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ORIGINAL ARTICLE

Identification of Quantitative Trait Loci Associated with the Skeletal Deformity LSK complex in Gilthead Seabream (*Sparus aurata* L.)

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Abstract Morphological abnormalities, especially skeletal deformities, are some of the most important problems affecting gilthead seabream (*Sparus aurata* L.) aquaculture industry. In this study, a QTL analysis for LSK complex deformity in gilthead seabream is reported. LSK complex is a severe deformity consisting of a consecutive repetition of three vertebral deformities: lordosis, scoliosis, and kyphosis. Seventy-eight offspring from six breeders from a mass-spawning were analyzed: five full-sibling families, three maternal, and two paternal half-sibling families. They had shown a significant association with the LSK complex prevalence in a previous segregation analysis. Fish were genotyped using a set of multiplex PCRs (ReMsa1-13), which includes 106 microsatellite markers. Two methods were used to perform the QTL analysis: a linear regression with the *GridQTL* software and a linear mixed model with the *Qxpak* software. A total of 18 QTL were identified. Four of them (QTLSK3, 6, 12, and 14), located in LG5, 8, 17, and 20, respectively, were the most solid ones. These QTL were significant at genome level and showed an extremely large effect (>35 %) with both methods. Markers close to the identified QTL showed a strong association with phenotype. Two of these molecular markers (DId-03-T and Bt-14-F) were considered as potential linked-to-this-deformity markers. The detection of these QTL supposes a critical step in the implementation of marker-assisted selection in this species, which could decrease the incidence of this deformity and other related deformities. The identification of these QTL also represents a major step towards the study of the etiology of skeletal deformities in this species.

Keywords MAS · QTL · Genetic improvement · Lordosis · Scoliosis · Kyphosis

Introduction

In gilthead seabream (*Sparus aurata* L.) industry, products are mainly commercialized as whole fish, so deformities altering fish appearance like skeletal deformities are considered the most important, since they affect directly to fish visual quality. The prevalence of anomalies in juveniles from hatchery companies, which ranges currently from 15 to 50 %, has to be reduced to 5 % prior to batch commercialization (Afonso and Roo 2007; Prestinicola et al. 2013). This reduction requires individual manual sorting and visual assessment (Divanach et al. 1996), which introduces an additional cost of about 10 % per sold healthy fingerling. However, many abnormalities are detected later and they persist in fish at harvest size. In this way, on-growing companies have to remove deformed fish prior to their commercialization as whole fish or to sell them below their production costs, as customers rarely accept malformation-showing fish (Bardon et al. 2009). This is the reason why deformed fish affect negatively to the turnover of hatcheries and on-growing companies.

Skeletal deformities affect neurocranium or head, vertebral column, and appendicular skeleton. The most frequent of which are lordosis, scoliosis, kyphosis, and vertebral fusion. They not only affect fish appearance but also lead to physiological alterations that result in a decrease of fish commercial

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trait value: a lower growth rate, a higher mortality during handling, and an increased difficulty of filleting (Boglione et al. 2013). A triple column deformity consisting of a consecutive repetition of three of these vertebral deformities, lordosis, scoliosis, and kyphosis (LSK complex), from head to caudal fin, was described for the first time by Afonso et al. (2000) in gilthead seabream. The prevalence of LSK complex is low (0–0.2 %) and can be easily diagnosed at early ages, so hatcheries can detect and eliminate LSK-deformed individuals before selling them to on-growing companies. However, a possible genetic link between this deformity and other significant skeletal deformities (lordosis and vertebral fusion) has been reported by Negrín-Báez et al. (2015a). In that study, breeders that had LSK-deformed descendants were also responsible for the 11.2 % of lordosis deformed fish and for the 75 % of vertebral fusion-deformed fish.

On the other hand, for LSK complex in gilthead seabream, Afonso et al. (2000) found a significant association between a family and the prevalence of this deformity in its offspring after a cross-breeding scheme. A significant relation between breeders and family showing this deformity was also obtained from a larger experiment in which only six out of 89 families from an industrial mass-spawning showed the total incidence of LSK-deformed individuals (Negrín-Báez et al. 2015a). These previous findings suggest that the use of genetic improvement tools to decrease the incidence of LSK complex in offspring could also decrease the incidence of lordosis and vertebral fusion deformities in this species, and consequently to contribute industry profits.

In breeding programs, the development of new genetic tools has allowed researchers to identify genomic regions associated with specific traits of interest (quantitative trait loci (QTL)), as well as variations of these regions that are responsible for phenotypic variations (Doerge 2002). Having an accurate linkage map is crucial to search for QTL, since the density of genetic markers is an important factor to detect them (Rodríguez-Ramilo et al. 2014). In gilthead seabream, a linkage map based on microsatellite markers is available (Franch et al. 2006). Microsatellites are useful molecular markers for medium-high density maps. Multiplex PCR is a highly effective tool to reduce cost per reaction and to minimize genotyping errors by reducing steps and introducing automation during the sample analysis process (Navarro et al. 2008). This is especially important in methodologies where a large number of markers has to be genotyped, such as QTL location and identification. In gilthead seabream, a set of 13 multiplex PCR assays formed by 106 specific microsatellite markers that cover all LG in the gilthead seabream genetic map has been developed by Negrín-Báez et al. (2015b).

