Kafkas Univ Vet Fak Derg
18 (2): 235-242, 2012
DOI:10.9775/kvfd.2011.5359

RESEARCH ARTICLE

eQTL Analysis and Association of *MYF6* mRNA Expression with Meat Quality Traits in Pigs

Mehmet Ulaş ÇINAR * Huitao FAN * Christiane NEUHOFF * Christine GROßE-BRINKHAUS *

* Institute of Animal Sciences, Animal Breeding and Husbandry Group, University of Bonn, 53115 Bonn - GERMANY

Makale Kodu (Article Code): KVFD-2011-5359

Summary

The aim of this research was to measure mRNA expression of porcine *MYF6* (myogenic factor 6, also known in the medical literature as herculin and myogenic regulatory factor 4, MRF4) and to perform association study with meat quality traits as well as to unravel the transcriptional regulation of this gene by expression QTL (eQTL) study. For this purpose, Duroc × Pietrain F2 resource population (DuPi; $n = 313$) were used for association and eQTL study. The mRNA levels in *Longissimus dorsi* muscle tissue of *MYF6* gene were evaluated by using qRT-PCR to identify association between gene expression and meat quality traits as well as to analyse eQTL. The mRNA expression of MYF6 associated with conductivity24L ($P<0.01$) and pH24L ($P<0.1$). Expression of *MYF6* gene was higher in animals with high pH and conductivity of muscle. Linkage analysis using GridQTL revealed 4 *trans*-regulated eQTL on four porcine autosomes. Significant eQTL [$P<0.01$, CW (chromosome-wide)] were found for *MYF6* on SSC2. A suggestive eQTL ($P<0.05$, CW) was identified on SSC8. These results revealed that gene expression of *MYF6* associated with the meat quality traits and this gene could be potential functional candidate gene for meat quality traits in pigs. However, the analysis of eQTL also suggested that additional genes encoding for transcription factors (TF) could be considered, via fine-mapping underlying the eQTL peaks, in order to understand interaction among these genes.

Keywords: *eQTL, Pig, MYF6, mRNA expression, Meat quality traits*

MYF6 mRNA İfadesinin Domuzlarda Et Kalitesi ile İlişkisi ve eQTL Analizi

Özet

Bu çalışmanın amacı literatürde herculin ve myogenic factor 4 *(MRF4)* olarak ta bilinen domuz *MYF6* (myogenic factor 6) geninin et kalite özellikleri ile ilişkisi belirlenmesi ve transkripsiyon seviyesinde gen ifadesinin kontrölünün QTL (eQTL) yöntemiyle incelenmesidir. Bu amaçla, Duroc × Pietrain F2 deneysel populasyonu (DuPi; $n = 313$) asosiyasyon ve eQTL analizleri icin kullanılmıştır. *MYF6* geninin mRNA düzeyleri *Longissimus dorsi* kas dokusu hücrelerinde asosiyasyon ve eQTL analizi icin qRT-PCR kullanılarak ölçülmüştür. *MYF6* genin mRNA düzeyi iletkenlik24L ($P<0.01$) ve pH24L ($P<0.1$) ile ilişkili bulunmuştur. *MYF6* ifade düzeyi iletkenliğin ve pH'nın yüksek olduğu kaslarda daha yüksek olarak bulunmuştur. GridQTL kullanılarak yapılan bağlantı analizinde dört domuz otozomal kromozomu üzerinde 4 *trans*-kontrollü eQTL tespit edilmiştir. SSC2 üzerinde *MYF6* ifadesi için istatistiki olarak önemli eQTL [$P<0.01$, KÇ (kromozomal çapta)] bulunmuştur. SSC8 üzerinde önerim düzeyinde bir eQTL tespit edilmiştir ($P<0.05$, KÇ). Bu sonuçlar *MYF6* ifadesinin et kalite özellikleri ile ilişkili olduğunu ve bu genin domuzlarda et kalitesinden sorumlu fonksiyonel bir aday gen olabileceğini göstermiştir. Ancak eQTL analizleri sonucunda, genler arasındaki etkileşimin anlaşılabilmesi için, detaylı haritalama çalışmaları ile eQTL bölgesindeki transkripsiyon faktörlerini (TF) kodlayan genlerin incelenmesi gerekmektedir.

Anahtar sözcükler: *eQTL, Domuz, MYF6, mRNA ifadesi, Et kalite kriterleri*

INTRODUCTION

A significant number of genes [1-4] and quantitative trait loci (QTL) (www.animalgenome.org/cgi-bin/QTLdb/SS) have been reported for meat and carcass quality traits in pig. The myogenic factor 6 (*MYF6*/myogenic regulatory factor 4, *MRF4*) gene codes for the basic helix-loop-helix (bHLH) transcription factor (TF) belonging to MyoD family [5]. Its expression accompanies the processes of differentiation and maturation of myotubes during embryogenesis and

İletişim (Correspondence)
+49 228 733583
ucin@itw.uni-bonn.de

continues on a relatively high level after birth, affecting the muscle phenotype [6]. The expressed *MYF6* is involved in the processes of differentiation and maturation of myotubes during embryogenesis and dominates quantitatively over the other myogenic regulatory factors (MRFs) in adult muscle [6-9]. Increases in MYF6 mRNA and protein may play a role in the differentiation of adult fibers [10]. This gene has 10-fold higher postnatal expression than the other genes of the MRF family [7]. The lack of active MYF6 protein causes an anomaly in the growth of muscle, known as myopathy. On the other hand, it was demonstrated that MYF6 also acts as a determining factor in the absence of myogenic factor 5 (*MYF5*) and myogenic differentiation 1 (*MYOD1*) during the onset of myogenesis in mice [11]. This is the main reason why *MYF6* is regarded as the principal factor influencing skeletal muscle phenotype [12]. Transcriptional regulation complexity of the genes encoding the myogenic regulatory factors has been reviewed in vertebrates and concluded as skeletal myogenesis is coordinated by the activation of the MRFs in response to signals that are interpreted by their associated regulatory elements in different precursor cells during development [13]. This explains the existence of numerous regulatory elements such as *cis*-, *trans*- or miRNAs at large distances to *MYF6* points make very complex pattern of the gene regulation with significant differences between species.

