

Detection Of Pleiotropic QTL Related To Protein Expression And Foie Gras Quality Traits

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ABSTRACT: The aim is to identify proteomic QTL and their relationships with quality traits in a backcross design optimized for foie gras quality traits detection. Five quality phenotypes and 326 protein spots expression levels were recorded on the fatty livers of 294 mule ducks. Eight QTL and 23 pQTL were identified at least at 5% chromosome wide significance on 2 linkage groups. Eight proteins were identified and were associated with phenotypes to perform multi-trait QTL detections. All the 32 combinations (20 on LG9 and 12 on LG2c) showed a multi-trait QTL at least at 5% chromosome wide significance and 14 out of the 32 combinations showed a pleiotropic QTL. Among them, 9 QTL with a 0.5% chromosome wide significance concerned TPI and ENO1, protein involved in glycolysis. These results suggest the linkage group 2c contains a gene involved in the lipid and/or glucose metabolism.

Keywords: Foie gras; Proteomic; pQTL; Quality

Introduction

In France, 95% of foie gras is produced by mule ducks, which are interspecific hybrids issued from the cross between a common female duck (*Anas Platyrhynchos*) and a Muscovy drake (*Cairina Moschata*). The main objective of the industry is to improve the foie gras quality. A first study for estimating the influence of the duck genome expression on foie gras quality traits identified several QTL (Kileh-Wais *et al.* (2013)). We now propose to go further by exploring the influence of the protein expression levels. The purpose is to identify proteomic QTL (pQTL) influencing liver protein expression and to find pleiotropic QTL between phenotypes and proteins.

Material and methods

So as to reduce the number of animals to analyze by proteomics, our pQTL backcross design was a reduction of the one used for all mule ducks traits (Kileh-Wais *et al.* (2013)). This cross was based on 7 F1 families with a total of 382 back-cross (BC) female common ducks. These BC females were progeny-tested by measuring traits in their mule duck sons and genotyped with 102 microsatellite markers associated in 17 linkage groups (LG) for QTL detections. The pQTL design focused on 3 of these families for which major QTL for liver quality traits had been detected (François *et al.* (2013)).

Animal Design. This optimized design is thus composed of 3 F1 sires, 98 BC pekin females and 294 grand-sire mule ducks (figure 1). The 3 F1 drakes are products of a cross between 2 common duck parental

lines (the heavy pekin INRA37 and the light Kaiya INRA444). These 3 F1 sires were backcrossed to 20 INRA444 female common ducks to produce BC female common ducks. To estimate their values, these BC were crossed with 23 Muscovy drakes to produce the mule ducks. To minimize proteomics work and to keep the design balanced, exactly 3 mule ducks sons per dam were kept for analysis, that is to say a total of 294 animals. Mule ducks were bred from 0 to 12 weeks of age, followed by an overfeeding period of 12 days. After slaughter, liver samples were collected and numerous liver phenotypic traits were measured.

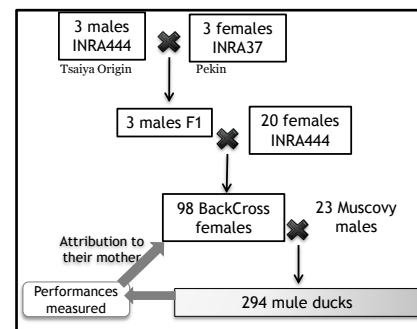


Figure 1: Backcross design and offspring testing for pQTL detections in the common duck

Genotyping and genetic map. All the common ducks of this design had been genotyped with 91 microsatellites markers (Kileh-Wais *et al.* (2013)). Only the 2 linkage groups - LG2c and LG9 - for which interesting traits segregating in the 3 families considered here, could be analyzed (figure 2).

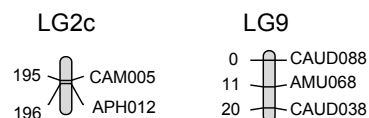


Figure 2: Microsatellite genetic map of the 2 linkage groups studied

Data recording. Five liver quality traits were measured on the 294 duck livers: the liver weight (LW), the melting rate (MR), corresponding to the fat loss during cooking, the protein and lipid percent in dry liver (LprotDc and LlipDc) and the liver redness (La). Liver soluble proteins were extracted from liver samples and separated using bi-dimensional gel electrophoresis. Computational analyzes of the 326 protein spots present on the gels produced level values for each spot and each 294 sample.