Marker-assisted selection (MAS) may be implemented through identification of QTL. The advantages of MAS are notable as compared with the traditional selective breeding. A wide number of studies using microsatellite markers have revealed the existence of QTL for several traits in different species. Among these, growth and morphometric parameters (total length, standard length, eyes diameter, and eyes cross) were related to QTL in Asian seabass (*Lates calcarifer*), European seabass (*Dicentrarchus labrax*), rainbow trout (*Oncorhynchus mykiss*), turbot (*Scophthalmus maximus*), Atlantic salmon (*Salmo salar*), Artic charr (*Salvelinus alpinus*), and common carp (*Cyprinus carpio* L.). QTL related with resistance to microorganism infection and survival to furunculosis have been reported in turbot and to infectious pancreatic necrosis in Atlantic salmon and rainbow trout. In addition, other traits with an increasing interest, like stress response and salinity tolerance, have shown an association to QTL in European seabass and salmonids (reviewed by Yue 2014). In gilthead seabream, several QTL related with commercial traits have been also reported for growth, sex determination, and several morphometric traits are influenced in this species by 4, 6, and 3 QTL, respectively (Loukovitis et al. 2011, 2012, 2013); two QTL for resistance to pasteurellosis were found in LG13 and LG21 (Massault et al. 2010); additionally, Boulton et al. (2011) detected a significant QTL for morphometric traits and two suggestive QTL for stress response to confinement.

LSK complex presents low prevalence, but it is considered highly important due to its high mortality and its relationship with other column deformities. MAS of this deformity could provide farmers with a useful tool to minimize the prevalence of other economically relevant and more difficult to identify at early age deformities, like lordosis and vertebral fusion. Moreover, the QTL identification of LSK complex would represent a major step towards the location of genes that determine the presence of skeletal deformities in this species. Considering all of the above, the main objective of the present study was to analyze and scan for QTL affecting LSK complex deformity in five families of gilthead seabream.

Material and Methods

Samples, Trait Measurement, and Parental Assignment

Gilthead seabream offspring from an industrial broodstock were reared until 509 days post-hatching (419.7±3.1 g). At 111 days post-hatching, fish were sorted by deformity to exclude fish showing severe skeletal anomalies. Deformed fish were culled with the exception of LSK-deformed fish that were easily identified, selected, and reared separately in a 1000-L fiberglass tank at Marine Science and Technology Park, property of the University of Las Palmas de Gran Canaria (PCTM-ULPGC). Non-deformed fish were reared in a cage of Playa de Vargas 2001 S.L. Company (PLV2001, Gran Canaria, Spain). Pedigree was determined by genetic characterization by using the microsatellite multiplex PCR

SMsa1 (Lee-Montero et al. 2013). At the end of their rearing period, 900 fish (LSK-deformed fish and PLV2001 fish) were genetically analyzed. The PLV2001 fish were also phenotypically identified as deformed fish (with other deformities) or normal fish (without deformities). Eighty-nine full-sibling families were represented, and all the LSK-deformed individuals were descendants of only six of these families. These six families were originated from two sires (♂1L and ♂2L) and five dams (♀1L, ♀2L, ♀3L, ♀5L, and ♀6L). Five of these families (FAM1 to FAM5) were selected for QTL mapping. Rearing conditions, fish sampling, parental assignment, and measurement methods of phenotypic traits (deformities) are described extensively by Negrín-Báez et al. (2015a). The number of individuals per family and their phenotype are shown in Table 1. LSK factor was measured in each fish as presence (1, LSK-fish) or absence (0, normal fish) of the deformity (binary trait).

Genotyping

Breeders and their offspring were genotyped by using 13 multiplex PCRs (ReMsa1 to ReMsa13), following the conditions previously described in Negrín-Báez et al. (2015b). These multiplex PCRs include 106 microsatellite markers which are all located on the genetic map of this species (Franch et al. 2006; Senger et al. 2006).

QTL Mapping

Two methods were used to detect QTL: a linear regression (LR) methodology and a linear mixed model (LMM) approach.

The LR method (Knott et al. 1996) was performed by using the *GridQTL* software (http://www.gridqtl.org.uk) (Seaton et al. 2006). Due to family structure, half-sibling (HS) and full-sibling (FS) regression analysis were carried out. Chromosome- and genome-wide significance thresholds for $P=0.05$ and for $P=0.01$ were estimated from a permutation test with 10,000 iterations (Churchill and Doerge 1994).

Table 1 Family structure, number of offspring analyzed in this study, and number of fish showing each phenotype

Sires	Dams	Families	Number[a]	LSK	Normal
♂1L	♀1L	FAM1	19	13	6
	♀2L	FAM2	25	20	5
♂2L	♀1L	FAM3	6	2	4
	♀2L	FAM4	13	8	5
	♀3L	FAM5	15	9	6
		Total	78	52	26

[a] Number of analyzed descendants in each family

Confidence intervals were calculated by bootstrapping the samples 10,000 times (Visscher et al. 1996).

The LMM was performed by using the *Qxpak* software v.2.16 (http://www.icrea.cat/Web/ScientificForm.aspx?key=255) (Pérez-Enciso and Misztal 2004). Chromosome- and genome-wide significance thresholds for $P=0.05$ and for $P=0.01$ were estimated following the method proposed by Nezer et al. (2002). The 95 % confidence intervals were calculated according to Mangin et al. (1994).

In both methods, according to a chromosome-wide level, QTL were considered suggestive when the significance was between 5 and 1 % and significant when it was below 1 %. According to a genome-wide level, QTL were considered significant when the significance was between 5 and 1 % and highly significant when it was below 1 % (Rodríguez-Ramilo et al. 2011; Vallejo et al. 2014). No fixed factor or covariates were included in the model.

