GUANINE NUCLEOTIDE BINDING PROTEIN (*GNAS* COMPLEX LOCUS) GENE PRODUCES BIALLELICALLY EXPRESSED AND PATERNALLY EXPRESSED TRANSCRIPTS IN PIGS*

Maria Oczkowicz^{1*}, Agata Piestrzyńska-Kajtoch², Katarzyna Ropka-Molik¹

¹Laboratory of Genomics,

²Department of Animal Cytogenetics and Molecular Genetics,
National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

*Corresponding author: maria.oczkowicz@izoo.krakow.pl

Abstract

Imprinted loci are a subset of genes expressed only from one parental allele. Guanine nucleotide binding protein (*GNAS* complex locus) produces a few different proteins and noncoding RNAs. It is imprinted in mice and humans, while in pigs imprinting status of only one of them – *NESP55* (neuroendocrine secretory protein-55) has been established. In the present study we aimed to establish imprinting status of the other two *GNAS* transcripts. We collected a panel of tissues (muscle, liver, kidney cortex, heart, fat, ovary, brain, blood) from the animals (aged 40–210 days) heterozygous in rs#333005482 polymorphism. We performed several RT-PCRs (Reverse Transcription Polymerase Chain Reactions) with variant specific primers and sequenced the cDNA products obtained. We observed only paternal allele in all tissues at all developmental stages after sequencing with primers specific to variant 8 of *GNAS* gene and both alleles when sequencing with primers specific to variant 6. Our results show for the first time that, in pigs, *GNAS* complex locus produces biallelically expressed and paternally expressed transcripts, in the same way as previously demonstrated in mice and humans

Key words: *GNAS*, imprinting, pigs, *NESP55*

Imprinted genes, contrary to the rest of the genes in the genome, are expressed monoallelically in the offspring. Among vertebrates, imprinting is present only in mammals and its evolutionary justification is still debated; however, the most convincing hypothesis is the so called “parental conflict” theory (Moore and Haig, 1991). Most of imprinted genes play a crucial role in growth and development. That

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is why this class of genes includes many interesting candidate genes for Marker Assisted Selection (MAS) in farm animals. Currently, most of the QTL (Quantitative Trait Locus) experiments include analysis of the manner of inheritance. What is more, polymorphism in the imprinted *IGF2* gene (Van Laere et al., 2003), affecting muscle growth is presently included in MAS by commercial companies.

GNAS gene codes for several different proteins. The best studied is $G\alpha$ – Guanine nucleotide-binding protein (G protein). It plays a crucial role in transmembrane signaling pathways and the mutations which disturb its expression are responsible for the occurrence of Albright's hereditary osteodystrophy in 60–70% of patients (Elli et al., 2013). One of the symptoms of this disorder is obesity, therefore *GNAS* is an interesting candidate gene for economically important traits in farm animals. Another protein produced by *GNAS* complex locus is *XLas* – a large form of $G\alpha$, which affects early postnatal growth and energy glucose metabolism and *NESP55* (neuroendocrine secretory protein 55), which probably regulates the behavior in a novel environment (Plagge et al., 2005).

In pigs, *GNAS* gene is located on chromosome 17 (66,304,009-66,423,430) (Sscrofa 10.2.). To date, eight different variants of *GNAS* transcripts (1, 3, 4, 6, 7, 8, 9, 10) have been identified in pigs (<http://www.ncbi.nlm.nih.gov/nucore>); however, as many as twenty three are predicted in Ensembl database (<http://www.ensembl.org/index.html>). In human and mice, different *GNAS* transcripts are oppositely imprinted in most tissues. The transcript, which codes for *NESP55*, is maternally expressed (Hayward et al., 1998 b; Peters et al., 1999), $G\alpha$ transcripts are biallelically expressed (Campbell et al., 1994; Hayward et al., 1998 a), while noncoding transcripts are paternally expressed (Liu et al., 2000). Recently, Congras et al. (2014) have found that methylation status of *GNAS* gene in infertile boars is different from their fertile counterparts, which suggests that *GNAS* gene may play a role in proper formation of male gametes. In pigs, only maternal expression of *NESP55* transcript has been experimentally proved (Oczkiewicz et al., 2012). Some projects aimed to establish the imprinting status of the *GNAS* gene yet did not succeed because of complex pattern of splicing (Bischoff et al., 2009). In the present study, we designed primers specific to porcine variant 6 (coding for $G\alpha$) and variant 8 (non-coding RNA corresponding to mouse variant 1A) and demonstrated for the first time that *GNAS* complex locus produces biallelically expressed and paternally expressed transcripts in pigs.