Growth rate and muscularity are economically important in pig breeding. The development of skeletal muscle depends on the number and size of muscle fibers. Because of its functions and supported by results of linkage studies, *MYF6* is considered as candidate gene for growth related traits in meat-producing animal species [14]. Wyszynska-Koko et al.[6] has shown the association between SNP in coding and promoter region of *MYF6* and production traits in pigs. Porcine *MYF6* was mapped to porcine chromosome 5 (SSC5) [15] where different QTLs for meat and carcass quality traits were identified in pigs (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index, August 2011). mRNA expression of *MYF6* was shown to be differentially regulated between different pig breeds due to genetic [16] or epigenetic factors [8]. Association between mRNA expression and meat quality traits were shown in pigs [17,18]. Moreover, expression variation of meat quality related genes enables us to identify the effects of QTL for gene expression called expression QTL (eQTL) [19]. However, to best of our knowledge, no study has been devoted to association between mRNA expression of *MYF6* and meat quality in pigs in a large animal population. Therefore, the aim of this work was to study *MYF6* through association and expression as well as eQTL study to prove its candidacy for meat quality traits.

MATERIAL and METHODS

Populations and Phenotypes

Genomic DNA and phenotypic data were obtained from a reciprocal cross of Duroc and Pietrain (DuPi, n = 313) F2. The structure and breeding of the DuPi population was described in a previous study [20]. All pigs were kept at the experimental research farm "Frankenforst", Institute of Animal Science, University of Bonn (Germany). All pigs were slaughtered in a commercial slaughter house. Meat quality traits analysed in this study cover indicators of water holding capacity including drip loss, thawing loss, cooking loss, pH at 45 min *post-mortem* (p.m.) in loin (pH_{1L}), pH at 24 h p.m. in loin (pH_{24L}), conductivity at 45 min p.m. in loin ($Conductivity_{1L}$) and conductivity at 24 h p.m. in loin ($Conductivity_{24L}$). Conductivity and pH-values were measured using Star-series equipment (Rudolf Matthaeus Company, Germany) in both the *longissimus dorsi* muscle between 13th/14th ribs. Drip loss was scored based on a bag-method with a size-standardized sample from *longissimus dorsi* collected at 24 h p.m. that was weighed, suspended in a plastic bag, held at 4°C for 48 h, and thereafter re-weighed. To determine cooking loss, a loin cube was taken from the *longissimus dorsi*, weighed, placed in a polyethylene bag and incubated in water at 75°C for 50 min. Shear force was recorded in Instron testing machine. The bag was then immersed in flowing water at room temperature for 30 min and the solid portion was re-weighed. Thawing loss was determined similarly after at least 24 h freezing at -20°C. Drip loss, cooking loss, and thawing loss were calculated as a percentage of weight loss based on the start weight of a sample. Carcass quality traits were collected according to guidelines of German performance test [21]. The numbers of records, mean values and standard errors are shown in *Table 1*.

Expression Study Using Quantitative Real-Time PCR (qRT-PCR)

Total RNA from the skeletal muscle samples of the 313 DuPi animals were isolated using TriZol® reagent (Sigma-Aldrich). cDNA was synthesised by reverse transcription PCR using 2 µg of total RNA, SuperScript II reverse transcriptase (Invitrogen) and oligo(dT)12 primer (Invitrogen). Gene specific primers for the qPCR were designed using the Primer Express software (Applied Biosystem); primers are listed in *Table 2*.

The qPCR was conducted using the following program: 95°C for 3 min, 40 cycles 95°C for 15 sec/60°C for 45 sec on the ABI Prism 7000 Sequence Detection System (Applied Biosystem). Each PCR reaction contained 10 µl iTaqTM SYBR® Green Supermix with ROX PCR core reagents (Bio-Rad), 2 µl of cDNA and an optimized amount of primer were mixed with double-distilled water to a final reaction volume of 20 µl. All samples were analysed twice (technical replication) and the arithmetic mean of the Ct values were further used for association analysis using SAS v9.2 (SAS Institute Inc., Cary, NC). The geometric mean of three housekeeping genes *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), *RPL32* (ribosomal protein 32) and *TBP* (TATA box binding protein) was used for normalisation of

Table 1. *Data collection in the DuPi population and traits measured with mean and standard errors*
Tablo 1. *DuPi populasyonundaki veri seti ve ölçülen özelliklerin ortalaması ve standart hatası*

Trait	N	Mean	Standard Error	Minimum	Maximum
pH_{1L}	313	6.56	0.21	5.91	7.02
pH_{24L}	313	5.49	0.08	5.30	5.84
Con_{1L} (ms)	313	4.35	0.63	2.80	6.00
Con_{24L} (ms)	313	2.79	0.81	1.60	9.20
Drip loss (%)	313	2.07	0.96	0.50	5.60
Thawing loss (%)	313	8.20	1.86	3.30	13.60
Cooking loss (%)	313	24.68	1.97	17.20	29.40
Shear force (N)	313	35.18	6.62	21.96	61.21

pH_{1L}, pH_{24L}: *pH in longissimus dorsi muscle at 13th/14th rib at 45 minutes and 24 h p.m., respectively;* ***Con_{1L}, Con_{24L}:*** *conductivity in longissimus dorsi muscle at 13th/14th rib at 45 min and 24 h p.m., respectively,* ***N:*** *Newton,* ***ms:*** *millisecond*

the target genes. The delta Ct (ΔCt) values were calculated as the difference between target gene and geometric mean of the reference genes: $\Delta Ct = Ct_{target\ gene} - Ct_{housekeeping\ gene}$. Ct values were Logarithm (Log) transformed and used for further analysis. The higher Ct value indicated lower expression and lower Ct value indicated higher gene expression.