The QTL effect, in terms of the percentage of variance explained (PVE) from the linear regression analysis, was estimated, according to Knott et al. (1996), by using the difference between the full and the reduced models as follows:

$$PVE_{HS}(\%) = 4 \times ((RMS\,red - RMS\,full)/RMS\,red) \times 100$$

$$PVE_{FS}(\%) = 2 \times ((RMS\,red - RMS\,full)/RMS\,red) \times 100$$

where HS is half-sibling analysis, FS is full-sibling analysis, RMS red is the residual mean square from the reduced model in which the QTL effect is excluded, and RMS full is the residual mean square from the model in which the QTL effect is fitted.

The QTL effect, in terms of the QTL heritability from variance component analysis (VCA) in the linear mixed model was estimated by using the *Qxpak* software v.2.16, and was expressed as percentage.

The magnitude of the QTL effect (PVE/VCA) was established, by following the classification of Massault et al. (2011), as small effect when the value was lower than 5 % and large effect when it was higher than 10 %, additionally, other magnitudes were included in the classification: medium effect when the value was between 5 and 10 % and extremely large effect when it was higher than 35 %.

Genotypic Association Analysis

In order to determine the association between phenotype (normal fish vs. LSK-deformed fish) and alleles from markers close to QTL, contingency tables and Pearson chi-square tests were performed by using the statistical software package SPSS (PASW Statistics v18). Alleles were codified to

discriminate between those coming from the sire (s1 and s2) and those coming from the dam (d1 and d2). The allelic segregation was tested separately, from the sires and from the dams, for each marker. The association was considered significant when $P \leq 0.05$.

Results

Genotyping

Microsatellite markers (106) were genotyped, and there were several no informative markers in each family because either the breeders were homozygous or null alleles were observed. The number of informative microsatellite markers per family, the map length covered, the average distance between microsatellite markers and the average number of microsatellite markers per linkage group are shown in Table 2.

QTL Mapping

A full-sibling regression analysis was carried out for FAM1 to FAM5, and a half-sibling regression analysis was carried out for families from ♂1L and ♂2L (paternal half-sibling) and families from ♀1L, ♀2L, and ♀3L (maternal half-sibling). A linear mixed model analysis was carried out also for FAM1 to FAM5, but also taking into account half-sibling family structure.

Eighteen QTL for LSK complex deformity were detected. Their positions, confidence intervals, and significances are shown in Table 3.

Four of these QTL (named QTLSK3, QTLSK6, QTLSK12, and QTLSK14) were significant in, at least, three of the analyses and showed significance also at genome-wide level in, at least, two analyses. Their percentages of variance explained (PVE) and their percentages of heritability from variance component analysis (VCA) by the QTL effect are shown in Table 4. Their plots with their LR (ratio of the likelihood of the data under the alternative hypothesis that assumes the existence of a QTL and the likelihood under the null hypothesis of no QTL) for each position and LG are shown in Fig. 1.

Genotypic Association Analysis

In LG where the QTLSK3, QTLSK6, QTLSK12, or QTLSK14 were localized, all microsatellite markers were analyzed, although only data from those that were closer to each QTL are shown. Microsatellite markers and their allelic significant association for breeders are shown in Table 4.

Markers BId-39-T (28.9 cm) and P96 (40.1 cm) were the closest-to-QTLSK3 markers, and a significant association between the allelic segregation of a dam and its LSK-deformed offspring was found. All the LSK-deformed individuals showed the alleles 74 for BId-39-T and 78 for P96, while none of the normal individuals showed these alleles. In LG8, the closest-to-QTLSK6 marker was DId-22-F (37.7 cm), which showed a significant association between the allelic segregation of a dam and its LSK-deformed offspring ($P=0.05$). Only one microsatellite marker was close to QTLSK12, Gt57 (0 cm). Significant Pearson chi-square test revealed a relationship between the allelic segregation of a sire and a dam and their LSK-deformed offspring ($P<5 \times 10^{-4}$ and $P \leq 4 \times 10^{-3}$, respectively). For this marker, LSK-deformed individuals had inherited the allele 150, while normal individuals did not show this allele. The closest-to-QTLSK14 microsatellite markers were DId-03-T and Bt-14-F (1.3 cm). For DId-03-T, the use of contingence tables and a significant Pearson X^2 test showed a high association between breeder's allelic segregation for this marker and LSK-deformed offspring in sires ($P \leq 4 \times 10^{-5}$) or in dams ($P \leq 0.01$). All the individuals showing this deformity had inherited the allele 126, while none of the normal fish had inherited this allele. Similar results were obtained for Bt-14-F, as a high significant association between LSK complex and allelic segregation of sires ($P \leq 0.01$) or dams ($P<5 \times 10^{-3}$) was observed. In this case, all the LSK-deformed offspring showed the allele 110, which did not appear in normal individuals.