QTL detections. QTL and pQTL detections were performed on the 98 females BC from the opti-

mized design, their performances being the mean value of that of their sons. Linkage analysis was performed wBotstein (1989)) with the QTLMap software (Elsen *et al.* (1999)) and the empirical chromosome-wide significance (CWS) level was estimated with 1,000 within-family permutations. First, single trait QTL and pQTL detections were carried out for the liver quality traits and for each of the 326 protein spots, respectively. Detections of multi-trait QTL (mQTL) were then performed by combining each pQTL with each of the significant quality phenotype QTL, in order to identify pleiotropic QTL.

Results

Single trait QTL. A total of 8 QTL - 3 on LG2c and 5 on LG9 - significant at 5% CWS and related to foie gras quality traits, were identified (table 1), confirming the results of Kileh-Wais *et al.* (2013). The 3 strongest QTL were identified on LG9 for LprotDc (1% CWS) and LW and LlipDc (0.5% CWS). Twenty-three pQTL - 15 on LG2c and 8 on LG9 - significant at 5% CWS, were detected for 22 out of the 326 spots tested (figure 3). As most of the 326 protein spots present on the gel have not yet been identified, only 8 out of the 22 significant pQTL are known accurately to date and we will focus analyses on them (table 1). Each LG counted 4 QTL related to the following proteins: Peroxiredoxin (PRDX6), Triose Phosphate Isomerase (TPI), destrin (DSTN), and alpha-enolase (ENO1 (1)) on LG2c and alpha-enolase (ENO1 (2) and (3)), NADH dehydrogenase iron-sulfur protein 3 (NDUFS3) and Proteasome Subunit alpha 1 (PSMA1) on LG9. The most significant pQTL was for TPI on LG2c (0.5% CWS).

Multi-traits QTL. For each LG, each pQTL was combined to each of the phenotypes presenting significant QTL. Twenty combinations (4 pQTL x 5 phenotypes) were tested for LG9 and 12 (4pQTL x 3 phenotypes) for LG2c. These all gave rise to significant mQTL at 5% CWS. The association of two traits never decrease the mQTL p-value compared to single QTL or pQTL. For 14 combinations the threshold reached by the mQTL is higher than the best of the 2 single QTL/pQTL (table 2) *i.e.* on LG9, the association between PSMA1 and La gives a very significant mQTL at 0.5% CWS, whereas both single QTL and pQTL only reached 5% CWS. On LG2c, the association between LlipDc and the proteins gave rise to 4 strong mQTL (0.5% CWS with PRDX6, DSTN and ENO1 and 0.1% CWS with TPI). On LG2c, all mQTL for ENO1 are stronger than the single QTL (0.5% CWS). On LG9, 4 mQTL for ENO1 follow the same trend (with MR and LprotDc).

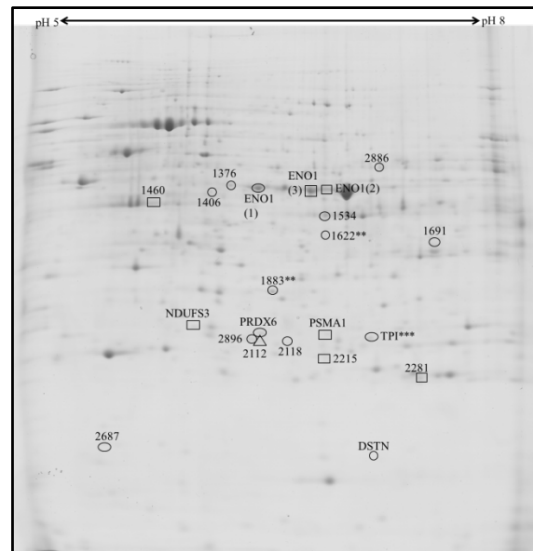


Figure 3: Two-dimensional gel electrophoresis representing a map of duck fatty liver soluble proteins. The 22 protein spots presenting a pQTL (at 5% Chromosome Wide Significance - CWS) for linkage groups 2c (circles) and 9 (squares) are represented. The triangle spot has a pQTL in both linkage groups. Stars next to spot numbers correspond to pQTL at 1% CWS () and 0.5% CWS (***). PRDX6 = Peroxiredoxin 6, TPI = TriosePhosphate Isomerase, DSTN = Destrin, ENO1 = α -enolase, NDUFS3=NADH dehydrogenase iron-sulfur protein 3, PSMA1 = Proteasome subunit alpha.**