Material and methods

Animals

Material for the study was selected from the samples collected previously for the other experiments (Rejduch et al., 2010 and unpublished data). All animals were kept in the Experimental Testing Station of the National Research Institute of Animals Production in Pawlowice. In total, 13 gilts of different breeds, slaughtered at different age (40–210 days of age (doa), were investigated as stated in Table 1.

Table 1. Alleles observed in rs#33005482 locus in DNA of the parents of analyzed animals and in the cDNA of these animals for two variants of GNAS complex locus. Doa – days of age, x – parental DNA was not available. Grey shaded rows indicate informative samples, evidencing paternal expression of variant 8

Sample	Variant	Father	Mother	Offspring	Muscle	Liver	Heart	Kidney	Brain	Fat	Blood	Ovary	Doa
1	v6	CT	X	CT	CT	CT	CT	CT	CT	^	^	^	60
1	v8	CT	X	CT	C	C	C	C	C	^	^	^	60
2	v6	CT	X	CT	CT	CT	CT	CT	CT	^	^	^	60
2	v8	CT	X	CT	T	T	T	T	T	^	^	^	60
3	v6	CT	CT	CT	CT	CT	CT	CT	CT	^	^	^	60
3	v8	CT	CT	CT	C	C	C	C	C	^	^	^	60
4	v6	TT	CT	CT	CT	CT	CT	CT	CT	^	^	^	210
4	v8	TT	CT	CT	T	T	T	T	T	^	^	^	210
5	v6	CT	X	CT	CT	CT	CT	CT	CT	^	^	^	210
5	v8	CT	X	CT	T	T	T	T	T	^	^	^	210
6	v6	TT	X	CT	CT	CT	CT	CT	CT	^	^	^	210
6	v8	TT	X	CT	T	T	T	T	T	^	^	^	210
7	v6	CC	TT	CT	CT	^	CT	CT	CT	CT	^	^	40
7	v8	CC	TT	CT	C	^	C	C	C	C	^	^	40
8	v6	CC	TT	CT	CT	^	^	^	CT	^	CT	CT	180
8	v8	CC	TT	CT	C	^	^	^	C	^	C	C	180
9	v6	CC	TT	CT	CT	^	^	^	CT	^	CT	CT	180
9	v8	CC	TT	CT	C	^	^	^	C	^	C	C	180
10	v6	CT	X	CT	CT	CT	^	^	^	^	^	^	90
10	v8	CT	X	CT	C	C	^	^	^	^	^	^	90
11	v6	TT	CT	CT	CT	CT	^	^	^	^	^	^	180
11	v8	TT	CT	CT	T	T	^	^	^	^	^	^	180
12	v6	CT	CT	CT	CT	CT	^	^	^	^	^	^	180
12	v8	CT	CT	CT	T	T	^	^	^	^	^	^	180
13	v6	CT	X	CT	CT	CT	^	^	^	^	^	^	180
13	v8	CT	X	CT	T	T	^	^	^	^	^	^	180

Tissue and blood sampling

Immediately after the slaughter, samples of tissues were frozen in liquid nitrogen or immersed in RNAlater® Stabilization Solution (Life Technologies, California, USA). The whole blood was collected into the tubes containing EDTA from all animals and from their parents, when available. Blood samples for RNA analysis were collected into the Tempus® tubes (Life Technologies). The summary of the collected samples is presented in Table 1.

RNA and genomic DNA extraction

In order to obtain the best quality of RNA, it was isolated with different methods, depending on the tissue type. RNA from the muscle, liver, kidney and heart was extracted with TRI Reagent® Solution (Life Technologies), as described by Chomczyński and Sacchi (1987), while from the brain, with SV Total RNA Isolation Kit (Promega, Madison, USA). Moreover, RNA from the ovary was extracted with PureLink RNA Mini Kit (Life Technologies), and from the blood with Mag-MAX™-96 Blood RNA Isolation Kit (Life Technologies). RNA samples were treated with DNase or purified by LiCl (Life Technologies). RNA quantity and quality was established with Nanodrop 2000 (Thermoscientific, Wilimington, USA) and 1.5% agarose gel electrophoresis.

