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Article

Expression pattern, polymorphisms and association analyses of the porcine *NREP* gene

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Simple Summary: Effective breeding is essential for economical and sustainable pig industry. The search for new genes and their variability involved in myogenesis, meat production and meat quality are part of new, more precise molecular approaches for selective breeding. In this study, differential expression of neuronal regeneration related protein homolog gene (*NREP*) in domesticated and wild pigs were analyzed. Moreover, genetic variability of nine polymorphisms of *NREP* gene were detected and their effects with meat production traits in pigs were statistically analyzed. The results revealed significant differences in expression of gene in skeletal muscles between the two type of pigs. The four polymorphisms were significantly associated with growth and carcass traits. The porcine *NREP* gene can be considered as a candidate gene for meat performance and its variability can be used in modern pig breeding programs.

Abstract: The expression microarray technique was performed to investigate the differences in gene expression between Czech Large White pigs and wild boars in the *longissimus lumborum et thoracis* and *biceps femoris* muscle tissues. The *NREP* gene (neuronal regeneration related protein homolog) was selected for detailed study as an expressional and functional candidate gene. *NREP* plays a role in the transformation of neural, muscle and fibroblast cells and in smooth muscle myogenesis. Quantitative real-time PCR results confirmed that the porcine *NREP* gene was expressed in both skeletal muscles and significantly overexpressed in Czech Large White pigs compared to wild boars ($P < 0.05$). We identified 9 polymorphic sites in genomic DNA of *NREP* gene. Six of these polymorphisms were in complete linkage disequilibrium and therefore only 4 polymorphisms were informative. Associations of these 4 polymorphisms (*HF571253:g.103G>A*, *HF571253:g.134G>A*, *HF571253:g.179T>C* and *HF571253:g.402_409delT*) with meat performance traits were assessed in Czech Large White pigs. New polymorphisms in *NREP* gene were significantly associated with parameters of daily weight gain, lean meat and backfat thickness in Czech Large White pigs. Our primary study suggested that porcine NREP may play an important role in skeletal muscle growth, fat metabolism and meat performance traits.

Keywords: pig; *NREP*; gene expression; polymorphism; SNP; meat performance

1. Introduction

Production traits in pigs, like growth rate, body composition, meat quality and carcass characteristics, play an important role in pig breeding, selection and meat production. The key for the livestock breeding is the identification of responsible genes and pathways that control meat and reproductive performance traits and the use of these genes through marker-assisted selection or genomic selection [1]. Expression analyses, especially comparison of gene expressions between different pig breeds, have a great potential to discover novel genes which affect traits important for

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animal production [2,3]. This approach streamlines the selection of this important genes for subsequent detection of polymorphisms (SNPs) using different breeds and modern molecular genetic methods [4].

The *NREP* gene, also referred to as *C5orf13* or *P311*, is mapped to the long arm of porcine chromosome 2 [5] and is consisted of 10 exons (NC_010444.4 Reference Sscrofa11.1 Primary Assembly). Current research data suggest that it could have a wide range of effects on endocrine factors and their receptors, on transcription factors of myogenesis, and a possible role in the transformation or morphogenesis of neural, muscle and fibroblast cells. In mouse, the mRNA of *NREP* encodes an 8-kDa polypeptide that is rapidly degraded in consequence of degradation by the ubiquitin/proteasome system and an unidentified metalloproteinase. The gene expression of *NREP* is decreased by several pathways that regulate cellular growth and transformation [6]. *NREP* protein was found in myofibroblasts present in human wounds and is involved in preventing fibrosis [7]. The *NREP* gene activate smooth muscle myogenesis and myofibroblast differentiation [8]. In addition, the gene is involved in regulating the accumulation of lipid droplets, evokes the up-regulation of several genes associated with lipid synthesis, and increases the level of intracellular cholesterol, triglyceride and intracellular lipid droplets [9]. *NREP* gene is highly expressed in striated muscles of pigs fed on a low protein and energy diet [10]. Therefore, its gene expression profile was investigated in murine C2C12 muscle cells and the results suggest that *NREP* may be involved in the differentiation of skeletal muscle. Gene expression profiling of piglet muscle with congenital splay leg syndrome showed *NREP* down-regulation. Thus, this gene may play a key role in muscle differentiation [11]. The down-regulation of *NREP* mRNAs was also linked to muscle atrophy in mice and rats [12]. The recent study demonstrated, that *NREP* gene is epigenetically regulated and modulate the expression of several genes involved in the regulation of hepatic lipid metabolism in mice [13].