Discussion

LG2c and LG9 segregated for 15 QTL related to “foie gras” quality in the first study using all 7 backcross families (Kileh-Wais *et al.* (2013)). Our reduction to 3 families with 3 mule ducks per backcross common female duck still allowed us to detect 12 out of the 15 QTL. Moreover, 23 pQTL were also identified for 22 protein spots. The fact that these pQTL allow the detection of very significant mQTL when combined with LlipDc, MR or LprotDc, suggests that genes involved in or regulating the lipid and/or glucose metabolism reside in these locations, especially on L2c. On LG9 there is a clear pleiotropic effect between MR and ENO1 with both single pQTL and QTL only at 5% CWS and the mQTL reaching 0.5% CWS. Among the spots identified, TPI, ENO1 (both enzymes of the glycolysis) and PSMA1 (a subunit of the proteasome) had already been highlighted as linked to the variability of the melting rate (Theron *et al.* (2011)). Moreover, François *et al.* (submitted paper) showed that ENO1 and PRDX6 (a stress response protein) were linked to liver weight and to liver protein rate variability. Even if these results comfort our hypothesis, the mQTL between DSTN (involved in actin de-polymerization) and LlipDc on LG9 bring a new hypothesis on a link between LlipDc and the hepatocyte structure.

Conclusion

The protein spots identification to come will probably help in indicating the pathways concerned by the QTL. Moreover, our LG are small (1cM for LG2c and 20 cM for LG9) so the QTL positions are not accu-

Table 1: LK1 and threshold reached by the pleiotropic QTL in each linkage group (LG)

MR=Melting rate, LlipDc=Dry liver lipid content, LprotDc=Dry liver protein content, La = Liver redness, LW = Liver Weight, PRDX6 = Peroxiredoxin 6, TPI = TriosePhosphate Isomerase, DSTN = Destrin, ENO1 = α -enolase, NDUFS3=NADH dehydrogenase iron-sulfur protein 3, PSMA1 = Proteasome subunit alpha. Stars next to the names of the traits indicate the significance of their single QTL or pQTL. All multi-trait QTL are significant at least at 5% Chromosome Wide Significance (CWS). Values presented correspond to the multi-trait QTL whose reached threshold is higher than the best of the 2 single QTL/pQTL. *: 5% CWS; **: 1% CWS; ***: 0.5% CWS; ****: 0.01 CWS.

LG2c					LG9				
TRAITS	PRDX6*	TPI***	DSTN*	ENO1* (1)	TRAITS	ENO1* (2)	ENO1* (3)	NDUFS3*	PSMA1*
					La*		15.85**		17.35***
					LW***				
MR*			15.95**	18.20***	MR*	17.36***	17.03**		
LlipDc*	16.78***	19.04****	18.44***	19.00***	LlipDc***				
LprotDc*			15.81**	16.79***	LprotDc**	17.70***	18.14***		

Table 2: QTL and pQTL identified on the linkage groups 2c and 9

LG	Traits	Threshold	Flanking Marker	Location (cM)	LRT	
2c	QTL	MR	5%	CAM005	0	8.53
		LlipDc	5%	CAM005	0	9.88
		LprotDc	5%	CAM005	0	7.39
	pQTL	DSTN	5%	CAM005	0	8.13
		PRDX6	5%	APH012	1	8.15
		ENO1 (spot1403)	5%	APH012	1	8.27
		TPI	0,5%	CAM005	0	14.09
9	QTL	La	5%	CAU088	0	8.76
		LW	0,5%	AMU068-CAU038	15	11.17
		MR	5%	CAU088	0	9.30
		LlipDc	0,5%	CAU088	0	11.56
		LprotDc	1%	CAU088	0	10.76
	pQTL	ENO1 (spot 1414)	5%	CAU038	20	9.71
		ENO1 (spot 1427)	5%	CAU038	20	8.58
		NDUFS3	5%	AMU068 et CAU038	11	9.01
		PSMA1	5%	AMU068 et CAU038	16	8.63

MR=Melting rate, LlipDc=Dry liver lipid content, LprotDc=Dry liver protein content, La = Liver redness, LW = Liver Weight, PRDX6 = Peroxiredoxin 6, TPI = TriosePhosphate Isomerase, DSTN = Destrin, ENO1 = α -enolase,

rate enough. In order to bring precision on the location, the same QTL, pQTL and mQTL detections are about to be performed on a genetic map composed of the these microsatellite markers and of SNP.

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