Discussion

The development of new genetic technologies has significantly increased in the last decades. From the emergence of the molecular-based knowledge as a useful tool in aquaculture genetic improvement, genetic technologies have gained importance and they will become increasingly important as aquaculture further develops (Dunham 2014). In the present study, genotyping of all the analyzed families was conducted by using 106 microsatellite markers from 13 multiplex PCRs with redesigned primer sets (Negrín-Báez et al. 2015b), located in the linkage map for this species (Franch et al. 2006;

Table 2 QTL mapping information in full-sibling analysis

Family	NM[a]	ML[b]	MD[c]	MM[d]
FAM1	95	936.7	15	3.8
FAM2	96	886.2	13.8	4
FAM3	94	932.5	15	3.8
FAM4	94	952.4	14.9	3.9
FAM5	94	936.9	15.1	3.9

[a] Number of microsatellite markers used per family

[b] Map length covered in centimeters

[c] Average distance between microsatellite markers in centimeters

[d] Average number of microsatellite markers per linkage group

Table 3 Position and confidence interval (in brackets) in centimeters for each detected QTL

QTL	LG[a]	Analysis[b]			
		FS	pHS	mHS	LMM
QTLSK1	1			0 (0–16)*	3 (3–8)*
QTLSK2	3				93 (92–94)**
QTLSK3	5	39 (11–45)**[+]		17 (0–47)**	5 (0–11)**[++]
QTLSK4	6			28 (6–48)*	34 (11–38)*
QTLSK5	7			13 (0–56)**	0 (0–1)**[+]
QTLSK6	8	29 (10–36)*	34 (17–39)**[+]	26 (3–39)**	34 (4–35)**[+]
QTLSK7	10			46 (0–46)*	46 (45–46)**[+]
QTLSK8	11			0 (0–51)*	37 (36–38)**
QTLSK9	12	94 (46–94)**			94 (93–94)*
QTLSK10	14				0 (0–3)**[+]
QTLSK11	15		54 (45–54)*		54 (48–54)*
QTLSK12	17	0 (0–20)**[++]	0 (0–17)**[++]	7 (0–129)*	0 (0–4)**[++]
QTLSK13	18		8 (0–28)*		30 (22–30)*
QTLSK14	20	2 (1–4)**[++]	0 (0–10)**[++]	2 (1–4)**[++]	2 (1–2)**[++]
QTLSK15	22				0 (0–0)**[+]
QTLSK16	23			0 (0–2)**	0 (0–2)**
QTLSK17	24				0 (0–3)**[+]
QTLSK18	25		4 (1–37)*		42 (40–42)*

*$P \leq 0.05$ at chromosome level (suggestive QTL); **$P \leq 0.01$ at chromosome level (significant QTL); [+] $P \leq 0.05$ at genome level (significant QTL); [++] $P \leq 0.01$ at genome level (highly significant QTL)

[a] Linkage group

[b] Type of analysis: full-sibling (FS), paternal half-sibling (pHS), maternal half-sibling (mHS), linear mixed model (LMM)

Senger et al. 2006). The use of multiplex PCR allows the automation of the genotyping process and entails significant reduction of costs and minimization of errors. In the present study, an average of 74.8 % (928.9 cm) of total length of the genetic linkage map (1241.9 cm) of gilthead seabream has been covered. In other studies, the covered length was significantly lower, ranging from 472 cm (Boulton et al. 2011) to 495.4 cm (Loukovitis et al. 2012). At the same time, average distance between the microsatellite markers was 14.8 cm, which is lower than the recommended maximum distance of

Table 4 QTL effect for LSK complex

QTL	LG[a]	PVE[b]		VCA[c]	Marker	$X^2(\male)$[d]	$X^2(\female)$[d]
		FS	HS				
QTLSK3	5	7.2	43.6	66.8	BId-39-T	(−)	$\female 3_L$ ($P < 2 \times 10^{-3}$)
					P96	(−)	$\female 3_L$ ($P < 6 \times 10^{-3}$)
QTLSK6	8	2.5	60.0	65.2	Did-22-F	(−)	$\female 1_L$ ($P = 0.05$)
QTLSK12	17	36.8	100.8	86.7	Gt57	$\male 2_L$ ($P < 5 \times 10^{-4}$)	$\female 2_L$ ($P < 4 \times 10^{-3}$)
QTLSK14	20	98.7	220.5	93.6	Did-03-T	$\male 2_L$ ($P < 4 \times 10^{-5}$)	$\female 1_L$ ($P < 0.01$)
							$\female 2_L$ ($P < 5 \times 10^{-8}$)
					Bt-14-F	$\male 1_L$ ($P < 0.01$)	$\female 3_L$ ($P < 5 \times 10^{-3}$)
						$\male 2_L$ ($P < 8 \times 10^{-6}$)	

[a] Linkage group

[b] Percentage of variance explained of QTL effect in each analysis; full-sibling (FS) an half-sibling (HS) analysis

[c] Percentage of variance explained of QTL effect refers to QTL heritability from variance component analysis in a linear mixed model