Tissue expression and the sequence and polymorphic analysis of porcine *NREP* gene to better understand the role of this gene in muscle growth and meat performance traits in pigs were analyzed in this study. For this purpose, the expression levels of *NREP* gene in *longissimus lumborum et thoracis* and *biceps femoris* muscle of the two pig types (domestic Czech Large White pigs and wild boars) were studied. The second aim was to determine whether there is an association between the polymorphisms identified in the gene and selected meat performance traits in Czech Large White pigs.

2. Materials and Methods

2.1. Expression analysis

Tissue samples from *longissimus lumborum et thoracis* and *biceps femoris* muscle were collected from 3 Czech Large White pigs and 3 wild boars. The tissues were immediately submerged in RNA*later* (Ambion, Austin, TX, USA) and stored at -20 °C until RNA isolation. Total RNA extraction was performed with RNeasy Mini Kit (Qiagen, Valencia, CA, USA). RNA concentration and integrity were analyzed using an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The samples were standardized to contain approximately 1 μg of total RNA. Reverse transcription was performed with Omniscript Reverse Transcriptase (Qiagen, Hilden, Germany) and oligo $(dT)_{20}$ primers (Invitrogen, Carlsbad, CA, USA). All steps were performed according to the standard protocol.

The microarray analysis (Affymetrix Genechip Porcine Genome Array) was performed including statistical evaluation using standard Affymetrix protocols at the Institute of Molecular Genetics of the ASCR, Functional Genomics and Bioinformatics Core, Prague, Czech Republic as a service. The porcine *NREP* gene mRNA expression was verified by quantitative real-time PCR with the *HPRT1*, *RPS18* and *OAZ1* genes as internal controls. The primers and PCR conditions to amplify the reference genes were applied according to Nesvadbova et al. [14]. Real-time PCR was performed using the 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) under the following conditions: 2 min of UNG incubation at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C,

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and 1 min at 60 °C. Each PCR 20-µl reaction mixture contained 1X Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 8.4 µl nuclease-free water (Top-Bio, Prague, Czech Republic), 0.2 µl Uracil N-glycosylase (UNG) (Applied Biosystems, Foster City, CA, USA), 0.2 µM forward and reverse primer (NREP_1A and NREP_1B, Table 1) and 1 µl of cDNA sample. For each sample, reactions were set up in triplicate to ensure the reproducibility of the results. The specificity of amplification products was verified by melting curve analysis, agarose gel electrophoresis, and direct sequencing using an ABI PRISM 3100 – Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The gene expression levels of porcine *NREP* transcripts were evaluated using the Relative Expression Software Tool (REST2009 V2.0.13) obtained from Qiagen [15].

2.2. Sequence analysis

Six Czech Large White pigs and wild boars (three each) gDNA samples were used in this study. Complete exon and partial intron sequences of *NREP* gene were amplified, sequenced, and compared. Primers were designed according to the porcine *NREP* sequence (NC_010444.3; Range 122373456..122393645). The polymerase chain reaction (PCR) was performed in 25 µl volumes containing 50 ng of genomic DNA, LA PCR reaction buffer included 2.25 mM $MgCl_2$, 200 µM of each dNTP (Fermentas, Vilnius, Lithuania), 0.2 µM of primer (NREP_2A and NREP_2B or NREP_3A and NREP_3B, Table 1) and 1U LA DNA Polymerases Mix (Top-Bio, Prague, Czech Republic). A PCR thermal profile consisted of pre-denaturation at 95 °C for 2 min; followed by 30 cycles of denaturation at 95 °C for 30 s, annealing temperature (Table 1) for 40 s, elongation at 68 °C for 60 s; and final extension at 68 °C for 7 min. The PCR products after purification were resequenced using the ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The acquired sequences were aligned to the reference sequence of the porcine *NREP* gene using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Table 1. Primers used in the study

Primer name	Primer sequence (5′-3′)	T_a (°C)	Product size (bp)	Amplified region
NREP_1A NREP_1B	ACTCCACTCGGCAGCAATG GACCTCATCAATACCCATACACCA	60	112	Partial cDNA
NREP_2A NREP_2B	ACCACTTTGCTTGCTAATGATAA TTTAGGATGTTCTTTTTCTTTTCTG	60	799	Partial intron 1, exon 2, partial intron 2
NREP_3A NREP_3B	GCCCAGCCATTAGAGAAACA ACTTACCACAAGGAAACAATGAGT	63	888	Partial intron 2, exon 3, partial 3′UTR region

2.3. Association analysis with meat performance traits

Direct sequencing was used to determine 8 polymorphic sites and PCR-RFLP was used to determine the one polymorphism. For this, the PCR primers and conditions were the same as those used for polymorphism identification by sequencing. Restriction analysis was performed in a total volume of 15 µl with 2U of *Dde*I (BioLabs, New England, MA, USA) at 37 °C overnight.

