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Genomic Structure and Expression of the Porcine *ACTC1* Gene

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ABSTRACT

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A partial cDNA (~1200 bp) of the porcine *ACTC1* gene was identified in the subtracted foetal hind limb muscle cDNA library (44 days of gestation; using *m. biceps femoris* cDNA as the driver). Using specific polymerase chain reaction (PCR) primers, a bacterial artificial chromosome (BAC) clone containing the genomic *ACTC1* gene was identified and the gene was sequenced. Specific PCR primers designed from the BAC and cDNA sequences were used for amplification and comparative sequencing of *ACTC1* of Pietrain and Meishan pigs. The gene is approximately 5.4 kb in length, is composed of 7 exons, and has a coding sequence containing 1134 bp. The gene was mapped using the INRA-Minnesota porcine radiation hybrid (IMpRH) panel to chromosome 1, with *SW65* as the closest marker (41 cR; LOD = 7.73). Differences were observed in tissue-specific expression of *ACTC1* that was studied by transcription profiling in 28 porcine tissues. Developmental differences in muscle and heart were analysed by real-time quantitative PCR (RT-qPCR). Two single nucleotide polymorphisms (SNPs) were found in intron 1. One adequately informative SNP (FM212567.1:g.901C>G) was genotyped by PCR-restriction fragment length polymorphism, and allele frequencies in eight pig breeds were calculated.

Keywords: actin, alpha; cardiac muscle 1; mRNA; pig; transcription profiling; RT-qPCR

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Alpha actins are present in muscle tissues as a major constituent of the contractile apparatus. Actin in muscle fibres comprises about 20% of total cellular protein. In human skeletal and cardiac muscles, two sarcomeric actins, ACTA1 (actin, alpha 1, skeletal muscle) and ACTC1 (actin, alpha, cardiac muscle 1) are co-expressed (Gunning et al. 1983; Ilkovski et al. 2005). These two human actins are highly homologous and their amino acid sequences differ by only four residues of the total 377 amino acids in each protein. Both human actins are 100% identical with the porcine orthologues. Homologies are lower, however, in nucleotide sequences of the two genes – in human, they share 85.6% and in the pig 86.8% identities of coding sequences (http://www.ensembl.org/).

As studied using microarray and RNA sequencing, mRNA expression of *ACTC1* in various human tissues is recorded in the Ensembl database (http://www.ensembl.org/index.html). The gene's expression is high in the heart, but lower in the skeletal muscle and some other tissues. Protein expression of human ACTA1 and ACTC1 was studied by Ilkovski et al. (2005). They showed that ACTA1 is a predominant skeletal muscle isoform from 25 to 27 weeks of gestation to adulthood, and ACTC1 is a predominant sarcomeric isoform in heart and embryonic and foetal skeletal muscles. Protein expression of ACTC1 in several other human tissues is also recorded in the Ensembl database.

Recently, *ACTC1* mRNA expression in pig tissues was studied using the porcine Affymetrix expression array (Snowball) (http://biogps.org; Freeman et al. 2012). The expressions in various tissues differed appreciably.

In the subtracted porcine foetal hind limb muscle cDNA library (44 days of gestation, using adult *biceps femoris* cDNA as the driver; Stratil et al. 2008) several clones were obtained that contained sequences orthologous to human *ACTC1* cDNA. In a preliminary study, Horak et al. (2008) estimated that *ACTC1* mRNA in the muscle tissue of pig foetuses (hind limb; 50 days of gestation) is overexpressed compared to the adult skeletal muscle. The overexpression of *ACTC1* mRNA in the foetal muscle may indicate an important role of the protein during development and early stages of myogenesis (http://www.ebi.ac.uk/QuickGO/GProtein?ac=P68032).

Numerous genes expressed in skeletal muscle at various stages of development in farm animals may be candidate genes that could play a role in myogenesis (Te Pas et al. 2005; Murani et al. 2007). Mutations in these genes can modify structure and function or expression of the proteins and can influence muscle growth and meat quality (Wimmers et al. 2007; Chalupova et al. 2014).

The aims of the present study were to determine the genomic sequence and organization of the porcine *ACTC1* gene, map the gene, study mRNA expression, and search for polymorphism. We also attempted to study associations of the polymorphism with carcass traits in a crossbred porcine population and as no significant associations were observed, the data are not presented.