[d] Pearson chi-square test for allelic association analysis in sire and dams for the closest microsatellite markers. Significance when $P \leq 0.05$; (−) no significance
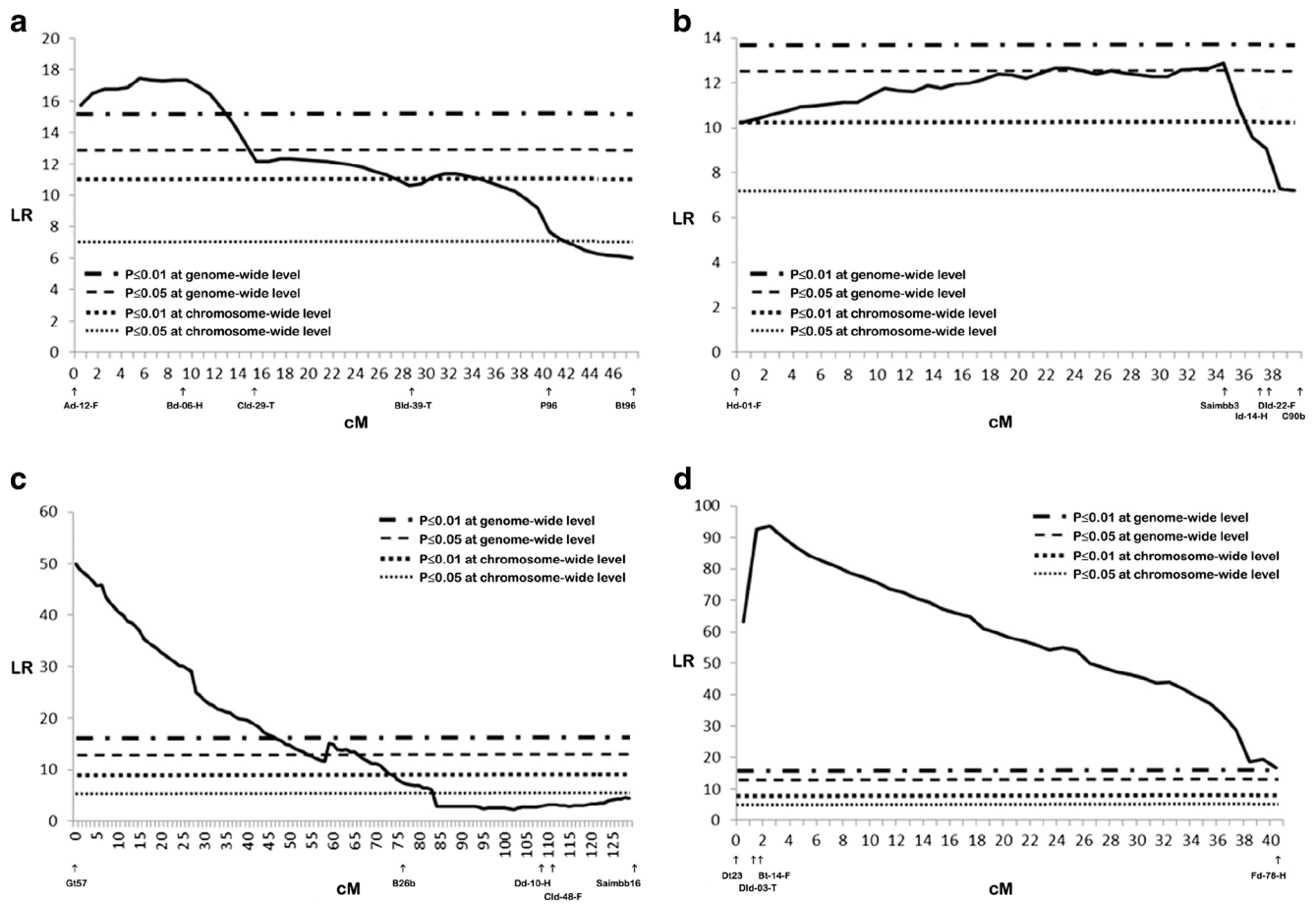
Fig. 1 Mapping for each solid QTL for LSK complex by linear mixed model analysis: QTLSK3 at LG5 (**a**), QTLSK6 at LG8 (**b**), QTLSK12 at LG17 (**c**), and QTLSK14 at LG20 (**d**). LR: ratio of the likelihood of the data under the alternative hypothesis that assumes the existence of a QTL and the likelihood under the null hypothesis of no QTL at each position of the LG

20 cm for QTL searching (Massault et al. 2008), and larger than in other QTL searching studies for this species (Boulton et al. 2011; Loukovitis et al. 2011, 2012, 2013).

In this study, for the first time in gilthead seabream, 18 QTLs for LSK complex deformity have been identified. These QTLs support the hypothesis of a genetic origin for LSK complex deformity, previously proposed by Afonso et al. (2000) and Negrín-Báez et al. (2015a), who found significant statistical associations between the prevalence of this deformity and certain specific families in gilthead seabream.

Two different methodologies for QTL detection were evaluated in the present study; LR and LMM methods. Three analyses using the LR method were performed taking into account the family structure. However, sample and the family size were reduced in this study, and consequently a family by family analysis could have a reduced accuracy. Despite this limitation, it is remarkable that significant QTL were detected. This can be explained because the five analyzed families were from a larger experiment in which only six out of 89 families contained the total incidence of LSK-deformed individuals and a significant relationship between breeders or families and their LSK-deformed offspring was obtained (Negrín-

Báez et al. 2015a). The comparison of LR analyses allowed also identifying the most solid QTL (QTLSK3, 6, 12, and 14), which were significant in, at least, two of the analyses at a chromosome level. It is remarkable that these four QTL also reached a genome-wide level of significance in at least one of the analyses. The LMM method provides a flexible and effective modeling tool, using all markers and pedigree information jointly (Pérez-Enciso and Misztal 2004), which seems to be more suitable than LR method given the family structure of the data analyzed in this study. This method can analyze simultaneously these five families and also can take family structure into account, improving the detection power. Using this method, more QTL have been localized and their confidence intervals have been narrowed. The major effect QTL were also the QTLSK3, 6, 12, and 14, being significant also at the genome-wide level, supporting the results from the LR analysis.

The percentage of phenotypic variance explained (PVE) from the linear regression analyses showed an extremely large effect in the half-sibling analysis, and also for QTLSK12 and QTLSK14 in the full-sibling analysis. Actually, PVE values of these two QTL in the half-sibling analysis were over 100 %.