A total of 98 DNA samples from unrelated animals of Czech Large White sows were analyzed and the genotype and allele frequencies were determined. Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by the chi-square test (package HardyWeinberg 1.6.6 in R v4.0.2 [16]).

Records from 98 randomly selected animals (born between 2003 and 2008) for meat performance traits obtained in a field test were as follows: backfat thickness (BFT, cm) and lean meat content (LM, %) measured with ultrasonic apparatus SonoMark SM-100 M at the end of the test; average daily gain (ADGb, g) calculated from birth to 90 kg of live weight; average daily gain in test (ADGt, g) calculated from the start to the end of the test; breeding value of average daily gain from birth (BVadg), breeding

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value for lean meat content (BVlm), breeding value for reproduction (BVr), and total breeding value (TBV) estimated using the BLUP Animal Model (the field test started when the animals were 12 weeks of age and lasted for approximately 57 days).

Association analysis was performed using a Mixed Linear Model (MLM Procedure with REML method) by SAS v9.4 statistical software [17] using this equation with fixed and random effects:

$y_{ijklmno} = \mu + G_i + G_j + G_k + G_l + F_m + M_n + e_{ijklmno}$,

where:

$y_{ijklmno}$ = the phenotypic value of the analyzed trait,

μ = the overall population mean,

G_i = the effect of the i^{th} genotype of the SNP HF571253:g.103G>A, (2 levels: GG, AG),

G_j = the effect of the j^{th} genotype of the SNP HF571253:g.134G>A, (2 levels: GG, AG),

G_k = the effect of the k^{th} genotype of the SNP HF571253:g.179T>C, (2 levels: TT, CT),

G_l = the effect of the l^{th} genotype of the polymorphism HF571253:g.402_409delT, (2 levels: 67, 77),

F_m and M_n = random effects of the gilt's father (21 levels) and the gilt's mother (52 levels), respectively,

$e_{ijklmno}$ = random residual effect of each observation.

Due to several independent trials in models, Bonferroni correction was performed. The statistical results were presented as the lest square mean ± SE.

3. Results

3.1. Gene expression analysis of porcine skeletal muscle tissues

To detect differences in the expression of the *NREP* gene between the two breeds, an expression analysis was carried out using microarray and quantitative real-time PCR. The microarray analysis showed that expression levels in the *musculus longissimus lumborum et thoracis* and *biceps femoris* were 12.78- and 12.03-fold higher ($P < 0.01$) in Czech Large White pigs compare to wild boars. These microarray results were subsequently verified by a real-time PCR method. The results of real-time PCR analysis revealed that the expression of the porcine *NREP* gene in the *longissimus lumborum et thoracis* and *biceps femoris* muscles of Czech Large White pigs was significantly higher than that in wild boars. This gene is up-regulated in Czech Large White pigs (in comparison to wild boars) by a mean factor of 14.50 ($P < 0.05$) and 11.64 ($P < 0.01$) for *musculus longissimus lumborum et thoracis* and *biceps femoris*, respectively.

3.2. Polymorphism identification and analysis of genetic variability

Results of the sequencing were deposited to GenBank under Acc. No. HF571253, HF571254, HF571255 and HF571256. Sequence comparisons between Czech Large White pigs and wild boars revealed 9 polymorphic sites in the porcine *NREP* gene. Five SNPs were identified in the intron 1 (*HF571253:g.103G>A*, *HF571253:g.134G>A*, *HF571253:g.179T>C*, *HF571253:g.181G>A*, *HF571253:g.210C>T*), two deletions and one substitution in intron 2 (*HF571253:g.380_388delT*, *HF571253:g.402_409delT*, *HF571253:g.401CA>TG*) and one SNP (*HF571256:g.295C>T*) in exon 3. We analyzed these polymorphisms in 247 unrelated Czech Large White pigs from three herds. Polymorphisms in the intron 1 and intron 2 were tested by means of sequencing (799 bp PCR amplicon). Polymorphism *HF571256:g.295C>T* was tested using PCR-RFLP (888 bp PCR amplicon, digestion with *Dde*I) resulting in allele *C* (83, 151, 167 and 487 bp) and allele *T* (83, 151, 167, 193 and 294 bp).