MATERIAL AND METHODS

Animals, isolation of DNA and RNA, and reverse transcription. Genomic DNA was isolated from blood of pigs (Czech Large White, Czech Landrace, Czech Meat Pig, Pietrain, Black Pied Prestice, Hampshire, Duroc, and Meishan) by conventional methods. DNA from bacterial artificial chromosome (BAC) clones was isolated using the QIAGEN Plasmid Mini or Midi Kit (QIAGEN, Germany).

Samples from the foetal hind limb muscle (Czech Large White; 42 days of gestation) and from the skeletal muscle and heart tissues of piglets (Czech Large White; 1, 7, and 14 days old) and 8 tissues of a 6-month-old pig (Czech Large White; *m. biceps femoris*, heart, tongue, lymph node, brain, lung, kidney, and backfat) were collected and stored in RNA*later* (QIAGEN) at –20°C. Another set of samples from 25 tissues (adrenal gland, aorta, bladder, caecum, colon (centrifugal coil and centripetal coil), diaphragm, duodenum, gallbladder, heart, ileum, jejunum, kidney, liver, lung, lymph node, *m. longissimus dorsi*, mesenterium, oesophagus, pancreas, rectum, spleen, stomach, tongue, and ureter) were taken from a commercial crossbred pig 10 days old immediately after slaughtering and were kept in liquid nitrogen. All pigs were slaughtered according to protocols for certified national slaughterhouses under the supervision of an independent veterinarian. Principles of ethical standards were adhered to during all sample collections.

Isolation of RNA and reverse transcription was performed basically as described by Van Poucke et al. (2009) or Svobodova et al. (2015).

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Sequencing of cDNA and genomic DNA of ACTC1. The mRNA and genomic sequences of porcine *ACTC1* were unknown at the time of this study. The cDNA sequence encompassing the whole coding sequence of porcine foetal muscle *ACTC1* (orthologous with human *ACTC1*) was obtained from the clones of a subtracted foetal hind limb muscle cDNA library (Stratil et al. 2008) and the sequence was deposited in the EMBL/GenBank database under accession No. FM212568.1 (submitted September 29, 2008). Sequences corresponding to individual exons were identified by alignment with the human genomic sequence of *ACTC1* (Ensembl; ENSG00000159251, transcript ACTC1-001; http://www.ensembl.org/Homo_sapiens/Info/Index).

For the study of the porcine genomic sequence of *ACTC1*, first sets of polymerase chain reaction (PCR) primers were designed on the basis of cDNA sequence (ACTC1-A, -B; -C, -D; -E; -G; -M; -R; Table 1). Further primers were designed from the sequences of the amplicons (Table 1). The 5' and 3' ends of the gene and 5' upstream and 3' downstream sequences were first obtained by sequencing a BAC clone, using primer walking (the primers for primer walking are not shown). Two positive BAC clones for *ACTC1* (PigI-258C6 and PigI-417B2) were identified in the porcine genomic BAC library (Rogel-Gaillard et al. 1999) using primers ACTC1-A/-B. The clone PigI-417B2 was used for sequencing by primer walking. PCR primers were designed from the obtained sequences (see Table 1) to amplify and sequence the fragments of genomic DNA of Pietrain and Meishan pigs.

The sequences of cDNA clones, PCR fragments of genomic DNA, and the BAC clone were obtained by Sanger sequencing (ABI PRISM 3130 Sequencer; Applied Biosystems, USA).

The obtained genomic sequences of *ACTC1* of Pietrain and Meishan were deposited in the EMBL/GenBank database under accession Nos. FM212566.1 and FM212567.1 (submitted September 29, 2008).

Polymorphism testing. Single nucleotide polymorphisms (SNPs) revealed by sequencing were tested by PCR-restriction fragment length polymorphism (RFLP). The DNA fragments were amplified using PCR primers ACTC1-Q/-R, and digestions were performed with restriction enzymes *Alw*26I and *Bsa*WI, respectively. Further details on primers, amplification conditions, and SNP genotyping are presented in Table 1. The restriction fragments were separated by electrophoresis on 1% agarose gel.

Radiation hybrid mapping. Radiation hybrid (RH) mapping was performed on the whole-genome 7000-rad IMpRH panel (Yerle et al. 1998; Hawken et al. 1999). A panel of 90 clones was screened by PCR using the ACTC1-A/-B primers (Table 1).