This is a common result in QTL mapping studies, as PVE tends to be overestimated (Wang et al. 2006). It can be explained by the Beavis effect (Xu 2003), which explains that the detected QTL effect is overestimated because QTL identification requires a genetic effect estimation and a statistical analysis in a shortened distribution. The overestimated QTL effect value is higher when the family size is small as in this study. On the other hand, given the reduced number of individuals and the family structure of this study, a linear mixed model can provide more accurate estimates of QTL effects, in terms of the QTL heritability from variance component analysis (VCA) (Rowe et al. 2006). VCA has the advantage of direct application to a pedigree regardless of family structure compared with PVE. In general, when a pedigree structure is available with half-sibling and full-sibling families, using of VCA is beneficial to detect alleles that are segregating differently in sires and dams and is not to be overestimated (Rowe et al. 2006). In fact, in this study, the effect of these four solid QTL according to their VCA values seems to be closer to the real magnitude, as these percentages do not exceed 100 %. The finding of a large number of QTL suggests that LSK complex deformity is influenced by a complex interaction between several regions of the genome, which was expected since this deformity consists of three different deformities. However, the results of this study also suggest that LSK complex deformity might be controlled mainly by these four large effect QTL (solid QTL).

A highly significant association between phenotype and genotype in solid QTL was observed. Indeed, QTL closer markers seem to be linked with LSK complex, so all LSK-deformed descendants of, at least, one breeder showed determined alleles, while normal individuals did not show these alleles, and all LSK-deformed descendants of, at least, one full-sibling family showed the determined genotypes, while normal individuals did not show them. This strong linkage between these solid QTL and their markers may have repercussions, like the linkage between the marker Id13 and a QTL for pasteurellosis resistance found by Massault et al. (2010), in which all the descendants of a full-sibling family that showed a determined allele died.

The minimum annual loss estimated for European aquaculture due to deformities is higher than 50 M€/year, so a 50 % reduction of deformities could save 25 M€/year (Hough 2009). LSK complex is a severe column deformity that is presented with a low prevalence in gilthead seabream fingerlings and it is associated with high mortality (Negrín-Báez et al. 2015a). Therefore, this deformity is a minor problem for aquaculture producers, so it has not been traditionally considered in deformities studies (Afonso et al. 2000; Ebrahimnezhad et al. 2009; Negrín-Báez et al. 2015a). However, lordosis and vertebral fusion are the most frequent deformities affecting vertebral column in gilthead seabream (Boglione et al. 2013), which are the major problems for

industry as they entail significant economic losses. A possible genetic link between these two deformities and LSK complex has been reported by Negrín-Báez et al. (2015a). In that study, breeders responsible for all LSK-deformed descendants of a commercial batch were also responsible for a large number of descendants with those deformities, when analyzed at commercial size. This means that if these breeders have not been selected in that batch, the prevalence of these deformities would have decreased by 17 %. Furthermore, this batch was sorted according to deformities at fingerling size with the consequent cost money, so if the batch had not been sorted, the decrease would have been greater, and even the sorting could have been avoided. Lordosis and vertebral fusion are deformities that are hardly diagnosed at early ages, can appear throughout the development of the fish, and even at commercial size the fish may appear normal but after filleted deformities are evident, resulting in consumer dissatisfaction (Negrín-Báez et al. 2015a). For this reason, these deformities are difficult to incorporate into a genetic program. However, the LSK complex is easily diagnosed at early ages and never appears after fingerling size. All this indicates that, if the LSK complex was genetically and negatively selected, the incidence of lordosis and vertebral fusion would be reduced, which would be of great economic importance for industry. In this way, marker-assisted selection (MAS) is a powerful strategy that can help to increase breeding program gains. Two cases of MAS-including programs have been successfully applied for diseases resistance in aquaculture species like Japanese flounder and Atlantic salmon (Ozaki et al. 2012), thus validating this methodology. In gilthead seabream, the project PROGENSA® has developed, at Spanish national scale, a genetic breeding program to improve growth traits (Afonso et al. 2012), where MAS selection would be implemented. In this study, in three solid QTL (QTLSK3, QTLSK6, and QTLSK12), the 95 % confidence intervals were large in LR analyses. This could have a negative effect in MAS application since it implicates that marker–QTL linkage phases are not stable over generation or population. So, the LSK-linked markers should be verified in other families to provide a reliable tool for MAS. However, these three QTL represent a major step in the location of genes that determine the presence of skeletal deformities in this species and focus the search in these three areas. With respect to QTLSK14, it showed a short 95 % confidence interval in both methods (4 or 10 cm in LR analyses and 2 cm in LMM), and a large effect, which highlights the importance of considering this QTL in a MAS selection program.

As the next step, it is proposed to perform a second more comprehensive screening in order to narrow the QTL position and to search candidate genes or markers linked to this deformity. This could be carried out through a study of comparative genomics in another species genetically close, as the complete genome of gilthead seabream is not yet available. As well as in

guppy (*Poecilia reticulata*), a major QTL for scoliosis was associated with MTNR1B, a candidate gene for human idiopathic scoliosis (Gorman et al. 2011). Given all this, the results of the present study represent a unique opportunity to study the etiology of skeletal deformities in gilthead seabream. Not only about the LSK complex but also about other deformities, as besides this deformity seems to be genetically linked to others.