Association of Expression Profiles with Meat Quality Traits

Association of gene expression levels with meat quality traits was analysed using generalized linear model (PROC GLM) in SAS. In this model, season of slaughter, family (Dam × Sire) and sex of the animal were assumed as fixed effects; slaughter weight and gene expressions were included as a linear covariate for meat quality traits. The following GLM model was used:

$$y_{ijk} = \mu + sex_i + ys_j + family_k + \beta_{SW} + \beta_{GE} + e_{ijk}$$

where y_{ijk} is the observation of the trait (meat quality); μ is the population mean; Sex_k is the effect of k-th sex (k = 1 for male and 2 for female); ys_j is the effect of j-th season of slaughter (j = 1 through 12; four seasons in 3 years); $Family_k$ is the fixed effect of k-th family (k = 1 through 19); β_{GE} is the linear effect of normalized gene expression as covariate (β_{GE} = *MYF6*) and β_{SW} is the linear effect of slaughter weight as covariate and e_{ijk} is the random residual error.

Marker Analysis and eQTL Study

A linkage map with the total length of 2159.3 cM and an average marker interval of 17.7 cM was constructed. F_0, F_1 and F_2 animals of the DuPi population were genotyped at 122 markers loci covering all porcine autosomes. Marker positions and details of genotyping procedures were described by Liu et al.[20] and for SSC1 by Große-Brinkhaus et al.[22]. F_2 QTL interval mapping was performed using the web-based program GridQTL [23] based on a least square method. Single line-cross QTL analysis was carried out. Relevant fixed effects for gene expression values including class effects and covariates were tested the using GLM procedure of SAS. Residuals of the GLM model were used for the QTL analysis. Therefore, the basic QTL regression model used for eQTL analysis was:

$$y_{ijk} = \mu + sex_i + ys_j + family_k + \beta_{SW} + e_{ijk}$$

where y_{ijk} is the observation of the trait (gene expression); μ is the population mean; sex_k is the effect of k-th sex (k = 1 for male and 2 for female); ys_j is the effect of j-th season of slaughter (j = 1 through 12; four seasons in 3 years); $family_k$ is the fixed effect of k-th family (k = 1 through 19); and β_{SW} is the linear effect of slaughter weight as covariate and e_{ijk} is the random residual error.

Chromosome-wide (CW) 5% significance thresholds were determined using 1000 permutations [24]. The confidence

Table 2. *Primer sequences used for qRT-PCR analysis*
Tablo 2. *qRT-PCR analizi için kullanılan primer dizileri*

Gene Name	Primers Sequence	Tm	Product Size	GenBank ID
MYF6	F: TGGATCAGCAGGACAAAATG R: TGTTTGTCCCTCCTTCCTTG	55°C	171 bp	AY188502
GAPDH	F: ACCCAGAAGACTGTGGATGG R: ACGCCTGCTTCACCACCTTC	60°C	247 bp	DQ845173
RPL32	F: AGCCCAAGATCGTCAAAAAG R: TGTTGCTCCCATAACCAATG	54°C	164 bp	AY550039
TBP	F: AGCAGCACAGTACGAGCAA R: GATGGACGTTCGGTTTAGG	60°C	124 bp	DQ178129

interval (CI) was calculated by bootstrapping in QTL Express. We considered only the QTL which cross LOD score ≥1.7 in this study. Percentage of F_2 variance explained by the model was calculated as

$$Var\% = \frac{MS_R - MS_F}{MS_R} \text{ x } 100 \qquad 25$$

Where MS_R is the residual MS from the reduced model, omitting QTL but including all fixed effects, and MS_F is the residual MS from the full model, including QTL and all fixed effects.

RESULTS

Association of Expression with Meat and Carcass Quality Traits

The gene expression as well as association study and eQTL analysis were conducted in 313 DuPi animals. Association between gene expression and meat quality traits were estimated with a GLM model. The main fixed effects influencing meat quality characteristics were season of slaughter followed by sex (except Con_{24L}, drip loss and cooking loss) and family (except pH_{24L}, con_{1L} and drip loss). Slaughter weight has no effect on meat quality traits except for cooking loss and shear force. Significant (*P* values) values of the class effects were given in *Table 3*.

According to the result of the linear model, estimated R-square value explained 58% of pH_{24L} and 58% of Con_{24L} variation by *MYF6* expression *(Table 3)*. Suggestive association was detected between *MYF6* expression with pH_{24L} (*P*<0.1). A significant association was detected between *MYF6* mRNA expression and Con_{24L} (*P*<0.01). Moreover, in the DuPi population, one of the important meat quality parameter drip loss was found to be negatively correlated with pH_{24L} and positively correlated with Con_{24L} *(Table 4)*.