Transcription profiling. Transcription profiles of *ACTC1* were studied by PCR using cDNA from the pig tissues (see Results). *ACTB* and *GAPDH* were used as reference genes, and the primers were from Erkens et al. (2006). For PCR amplification, primer pairs ACTC1-C/-D (exons 3 and 5) and ACTC1-A/-B (exons 6 and 7) (Table 1) were used. The analyses were performed in duplicates. The PCR products were separated on 1% agarose gel.

Relative quantification of ACTC1 expression (RT-qPCR). The mRNA expression of *ACTC1* was studied by real-time quantitative PCR (RT-qPCR) in foetal hind limb muscle (42 days of gestation), skeletal muscle (*m. biceps femoris*), and heart muscle of 1-, 7-, and 14-day-old piglets and adult pigs. PCR primers were: Forward: 5'CCAGCACCATGAAGATCAAGA 3' (from exon 6; one nt from exon 7); Reverse: 5'AAAGAAGGGTGGGTTGGAAG 3' (from exon 7; 3'untranslated region). A 232-bp fragment was amplified, and the specificity was verified by sequencing. The reference gene was *HPRT1*, which has been found to be stable in various adult tissues and piglet muscles, but less stable in foetal muscle (Svobodova et al. 2015). RT-qPCR was conducted in the 7500 Real Time PCR System, using Power SYBR® Green PCR Master Mix (Applied Biosystems). The programme started with 2 min of the AmpErase Uracil N-glycosylase incubation step at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. A melting curve was constructed for verification of the specificity of PCR products. The efficiency of the reaction was calculated from the slope of the standard curve determined in the 7500 System SDS software v1.2 application (Applied Biosystems). The efficiency of the reactions in all samples was in the range of 93–105%, and the values of the coefficient of determination (R^2) were in the range of 0.98–1.00.

The values of the relative quantification were determined using 7500 SDS software v1.2, where calculations are based on the $\Delta\Delta C_T$ method (http://www3.appliedbiosystems.com/cms/groups/

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Table 1. Polymerase chain reaction (PCR) primers for sequencing, single nucleotide polymorphism (SNP) analysis, radiation hybrid (RH) mapping and transcription profiling, and amplification conditions for porcine *ACTC1*

Accession No.; source sequence	Primer name	Primer sequences (5'-3')	Location in genomic sequence	Amplicon size (bp)	$MgCl_2$ (mM)	T_a (°C)	Comments
PW[1] on BAC PigI-417B2	ACTC1-5F ACTC1-5R	atccaggttggtgagggctaca agggcaggggagaggatcag	5' upstream 5´UTR	467	1.0	58	LA DNA polymerase[2]; sequencing
PW on BAC PigI-417B2 FM212568.1	ACTC1-Q ACTC1-R	ggtgggctggcgtcacttagtct cctcgtcgtcgcacatctttgtac	5' upstream exon 2	935	1.0	60	LA DNA polymerase; sequencing; SNP analysis (FM212566.1:g.889G>A; *Bsa*WI PCR-RFLP: allele *G*: 935 bp; allele *A*: 704 + 231 bp; FM212567.1:g.901C>G; *Alw*26I PCR-RFLP: allele *C*: 935 bp; allele *G*: 721 + 214 bp)
PW on BAC PigI-417B2 Amplicon MN	ACTC1-Sf ACTC1-Tr	cgaggccagttccgcatcac agacgtgccaggaggggttgtg	intron 1 intron 2	273	1.0	58	LA DNA polymerase; sequencing
FM212568.1 Amplicon GH	ACTC1-M ACTC1-N	acggctccgggctggtaaa ttccggggaccattccttttg	exon 2 intron 3	1677	1.5	56	LA DNA polymerase; sequencing
Amplicon MN Amplicon MN	ACTC1-O ACTC1-P	acccagggccaagccacatt cctgccaaagtcaagggacatctta	intron 2 intron 2	667	1.5	58	LA DNA polymerase; sequencing
FM212568.1 Amplicon EF	ACTC1-G ACTC1-H	gcccagagcaagcgaggtattc tgagggccccataatcgttgtt	exon 3 intron 5	1551	2.0	56	LA DNA polymerase; sequencing
FM212568.1 FM212568.1	ACTC1-C ACTC1-D	aggccaaccgcgagaagatga ggaagaagcagctgtagccatctca	exon 3 exon 5	1195 (cds 362)	1.5	66	LA DNA polymerase; sequencing, transcription profiling
Amplicon CD Amplicon CD	ACTC1-K ACTC1-L	gtccccggaaaggtgtctgtg tggattcgctggtggagacaa	intron 3 intron 4	837	2.0	62	LA DNA polymerase; sequencing
FM212568.1 Amplicon AB	ACTC1-E ACTC1-F	tgccctggattttgagaatgagatg ccaacacaccccacccacaaa	exon 5 intron 6	1137	1.5	56	LA DNA polymerase; sequencing
FM212568.1 FM212568.1	ACTC1-A ACTC1-B	tccatgaaacgacttacaac catcctgaacgtaaagtagact	exon 6 exon 7	899 (cds 345)	2.0	59	LA DNA polymerase; sequencing; BAC screening, RH mapping, transcription profiling
Amplicon AB PW on BAC PigI-417B2	ACTC1-U ACTC1-V	gcctctaccatgtaccaagcttattg tggcctcagtgtttcaccattaac	intron 6 3' downstream	1089	1.5	58	LA DNA polymerase; sequencing
PW on BAC PigI-417B2	ACTC1-X ACTC1-Y	ggccaatgtttgcttactcagtgta gcctgccctgtgctttatatga	3' downstream 3' downstream	1113	1.5	60	LA DNA polymerase; sequencing
PW on BAC PigI-417B2	ACTC1-W ACTC1-Z	ggctcgggtcgctgctatg tccgggatcaagagatgttttatga	3' downstream 3' downstream	907	HotStar[2]	60	HotStar*Taq* polymerase; sequencing