In summary, the results obtained in this study confirm the genetic origin of this severe skeletal deformity and show four solid QTL associated with LSK complex, which should be verified in other families. The strongest of them (QTLSK12) presented a short confidence interval, an extremely large effect, and two markers (DId-03-T and Bt-14-F) as potential candidates to be included in a MAS selection for this species. Genetically selecting the appropriate breeders for non-LSK-deformed individuals would decrease the incidence of this deformity in hatcheries and, at the same time, could also decrease the incidence of other frequent skeletal deformities (lordosis and vertebral fusion), thus, increasing company gains. Moreover, these QTL represent the first step to research the etiology of skeletal deformities in this species.

References

Afonso JM, Roo FJ (2007) Anomalías morfológicas en peces cultivados: heredabilidad y selección. In: Espinosa J, Martínez P, Figueras A (eds) Genética y genómica en acuicultura. Consejo Superior De Investigaciones Científicas, Madrid, pp 215–240

Afonso JM, Montero D, Robaina L, Astorga N, Izquierdo MS, Ginés R (2000) Association of a lordosis-scoliosis-kyphosis deformity in gilthead seabream (*Sparus aurata*) with family structure. Fish Physiol Biochem 22:159–163

Afonso JM, Manchado M, Estévez A, Ramis G, Lee-Montero I, Ponce M, Sánchez JA, Armero E, Navarro A, Puertas MA, Borrell Y, García M, Negrín-Báez D, Crespo AM, Blanco G, Mariadolores E, Sánchez JJ, Martín N, Aguilera C, Roo FJ, Zamorano MJ and Toro MA (2012) PROGENSA®: development of a genetic improvement program in gilthead sea bream Sparus aurata L between industry and research centers in Spain. In: Proceedings of AQUA 2012, September 1–5, Prague, Czech Republic

Bardon A, Vandeputte M, Dupont-Nivet M, Chavanne H, Haffray P, Vergnet A, Chatain B (2009) What is the heritable component of spinal deformities in the European seabass (*Dicentrarchus labrax*)? Aquaculture 294:194–201

Boglione C, Gisbert E, Gavaia P, Witten PE, Moren M, Fontagné S, Koumoundouros G (2013) Skeletal anomalies in reared European fish larvae and juveniles. Part 2: main typologies, occurrences and causative factors. Rev Aquac 5(suppl 1):S121–S167

Boulton K, Tsigenopoulos CS, Massault C, Houston RD, de Koning DJ, Haley CS, Bovenhuis H, Batargias C, Canario AVM, Kotoulas G (2011) QTL affecting morphometric traits and stress response in the gilthead seabream (*Sparus aurata*). Aquaculture 319:58–66

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Divanach P, Boglione C, Menu B, Koumoundourus G, Kentouri M, Cataudella S (1996) Abnormalities in finfish mariculture: an overview of the problem, causes and solutions. In: Chatain B, Saroglia M, Sweetan J, Lavens P (eds) International workshop on seabass and seabream culture: problems and prospects. European Aquaculture Society, Oostende, pp 45–66

Doerge RW (2002) Mapping and analysis of quantitative trait loci in experimental populations. Nat Rev Genet 3:43–52

Dunham (2014) Introduction to genetics in aquaculture XI: the past, present and future of aquaculture genetics. Aquaculture 420–421:S1–S2

Ebrahimnezhad H, Modarres Mousavi M, Tabatabaei Naeini A, Bozorgi H (2009) Coincidence of lordosis, kyphosis and scoliosis syndrome (LSK) in parrot cichlid (*Cichlasoma*). In: Proceedings of 1st International Congress on Aquatic Animal Health Management and Diseases. Tehran (Iran), abstract 1296

Franch R, Louro B, Tsalavouta M, Chatziplis D, Tsigenopoulos CS, Sarropoulou E, Antonello J, Magoulas A, Mylonas CC, Babbucci M, Patarnello T, Power DM, Kotoulas G, Bargelloni L (2006) A genetic linkage map of the hermaphrodite teleost fish *Sparus aurata* L. Genetics 174:851–861

Gorman KF, Christians JK, Parent J, Ahmadi R, Weigel D, Dreyer C, Breden F (2011) A major QTL controls susceptibility to spinal curvature in the curveback guppy. BMC Genet 12:16

Hough C (2009) Improving the sustainability of European fish aquaculture by the control of malformations. Ghent University, Belgium

Knott SA, Elsen JM, Haley CS (1996) Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor Appl Genet 93:71–80

Lee-Montero I, Navarro A, Borrell Y, García-Celdran M, Martín N, Negrín-Báez D, Sánchez JA, Armero E, Berbel C, Zamorano MJ, Sánchez J, Estévez A, Ramis G, Manchado M, Afonso JM (2013) Development of the first standardized panel of two new microsatellites multiplex PCRs for gilthead seabream (*Sparus aurata* L.). Anim Genet 44:533–546

Loukovitis D, Sarropoulou E, Tsigenopoulos CS, Batargias C, Magoulas A, Apostolidis AP, Chatziplis D, Kotoulas G (2011) Quantitative trait loci involved in sex determination and body growth in the gilthead sea bream (*Sparus aurata* L.) through targeted genome scan. PLoS One 6:e16599

Loukovitis D, Sarropoulou E, Batargias C, Apostolidis A, Kotoulas G, Tsigenopoulos C, Chatziplis D (2012) Quantitative trait loci for body growth and sex determination in the hermaphrodite teleost fish *Sparus aurata* L. Anim Genet 43:753–759

Loukovitis D, Batargias C, Sarropoulou E, Apostolidis AP, Kotoulas G, Magoulas A, Tsigenopoulos CS, Chatziplis D (2013) Quantitative trait loci affecting morphology traits in gilthead seabream (*Sparus aurata* L.). Anim Genet 44:480–483