Table 3. *Association between MYF6 mRNA expression and meat quality traits in the DuPi population*
Tablo 3. *DuPi populasyonunda MYF6 mRNA ekspresyonu ve et kalite kriterleri arasindaki asosiyasyon*

Traits	Class Effects					
	R^2	Gene Expression	Season of Slaughter	Sex	Slaughter Weight	Family
pH_{1L}	0.60	ns	***	*	ns	***
pH_{24L}	0.58	*	***	***	ns	ns
Con_{1L}	0.48	ns	**	***	ns	ns
Con_{24L}	0.58	***	***	ns	ns	***
Drip loss	0.47	ns	***	ns	ns	ns
Cooking loss	0.65	ns	***	ns	***	***
Thawing loss	0.54	ns	***	*	ns	***
Shear force	0.63	ns	***	**	**	***

ns *= not significant*
*Three significance levels were used: *** (P<0.01); ** (P<0.05); * (P<0.1)*
P-values showed by the star, derived from total variation explained by the class effects (R^2)

Table 4. *Correlation among meat quality traits in the DuPi population*
Tablo 4. *DuPi populasyonunda et kalite özellikleri arasındaki korrelasyon*

Trait r, (p)	pH_{1L}	pH_{24L}	Con_{1L}	Con_{24L}	Drip Loss	Thawing Loss	Cooking Loss	Shear Force
pH_{1L}	1	0.14 (0.007)	-0.28 (<.0001)	-0.50 (<.0001)	-0.43 (<.0001)	0.03 (0.57)	-0.14 (<.0001)	-0.16 (0.0041)
pH_{24L}		1	0.01 (0.72)	0.00073 (0.98)	-0.20 (0.0002)	-0.15 (0.0051)	-0.16 (0.0022)	0.02 (0.71)
Con_{1L}			1	0.20 (0.0002)	0.23 (<.0001)	-0.01 (0.82)	0.04 (0.37)	0.13 (0.01)
Con_{24L}				1	0.38 (<.0001)	0.01 (0.81)	0.10 (0.06)	0.25 (<.0001)
Drip loss					1	0.14 (0.007)	0.15 (0.003)	0.15 (0.0067)
Thawing loss						1	-0.03 (0.50)	0.04 (0.44)
Cooking loss							1	0.23 (<.0001)
Shear force								1

Differential Expression of MYF6

In the animals with extreme phenotype of pH_{1L}, pH_{24L} and Con_{24L} (n = 10) selected for differential expression, the gene expression was significantly higher in animals with low pH_{1L} compared to animals with high pH_{1L} (data not shown) *(Fig. 1)*. Animals with higher pH_{24L} had higher *MYF6* gene expression compared to animals with low pH_{24L} *(Fig. 1)*. The *MYF6* mRNA expression was higher in animals with high conductivity at 24 h p.m. value compared to animals with low conductivity at 24 h p.m. *(Fig. 1)*.

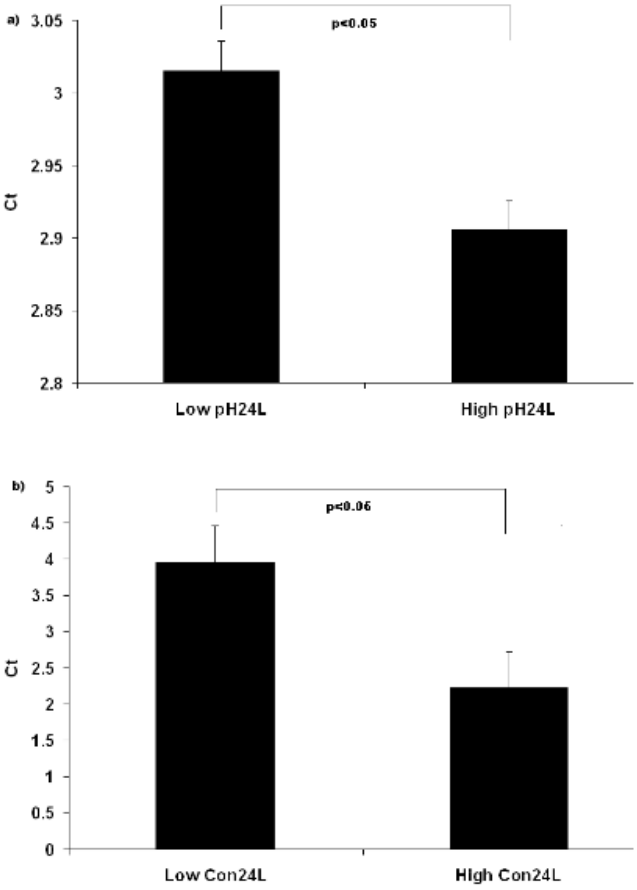

Fig 1. Differential expression of *MYF6* mRNA (a) animals with high and low pH_{24L}, (b) animals with high and low Con_{24L}. Lower Ct values represent higher gene expression and vice versa

Şekil 1. *MYF6* mRNA'sının ifade farklılığı (a) yüksek ve düşük pH_{24L} özelliğe sahip hayvanlar, (b) yüksek ve düşük Con_{24L} özelliğine sahip olan hayvanlar. Düşük Ct değerleri yüksek gen ifadesini göstermektedir

Expression QTL (eQTL)

A total of four eQTL were detected for *MYF6* expression on four porcine autosomes. On SSC2, a 1% chromosome-wide significant eQTL at 26 cM close to the marker *S0141* explained 5.12% of the phenotypic variation *(Table 5)*. A suggestive eQTL was identified on SSC5 at 116 cM close to marker *IGF1*. Moreover, a 5% chromosome-wide significant eQTL was detected on SSC8 between the markers *SW2611* and *S0086* which explains 3.14% of total variation. Another suggestive eQTL for *MYF6* was detected on SSC10 at 133 cM close to marker *SW951 (Table 5)*. Experiment-wide thresholds (8.36 and 9.80, $P<0.05$ and $P<0.01$, respectively) were also calculated; however no eQTL in this study reached the experiment-wide thresholds.