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Table 1 to be continued

Accession No.; source sequence	Primer name	Primer sequences (5'-3')	Location in genomic sequence	Amplicon size (bp)	$MgCl_2$ (mM)	T_a (°C)	Comments
PW on BAC PigI-417B2	ACTC1-IA ACTC1-IB	tgccacagctcacgacaacat ggtcgaagatgtggctcagatctac	3' downstream 3' downstream	1131	1.5	64	LA DNA polymerase; sequencing
PW on BAC PigI-417B2	ACTC1-3A ACTC1-3B	ctggctgggtattctgtgagtga cgattagtatccctgaggatgtg	3' downstream 3' downstream	484	1.0	55	LA DNA polymerase; sequencing

T_a = annealing temperature, cds = coding sequence

[1]primers were designed on the sequence that was obtained by sequencing (primer walking, PW) of bacterial artificial chromosome (BAC) clone PigI-417B2 (Jouy-en-Josas, France)

[2]LA DNA Polymerases Mix (Top-Bio, Czech Republic); HotStar*Taq* Master Mix Kit (QIAGEN, USA)

mcb_support/documents/generaldocuments/cms_040980.pdf). Amplifications were performed in triplicate and a no-template negative control was included for each RNA isolate and gene.

RESULTS AND DISCUSSION

Comparative sequencing of ACTC1 of Pietrain and Meishan pigs. The obtained genomic sequences, including 5´ and 3´ flanking sequences of *ACTC1* of Pietrain and Meishan pigs, were deposited in the EMBL/GenBank database under accession Nos. FM212566.1 and FM212567.1 (submitted September 29, 2008). The gene encompasses approximately 5.4 kb and is composed of 7 exons. The coding sequence encompasses 1134 bp and the deduced protein contains 377 amino acids (FM212568.1). The genomic sequence is virtually identical with that of ENSSSCG00000004803 (Sscrofa10.2; GenBank Assembly ID GCF_000003025.5) (Groenen et al. 2012), INSDC coordinates CM000812.4, location on SSC1: 152,412,334–152,417,610; Ensembl release 83 – December 2015 (http://www.ensembl.org/index.html). We confirmed the assignment to chromosome 1 by IMpRH mapping. The closest marker (2pt analysis) was *SW65* (41 cR; logarithm of the odds (LOD) = 7.73).

In both Pietrain and Meishan pigs, approximately 8.5 kb were sequenced, including 5' and 3' flanking sequences. The two sequences were identical. One SNP was found in each pig – in Pietrain FM212567.1:g.901C>G and in Meishan FM212566.1:g.889G>A.

The BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) showed the amino acid sequences of ACTC1 in various animal species to be highly homologous. Most vertebrate species studied share 99–100% identity of the amino acid sequences. Similarities of the coding nucleotide sequences are lower, however, mostly in the range of 92–95%. For example, porcine and human ACTC1 amino acid sequences are 100% identical, while the coding nucleotide sequences share 93% similarity.