In our population comprising 98 individuals, the polymorphic sites *HF571253:g.103G>A*, *HF571253:g.181G>A*, *HF571253:g.210C>T*, *HF571253:g.380_388delT*, *HF571253:g.401CA>TG* and *HF571256:g.295C>T* were in complete genetic linkage disequilibrium. Therefore, only 4 polymorphisms in the *NREP* gene were considered informative: *HF571253:g.103G>A*, *HF571253:g.134G>A*, *HF571253:g.179T>C* and *HF571253:g.402_409delT*. The frequencies of genotypes and alleles, and compatibility with Hardy-Weinberg equilibrium are shown in Table 2. Analysis of genetic variation in four SNPs revealed that genotype and allele frequencies in herd of Czech Large

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White pigs were comparable. The frequency of one of the homozygous genotypes in all loci was very low (0–0.03). Heterozygous individuals were from 0.08 to 0.33 and the second type of homozygous genotype had a significant incidence (0.64–0.91). Except for locus *HF571253:g.103G>A*, where the allele frequencies were 0.05 and 0.949; the allele frequencies at the other loci *HF571253:g.134G>A*, *HF571253:g.179T>C* and *HF571253:g.402_409delT* were similar: 0.168, 0.133 and 0.179, respectively. and 0.832, 0.867 and 0.821, respectively. Results show that the population was in the HWE for all polymorphisms. However, this may reflect the actual genetic constitution for corresponding locus in commercial herd.

Table 2. Genotypic and allelic frequencies and χ^2 test of HWE with p-value of SNPs

SNP locus	Genotype frequencies (number)			Allele frequencies		χ^2 HWE (p-value)
HF571253:g.103G>A	*AA*	*AG*	*GG*	*A*	*G*	0.3390
	0.01 (1)	0.08 (8)	0.91 (89)	0.051	0.949	(0.5604)
HF571253:g.134G>A	*AA*	*AG*	*GG*	*A*	*G*	0.02909
	0.03 (3)	0.28 (27)	68 (0.69)	0.168	0.832	(0.8646)
HF571253:g.179T>C	*CC*	*TC*	*TT*	*C*	*T*	1.2754
	0 (0)	26 (0.265)	72 (0.735)	0.133	0.867	(0.2587)
HF571253:g.402_409delT	*66*	*67*	*77*	*6*	*7*	1.3741
	1 (0.01)	33 (0.34)	64 (0.65)	0.179	0.821	(0.2411)

χ^2, Chi-square value; HWE, Hardy–Weinberg equilibrium.

3.3. Association of genotypes of SNPs with meat performance

We performed an association study to determine the effect of these polymorphisms on some traits in pigs. The one of the homozygous genotypes was exclude from analysis due to a very small number of animals (< 5). In Table 3, all SNPs had significant associations with at least one analyzed trait. We found significant associations *HF571253:g.103G>A* (informative SNP representing polymorphic sites in complete linkage disequilibrium) with backfat thickness and lean meat ($P < 0.05$). Animals with *GG* genotypes had significantly higher LM and lower BFT then with *AG* genotypes. The SNP *HF571253:g.134G>A* was associated with ADGb ($P < 0.05$), ADGt ($P < 0.01$), and BVadg ($P < 0.05$). Animals with *GG* genotypes had better parameters of traits. The polymorphisms of *HF571253:g.179T>C* and *HF571253:g.402_409delT* were statistically significantly associated ($P < 0.05$) with BFT, LM, ADGb, BVadg, and TBV, where animals with *TT* and *67* genotypes had better performance.