DNA was isolated from the whole blood using Wizard Genomic Purification Kit (Promega) in accordance with the manufacturer's protocol with minor modifications.

PCR-RFLP genotyping

GNAS AM490165:g.324C>T polymorphism (*rs#333005482* – accession number of dbSNP NCBI) was genotyped in DNA samples from all animals and available parents (23 samples) by PCR-RFLP using primers designed by Stratil *et al.* (2008) and AmpliTaq Gold® 360° DNA Polymerase (Life Technologies) according to the manufacturer's protocols. PCR thermal conditions were as follows: 95° for 10 min, 30 cycles of 95° for 30 sec, 54° for 30 sec and 72° for 1 min and 10 min of final elongation in 72°. After 24 h of PCR product digestion with *Hpy*CH4IV enzyme (Biolabs, Ipswich, USA), the reaction products (fragments of 549bp+151bp for allele T and 387bp+162bp+151bp for allele C) were subjected to the electrophoresis in a 3% agarose gel.

cDNA synthesis and sequencing

High capacity cDNA Archive Kit (Life Technologies), containing random hexamers was used to produce first strand of cDNA from 1000 ng of each RNA sample, according to the manufacturer's protocol. Control without reverse transcriptase was included in the experiment and revealed lack of contamination.

Fragments of *GNAS* gene (chr17: 66,421,590-66,422,289), containing *rs#333005482* polymorphism, were sequenced in DNA from one triplet (offspring, mother, father), with the same primers as used for PCR-RFLP (Stratil *et al.*, 2008).

Two PCR primer pairs were designed in order to separately amplify putatively paternally expressed variants (PCR1) and biallelic variants (PCR2). It was impossible to design the primers separately for each transcript, because they have only small unique fragments of coding sequence (Figure 1). PCR1 primers (F1 – ACTGC-CTTGCGTGTGAGT, start: chr17:66,350,405, Rev1 – CCGAATGACCATGTTGTAGC, start: chr17:66,421,938) were specific only to variants 8, 9 and 10 of *GNAS* complex locus – accession numbers: NR_033291.2, NR_033292.2, NR_073434 respectively. PCR2 primers were complementary only to variants: 1- (NM_214312.3), 3- (NM_001171983.2), 6- (NM_001171984.2) and 7- (NM_001171985.2). Their sequences were as follows: F2 – CCGCCATGGGCTGTCT (start: chr17:66,352,835), R1 – CCGAATGACCATGTTGTAGC (start: chr17: 66,421,938) (Figure 1).

Figure 1. Distinction of *GNAS* transcripts; exons are indicated; Access No NC_010459.4; v1 – variant, 1 NM_214312.3; v3 – variant 3, NM_001171983.2; v6 – variant 6, NM_001171984.2; v7 – variant 7, NM_001171985.2; v8 – variant 8, NR_033291.2; v9 – variant 9, NR_033292.2; v10 – variant 10, NR_073434.1; F1 – forward primer for PCR1; F2 – forward primer for PCR2; R1 – reverse primer for PCR1 and 2; v6F – forward primer for sequencing transcript variant 6; v8F – forward primer for sequencing transcript variant 8; dark grey – transcripts amplified in PCR1; light grey – transcripts amplified in PCR2

Two PCRseq forward primers complementary only to variant 6 (v6F- GCAA-GGAGCAACAGCGATGGTAG, start: chr17: 66,411,891) or only to variant 8 (v8F-GACCCGCGAGGCTGCAAGGAG, start:chr17: 66,411,879) were designed for sequencing distinct variants (Figure 1). Primers of beta actin were as follows: F: 5'-TCCCTGGAGAAGAGCTACGA -3', R: 5'-CGCACTTCATGATCGAGTTG -3' (Bratuś and Słota, 2009). All primers were designed with Primer 3 software (<http://primer3.wi.mit.edu/>).