DISCUSSION

Expression of MYF6

In recent few decades, pig breeding focused on improving growth rate and muscularity. This causes gradual decrease in meat quality and deterioration of carcass traits such as intramuscular fat content, colour, hardness or water content in meat [16]. The commercial Duroc and Pietrain breeds differ extremely for their muscle phenotypes (i.e., myofiber numbers and myofiber types) [26]. In vertebrates skeletal myogenesis is coordinated by the activation of the myogenic regulatory factors (MRFs) in response to signals that are interpreted by their associated regulatory elements in different precursor cells during

Table 5. *Expression QTL (eQTL) mapping results for the mRNA expression of MYF6 gene on swine autosomes identified in the DuPi population*
Tablo 5. *Domuz otozomlarında tanımlanan DuPi populasyonunda MYF6 geninin mRNA ifadesi için ekspresyon QTL (eQTL) haritalama sonuçları*

SSC[a]	Trait[b]	*F*-ratio[c]	Position (CI_{95})[d]	Closest Markers[e]	Additive (SE)[f]	Dominance (SE)[g]	Variation (%)[h]
2	*MYF6*	8.1**	26 (0 – 202)	SW263 – S0141	0.14 (0.21)	2.43 (0.60)	5.12
5	*MYF6*	3.28	116 (0 – 113)	IGF1	-0.05 (0.17)	0.82 (0.32)	1.71
8	*MYF6*	5.26*	41 (16.5 – 113.5)	SW2611 – S0086	-0.24 (0.19)	0.71 (0.27)	3.14
10	*MYF6*	3.98	133 (0 – 129)	SW951 – SWR67	-0.55 (0.21)	0.55 (0.44)	2.22

[a] *Sus scrofa chromosome*
[b] *Gene abbreviations according to NCBI database*
[c] *Significance level was used: 5% chromosome-wide significance level, as suggestive level. The eQTL those can reach the level marked by star*
[d] *Positions of detected eQTL in Kosambi cM with the 95% confidence interval (CI_{95}) given in parentheses according to the bootstrapping approach*
[e] *The closest markers were those markers around the peak, as near as possible*
[f] *Additive effects, expressed as the deviation of the Duroc-Pietrain alleles. SE = standard error*
[g] *Dominance effects, expressed as the deviation of the Duroc-Pietrain alleles. SE =standard error*
[h] *Fraction of phenotypic variance explained by each eQTL as a percentage of the residual variance in the F_2 population*
** Significant on a chromosome-wide level with P≤0.05*
*** Significant on a chromosome-wide level with P≤0.01*

development [27]. *MYF6* is the most abundantly expressed myogenic factor in postnatal muscle where it quantitatively predominates over the other MyoD family transcripts [28]. *MYF6* is considered as one promising candidate gene for growth- and meat quality-related traits in adult pigs [5]. Recently, MYF6 mRNA and protein expression was shown to be significantly up-regulated in purebred Pietrain pigs compared to purebred Duroc pigs in the founder family of DuPi F_2 animals [8]. Polymorphism in *MYF6* in the promotor region and exon 1 are signicantly correlated with weight of loin and ham, right half-carcass weight and daily gain [29]. The same researchers did not observe any mRNA expression differences among genotype classes. However, in our study, we found that *MYF6* expression was associated with meat pH at 24 h *post-mortem (Table 3)* and showed that expression was higher in animal with high muscle pH and conductivity indicating that this gene was expressed higher in muscle with lower drip loss because in the DuPi population, muscle pH was negatively correlated with drip loss (r = -0.20, P = 0.0002). The same negative correlation between muscle pH and drip loss has also been reported [30]. Similar results were also obtained by Liu et al.[31]; they also identified association between *MYF5* SNP and *longissimus dorsi* pH in pigs. Te Pas et al.[32] stated that genetic variation at the porcine *MYF5* gene locus could also be used as a marker to study association of the *MYF6* locus with muscle development-related meat traits. SNP in bovine *MYF5* were found to be associated with meat traits in cattle [33]. In chicken, although *MYF5* found to be associated with several carcass traits, no polymorphism was detected in *MYF6* gene [34]. In the study of Ropka-Molik et al.[16] expression for the *MYF6* gene did not show statistically significant differences between ages of development in analyzed Poland Landrace and Pietrain breeds. The highest mRNA level of *MYF6* gene was observed in gracilis muscle in this study among two breeds, but a statistically significant (P<0.01) difference was only found in Pietrain pigs.

Expression QTL Analysis

Analysis of expression quantitative trait loci (eQTL) provides a means for detecting transcriptional regulatory relationships at a genome-wide scale [35]. Many investigations have reported the successful mapping of eQTL in rats, mice, humans or pigs with different sample sizes [36-39]. But experimental data from genetic investigations of gene expression in farm animals are still limited [37]. Mapping of eQTL to the specific gene indicates that *cis*- changes are responsible for the different expression levels, whereas mapping positions of eQTL that are different from the positions of the corresponding genes indicate *trans*-regulation. In this study, four eQTLs on four different porcine autosomes were detected. Different Lod scores were reported as threshold for a QTL [25,40,41]. Here in our study a Lod score value of 1.7 was chosen based on the median of presented thresholds in literature [40,41] to indicate an eQTL as suggestive. Among them, an eQTL on SSC5 might be a putative *cis*-regulated eQTL since the confidence interval of this eQTL did incorporate the chromosomal location of the *MYF6* gene. Moreover, the highest peak of this eQTL (116 cM) was closed to the marker *IGF1*, very close to the chromosomal location of *MYF6*. The power of this putative eQTL on SSC5 is low which might be due to low number of animals in the population. However, the DNA variations of this gene in promoter and exon 1 were not affecting the expression of the *MYF6* gene in a previous study [16] and the confidence interval of this eQTL is very wide indicating that the eQTL on SSC5 is better to be considered as a *trans*-eQTL. The other three eQTLs showed *trans*-regulation of this gene which were consistent with the previous reports for pigs [42] and other species [35,43].