ACTC1 polymorphism. Both SNPs (FM 212566.1: g.889G>A; FM212567.1:g.901C>G) are located in intron 1. SNP g.889G>A was genotyped after digestion with *Bsa*WI, and SNP g.901C>G after digestion with *Alw*26I. As for SNP g.889G>A, of 34 pigs (Meishan, Pietrain, and crosses Meishan × Pietrain) only one (Meishan) was heterozygous; all

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other pigs were homozygotes *GG*. SNP g.901C>G was more polymorphic. Unrelated pigs of eight breeds were genotyped and allele frequencies are presented in Table 2. The most balanced distribution of alleles was in Czech Large White (allele *C* – 0.68), while in Duroc and Meishan allele *C* was fixed. Other breeds had frequencies of allele *C* from 0.85 to 0.96.

Transcription profiling. When the cDNA samples from the porcine tissues were analysed using PCR primers ACTC1-C/-D and -A/-B, there were some differences in the results, namely that some samples amplified with one primer pair and did not amplify with the other primer pair. Some of these ambiguities were resolved in another experiment, where some questionable tissues were analysed. Altogether, strong expressions with both primer pairs, or with the qPCR primers, were for *m. longissimus dorsi*, *m. biceps femoris*, heart, tongue, diaphragm, jejunum, liver, and spleen; low or very low expressions were for back fat, brain, lymph node, lung, kidney, ileum, colon ascendens (centripetal coil), bladder, pancreas, ureter, aorta, and adrenal gland. In all samples fragments of expected sizes were observed. No expression was determined for oesophagus, stomach, duodenum, appendix, colon ascendens (centrifugal coil), rectum, gall bladder, and mesenterium. Although this approach can be considered as at best semi-quantitative, it does provide information as to whether the gene is or is not expressed.

The *ACTC1* mRNA expressions in pig tissues were studied previously using the porcine Affymetrix expression array (Snowball) (http://biogps.org; Freeman et al. 2012). Those analyses were performed on the mRNAs of two pigs of different sex, and the expressions in some tissues differed appreciably. In the probeset SNOWBALL_000044_s_st, the highest expression was in the heart, lower expression was observed in the retina/sclera of one animal, and the expression in the skeletal muscle (leg) was less than half that of the heart's muscle. Similar or slightly lower expressions were observed in the tongue (dermal layer), oesophagus (upper third), pylorus (smooth muscle), ileum, bladder, gall bladder, abdominal aorta, bone marrow, and spleen. The expression levels in all other tissues were still lower. Compared with other probesets (SNOWBALL_000041_st and SNOWBALL_000047_st) those expressions for individual tissues were quite variable.

Table 2. Allele frequencies at single nucleotide polymorphism FM212567.1:g.901C>G in eight pig breeds

Breed	*n*	*C*	*G*	HWE	
				χ^2	*P*
Czech Large White	20	0.68	0.32	4.72	< 0.05
Czech Landrace	26	0.96	0.04	0.04	> 0.80
Czech Meat Pig	15	0.87	0.13	2.86	> 0.05
Piétrain	26	0.85	0.15	0.37	> 0.50
Prestice Black Pied	11	0.95	0.05	0.03	> 0.80
Hampshire	9	0.94	0.06	0.03	> 0.80
Duroc	15	1.00	0.00	–	–
Meishan	15	1.00	0.00	–	–

HWE = Hardy-Weinberg equilibrium

Gene expression profiling simultaneously compares the expression levels of a gene in many tissues. There can be differences in expression levels depending on the method used, experimental details of the analysis, animal, breed, age, health status, and environmental conditions. It nevertheless provides the first information on the expression of a gene in different tissues, and this can serve as a starting point for more detailed study of expressions, as well as for unraveling the functions of the protein in different tissues. Although the expression results can be validated using RT-qPCR or mRNA sequencing, these methodologies are more costly and labour-intensive and so only a limited number of samples can be analysed.

RT-qPCR of ACTC1 mRNA. Using RT-qPCR, the level of *ACTC1* expression was estimated in foetal muscle, as well as in skeletal and heart muscle of piglets and adult pigs (Figure 1). It can be seen that the expression of *ACTC1* in muscle of foetus and piglets (1–14 days of age) was much higher compared to that of adult pig skeletal muscle. In heart muscle, the *ACTC1* expression in adult pigs was much higher than that in adult skeletal muscle, and the expressions in the piglet heart was clearly higher than that in adult pig heart.