The PCRs were performed using 1 µL of cDNA, REDTaq® ReadyMix™ PCR Reaction Mix (Sigma-Aldrich, Saint Louis, MO, USA) and 10 pmol of primers. Thermal conditions were as recommended by the manufacturer. The annealing temperature was 59°C for both primer pairs. According to the current databases, the first

PCR should provide the mix of three products 668 bp (v8), 623 bp (v9) and 626 bp (v10) and we have used it as a matrix for PCRseq with primers complementary to variant 8, while the second PCR should provide the mix of four products: 779 (v1), 737 (v3), 782 bp (v6) and 734 bp (v7) and was a matrix for PCRseq with primers complementary to variant 6. The electrophoresis was carried out on agarose gel 3%, Midori Green stained (Nippon Genetics, Dueren, Germany), for 1 hour at 75V. Obtained PCR products were treated with ExoSAP-It® (Affymetrix, California, USA) and 1.5 µl of purified products was used for PCRseq. PCRseq was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and 8 pmols of primer specific to variant 6 or 8 according to the manufacturer's recommendation. The fast thermal conditions and annealing temperature of 57°C were applied. Next, reaction products were purified with BigDye XTerminator® Purification Kit (Life Technologies) and analyzed on Genetic Analyzer 3130xl sequencer (Life Technologies). The same procedure was implemented for DNA sequencing. The sequencing results were analyzed with Finch TV software (<http://www.geospiza.com/Products/finchtv.shtml>).

Results

For the experiment, we have chosen thirteen animals, heterozygous at rs#333005482. Both parents of three animals (samples 7, 8, 9) were opposite homozygotes, while only the father of other three of them was homozygotic (samples 4, 6, 11) (Table 1). We were able to establish the parental origin of expressed allele for six animals (Table 1, grey shaded), while for the other animals we could only discriminate between monoallelic and biallelic expression because their parents were heterozygous or parental samples were not informative. In total we analyzed nine tissues (muscle, liver, brain, blood, ovary, kidney cortex, heart, fat), however not all tissues were available for all animals (Table 1). PCRs with primers specific to putatively paternally expressed transcripts (PCR1) and with primers specific to biallelic transcripts (PCR2) revealed the presence of strong products in all tissues for both PCRs (Figure 2), which suggests that *GNAS* transcripts are ubiquitously expressed. After 4 hours of electrophoresis, the *GNAS* bands started to separate into individual transcripts (data not shown), however the differences between individual transcripts were too small to visibly discriminate bands. After cDNA sequencing of PCR1 product with primer complementary only to variant 8 we observed monoallelic expression in all samples. In the samples, for which parental genotypes were available (samples 4, 6, 7, 8, 9, 11) we noted that expressed allele comes from the father (Figure 3 a–c). cDNA sequencing of PCR2 product with primer specific to variant 6 revealed that in all analyzed samples biallelic expression of analyzed transcript was present. We investigated samples at different developmental stages (40–210 doa) and found no difference between young and adult animals as well as between analyzed tissues.

Figure 2. Electrophoresis of *GNAS* PCR products; agarose gel 3%, Midori Green stained (Nippon Genetics), 75V, 1 hour electrophoresis; m – muscle, h – heart, k – kidney, l – liver, o – ovary; b – brain, bl – blood, the numbers after the letters indicates developmental stage (60–60 days of age, 180–180 days of age, 210–210 days of age), DNA – control porcine DNA sample, nc – negative control, M – 100bp DNA Ladder (Promega); PCR1 – PCR for transcript variants 8, 9, 10; PCR2 – PCR for transcript variants 1, 3, 6, 7; beta-actin – control PCR with porcine beta-actin primers

Discussion

GNAS complex locus produces many alternative splicing variants in pigs. To date, eight different variants have been deposited to the nucleotide NCBI database (variants 1, 3, 4, 6, 7, 8, 9, 10). The imprinting status of variant 4 was established previously (Oczkiewicz et al., 2012), while the other variants were not investigated to date. In order to establish imprinting status of two other variants (variants 6 and 8) we have chosen rs#333005482, which was highly polymorphic in the material collected for the previous experiments (data not shown). Polymorphism rs#333005482 mutation is located in the 10th exon of *GNAS* variant 1 and its location enabled us to evaluate the imprinting status of two different *GNAS* variants. Recently, we have shown that this polymorphism, together with neighboring mutation is associated with growth and carcass traits in pigs (Oczkiewicz et al., 2013).