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Table 3. Association analysis of porcine *NREP* polymorphisms with meat performance traits (Least square means LSM ± standard error SE)

SNP	BFT	LM	ADGb	ADGt	BVadg	BVlm	BVr	TBV
HF571253:g.103G>A								
AG (n = 8)	0.86 ± 0.09^{a}	62.02 ± 1.02	542.86 ± 46.53	916.85 ± 93.69	19.12 ± 7.21	1.49 ± 0.31	1.26 ± 0.30	977.87 ± 174.76
GG (n = 86)	0.65 ± 0.02^{a}	63.69 ± 0.24	610.87 ± 9.62	996.06 ± 19.82	25.45 ± 2.96	1.29 ± 0.10	1.34 ± 0.10	1187.73 ± 75.57
HF571253:g.134G>A								
AG (n = 27)	0.75 ± 0.05	62.87 ± 0.57	562.39 ± 25.68^{a}	920.67 ± 51.90^{A}	19.83 ± 4.60^{a}	1.38 ± 0.18	1.30 ± 0.18	1056.63 ± 112,27
GG (n = 67)	0.76 ± 0.05	62.85 ± 0.55	591.34 ± 25.45^{a}	992.24 ± 49.60^{A}	24.74 ± 4.43^{a}	1.40 ± 0.18	1.29 ± 0.17	1108.97 ± 109.06
HF571253:g.179T>C								
TT (n = 68)	0.63 ± 0.02^{a}	64.06 ± 0.29^{a}	627.52 ± 12.03^{a}	1032.29 ± 24.87	31.72 ± 3.20^{a}	1.42 ± 0.11	1.29 ± 0.10	1233.37 ± 80.28^{a}
TC (n = 26)	0.88 ± 0.09^{a}	61.66 ± 1.05^{a}	526.21 ± 47.34^{a}	880.62 ± 95.92	12.85 ± 7.54^{a}	1.37 ± 0.32	1.31 ± 0.31	932.24 ± 179.13^{a}
HF571253:g.402_409delT								
67 (n = 33)	0.64 ± 0.02^{a}	63.92 ± 0.30^{a}	625.59 ± 12.79^{a}	1028.52 ± 26.34	30.90 ± 3.29^{a}	1.35 ± 0.12	1.30 ± 0.11	1216.17 ± 82.05^{a}
77 (n = 61)	0.87 ± 0.09^{a}	61.80 ± 1.05^{a}	528.14 ± 47.62^{a}	884.38 ± 96.41	13.68 ± 7.55^{a}	1.44 ± 0.32	1.30 ± 0.31	949.44 ± 179.82^{a}

n total number of investigated pigs, *BFT* backfat thickness (cm), *LM* lean meat content (%), *ADGb* average daily gain from birth (g), *ADGt* average daily gain in test (g), *BVadg* breeding value for average daily gain, *BVlm* breeding value for lean meat content, *BVr* breeding value for reproduction, *TBV* total breeding value; the same superscripts in a column show significant differences: ^{A} $P < 0.01$, ^{a} $P < 0.05$ between genotypes.

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4. Discussion

The identification of differentially expressed genes related to muscle development and growth is very important for genetic and physiological studies related to pig meat quality [18]. To better understand the development and growth of muscle, gene expression profiling was performed in *longissimus lumborum et thoracis* and *biceps femoris* skeletal muscle of Czech Large White pigs and wild boars using the Affymetrix Porcine Genechip (unpublished data). Comparative expression analysis showed significantly higher gene expression in Czech Large White pigs compared to wild boards in the *longissimus lumborum et thoracis* and *biceps femoris* muscle. These findings were confirmed using quantitative real-time PCR. The relatively high expression level of the porcine *NREP* gene in the skeletal muscle of Czech Large White pigs may indicate its important role in regulating skeletal muscle growth. This is a similar finding to that of another study in which the *NREP* gene was highly expressed in striated muscles of diet-restricted growing pigs [10]. The *NREP* gene was relatively down-regulated in the muscles of piglets with porcine congenital splay leg syndrome, which is characterized by lameness and immobility immediately after birth. These piglets also exhibited smaller muscle fibre size [11]. In accordance with this study, the *NREP* gene had a lower relative expression in the muscles of wild boars, where the size of muscle fibers is smaller compared to commercial breeds of pigs [19].