Most genes are regulated through complex networks, and function and expressions are influenced by itself as well as different other genes, transcription factors and miRNAs [34]. Moreover, distant eQTL may affect their target genes in many possible ways such as protein-coding regions, *cis*-regulatory DNA motifs, transcription factor that is polymorphic in its DNA binding region, or other functional nucleotide sequences [35]. The MRFs trigger a cascade of transcription factors and downstream structural genes. Due to the close vicinity of *MYF6* and *MYF5* genes on SSC5, these two genes have a common regulatory region and the expression of both genes is in part activated together [44]. Analyses of the mechanisms behind promoter-enhancer specificity in the *MYF6*/*MYF5* locus have unveiled a new class of element, termed Transcription balancing sequences which explains the *cis*-acting effects observed in the various *MYF6* and *MYF5* alleles [13]. On the other hand, Black et al.[45] showed that *MYF6* promoter is *trans*-activated by myogenin, *MyoD*, *MYF5* and by the *myocyte enhancer factor-2 (MEF2)* factors in mice. In our study a significant eQTL *(Table 5)* was detected close to marker *S0141*. An important gene called insulin-like growth factor 2 *(IGF2)* locates distal part of the p arm on SSC2. Regulatory mutation of *IGF2* and relation to muscle growth in pigs gene was shown previously [46]. Alzhanov et al.[47] identified distal transcriptional enhancer directly stimulates the transcriptional activity of an *IGF2* promoter-reporter gene in differentiating myoblasts.

Ponsuksili et al.[37] stated that the expression of genes associated with traits that have low heritability, like drip loss or meat pH is under the control of several *trans*-regulated genes. The detected eQTL in this study explain a low proportion of phenotypic variation. *Trans*-eQTL usually reflects genetic regulation that is dispersed across many loci with small effects and each explains small phenotypic variance [48]. Moreover, *trans*-regulated genes appear more in complex traits (i.e., under polygenic control) than the *cis*-regulated genes and are likely to reflect the additive outcome of genetic, epigenetic, and environmental regulation [49]. In our study, all detected

eQTLs were found to have higher dominance effects *(Table 5)*. Duroc and Pietrain pig breeds were divergent for meat quality and growth traits, recently their mRNA expression for *MYF6* gene was found to be differentially regulated [8]. Heterosis might be one of the possible reasons for this over dominancy. However, it is not possible to proof this with the available data in our hand. To link an eQTL to the genetic background of a classical phenotypic trait of interest, it is necessary to establish a relationship between the variation of that classical phenotypic trait and its corresponding 'pQTL' (pheneQTL) position on one hand and the expression level of that particular transcript or the mapping position of the eQTL on the other hand [37]. Among these QTL, phenotypic QTL for pH and conductivity reported on SSC2 [20,50] are close to our detected eQTL for *MYF6* in the DuPi population.

As a conclusion, significant and suggestive associations were found between gene expression and meat quality traits in the DuPi animals and higher expression of the *MYF6* might contribute to lower drip loss and higher muscle pH. According to eQTL analyses it is difficult to say that our candidate gene is *cis*-regulated. Moreover, DNA variations in promoter of *MYF6* including *MYF5* were requested as further study to test whether SNPs affecting its transcription abundance. Therefore, *MYF6* could be a *trans*-regulated gene which might be due to the presence of the widely distributed genetic regulators such as transcription factors and non-coding RNAs in the whole genome as well as non-genetic mechanisms (epigenetics). Finally, *MYF6* could be suggested as a potential candidate gene for meat quality traits in pigs. However, further studies are necessary to validate these results with other breeds especially in larger outbreed populations and further analysis of detected overlapping eQTLs and QTL regions can dissect genes and regulators that are responsible for meat quality traits in pigs.

Acknowledgements

This project was supported by the German Research Foundation (DFG), "Functional Genetic Principles of Water Binding Capacity in Pork "DRIP FOR 753" Germany. Authors are indebted to Ms. Nadine Leyer for technical assistance during the experiments.

REFERENCES

1. Davoli R, Fontanesi L, Cagnazzo M, Scotti E, Buttazzoni L, Yerle M, Russo V: Identification of SNPs, mapping and analysis of allele frequencies in two candidate genes for meat production traits: The porcine myosin heavy chain 2B (MYH4) and the skeletal muscle myosin regulatory light chain 2 (HUMMLC2B). *Animal Genet*, 34 (3): 221-225, 2003.

2. Fontanesi L, Colombo M, Tognazzi L, Scotti E, Buttazzoni L, Dall'Olio S, Davoli R, Russo V: The porcine TBC1D1 gene: Mapping, SNP identification, and association study with meat, carcass and production traits in Italian heavy pigs. *Mol Biol Rep*, 38 (2): 1425-1431, 2011.

3. Srikanchai T, Murani E, Phatsara C, Schwerin M, Schellander K, Wimmers K, Ponsuksili S: Association of ZYX polymorphisms with carcass and meat quality traits in commercial pigs. *Meat Sci*, 84 (1): 159-164, 2010.

4. Rothschild MF, Fan B, Lkhagvadorj S, Cai W, Young J, Smith RM, Dekkers JCM, Huff-Lonergan E, Lonergan SM: Identification of genetic markers associated with residual feed intake and meat quality traits in the pig. *Meat Sci*, 84 (4): 645-650, 2010.

5. Maak S, Neumann K, Swalve HH: Identification and analysis of putative regulatory sequences for the MYF5/MYF6 locus in different vertebrate species. *Gene*, 379, 141-147, 2006.

6. Wyszynska-Koko J, Kuryl J: Porcine MYF6 gene: sequence, homology analysis, and variation in the promoter region. *Anim Biotech*, 15 (2): 159-173, 2004.

