QTLs in this region cannot be ruled out and should be tested with multi-trait analyses. Two important QTLs (HW and AFWadj) were mapped to a shorter interval. Searching the genome sequence for candidate genes in this interval could be recommended.

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