As mentioned above, NREP was of particular interest because of its putative role in the induction of myofibroblast phenotype and muscle myogenesis [9,11]. The *NREP* gene is assigned to porcine chromosome 2 [5]. Several studies in different populations reported quantitative trait loci (QTLs) affecting production traits [20] and meat quality traits [21] on porcine chromosome 2. So far, several studies have also shown that this chromosome hides important genes for meat production traits. e.g., *MyoD*, *CAST* or *IGF2*. Moreover, *NREP* is involved in gene expression stimulation of muscle-related transcription factors, like *MyoD*, *myogenin*, *SRF*, and *MEF2*, which are involved in smooth and striated muscle differentiation [8]. Several experiments have proved that these genes may affect the growth and other production traits of pigs. The gene expression of *MyoD* suggests that higher muscularity in Pietrain pigs is associated with the presence of a larger number of active satellite stem cells in postnatal porcine skeletal muscles [22]. Satellite cells play a primary role in hyperplastic and hypertrophic growth in skeletal muscle and their proliferation and differentiation is regulated by the genes of the *MyoD* gene family. For this reason, postnatal expression levels of gene *MyoD* and *myogenin* can also related to growth rate [23]. Some recent publications also demonstrate that the *NREP* gene downregulates *TGF-β1* and *TGF-β2* in part by blocking TGF-β autoinduction [8,24]. As is generally known, TGF-β superfamily members have been shown to have potent effects on both muscle development and postnatal skeletal muscle mass [25].

Previous studies have also shown that the *NREP* gene regulates lipid droplet synthesis and accumulation [9]. In our study, the allele *G* at locus *HF571253:g.103G>A*, allele *T* at locus *HF571253:g.179T>C* and allele *6* at locus *HF571253:g.402_409delT* had a positive effect on lower level of backfat thickness. Furthermore, allele *T* at locus *HF571253:g.179T>C* and allele 6 at locus *HF571253:g.402_409delT* were associated with increased lean meat content. Also allele *G* at locus *HF571253:g.103G>A* showed an increased tendency for this parameter ($P < 0.10$). The allele *G* at locus *HF571253:g.134G>A* was associated with higher values of all parameters of daily gain ($P < 0.05$). Also, allele *T* at locus *HF571253:g.179T>C* and allele *6* at locus *HF571253:g.402_409delT* were associated with higher level of ADGb and BVadg.

Homozygous animals (*GG*, *GG* and *TT*) had better meat performance in most polymorphisms of *NREP* gene (*HF571253:g.103G>A*, *HF571253:g.134G>A* and *HF571253:g.179T>C*, respectively), than those of heterozygotes. On the contrary, in *HF571253:g.402_409delT* polymorphism, heterozygotes give better results.

Meat performance traits of pigs are complex characteristics which are influenced by other genetics, environmental and probable also epigenetic factors, as suggest recent research of epigenetic regulation by DNA methylation in the TGF-β family members in mice [13]. In our study, it was found that polymorphic sites of porcine *NREP* are in intron 1, intron 2 and exon 3, although we found

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significant associations with meat performance. SNP *HF571256:g.295C>T* in exon 3 is silent mutation and, therefore, this polymorphism is also unlikely to have a direct effect. Although mutations in introns causing the phenotype manifestation of a gene are today known in animals (e.g. SNP in the *IGF2* gene in pigs [26]), it is likely that our polymorphisms are not causative, but linked with another polymorphisms.

5. Conclusions

Based on these results, we can deduce that the porcine *NREP* gene is a candidate gene for meat performance traits of pigs, especially for backfat thickness, lean meat, and average daily gain. Notably growth rate measured as average daily gain is often used as one of the main selection criteria of farm animals. Polymorphic sites could be used as genetic markers that are in linkage disequilibrium with an unknown causative mutation in coding sequences affecting meat performance traits of pig. However, to confirm these results, further studies are required to investigate the associations of these polymorphisms in the *NREP* gene with meat performance traits in larger groups of pigs. Finally, the results of our study provide first important information about the variability and expression in porcine *NREP* gene and useful knowledge for pig breeding.

Author Contributions: Experimental design and concept, A.K.; sampling, M.N.; data and statistical analysis, A.K:, T.U.; gene expression M.N., writing first draft of the manuscript, all authors; final revision of the manuscript, A.K., T.U. All authors have read and agreed with to the published version of the manuscript.

Funding: This research was funded by project GACR 523/09/0844 and realized in project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II.

Acknowledgments: The authors thank prof. Antonín Stratil (Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, v.v.i, Czech Republic) and dr. Pavel Horák for providing DNA samples for microarray analysis and the Institute of Molecular Genetics of the ASCR, Functional Genomics and Bioinformatics Core, Prague, Czech Republic for microarrays analysis.

Conflicts of Interest: The authors declare that they have no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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