To date, there are four protein coding reference *GNAS* transcripts in the NCBI Nucleotide database. All of them code for different isoforms of Gs α protein. Our experimental approach aimed to investigate only one of the reference *GNAS* transcripts (Variant 6). Another *GNAS* transcript – variant 1 (NM_214312.3) uses alternate splice site and is only three nucleotides longer, therefore we cannot exclude that it was present in our sample. Human Gs α is biallelically expressed in most tissues while predominantly maternally expressed in thyroid, pituitary and gonads (Mantovani et al., 2002). Recently, preferential expression of maternal allele of Gs α in some tissues (lymphoblasts, peripheral blood mononuclear cell, mammary adipose tissue, heart) has been shown by pyrosequencing (Klenke et al., 2011). The advantage of

maternal allele did not exceed 60% of total expression; however, it was statistically significant. In our study, we did not statistically compare the area of peaks on chromatograms – in most tissues both parental alleles were equally expressed, including fat (adipose tissue). Nevertheless, when the cDNA from the ovary was sequenced we always observed that the area of maternal allele is larger than the area of the paternal allele (Figure 3 c). In mice, *Gsa* is maternally expressed in thyroid, white and brown adipose tissue, and renal cortex but biallelically expressed in renal medulla (Yu et al., 1998). Contrary to previous experiments performed in mice, we found biallelic expression of variant 6 (which produces *Gsa*) in all samples of kidney cortex. This finding suggests that imprinting of *Gsa* is not conserved in porcine kidney cortex; however, a detailed examination of the inner and outer parts of the kidney would give the final conclusions.

Porcine variant 8 of *GNAS* complex locus is a noncoding RNA. Currently two other transcripts produced by the same promoter are deposited in the NCBI Nucleotide database (variant 9 and 10) in pigs. Our assay was designed to sequence only one reference NCBI *GNAS* transcript. On the other hand, in the Ensembl database there are 10 transcripts predicted to be a noncoding RNA. Among them, there are three transcripts which could be amplified in our assay. Therefore, most probably our results are not restricted only to variant 8. In human and mice, noncoding transcripts are called A/B transcripts. It was shown that murine maternal DMR (Differentially Methylated Region) of A/B transcripts is methylated while the paternal allele is unmethylated and active (Liu et al., 2000). Our results are in concordance with previous findings since we observed only paternal allele in the samples in which parental origin of the expressed allele could be estimated. This suggests that imprinting status of investigated transcripts is conserved among mammals. It is supposed that noncoding variants may regulate expression of other transcripts of *GNAS* complex locus. Paternal ablation of the A/B DMR results in a derepression of *Gsa* paternal allele in tissues, where it is normally silenced (Williamson et al., 2004; Liu et al., 2005, reviewed in Bastepe 2007). Recently, it has been shown that the administration of short-interfering *GNAS* RNA supports bone regeneration in vitro and in an in vivo model sheep (Rios et al., 2012).

The knowledge about imprinted genes in pigs is still only fragmentary. Currently, imprinting status of 29 porcine genes is established (<http://www.geneimprint.com/site/genes-by-species.Sus+scrofa>), which is a small subset when compared to human or mice databases. The most comprehensive survey of imprinted genes in pigs was performed by Bischoff et al., (2009), who identified 25 differentially expressed genes by expression profiling of parthenote fetuses. Hopefully, the number of examined genes will increase soon since only in the last year imprinting status of *NECD*, *SNRPN*, *UBE3A* (Wang et al., 2012), *CDKN1C*, *NAP1L4* (Li et al., 2012), *NDN*, *MAGEL2* and *MEST* genes was proved (Zhang et al., 2012). On the other hand, Next Generation Sequencing (NGS) method may be a very valuable tool for the efficient identification of imprinted genes. This approach has already been implemented in mice imprinting experiments (Gregg et al., 2010). Nevertheless, some results are conflicting (Hayden, 2012, DeVeale et al., 2012), so the implementation of this method in imprinting experiments in farm animals may give profitable feedbacks.