7. Bober E, Lyons GE, Braun T, Cossu G, Buckingham M, Arnold HH: The muscle regulatory gene, Myf-6, has a biphasic pattern of expression during early mouse development. *J Cell Biol*, 113 (6): 1255-1265, 1991.

8. Fan H, Cinar MU, Phatsara C, Tesfaye D, Tholen E, Looft C, Schellander K: Molecular mechanism underlying the differential MYF6 expression in postnatal skeletal muscle of Duroc and Pietrain breeds. *Gene,* 2011.

9. Hinterberger TJ, Sassoon DA, Rhodes SJ, Konieczny SF: Expression of the muscle regulatory factor MRF4 during somite and skeletal myofiber development. *Dev Biol*, 147 (1): 144-156, 1991.

10. Lowe DA, Lund T, Alway SE: Hypertrophy-stimulated myogenic regulatory factor mRNA increases are attenuated in fast muscle of aged quails. *Am J Physiol*, 275 (1 Pt 1): C155-162, 1998.

11. Kassar-Duchossoy L, Gayraud-Morel B, Gomes D, Rocancourt D, Buckingham M, Shinin V, Tajbakhsh S: Mrf4 determines skeletal muscle identity in Myf5: Myod double-mutant mice. *Nature*, 431 (7007): 466-471, 2004.

12. Buonanno A, Apone L, Morasso MI, Beers R, Brenner HR, Eftimie R: The myod family of myogenic factors is regulated by electrical-activity - Isolation and characterization of a mouse Myf-5 Cdna. *Nucleic Acids Res*, 20 (3): 539-544, 1992.

13. Carvajal JJ, Rigby PWJ: Regulation of gene expression in vertebrate skeletal muscle. *Exp Cell Res*, 316 (18): 3014-3018, 2010.

14. Pas MFWT, Harders FL, Soumillion A, Born L, Buist W, Meuwissen THE: Genetic variation at the porcine MYF-5 gene locus. Lack of association with meat production traits. *Mamm Genome*, 10 (2): 123-127, 1999.

15. Vykoukalova Z, Knoll A, Dvorak J, Rohrer GA, Cepica S: Linkage and radiation hybrid mapping of the porcine MYF6 gene to chromosome 5. *Animal Genet*, 34 (3): 238-240, 2003.

16. Ropka-Molik K, Eckert R, Piorkowska K: The expression pattern of myogenic regulatory factors MyoD, Myf6 and Pax7 in postnatal porcine skeletal muscles. *Gene Exp Patt*, 11 (1-2): 79-83, 2011.

17. Gandolfi G, Cinar MU, Ponsuksili S, Wimmers K, Tesfaye D, Looft C, Jungst H, Tholen E, Phatsara C, Schellander K: Association of PPARGC1A and CAPNS1 gene polymorphisms and expression with meat quality traits in pigs. *Meat Sci,* 89 (4): 478-485, 2011.

18. Kayan A, Cinar MU, Uddin MJ, Phatsara C, Wimmers K, Ponsuksili S, Tesfaye D, Looft C, Juengst H, T holen E, Schellander K: Polymorphism and expression of the porcine Tenascin C gene associated with meat and carcass quality. *Meat Sci*, 89 (1): 76-83, 2011.

19. Jansen RC, Nap JP: Genetical genomics: The added value from segregation. *Trends Genet*, 17 (7): 388-391, 2001.

20. Liu G, Jennen DGJ, Tholen E, Juengst H, Kleinwachter T, Holker M, Tesfaye D, Un G, Schreinemachers HJ, Murani E, Schellander K, Wimmers K: A genome scan reveals QTL for growth, fatness, leanness and meat quality in a Duroc-Pietrain resource population. *Animal Genet*, 38 (3): 241-252, 2007.

21. ZDS: Richtlinie für die Stationsprüfung auf Mastleistung, Schlachtkörperwert und Fleischbeschaffenheit beim Schwein. *Zentralverband der Deutschen Schweineproduktion eV, Ausschussfür Leistungsprüfung und Zuchtwertschätzung, Bonn*, 2003.

22. Grosse-Brinkhaus C, Phatsara C, Tholen E, Schellander K, Jonas ES: Finemapping of QTL for meat quality traits on porcine chromosome 1.

Zuchtungskunde, 81 (1): 63-68, 2009.

23. Seaton G, Grunchec JA, White I, Allen J, De Koning DJ, Wei W, Berry D, Haley C, Knott S: 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 2006; Belo Horizonte, Brazil*, 2006.

24. Churchill GA, Doerge RW: Empirical threshold values for quantitative trait mapping. *Genetics*, 138, 963-971, 1994.

25. Uemoto Y, Soma Y, Sato S, Shibata T, Kadowaki H, Katoh K, Kobayashi E, Suzuki K: Mapping QTL for fat area ratios and serum leptin concentrations in a Duroc purebred population. *Anim Sci J*, doi: 10.1111/j.1740-0929.2011.00951.x, 2011.

26. Cagnazzo M, te Pas MF, Priem J, de Wit AA, Pool MH, Davoli R, Russo V: Comparison of prenatal muscle tissue expression profiles of two pig breeds differing in muscle characteristics. *J Anim Sci*, 84 (1): 1-10, 2006.

27. Pownall ME, Gustafsson MK, Emerson CP Jr: Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. *Annu Rev Cell Dev Biol*, 18, 747-783, 2002.

28. Miner JH, Wold B: Herculin, a fourth member of the MyoD family of myogenic regulatory genes. *Proc Natl Acad Sci USA*, 87 (3): 1089-1093, 1990.

