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# Discovery of single nucleotide polymorphisms in *FABP3* and *leptin* gene in pig (Brief Report)

**Identifizierung von Einzelnukleotid-Polymorphismen im FABP3 und Leptin Gen beim Schwein** (Brief report)

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## Background

Knowledge about structural variations in genes and proteins relevant for fat traits is very important information to improve selection of breeding lines and preserve genetic variability in pig industry. The classical proteomics is a useful tool to separate and measure differentially expressed proteins in fat tissue of phenotypic different individual pigs. Single nucleotide polymorphisms (SNP) in the swine adipocyte *fatty-acid binding protein 3* (*FABP3*) and *leptin* gene (*LEP*) were related to adipocyte accumulation and associated with meat quality in pigs (NECHTELBERGER *et al.* 2001, KULIG *et al.* 2001, KMIEC *et al.* 2003).

## Procedure

*Detection of single nucleotide polymorphisms*

A total of 40 commercial pigs (Landrace × Duroc × Yorkshire) in 2 growth stages (150 and 210 days) were used from Swine Production Division at National Institute of Animal Science (NIAS). Back fat samples were collected and the samples were pooled after adjusting concentration to 6 mg/ml per individual. Isoelectric focusing (IEF) with 24 cm of IPG strips (pH 3-10 non-linear) with 8000 v/h and 12% of SDS-PAGE gels stained with Commassie G-250 were used. After characterization and identification of differentially expressed spots between growth stages, 2 primers were designed from known nucleotide sequences of the corresponding genes *FABP3* (AJ416019) and *LEP* (NM213840). Specific primers were used with 2 ul 10 x reaction buffer, 2.5 mM dNTP, 50 ng of genomic DNA, and 0.2 U of DNA polymerase in a final volume of 20 ul. After heating at 95°C for 2 min, a total of 35 cycles were adapted for denaturation at 94°C / 1 min, annealing at 54~57°C/ 1 min, and polymerization at 72°C/ 2 min. The PCR products for 235 individuals were sequenced directly, and sequences were aligned to find SNPs by DNAstar version 7.0.

*Primer sequences*

*LEP-F*: 5'-CCTGGTTTGGGATTTGTATGC-3'; *LEP-R*: 5'-TGCCTCCTTGTTTGACCTATTG-3'
*FABP3-F*: 5'-GCCCATCCCTTCGACTGTTCC-3'; *FABP3-R*: 5'-TTTTGCCTTGGCTATTTTATTG-3'

## Results

After separation of differentially expressed spots between growth stages by IEF and 2 dimensional electrophoresis, mass spectrometer analysis revealed *LEP* and *FABP3*. Comparative sequencing of amplicons of 235 animals revealed a total of 23 SNPs in *FABP3* containing 1 deletion (at nt121 in acc. no. EU981814) and 3 insertions (at nt127, nt271, and nt328) in Table 1.

Table 1
Detection of SNPs in gene fragments of *FABP3* and *LEP* gene
*Nachweis von SNPs in Genfragmenten vom FABP3- und LEP-Gen*

| *FABP3* | | | | | | | | | | | | *Leptin* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | S | AA | P | S | AA | P | S | AA | P | S | AA | P | S | AA |
| 53 | T/C | S/L | 149 | A/C | Q/R | 256 | T/G | L/V | 306 | T/C/G | – | 256 | T/C | S/X |
| 57 | A/G | – | 155 | A/G | Q/R | 265 | A/C | N/H | 309 | A/T | – | 352 | G/A | – |
| 77 | T/C | L/S | 195 | A/G | – | 271 | –/T | – | 323 | A/G | R/K | | | |
| 108 | A/G | - | 208 | A/C | T/P | 287 | T/C | T/M | 327 | T/C | – | | | |
| 121 | T/D | - | 215 | A/G | K/R | 291 | A/G | – | 328 | –/G | – | | | |
| 127 | –/A | - | 246 | T/C | - | 302 | T/C | A/N | | | | | | |

The obvious point in this analysis was 28 bp insertions (TTGTTTGGGGAACTAGTTAA GACAATAA) between positions 240 and 241, assuming to be repetitive sequences, and 3 nucleotides (T /C /G) at 306 in EU981814. Interesting findings were observed possible haplotypes with 8 SNPs that are always paired with others at positions 256 (G), 265 (C), 271 (T), 287 (T), 291 (G), 306 (G), 323 (A), and 327 (T). Two SNPs were observed in *LEP* with an amino acid change (S/X) at nt 256 of acc. no. FJ154077. In particular the polymorphisms causing amino acid exchanges, which are reported here first, potentials affect the phenotype. However, the further study will be needed to understand genetic functions of the identified SNPs with changing of protein expression in growth stages.

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