Figure 3. Chromatograms after sequencing of DNA and cDNA of fragments of GNAS complex locus, containing rs#333005482. A – chromatograms for the animal no 4, B – chromatograms for animal no 7, chromatograms for animal no 8

In conclusion, our results suggest that *GNAS* complex locus produce biallelically expressed transcripts and paternally expressed transcripts in pigs as it was shown previously in mice and human (Hayward et al., 1998 b; Peters et al., 1999). Moreover, imprinting of *GNAS* complex locus in pigs resembles the pattern observed in other mammals, since another *GNAS* transcript – *NESP55* is maternally expressed in pigs, mice and humans (Oczkowicz et al., 2012; Hayward et al., 1998 b; Peters et al., 1999).

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References

- Bastepe M. (2007). The *GNAS* Locus: Quintessential complex gene encoding *Gsa*, *XLas*, and other imprinted transcripts. *Curr. Genomics*, 8: 398–414.
- Bischoff S.R., Tsai S., Hardison N., Motsinger-Reif A.A., Freking B.A., Nonneman D., Rohrer G., Piedrahita J.A. (2009). Characterization of conserved and nonconserved imprinted genes in swine. *Biol. Reprod.*, 81: 906–920.
- Bratuś A., Słota E. (2009). Comparative cytogenetic and molecular studies of DM domain genes in pig and cattle. *Cytogenet. Genome Res.*, 126: 180–185.
- Campbell R., Gosden C.M., Bonthron D.T. (1994). Parental origin of transcription from the human *GNAS1* gene. *J. Med. Genet.*, 31: 607–614.
- Chomczyński P., Sacchi N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, 162: 156–159.
- Congras A., Yerle-Bouissou M., Pinton A., Vignoles F., Liaubet L., Ferchaud S., Aclouque H. (2014). Sperm DNA methylation analysis in swine reveals conserved and species-specific methylation patterns and highlights an altered methylation at the *GNAS* locus in infertile boars. *Biol. Reprod.*, 91: 137.
- De Veale B., van der Kooy D., Babak T. (2012). Critical evaluation of imprinted gene expression by RNA-Seq: a new perspective. *PLoS Genet.*, 8(3): e1002600.
- Elli F.M., Desanctis L., Ceoloni B., Maria Barbieri A., Bordogna P., Beck-Pecoz P., Spada A., Mantovani G. (2013). Pseudohypoparathyroidism type Ia and pseudopseudohypoparathyroidism: The growing spectrum of *GNAS* inactivating mutations. *Hum. Mutat.*, 34: 411–416.
- Gregg C., Zhang J., Butler J.E., Haig D., Dulac C. (2010). Sex-specific parent-of-origin allelic expression in the mouse brain. *Science*, 329: 682–685.
- Hayden E.C. (2012). RNA studies under fire. *Nature*, 484: 428.
- Hayward B.E., Kamiya M., Strain L., Moran V., Campbell R., Hayashizaki Y., Bonthron D.T. (1998 a). The human *GNAS1* gene is imprinted and encodes distinct paternally and biallelically expressed G proteins. *Proc. Natl. Acad. Sci. USA*, 95: 10038–10043.
- Hayward B.E., Moran V., Strain L., Bonthron D.T. (1998 b). Bidirectional imprinting of a single gene: *GNAS1* encodes maternally, paternally, and biallelically derived proteins. *Proc. Natl. Acad. Sci. USA*, 95: 15475–15480.
- Klenke S., Siffert W., Frey U.H. (2011). A novel aspect of *GNAS* imprinting: higher maternal expression of *Gas* in human lymphoblasts, peripheral blood mononuclear cells, mammary adipose tissue, and heart. *Mol. Cell. Endocrinol.*, 341: 63–70.
- Li S., Li J., Tian J., Dong R., Wei J., Qiu X., Jiang C. (2012). Characterization, tissue expression, and imprinting analysis of the porcine *CDKN1C* and *NAP1L4* genes. *J. Biomed. Biotechnol.*, 946527.