Mangin B, Goffinet B, Rebai A (1994) Constructing confidence intervals for QTL location. Genetics 138:1301–1308

Massault C, Bovenhuis H, Haley CS, Koning DJ (2008) QTL mapping designs for aquaculture. Aquaculture 285:1–4

Massault C, Hellemans B, Louro B, Batargias C, Van Houdt JKJ, Canario A, Volckaert FAM, Bovenhuis H, Haley C, de Koning DJ (2010) QTL for body weight, morphometric traits and stress response in European seabass *Dicentrarchus labrax*. Anim Genet 41:337–345

Massault C, Franch R, Haley C, De Koning DJ, Bovenhuis H, Pellizzari C, Patarnello T, Bargelloni L (2011) Quantitative trait loci for resistance to fish pasteurellosis in gilthead sea bream (*Sparus aurata*). Anim Genet 42:191–203

Navarro A, Badilla R, Zamorano MJ, Pasamontes V, Hildebrandt S, Sánchez JJ, Afonso JM (2008) Development of two new microsatellite multiplex PCRs for three sparid species: Gilthead seabream (*Sparus auratus* L.), red porgy (*Pagrus pagrus* L.) and redbanded seabream (*P. auriga*, Valenciennes, 1843) and their application to paternity studies. Aquaculture 285:30–37

Negrín-Báez D, Navarro A, Lee-Montero I, Soula M, Afonso JM, Zamorano MJ (2015a) Inheritance of skeletal deformities in gilthead seabream (*Sparus aurata*) –lack of operculum, lordosis, vertebral fusion, and LSK complex. J Anim Sci 93:53–61

Negrín-Báez D, Navarro A, Lee-Montero I, Afonso JM, Sánchez JJ, Elalfy IS, Manchado M, Sánchez JA, García-Celdrán M, Zamorano MJ (2015b) A set of 13 multiplex PCRs of specific microsatellite markers as a tool for QTL detection in gilthead seabream (*Sparus aurata* L.). Aquac Res 46(suppl 1):45–58

Nezer C, Moreau L, Wagenaar D, Georges M (2002) Results of a whole genome scan targeting QTL for growth and carcass traits in a Piétrain x Large White intercross. Genet Sel Evol 34:371–387

Ozaki A, Araki K, Okamoto H, Okauchi M, Mushiake K, Yoshida K, Tsuzaki T, Fuji K, Sakamoto T, Okamoto N (2012) Progress of DNA marker-assisted breeding in maricultured finfish. Bull Fish Res Agen (Jpn) 35:31–37

Pérez-Enciso M, Misztal I (2004) Qxpak: a versatile mixed model application for genetical genomics and QTL analyses. Bioinformatics 20:2792–2798

Prestinicola L, Boglione C, Makridis P, Spanò A, Rimatori V, Palamara E, Scardi M, Cataudella S (2013) Environmental conditioning of skeletal anomalies typology and frequency in gilthead seabream (*Sparus aurata* L.) juveniles. PLoS One 8:e55736

Rodríguez-Ramilo ST, Toro MA, Bouza C, Hermida M, Pardo BG, Cabaleiro S, Martínez P, Fernández J (2011) QTL detection for Aeromonas salmonicida resistance related traits in turbot (*Scophthalmus maximus*). BMC Genomics 12:541

Rodríguez-Ramilo ST, Martínez P, Fernández J, De La Herrán R, Ruiz-Rejón C, Hermida M, Fernández C, Pereiro P, Figueras A, Bouza C, Toro MA (2014) Identification of quantitative trait loci associated with resistance to viral haemorrhagic septicaemia (VHS) in turbot (*Scophthalmus maximus*): a comparison between bacterium, parasite and virus diseases. Mar Biotechnol 16:265–276

Rowe SJ, Windsor D, Haley CS, Burt DW, Hocking PM, Griffin H, Vincent J, De Koning DJ (2006) QTL analysis of body weight and conformation score in commercial broiler chickens using variance component and half-sib analyses. Anim Genet 37:269–272

Seaton G, Hernandez J, Grunchec JA, White I, Allen J, de Koning DJ, Wei W, Berry D, Haley C, Knott S (2006) *GridQTL*: a grid portal for QTL mapping of compute intensive datasets. In: Proceedings of the 8th World Congress on Genetics Applied to Livestock Production. August 13–18, Belo Horizonte

Senger F, Priat C, Hitte C, Sarropoulou E, Franch R, Geisler R, Bargelloni L, Power D, Galibert F (2006) The first radiation hybrid map of a perch-like fish: the gilthead seabream (*Sparus aurata* L). Genomics 87:793–800

Vallejo RL, Palti Y, Liu S, Evenhuis JP, Gao G, Rexroad CE III, Wiens GD (2014) Detection of QTL in rainbow trout affecting survival when challenged with *Flavobacterium psychrophilum*. Mar Biotechnol 16:349–360

Visscher PM, Thompson R, Haley CS (1996) Confidence intervals in QTL mapping by bootstrapping. Genetics 143:1013–1020

Wang CM, Lo LC, Zhu ZY, Yue GH (2006) A genome scan for quantitative trait loci affecting growth-related traits in an F1 family of Asian seabass (*Lates calcarifer*). BMC Genomics 7:274

Xu S (2003) Theoretical basis of the Beavis effect. Genetics 165:2259–2268

Yue GH (2014) Recent advances of genome mapping and marker-assisted selection in aquaculture. Fish Fish 15:376–396