



CHARACTERISATION OF POLYMORPHISMS IN THE PORCINE MC3R GENE

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Introduction :

(1)

The **central melanocortin system** is critical for the long-term regulation of energy homeostasis. Important members: *MC1R* (pigmentation regulation) *MC4R*, *MC5R* (complex control of appetite and body weight). Disfunction / over expression - obesity, disturbances of food intake and energy imbalance (human, mice)

However, little is known about the function, localisation and structure of another porcine melanocortin receptor - **MC3R**.

Main Goals to Achieve :

(2)

Structure analysis of the porcine gene *MC3R* using the direct sequencing method of purified PCR product

Detection of new polymorphisms

Verification of polymorphisms by PCR-RFLP method

Material and Methods:

(3)

Partial primary structure: Primer pair A designed from mRNA sequence of the human gene (NM_019888):

Forward: 5'-CTTCGTGCTGCTGCCCTCT-3'

Reverse: 5'-GGGTCGATGACGGAGTTGCAC-3'

Amplicon: 880 bp (EMBL AJ744762)

Analysis of detected SNP polymorphisms

Primer pair B designed:

Forward: 5'-CATCTTCTACGCGCTGCGCTA-3'

Reverse: 5'-CCAGCAGGAGGGAGATGGTCA-3'

Amplicon: 311 bp

PCR conditions:

genomic DNA (50-100 ng), standard PCR buffer, 200 μ M each dNTP, 0,2 μ M

each primer (10 pmol/ μ l), 2.2 mM Mg^{2+} , 1U LA polymerase; cycling

conditions: 95°C/2min; 30 cycles: 95°C/20s, 65°C/20s, 68°C/60s; 68°C/7min

The identity of 880 bp amplicon verified by **direct PCR fluorescent**

terminator sequencing (ABI PRISM 3100 Avant Genetic Analyser, Applied Biosystems) and two polymorphisms were detected (**figure 1**)

RFLP digestion of 311 bp PCR product performed with 5U of *MnII*

or *DdeI* restriction enzyme at 37°C overnight (**figure 2**)

Allele frequencies in five pig breeds are given in **table 1**

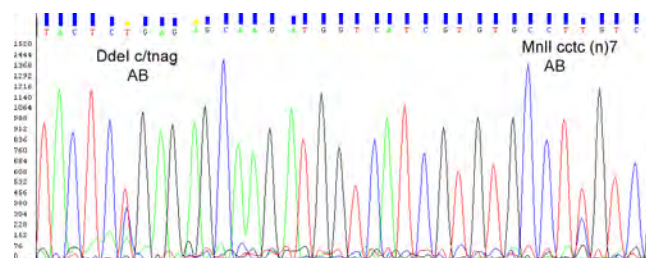


Fig.1: Electroforetogram showing polymorphisms in heterozygous sample

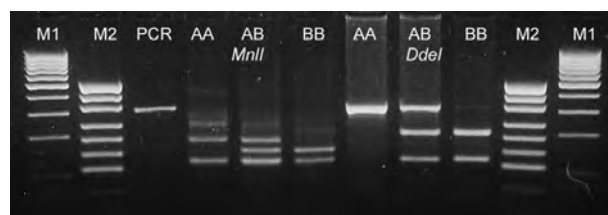


Fig.2: Agarose gel (4%) showing *MnII* and *DdeI* genotypes in porcine *MC3R* gene. *MnII* RFLP: AA (179, 120, 12 bp), AB (179, 149, 120, 30, 12 bp), BB (149, 120, 30, 12 bp); *DdeI* RFLP: AA (311 bp), AB (311, 197, 114 bp), BB (197, 114 bp).

Results :

(4)

Partial primary structure of porcine *MC3R* gene was determined

Comparative sequencing of 880 bp amplicons revealed two C/T substitutions located at positions 552 and 549 bp (silent mutations)

839 bp sequence AJ744762 (without A primers) was deposited in the EMBL/GenBank/DDJ databases

Codominant mendelian inheritance of polymorphic loci was confirmed in USDA-MARC backcross pedigree

Two point linkage analysis assigned porcine *MC3R* gene to **chromosome 17** (*in press*)

The porcine *MC3R* maps to chromosome region harbouring QTL fat traits in German MeishanXPietrain pedigree

Breed	No. of animals	<i>MnII</i>		<i>DdeI</i>	
		A	B	A	B
Large White	10	0.65	0.35	1	0
Landrace	13	0.85	0.15	1	0
Pietrain	11	0.7	0.3	0.77	0.23
Hampshire	3	0.83	0.17	1	0
Duroc	10	1	0	1	0

Tab.1: Allele frequencies at *MnII* and *DdeI* loci in different breeds