29. Wyszynska-Koko J, Pierzchala M, Flisikowski K, Kamyczek M, Rozycki M, Kuryl J: Polymorphisms in coding and regulatory regions of the porcine MYF6 and MYOG genes and expression of the MYF6 gene in m. longissimus dorsi versus productive traits in pigs. *J Appl Genet*, 47 (2): 131-138, 2006.

30. Essén-Gustavsson B, Karlström K, Lundström K: Muscle fibre characteristics and metabolic response at slaughter in pigs of different halothane genotypes and their relation to meat quality. *Meat Sci*, 31 (1): 1-11, 1992.

31. Liu M, Peng J, Xu DQ, Zheng R, Li FE, Li JL, Zuo B, Lei MG, Xiong YZ, Deng CY: Association of MYF5 and MYOD1 Gene polymorphisms and meat quality traits in Large White x Meishan F2 Pig Populations. *Biochem Genet*, 46 (11-12): 720-732, 2008.

32. te Pas MF, Verburg FJ, Gerritsen CL, de Greef KH: Messenger ribonucleic acid expression of the MyoD gene family in muscle tissue at slaughter in relation to selection for porcine growth rate. *J Anim Sci*, 78 (1): 69-77, 2000.

33. Robakowska-Hyzorek D, Oprzadek J, Zelazowska B, Olbromski R, Zwierzchowski L: Effect of the g.-723G -> T polymorphism in the bovine myogenic factor 5 (Myf5) gene promoter region on gene transcript level in the longissimus dorsi muscle and on meat traits of Polish Holstein-Friesian Cattle. *Biochem Genet*, 48 (5-6): 450-464, 2010.

34. Yin HD, Zhang ZC, Lan X, Zhao XL, Wang Y, Zhu Q: Association of MyF5, MyF6 and MyOG gene polymorphisms with carcass traits in Chinese Meat Type Quality chicken populations. *J Anim Vet Adv*, 10 (6): 704-708, 2011.

35. Michaelson JJ, Loguercio S, Beyer A: Detection and interpretation of expression quantitative trait loci (eQTL). *Methods*, 48 (3): 265-276, 2009.

36. Morley M, Molony CM, Weber TM, Devlin JL, Ewens KG, Spielman RS, Cheung VG: Genetic analysis of genome-wide variation in human gene expression. *Nature*, 430 (7001): 743-747, 2004.

37. Ponsuksili S, Murani E, Phatsara C, Schwerin M, Schellander K, Wimmers K: Expression quantitative trait loci analysis of genes in porcine muscle by quantitative real-time RT-PCR compared to microarray data. *Heredity*, 105 (3): 309-317, 2010.

38. Stranger BE, Forrest MS, Clark AG, Minichiello MJ, Deutsch S, Lyle R, Hunt S, Kahl B, Antonarakis SE, Tavare S: Genome-wide associations of gene expression variation in humans. *PLoS Genet*, 1 (6): e78, 2005.

39. Wimmers K, Ponsuksili S, Murani E, Schwerin M, Schellander K: Identification of expression QTL (eQTL) of genes expressed in porcine M. longissimus dorsi and associated with meat quality traits. *BMC Genomics*, 11 (1): 572, DOI: 10.1186/1471-2164-11-572.

40. Soma Y, Uemoto Y, Sato S, Shibata T, Kadowaki H, Kobayashi E, Suzuki K: Genome-wide mapping and identification of new quantitative trait loci affecting meat production, meat quality, and carcass traits within a Duroc purebred population. *J Anim Sci*, 89 (3): 601-608, 2011.

41. Van Ooijen JW: LOD significance thresholds for QTL analysis in experimental populations of diploid species *Heredity,* 83, 613-624, 1999.

42. Ponsuksili S, Jonas E, Murani E, Phatsara C, Srikanchai T, Walz C, Schwerin M, Schellander K, Wimmers K: Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. *BMC Genomics*, 9, 367, doi:10.1186/1471-2164-9-367, 2008.

43. Bystrykh L, Weersing E, Dontje B, Sutton S, Pletcher MT, Wiltshire T, Su AI, Vellenga E, Wang J, Manly KF: Uncovering regulatory pathways that affect hematopoietic stem cell function using 'genetical genomics'. *Nat Genet*, 37 (3): 225-232, 2005.

44. Carvajal JJ, Cox D, Summerbell D, Rigby PWJ: Control of the expression of the Mrf4 and Myf5 genes: A BAC transgenic approach. *Inter J Dev Biol*, 45, S139-S140, 2001.

45. Black BL, Martin JF, Olson EN: The mouse Mrf4 promoter is trans-activated directly and indirectly by muscle-specific transcription factors. *J Biol Chem*, 270 (7): 2889-2892, 1995.

46. Georges M, Van Laere AS, Nguyen M, Braunschweig M, Nezer C, Collette C, Moreau L, Archibald AL, Haley CS, Buys N: A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. *Nature*, 425 (6960): 832-836, 2003.

47. Alzhanov DT, Rotwein P, McInerney SF: Long range interactions regulate Igf2 gene transcription during skeletal muscle differentiation. *J Biol Chem*, 285 (50): 38969-38977, 2010.

48. Gibson G, Weir B: The quantitative genetics of transcription. *Trends Genet*, 21 (11): 616-623, 2005.

49. Petretto E, Mangion J, Dickens NJ, Cook SA, Kumaran MK, Lu H, Fischer J, Maatz H, Kren V, Pravenec M: Heritability and tissue specificity of expression quantitative trait loci. *PLoS Genet*, 2 (10): e172, 2006.

50. Lee SS, Chen Y, Moran C, Cepica S, Reiner G, Bartenschlager H, Moser G, Geldermann H: Linkage and QTL mapping for Sus scrofa chromosome 2. *J Anim Breed Genet*, 120, 11-19, 2003.