When studying expression of α-cardiac actin mRNA in hearts of neonatal and young rats, Carrier et al. (1992) found that in young rats the expression was higher when compared with more aged hearts. The expression of α-cardiac actin during human muscle development was studied by Ilkovski et al. (2005). They used Western blotting with the specific α-cardiac actin antibodies. The protein was expressed throughout embryonic and

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foetal development until birth, after which expression was markedly downregulated. The α-cardiac actin was also detected in skeletal muscle about 1 month after birth, while it was not detected in the muscle at 6 months and in adults. In the mouse skeletal muscle, Ilkovski et al. (2005) observed low expression of cardiac actin at postnatal day 7, but no expression at 6 months. McHugh et al. (1991) studied the epression of α-cardiac actin in rat muscle and heart. In the skeletal muscle, α-cardiac actin was expressed at low levels during embryonic development and during first postnatal days. This expression then decreased to almost undetectable level in the adult. In the heart, α-cardiac actin was significantly expressed during embryonic and foetal development, as well as during 8 days postnatal, and decreased slightly in adults. Moreover, α-cardiac actin mRNA ratios in the skeletal muscle and heart appeared to be similar to the ratios of the respective proteins in the same tissues.

Although different methodological approaches have been used, it appears that in skeletal muscles of mammals, ACTC1 (both mRNA and protein) is highly expressed during embryonic and foetal development, and then is gradualy downregulated. Appreciable expression continues in the early period of postnatal development but expression is low in adult human and animals. Earlier reported absence of ACTC1 expression in adult humans and mouse may be due to the use of less-sensitive methodologies.

While there is no doubt as to the structural and functional role of ACTC1 in heart and skeletal muscle (e.g. cardiac muscle tissue morphogenesis, cardiac myofibril assembly, heart contraction, cardiac muscle contraction, actomyosin structure organization, and actin-myosin filament sliding), *ACTC1* mRNA is expressed also in several other tissues. In humans, the protein has been found in other tissues, too (Ensembl) wherein the ACTC1 canonical role cannot be expected. It should have other functions, as indicated in the list of gene ontologies (e.g. ATP binding, ATPase activity, myosin binding, positive regulation of gene expression, and several others) (genecards.org/cgi-bin/carddisp.pl?id_type=hgnc&id=143).

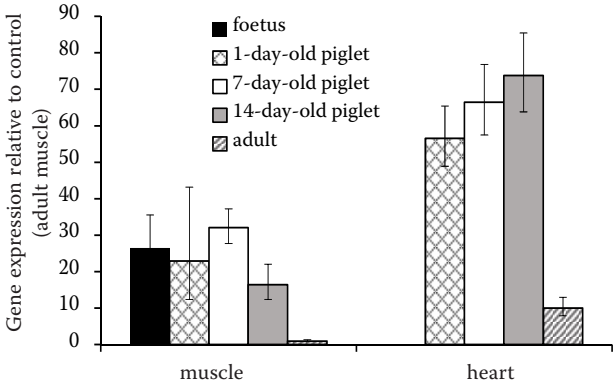

Figure 1. Relative quantification by RT-qPCR of *ACTC1* mRNA expression in hind limb muscle of porcine foetus, in *m. biceps femoris* and heart of 1-, 7-, and 14-day-old piglets, and adult pigs. Expression values are rescaled against the value of the muscle of adult pig (= 1). Bars represent standard error

CONCLUSION

In this study we report on the porcine mRNA and genomic sequences of the *ACTC1* gene. The gene is composed of 7 exons and coding sequence contains 1134 bp. The gene was mapped by IMpRH mapping to chromosome 1 (the closest marker was *SW65*). Transcription profiling was studied in 28 tissues. The expression was high in some tissues (*m. longissimus dorsi*, *m. biceps femoris*, heart, tongue, diaphragm, jejunum, liver, and spleen), low in others (back fat, brain, lymph node, lung, kidney, ileum, colon ascendens (centripetal coil), bladder, pancreas, ureter, aorta, and adrenal gland), and in 8 tissues (oesophagus, stomach, duodenum, appendix, colon ascendens (centrifugal coil), rectum, gall bladder, and mesenterium) there was no expression of *ACTC1*. By using RT-qPCR, in foetal and piglet muscle (1, 7, and 14 days of age) the expression was higher compared to that in adult muscle; *ACTC1* expression in adult porcine heart was higher than that in the skeletal muscle, and the expression in piglets' heart was much higher than that in the heart of adult pigs.

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