- Liu J., Yu S., Litman D., Chen W., Weinstein L.S. (2000). Identification of a methylation imprint mark within the mouse *GNAS* locus. *Mol. Cell. Biol.*, 20: 5808–5817.
- Liu J., Chen M., Deng C., Bourc'his D., Nealon J.G., Erlichman B., Bestor T.H., Weinstein L.S. (2005). Identification of the control region for tissue-specific imprinting of the stimulatory G protein α -subunit. *Proc. Natl. Acad. Sci. USA*, 102: 5513–5518.
- Mantovani G., Ballare E., Giammona E., Beck-Peccoz P., Spada A. (2002). The *Gsa* gene: predominant maternal origin of transcription in human thyroid gland and gonads. *J. Clin. Endocrinol. Metab.*, 87: 4736–4740.
- Moore T., Haig D. (1991). Genomic imprinting in mammalian development: a parental tug-of-war. *Trends. Genet.*, 7: 45–49.
- Oczkiewicz M., Piestrzyńska-Kajtoch A., Ropka-Molik K., Rejduch B., Eckert R. (2012). Expression and imprinting analysis of the *NESP55* gene in pigs. *Gene Expr. Patterns*, 12: 18–23.
- Oczkiewicz M., Ropka-Molik K., Tyra M. (2013). Analysis of the associations between polymorphisms in *GNAS* complex locus and growth, carcass and meat quality traits in pigs. *Mol. Biol. Rep.*, 40: 6419–6427.
- Peters J., Wroe S.F., Wells C.A., Miller H.J., Bodle D., Beechey C.V., Williamson C.M., Kelsey G. (1999). A cluster of oppositely imprinted transcripts at the *GNAS* locus in the distal imprinting region of mouse chromosome 2. *Proc. Natl. Acad. Sci. USA*, 96: 3830–3835.
- Plagge A., Isles A.R., Gordon E., Humby T., Dean W., Gritsch S., Fischer-Colbrie R., Wilkinson L.S., Kelsey G. (2005). Imprinted *NESP55* influences behavioral reactivity to novel environments. *Mol. Cell. Biol.*, 25: 3019–3026.
- Rejduch B., Oczkiewicz M., Piestrzyńska-Kajtoch A., Piórkowska K., Witoń M., Rogoz M., Różycki M. (2010). Expression of *IGFBP-3* and *IGFBP-5* genes in muscles of pigs representing five different breeds. *J. Anim. Feed Sci.*, 19: 554–563.
- Ríos C.N., Skoracki R.J., Mathur A.B. (2012). *GNAS1* and *PHD2* short-interfering RNA support bone regeneration in vitro and in an in vivo sheep model. *Clin. Orthop. Relat. Res.*, 470: 2541–2553.
- Stratil A., Knoll A., Horák P., Bílek K., Bechynová R., Bartenschlager H., Van Poucke M., Peelman L.J., Svobodová K., Geldermann H. (2008). Mapping of the porcine *FBN2*, *YWHAQ*, *CNN3*, *DCN*, *POSTN*, *SPARC*, *RBM39* and *GNAS* genes, expressed in foetal skeletal muscles. *Anim. Genet.*, 39: 204–205.
- Van Laere A.S., Nguyen M., Braunschweig M., Nezer C., Collette C., Moreau L., Archibald A.L., Haley C.S., Buys N., Tally M., Andersson G., Georges M., Andersson L. (2003). A regulatory mutation in *IGF2* causes a major QTL effect on muscle growth in the pig. *Nature*, 425: 832–836.
- Wang M., Zhang X., Kang L., Jiang C., Jiang Y. (2012). Molecular characterization of porcine *NECD*, *SNRPN* and *UBE3A* genes and imprinting status in the skeletal muscle of neonate pigs. *Mol. Biol. Rep.*, 39: 9415–9422.
- Williamson C.M., Ball S.T., Nottingham W.T., Skinner J.A., Plagge A., Turner M.D., Powles N., Hough T., Papworth D., Fraser W.D., Maconochie M., Peters J. (2004). A cis-acting control region is required exclusively for the tissue-specific imprinting of *GNAS*. *Nat. Genet.*, 36: 894–899.
- Yu S., Yu D., Lee E., Eckhaus M., Lee R., Corria Z., Accili D., Westphal H., Weinstein L.S. (1998). Variable and tissue-specific hormone resistance in heterotrimeric G_s protein α -subunit ($G_s\alpha$) knockout mice is due to tissue-specific imprinting of the $G_s\alpha$ gene. *Proc. Natl. Acad. Sci. USA*, 95: 8715–8720.
- Zhang F.W., Han Z.B., Deng C.Y., He H.J., Wu Q. (2012). Conservation of genomic imprinting at the *NDN*, *MAGEL2* and *MEST* loci in pigs. *Genes Genet. Syst.*, 87: 53–58.

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